

Original Research Article

Determination of the execution time of down tapping allowing a good performance of agrophysiological parameters in reverse tapping of fast metabolizing rubber clones PB 260 and IRCA 18

Abstract

The classic latex harvesting system consists of top-down tapping for nine years before being immediately followed by reverse tapping. This period is often considered too long by the farmers. To address this concern, a study was conducted to determine the best period for down tapping to ensure good performance of agrophysiological parameters in reverse tapping of fast metabolising rubber clones. For this purpose, the PB 260 and IRCA 18 clones were used as plant material in Divo and Daoukro respectively, where the experiments were conducted. Five time frames for down-bleeding (5, 6, 7, 8 and 9 years = control) and two concentrations of ethephon (ET) stimulating paste (2.5 and 5% ET), except for the control which is stimulated only at 5% ET, were tested. The experimental design was a Fisher block design with 9 treatments and 4 replicates. Rubber productivity results (5262 ± 205 and 4951 ± 351 kg.ha⁻¹.yr⁻¹) showed that reverse tapping of these metabolically active clones preceded by 5 and/or 6 years of down tapping was the best (3723 ± 29 kg.ha⁻¹.yr⁻¹ ; control). Rubber production and average annual increment from these tapping periods (4.00 ± 0.42 and 3.60 ± 0.00 cm.yr⁻¹ ; control) were the highest. Productivity gains were 41% for reverse tapping at 6 years stimulated at 5%. The dry notch rate was relatively low (3.30% LEM and 0% dry trees). The physiological profile was generally good. These results indicate that downward bleeding for 5 and/or 6 years allows these clones to express their best potential in reverse bleeding. These results are satisfactory and respond exactly to the concerns of the farmers.

Keywords: *Hevea brasiliensis*; PB 260 and IRCA 18; rubber production; vegetative growth; downward tapping; reverse tapping; Ivory Coast.

Introduction

Rubber production in rubber trees, unlike other plants that produce fruits, seeds, roots or tubers, etc., is derived from the cytoplasm of the laticifier cells, the latex, following the application of tapping (1). This consists of making an incision or notch in the bark of the tree trunk, which results in the flow of latex (2; 3), the processing of which produces natural rubber. Tapping trees in a plantation does produce rubber, but this production is limited (4). Hormonal stimulation alone does not allow for better exploitation of the production potential of different rubber clones (5; 6). To take into account the production potential of different rubber clones, several studies have shown that it is important to add hormonal stimulation to the conventional tapping system (7; 6). The stimulating agent, Etephon, stimulates the metabolism of the laticifier cells and increases the time and volume of latex flow and thus the rubber production of the tree. Hormonal stimulation of rubber production is thus becoming an essential component of latex harvesting systems in rubber farming (4; 8) to the point that it is widespread in Côte d'Ivoire. Indeed, tapping causes traumatism to the tree. The same applies to hormonal stimulation. The combination of these two types of trauma improves the rubber productivity of the plantation provided that the sum of their intensity is not excessive (9). Otherwise, the tapping notch becomes dry, i.e. the tree stops producing latex although it is alive (5; 10). The intensity of the bleeding regime (strong, moderate and weak) and the associated stimulation or not, is strongly dependent on the intracellular metabolism of the clone as shown by Jacob et al. Consequently, the linkage of tapping and stimulation of a given rubber clone increasingly requires perfect knowledge of this clone, both from an agronomic and physiological point of view. This concern has led to the integration of Micro Latex Diagnostics (MDL) in such a latex harvesting system. Latex microdiagnosis is a method that makes it possible to assess the physiological state of the rubber tree's laticifier system at a given time (5). Indeed, it allows early detection and prevention of physiological disorders leading to a drop in rubber production. Thus, latex microdiagnosis has become an indispensable tool in rubber farming (5; 4). Improving productivity necessarily requires optimal exploitation of the tree, which consists of practising a much more productive inverted tapping after the downward tapping (11). The performance of inverted tapping in terms of rubber productivity is well documented (12; 11). However, the influence of the low panel, tapped downwards, on the high panel, tapped upwards (inverted), is very rarely mentioned (10). This is all the more true since inverted bleeding can be practised early and/or late (11). Therefore, there is a time frame for the execution of down tapping that would allow for good

and sustainable rubber production in reverse tapping. In the case of this study, and in view of all the above, we set ourselves the objective of determining the time required for top-down tapping in order to achieve efficient rubber productivity in reverse tapping of the PB 260 and IRCA 18 clones.

UNDER PEER REVIEW

MATERIALS AND METHODS

Plant material

Clone PB 260

Hevea brasiliensis clone PB 260 (Prang Besar 260) originates from Malaysia (Prang Besar). It was first planted in Côte d'Ivoire in 1983. It is a cross between PB 5/51 and PB 49. PB 260 is a very metabolically active clone characterised by easy latex flow and good production increase. In the absence of hormonal stimulation, rubber production and inorganic phosphorus levels are high while sucrose levels are very low. It is characterised by good vigour, higher than that of IRCA 18 but lower than PB 235, RRIC 100 and AVROS 2037. Clone PB 260 is resistant to *Colletotrichum* but very susceptible to dry rot and *Corynespora* (13).

Clone IRCA 18

This clone originates from the Rubber Research Institute in Ivory Coast. It is a genetic cross between PB5/51 and RRIM605. It is a vigorous and very homogeneous clone. It is characterised by a low sucrose reserve in the latex, a risk of physiological imbalance in the event of oversimulation, relatively late defoliation, a reduced size at adulthood and by its sensitivity to dry notching and wind breakage (13).

Methods

2.1- Choice of trees

The experiment was conducted in a non-industrial rubber plantation in Daoukro and Divo. Two plots were chosen in each of these sites. The clones were distributed by locality, namely IRCA 18 in Daoukro and PB 260 in Divo. The elementary plots consisted of 20 trees. At the start of this study, the trees were at the end of their 5th year of downward bleeding on the panel (BO-2). On each plot per site, the selection of trees was made on the basis of :

- the homogeneity of their circumference (≥ 55 cm), after elimination of border trees;
- the homogeneity of their unstimulated production (edge weight) previously determined with 2 to 3 coagulums corresponding to the number of bleedings;
- the homogeneity of the downward bleeding history over the last 5 years;

- the year of opening or of tapping at a height of 1.40 m from the ground, for inverted tapping patterns in accordance with the established protocol.

2.3- Treatments

A total of nine treatments (A, B, C, D, E, F, G, H and I: the control) were set up and the experimental set-up was a Fisher block with 4 replicates. The latex from the bleedings was collected with a knife in a plastic cup. The downward bleeding was done in a half spiral and the reverse bleeding in a quarter spiral. Bleeding was carried out every three days, six days a week. Sunday was the day of rest for bleeding. The bleeding was done 12 months out of 12. The coagulum, removed at the next bleeding, was collected, weighed monthly (fresh weight, F.W.) and stored. The trees were stimulated on the bleeding panel, on a 1 cm wide strip, at a rate of 1 g of stimulant per tree (14). The stimulation product used was obtained by mixing Ethrel and palm oil. Ethrel is the commercial name of the active ingredient, Etephon (2-chloroethyl phosphonic acid). Ethrel contains 480 g/l of active ingredient. The density of Ethrel at 480 g/l is 1.2; this gives 400 g/kg of active ingredient, or 40%. The stimulant pastes used in the experiments in this study have concentrations of 2.5 and 5% Etephon. The stimulation frequencies used varied from 6 to 13 per year (6-13/y) depending on the treatment.

The treatments are as follows:

- A: Inverse tapping at 6 years (down tapping to 5 years), stimulated at 5
- B: Inverse tapping at 6 years (downward tapping to 5 years), stimulated at 2.5
- C: Inverse tapping at 7 years (downward tapping up to 6 years), stimulated at 5
- D: Inverse tapping at 7 years (downward tapping to 6 years), stimulated at 2.5
- E: Inverse tapping at 8 years (downward tapping to 7 years), stimulated at 5
- F: Inverse tapping at 8 years (downward tapping to 7 years), stimulated at 2.5
- G: Inverse tapping at 9 years (downward tapping up to 8 years), stimulated at 5
- H: Inverse tapping at 9 years (downward tapping to 8 years), stimulated at 2.5
- I: Control, classic inverse tapping at 10 years (downward tapping to 9 years), 5% (current panel tapping scheme).

1.2.3. Measurements carried out on agronomic parameters

1.2.3.1. Rubber production

Rubber production was recorded per treatment. The processing coefficient (PC), the percentage of dry rubber in a given sample of fresh rubber, was used to calculate the dry rubber production (dry weight, DW) for each pattern. It was calculated from one coagulum sample per treatment. Each sample was weighed (fresh weight), creped, oven dried at 80°C for 24 hours and reweighed (creped dry weight). The P.C. is defined by the following formula:

$$P.C = D.W \times (F.W)^{-1} \times 100$$

1.2.3.2. Radial vegetative growth

The growth in thickness of the rubber tree trunk was assessed by measuring the circumference at 1.70 m above the ground, using a tape measure. Measurements were taken at the beginning of the experiment and then at the end of each physiological cycle just before the onset of natural defoliation (January to February), which coincides with very weak growth of the rubber trees. The average annual increase in girth was determined by the following relationship:

$$G_n = G_n - G_{n-1}$$

G_n: average annual increase in circumference ;

G_n: Circumference of trees in the current season;

G_{n-1}: circumference of trees from the previous season.

2.5- Sensitivity to dry rot syndrome

The rapid visual assessment method is used to report on the occurrence and progress of dry rot (15). For each bled tree, a number between 0 and 6 was assigned with the following meaning:

- 0, for a healthy bleeding notch,
- 1, for a notch that is dry over 1 to 20% of its length (10% on average),
- 2, for a notch that is dry over 21 to 40% of its length (average 30%),
- 3, for a dry notch over 41 to 60% of its length (average 50%),

- 4, for a dry notch over 61 to 80% of its length (average 70%),
- 5, for a dry notch 81-99% of its length (average 90%),
- 6, for a completely dry bleeding notch.

For each plot, an accurate count of the condition of the trees was made and the percentage of total diseased notch length (DNL %) for each treatment was calculated as follows

$$\text{DNL (\%)} = (0n_0 + 0.1 n_1 + 0.3 n_2 + 0.5 n_3 + 0.7 n_4 + 0.9 n_5 + n_6 + \text{DN}) \times N^{-1}$$

0.1; 0.3; 0.5; 0.7; 0.9 and **1** are coefficients expressing the average percentage of diseased notches of the class considered (score attributed); **N**: Total number of trees; **n_i**: Number of trees per dry notch class; **DN**: Number of trees whose bleeding has already been stopped for total dry notching

For each treatment, the percentage of live trees and totally dry trees was determined by the following formula

$$\text{Dry trees (\%)} = 100 \times (n_6 + \text{DN}) \times N^{-1}$$

DN: Number of trees already stopped for total dry notching; **N**: Total number of trees; **n₆**: Number of trees with the dry notching class noted 6 totally dry trees not yet stopped.

2.6- Physiological parameters of latex

Latex samples were used for the determination and measurement of physiological parameters using the latex microdiagnostic method (MDL). The parameters taken into account in the MDL were the dry extract rate (Ex.S, %), the contents (mmol.l⁻¹) of sucrose (Sac), inorganic phosphorus (Pi) and thiol compounds (RSH) in the latex. The dry extract content was determined according to the method described by (16), while the methods of (17), (18), (19) were used to determine the sucrose, inorganic phosphorus, and thiol group contents of latex, respectively.

The values of the physiological parameters thus determined were then compared with the reference values (**Table 1**) established by (20).

Table 1: Reference values of the four physiological parameters of the latex microdiagnosis (20)

	DRC (%)	Suc (mmol.l⁻¹)	P.i (mmol.l⁻¹)	R-SH (mmol.l⁻¹)
Très élevé	> 43	> 12	> 25	> 0,90
Elevé	38 à 43	9 à 12	20 à 25	0,80 à 0,90
Moyen	33 à 43	6 à 9	15 à 20	0,6 à 0,80
Faible	29 à 33	4 à 6	10 à 15	0,5 à 0,60
Très faible	≤ 29	≤ 4	≤ 10	≤ 0,50

DRC: dry extract; **Suc:** sucrose; **Pi:** inorganic phosphorus; **R-SH:** thiol compounds

1.2.4. Statistical analysis

The statistical analysis focused on tests for comparison of means. In the present study, where means of variables tested on more than two groups were to be compared, the one-factor analysis of variance (ANOVA 1) was used. The degree of liberty is n-k where n and k are the number of observations and groups respectively. The significance level chosen for these analyses is 5% (p-value ≤ 0.05). When the difference is significant, the Student-Newman-Keuls test was performed to classify and indicate which groups are different with the mean values of production, radial vegetative growth, sucrose, inorganic phosphorus and thiol compounds. For values expressed as a percentage (dry notch sensitivity; dry extract), the Kruskal Wallis test at the 5% threshold was performed. These analyses were performed with the XLSTAT-Pro 7.5 statistical software.

2- RESULTS

Agronomic parameters

Dry rubber production of PB 260 and IRCA 18 clones

The analysis of **Table 2** reveals that the average annual production per tree and per tapping of the inverse tapping was significantly influenced by the execution time of the downward tapping. Trees reverse tapped at 6 or 7 years (down tapping for 5 or 6 years) were significantly more productive respectively (126 ± 130 ; 119 ± 19 g.tr⁻¹.ta⁻¹) than those reverse tapped at 10 years (down tapping for 9 years) which is 89 ± 62 g.tr⁻¹.ta⁻¹

The average annual rubber production per hectare shown by treatment A (inverse tapping at 6 years and stimulated with 5% concentrated ethephon), with a gain (2695 ± 79 kg.ha⁻¹.yr⁻¹) in

productivity of 41% compared to the control, is the highest. This was not statistically different from those generated by treatments B to G, with statistically identical productions varying from 4088 ± 277 to $5158 \pm 234 \text{ kg.ha}^{-1}.\text{yr}^{-1}$. Conversely, the average annual production recorded by treatment J [control (classic inverse tapping at 10 years)] is the lowest ($3723 \pm 29 \text{ kg.ha}^{-1}.\text{yr}^{-1}$) without, however, being different from those expressed by the treatments from B to H despite the fact that they varied from 3967 ± 212 to $4951 \pm 351 \text{ kg.ha}^{-1}.\text{yr}^{-1}$ (**Table 2**)

UNDER PEER REVIEW

Table 2: Dry rubber production of the fast metabolizing clones IRCA 18 and PB 260 during the eight years of experimentation in Divo and Daoukro

Dry rubber production (g.tr ⁻¹ .ta ⁻¹)									Dry rubber production (kg.ha ⁻¹ .yr ⁻¹)								
Treatments		PB 260		IRCA 18		Average			PB 260		IRCA 18		Average		Gain %		
A		123 a		130 a		126 ± 130 a			5117 a		5408 a		5262 ± 205 a		41		
B		120 a		128 a		124 ± 58 a			4992 a		5324 ab		5158 ± 234 ab		38		
C		113 ab		125 a		119 ± 19 ab			4703 b		5200 ab		4951 ± 351 ab		33		
D		99 b		118 ab		108 ± 29 ab			4120 c		4908 b		4514 ± 557 ab		21		
E		99 b		113 ab		106 ± 48 ab			4129 c		4701 bc		4415 ± 404 ab		18		
F		96 b		105 ab		100 ± 105 ab			3990 c		4368 c		4179 ± 267 b		12		
G		93 b		103 ab		98 ± 81 b			3892 c		4284 c		4088 ± 277 bc		10		
H		92 b		99 ab		95 ± 28 b			3817 c		4118 c		3967 ± 212 bc		6		
I (control)		89 b		90 b		89 ± 62 b			3702 c		3744 d		3723 ± 29 c		0		
Mean values		with the same letter in a column are not significantly different (Newman-Keuls test at 5%)															

Radial vegetative trunk growth of trees of the fast metabolising rubber clones PB 260 and IRCA 18

The results of the mean annual girth increment of the fast metabolising clones are recorded in **Table 3**. The best girth increments are shown by the reverse tapped trees at 6 and 7 years (**Table 3**). Overall, treatment A (inverse tapping at 6 years and stimulated with 5% concentrated ethephon) produced the greatest average annual increase in tree trunk circumference ($4.55 \pm 0.21 \text{ cm.yr}^{-1}$). This is statistically identical to the other treatments, except for the control (classic inverse tapping at 10 years), which is the lowest ($3.60 \pm 0.00 \text{ cm.yr}^{-1}$). However, clone by clone, the analysis of the results in Table II shows that, whatever the fast metabolising clone (IRCA 18 and PB 260), the period of downward tapping had no significant effect on the radial vegetative growth of the inverse tapped trees. Over this period, it appears that radial vegetative growth (isodiametric) was very good (increase in circumference greater than 3 cm.yr^{-1}).

Table 3: Average annual increase in trunk circumference of trees of the fast-metabolizing rubber clones IRCA 18 and PB 260 during the eight years of experimentation at Divo and Daoukro

Treatments	Circumference increase (cm.yr^{-1})		
	PB 260	IRCA 18	Average
A	3,7 a	4,3 a	$4,00 \pm 0,42 \text{ ab}$
B	4,4 a	4,7 a	$4,55 \pm 0,21 \text{ a}$
C	3,8 a	4,2 a	$4,00 \pm 0,28 \text{ ab}$
D	3,8 a	4,1 a	$3,95 \pm 0,21 \text{ ab}$
E	3,5 a	3,9 a	$3,70 \pm 0,28 \text{ ab}$
F	3,8 a	3,8 a	$3,80 \pm 0,00 \text{ ab}$
G	3,8 a	3,5 a	$3,65 \pm 0,21 \text{ ab}$
H	3,9 a	3,8 a	$3,85 \pm 0,07 \text{ ab}$
I (control)	3,6 a	3,6 a	$3,60 \pm 0,00 \text{ b}$

Mean values with the same letter in a column are not significantly different (Newman-Keuls test at 5%)

Dry notch syndrome

The results of dry notch susceptibility in relation to the dry tree rate and diseased notch rate during the experiment for all treatments are shown in **Table 4**. Analysis of these results showed that the period of downward tapping had a significant effect on the dry tree rate and the diseased notch rate, which ranged from 0.00 to 9.65% for the dry tree rate and from 2.75 to 22.85% for the diseased notch rate. The trees undergoing inverse tapping at 6 years (5 years of downward tapping) had a dry tree rate of 0.00% and a diseased notch rate of 3.30%. In contrast, the control (treatment I) which is reverse tapped at year 10 had the highest dry tree (9.65%) and diseased notch (22.85%) rates.

Table 4: Susceptibility to dry notch syndrome of fast metabolizing clones IRCA 18 and PB 260 during the eight years of experimentation in Divo and Daoukro

Treatments	Dry tree rate in %			Rate of diseased notches in %		
	PB 260	IRCA 18	Average	PB 260	IRCA 18	Average
A	0,0 e	0 c	0,00 ± 0,00 b	5,0 d	1,6 c	3,30 ± 2,40 ab
B	5,4 c	4,2 b	4,80 ± 0,84 ab	8,8 b	4,9 b	6,85 ± 2,75 ab
C	5,1 c	3 b	4,05 ± 1,48 ab	9,6 b	4,6 b	7,10 ± 3,53 ab
D	2,0 d	4,1 b	3,05 ± 1,48 ab	6,6 c	4,7 b	5,65 ± 1,34 ab
E	0,4 e	0 c	0,20 ± 0,28 ab	4,1 d	1,4 c	2,75 ± 1,90 b
F	4,1 c	0 c	2,05 ± 2,89 ab	9,3 b	0,5 d	4,90 ± 6,22 ab
G	8,1 b	0 c	4,05 ± 5,72 ab	13,5 b	1,1 c	7,30 ± 8,76 ab
H	2,8 d	0 c	1,40 ± 1,97 ab	9,6 b	1,8 c	5,70 ± 5,51 ab
I (control)	10,8 a	8,5 a	9,65 ± 1,62 a	27,8 a	17,9 a	22,85 ± 7,00 a

Mean values with the same letter in a column are not significantly different (Newman-Keuls test at 5%)

Physiological parameters

Latex dry matter of the slow metabolism clones PB 260 and IRCA 18

The results of the average latex dry matter content of the fast metabolising rubber clones are shown in **Table 5**. These results show that there was no significant difference between the different treatments for both clones. However, the latex dry matter content was very high regardless of the treatment (> 43%). For both clones, it increased on average from 57.40% to 60.50%. Overall, down tapping had no effect on the inverse tapping rubber rate of the fast metabolising clones.

Table 5: Latex dry matter content of fast metabolising rubber clones IRCA 18 and PB 260 during the eight years of experimentation at Divo and Daoukro

Treatments	DRC (%)		
	PB 260	IRCA 18	Average
A	59 a	61,4 a	60,20 ± 1,69 a
B	56 a	58,8 a	57,40 ± 1,97 a
C	60 a	61 a	60,50 ± 0,70 a
D	62 a	58,8 a	60,40 ± 2,26 a
E	59 a	61,1 a	60,05 ± 1,48 a
F	60 a	59,5 a	59,75 ± 0,35 a
G	62 a	59,5 a	60,75 ± 1,76 a
H	60 a	58,2 a	59,10 ± 1,27 a
I (control)	58 a	59,3 a	58,65 ± 0,91 a

Mean values with the same letter in a column are not significantly different (Newman-Keuls test at 5%)

Sucrose content of latex of PB 260 and IRCA 18 clones

The analysis of the average latex sucrose contents of the fast metabolising clones shows that the period of down-bleeding did not influence the average sucrose content of any clone (**Table 6**). Overall, the mean sucrose contents of the latex varied from 4.05 to 7.45 mmol.l⁻¹. These contents varied from low (≤ 4 to 6 mmol.l⁻¹) to medium (≤ 6 to 9 mmol.l⁻¹) for the different treatments. Trees reverse tapped at 6 and 7 years (down tapped for 5 and 6 years) recorded the highest average latex sucrose contents compared to the control reverse tapped at 10 years (down tapped for 9 years). Furthermore, individually, for clone PB 260, the average sucrose content ranged from low (≤ 4 to 6 mmol.l⁻¹) to high (≤ 9 to 12 mmol.l⁻¹) while for clone IRCA 18 it ranged from very low (≤ 4 mmol.l⁻¹) to low (≤ 4 to 6 mmol.l⁻¹).

Table 6: Sucrose content of latex of fast metabolizing rubber clones IRCA 18 and PB 260 during the eight years of experimentation in Divo and Daoukro

Treatments	Sucrose content (mmol.l ⁻¹)		
	PB 260	IRCA 18	Average
A	5,1 b	5,1 a	5,10 ± 0,00 a
B	9,8 a	4,1 a	6,95 ± 4,03 a
C	7,3 ab	5,1 a	6,20 ± 1,55 a
D	8,3 ab	4,7 a	6,50 ± 2,54 a
E	10,4 a	4,5 a	7,45 ± 4,17 a
F	8 ab	3,6 ab	5,80 ± 3,11 a
G	6,6 b	5,6 a	6,10 ± 0,70 a
H	5,6 b	3,4 ab	4,50 ± 1,55 a
I (control)	5,2 b	2,9 b	4,05 ± 1,62 a

Mean values with the same letter in a column are not significantly different (Newman-Keuls test at 5%)

Inorganic phosphorus content of latex of PB 260 and IRCA 18 clones

The results of the average inorganic phosphorus content of the latex of the fast-metabolising rubber clones are shown in **Table 7**. The analysis of the results in this table shows that the average inorganic phosphorus content varies from one treatment to another. Its variation ranges from low (10 mmol.l⁻¹) to high (24.6 mmol.l⁻¹) for clone IRCA 18, while for clone PB 260 it varies from average (18.8 mmol.l⁻¹) to very high (31.5 mmol.l⁻¹). The period of execution of downward tapping had no significant influence on the average inorganic phosphorus content of the latex of reverse tapped trees. Overall, this content varied from medium (≤ 15 to 20 mmol.l^{-1}), high (≤ 20 to 25 mmol.l^{-1}) and to very high ($> 25 \text{ mmol.l}^{-1}$) all clones combined.

Table 7: Inorganic phosphorus content of latex of fast metabolizing rubber clones IRCA 18 and PB 260 during the eight years of experimentation at Divo and Daoukro

Treatments	Inorganic phosphorus content (mmol.l ⁻¹)		
	PB 260	IRCA 18	Average
A	31,5 a	17,3 ab	24,40 ± 10,04 a
B	28 ab	17,6 ab	22,80 ± 7,35 a
C	21,8 b	22,2 a	22,00 ± 0,28 a
D	22,9 b	19,5 ab	21,20 ± 2,40 a
E	19,8 b	17,7 ab	18,75 ± 1,48 a
F	18,8 b	10 b	14,40 ± 6,22 a
G	25,2 ab	21,6 a	23,40 ± 2,54 a
H	26 ab	24,6 a	25,30 ± 0,98 a
I (control)	27 ab	13,6 ab	20,30 ± 9,47 a

Mean values with the same letter in a column are not significantly different (Newman-Keuls test at 5%)

Thiol group content of latex of PB 260 and IRCA 18 clones

The results of the average content of thiol groups in the latex of the fast metabolising rubber clones are shown in **Table 8**. The analysis of this table indicates that the period of downward tapping had no significant influence on the average thiol group content of the reverse tapped trees, all clones included. Overall, this content varies from very low (≤ 0.50 mmol.l⁻¹) to very high (> 0.90 mmol.l⁻¹) for all clones combined. Trees that have been bled while descending for 5 and 6 years have high thiol group contents. Individually, the average content of thiol groups varies from very low (0.39 mmol.l⁻¹) to medium (0.67 mmol.l⁻¹) for the IRCA 18 clone, whereas for the PB 260 clone it varies from low (0.59 mmol.l⁻¹) to very high (1.35 mmol.l⁻¹)

Table 8: Thiol group contents in the latex of the fast-metabolising rubber clones IRCA 18 and PB 260 during the eight years of experimentation at Divo and Daoukro

Treatments	Thiol group content (mmol.l ⁻¹)		
	PB 260	IRCA 18	Average
A	1,35 a	0,67 a	1,01 ± 0,48 a
B	0,95 b	0,47 ab	0,71 ± 0,33 a
C	0,64 c	0,52 ab	0,58 ± 0,08 a
D	0,64 c	0,18 c	0,41 ± 0,32 a
E	0,66 c	0,26 d	0,46 ± 0,28 a
F	0,59 c	0,39 b	0,49 ± 0,14 a
G	0,79 cc	0,48 ab	0,63 ± 0,21 a
H	0,62 c	0,50 ab	0,56 ± 0,08 a
I (control)	0,66 c	0,43 ab	0,54 ± 0,16 a

Mean values with the same letter in a column are not significantly different (Newman-Keuls test at 5%)

DISCUSSION

Agronomic parameters

The best annual productivity results were obtained with treatments A, B and C, whose down tapping execution times were shorter than all the others. The results, obtained in our study, show that the rubber production performance of up- or down-tilled latex harvesting systems are related to previous down-tilting as the findings of (11) had noted. Indeed, these results indicate the existence of a strong presumption that a minimum delay in years of downward tapping is necessary to make inverse or upward tapping efficient. The nature and extent of our results are such that the existence of a minimum delay in downward tapping for better performance of inverse tapping is proven. Our results show that this delay is at least equal to five (5) years of downward bleeding. This indicates that all the rubber trees, under a given latex harvesting technology, have a significantly similar or identical level of activation at the end of the 5th down tapping campaign. In addition, the high yields of treatments A and B highlight the activating effect of metabolism by bleeding and stimulation, as shown by (21 ; 22). This illustrates that the overall activation of the tree under tapping becomes effective after five years of downward tapping. Thus, practising downward tapping for five years has a positive effect on the production of the inverse tapping, which is up to 41% of that of the

downward tapped control for 9 years. This fact meets the expectations of rubber farmers who are looking for a rapid and significant return on their investment. With this work, there is no need to wait until the tenth year to switch to inverse tapping, which is much more productive. The production gains, expressed as a percentage in this study, were high (41%) for treatment A (inverse tapping at six years stimulated at 5% with five years of downward tapping). Indeed, this high level of production could be explained by the fact that the metabolic activation of the clones is ensured by the intrinsic energy whose initial availability determines their rubber productivity. This energy, which varies according to the metabolism of the clone, is essential for good to better metabolic functioning. It is partly responsible for the productivity of the different clone metabolisms and characterises them (23; 1). Our results corroborate those of (5) and (24), who showed that in their work on the clonal typology of laticifier function in rubber, clones of the fast metabolic activity class have high production.

Sensitivity to dry notch syndrome

The study of the sensitivity to dry notch syndrome showed that the period of downward tapping had a positive effect on it in inverse tapping, as long as the results obtained were statistically lower than or equal to those of the control with a downward tapping period of 9 years. The trees subjected to inverse tapping at 6 years (downward tapping for 5 years) expressed a priori less sensitivity to dry notch. This is a good sign that the 5-year downward bleeding expressed relatively less sensitivity to dry notch than the 9-year downward bleeding. The trees reverse tapped at 6 years, all clones combined, recorded fewer dry trees than the control reverse tapped at 10 years. This represents a very good level of resistance (0% dry tree rate) for these clones, which are known to be very susceptible to dry notch syndrome. Indeed, as saccharose is the main material for rubber biosynthesis (1), its use for this biosynthesis can be intensified by the latex harvesting system (25). If over-harvesting occurs, a dry notch will appear (5). This dry notch is due to the fact that they have low levels of thiol compounds as indicated by (9). According to the same author, antioxidants favour the stability of lutoids and their low content is a probable indication of their decompartmentization, which is destroyed in situ, probably leading to the occurrence of the dry notch. Indeed, the subsequent lysis of the lutoids leads to coagulation of the latex within the laticifers, which subsequently stops the flow of latex and causes dry notch. The overall low dry notch rates indicate a very good level of resistance to the dry notch syndrome, especially for fast metabolising clones (20). Thus, the different dry notch rates express a good adaptation to this new latex harvesting system of the

clones studied. Inverse tapping preceded by 5 years of downward tapping with high rubber productivity and high resistance to dry notch syndrome would be an asset in recommending this latex harvesting technology for rubber farming.

Physiological parameters

The physiological state of the trees expressed by the physiological profile is the state of health of the rubber trees at a given moment. This state can be very good, good, average or poor. This means that the rubber trees are respectively in a good or optimal latex harvesting condition (normal exploitation of the trees) or in a poor condition (overexploitation or under-exploitation of the trees). The physiological profile is assessed by analysing biochemical parameters such as dry extract (Ex.S), saccharose (Sac), inorganic phosphorus (Pi) and thiol groups (R-SH). The dry extract, reflecting the efficiency of isoprenic biosynthesis or rubber production, for all clones combined is very satisfactory (rate > 43%, reference value). Indeed, these rates were very high for trees bled while descending for 5 and 6 years. This is a sign of a very good activation of the laticifere metabolism and of a good yield, which is confirmed by the good productions per hectare observed, i.e. the intra-laticifere saccharose is sufficiently available and very efficiently transformed into rubber from 5 years of downward tapping. This also reflects good regeneration of the latex exported during tapping (26). Furthermore, with average dry matter contents above 50%, this indicates that rubber biosynthesis is normally carried out by the laticifers (5), cis-polyisoprene biosynthesis is efficient: i.e. effective latex regeneration (26).

The saccharose content of the latex depends on the activity and the level of production imposed on the clone, notably by the use of exogenous hormonal stimulation of production. For clones in the fast metabolic activity classes, the average annual saccharose content of the latex, the initial molecule essential for the laticogenic metabolism, is globally of the same importance and its moderate level does not a priori constitute a limit to the productivity of the different latex harvesting systems applied during the period of downward bleeding. This is in addition to the fact that clones of the fast metabolic activity class are known to have low latex saccharose contents. The level of average sucrose content is, on the whole, a good indication of the availability of sucrose, the raw material for isoprenic synthesis (27). This high content suggests non-limiting carbohydrate availability in the laticifers, indicating a good supply (28; 29) and also guaranteeing good subsequent production. Thus, the execution of downward

tapping for 5 or 6 years is sufficient to strongly activate the metabolism leading to a strong transformation of saccharose, present in the laticifers, into rubber.

For both clones, the level of latex inorganic phosphorus (Pi), the main biochemical energy source of metabolism, is overall good, compared to the reference values of this parameter. It reflects and characterises the metabolism of the tested class (≤ 20 to 25 mmol.l^{-1}): high, reference value of fast metabolism **(30)**. These results reflect the fact that biochemical energy within the laticifers for rubber biosynthesis is available and is increasing well in relation to each clone, especially its metabolism. This is closely related to the increased activation of the rubber production metabolism. This is illustrated by the high dry extract values of all treatments. In these clones, the inorganic phosphorus content at the level of the trees bled downwards for 5 and 6 years is of the same order as that of the control, and is an advantage.

The levels of thiol groups reflect the intensity of the stress to which the trees are subjected in relation to latex harvesting **(5)** and, above all, the level of resilience developed by these trees to the different treatments. The physiological state of the laticifers, the cells that produce latex and therefore rubber, expressed by the content of thiol groups in the latex, is generally good. Whatever the clone and the treatment, no inconvenience linked to the R-SH content of the latex was noted and likely to be noted in the future. This is a sign of good biological protection of the lactiferous systems and the expression of an equally good resilience of the lactiferous cells of the rubber trees of the different technologies, as several authors have shown **(9; 5; 21)**. Furthermore, the average HR content of the control, tapped in reverse at 10 years (9 years of downward tapping), is, on the whole, comparable to that of the reverse tapping at 6, 7, 8 and 9 years (treatments A to H) during the experiment. The level of SH-R levels in all treatments reflects the fact that down-breeding for 5 and 6 years did not have any negative impact on the protective mechanisms of the lactiferous system. It further indicates an increased stability of the latex lutoids. This corroborates the conclusions drawn from the work of **(11)**. This stability seems to explain the high rubber production of the 6 and 7 year old inverse tapplings preceded by 5 and 6 years of downward tapping. At the very least, the state of the protective systems of the latex-producing laticifier cells is not a limiting factor in rubber production.

CONCLUSION

The study undertaken to determine the influence of down tapping on the inverse tapping productivity of fast metabolising rubber clones yielded satisfactory results. Overall, all the results obtained at the end of this study allow us to say with certainty that the period of execution of down tapping positively influences the productivity in inverse tapping. These results showed that downward tapping for 5 and 6 years allows these clones to express their full potential in inverse tapping. After 5 or 6 years of downward tapping, inverse tapping can be practised without any prejudice, on the contrary. These results respond exactly to the concerns of the growers, who want to go, in a short time, to inverse tapping, which is much more productive, in order to have an important and rapid return on investment.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Jacob J. L., D'Auzac J., Provost J. C & Serier J. B. 1995a. A natural rubber factory: the rubber tree. *Research*, 26 (276): 538-545.
2. Gomez J. B., 1982. Anatomy of Hevea and its influence on latex production. Malaysian Rubber Research and Development Board, monograph n°7, Kuala Lumpur, 76 p.
3. Obouayeba S., Boa D., Gohet E., Dian K., Ouattara N. & Kéli J., 2000. Dynamics of vegetative growth of Hevea brasiliensis in the determination of tapping norms. *Journal of Rubber Research*, 3 (1): 53-62
4. Traore M. S. 2014. Effects of different annual frequencies of ethylenic stimulation on the agrophysiological parameters of Hevea brasiliensis Müll clones. Arg. (Euphorbiaceae), PB 235, PB 260, GT 1 and PB 217 grown in the south-east of Côte d'Ivoire. Doctoral thesis from Félix Houphouët-Boigny University, Abidjan (Ivory Coast), 150 p.
5. Jacob J. L., Serres E., Prévôt J. C., Lacrotte R., Clement-Vidal A., Eschbach J. M. & D'Auzac J., 1988. Development of latex diagnostics. *Agritrop*, 12(2): 97-118.
6. Soumahin E. F., 2010: Optimization of latex harvesting systems in rubber cultivation by reducing tapping intensities. Doctoral thesis from Felix Houphouët Boigny University, Abidjan

(Ivory Coast), 189 p.

7. Obouayeba S., 2009. Operating systems in downward bleeding of the clone of *Hevea brasiliensis* PB 235. Technical sheet, 4 p.

8. Obouayeba S., 1993. Estimation of the quantity of stimulating paste applied to rubber trees according to their circumference in the south-east of Côte d'Ivoire. *African Agronomy*, 1: 26-32.

9.

10. Chrestin H., 1985. Etherel stimulation of rubber trees; how far not to go too far. *Rubber Plastic*, 647: 75-78.

11. Dian K., Sangaré A., Obouayeba S. & Boa D. 1999. Intensive exploitation of a few clones of *Hevea brasiliensis* Müll. Arg. in Ivory Coast. *African Agronomy*, 10 (1): 7-17.

12. Obouayeba S., Boko A. M. C., Soumahin E. F., Elabo A. E. A., Dea G. B., N'guessan B. E. A., Kouamé C., Zéhi B., & Kéli Z. J., 2016. *Hevea brasiliensis*, component of stable rural production systems: summary of forty years of work, IRRDB Regional Workshop, September 28-30, 2016, Yamoussoukro, Côte d'Ivoire, 27 p.

13. Obouayeba S., Soumahin E., Boko A. M. C., Goue B. D., Gnagne Y. M. & Dian K. 2008. Improvement of rubber trees' productivity in smallholding by the introduction of upward tapping. *Journal of Rubber Research* 11(3): 163 – 170

14. Chapuset T., 2001. Description of the clones studied on a large scale. CNRA-HEVEA report n°01/01–A- May 2001, 36 p.

15. Compagnon P., 1986. Natural rubber. Coste R., edition G.P. Maisonneuve and Larose, Paris, 595 p.

16. Van De Syne H., 1984. The dry cut syndrome in *Hevea brasiliensis*, evolution, agronomical and physiological aspects. In C.R. Coll. IRRDB. Physiology, Exploitation, Improvement. rubber tree. IRCA-CIRAD, Montpellier edition, p. 227-249.

17. Eschbach J. M., Roussel D., Van De Syne H., Jacob J. L. & D'Auzac J., 1984. Relationships between yield and clonal physiological characteristics of latex from *Hevea brasiliensis*. *Plant Physiology*, 22: 295 - 304.

18. Ashwell G., 1957. Colorimetric analysis of sugar. *Methods in Enzymology*, 3: 73-105.

19. Taussky H. H. & Shorr E., 1953. A micro colorimetric method for the determination of

inorganic phosphorus. *Journal of Biology and Chemistry*, 20: 675 - 685.

20. Boyne A.F. & Ellman G.I., 1972. A methodology for analysis of tissue sulphydryl components. *Analytical Biochemistry*, 46: 639-653.

21. Jacob J. L., Lacrotte R., Serres E. & Roussel D., 1987. The physiological parameters of the latex of *Hevea brasiliensis*, latex diagnosis, its bases and its development. Ekoma, Ivory Coast, February, 1987, 64-74.

22. Obouayeba S., Boa D. & Keli Z. J. 1996. Adequacy between quantity of stimulating paste and production of rubber from *Hevea brasiliensis* in the south-east of Côte d'Ivoire. *Tropicicultura*, 14 (2): 54-58

23. Silpi U., Chantuna P., Kasemsap P., Thanisawanyangkura S., Lacointe A., Ameglio T. & Gohet, E., 2006. Sucrose metabolism distribution patterns in the lattices of three *Hevea brasiliensis*: effects of tapping and stimulation on the tree trunk. *Journal Rubber Research*, 9: 115 – 131.

24. Jacob J. L., Provost J. C., Lacrotte R. & Eschbach J. M. 1995b. Latex diagnosis. *Plantations, Research, Development*, 2(2): 33-37.

25. Lacrotte R., Van de sype H. & Chrestin H., 1985. Influence of ethylene on the use of exogenous sucrose by laticifers of *Hevea brasiliensis*: proposal of a mechanism of action. *Plant Physiology*, 23: 187-198.

26. Milford G.F.J., Paardekooper E.C. & Ho C.Y., 1969. Latex vessels plugging, its importance to yield and clonal behavior. *Journal of Rubber Research Institute. Malaysia*, 21: 274 – 282.

27. Tupy J. & Primot L., 1976. Control of carbohydrate metabolism by ethylene in latex vessels of *Hevea brasiliensis* Muell. Arg. in relation to rubber production. *Plant Biology*, 18: 373-384.

28. Provost J.C., Jacob J. L., Vidal A. & Irrchidi S., 1987. Demonstration of a pyrophosphate: fructose-6-phosphate-1-phosphotransferase in the latex of *Hevea brasiliensis*. *Proceedings of the Academy of Sciences, Paris (France)*, Volume 305 (Series III), pp. 405-410.

29. Lacrotte R., 1991. Study of the relationship between the sugar content of latex and production: approach to the mechanisms of sucrose loading in laticifers of *Hevea brasiliensis* Muell. Arg. University Doctorate thesis, University of Sciences and Techniques of Languedoc, Montpellier II (France), 266 p.

30. Gohet E., 1996. Latex production by *Hevea brasiliensis*. relationship with growth. Influence

of different factors: clonal origin, hormonal stimulation, hydrocarbon reserves. 3rd cycle thesis from the University of Sciences and Techniques of Languedoc, Montpellier II (France), 343 p.

UNDER PEER REVIEW