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Effect of latex harvesting techniques on agronomic and physiological parameters of IRCA 111 and IRCA130 clones of Hevea Brasiliensis (Muell. Arg.) West of Côte D'ivoire

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Abstract
Lack of knowledge of latex harvesting techniques from Hevea brasiliensis clones results in a high rate of unproductive trees and a drop in rubber production. In order to improve these techniques and to determine the stimulation regime allowing the clones to express their production potential, a study was undertaken for 6 years in the south-eastern part of the Ivory Coast. It focused on clones of Hevea brasiliensis with active metabolism (IRCA 111 and IRCA 130) planted at a density of 510 trees / ha (7 m X 2.80 m), bled in a descending semi-spiral every 4 days (S / 2 d4) with a day of rest (Sunday) and stimulated with Ethephon at 2.5% at frequencies varying from 0 to 26 stimulations per year. The parameters measured were rubber production, isodiametric trunk growth, physiological profile, sensitivity to dry notching and tree metabolic index. The results of this experiment showed that beyond six and four annual stimulations respectively for the clones IRCA 111 and IRCA 130, any increase in the frequency of stimulation becomes very stressful for the tree, leading not only to a drop in production. Of rubber, but also that of the growth in thickness. Also, the stimulation had a detrimental effect on the sugar content of the IRCA 130 clone. It had no significant effect on the inorganic phosphorus content of the clones studied, but on the other hand it caused a decrease. Their content of thiol compounds. As for the sensitivity to dry notch, it is noticeable in these two clones of the rapid metabolic activity class. However, the clones displayed a good physiological profile with optimal stimulation.

Keywords: *Hevea brasiliensis*, latex harvesting technique, ethylenic hormonal stimulation, Ethephon, descending half-spiral.

1. Introductions

The technology of harvesting latex for the production of natural rubber is carried out by the operations related to the bleeding system and the hormonal simulation strategy. The main component of the first operation, which is tapping, consists of making a notch called a bleeding notch in the bark of the tree in order to extract the latex (Gomez, 1982; Thomas *et al*., 1995) [14, 35]. The second, which is carried out by applying the stimulating product to the bleeding notch, makes it possible to substantially increase the yield of trees and consequently that of plantations (Eschbach and Tonnelier, 1984; Obouayeba, 1993) [8, 28]. However, not all clones have the same response to this stimulation. Some react well, others respond less well and still others tolerate little stimulation (Eschbach *et al.*, 1984, Prévôt *et al*., 1986, Lacrotte, 1991) [8, 31, 20]. To this end, the work of Jacob *et al*. In 1988 [15] on the clonal typology of metabolic functioning of the laticiferous system defined three classes of metabolic activities: fast or strong, moderate or intermediate and slow. These correspond respectively to the classes which respond poorly, moderately and well to hormonal stimulation. In addition, the practice of stimulation is poorly mastered. Indeed, it appears more and more with the use, an abusive use of stimulants to hope to increase the production of rubber. However, overstimulation can in the long term stop the flow of latex from trees with dry notches and lead to a drop in rubber production. The resurgence of this phenomenon is said to result from the lack of knowledge of the metabolism of the *Hevea brasiliensis* clones and of their potential for rubber production. Therefore, determining the stimulation regime suitable for each Hevea brasiliensis clone is essential for mastering the latex harvesting technique. Thus, for 6 years, a study of the effects of different annual frequencies of ethylenic stimulation on the rubber productivity of the IRCA 111 and IRCA 130 clones of Hevea brasiliensis, belonging to the class of rapid metabolic activity, was it. business.

2 Methods

2.2 Materials and methods

2.2.1 Plant material

The plant material used consists of two clones of *Hevea brasiliensis* (Mueller Argoviensis Euphorbiaceae), belonging to the class of rapid metabolic activity. These are the IRCA 111 and IRCA 130 clones.

2.2.2 Experimental protocol

Two experiments were carried out in descending bleeding over nine years with the IRCA 111 and IRCA 130 clones :

- The IRCA 111 clone was planted in June 1999 and tapped in March 2005, at the age of 4 years and 10 months, with a circumference between 48.8 and 51.2 cm,
- The IRCA 130 clone was planted in June 2001 and tapped in December 2007, at the age of 5 years and 6 months, with a circumference varying from 50.0 to 54.7 cm.

The statistical device used is the One tree plot design, a tree constituting a repetition. In each experiment, the trees selected are divided into 9 distinct treatments (Table I), at the rate of 33 trees per treatment. They are taped in a semi-spiral every 4 days, with a rest day (Sunday) in the week, 12 months out of 12 (S / 2 d4 6d / 7 12m / 12) and are subjected to different hormonal stimulation regimes as shown in Table I (Lukman, 1983; Vijayakumar, 2008 and Vijayakumar *et al*., 2009) [22, 37, 38].

Table I: List of the different treatments applied to the IRCA 111 and IRCA 130 clones

N ° Treatments	Latex harvesting technology	Description
1	Absolute witness	Not taped
2	S/2 d4 6d/7 12 m/12.and 2,5% Pa1(1) 0/y*	tapping in a descending semi-spiral every 4 days with 1 day of rest per week, 12 months out of 12; 2.5% Ethephon stimulation at à rate of 1 g of stimulating mixture *per tree on a 1 cm wide strip, zero annual application.
3	S/2 d4 6d/7 12 m/12.and 2,5% Pa1(1) 1/y*	tapping in a descending half-spiral every 4 days with 1 day of rest per week, 12 months out of 12; stimulation with 2.5% Ethephon at a rate of 1 g of stimulating mixture *per tree on a strip 1 cm wide, one application per year.
4	S/2 d4 6d/7 12 m/12. And 2,5% Pa1(1) 2/y*	tapping in a descending half-spiral every 4 days with 1 day of rest per week, 12 months out of 12; 2.5% Ethephon stimulation at a rate of 1 g of stimulating mixture *per tree on a 1 cm wide strip, 2 applications per year.
5	S/2 d4 6d/7 12 m/12.and 2,5% Pa1(1) 4/y (m)	tapping in a descending half-spiral every 4 days with 1 day of rest per week, 12 months out of 12; 2.5% Ethephon stimulation at a rate of 1 g of stimulating mixture *per tree on a 1 cm wide strip, 4 applications per year.
6	S/2 d4 6d/7 12 m/12.and 2,5% Pa1(1) 6/y (3w)	tapping in a descending half-spiral every 4 days with 1 day of rest per week, 12 months out of 12; 2.5% Ethephon stimulation at a rate of 1 g of stimulating mixture *per tree on a 1 cm wide strip, 6 applications per year.
7	S/2 d4 6d/7 12 m/12.and 2,5% Pa1(1) 8/y (2w)	tappinging in a descending half-spiral every 4 days with 1 day of rest per week, 12 months out of 12; 2.5% Ethephon stimulation at a rate of 1 g of stimulating mixture *per tree on a 1 cm wide strip, 8 applications per year.
8	S/2 d4 6d/7 12 m/12.and 2,5% Pa1(1) 13/y (w)	tapping in a descending half-spiral every 4 days with 1 day of rest per week, 12 months out of 12; 2.5% Ethephon stimulation at a rate of 1 g of stimulating mixture *per tree on a 1 cm wide strip, 13 applications per year.
9	S/2 d4 6d/7 12 m/12.and 2,5% Pa1(1) 26/y (4d)	tapping in a descending half-spiral every 4 days with 1 day of rest per week, 12 months out of 12; 2.5% Ethephon stimulation at a rate of 1 g of stimulating mixture *per tree on a 1 cm wide strip, 26 applications per year.

2.2.3 Measurements taken

Rubber production was determined from rubber weighings, tree by tree, carried out every 4 weeks. The transformation coefficient was determined by treatment. This coefficient made it possible to obtain, from the fresh weight, the production of dry rubber in grams per tree and per taping (g / t / t). By considering the variation in circumference at different levels of the tree trunks, it was possible to express the rubber production as a function of the tapping notch and this, thanks to the estimation of the length of the tapping notch (L.E.S.E) as a function of the circumference at 1.70 m from the ground (Obouayeba *et al*., 1996) [27].

L.E.S.E (cm) = Circumference at the end of the experiment (cm) / 2 Cos 30 °

The dry rubber production was therefore expressed in $g.t^{-1}.t^{-1}.cm^{-1}$ of the tapping notch, over a period of 6 years. The following measurements were taken annually and more precisely every month of November:

- Circumference at 1.70 m from the ground, expressed in centimeter (cm)
- Dry notch survey (Van de Sype, 1984), in order to determine the percentages of diseased notch length (% LEM) and totally dry trees (% Arb.secs).

The latex taken was used for the assay and measurement of physiological parameters by the latex micro-diagnostic method (MDL, Jacob *et al*., 1988) [15]. These were also measured annually. The parameters taken into account in the MDL were the solids content (Ex.S), the sucrose (Sac), inorganic phosphorus (Pi) and thiol compounds (RSH) contents of the latex. In practice, the dry extract is determined from one (1) ml of latex taken and weighed before and after placing in an oven for 24 hours. The difference in weight expressed as a percentage indicates the dry extract. Inorganic phosphorus, sugars and thiol compounds were measured from serum trichloroacetic acid (TCA). This serum was obtained by mixing 1 ml of latex and 9 ml of 2.5% TCA. Using a rod, the coagulated rubber was squeezed out and then separated from the 2.5% TCA. Finally, the various dosages were carried out:

- Sucrose (Sac) was determined by the anthrone method of Ashwell (1957) [2] in the presence of concentrated acid,

the hexoses dehydrate to give a furfural which reacts with the anthrone giving a blue-green color whose optical density (OD) is read at the wavelength of 627 nm;
- Inorganic phosphorus (Pi) was determined by the method of Taussky and Shorr (1953) [33], a method using ammonium molybdate: the phosphorus is complexed by an excess of ammonium molybdate. The complex formed is then reduced with ferrous sulphate, giving a blue coloration whose optical density (O.D.) is read at the wavelength of 680 nm;
- The assay of thiol compounds (R-SH) was carried out by the method of Boyne and Ellman (1972) using dinitro-2,2'-dithio-5-5'-dibenzoic acid (DTNB): compounds R - SH react with this acid to give thio-nitro-benzoic acid (TNB) absorbing strongly at 412 nm (Ellman reaction); TNB is revealed by Tris buffer (yellow coloration).

Then, the variation (Δ) between the parameters zero (parameter measured without stimulation) and max (parameter measured with the stimulation frequency assumed to give the highest production of rubber), was determined. This represents the effect of the number of annual stimuli on the metabolism of laticifers and the increase in rubber production. It is calculated as follows:
- ΔProduction = Pmax - P0 = ΔP
- ΔIncrease = AccP0 - AccPmax = ΔAcc
- ΔSecret extract = EXSPmax - EXSP0 = ΔEXS
- ΔSucrose = SacP0 - SacPmax = ΔSac
- ΔInorganic phosphorus = PiPmax - PiP0 = ΔPi
- ΔThiol compounds = RSHPmax - RSHP0 = ΔRSH

Thus, the metabolic index, which is the product of the content of inorganic phosphorus and the content of thiol compounds, was determined. It gives information on the physiological profile of the clones after stimulation (Gohet, 1996) [13]. Indeed, the use of ethylene stimulation has the effect of increasing the metabolic activation of the laticigen system and therefore increasing its yield. However, its use must be reasoned because of the danger it can represent by activating the processes, namely, the fall of thiol compounds. Moreover, as some previous results have shown, overstimulation results in decreased production and the onset of dry notch syndrome (Jacob *et al*., 1994) [32]. The metabolic index that we define as the product of the average Pi and R-SH contents (Pi x R-SH), makes it possible to identify the stimulation intensities making it possible to obtain a good metabolic activation of the laticiferous tissues (high Pi : energized laticiferous cells) while maintaining good latex stability (high R-SH: membrane stability provided by lutoids and sign of sufficient metabolic energy to induce optimal regeneration of R-SH). The physiological optimum will thus be obtained for a stimulation intensity making it possible to obtain a maximum metabolic index, ensuring the best compromise between metabolic activation and membrane protection.

2.2.4 Statistical analysis
Data on rubber production ($g.t^{-1}.t^{-1}.cm^{-1}$), isodiametric growth of the trunk (cm), latex micro-diagnosis and metabolic index were processed using the XL-statistical software. STAT. An analysis of variance was carried out and the level of significance of the differences between the means was estimated by the Newman-keuls test for a threshold of 5%.

3. Results
3.1 Under regime without hormonal stimulation of rubber production
3.1.1 Rubber production
After 6 years of experimentation without stimulation, the IRCA 111 clone had the highest rubber production. However, that of the IRCA 130 clone was at a good level (Table II).

Clone IRCA 111

The stimuli did not have a marked effect on rubber production. However, it varied very little between treatments and reached its maximum with six stimulations per year. Rubber productions were similar for all trees in all treatments. However, the 6 / y trees showed the best rubber yields with 1.31 $g.t^{-1}.t^{-1}.cm^{-1}$ tapping notch.

Clone IRCA 130

Rubber production increased gradually with the number of annual stimulation. It reached its maximum with eight stimulations per year, then gradually declined. The trees from the 8 / y treatment gave the highest rubber production (1.71 $g.t^{-1}.t^{-1}.cm^{-1}$) which was similar to the yields from the trees from the 1 / y, 2 / y, 4 / y, 6 / y and 13 / y and different from those of the processing trees 0 / y and 26 / y. The trees in treatment 0 / y recorded the lowest rubber production. But, there was no significant stimulation effect after four annual applications.

Table II: Average annual rubber production in $g.t^{-1}\ t^{-1}cm^{-1}$ and average increase in circumference in cm of clones IRCA 111 and IRCA 130 after 6 years of latex harvest with ethylenic stimulation

	Production g/t/t/cm		Increase (cm)	
Traitments	**IRCA 111**	**IRCA 130**	**IRCA 111**	**IRCA 130**
Non saigné	/	/	50,72 a	38,84 a
0/y	1,11 a	1,40 c	26,68 b	21,38 b
1/y	1,14 a	1,49 abc	25,38 bc	20,53 b
2/y	1,14 a	1,57 abc	26,69 b	18,96 b
4/y	1,22 a	1,65 ab	23,56 bc	19,84 b
6/y	1,31 a	1,63 ab	23,87 bcd	14,44 c
8/y	1,27 a	1,71 a	21,68 bcd	14,27 c
13/y	1,24 a	1,52 abc	20,61 cd	13,1 c
26/y	1,22 a	1,44 bc	19,57 d	12,8 c
Δ	0,2	0,25	2,81	1,54

In each column, the means assigned the same letter are not significantly different (Newman-Keuls test at 5%). (g / t / t / cm): gram per tree per centimeter of bleeding notch

3.2.2 Increase in the circumference of the tree trunk
The average annual increases in the circumference of the trunk of the trees of the various clones studied, expressed in cm, are given in Table III. Whatever the clone, three cases arise:
- Non-tapped trees have mean increases in circumference significantly greater than those of bled trees.
- Tapped and unstimulated trees have average increases in circumference greater than those of stimulated trees.
- Stimulated trees show less average increases in trunk circumference with the increase in the number of annual stimulations.

It is noted, however, in the case of the IRCA 130 clone that from 0 to 4 / y and from 6 to 26 / y distinctly, this decrease is statistically of the same order of importance. The growth displayed by the trees of treatments 6 to 26 / y is significantly lower than that of the trees of treatments 0 to 4 / y.

3.2.3 Physiological profile
The results of the physiological parameters are reported in Table IV.

3.2.3.1 Dry Extract Rate
The dry extract levels of the trees from the different treatments are expressed as a percentage (%).

Clone IRCA 111

There was a sharp drop in the solids content in the trees of the 13 / y and 26 / y treatments. Trees from treatment 2 / y exhibited the highest solids content. It was statistically similar to tree rates for 0 / y, 1 / y, 4 / y, 6 / y, and 8 / y treatments, and higher than for other treatments. The trees in the 13 / y treatment showed a higher dry extract rate than the trees in the 26 / y treatment, which had the lowest level. But, on the whole, the dry extract rates observed are high.

Clone IRCA 130

The dry extract levels are high and did not vary regardless of the intensity of stimulation.

Table III: Evolution of physiological parameters of IRCA 111 and IRCA 130 clones after 6 years of ethylene stimulation

		IRCA111				IRCA 130		
Treatments	EXS	SAC	Pi	RSH	EXS	SAC	PI	RSH
	%	mmol.l^{-1}	mmol.l^{-1}	mmol.l^{-1}	%	mmol.l^{-1}	mmol.l^{-1}	mmol.l^{-1}
0/y	48,54 a	8,00 a	16,34 a	0,40 a	58,50 a	12,15 a	11,48 a	0,58 ab
1/y	49,40 a	6,44 a	13,79 a	0,37 a	58,78 a	15,37 a	14,41 a	0,67 a
2/y	50,25 a	5,06 bc	14,92 a	0,40 a	56,42 a	12,39 a	14,58 a	0,63 ab
4/y	47,44 a	4,41 bc	16,10 a	0,33 abc	59,69 a	9,56 a	14,15 a	0,54 abc
6/y	46,15 ab	5,24 bc	16,31 a	0,30 bcd	58,09 a	11,62 a	13,29 a	0,5 bc
8/y	45,41 ab	2,72 c	14,36 a	0,26 cd	59,99 a	8,86 a	11,18 a	0,38 c
13/y	41,41 b	2,65 c	12,19 a	0,25 d	59,52 a	8,18 a	11,86 a	0,40 c
26/y	36,38 c	2,70 c	13,01 a	0,24 d	57,54 a	9,2 a	14,18 a	0,42 c
Δ	-2,39	2,76	-0,03	-0,1	1,19	2,59	2,67	-0,04

Dans chaque colonne, les moyennes affectées de la même lettre ne sont pas significativement différentes (test de Newman-Keuls à 5 %).
(mmol.l-1): millimoles par litre; (%) : pourcentage; Ex.S: extrait sec; Sac: saccharose Pi: phosphore inorganique; RSH: composés de thiols.

3.2.3.2 Sucrose content
The sucrose contents of different trees can be expressed in millimoles per Liter (mmol.l^{-1}).

Clone IRCA 111

The sucrose content of the trees experienced a steady decrease as the stimulation intensity increased. It was more remarkable from 8 / y. All of the stimulated trees exhibited a lower sucrose content lower than that of the unstimulated control trees. Sucrose contents of trees from all treatments were low but characteristic of clone IRCA 111. Trees from treatment 0 / y showed the highest content. It was similar to that of the trees in the 1 / y treatment and greater than that of the other treatments. The trees from the 2 / y, 4 / y and 6 / y treatments recorded sucrose contents similar but different from those of the trees from the 8 / y, 13 / y and 26 / y treatments. The latter had the lowest and equivalent contents.

Clone IRCA 130

The sucrose contents of the trees of the different treatments are good, they did not vary significantly with the increase in the number of annual stimulation. The average content obtained is 10.92 mmol.l^{-1}. However, the trees from the 0 / y, 1 / y, 2 / y and 6 / y treatments showed grades above this average.

3.2.3.3 Inorganic phosphorus content
The inorganic phosphorus contents of the trees from the different treatments are expressed in millimoles per liter (mmol.l^{-1}).

Clone IRCA 111

The inorganic phosphorus contents of the tree latex from the different treatments were all equivalent and average (14.63 mmol.l^{-1}) relative to the characteristic values of the clone. The trees from the 0 / y, 4 / y and 6 / y treatments showed the highest levels.

Clone IRCA 130

The inorganic phosphorus contents of the latex of the trees of the different treatments were all similar and average (13.14 mmol.l^{-1}) compared to the characteristic values of the clone. However, the trees from the 1 / y, 2 / y and 4 / y treatments showed the highest levels.
In general, after 6 years under a stimulation regime, the inorganic phosphorus content of the latex of the clones studied was not significantly influenced by the frequency of stimulation applied.

3.2.3.4 Content of thiol compounds
The contents of thiol compounds in the trees of the various treatments are expressed in millimoles per liter (mmol.l^{-1}).

Clone IRCA 111

The average content of thiol compounds in the latex is moderate (0.31 mmol.l^{-1}). It varies significantly depending on the frequency of hormonal stimulation. The trees of the 0 / y, 1 / y, 2 / y and 4 / y treatments presented equivalent contents, but higher than those of the trees of the other treatments. The 6 / y treatment trees showed a different thiol content than the 8 / y, 13 / y and 26 / y treatment trees. The thiol content of the trees from the 8 / y treatment was higher than those from the trees from the 13 / y and 26 / y treatments, which had the lowest levels. A decrease in the content of thiol compounds was observed with the increase in the number of annual stimulations.

Clone IRCA 130

The average content of thiol compounds in the latex is average

(0.51 mmol.l^{-1}). It is influenced by the frequency of hormonal stimulation. Trees from treatment 1 / y showed the highest content of thiol compounds. It was similar to those of the 0 / y, 2 / y and 4 / y processing trees. The thiol content of the trees from the 6 / y treatment was higher than those from the trees from the 8 / y, 13 / y and 26 / y treatments, which showed the lowest levels, but statistically equivalent to each other. The content of thiol compounds has fallen steadily with the increase in the number of annual stimulations.

Under the stimulation regime, the various clones studied showed a decrease in their content of thiol compounds linked to the increase in the annual number of stimulation.

3.2.3.4 Evolution of sensitivity to dry notch

The average values of the percentages of diseased notch length (LEM%) and dry trees (dry Arb%) Are given in Table V.

Clone IRCA 111

The rate of diseased notch length (LEM), the highest (27.3%) was obtained with the shafts of the 26 / y treatment and the lowest (2.5%) with the shafts of the 8 / y treatment.. The 2 / y and 13 / y treatment trees showed high percentages of LEM (12.3% and 18.86% respectively). At the level of the 0 / y treatment trees (control), an LEM rate of 3.2% was recorded. This rate is higher than that of the trees in the 8 / y treatment. The highest rate of dry trees (6.7%) was observed with trees from treatment 2 / y and the lowest (0%) with trees from treatments 1 / y, 6 / y, and 8 / y. Unstimulated trees (0 / y treatment) showed a dry tree rate of 3.2%.

Clone IRCA 130

The highest rate of diseased notch length (38%) was observed in trees on the 26 / y treatment and the lowest (4.4%) on trees on the 4 / y treatment. Treatment 13 / y trees exhibited a high percentage of LEM (21%). The level of LEM of the trees of the 0 / y treatment (control, unstimulated) is high (9.7%). This rate is higher than those of trees stimulated annually 1 / y, 2 / y, 4 / y, 6 / y and 8 / y. The highest rate of completely dry trees (6.5%) was obtained with trees from the 13 / y treatment and the lowest (0%) with trees from 2 / y and 6 / y treatments. The control trees (0 / y treatment) presented a rate of dry trees of 3.2%, identical to those of the trees of the 1 / y and 8 / y treatments and very pocketed of those of the trees of the 4 / y and 26 / y.

The highest percentages of diseased notch length were obtained with the maximum stimulation frequencies:

- 27.3% of LEM with 26/ y for the PB 235 clone (Table V).
- 38% of LEM with 26 / y for the PB 260 clone (Table V).
- Both experiments show a proportion of completely dry trees in all trees from all treatments, including controls.

Table IV: Percentages of diseased notch length and dry trees of clones IRCA 111 and IRCA 130 after 6 years of Stimulation

	IRCA 111		IRCA 130	
Treatment	**LEM (%)**	**Dry trees (%)**	**LEM (%)**	**Dry trees (%)**
0/y	3,2	3,2	9,7	3,2
1/y	4	0	6,5	3,2
2/y	12,3	6,7	5,3	0
4/y	5,2	3	4,4	3,1
6/y	5,7	0	5,8	0
8/y	2,5	0	5,5	3,2
13/y	18,6	3,4	21	6,5
26/y	27,3	3,8	38	3,1
(%): parentage				

3.2.3.5 Metabolic index

The mean values of the metabolic indices of the IRCA 111 and IRCA 130 clones are given in Table VI.

Clone IRCA 111

The best metabolic index was obtained with the trees of the 0 / y treatment. It is statistically comparable to those of the trees of the 1 / y, 2 / y, 4 / y and 6 / y treatments and higher than those of the trees of the 8 / y, 13 / y and 26 / y treatments. The metabolic index of the 1 / y, 4 / y, 6 / y and 8 / y treatment trees are of the same order. Those of the trees of the 13 / y and 26 / y treatments are of the same importance and significantly lower than the metabolic indices of the other treatments. The detrimental effect of stimulation on the metabolic index of clone IRCA 111 was expressed from eight stimulations per year.

Clone IRCA 130

The trees from the 1 / y and 2 / y treatments showed the highest metabolic indices. It was of the same order of magnitude from 0 / y to 6 / y, then weakened with the intensity of the stimulation. But, the trees from the 26 / y process showed a similar index to those from the control trees and from the 1 / y, 2 / y, 4 / y, and 6 / y treatments. The depressive effect of the stimulation on the metabolic index of clone IRCA 130, was noticeable from eight annual stimulations.

The detrimental effect of the stimulation of the stimulation on the metabolic index is marked in clones IRCA 111 and IRCA 130 beyond six stimulations per year.

Table V: Metabolic index of IRCA 111 and IRCA 130 clones after 6 years of stimulation

Treatments	IRCA 111	IRCA 130
0/y	6,77 a	6,83 ab
1/y	5,14 ab	9,85 a
2/y	6,01 a	9,37 a
4/y	5,31 ab	7,82 ab
6/y	4,96 ab	7,03 ab
8/y	3,90 bc	4,24 b
13/y	3,08 c	4,84 b
26/y	3,08 c	6,34 ab

In each column, results assigned the same letter are not significantly different (5% Newman-Keuls test).

4. Discussion

4.1 Behavior of clones in the absence of stimulation

4.1.1 Production

The observation of physiological and rubber production parameters, obtained in a bleeding system without stimulation, supports the notion of clonal typology of metabolic functioning (Eschbach *et al*., 1984; Jacob *et al*., 1995 and Gohet *et al*., 1996) [8, 17, 13]. The results of our work on the IRCA 111 and IRCA 130 clones indicate that in the absence of hormonal stimulation, they display:

- A high production of rubber, indicative of a high intrinsic metabolic activity,
- A high content of inorganic phosphorus (Pi) in the latex, a sign of energized cells,
- A low to very low sugar content,
- A high dry extract rate.
- These characteristics obtained with these clones confirm their belonging to the class of clones with active metabolism.

4.1.2 Increase in trunk circumference

In the regime without stimulation, the trunk of the IRCA 111 clone showed a greater increase in circumference than that of the IRCA 130 clone. This is probably due to the fact that the IRCA 111 clone has a stronger vegetative growth before bleeding than that of the IRCA 111 clone. Clone IRCA 130 (Anonymous, 1988) [1]. Also, the rubber productions recorded in these clones give indications as to the distribution of photosynthetic assimilates. The latter in clone IRCA 111 were oriented towards the creation of primary biomass (growth) while in clone IRCA 130, they favored the creation of secondary biomass (rubber). Indeed, as Templeton (1969) [34] have shown, primary biomass losses are not only caused by the production of rubber but also, they can only be explained by this production alone.

4.1.3 Physiological profile

The study of physiological parameters focused on the level of dry extract, the contents of sucrose, inorganic phosphorus and thiol compounds.

The dry extract of the latex, which corresponds to its rubber content, reflects the biosynthetic activity of laticifers. The respective levels of the two clones were raised as part of the bleeding without stimulation of production. However, the IRCA 130 clone has a higher solids content than that of the IRCA 111 clone. This is explained by the fact that the clones with active metabolism have the capacity to rapidly regenerate their cellular content exported during bleeding, thanks to their rapid metabolism (Eschbach *et al*., 1984; Serres *et al*., 1994; Jacob *et al*., 1995 and Gohet *et al*., 1996) [8, 32, 17, 13]. Also, there could be differences in the metabolic functioning of these clones (Koffi *et al*., 2007) [20]. This situation probably explains the high regeneration capacity of the IRCA 130 clone compared to the IRCA 111 clone.

With regard to sucrose, it is the major product of photosynthesis and constitutes the raw material necessary for all cellular activity. The sucrose content is admittedly low for the two clones, but it is in conformity with that of the clones of this metabolic class. In fact, sucrose can be a limiting factor in rubber production (Lacrotte, 1991) [21]. In clone with rapid metabolism (IRCA 111 and IRCA 130), its low content is explained by its rapid use for metabolism with a view to high rubber production and / or better vegetative growth (Eschbach *et al*. 1986) [31]. This is reflected by their rapid entry into production which characterizes them (Anonymous, 1988). Also, the difference in sucrose content of these clones was expressed by their respective productions and increases.

The content of inorganic phosphorus, which includes the energy necessary for the metabolic activity of the clones, is lower the more the metabolism of the clone is slow and vice versa. Both IRCA 111 and IRCA 130 clones displayed high levels of inorganic phosphorus due to their very active metabolism. They therefore have an internal energy reserve allowing them to have a strong metabolic activity. Nevertheless, that of the IRCA 111 clone was stronger than that of the IRCA 130 clone. This indicates that there was a better availability of the energy necessary for the metabolism (Jacob *et al*., 1988) [15] in the two clones. The inferiority of the Pi content of the IRCA 130 clone compared to that of the IRCA 111 clone is explained by the respective productions recorded in these clones.

4.1.4 Sensitivity to dry notch

At zero stimulation, the sensitivity to dry notch of the studied clones is important. The IRCA 130 clone displayed the highest percentage of LEM (9.7%) compared to the IRCA 111 clone (3.2%). This is in accordance with the observations made on these clones that clone IRCA 130 is more sensitive to dry notch than clone IRCA 111 (Anonymous, 1993) [1]. The IRCA 130 clone, despite its good content of sucrose and thiol compounds, however, showed a greater sensitivity to dry notch than the IRCA 111 clone. This observation is therefore in contradiction with that of Gohet (1996) [13]. Also, it reveals that the contents of sucrose and thiol compounds of a given clone may be higher than those of another clone of the same class of metabolic activity, but they would be insufficient for this clone. Indeed, the sensitivity of the clones to the dry notch is specific to the class of metabolic activity (Eschbach *et al*., 1984; Serres *et al*., 1994; Jacob *et al*., 1995 and Gohet *et al*., 1996) [8, 32, 17, 13]. The clones with active metabolism (IRCA 111 and IRCA 130) show a high sensitivity to this disease due to their low content of thiol compounds (sign of the destabilization of lutoids), as indicated by Chrestin in 1984 [5].

4.2 Behavior of clones with stimulation

4.2.1 Production

In clone IRCA 111, statistically, stimulation did not have a marked effect on rubber production. Nevertheless, this, taken in absolute value, varied very little between the treatments. She reached her maximum with six stimulations per year, then began to fall. For the IRCA 130 clone, rubber production increased gradually with the number of annual stimulation. It reached its optimum with four annual stimuli, then gradually declined. However, from one to thirteen stimulations per year the effect on tree production remained the same in this clone. These observations reflect the functional dysfunction of the laticiferous system which results in the impossibility of any activation of the metabolism by hormonal stimulation. This metabolic inactivation is obtained for stimulation intensities as much lower as the intrinsic laticiferous metabolism is active and vice versa (Gohet, 1996) [13]. This is the confirmation that these clones belong to the class of clones with a very active metabolism which only require little stimulation for the activation of their metabolism (Eschbach *et al*., 1984; Serres *et al*., 1994; Jacob *et al*., 1995 and Gohet *et al*., 1996) [8, 32, 13].

This information obtained on the production of rubber constitutes clues which can lead to the determination of the optimum frequency of ethylenic stimulation of the clones studied. However, studying the other parameters will give more precision.

4.2.2 Isodiametric growth of the trunk

The mean gain in girth of non-bled trees was greater than that of bled trees. It is the fact that the tapping of the rubber trees which is accompanied by a flow of latex, is translated by the diversion of a fraction of the photosynthetic assimilates, strictly affected to the general metabolism of the tree and to the creation of primary biomass (wood and bark). Therefore, the metabolism is oriented towards a regeneration of the cellular content exported during this bleeding and which corresponds to the synthesis of secondary biomass (rubber) as shown by Jacob *et al*. in 1995. As a result, there is a reduction in vegetative growth. This is materialized by a reduction in the circumference of the trunk of trees bled compared to that of non-bled trees (Gohet, 1996) [13]. Our results thus obtained are analogous to the observations of Paardekooper and Obouayeba and Boa (1993) [28]. These authors have shown that bleeding is inevitably accompanied by a reduction in the rate of vegetative growth.

The intensification by ethylenic stimulation of the technique of harvesting latex from a tree bled 3 times a fortnight (d4 6d / 7) was manifested by a reduction in the circumference of the trunk, compared to an unstimulated control. This is because the available tree energy and photosynthetic assimilates have been used for latex production to the detriment of vegetative growth (Gohet, 1996) [13]. Thus, the average increase in circumference of unstimulated trees (0 / y treatment) was greater than that of stimulated trees. In fact, hormonal stimulation, by its effect, increases the production of rubber by strongly orienting the hydrocarbon assimilates towards it to the detriment of the growth in thickness. This results in a decrease in the trunk circumference of stimulated trees compared to unstimulated trees (Templeton 1969; Gohet 1996; Obouayeba *et al*., 2002 and Obouayeba, 2005) [34, 13, 25].

4.2.3 Physiological profile

Dry extract rate

In the IRCA 111 clone, there was a strong drop in the dry extract level at the level of the trees of the 13 / y and 26 / y treatments while in the IRCA 130 clone, the dry extract levels were high and did not vary regardless of the intensity of stimulation. This is explained by the fact that there was an efficient regeneration of the latex in the IRCA 130 clone (Milford *et al*., 1969) [23], while the IRCA 111 clone showed metabolic dysfunction due to physiological fatigue (Gohet, 1996) [13]. In addition, these observations attest that the IRCA 130 clone regenerates its cellular content more efficiently than the IRCA 111 clone.

Sucrose happy

In the IRCA 111 clone, the sucrose content of the trees decreased steadily as the stimulation intensity increased. It was more remarkable from 8 / y. All of the stimulated trees had a lower sucrose content than the trees of the unstimulated control. On the other hand, in the IRCA 130 clone, the sucrose contents of the trees from the different treatments were good and did not vary with the increase in the number of annual stimulation. There was therefore a consumption of intralaticiferous sugar for the biosynthesis of rubber (Lacrotte, 1991) [21] in the case of the IRCA 111 clone. In fact, the IRCA 111 clone, a clone with a very active metabolism, has a relatively low sucrose content and the ethylenic stimulation only slightly activates its metabolism (Eschbach *et al*., 1984; Serres *et al*., 1994; Jacob *et al*., 1995 and Gohet *et al*., 1996) [8, 32, 15, 13]. There is a rapid transformation into rubber of the sugar present in laticifers, which is done in the direction of increasing the frequency of stimulation. This is the result of the level of production imposed on this clone, in particular by the use of this stimulation (Lacrotte, 1991) [21]. If the stimulation frequency is high, then the consumption of intralaticiferous sugar is high. Because of its limited quantity, it is easy to understand the fact that from 1 / y to 6 / y, sugar consumption is average, and that at 26 / y, it is high.

Inorganic phosphorus content

In the two clones, the inorganic phosphorus contents of the latex of the trees of the different treatments were all equivalent and average (14.63 mmol.l-1 for the clone IRCA 111 and 13.14 mmol.l-1 for the clone IRCA 130). The inorganic phosphorus content of the latex of the studied clones was not significantly influenced by the frequency of stimulation applied. These observations are a sign of a good availability of the energy necessary for the metabolism as shown by Jacob *et al*., In 1988 [15].

Content of thiol Compounds

The average content of thiol Compounds in the latex is moderate. It varies significantly depending on the frequency of hormonal stimulation. For the two clones studied, the tree contents of these compounds are acceptable from 1 / y to 6 / y. Under the stimulation regimen, the different clones studied showed a decrease in the number linked to the increase in the annual number of stimulation. In fact, the stimulation leads to a reduction in the contents of thiol compounds by virtue of its intensity. This decrease is slower and smaller as the concentrations of intralaticiferous sucrose without stimulation are high. A high availability of sugar in the latex therefore seems to allow resistance to the mechanisms leading to early senescence. And therefore, it could explain the predisposition to dry notch syndrome observed in these clones (Gohet, 1996) [13].

4.2.4 Sensitivity to dry notch

Our study revealed that the studied clones have a high sensitivity to the dry notch. The highest percentages of diseased notch length were obtained with the maximum stimulation frequencies (27.3% LEM with 26 / y for the IRCA 111 clone and 38% with 26 / y for the IRCA 130 clone). Indeed, the sensitivity of the clones to the dry notch is specific to the class of metabolic activity as shown by Eschbach *et al*., In 1984, Serres *et al*., In 1994, Jacob *et al*., 1995 and Gohet. *et al*., 1996 [8, 32, 15]. Our results corroborate the empirical observations of the producers and those obtained by De Fäy in 1981, Chrestin in 1985 [4], Omokhaf in 2001 and 2004. The sensitivity to dry notch of clones with very active metabolism is due to the fact that, these clones have low contents of thiol compounds (sign of the destabilization of lutoids), as indicated by Chrestin in 1984

[5]. Indeed, thiol compounds allow the stability of lutoids, their low content leads to the decompartmentation of these. These which are destroyed in situ. This lysis of the lutoids leads to the coagulation of the latex within the laticifera, causing the flow to stop and causing the dry notch (Chrestin, 1984) [5]. Under the effect of stimulation, these lutoids are much more degraded as a result of the resulting drop in thiol compounds. In fact, the stimulation leads to a reduction in the contents of thiol compounds by virtue of its intensity. This is slower and lower the higher the concentrations of intralaticiferous sucrose without stimulation.

5. Conclusion

The 6-years study on Hevea brasiliensis clones IRCA 111 and IRCA 130 gave convincing results. It focused on the effect of different frequencies of annual ethylene stimulation on the rubber productivity of these clones, with a single bleeding system (descending half-spiral every 4 days or S / 2 d4). The results of this study showed that the annual stimulation frequency best suited to each clone varies within the same class of metabolic activity. The strong response to stimulation, hence the increase in rubber production, is dependent on the available intralaticiferous sucrose. The activation of the metabolism and the anti-senescent protection depend respectively on the contents of inorganic phosphorus and thiol compounds. Indeed, within the framework of the intensification of the harvest of latex by the use of the stimulation, the analysis of the parameters of rubber production, vegetative and physiological indicated that, the clones IRCA 111 and IRCA 130 did not need only six and four stimuli per year, respectively. Stimulation has a depressive effect on the isodiametric growth of tree trunks, as it promotes production at the expense of growth. It also has effects on physiological parameters either increasing or decreasing their content in the latex. As for the sensitivity of the clones to the dry notch, it is specific to this class of metabolic activity (rapid). These optimal stimulation frequencies of the IRCA 111 and IRCA 130 clones of *Hevea brasiliensis* allow for the best rubber production while keeping the trees in a good physiological and vegetative state and in an acceptable state of health.

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