ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2020; 6(9): 05-13
www.allresearchjournal.com
Received: 05-07-2020
Accepted: 12-08-2020

Beugre Manéhonon Martine
University Jean Lorougnon Guédé, UFR (Faculty) of Agroforestry, Laboratory for improving agricultural production BP 150 Daloa, Côte d'Ivoire

Ayolie Koutoua
University Jean Lorougnon Guédé, UFR (Faculty) of Agroforestry, Laboratory for improving agricultural production BP 150 Daloa, Côte d'Ivoire

Kouadio Yatty Justin
University Jean Lorougnon Guédé, UFR (Faculty) of Agroforestry, Laboratory for improving agricultural production BP 150 Daloa, Côte d'Ivoire

Obouayeba Samuel
CNRA (National Agronomic Research Center), Bimbresso Research Station, 01 BP 1536 Abidjan 01, Cote d'Ivoire

Corresponding Author:
Beugre Manéhonon Martine
University Jean Lorougnon Guédé, UFR (Faculty) of Agroforestry, Laboratory for improving agricultural production BP 150 Daloa, Côte d'Ivoire

Effect of latex harvesting technologies on agrophysiological parameters of the bpm 24 clone of *Hevea brasiliensis* south-west of cote d'ivoire

Beugre Manéhonon Martine, Ayolie Koutoua, Kouadio Yatty Justin and Obouayeba Samuel

Abstract
A prospective study of technologies for harvesting the latex of the *Hevea brasiliensis* clone BPM 24, with moderate metabolism, at the opening, was undertaken for 7 years in the south-west of the Côte d'Ivoire. The rubber trees were planted at a density of 510 trees / ha (7 m x 2.80 m) according to a completely randomized scheme. They were bled using ten different latex harvesting systems. The parameters measured were rubber production, isodiametric trunk growth, physiological profile and sensitivity to dry notching. The results showed that bloodletting, like hormonal stimulation, strongly activates the metabolism of laticifers. They indicated that, tapping at 1.20 m from the ground (descending tapping) gives better results compared to that at 0.70 m (ascending tapping) not preceded by a descending tapping for a minimum delay equivalent to 4 years. The tapping in a half-spiral ascending from the fifth year with a high production and a high rate of dry notching shows signs of the onset of physiological fatigue probably linked to overuse. The different hormonal stimulation strategies had little damage to trunk growth and physiological parameters of trees. To best express and maintain its long-term agronomic potential, the latex harvesting system best suited to the BPM 24 clone, when opened, is S / 2 d4 6d / 7 12 m / 12 ET 2.5% Pa1 (1) 4 / y at 1.20 m from the ground (without gradient). In addition, our results indicate that reverse tapping can be introduced at least as early as the fifth, possibly from the sixth, year of latex harvest.

Keywords: *Hevea brasiliensis*, descending half-spiral, Ascending half-spiral, Isodiametric growth, Côte d'Ivoire

1. Introduction

The production of rubber in hevea, unlike other plants which give fruits, seeds, roots or tubers, etc., comes from the cytoplasm of laticiferous cells, the latex, following the application of bloodletting (Jacob *et al*., 1995a) [18]. This involves making an incision or a notch called a tapping notch in the bark of the tree trunk; which leads to the flow of latex (Frey-wyssling, 1932; Bouychou, 1962; Gomez, 1982; Thomas *et al*., 1995; Obouayeba, 1995; Obouayeba *et al*., 2000) [13, 5, 17, 40, 27, 30], the treatment of which gives natural rubber tapping trees on a plantation does produce rubber, but this production is relatively limited. Neither can it be modulated to the needs of users and, above all, tapping alone cannot make it possible to better exploit the rubber production potential of all the groups of clones cultivated because of their different laticigenic metabolism (Jacob *et al*. 1988) [21]. Cultured clones do not in fact have the same reaction to hormonal stimulation of production (Jacob *et al.*, 1988; Diarrassouba, 2013; Diarrassouba *et al*., 2012) [21, 10, 11]. Nowadays, a strategy for hormonal production stimulation is systematically added to the tapping system (Gallois, 1998; Obouayeba, 1995; Soumahin *et al*., 2009; Soumahin, 2010) [14, 19, 27, 36, 37]. Hormonal stimulation of production consists in applying to the tapping panel, a stimulating chemical substance which significantly increases the level of rubber production (Eschbach and Tonnelier, 1984; Obouayeba, 1993; Obouayeba *et al*., 1996) [12, 25, 26, 29]. This practice developed considerably after the discovery of the significant stimulating power of Ethephon, a growth regulator releasing ethylene in situ (Abraham *et al*., 1968; d'Auzac and Ribaillier, 1969) [1]. Commonly used stimulants have as active ingredient 2-chloro-ethylphosphonic acid or Ethephon, which generates ethylene in laticifers, specialized cells that make latex. The presence of ethylene in these fabrics allows for an extension of the latex flow time and a high rubber yield. More recently, Silpi *et al.* (2006) [35] showed that ethylene stimulation with Ethephon significantly altered the sugar balance between supply and demand in the latex-producing bark, while increasing metabolic activation. In addition, very recent work (Obouayeba *et al.*, 2008) have shown that reverse tapping on virgin bark preceded by nine years of descending tapping significantly improves the rubber productivity (35%) of rubber trees compared to those bled in descending on regenerated bark. To take into account, the proven productivity of reverse tapping relative to descending tapping, the positive effects of ethylenic stimulation in order to achieve the objective of determining the latex harvesting systems likely to improve the productivity of the BPM 24 clone. *Hevea brasiliensis* from the tapping (opening), the present study introduced the tapping in an upward semi-spiral from the tapping as well as the application of different strategies of ethylenic stimulation. It was carried out on the experimental plantation of the CNRA / Bimbresso research station in the south-west of Côte d'Ivoire. This article reviews and analyzes the results of seven (7) years of experimentation on the different technologies or systems for harvesting the latex of the BPM 24 clone at the opening in order to determine the best one or the best.

2. Material And Methods

2.1. Plant material and experimental design

The BPM 24 clone was planted in June 2000 at a density of 510 trees per hectare (7 mx 2.8 m). The experiment began in April 2006 with the tapping of trees. It has an average growth, but weak after the tapping (Anonymous, 1993; Obouayeba *et al.*, 2000; Obouayeba, 2005) [2, 30, 28]. It is one of the most planted clones in the world, occupying 50% of the rubber plantations in Côte d'Ivoire and 95% in villages. It is characterized by low sensitivity to dry notch and relatively low wind breakage (Chapuset *et al*. 2001) [6]. The latex was collected in polyethylene bags (polybag). The trees that were the subject of the experiment were chosen so as to obtain homogeneity of circumference (average circumference of 48.9 cm), and regularity of the trunk within each treatment. The experimental setup is completely randomized where a tree is a repetition, with thirty-three (33) trees per repetition (One tree plot design).

2.3. Treatments

The different treatments (Tableau 1) were as follows (Lukman, 1983; Vijayakumar, 2008 and Vijayakumar *et al.*, 2009) [23, 32, 42]:

A. Witness not bled.
B. S / 2 d4 6d / 7 12 m / 12.ET2.5% Pa1 (1) 0 / y * at 1.20 m from the ground.
C. S / 2 d4 6d / 7 12 m / 12.ET2.5% Pa1 (1) 4 / y * at 1.20 m from the ground (without gradient).
D. S / 2 d4 6d / 7 12 m / 12.ET2.5% Pa1 (1) 8 / y at 1.20 m from the ground (without gradient).
E. S / 2 d4 6d / 7 12 m / 12. ET2.5% Pa1 (1) at 1.20 m from the ground (increasing gradient):
   4 / y for years 1 and 2 (2 years),
   8 / y from year 3 to 7 (6 years).
F. S / 2 d4 6d / 7 12 m / 12. ET2.5% Pa1 (1) at 1.20 m from the ground (decreasing gradient):
   13 / y for year 1 on panel B0-1,
   8 / y, year 2 on panel B0-1,
   10 / y, year 3 on B0-2,
   6 / y, years 4 (B0-1) and 5 (B0-2),
   4 / y, years 6 (B0-1) and 7 (B0-2).
G. S / 2 d4 6d / 7 12 m / 12.ET2.5% Pa1 (1) at 0.70 m from the ground, with decreasing gradient:
   13 / y for year 1 on panel B0-1,
   8 / y, year 2 on panel B0-1,
   10 / y, year 3 on B0-2,
   6 / y, year 4 (B0-2),
   then, S / 2U d4 6d / 7 12 m / 12.ET2.5% Pa1 (1) 8 / y, years 5 to 7, annual alternation.
H. S / 2U d4 6d / 7 12 m / 12. ET2.5% Pa1 (1) not stimulated, years 1 to 7, annual alternation.
I. S / 2U d4 6d / 7 12 m / 12.ET2.5% Pa1 (1) 4 / y, years 1 to 7, annual alternation.
J. S / 2U d4 6d / 7 12 m / 12.ET2.5% Pa1 (1) 8 / y, years 1 to 7, annual alternation.
K. S / 2U d4 6d / 7 12 m / 12.ET2.5% Pa1 (1) 13 / y, years 1 to 7, annual alternation.

Table 1: Description of the different treatments

N° Treatments	Latex Harvesting Technique	Description
1	Absolute witness	not bled
2	S/2 d4 6d/7 12 m/12.ET2,5% 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 based on 1 g of Stimulant Mix * per tree on a 1 cm wide strip, zero annual application.
3	S/2 d4 6d/7 12 m/12.ET2,5% Pa1(1) 2/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 based on 1 g of Stimulant Mix * per tree on a 1 cm wide strip, 2 applications per year.
4	S/2 d4 6d/7 12 m/12.ET2,5% Pa1(1) 4/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 based on 1 g of Stimulant Mix * per tree on a 1 cm wide strip, 4 applications per year.
5	S/2 d4 6d/7 12 m/12.ET2,5% Pa1(1) 8/y (m)	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 the rate of 1 g of Stimulant Mix sur per tree on a 1 cm wide strip, 8 applications per year.
6	S/2 d4 6d/7 12 m/12.ET2,5% Pa1(1) 13/y (3w)	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 1 g of Stimulant Mix per tree on a 1 cm wide strip, 13 applications per year.
7	S/2 d4 6d/7 12 m/12.ET2,5% Pa1(1) 26/y (2w)	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 1 g of Stimulant Mix * per tree on a 1 cm wide strip, 26 applications per year.

8	S/2 d4 6d/7 12 m/12.ET2,5% Pa1(1) 39/y (w)	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 based on 1 g of Stimulant Mix * per tree on a 1 cm wide strip, 39 applications per year.
9	S/2 d4 6d/7 12 m/12.ET2,5% Pa1(1) 78/y (4d)	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 1 g of Stimulant Mix * per tree on a 1 cm wide strip, 78 applications per year.

2.4. Measurements made

The production was determined from rubber weighings, tree by tree, carried out every four weeks. The transformation coefficient was determined by treatment. This coefficient was used to obtain from the fresh weight, the dry rubber production in grams per tree and per bleed ($g.a^{-1}. s^{-1}$). In order to limit the effect of variable circumferences of the tree trunk, the rubber production was also expressed in $g. a^{-1}. s^{-1}.cm^{-1}$ ($g.a^{-1}. s^{-1}.cm^{-1}$) thanks to the estimation of the latter (LESE), depending on the circumference at 1.70 m from the ground (Obouayeba *et al*., 1996) [29]:

L.E.S.E (estimated bleeding notch length in cm) = Circumference at the end of the experiment (cm) / 2 Cos 30 °. This dry rubber production was expressed in grams, per tree, per bleed and per centimeter of bleeding notch ($g.a^{-1}. s^{-1}.cm^{-1}$) ($g.a^{-1}. s^{-1}.cm^{-1}$) over a period of seven (7) years. The following measurements were taken annually:

- Circumference at 1.70 m from the ground,
- Reading of dry notch, 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). These were also measured annually. The parameters taken into account in the MDL were the solids content (Ex.S), the contents ($mmol.l^{-1}$) of sucrose (Sac), of inorganic phosphorus (Pi) and of thiol compounds (RSH) 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) [3]: 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) [38], method using ammonium molybdate: Phosphorus is complexed by an excess of ammonium molybdate. The complex formed is then reduced by 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) [4] using dinitro-2,2'-dithio-5-5'-dibenzoic acid (DTNB): The compounds R -SH react with this acid to give thio-nitro-benzoic acid (TNB) which absorbs strongly at 412 nm (Ellman reaction). TNB is revealed by Tris buffer (yellow coloration).

3. Statistical analysis

Data for rubber production, trunk isodiametric growth (circumference), MDL were subjected to analysis of variance. For this purpose, we only considered the final values of the physiological parameters to assess the influence of the treatments on the physiological state of the trees. The level of significance of the differences between the means was estimated by the Newman-Keuls test at the 5% level. Principal component analysis was used to determine the best latex harvesting system.

4. Results

4.1. Agronomic parameters

Regardless of the opening standard, in the case of production expressed in $g.a^{-1}. s^{-1}$, five groups of latex harvesting technologies or systems have been revealed. Group 1, consisting of treatment G, recorded a production of 60 $g.a^{-1}. s^{-1}$. Group 2, with treatments C, D and E, gave a production which oscillated between 54 and 57 $g.a^{-1}. s^{-1}$. Group 3, consisting of treatment F, gave a production of 52 $g.a^{-1}. s^{-1}$. Group 4, formed from treatment K, produced a production of 46 $g.a^{-1}. s^{-1}$. Treatments B, H, J and L, which made up group 5, recorded productions of between 39 and 44 $g.a^{-1}. s^{-1}$. The trees of treatment G, bled in a descending half-spiral and stimulated with a decreasing gradient (13, 8, 10 and 6 / y) the first four years, alternated by a bleeding in a half-spiral upwards in years 5 to 7, gave the most important rubber production. It was equivalent to that of the trees of treatments C, D and E, bled in a descending half-spiral and stimulated respectively 4 and 8 times per year and with increasing gradient (2, 6 and return to 2 / y). The trees of treatment F, bled in a descending half-spiral and stimulated with decreasing gradient (13, 8, 10, 6, 4 and 2 / y), presented a rubber production equivalent to that of the trees of treatments C, D, E and K (bled respectively in an ascending half-spiral, stimulated 8 times a year and opened at 0.70 m from the ground). The K treatment displayed similar rubber production to those of the H, J and L treatments, bled in an upward semi-spiral and stimulated respectively 0, 4 and 13 / y and open at 0.70 m from the ground, as well as than that of the bled control treatment, in a descending semi-spiral, unstimulated and open at 1.20 m (Table 2). With regard to rubber production expressed in $g.a^{-1}. s^{-1}.cm^{-1}$, eight groups of latex harvesting technologies or systems have been identified. Groups 1 and 2, represented by treatments G and E respectively, showed a production of 3.14 and 2.82 $g.a^{-1}. s^{-1}.cm^{-1}$, respectively. Group 3, consisting of treatments C and D, gave a rubber production between 2.69 and 2.73 $g.a^{-1}. s^{-1}.cm^{-1}$. Groups 4, 5, 6 and 7, composed respectively of treatments K, L, J and B, recorded respectively productions of 2.50; 2.42; 2.29 and 2.02 $g.a^{-1}. s^{-1}.cm^{-1}$. Group 8 was formed from treatment H with a yield of 1.89 $g.a^{-1}. s^{-1}.cm^{-1}$. The trees in treatment G gave the highest rubber production. It was equivalent to that of the trees in treatment E. The trees in the treatments, bled in a downward half-spiral and stimulated with a decreasing gradient (13, 8, 10, 6, 4 and 2 / y), exhibited rubber production. equivalent to those of the trees of treatments C, D, E and K (bled respectively in an upward semi-spiral, stimulated 8 times a year and open at 0.70 m from the ground). The K treatment displayed similar rubber production to those of the H, J and L treatments, bled in an upward semi-spiral and stimulated respectively 0, 4 and 13 / y and open at 0.70 m from the ground, as well as than that of the control treatment, bled in a descending semi-spiral, unstimulated and open at 1.20 m (Table 2).

Table 2: Agronomic parameters of the BPM 24 clone at opening according to different latex harvesting systems during 7 years of experimentation

Treatments	$g.a^{-1}. s^{-1}$	$g.a^{-1}. s^{-1}.cm^{-1}$	Acc. Ann. $cm.an^{-1}$
A. Witness not bled	-	-	5,14 a
B. S / 2 d4 6d / 7 12 m / 12.ET2.5% Pa1 (1) 0 / y * at 1.20 m from the ground.	44 d	2,02 ef	3,03 b
C. S / 2 d4 6d / 7 12 m / 12.ET2.5% Pa1 (1) 4 / y * at 1.20 m from the ground (without gradient).	54 ab	2,69 bc	2,29 cd
D. S / 2 d4 6d / 7 12 m / 12.ET2.5% Pa1 (1) 8 / y at 1.20 m from the ground (without gradient).	55 ab	2,73 bc	2,22 cd
E. S/2 d4 6d/7 12 m/12.ET2.5% Pa1(1) at 1,20 m of the ground (increasing gradient): 4 / y for years 1 and 2 (2 years), 8 / y from year 3 to 7 (6 years)	57 ab	2,82 ab	2,25 cd
F. S/2 d4 6d/7 12 m/12.ET2.5% Pa1(1) at 1,20 m of the ground (increasing gradient): 13 / y, for year 1 on panel B0-1, 8 / y, year 2 on panel B0-1, 10 / y, year 3 on B0-2, 6 / y, years 4 (B0-1) and 5 (B0-2), 4 / y, years 6 (B0-1) and 7 (B0-2)	52 bc	2,66 bcd	2,10 d
G. S/2 d4 6d/7 12 m/12.ET2.5% Pa1(1) at 0,70 m of the ground (increasing gradient): 13 / y for year 1 on panel B0-1, 8 / y, year 2 on panel B0-1, 10 / y, year 3 on B0-2, 6 / y, year 4 (B0-2), then, S / 2 in an ascending half-spiral d4 6d / 7 12 m / 12.ET2.5% Pa1 (1) 8 / y, years 5 to 7, annual alternation	60 a	3,14 a	1,76 e
H. S / 2 U d4 6d / 7 12 m / 12.ET2.5% Pa1 (1) 0 / y, at 0.70 m from the ground, years 1 to 7, annual alternation	39 d	1,89 f	2,49 c
J. S/2 U d4 6d/7 12 m/12.ET2.5% Pa1(1) 4/y*, at 0,70 m from the ground, years 1 to 7, annual alternation	44 d	2,29 de	1,79 e
K. S/2 U d4 6d/7 12 m/12.ET2.5% Pa1(1) 8/y, at 0,70 m from the ground, years 1 to 7, annual alternation	46 cd	2,50 bcd	1,70 e
L. S/2 U d4 6d/7 12 m/12.ET2.5% Pa1(1) 13/y, at 0,70 m from the ground, years 1 to 7, annual alternation	44 d	2,42 cd	1,56 e

4.2. Physiological parameters

Regarding the physiological parameters (Table 3), after 7 years, the dry extract levels of the trees from the different treatments were all of the same order of magnitude. At the level of sucrose content, four groups have identified. Group 1, consisting of treatment H, showed a content of 46.27 $mmol.l^{-1}$. Group 2, identified by treatment B, gave a content of 35.24 $mmol.l^{-1}$. The trees of the C, D, E, F, J, K and L treatments, which formed group 3, gave sucrose contents between 22.63 and 28.91 $mmol.l^{-1}$. Group 4, represented by treatment G, displayed a sucrose content of 19.67 $mmol.l^{-1}$. Overall, the sucrose content was good for all trees in all treatments. Unstimulated trees exhibited the highest sucrose contents. All of the stimulated trees had similar sucrose contents. The trees in treatment G have the lowest sucrose content. For the content of inorganic phosphorus, five groups have been revealed. Group 1, consisting of treatments B, C, E and F, showed an inorganic phosphorus content which ranged from 17.00 and 19.63 $mmol.l^{-1}$. Group 2, consisting of treatment D, gave a content of 15.82 $mmol.l^{-1}$. Group 3, formed from treatments G, H and J, recorded an inorganic phosphorus content of between 11.80 and 13.72 $mmol.l^{-1}$. Treatment L, which gave a content of 10.10 $mmol.l^{-1}$, formed group 4 and group 5 with an inorganic phosphorus content of 8.45 $mmol.l^{-1}$ was formed from treatment K. Trees from all treatments gave good inorganic phosphorus content except those from treatment K. For thiol content, eight groups were identified. Group 1, represented by treatment B, showed a thiol content of 0.88 $mmol.l^{-1}$. Group 2, formed from treatment C, recorded a content of 0.76 $mmol.l^{-1}$. The trees of treatment H, with a content of 0.63 $mmol.l^{-1}$, made up group 3. Treatment E, with a content of 0.58 $mmol.l^{-1}$, made up group 4. The group 5, composed of treatments D and F, was characterized by a content of the order of 0.54 $mmol.^{l-1}$. With a content of 0.48 $mmol.l^{-1}$, the J treatment materialized group 6. Group 7, consisting of the K and L treatments, gave respectively contents of thiol compounds of 0.38 and 0.35 $mmol..l^{-1}$. Depending on the opening height, unstimulated trees displayed higher levels of thiol compounds than stimulated trees. Trees opened and stimulated at 1.20 m from the ground showed higher contents than trees opened and stimulated at 0.70 m. The same is true for unstimulated trees.

Table 3: Physiological parameters of the BPM 24 clone at opening according to different latex harvesting systems during 7 years of experimentation

Treatments: Latex harvesting systems or technologies	Ex. sec (%)	Sac ($mmol.l^{-1}$)	Pi fin ($mmol.l^{-1}$)	RSH fin ($mmol.l^{-1}$)
B. S / 2 d4 6d / 7.ET2.5% Pa1 (1) 0 / y * at 1.20 m from the ground	55,96 a	35,24 ab	19,63 a	0,88 a
C. S / 2 d4 6d / 7.ET2.5% Pa1 (1) 4 / y * at 1.20 m from the ground (without gradient)	51,98 a	28,91 bc	17,74 a	0,76 ab

D. S / 2 d4 6d / 7.ET2.5% Pa1 (1) 8 / y at 1.20 m from the ground (without gradient)	58,14 a	27,44 bc	15,82 ab	0,54 cd
E. S / 2 d4 6d / 7.ET2.5% Pa1 (1) at 1.20 m from the ground (increasing gradient): 4 / y for years 1 and 2 (2 years), 8 / y from year 3 to 7 (6 years)	52,54 a	27,55 bc	17,57 a	0,58 c
F. S / 2 d4 6d / 7.ET2.5% Pa1 (1) at 1.20 m from the ground (decreasing gradient): 13 / y, for year 1 on panel B0-1, 8 / y, year 2 on panel B0-1, 10 / y, year 3 on B0-2, 6 / y, years 4 (B0-1) and 5 (B0-2), 4 / y, years 6 (B0-1) and 7 (B0-2)	49,78 a	24,60 bc	17,00 a	0,55 cd
G. S / 2 d4 6d / 7.ET2.5% Pa1 (1) at 0.70 m from the ground, with decreasing gradient: 13 / y for year 1 on panel B0-1, 8 / y, year 2 on panel B0-1, 10 / y, year 3 on B0-2, 6 / y, year 4 (B0-2), then, S / 2 U d4 6d / 7 12 m / 12.ET2.5% Pa1 (1) 8 / y, years 5 to 7, annual alternation	55,16 a	19,67 c	12,58 abc	0,34 e
H. S / 2 U d4 6d / 7.ET2.5% Pa1 (1) 0 / y, 0.70 m from the ground, years 1 to 7, annual alternation	51,72 a	46,27 a	11,80 abc	0,63 bc
J. S / 2 U d4 6d / 7 12 m / 12.ET2.5% Pa1 (1) 4 / y *, 0.70 m from the ground, years 1 to 7, annual alternation	57,03 a	25,22 bc	13,72 abc	0,48 cde
K. S / 2 U d4 6d / 7.ET2.5% Pa1 (1) 8 / y, 0.70 m from the ground, years 1 to 7, annual alternation	52,89 a	27,12 bc	8,45 c	0,38 de
L. S / 2 U d4 6d / 7.ET2.5% Pa1 (1) 13 / y, 0.70 m from the ground, years 1 to 7, annual alternation	59,18 a	22,63 bc	10,10 bc	0,35 de

In the same column, the means assigned the same letter are not statistically different.

4.3. Determination of the latex harvesting technology suitable for the BPM 24 clone upon opening

The correlation matrix between the parameters studied (Table 4), revealed that the increase in tree trunk circumference was positively correlated with the contents of sucrose, inorganic phosphorus and thiol compounds in the latex. Rubber production increased inversely with the sucrose content of the latex and diseased notch length. The contents of inorganic phosphorus and thiol compounds evolved in the same direction as the length of diseased notch and the rate of completely dry trees. The screening of these studied parameters made it possible to obtain the Biplot dispersion plan (fig 1). The parameters of increased trunk circumference, sucrose and thiol compound contents were strongly and positively correlated with the F1 axis (Table IV). Rubber production, diseased notch length and rate of dry trees were negatively correlated to the same axis which contributed 59.01% to the total expressed variance. The inorganic phosphorus content was positively correlated with the F2 axis which accounted for 23.49% of the total variance (Table 5). These two axes made it possible to characterize the different latex harvesting systems. The J, K and L treatments located in the quarter plane not including any studied parameter, showed low values for each of the studied parameters. The H treatment in the quarter plan with the sucrose content showed a high content of this parameter, low production, as well as low increases in circumference, contents of inorganic phosphorus and thiol compounds, low values of LEM and completely dry trees. In the quarter plan with rubber production, diseased notch length and dry tree rate, treatment G had the highest production, high LEM and high dry tree rates. However, the contents of inorganic phosphorus and thiol compounds, as well as the increase in trunk circumference were of less importance. Treatments B (unstimulated), C, D, E and F produced good average annual increases in trunk circumference, good levels of inorganic phosphorus and thiol compounds. Sucrose content is also good, while rubber production is less important with high rates of diseased notch length and dry trees. Their membership in the quarter plan containing these parameters clearly indicates this. All of these observations made it possible to retain treatment C as that allowing the BPM 24 clone to better express its agronomic potential on opening. In addition, it differs from the others by its very good content of inorganic phosphorus and thiol compounds, close to that of the unstimulated control, the reference.

Table 4: Correlation matrix between parameters studied

	Acc	Prod	Sacc	Pi	RSH	LEM	Arb. secs
Acc	1						
Prod	-0,399	1					
Sacc	0,696	-0,772	1				
Pi	0,750	0,066	0,111	1			
RSH	0,922	-0,436	0,611	0,792	1		
LEM	-0,226	0,651	-0,454	-0,103	-0,406	1	
Arb secs	-0,509	0,506	-0,492	-0,431	-0,609	0,896	1

In bold, significant values (excluding diagonal) at the alpha threshold = 0.050 (two-tailed test)

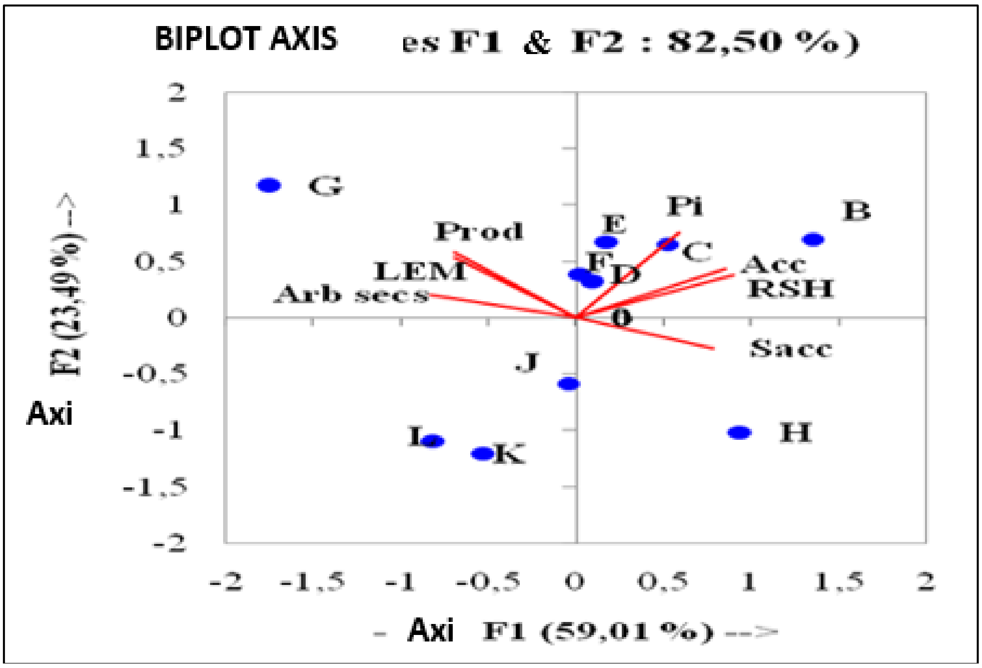

Fig 1: Distribution of the different latex harvesting systems when opening the BPM 24 clone

Table 5: Correlation of variables with axes F1 and F2

Variables studied	F1	F2
Acc	0,851	0,432
Prod	-0,693	0,578
Sacc	0,786	-0,268
Pi	0,581	0,761
RSH	0,898	0,379
LEM	-0,692	0,539
Arb secs	-0,827	0,200

5. Discussion

5.1. Agronomic parameters

The downward half-spiral tappding systems performed better than the upward half-spiral ones, with the exception of the latex harvesting system, bled down four years and then up the rest of the time, which gave the maximum of rubber production. These results, especially that of the latex harvesting system G, show that the rubber production performance of the upward or reverse bled latex harvesting systems is related to a previous downward tapping. These results also indicate that a minimum time in years of descending tapping is necessary to make reverse or ascending tapping efficient. This period is at least equal to four years of descending tapping, with regard to our results. We are even justified in saying that the minimum delay in the case of this study is, concerning the BPM 24 clone, five years of descending tapping. This thesis is supported by the fact that the work of Traoré, (2014) and (Obouayeba, personal communication) have shown that the trees of the BPM 24 clone under unstimulated rubber production have a production level statistically equivalent to that of all other treatments boosted in the 5th year of latex harvest. This is a sign that all the stimulated and unstimulated rubber trees (control) have, at the end of the 5th campaign, a level of activation significantly of the same or identical order of magnitude. Indeed, observation of the evolution curves of this parameter makes it possible to note that with the exception of the control not stimulated in reverse, whose tendency is sawtooth with a peak of production in the 6th year of harvest of the latex, all other latex harvesting systems showed maximum production in year 5. This illustrates the fact that regardless of the direction of tapping and the stimulation regimen, the overall activation of the bleeding tree (BPM 24 clone) occurs after five years. And as at this stage, no reverse tapping latex harvesting technology from the opening has been shown to be effective relative to latex downhill harvesting technologies, this implicitly suggests the exclusion of all latex harvesting technologies from. This nature in the management of the BPM 24 clone and probably of the moderately metabolized clones. This assertion is supported by the rubber production results of the G Latex Harvesting System which we rate as good. However, the high dry notch rate displayed by the G treatment is the expression of what he probably must have suffered, from an increase in metabolic activation linked to an excess of energy probably coming from the precocity of reverse tapping (Gohet *et al.*, 1991) [15] and especially the intensification of the latex harvesting system by a stimulation regime can be strong for the latex harvest years concerned. These results highlight the very important role of the activation of the rubber production metabolism at the origin of the performance of laticifers, as demonstrated by several authors (Jacob *et al*. 1988, 1994, 1995 a and b; Obouayeba *et al*. 1996 and Silpi *et al*., 2006) [21, 18, 20, 29, 35]. This is because the tapping and hormonal stimulation of rubber production provide energy to the rubber plant that is the laticiferous cell (laticifer; Jacob *et al*., 1995a) [18]. This process, which is essential in the efficiency of isoprene synthesis, is amplified over time and probably explains the poor performance of upward bleeding upon opening, yet effective as noted by Obouayeba *et al*. (2008) [32].

Our results also show the efficiency in rubber production of systems with increasing gradient of stimulation over those with decreasing gradient. In fact, the bloodletting, by stress, resulting from the trauma (Chrestin, 1985) [9] that it induces, would activate the metabolism of laticifers in the same way as hormonal stimulation, by production of ethylene. This ethylene is produced and released during stress in the tissues to act by activating the entire process of synthesis of the cis-polyisoprene or rubber molecule (Lacrotte, 1991) [22]. In addition, the presence of this hormone in these tissues allows an extension of the flow time of the latex and a high yield of rubber. Due to the action of ethylene on glycolysis, there is a production of energy necessary for the transformation of the cis-polyisoprene molecule into rubber (Jacob *et al*., 1998) [19]. This clearly explains the fact that hormonal stimulation with increasing gradient is more efficient than that at single intensity, reflecting an activation of the laticiferous metabolism. The tapping in an upward semi-spiral being very productive, it requires good activation by the exogenous energy associated with the bleeding and the stimulation before its implementation. Hence, the downward half-spiral tapping for four years strongly activated the metabolism through its action and promoted better production when it was alternated by the upward half-spiral bleeding. This probably explains the fact that, the alternation between tapping in a descending half-spiral and tapping in an ascending half-spiral after 4 years, allowed to have the highest production of rubber.

The average annual increase in girth of non-bled trees is greater than that of bled trees. This situation comes from this This is due to the fact that the tapping of rubber trees results in the diversion of a fraction of the photosynthetic assimilates and of the necessary energy, strictly assigned to the general metabolism of the tree and to the primary biomass (leaves, wood and bark). It thus directs the metabolism towards regeneration of the cellular material exported during tapping and corresponding to the synthesis of secondary biomass, as demonstrated by Jacob *et al*. (1995a) [18]. The consequence is a reduction in radial vegetative growth which resulted in a decrease in the trunk circumference of bleeding trees compared to that of non-bled trees (Gohet, 1996) [16]. Our results are analogous to the observations of Paardekooper *et al*. (1975), from Obouayeba and Boa (1993) [25, 26] and Gohet, (1996) [16], Obouayeba *et al*. (2002) and Obouayeba, (2005) [28]. These authors have shown that tapping is inevitably accompanied by a reduction in the annual rate of vegetative growth. The ethylenic stimulation intensification of the technique of harvesting latex from a tree resulted in a decrease in trunk circumference compared to an unstimulated control. In fact, the orientation of energy and photosynthetic assimilates towards the production of latex comes at the expense of vegetative growth (Gohet, 1996) [16]. It is the fact that, the hormonal stimulation, by its effect, increases the production of rubber by strongly orienting the hydrocarbon assimilates towards it to the detriment of the vegetative growth. This results in a decrease in the thickness growth of the trunk of stimulated trees compared to unstimulated trees (Templeton, 1969; Gohet, 1996; Obouayeba *et al*., 2002; Obouayeba, 2005) [39, 16, 28]. Therefore, in stimulated trees, there is a decrease in trunk circumference with the increase in the number of annual stimulations, partly responsible for the increase in production.

5.2. Physiological parameters

The different treatments had no impact on the dry extract level of the latex. This reflects good regeneration of the latex exported during tapping due to the dry extract level which reflects the biosynthetic activity of laticifers (Milford *et al*., 1969) [24]. Sucrose content was good for all trees in all treatments. Unstimulated trees exhibited the high levels of sucrose. All the trees stimulated had similar productions. The trees of treatment G have the lowest sucrose content, probably because of the high rubber production displayed. This is a sign of a good supply of sucrose in laticifera, a raw material necessary for rubber biosynthesis (Prévôt *et al*., 1986). This supply indicates that there is permanently available sucrose in the laticifers, allowing good rubber production. The sucrose content of the G treatment is the result of the good rubber production it generated. Indeed, this sucrose has been heavily used for the production of rubber as shown by Lacrotte (1991) [22], Gohet (1996) [16] and Jacob *et al*. (1998) [19]. The inorganic phosphorus contained in the latex can be considered as an indicator of the intensity of the energy metabolism of laticiferous cells (Jacob *et al*., 1988) [21]. Trees from all treatments gave good inorganic phosphorus content except those from the K treatment, reflecting better availability of the energy required for rubber biosynthesis. This exception is certainly due to a metabolic dysfunction caused by this latex harvesting system in the sense that the trees subjected to this treatment have retained a good sucrose content (Gohet, 1996) [16].

Thiol groups, in their reduced form R-SH, are capable of neutralizing toxic forms of oxygen (Chrestin, 1984) [8]. These are antioxidants which protect the cellular compartmentalisation of the latex including that of lutoids, and consequently the functioning of laticifers. Unstimulated trees displayed higher levels of thiol compounds than stimulated trees. This is explained by the fact that, the intensification of the metabolism of the clones by the stimulation leads to an increasingly intense stress, resulting in a weakening of the protective systems and resulting in "physiological fatigue".

6. Conclusion

A 7-year study of *Hevea brasiliensis* clone BPM 24 latex harvesting systems at opening has yielded convincing results. They indicated that the bleeding at 1.20 m from the ground (descending tapping) gives better results compared to that at 0.70 m (ascending tapping) not preceded by a descending tapping for a minimum delay equivalent to 4 years. The latex C harvesting system (S / 2 d4 6d / 7 12 m / 12.ET2.5% Pa1 (1) 4 / y at 1.20 m from the ground (no gradient)) is probably best suited to the clone GT 1 at the opening. Even though, the downward half-spiral bleeding for four years from 1.20 m above the ground, alternated by a half-spiral tapping up to 0.70 m, has been shown to be more productive, with little effect. harmful to radial vegetative growth, good levels of sucrose and inorganic phosphorus in tree latex. In addition, our results indicate that inverted tapping (upward half-spiral or quarter-spiral) can be introduced at least from the fifth, probably from the sixth, year of harvesting the latex.

6. Conflict Of Interest

The authors do not declare any conflict of interest.

7. Author Contributions

For this work, BMM and AK carried out the entire sampling and took an active part in the data processing and the preparation of the document. As for KYJ and BMM, they were very present in the data processing and the preparation of the final document. OS made its research laboratory available to the team and provided the working equipment.

8. References

1. Abraham PD, Wycherly PR, Pakianathan SW. Stimulation of latex flow in Hevea brasiliensis by 4-amino-3,5,6-trichloropicolinic and 2-chloroethane phosphonic acid. J. Rubb. Res. Inst. Malaya, 1968; 20:291-305.
2. Anonymous. Collection of Hévéa clone files. CIRAD-Cultures Pérennes, ed., Montpellier, France, 1993, 20.
3. Ashwell G. Colorimetric analysis of sugar. Methods Enzymol 1957; 3:73-105.
4. Boyne AF, Ellman GL. A methodology for analysis of tissue sulphydrylcomponents. Anal. Biochem., 1972; 46:639-653.
5. Bouychou JG. Hevea tading, a rubber planter's manual, Soc. Techn. Contin., Paris, 1962, 50.
6. Chapuset TH. Description of the clones studied on a large scale. CNRA-HEVEA report n ° 01/01, 2001, 36.
7. Chrestin H. The vacuo - lysosomal compartment (lutoids) of Hevea brasiliensis latex, its role in the maintenance of homeostasis and in the processes of senescence of laticiferous cells. Doctoral thesis Sci State. Nat., Sci University. Tech. Languedoc, Montpellier, France, 1984, 575.
8. Chrestin H, Bangratz J, (d') Auzac J, Jacob JL. Role of the lutoidic tonoplast in the senescence and degradation of the laticifers of Hevea brasiliensis. Z. Pflanzen Physiol., 1984; 114:261-268.
9. Chrestin H. The ethrel stimulation of the rubber tree; as far as not to go too far. Caouth. Plast. 1985; 647-648: 75-78.
10. Diarrassouba M. Contribution to the definition of latex harvesting technologies adapted to Hevea brasiliensis Muell clones. Arg. (euphorbiaceae) of the metabolic, active, moderate and slow activity classes. Unique doctoral thesis, UFR Biosciences, University of Cocody, Ivory Coast, 2013, 207.
11. Diarrassouba M, Soumahin EF, Coulibaly LF, N'guessan AEB, Dick KE, Kouamé C *et al*. Latex harvesting technologies adapted to clones PB 217 and PR 107 of Hevea brasiliensis Muell. Arg of the slow metabolism class and to the socio-economic context of Côte d'Ivoire. International Journal of Biosciences (IJB), 2012; 2(12):125-138.
12. Eschbach JM, Tonnelier M. Influence of the method of stimulation, the concentration of the stimulant and the frequency of its application on the production of the GT 1 clone in the Ivory Coast. In: C.R. Coll. Expl. Physiol. Amel. Hévéa, IRCA-CIRAD, ed., Montpellier, France, 1984, 295-306.
13. Frey-Wyssling A. Studies on the dilution reaction and the mouvement of the latex of Hevea brasiliensis during tapping. Arch. Rubbercult., 1932; 16:285-327.
14. Gallois R. Métabolisme des nucléotides adényliques dans le latex d'Hevea brasiliensis. Effet de l'éthylène. Thèse Université de Montpellier II, Faculté des Sciences et Techniques du Languedoc, France, 1998, 154.
15. Gohet E, Lacrotte R, Obouayeba S, Commère J. Tapping system recommended in West Africa. Proc., RRIM Rubber grower's conf. 1991, 235-254.
16. Gohet E. The production of latex by Hevea brasiliensis. Relationship with growth. Influence of different factors: clonal origin, hormonal stimulation, hydrocarbon reserves. PhD thesis, University of Montpellier II, France, 1996, 343.
17. Gomez JB. Anatomy of Hevea and its influence on latex production. Malaysian Rubber Research and Board (MRRDB), monograph n°7, Kuala Lumpur, 76 p. IRRDB Workshop of Latex Harvesting Technologies, Sungai Buloh, Selangor, 1982, 2008, 20.
18. Jacob JL, D'Auzac J, Prévôt JC, Sérier JB. A natural rubber factory: L'Hévéa. Research, 1995a; 276:538 - 545.
19. Jacob JL, Prévôt JC, Lacote R, Gohet E, Clément A, Gallois R *et al*, Biological mechanisms of rubber production by Hevea brasiliensis. Communication from IRRDB 1997 Workshop, Ho Chi Minh City, Vietnam, 1997, 1998, 13.
20. Jacob JL, Prévôt JC, Lacrotte R, Eschbach JM. The latex diagnosis. Plantations, research, development, 1995b; 2:34-37.
21. Jacob JL, Serrès E, Prévôt JC, Lacrotte R, Clément-Vidal A, Eschbach JM *et al*. Mise au point du diagnostic latex. Agritrop, 1988; 12:97-118.
22. Lacrotte R. Study of the relationship between the sugar content of the latex and the production. Approach to the mechanisms of sucrose loading of laticifers of Hevea brasiliensis Muell. Arg. University Doctorate thesis, Languedoc University of Sciences and Techniques, Montpellier II, 1991, 266.
23. Lukman HJG. Revised international notation for exploitation systems. Journal of Rubber Research Institute of Malaysia, 1983; 31:130-140.
24. Milford GFJ, Paardekooper EC, Ho CY. Latex vessels plugging, its importance to yield and clonal behaviour. Journal of Rubber Research Institute of Malaysia, 1969; 21:274 - 282.
25. Obouayeba S, Boa D. Frequency and annual rest of bleeding of Hevea brasiliensis, clone PB 235, in the south-eastern part of the Ivory Coast. Cahiers Agricultures. 1993; 2(6):387-393.
26. Obouayeba S. Estimation of the amount of stimulating paste applied to rubber trees according to their circumference in the south-east of Côte d'Ivoire. African Agronomy, 1993; 1:26-32.
27. Obouayeba S. Harvesting latex from rubber trees in a non-industrial environment. Training document for supervisors in the non-industrial rubber industry, Hévéa Project 5 bis 1995, 14.
28. Obouayeba S. Contribution to the determination of the physiological maturity of the bark for bleeding of Hevea brasiliensis Muell. Arg. (euphorbiaceae): opening standards. Unique doctoral thesis, UFR Biosciences, University of Cocody, Ivory Coast, 2005, 225.
29. Obouayeba S, Boa D, Kéli JZ. Adequacy between quantity of stimulating paste and rubber production of Hevea brasiliensis in the south-east of the Ivory Coast. Tropicultura, 1996; 14(2):54-58.
30. Obouayeba S, Boa D, Aké S. Critical age, bark growth and latex vessel formation as attributes for determination of tapping norms. Indian Journal of Natural Rubber Research, 2000; 13(1, 2):38-45.
31. Obouayeba S, Boa D, Aké S, Lacrotte R. Influence of age and girth at opening on growth and productivity of Hevea. Indian Journal of Rubber Research, 2002; 15(1):38 - 45.

32. Obouayeba S, Soumahin EF, Boko AMC, Dea GB, Dian K, Gnagne YM. Improvement of productivity of rubber trees in smallholding by the introduction of upward tapping in the south-east of Côte d'Ivoire. J. Rubb. Res. 2008; 11(3):163-170.
33. Paardekooper EC, Langlois SJC, Sompong S. Influence of tapping intensity and stimulation on yield, girth and latex constitution. Proc. Int. Rubb. Conf. Kuala Lumpur, 1975; 2:290.
34. Prévôt JC, Jacob JNL, Lacrotte R, Vidal A, Serrès E, Eschbach JM *et al*. Physiological parameters of latex from Hevea brasiliensis. Their use in the study of the laticiferous system. Typology of functioning production mechanisms. Effects of stimulation. In: IRRDB physiology and Récolte de latex Meeting, Hainan, 1986, Pan Yanqing and Lhao Canwen Eds, South China Academy of Tropical Crops of functioning (Hainan), 1986, 136-157.
35. Silpi U, Chantuna P, Kasemsap P, Thanisawanyangkura S, Lacointe A, Ameglio T *et al*. Sucrose metabolism distribution patterns in the lattices of three Hevea brasiliensis: effects of tapping and stimulation on the tree trunk. J. Rubb. Res., 2006; 9:115-131.
36. Soumahin E, Obouayeba S, Anno AP. Low tapping frequency with hormonal stimulation on Hevea brasiliensis clone PB 217 reduces tapping manpower requirement. Journal of Animal & Plant Sciences, 2009; 2 (3):109-117.
37. Soumahin EF. Optimisation des systèmes d'exploitation en hévéaculture par la réduction des intensités de saignée. Thèse unique de Doctorat, Ufr Biosciences, Université Cocody, Côte d'Ivoire, 2010, 189.
38. Taussky HH, Shorr E. A micro colorimetric method for the determination ofinorganic phosphorus. J. Biol. Chem., 1953; 202:675-685.
39. Templeton JK. Partition of assimilates. Journal of Rubber Research Institute of Malaysia, 1969; 21:259-273.
40. Thomas V, Premakumari D, Reghu CP, Panikkar AON, Saraswathy ACK. Anatomical and histochimical aspects of bark regeneration in Hevea brasiliensis. Annals of Botany Company, 1995; 75:421-426.
41. Vijayakumar KR. Revised international notation for latex production technology, 2008.
42. Vijayakumar KR, Gohet E, Thomas KU, Sumarmadji XW, Lakshman R, Thanh DK *et al*. Revised international notation for latex harvest technology. IRRDB Workshop of Latex. Harvesting Technologies, 2009, 19