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Effect of ethephon stimulation frequency in downward tapping on the production potential in upward tapping among PB 260 clone of *Hevea brasiliensis* in Ivory Coast

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Abstract

The present study evaluates the impact of different stimulation frequencies of Ethephon in downward tapping on the production potential of trees in upward tapping of the clone PB 260 of *Hevea brasiliensis*. Six parameters are measured: production per tree, tapping panel dryness percentage and four latex biochemical parameters which were dry rubber content, inorganic phosphorus, sugar and thiols. The results show that in downward tapping, generally, the biochemical parameters level is inversely proportional to the stimulation frequency during years 9 and 10. Regarding the production, beyond four stimulations per year, it is inversely proportional to the stimulation frequency. When the trees are tapped upward with a four stimulation uniform per year, the production continues to be low according to the preceding frequency stimulation in downward tapping beyond four stimulations per year. However, the factors which caused this decrease don't seem to be the same in the first and the second years of upward tapping. In the first year of upward tapping, according to the evolution of the biochemical parameters, the decrease in production is linked to physiological tiredness due to the stimulation in downward tapping. On the other hand, in the second year, it is a phenomenon of familiarization to Ethephon which could explain the decrease in production on treatments which received more than four stimulations per year in downward tapping. In second year of upward tapping, the trees need more than four times per year Ethephon stimulations to reach their production potential.

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Introduction

In Ivory Coast, the production of latex from *Hevea brasiliensis* at maturity is done in three phases. The first step consists of slashing the bark of the tree up to 1.2 meter above the ground in a downward direction; this phase is therefore called downward tapping. In the second phase, the tapping starts above the first opening (1.3 meters) and is directed toward the uplink of the trunk; it is called upward tapping or ascending tapping. Finally, the third phase is conducted in the same way as the first but on the new formed bark; for this reason, it is called tapping on regenerated bark (Attobra, 2011).

During these three phases, the Ethephon, a phytohormone ethylene generator is used as an exogenous latex stimulant production. This process widely adopted in rubber tree plantations extends the flow of latex and stimulates the metabolism of the cells responsible for latex regeneration (Jacob *et al.*, 1983; Eschbach *et al.*, 1984; Gohet, 1996; Duan, 2011). However, it has been shown that in downward tapping, its misuse has negative effects on the physiology of the tree (Chrestin, 1985; Jacob *et al*, 1989; Sivakumaran and Hardas, 1989; Dian *et al.*, 1999). In short or medium term, depending on the frequency of stimulation, the production capacity of the trees still called production potential is strongly affected in downward tapping. It should be noted that the effect of this stimulant which is commonly used in downward tapping is not well known in the production potential in upward tapping. This study analyzes the effect of different frequency stimulation of Ethephon when applied in downward tapping on the fluctuation of latex metabolism indicator of the production potential of trees in upward tapping on clone PB 260 of *Hevea brasiliensis*. The objective is to know the effect of these frequency stimulations on the production capacity of trees in ascending tapping.

Materials and methods

Plant Material

The study focused on the PB 260 clone of *Hevea brasiliensis* planted at the experimental station of Anguededou located in the southeast part of Ivory Coast. The growth of this clone is fast in the conditions of the southeast of Ivory Coast on tertiary sands. The first tapping of trees was made at the five years and ten months mark.

Methods

Experimental device

The experiment was performed using a completely randomized design in single shaft "One tree plot design" with nine treatments in downward tapping which are frequency stimulations and thirty three trees per treatment; making a total of two hundred and ninety seven trees in the experiment. Edge trees and the neighboring trees empty slots on the line were eliminated from the test to minimize edge effects.

The production stimulation was made with a mixture (stimulating paste) Ethrel and palm oil (adjuvant). The Ethrel is a commercial product whose active ingredient (ai) is the 2-chloroethyl-phosphonic acid or Ethephon (ET).

The production system used in downward tapping is half-spiral panel all four days except Sunday, stimulated with one gram mixture to 2.5% active ingredient applied to the panel regeneration over the tapping notch (S/2 d/4 6d/7 Et 2,5 % Pa 1(1)). Treatments that are frequency stimulation are shown in Table 1.

Table 1. Treatments applied in downward tapping.

Treatments	Modalities
A	Not tapped Trees
B	Tapped trees unstimulated, 0/y
C	Tapped trees stimulated, 1/y
D	Tapped trees stimulated, 2/y
E	Tapped trees stimulated, 4/y
F	Tapped trees stimulated, 6/y
G	Tapped trees stimulated, 8/y
H	Tapped trees stimulated, 13/y
J	Tapped trees stimulated, 26/y

Y : year

In upward tapping, one stimulation frequency was applied to all treatments. This is the frequency 4/y. The production system used is the spiral of each quarter tapping four days except Sunday, stimulated

with one gram of mixture to 5% of active ingredient applied to the panel regeneration below the notching slot (S/4 U d/4 6d/7 Pa ET 5% 1 (1)).

Measured parameters

Latex biochemical parameters

Four major biochemical latex parameters indicators of metabolic activity were measured in November of each year. They are the dry rubber content, sucrose content, thiols content, and inorganic phosphorus content. The determination of the dry rubber content was made according to the method described by Eschbach *et al*. (1984). Sucrose, thiol groups and inorganic phosphorus contents were obtained, respectively according to Ashwel (1957), Boyne and Ellman (1972), Taussky and Shorr (1953).

Tapping panel dryness percentage

The tapping panel dryness (TPD) percentage at each treatment was estimated from a dry notch length measure (DNLM). The DNLM is a visual assessment of the exudation of the latex at the indented area called notch. During the DNLM, trees are considered "healthy" when it exudes latex over the entire length of the notch after the tapping. They are scored zero (0). Others who do not exude any latex on a portion of the notch after tapping are considered diseased trees and are rated from 1 to 6 along the length of non-production of latex notch (Table 2).

Table 2. Rating of trees with dry notch in *Hevea brasiliensis*.

Non notch producing latex (%)	Notation
01 to 20	1
21 to 40	2
41 to 60	3
61 to 80	4
81 to 99	5
100	6

The percentage of TPD is obtained by the following formula:

$$TPD = \frac{0.1 \times n1 + 0.3 \times n2 + 0.5 \times n3 + 0.7 \times n4 + 0.9 \times n5 + 1 \times n6}{N} \times 100$$

Where N represents the number of trees per treatment and the coefficients 0.1, 0.3, 0.5, 0.7, 0.9 and 1 are the mean percentages of classes of non-production notch length latex.

The numbers n1, n2, n3, n4, n5 and n6 represent the number of trees observed by length class percentage of non-producing latex notch.

As part of this study, a DNLM was made in November of each year on all living trees of experimentation and TPD percentage of each treatment was calculated year by year.

Production

The production of each treatment was obtained by weighing the shaft by shaft coagulated latex in polybags after 6 tappings. A fresh rubber processing coefficient (C) in dry rubber was determined for each treatment by the following formula:

$$C = \frac{DW}{FW}$$

Where FW represents the Fresh Weight of a sample of the coagulum, and DW is the Weight of the same sample after draining and drying. The calculated coefficient for each treatment is multiplied by the total weight of fresh rubber after 6 taps for the monthly production in dry rubber of the treatment. This weight was estimated on average by tapping and by dividing the total annual number of tapping (gram per tree by tapping (g/t/t)) to reduce the effect of the number of tapping. The cumulative productions to the current year were calculated.

Statistical methods

Studied parameter values were subjected to a one-way ANOVA and different groups have been identified using the statistical Student-Newman-Keuils risk α = 5%. The statistical software used was the Statistical Package for Social Sciences (SPSS) version 17.0 (2008). TPD percentages which are proportions were transformed into Arc Sine Square Root (ASIN DNLM) to make the normal distribution and stabilize the variances in order to enable statistical analysis (Dagnelie, 1994).

Results

The data analyzed are those of the last two years of the downward tapping (year nine and year ten) and the first two years of upward tapping (years eleven and twelve); or four years of observation for biochemical parameters and the tapping panel dryness percentage. For production, in addition to these four years data, the cumulative values since the year 1 were also analyzed.

Latex biochemical parameters

Biochemical parameters in downward tapping

Newman and Keuils test risk α = 5% was used to assess changes in latex biochemical parameters, major indicators of the state of metabolism of laticiferous cells according to the frequency stimulation during the past two years of downward tapping (Tables 3 and 4).

Table 3. Evolution of latex biochemical parameters depending on the frequency stimulation on the 9th year downward tapping in the PB 260 clone *Hevea brasiliensis*.

Treatments	Latex Biochemical parameters			
	DRC (%)	Pi (mM)	SUC. (mM)	R-SH (mM)
S/2 d/4 6d/7 ET 2.5 % 0/y	49.2 ab	12.8 c	9.2 a	0.70 ab
S/2 d/4 6d/7 ET 2.5 % 1/y	50.1 a	15. 4 ab	8.9 a	0.76 a
S/2 d/4 6d/7 ET 2.5 % 2/y	49.5 ab	15 abc	6.4 b	0.74 ab
S/2 d/4 6d/7 ET 2.5 % 4/y	49.2 ab	16.1 a	5.7 bc	0.70 ab
S/2 d/4 6d/7 ET 2.5 % 6/y	45.5 cd	15.6 a	4.4 cd	0.67 bc
S/2 d/4 6d/7 ET 2.5 % 8/y	46.9 bc	16.1 a	3.7 d	0.62 c
S/2 d/4 6d/7 ET 2.5 % 13/y	44.5 cd	16.2 a	4.8 bcd	0.61 c
S/2 d/4 6d/7 ET 2.5 % 26/y	43.5 d	13.1 bc	4.6 bcd	0.44 d

DRC: Dry Rubber Content, Pi: inorganic Phosphorus, SUC: Sucrose, R-SH Thiols Groups, mM: millimolar. a, b, c, d: homogenous group according to the test of Newman and Keuil alfa risk of 5%, y: year, S/2 d/4 6d/7 ET 2.5% 4/y: half spiral downward tapping every 4 days except Sunday Ethephon stimulated with 2.5% 4 times a year.

Table 4. Evolution of latex biochemical parameters depending on the stimulation frequency on the 10th year downward tapping in the PB 260 clone *Hevea brasiliensis*.

Treatments	Latex Biochemical parameters			
	DRC (%)	Pi (mM)	SUC. (mM)	R-SH (mM)
S/2 d/4 6d/7 ET 2.5 % 0/y	48.6 ab	13.4 a	6.6 a	0.41 a
S/2 d/4 6d/7 ET 2.5 % 1/y	49.7 a	13.9 a	4.9 b	0.38 ab
S/2 d/4 6d/7 ET 2.5 % 2/y	49.4 a	12.8 a	5.5 ab	0.34 bc
S/2 d/4 6d/7 ET 2.5 % 4/y	49.0 ab	13.0 a	3.0 c	0.32 c
S/2 d/4 6d/7 ET 2.5 % 6/y	48.6 ab	13.4 a	2.8 c	0.27 d
S/2 d/4 6d/7 ET 2.5 % 8/y	47.9 abc	12.5 a	2.1 c	0.24 de
S/2 d/4 6d/7 ET 2.5 % 13/y	45.4 bc	12.7 a	2.4 c	0.21 e
S/2 d/4 6d/7 ET 2.5 % 26/y	44.2 c	9.5 b	2.4 c	0.14 f

DRC: Dry Rubber Content, Pi: inorganic Phosphorus, SUC: Sucrose, R-SH Thiols Groups, mM: millimolar. a, b, c, d: homogenous group according to the test of Newman and Keuil alfa risk of 5%, y: year, S/2 d/4 6d/7 ET 2.5% 4/y: half spiral downward tapping every 4 days except Sunday Ethephon stimulated with 2.5% 4 times a year.

In year nine before last the year of downward tapping (Table 3), latex biochemical parameters evolved as follows:

The highest dry rubber content (DRC) values are those treatments stimulated 0, 1, 2 and 4 times a year. Lower values are those treatments stimulated 13 and 26 times a year. There is a decreasing gradient of the DRC from the treatment stimulated 1 per year (50.1%) to the treatment stimulated 26 times a year (43.5%).

For the inorganic phosphorus content, the results show that it significantly increases the non-stimulated treatment (12.8 mM) to achieve its maximum value to four stimulations per year (about 16 mM). It remains to this maximum level up to 13 stimulations per year and significantly lower with 26 stimulations per year (13.1 mM).

The sucrose content of the non-stimulated and stimulated latex treatments 1 time per year are statistically of the same order and higher. The content reduces significantly from 2 to 8 stimulations per year. Often the sucrose content reaches its lowest level.

For the thiol groups, the highest values were observed between the unstimulated treatment to the treatment stimulated 4 times a year and the lowest values were observed during the treatment stimulated 26 times per year. The evolution of the latex thiol content during the stimulated treatment is inversely proportional to the frequency stimulation. It starts from 0.76 mM once a year for the stimulated treatment to finish at 0.44 mM for the treatment stimulated 26 times per year. The latex biochemical parameter values according to the frequency stimulation for the last year of downward tapping (year 10) are shown in Table 4. DRC of the latex has the same evolution in year 9. For stimulated treatments, the values are even higher than the frequency stimulation which is low. The percentage rises to 49.7 for the treatment stimulated 1 time per year to 44.2 for the treatment stimulated 26 times per year.

The latex inorganic phosphorus content remains statistically at the same order of magnitude for non-stimulated treatment until treatment which is done 13 times a year and it's significantly lower at the treatment stimulated 26 times per year to reach a concentration of 9.5 mM. Compared to the 9 years experiment, the values are lower.

Regarding the latex sucrose concentration of trees, it's significantly lower for non-stimulated treatment (6.6 mM) and treatment stimulated 4 times a year from which the values are statistically equivalent to the treatment stimulated 26 times per year (about 2.5 mM). The values of the sucrose concentration are generally low compared to those of 9 years.

As for the thiol groups, their concentration in the latex is significantly reduced for non-stimulated treatment (0.41 mM) to reach its lowest value (0.14 mM) in the treatment stimulated 26 times per year. Thiol groups have a concentration in the latex which is inversely proportional to the frequency stimulation. Thiols content values are lower than those of the year 9 as those of sucrose.

Biochemical parameters in upward tapping

The changes in the biochemical parameters of latex in the first and second year of upward tapping were collected and compared with the ones observed during the preceding stimulation done in downward tapping. The test of Newman and Keuils was used to compare the variation of these parameter levels (Tables 5 and 6).

Table 5. Evolution of latex biochemical parameters in the 1st upward tapping year depending on the downward tapping frequency stimulation in PB 260 clone of *Hevea brasiliensis*.

Treatments		Latex biochemical parameters			
Downward tapping	Upward tapping	DRC (%)	Pi (mM)	SUC (mM)	R-SH (mM)
S/2 d/4 6d/7 ET 2.5 % 0/y	S/4 U d/4 6d/7 ET 5 % 4/y	48.9 de	16.1 ab	08.5 ab	0.94 a
S/2 d/4 6d/7 ET 2.5 % 1/y	S/4 U d/4 6d/7 ET 5 % 4/y	48.5 e	17.4 a	07.8 ab	1.02 a
S/2 d/4 6d/7 ET 2.5 % 2/y	S/4 U d/4 6d/7 ET5 % 4/y	49.1 cde	17.1 a	10.0 a	0.94 a
S/2 d/4 6d/7 ET 2.5 % 4/y	S/4 U d/4 6d/7 ET 5 % 4/y	51.2 abc	15.2 ab	06.4 b	0.76 b
S/2 d/4 6d/7 ET 2.5 % 6/y	S/4 U d/4 6d/7 ET 5 % 4/y	49.8 bcde	15.4 ab	06.8 ab	0.75 b
S/2 d/4 6d/7 ET 2.5 % 8/y	S/4 U d/4 6d/7 ET 5 % 4/y	51.4 ab	14.8 b	06.3 b	0.73 b
S/2 d/4 6d/7 ET 2.5 % 13/y	S/4 U d/4 6d/7 ET 5 % 4/y	50.9 abcd	15.2 ab	05.6 b	0.68 b
S/2 d/4 6d/7 ET 2.5 % 26/y	S/4 U d/4 6d/7 ET 5 % 4/y	52.0 a	10.2 c	07.2 ab	0.55 c

DRC: Dry Rubber Content, Pi: inorganic phosphorus, SUC: Sucrose, R-SH Thiols Groups, mM: millimolar. a, b, c, d, e: homogeneous group according to the test of Newman and Keuil alfa risk of 5%, y: year, S/4 U d/4 6d/7 ET 5% 4/y: tapping ascending spiral quarter every 4 day except Sunday stimulated with 5% Ethephon 4 times a year.

Table 6. Evolution of latex biochemical parameters in the 2nd upward tapping year depending on the downward tapping frequency stimulation in PB 260 clone of *Hevea brasiliensis*.

Treatments		Latex biochemical parameters			
Downward tapping	Upward tapping	DRC (%)	Pi (mM)	SUC (mM)	R-SH (mM)
S/2 d/4 6d/7 ET 2.5 % 0/y	S/4 U d/4 6d/7 ET 5 % 4/y	58.5 ab	11.5 c	12.2 ab	0.59 abc
S/2 d/4 6d/7 ET 2.5 % 1/y	S/4 U d/4 6d/7 ET 5 % 4/y	58.9 ab	14.5 a	15.4 a	0.67 a
S/2 d/4 6d/7 ET 2.5 % 2/y	S/4 U d/4 6d/7 ET 5 % 4/y	56.4 b	14.6 a	12.4 ab	0.63 ab
S/2 d/4 6d/7 ET 2.5 % 4/y	S/4 U d/4 6d/7 ET 5 % 4/y	59.7 a	14.2 ab	9.6 b	0.55 bc
S/2 d/4 6d/7 ET 2.5 % 6/y	S/4 U d/4 6d/7 ET 5 % 4/y	58.1 ab	13.3 bc	11.6 ab	0.50 cd
S/2 d/4 6d/7 ET 2.5 % 8/y	S/4 U d/4 6d/7 ET 5 % 4/y	60.0 a	11.2 c	8.9 b	0.38 d
S/2 d/4 6d/7 ET 2.5 % 13/y	S/4 U d/4 6d/7 ET 5 % 4/y	59.5 a	11.9 bc	8.2 b	0.40 d
S/2 d/4 6d/7 ET 2.5 % 26/y	S/4 U d/4 6d/7 ET 5 % 4/y	57.5 ab	14.2 ab	9.2 b	0.42 d

DRC: Dry Rubber Content, Pi: inorganic phosphorus, SUC: Sucrose, R-SH Thiols Groups, mM: millimolar. a, b, c, d, e: homogeneous group according to the test of Newman and Keuil alfa risk of 5%, y: year, S/4 U d/4 6d/7 ET 5% 4/y: tapping ascending spiral quarter every 4 day except Sunday stimulated with 5% Ethephon 4 times a year.

In the first year of upward tapping (Table 5): The evolution of DRC in general depends on the downward tapping frequency stimulation. The highest value is that of the treatment which was preceded by a stimulation of 26 times a year and the lowest values are those of unstimulated and stimulated one time annually in downward tapping. The highest levels of inorganic phosphorus are those of treatments preceded by stimulation 1 and 2 times a year, and the lowest is that of the treatment that had been stimulated 26 times a year in downward tapping. The inorganic phosphorus content is lower when the trees were strongly stimulated in downward tapping.

Sucrose has its highest value at for the treatment which was preceded by stimulation 2 times a year, and the lowest values are those of the previous treatments by stimulation in downward tapping 4, 8 and 13 times year. In general, higher levels of sugars are observed in trees that were slightly stimulated in downward tapping.

The level of thiol groups observed in treatments that were not stimulated and those stimulated 1 or 2 times a year in downward tapping were statistically equivalent and higher. The lowest value was observed in the treatment that was stimulated 26 times a year in downward tapping.

In the second year of upward tapping: The highest DRC values were observed in treatments were the preceding treatment was stimulated in downward tapping 4, 8 and 13 times per year and the lowest value was observed in the stimulated treatment 2 times a year. The change does not follow a particular gradient. These values are very high in general compare to the first year of upward tapping. They are between 56.4 and 60% compare to 48.5 and 52% in the first year of upward tapping (Table 6). The highest levels of inorganic phosphorus were observed in the trees who received stimulated treatment in downward tapping 1 and 2 per year as well as in the first year of upward tapping. In contrast, the lowest content was not observed in the treatment stimulated 26 times a year in downward tapping but in those of non-stimulated and stimulated 8 times a year in downward tapping. The inorganic phosphorus levels are generally low compare to those of the first year in upward tapping.

The highest rate of sucrose was observed in the treatment that was stimulated 1 times a year in downward tapping and the lower rate was observed in the treatments that received 8 stimulations per year and more. The sucrose concentration of the latex compared to those of the first year in upward tapping was high. They varied between 8.2 and 15.4 mM compare to between 5.6 and 10 mM in year 1 in upward tapping.

Thiol groups have the same evolution as sucrose. The maximum level was observed in the treatment that received only one stimulation in downward tapping and the lowest level was observed in the treatments that were stimulated 8, 13 and 26 times a year in downward tapping. In contrast of the sugar, the thiol values were generally observed to be lower than those of the first year in upward tapping.

Tapping panel dryness percentage

Tapping panel dryness percentage in downward tapping

Tapping panel dryness (TPD) percentage estimated during the last two years in downward tapping are reported in Table 7. The same evolution is observed. TPD percentage values stimulated treatments 13 and 26 times a year are statistically significantly different and higher than those treatments stimulated 0 to 8 times per year which have statistically similar important values.

TPD percentage in upward tapping

During the two years of upward tapping, the TPD percentages calculated are shown in Table 8. The values of TPD treatment stimulated 13 and 26 times a year are significantly higher than those of the stimulated treatments 0-8 times per year which have statistically important values. Changes in TPD percentage for year 1 and 2 in upward tapping are similar to those observed in year 9 and 10 in downward tapping despite the application of a single stimulation plan (4/y). However, the amplitudes are lower in upward tapping compared to those observed in downward tapping.

Table 7. Evolution of tapping panel dryness (TPD) percentage depending on the downward tapping frequency stimulation in the clone PB 260 of *Hevea brasiliensis*.

	T.P.D. (%)	
Treatments	Year 9	Year 10
S/2 d/4 6d/7 ET 2.5 % 0/y	9.7 c	8.7 c
S/2 d/4 6d/7 ET 2.5 % 1/y	6.5 c	6.5 c
S/2 d/4 6d/7 ET 2.5 % 2/y	5.3 c	3.2 c
S/2 d/4 6d/7 ET 2.5 % 4/y	4.4 c	3.1 c
S/2 d/4 6d/7 ET 2.5 % 6/y	5.8 c	3.2 c
S/2 d/4 6d/7 ET 2.5 % 8/y	5.5 c	8.4 c
S/2 d/4 6d/7 ET 2.5 % 13/y	21.0 b	18.4 b
S/2 d/4 6d/7 ET 2.5 % 26/y	38.0 a	34.5 a

T.P.D. : Tapping Panel Dryness, S/2 d/4 6d/7 ET 2.5 % 4/y: Half downward spiral tapped every 4 days except Sunday stimulated with 5% Ethephon 4 times a year. a, b, c: homogeneous group according to the test of Newman and Keuil 5% risk Alfa, y: year.

Table 8. Evolution of tapping panel dryness (TPD) percentage in upward tapping depending on the downward tapping stimulation frequency in the clone PB 260 of *Hevea brasiliensis*.

Treatments		TPD (%)	
Downward tapping	Upward tapping	First year	Second year
S/2 d/4 6d/7 ET 2.5 % 0/y	S/4 U d/4 6d/7 ET 5 % 4/y	4.8 c	3.8 c
S/2 d/4 6d/7 ET 2.5 % 1/y	S/4 U d/4 6d/7 ET 5 % 4/y	4.8 c	3.5 c
S/2 d/4 6d/7 ET 2.5 % 2/y	S/4 U d/4 6d/7 ET 5 % 4/y	2.1 c	1.0 c
S/2 d/4 6d/7 ET 2.5 % 4/y	S/4 U d/4 6d/7 ET 5 % 4/y	3.3 c	2.3 c
S/2 d/4 6d/7 ET 2.5 % 6/y	S/4 U d/4 6d/7 ET 5 % 4/y	3.4 c	2.3 c
S/2 d/4 6d/7 ET 2.5 % 8/y	S/4 U d/4 6d/7 ET 5 % 4/y	8.5 bc	6.5 c
S/2 d/4 6d/7 ET 2.5 % 13/y	S/4 U d/4 6d/7 ET 5 % 4/y	14.2 b	12.1 b
S/2 d/4 6d/7 ET 2.5 % 26/y	S/4 U d/4 6d/7 ET 5 % 4/y	20.6 a	18.6 a

TPD: Tapping Panel Dryness, S/2 d/4 6d/7 ET 2.5 % 4/y : Half downward spiral tapped every 4 days except Sunday stimulated with 5% Ethephon 4 times a year, S/4 U d/4 6d/7 ET 5 % 4/y: tapping ascending spiral quarter every 4 day except Sunday stimulated with 5% Ethephon 4 times a year. a, b, c: homogeneous group according to the test of Newman and Keuil Alfa risk of 5%, y: year.

Production

Productions in downward tapping

The production resulting from frequency stimulations during the last two years in downward tapping (year 9 and 10) are shown in Tables 9 and 10, respectively. In year 9 before the last year in downward tapping, productions in gram/tree/tapping (g/t/t) allow us to distinguish three treatment groups (Table 9). The first group made up of stimulated treatments 0, 1, 2 and 4 per year has g/t/t identical statistics and they are higher. The second group formed by stimulated treatments 6 and 8 times a year has g/t/t identical statistics and they are significantly lower than those in Group 1. The third group consists of stimulated treatments 13 and 26 per years with identical statistics and they are significantly lower than the first two groups. For the cumulative production over the nine years, the highest value was observed during stimulated treatment 4 times per year and the lowest during stimulated treatment 26 times per year.

Table 9. Evolution of latex production based on frequency stimulation on the 9th year in downward tapping in the PB 260 clone of *Hevea brasiliensis*.

Treatments	Productions	
	g/t/ t year 9	g/t.cum. year 1 to 9
S/2 d/4 6d/7 ET 2.5 % 0/y	81.92 a	37133.8 de
S/2 d/4 6d/7 ET 2.5 % 1/y	83.15 a	39746.4 bcd
S/2 d/4 6d/7 ET 2.5 % 2/y	82.90 a	41421.9 abc
S/2 d/4 6d/7 ET 2.5 % 4/y	83.53 a	43346.6 a
S/2 d/4 6d/7 ET 2.5 % 6/y	69.56 b	40135.1 abcd
S/2 d/4 6d/7 ET 2.5 % 8/y	73.16 ab	42551.9 ab
S/2 d/4 6d/7 ET 2.5 % 13/y	54.32 c	38227.4 cd
S/2 d/4 6d/7 ET 2.5 % 26/y	42.36 c	34689 e

g/t/t: gram/tree/tapping, g/t.cum.: gram/tree cumulated, S/2 d/4 6d/7 ET 2.5% 4/y: half spiral down tapping every 4 days except Sunday Ethephon stimulated with 2.5% 4 times a year. a, b, c, d, e: homogenous group according to the test of Newman and Keuil Alfa risk of 5%, y: year.

In the last year of downward tapping (10th year), changes in production are essentially similar to the production in year 9 (Table 10). Based on the g/t/t, three groups stand out. Group 1 consisting of non-stimulated and stimulated treatments 1, 2 and 4 times per year which are identical and statistically superior to others. The 2nd group formed by the stimulated treatments 6 and 8 times a year is statistically identical with significantly lower values than the first group. Group 3 (stimulated treatment 13 and 26 times a year) are statistically identical with significantly lower values than the first two groups. Cumulatively over the 10 years in downward tapping, it is the treatment stimulated 4 times a year which statistically produces the highest while the lowest production was observed at the stimulated treatment 26 times (Table 10).

Table 10. Evolution of latex production based on the frequency stimulation on the 10th year in downward tapping in the PB 260 clone of *Hevea brasiliensis*.

Treatments	Productions	
	g/t/t year 10	g/t.cum. year 1 to 10
S/2 d/4 6d/7 ET 2.5 % 0/y	77.19 ab	43336.2 cd
S/2 d/4 6d/7 ET 2.5 % 1/y	87.48 a	46569.7 bc
S/2 d/4 6d/7 ET 2.5 % 2/y	82.65 a	47869.0 ab
S/2 d/4 6d/7 ET 2.5 % 4/y	86.34 a	50782 a
S/2 d/4 6d/7 ET 2.5 % 6/y	69.83 b	45680 bcd
S/2 d/4 6d/7 ET 2.5 % 8/y	65.87 b	47690.2 ab
S/2 d/4 6d/7 ET 2.5 % 13/y	45.71 c	41988.7 d
S/2 d/4 6d/7 ET 2.5 % 26/y	39.03 c	37803.5 e

g/t/t: gram/tree/tapping, g/t.cum.: gram/tree cumulated, S/2 d/4 6d/7 ET 2.5% 4/y: half spiral down tapping every 4 days except Sunday Ethephon stimulated with 2.5% 4 times a year. a, b, c, d, e: homogenous group according to the test of Newman and Keuil alfa risk of 5%, y: year.

Productions in upward tapping

The production obtained during the first two years in upward tapping after applying different frequency stimulations in downward tapping are reported in Tables 11 and 12. In the first year of upward tapping (Table 11) statistical analysis classifies production in g/t/t in four treatment groups. Group 1 consists of previous stimulated treatments in downward tapping 0, 1, 2 and 4 times a year with statistically similar levels of production and higher. Group 2 is composed of 2 treatments that were stimulated 6 and 8 times a year in downward tapping. Their productions are

statistically identical and significantly lower than those in group 1 and superior to others. Group 3 consists of the treatment that was stimulated 13 times a year in downward tapping. In this case, production is significantly lower than those of Groups 1 and 2 and superior to that of group 4 which is formed by stimulated treatment 26 times per year in downward tapping. This stimulated treatment 26 times a year in downward tapping has the lowest production in g/t/t.

Table 11. Evolution of latex production in the 1st upward tapping year based on downward tapping frequency stimulation in PB 260 clone of *Hevea brasiliensis*.

Treatments		Productions	
Downward tapping	Upward tapping	g/t/t year 1	g/t.cum.
S/2 d/4 6d/7 ET 2.5 % 0/y	S/4 U d/4 6d/7 ET 5 % 4/y	113.42 ab	52182.9 b
S/2 d/4 6d/7 ET 2.5 % 1/y	S/4 U d/4 6d/7 ET 5 % 4/y	155.93 a	58732.7 a
S/2 d/4 6d/7 ET 2.5 % 2/y	S/4 U d/4 6d/7 ET 5 % 4/y	152.47 a	59762 a
S/2 d/4 6d/7 ET 2.5 % 4/y	S/4 U d/4 6d/7 ET 5 % 4/y	128.93 a	60838.5 a
S/2 d/4 6d/7 ET 2.5 % 6/y	S/4 U d/4 6d/7 ET 5 % 4/y	86.77 b	52447.9 b
S/2 d/4 6d/7 ET 2.5 % 8/y	S/4 U d/4 6d/7 ET 5 % 4/y	92.90 b	54936.1 ab
S/2 d/4 6d/7 ET 2.5 % 13/y	S/4 U d/4 6d/7 ET 5 % 4/y	57.87 c	46501.8 c
S/2 d/4 6d/7 ET 2.5 % 26/y	S/4 U d/4 6d/7 ET 5 % 4/y	39.28 d	39301.3 d

g/t/t: gram/tree/tapping, g/t.cum.: gram/tree cumulated 10 years down tapping and 1 year upper tapping, S/2 d/4 6d/7 ET 2.5% 4/y: half spiral down tapping every 4 days except Sunday Ethephon stimulated with 2.5% 4 times a year, S/4 U d/4 6d/7 ET 5 % 4/y: tapping ascending spiral quarter every 4 day except Sunday stimulated with 5% Ethephon 4 times a year. a, b, c, d: homogenous group according to the test of Newman and Keuil Alfa risk of 5%, y: year.

For the cumulative productions in grams per tree (g/t.cum) over 11 years of tapping, 4 treatment groups were observed. Group 1 consists of treatments that have been stimulated in downward tapping 1, 2 and 4 times a year. Group 2 is formed by stimulated treatments 0, 6 and 8 times per year in downward tapping. Groups 3 and 4 are formed by stimulated treatment 13 times per year and stimulated treatment 26 times per year, respectively. Production levels were statistically similar within each group and significantly decreasing from group 1 to group 4.

In the second year of upward tapping (Table 12), production in g/t/t significantly varied based on treatment. Three treatment groups appear. The first group consists of the stimulated treatments 0, 1, 2 and 4 times a year in downward tapping with g/t/t equally important and statistically significant and higher than those of other treatments. The second group is formed by the treatments which received 8 and 6 stimulations per year in downward tapping. These have g/t/t statistically similar and significantly higher than those of stimulated treatments 13 and 26 annually. The third group consists of the treatments which received 13 and 26 stimulations per year in downward tapping with the value of g/t/t significantly weaker.

Cumulatively over the 12 years it appears that treatments that were most frequently stimulated in downward tapping (13 and 26 times a year) have cumulated g/t significantly weaker. In contrast, the cumulative g/t significantly higher was observed in stimulated treatments 1, 2 and 4 times a year, downward tapping. The stimulated treatments 0, 6 and 8 times a year in downward tapping have cumulative g/t which are intermediaries.

Table 12. Evolution of latex production in the 2nd upward tapping year based on downward tapping frequency stimulation in PB 260 clone of *Hevea brasiliensis*.

Treatments		Productions	
Downward tapping	Upward tapping	g/t/t year 1	g/t.cum.
S/2 d/4 6d/7 ET 2.5 % 0/y	S/4 U d/4 6d/7 ET 5 % 4/y	129.1 a	62124.3 bcd
S/2 d/4 6d/7 ET 2.5 % 1/y	S/4 U d/4 6d/7 ET 5 % 4/y	129 a	68671.8 ab
S/2 d/4 6d/7 ET 2.5 % 2/y	S/4 U d/4 6d/7 ET 5 % 4/y	120 a	68998.3 ab
S/2 d/4 6d/7 ET 2.5 % 4/y	S/4 U d/4 6d/7 ET 5 % 4/y	116.1 a	69779.4 a
S/2 d/4 6d/7 ET 2.5% 6/y	S/4 U d/4 6d/7 ET 5 % 4/y	88.8 b	59289 d
S/2 d/4 6d/7 ET 2.5 % 8/y	S/4 U d/4 6d/7 ET 5 % 4/y	89.4 b	61820 cd
S/2 d/4 6d/7 ET 2.5 % 13/y	S/4 U d/4 6d/7 ET 5 % 4/y	66.9 c	51650.7 e
S/2 d/4 6d/7 ET 2.5 % 26/y	S/4 U d/4 6d/7 ET 5 % 4/y	75.8 bc	45139.6 e

g/t/t: gram/tree/tapping, g/t.cum.: gram/tree cumulated 10 years down tapping and 2 years upper tapping, S/2 d/4 6d/7 ET 2.5% 4/y: half spiral down tapping every 4 days except Sunday Ethephon stimulated with 2.5% 4 times a year, S/4 U d/4 6d/7 ET 5 % 4/y: tapping ascending spiral quarter every 4 day except Sunday stimulated with 5% Ethephon 4 times a year. a, b, c, d: homogenous group according to the test of Newman and Keuil Alfa risk of 5%, y: year.

Discussion

The results of this study show that when the PB 260 clone is stimulated in downward tapping, in general the level of biochemical parameters is inversely proportional to the frequency stimulation during the last two years (years 9 and 10). The production is evolving in the same direction as the biochemical parameters. In fact, downward tapping production data showed that beyond 4 stimulations per year it is inversely proportional to the frequency stimulation which demonstrates the good prediction of production in downward tapping by biochemical parameters in the clone PB 260 as reported by Jacob *et al.* (1989).

The level of biochemical parameters of latex, especially in the 9th year of downward tapping reveals that 4 stimulations per year are sufficient to achieve the physiological optimum production with the production system S/2 d/4 6d/7 ET 2.5%. This is confirmed by production data especially in grams per tree cumulative treatment stimulated 4 times a year.

The clone PB 260 is ranked among the clones active metabolism (Gohet, 1996). Under normal circumstances, the main biochemical characteristics latex clones of this class are low in sugar (usually not exceeding 10 mM) and high content of inorganic phosphorus (greater than 12 mM) in downward tapping (Jacob *et al.*, 1989). This type of clones does not support high frequency stimulations in downward tapping. The results of this study confirm these characteristics. The higher sucrose content in downward tapping is 9.2 mM which was observed during the ninth year of non-stimulated treatment. The lowest inorganic phosphorus content is 9.5 mM which was observed in the treatment stimulated 26 times a year in year 10. The physiological optimum in downward tapping during production is achieved with only a frequency of 4 stimulations per year. Beyond 4 stimulations per year biochemical parameters indicate that it is in full harvesting regime. This results in an increase in TPD percentage (Eschbach *et al.*, 1989, Okoma *et al.*, 2009 and Obouayeba *et al.*, 2011). Similar results were obtained by Traore *et al.* (2011) in the GT 1 clone belonging to the middle metabolic class which is metabolically different from the clone PB 260. The decline in output in downward tapping when the frequency stimulation is too high seems to be a common feature of clones of intermediate metabolism and active metabolism.

When the trees are upward tapped with a uniform stimulation plan (4/year), in the first year, the

biochemical parameters continue to vary depending on the downward tapping stimulation plan. Thiols, sugar and inorganic phosphorus latex are inversely proportional to the stimulation frequency received in downward tapping. In contrast, the DRC is even higher when the frequency of stimulation was strong in downward tapping. There is no leveling of the values of the biochemical parameters despite standardization of the frequency stimulation. The maximum productions during the first year in upward tapping are those stimulated treatments 1, 2 and 4 times a year in downward tapping first. Beyond the previous stimulation in downward tapping 4 times a year, the production is even lower than the frequency stimulation which was high. The lower production was observed in the treatment that has been boosted 26 times a year in downward tapping. However, given the evolution of the DRC, the drop in production cannot be linked to physiological accumulation of fatigue due to strong stimulation in downward tapping. Also it is worth noting that the content of inorganic phosphorus increases in latex with treatment that has not been stimulated and treatment that was boosted 2 times a year in downward tapping then begins to fall from a previous treatment that has stimulation in downward tapping 4 times a year. This indicates that there is a strong activation of latex metabolism treatment that has not been stimulated to treatment that was boosted 2 times a year in downward tapping and decreases the activation from treatment that has a previous stimulation in downward tapping 4 times per year (Jacob *et al.*, 1989).

During the second year upward tapping, while the inorganic phosphorus levels are low compared to those of the first year of upward tapping, the DRC and especially the sugar concentrations are very high for this clone. This combination of data shows that trees are in a situation of under-utilization (Eschbach *et al.*, 1984, Lacote *et al.*, 2010) for this clone active metabolism. So the 4 stimulations per year applied to clone PB 260 in upward tapping are not sufficient to achieve the optimum physiological production even though they would have received 26 stimulations per year in downward tapping.

However, this study shows that for the clone PB 260 stimulated in downward tapping, the lesser production is in upward tapping. The evolution of production as well as biochemical parameters indicate that the trees that received 4 stimulations and more a year in downward tapping need more stimulation in upward tapping to achieve physiological optimum. This reaction trees resembles a phenomenon of habituation to Ethephon used to stimulate the production of latex from rubber trees.

A specific study should be conducted to determine the optimal stimulation frequency which allows the PB clone 260 to achieve the physiological optimum production in upward tapping.

Conclusion

This study showed that in the PB 260 clone harvest according to the downward half-spiral system tapped every 4 days, 6 days out of 7 stimulated with 2.5% Ethephon (S/2 d/4 6d/7 ET 2.5%), the frequency stimulation beyond 4 times a year have a depressive effect on the production of long shafts. This depressive effect remains in trees in upward tapping in the production system standardized spiral quarter every 4 days, 6 days out of 7 and stimulated with 5% Ethephon 4 times a year (S/4 U d/4 6d/7 ET 5 % 4/Y). Trees which have been more stimulated in downward tapping produce less in upward tapping.

However, if the decline in production in year 1 in upward tapping is due to a residual effect of physiological fatigue due to strong stimulation in downward tapping, the production in the second year in upward tapping seems more related to the phenomenon of habituation. Trees that received more stimulation in downward tapping need more stimulation to achieve their production potential in upward tapping.

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