Effect of Exogenous Hormonal Stimulation on the Metabolic Partition between Plant Growth and Rubber Production of *Hevea brasiliensis* Clones According to the Class of Metabolic Activity in Cote d’Ivoire

Lehi Malidy Irénée¹,²*, Konan Djezou³, Okoma Koffi Mathurin³, Yoboue Ange Naté¹, Konan Kouakou Séraphin¹,², Zouzou Michel¹,² and Obouayeba Samuel³

¹Laboratory of Biotechnology, Agriculture and Resource Development, UPR of Plant Physiology, Option Agrophysiology, Faculty of Biosciences, Félix Houphouët-Boigny University, 22 B.P. 582 Abidjan 22, Côte d’Ivoire.

²African Centre of Excellence - Climate Change, Biodiversity and Sustainable Agriculture (CEA-CCBAD), Félix Houphouët-Boigny University, 22 B.P. 582 Abidjan 22, Côte d’Ivoire.

³CNRA (Centre National de Recherche Agronomique), Station de Recherche de Bimbresso, 01 BP 1536 Abidjan 01, Côte d’Ivoire.

**Authors’ contributions**

This work was carried out in collaboration among all the authors. Author LMI designed the study, performed the statistical analysis, wrote the protocol, managed the bibliographical research and wrote the first draft of the manuscript. Authors KD and OKM made corrections for the finalization of the manuscript. Author OS contributed to the writing of the research project and the revision of the manuscript. All authors have read and approved the final manuscript.

**Article Information**

DOI: 10.9734/ARJA/2020/v13i430112

Editor(s):

(1) Dr. M. Yuvaraj, Adhiparasakthi Agricultural College, India.

Reviewers:

(1) P. Basuchaudhuri, Indian Council of Agricultural Research, India.

(2) Imene Rajhi, Center of Biotechnology of Borj Cedria, Tunisia.

Complete Peer review History: http://www.sdiarticle4.com/review-history/65195

**ABSTRACT**

**Aims:** In order to achieve a good and sustainable harvest of *Hevea brasiliensis* rubber, the effect of exogenous hormonal stimulation on the metabolic partition growth-production of clones according to the class of metabolic activity was made.
Place and Duration of Study: The study was conducted for 9 years in the San-Pedro region in south-west Ivory Coast, precisely in the experimental industrial plantations of the former HEVEGO, now Southwestern Agricultural Civil Society Société (SCASO).

Methodology and Results: The rubber trees were planted at a density of 510 trees/ha in a completely randomised system. They were bled in S/2 d4 and stimulated to 2.5% Etephon at annual frequencies of 0, 1, 2, 4, 6, 8, 13, 18, 26, 39 and 78. The parameters measured were rubber production, trunk circumference, sucrose content, inorganic phosphorus, thiol groups and dry notch rate. The results showed that productivity increased with the intensity of stimulation (58.96 - 68.49 g.a \(^{-1}\)s \(^{-1}\); 39.83 - 66.69 g.a \(^{-1}\)s \(^{-1}\)) over the intervals [0.6] and [0.26] stimulations respectively in clones with active and slow metabolism. The circumference of the trunk was marked by a less pronounced decrease in growth in slow metabolizing clones (0.52%) with good protection of the laticigene system (R-SH: 0.815 mmol.l-1) and low sensitivity to dry notching (1.6). Whatever the classes of metabolic activity of the clones, the agrophysiological parameters were strongly correlated with each other by a degree 2 polynomial function.

Conclusion: This polynomial function seems to reflect the existence of a good state of equilibrium between laticigenic metabolism and vegetative growth according to the class of metabolic activity of the *Hevea brasiliensis* clones.

Keywords: *Hevea brasiliensis*; vegetative growth; rubber production; stimulation; metabolic class of the clones.

1. INTRODUCTION

Rubber cultivation is of considerable socio-economic interest and is generating ever-increasing enthusiasm among producers in many tropical countries, particularly in Ivory Coast. This interest is linked to the production of natural rubber, which is a product that is increasingly prized for its specific characteristics of use. Furthermore, the rubber tree is distinguished from other crops by the way it is harvested and its particularity. Thus, unlike other plants whose fruits, seeds or tubers are harvested, in the rubber tree, natural rubber is obtained by processing latex. This milky liquid is produced in specialized cells, the laticifers, located in the bark of the tree [1]. By incising the bark of the tree, through the practice of bleeding, the latex flows out and is collected [2,3]. The volume of latex collected after successive cuts depends on the ease with which the latex flows, the duration of the flow and the rate of latex regeneration in the tree [4,5]. The bark of the tree thus serves as agricultural capital, since its quality conditions the quantity of latex regenerated during successive sampling [5,3].

The production of natural rubber is an energy-intensive activity, for one (1) kg of rubber (secondary biomass), it is worth 2.5 times more than the production of one (1) kg of primary or vegetative biomass [6]. This high energy demand is probably at the origin of the antagonism between rubber production and radial vegetative growth [3,6-9]. The antagonism between rubber production and vegetative growth is characterised by the fact that high rubber production negatively impacts radial vegetative growth and vice versa [3,6-9].

Low tapping frequencies are widely recommended for latex harvesting. Moreover, in the absence of stimulation, rubber tree productivity increases with the intensity of latex harvesting up to a certain limit. However, the production from bleeding alone cannot be adjusted to the needs of the users, nor can it be used to make better use of the rubber production potential of all the groups of clones cultivated because of their different laticigenic metabolism. Thus, in a systematic way, the actors add to the tapping system a strategy of hormonal stimulation of production in order to achieve sufficient production [10-14].

The response to exogenous hormonal stimulation of production differs from one class of clone metabolic activity to another. Metabolically active clones have a low response to hormonal stimulation, those with moderate metabolism have a medium response and slow metabolizing clones have a high response [15,16]. Modulation of the activity of the laticigene well, and consequently the production of latex, by ethylenic hormonal stimulation makes it possible to evaluate the metabolic partitioning of photosynthates between primary and secondary biomass and thus to specify the impact of the increase in production level on growth. This study aims to evaluate the importance of the
reduction in vegetative growth linked to exogenous ethylenic stimulation. Thus, does the practice of ethylenic stimulation, at various frequencies, modify the metabolic partition equilibria according to the different metabolic classes of the clones? It is to answer this essential concern that this study was undertaken. Its objective is to contribute to the sustainable improvement of the productivity of Hevea brasiliensis clones. It will involve comparing the growth and production of unstimulated tapped trees and trees subjected to different frequencies of exogenous hormonal stimulation and then relating growth, rubber production, dry notch and physiological parameters by class of metabolic activity of Hevea brasiliensis clones.

2. STUDY ENVIRONMENT

The study was conducted in the San-Pedro region in south-west Ivory Coast, precisely in the experimental industrial plantations of the former HEVEGO, now Southwestern Agricultural Civil Society (SCASO), located between 6°30’ and 6°50’ north latitude and 5°00’ and 5°20’ west longitude (Fig. 1). This region is characterised by an annual rainfall varying between 1200 and 1800 mm, with an annual insolation of 1500 hours [17]. The average annual temperature in this region is around 25.6°C. The San Pedro region is subject to four seasons: A long dry season from December to February, followed by a long rainy season from March to June; a short dry season from July to August and a short rainy season from September to November. The relief is characterised by vast plateaus topped in places by a few elevations. The soil profile is of ferralitic type, relatively altered and with a texture varying between clayey silt and silty sand. Deep and permeable, these soils are generally well adapted to all types of food and industrial crops [18].
3. PLANT MATERIAL

The plant material used in this study consisted of 16 clones of *Hevea brasiliensis* (Muel. Arg, Euphorbiaceae) distributed between the three classes of vegetative growth and metabolic activity [19,20,9]. The choice of these clones was based on their hardiness and the fact that they are more or less used in the country (Table 1).

4. STUDY METHODS

4.1 Conduct of the Trial and Data Collection

In order to evaluate the effect of the frequency of exogenous hormonal stimulation on the metabolic partition between vegetative growth and rubber production of *Hevea brasiliensis*, circumference measurements were made on the trees of the clones of the different classes of vegetative growth and metabolic activity planted at a density of 510 trees/ha, i.e. a 7 m x 2.80 m planting arrangement. Each monoclonal plot consists of randomised groups of 33 trees, representing an experimental design. The following patterns: one unstimulated bleeding treatment (0y) and nine stimulated treatments respectively 2, 4, 6, 8, 13, 18, 26, 39 and 78 times a year (y) were applied to each clone. The bleeding was carried out in a descending half spiral (S/2). It was done every four days, six days out of seven with one day of rest per week (d4 6d/7) and 12 months out of 12 (12 m/12), i.e. 78 bleedings per year. This latex harvesting technology is formulated as follows : S/2 d4 6d/7 12 m/12. The quantity of Ethephon applied at each stimulation was the same for each tree (25 mg) and corresponds to the intake of 1 g of paste containing 2.5% active ingredient. The rubber production of the bled trees was recorded per tree and per bleed and per clone during the experimental period. The main physiological parameters of the latex, i.e. dry extract, sucrose, inorganic phosphorus and thiol group contents, were evaluated tree by tree each year according to methods developed by CIRAD and CNRA [19,2] adapted in 1995 by IRRDB. The contents of sucrose, inorganic phosphorus and thiol groups were expressed in millimoles per litre of latex (mmol.l⁻¹). A complete dry notch survey (DRS) was carried out once a month on all unstimulated and stimulated tapped trees of all clones. During the DDR, all trees showing latex flow along the entire length of the notch after tapping were considered healthy and scored zero (0). The others were considered to have dry notches and scored from 1 to 6 depending on the length of the non-latex producing notch [21]. The data collected represent an average of nine years of latex harvesting.

4.2 Data Processing

4.2.1 Increasing the circumference

During this study, the average annual circumference increase was determined from measuring the circumference of the trunk of rubber trees using a tape measure. Thus, the average annual increase in circumference was obtained according to the formula:

\[ \text{Accn (cm/an)} = \text{Circn} - \text{Circn-1} \]

Accn: Average annual increase in circumference; Circn: Average circumference of trees from the current season; Circn-1: Average circumference of trees from the previous season.

4.2.2 Dry rubber production

Average dry rubber production was measured by weighting the coagulated rubber from each tree every four weeks. Thus, after tapping, the rubber in the cups was weighed and made up the fresh weight (Pf). A sample of each treatment was then crimped and formed the crimped weight (Pc) and dried in an oven at 80°C for 24 hours and then reweighted (dry weight). The creping consisted of crushing the coagulum between two metal rollers rotating in opposite directions. The coagulum thus flattened is easier to dry. This process allowed a large part of the water contained in the coagulum to be removed. The average dry rubber production was obtained by converting the fresh weight to dry weight by the transformation coefficient (CT) determined as follows:

\[ \text{CT} = \left( \frac{\text{Pc}}{\text{Pf}} \right) \times 100 \]

CT : Processing coefficient, Pc : Crepe weight, Pf : Fresh weight.

The determined processing coefficient was used to evaluate the dry weight (Ps) of the rubber produced. It was calculated according to the following formula:

\[ \text{Ps} = \text{CT} \times \text{Pf} \]

Ps : Dry weight
Rubber production is expressed in grams per tree per bleed (g.a⁻¹.s⁻¹). This gives an indication of the amount of rubber a tree produces when tapping.

4.2.3 Evaluation of the dry notch sensitivity coefficient according to the class of metabolic activity of *Hevea brasiliensis* clones

Raw data from comprehensive dry-notch surveys were used to determine dry-notch rates (Anonymous, 1993). The sensitivity coefficient was determined from the ratio of the difference in dry notch rate between unstimulated and maximally stimulated trees and the difference in latex sugar content of unstimulated and maximally stimulated trees according to the following formula:

\[
\text{Coef S} = \frac{\text{DCLd}}{\text{SUCd}}
\]

Coef S: Sensitivity coefficient; DCLd: difference in dry notch rate between unstimulated and stimulated trees; SUCd: difference in latex sugar content of unstimulated and stimulated trees.

4.2.4 Physiological parameters

The analysis of the physiological state of the rubber trees by the Micro-Diagnostic Latex (MDL) method was carried out every year on the various most important physiological parameters of latex. For this purpose, latex was taken by puncture under the bleeding notch (descending bleeding) using the Micro-Diagnostic Latex method [19]. The dry extract (% ExS), sucrose (Sac), inorganic phosphorus (Pi) and thiol groups (RSH) were the physiological parameters evaluated. They were determined from the solution obtained after coagulation of the latex in trichloroacetic acid (TCA). Latex sucrose was determined using the anthrone method developed by Ashwell (1957). Inorganic phosphorus was determined using the ammonium molybdate and ammonium vanadate method developed by Taussky and Shorr (1953). The determination of R-SH was carried out by the method of Boyne and Ellman (1972) using DTNB.

4.3 Statistical Analysis of the Data

The data collected was subjected to analysis of variance (Anova) using SAS version 9.3 software. The Newman-Keuls test at the 5% threshold was used to compare the means in case of significant differences between the variables studied. The determination of the analytical expressions of the growth curves was carried out using the SAS programme with the programme’s non-linear regression procedure (Proc Nlin). The trend curves were produced using Excel 2013 software.

5. RESULTS

5.1 Effect of the Frequency of Exogenous Hormonal Stimulation on the Average Annual Increase in Circumference of Clones of Different Metabolic Classes

Evaluation of the effect of the frequency of exogenous hormonal stimulation on the mean annual increase in tree circumference indicates a highly significant difference (p < 0.0001) within all three classes of metabolic activity (Table 2).

Analysis of the table reveals that increases ranged from 2.1 to 3 cm.yr⁻¹ for active metabolism clones (AMC), from 1.6 to 2.8 cm.yr⁻¹ for moderate metabolism clones (MMC) and from 2 to 3.7 cm.yr⁻¹ for slow metabolism clones (SMC).

In clones with active and moderate metabolism, non-stimulated trees (0y) had a statistically higher mean annual circumference increase (3 and 2.8 cm.yr⁻¹) than stimulated trees (2 to 78y) with values ranging from 2.1 to 2.8 for AMC and 1.6 to 2.4 for MMC.

At the level of slow metabolism clones, the mean annual circumference increase of unstimulated trees (0y) and trees stimulated 2 to 4 times in the year (2 and 4y) was higher and statistically identical (3.1 and 3.7 cm.yr⁻¹). Trees stimulated more than 4 times in the year (6, 8, 13, 18, 26, 39 and 78y) gave the lowest mean annual increases in circumference with the respective values (2.7;2.6;2.4; 2.3 and 2 cm.y⁻¹).

The importance of the reduction in growth, expressed as a percentage of the growth of the unstimulated control (% Accs) is low for MACs (20%), medium for SMCs (29.73%) and high for MMCs (32.14%). For the magnitude of the combined effect of bleeding at high stimulation frequency, the reduction in increase (% Red AccnS (Stim)) is more pronounced in MMC (61.69%) and AMC (56.04%), and average in SMC (45.72%). The ratio between the percentages due to tapping and stimulation (% tapping / % Stim) revealed that AMCs had a higher ratio (0.80) than other classes.
| Clones   | Geographical origin | Genetic origin                  | Production  | Class of activity metabolic |
|----------|---------------------|---------------------------------|-------------|-----------------------------|
| IRCA 109 | Ivory Coast         | PB5/51 x IR22                   | High production | Fast                       |
| IRCA 111 | Ivory Coast         | PB 5/51 X RRIM 605              | Top producuteur | Fast                       |
| IRCA 130 | Ivory Coast         | PB 5/51 X IR 22                 | Top producuteur | Fast                       |
| IRCA 18  | Ivory Coast         | PB 5/51 x RRIM605               | High production | Fast                       |
| IRCA 209 | Ivory Coast         | GT1 x PB5/51                    | High production | Fast                       |
| IRCA 230 | Ivory Coast         | GT1 x PB5/51                    | Very high production | Fast                       |
| PB 255   | Ivory Coast         | PB5/51 x PB32/36                | Very good initial productions | Fast                       |
| PB 260   | Malaysia            | PB 5/51 X PB 49                 | Top producer  | Fast                        |
| PB 280   | Malaysia            | PB 5/54 and PB 32/36            | Good production | Fast                       |
| PB 310   | Malaysia            | PB5/51 x RRIM600                | Quite productive | Very fast                  |
| PB 330   | Malaysia            | PB 5/51 X PB 32/36              | Good production | Fast                       |
| GT 1     | Indonesia           | Primary clone                   | Average production | Medium                     |
| BPM 24   | Malaysia            | GT 1 X Avros 1734               | Good production | Medium                     |
| RRIM 712 | Malaysia            | RRIM605 x RRIM71                | Quite productive | Medium                     |
| PB 217   | Malaysia            | PB 5/51 X PB 6/9                | Top producer   | Slow                        |
| PR 107   | Malaysia            | Primitiveclone                  | High productivity | Slow                       |
It appears from this analysis that the average annual increase in tree circumference in AMC and MMC trees decreased as the annual frequency of exogenous hormonal stimulation increased. On the other hand, in SMCs, the average annual growth rate decreased beyond 4 stimulations of annual frequency. The ratio between the percentages due to tapping and stimulation (% Tapping / % Stim), all classes combined, is less than 1.

5.2 Effect of Different Annual Frequencies of Exogenous Hormonal Stimulation on Rubber Production

The average rubber production of AMC and SMC was highly influenced by the annual frequency of exogenous hormonal stimulation (p < 0.0001). Production ranged from 53.34 to 68.49 g.a⁻¹.s⁻¹ for AMC and from 39.83 to 66.69 g.a⁻¹.s⁻¹ for SMC (Table 3).

The highest production (68.49 g.a⁻¹.s⁻¹) was obtained with the 6y treatment. It was statistically different from the unstimulated treatment (0y) with an average production of 58.96 g.a⁻¹.s⁻¹ for AMC. Treatment 78y gave the lowest production with an average value of 53.34 g.a⁻¹.s⁻¹.

At the SMC level, all stimulated trees gave higher rubber production (47.23 to 66.69 g.a⁻¹.s⁻¹) than non-stimulated trees (39.83 g.a⁻¹.s⁻¹). The highest rubber productions of statistically identical values (66.44 and 66.69 g.a⁻¹.s⁻¹) were obtained from treatments 26 and 39y.

On the other hand, no difference was observed in the production of MMCs (p > 0.05).

The analysis showed that rubber production increased with the annual number of exogenous hormonal stimulation up to 6 and 26 annual stimulations respectively for AMC and SMCs except for MMCs where production was statistically identical regardless of the frequency of annual stimulation applied. The response to the annual frequency of stimulation was not the same for the class of metabolic activity of the clones.

5.3 Evaluation of the Level of Thiol Group Content in Latex According to the Intensity of Stimulation according to the Metabolic Activity Class of the Hevea brasiliensis Clones

The level of latex thiol group content (RSH) as a function of the intensity of stimulation according to the class of metabolic activity of Hevea brasiliensis clones is presented in Table 4.

It appears from the analysis of Table 4 that, according to the metabolic activity class, no significant difference was observed between the RSH content of unstimulated and stimulated trees. According to the reference values, the RSH concentration in SMCs was high (0.815 mmol.l⁻¹), in MMCs was medium (0.619 mmol.l⁻¹) and in AMCs was low (0.593 mmol.l⁻¹).

5.4 Evaluation of the Coefficient of Sensitivity to Dry Notching according to the Class of Metabolic Activity of Hevea brasiliensis Clones

The sensitivity coefficient is determined from the ratio of the difference in dry notch rate between unstimulated and maximally stimulated trees and the difference in latex sugar content of unstimulated and maximally stimulated trees. Table 5 shows the result of this calculation.

The analysis of this table shows that the level of sensitivity is low for the slow metabolic activity class, lower for the moderate class and high for the active metabolic class.

5.5 Relationship between the Average Annual Increase in Circumference, Rubber Production and Physiological Parameters

5.5.1 Relationship between growth and production according to clone metabolic activity class

Figs. 2, 3 and 4 show the evolution of production as a function of the increase for each class of metabolic activity of Hevea brasiliensis clones.

It appears that the increase in production is strongly correlated with the value of the average annual increase in circumference of the clones per metabolic activity class (R² > 0.5). A study of the non-linear regression of polynomial type gives the following analytical expressions:

AMC: \[ P = -39.186 \text{Acc}^2 + 207.11 \text{Acc} - 209.47 \text{; } R^2 = 0.8004 \]

MMC: \[ P = -21.578 \text{Acc}^2 + 93.499 \text{Acc} - 38.256 \text{; } R^2 = 0.8027 \]

SMC: \[ P = -11.858 \text{Acc}^2 + 51.613 \text{Acc} + 7.849 \text{; } R^2 = 0.9205 \]
AMC: Active Metabolism Clones; MMC: Moderate Metabolism Clones; SMC: Slow Metabolism Clones.

5.5.2 Relationship between circumference increase and sucrose according to the class of clone metabolic activity

The evolution of the average annual circumference growth (cm yr\(^{-1}\)) of the trees of the clones according to the class of metabolic activity as a function of latex sucrose concentration is shown in Figs. 5, 6 and 7. The different figures show that whatever the class of metabolic activity of the clone, there is a very close relationship between the average annual circumference increase and the sucrose content of the latex within the drained area (\(R^2 > 0.5\)). This is described by the following polynomial function of analytical expression:

\[
\text{AMC: Acc (cm yr}^{-1}) = -0.0265 \text{Sac}^2 + 0.8218 \text{Sac} - 3.578; \quad R^2 = 0.8984
\]

\[
\text{MMC: Acc (cm yr}^{-1}) = 0.039 \text{Sac}^2 - 0.6409 \text{Sac} + 4.118; \quad R^2 = 0.8344
\]

\[
\text{SMC: Acc (cm yr}^{-1}) = -0.0132 \text{Sac}^2 + 0.6462 \text{Sac} - 4.4111; \quad R^2 = 0.8309
\]

Figs. 8 and 9 show the evolution of the average annual circumference growth (cm yr\(^{-1}\)) of trees in clones with moderate and slow metabolism as a function of the average latex inorganic phosphorus content expressed in millimoles per litre (mmol.l\(^{-1}\)).

5.5.3 Relationship between circumference increase and inorganic phosphorus according to clone metabolic activity class

Analysis of the figures shows a strong and significant correlation between the average annual circumference increase and the latex inorganic phosphorus content of the latex of the moderately and slowly metabolizing clones. The study of the non-linear regression gives the following analytical equations:

\[
\text{MMC: Acc (cm yr}^{-1}) = 0.0304 \text{iP}^2 - 1.4507 \text{iP} + 18.626; \quad R^2 = 0.7253
\]

\[
\text{SMC: Acc (cm yr}^{-1}) = 0.0206 \text{iP}^2 - 1.1219 \text{iP} + 17.35; \quad R^2 = 0.7034
\]

For metabolically active clones, the regression is not significant.

Table 2. Average annual circumference increase (cm yr\(^{-1}\)) of rubber trees of different classes of metabolic activity as a function of the annual frequency of exogenous hormonal stimulation and ratio between the percentages of reduction in growth related to tapping and the annual frequency of exogenous hormonal stimulation

| Stimulation frequency | AMC | MMC | SMC |
|----------------------|-----|-----|-----|
| 0y                   | 3,0 a | 2,8 a | 3,7 a |
| 2y                   | 2,8 ab | 2,4 b | 3,2 a |
| 4y                   | 2,7 ab | 2,2 bc | 3,1 a |
| 6y                   | 2,6 abc | - | - |
| 8y                   | 2,5 abc | 2,1 bc | 2,7 b |
| 13y                  | 2,5 abc | 1,9 c | 2,6 b |
| 18y                  | 2,4 abc | 1,8 c | 2,4 bc |
| 26y                  | 2,2 bc | 1,7 c | 2,4 bc |
| 39y                  | 2,3 bc | 1,6 d | 2,3 bc |
| 78y                  | 2,1 c | 1,6 d | 2,0 c |
| MG (cm yr\(^{-1}\)) | 2,5 | 2 | 2,7 |
| Pr > F                | <0,0001 | <0,0001 | <0,0001 |
| %RedAccnS (Tapping)  | 45,05 | 43,55 | 23,59 |
| % of RedAccnS (Stim) | 56,04 | 61,69 | 45,72 |
| %RedAccS             | 20 | 32,14 | 29,73 |
| Ratio (%Tap/%Stim)   | 0,8 | 0,71 | 0,52 |

AMC: active metabolism clones; MMC: moderate metabolism clones; SMC: slow metabolism clones. MG: Overall average; RedAcc (%): reduction in growth, expressed as a percentage of growth potential. The values followed by the same letter in the same column are not significantly different according to the Newman-Keuls Test at the 5% threshold.
Table 3. Average rubber production of the clones in g.a⁻¹.s⁻¹ according to the class of metabolic activity as a function of the annual frequency of exogenous hormonal stimulation during nine years of experimentation

| Stimulation frequency | AMC  | MMC  | SMC  |
|-----------------------|------|------|------|
| 0y                    | 58.96bcd | 55.19 a | 39.83 c |
| 2y                    | 62.54 ab  | 59.77 a  | 47.23 bc |
| 4y                    | 64.01 ab  | 61.92 a  | 53.76 abc |
| 6y                    | 68.49 a   | -        | -      |
| 8y                    | 62.62 ab  | 64.49 a  | 57.62 ab |
| 13y                   | 62.49 ab  | 60.78 a  | 62.43 ab |
| 18y                   | 60.44 bc  | 61.32 a  | 63.48 ab |
| 26y                   | 57.85 bcd | 60.99 a  | 66.69 a  |
| 39y                   | 55.5 cd   | 56.47 a  | 66.44 a  |
| 78y                   | 53.34 d   | 52.81 a  | 60.58 ab |
| MG (g.a⁻¹.s⁻¹)        | 60.34    | 59.3    | 57.45  |
| Pr > F                | <.0001   | 0.0602  | <.0001  |
| CV (%)                | 23.64    | 23.56   | 31.56   |

AMC: active metabolism clones, MMC: moderate metabolism clones, SMC: slow metabolism clones, MG: General Mean; CV: coefficient of variation. Values followed by the same letter in the same column are not significantly different according to the Newman-Keuls Test at the 5% threshold.

Fig. 2. Polynomial relationship between mean annual circumference increase (cm.yr⁻¹) and mean rubber production (g.a⁻¹.s⁻¹) of metabolically active clones during 9 years of latex harvest according to the Pearson test at the 5% threshold.
Fig. 3. Polynomial relationship between mean rubber production (g.a⁻¹.s⁻¹) and mean annual circumference increase (cm.yr⁻¹) of moderately metabolised clones during 9 years of latex harvest according to the Pearson test at the 5% threshold

\[ y = -21.578x^2 + 93.499x - 38.256 \]
\[ R^2 = 0.8027 \]

Fig. 4. Polynomial relationship between mean annual circumference increase (cm.yr⁻¹) of slow metabolizing clones during 9 years of latex harvest according to the Pearson test at the 5% threshold

\[ y = -11.858x^2 + 51.613x + 7.849 \]
\[ R^2 = 0.9205 \]
Fig. 5. Polynomial relationship between the average annual increase (cm yr$^{-1}$) in circumference and the average sucrose concentration (mmol l$^{-1}$) in the latex of metabolically active clones.

Table 4. Value of latex thiol group content as a function of stimulation intensity according to metabolic activity class of *Hevea brasiliensis* clones

| Stimulation frequency | CMA         | CMM         | CML          |
|-----------------------|-------------|-------------|--------------|
| 0y                    | 0.583 a ± 0.032 | 0.664 a ± 0.051 | 0.793 a ± 0.127 |
| 2y                    | 0.569 a ± 0.032 | 0.631 a ± 0.051 | 0.821 a ± 0.127 |
| 4y                    | 0.592 a ± 0.032 | 0.637 a ± 0.051 | 0.770 a ± 0.127 |
| 6y                    | 0.610 a ± 0.047 | -           | -             |
| 8y                    | 0.609 a ± 0.032 | 0.655 a ± 0.051 | 0.781 a ± 0.127 |
| 13y                   | 0.584 a ± 0.032 | 0.590 a ± 0.051 | 0.857 a ± 0.127 |
| 18y                   | 0.604 a ± 0.032 | 0.609 a ± 0.051 | 0.798 a ± 0.127 |
| 26y                   | 0.612 a ± 0.032 | 0.600 a ± 0.051 | 0.831 a ± 0.127 |
| 39y                   | 0.581 a ± 0.034 | 0.581 a ± 0.051 | 0.873 a ± 0.127 |
| 78y                   | 0.588 a ± 0.040 | 0.602 a ± 0.051 | 0.815 a ± 0.127 |
| Average               | 0.593 ± 0.102  | 0.619 ± 0.079  | 0.815 ± 0.135  |
| Pr > F                | 0.997        | 0.961        | 1.000         |

*AMC: active metabolism clones, MMC: moderate metabolism clones, SMC: slow metabolism clones. Values followed by the same letter in the same column are not significantly different according to the Newman-Keuls Test at the 5% threshold.

Table 5. Dry notch sensitivity coefficient according to the metabolic activity class of *Hevea brasiliensis* clones

| Metabolic class | Degree of sensitivity | Level of sensitivity |
|-----------------|-----------------------|----------------------|
| Active          | 2                     | high                 |
| Moderate        | 1.7                   | lower                |
| Slow            | 1.6                   | Not very weak        |

5.5.4 Relationship between production and sucrose according to the class of metabolic activity of the clone.

The evolution of rubber production as a function of the sucrose concentration in the latex of fast- and slow moving clones is shown in Figs. 10 and 11.
Fig. 6. Polynomial relationship between the average annual increase (cm.yr$^{-1}$) in circumference and the average sucrose concentration (mmol.l$^{-1}$) in the latex of moderately metabolised clones.

Fig. 7. Polynomial relationship between the average annual increase (cm.yr$^{-1}$) in circumference and the average sucrose concentration (mmol.l$^{-1}$) in the latex of slow metabolising clones.
Fig. 8. Polynomial relationship between the average annual increase (cm.yr\(^{-1}\)) in circumference and the average inorganic phosphorus concentration (mmol.l\(^{-1}\)) in the latex of moderately metabolised clones

\[
y = 0.0304x^2 - 1.4507x + 18.626 \\
R^2 = 0.7253
\]

Fig. 9. Polynomial relationship between the average annual increase (cm.yr\(^{-1}\)) in circumference and the average inorganic phosphorus concentration (mmol.l\(^{-1}\)) in the latex of slow metabolism clones

\[
y = 0.0205x^2 + 1.1218x + 17.55 \\
R^2 = 0.7034
\]
Fig. 10. Polynomial relationship between the average rubber production (g.a⁻¹.s⁻¹) and the average sucrose concentration (mmol.L⁻¹) of the latex of metabolically active clones

Fig. 11. Polynomial relationship between the average rubber production (g.a⁻¹.s⁻¹) and the average sucrose concentration (mmol.L⁻¹) in the latex of slow metabolizing clones
The analysis of the different figures shows a strong correlation between the production expressed in grams per tree per bleed \((\text{g.a}^{-1}.\text{s}^{-1})\) and the sucrose latex concentration of actively metabolising clones \((R^2 = 0.7717)\) and slowly metabolising clones \((R^2 = 0.7731)\). This polynomial relationship is described by the following analytical expressions:

**AMC:** \[ P (\text{g.a}^{-1}.\text{s}^{-1}) = -3.0295 \text{Sac}^2 + 70.828 \text{Sac} - 348.83 ; R^2 = 0.7717. \]

**SMC:** \[ P (\text{g.a}^{-1}.\text{s}^{-1}) = -0.7821 \text{Sac}^2 + 20.891 \text{Sac} - 75.492 ; R^2 = 0.7731. \]

### 5.5.5 Relationship between production and inorganic phosphorus according to the class of metabolic activity of the clone

Figs. 12 and 13 show the evolution of rubber production expressed in grams per tree per bleed \((\text{g.a}^{-1}.\text{s}^{-1})\) as a function of the inorganic phosphorus content of the latex.

**Fig. 12.** Polynomial relationship between the average rubber production \((\text{g.a}^{-1}.\text{s}^{-1})\) and the average inorganic phosphorus concentration \((\text{mmol.L}^{-1})\) in the latex of metabolically active clones

**Fig. 13.** Polynomial relationship between the average rubber production \((\text{g.a}^{-1}.\text{s}^{-1})\) and the average inorganic phosphorus concentration \((\text{mmol.L}^{-1})\) in the latex of slow metabolizing clones
These figures show a very strong correlation between rubber production and inorganic phosphorus content in slow metabolising clones ($R^2 = 0.9548$) and a medium correlation in fast metabolising clones ($R^2 = 0.5091$). The analytical expressions describing these relationships are as follows:

$$AMC: \quad P (g.a^{-1.s^{-1}}) = -5.199 \cdot iP^2 + 298.6 \cdot iP - 4222.8 \quad ; \quad R^2 = 0.5091$$

$$SMC: \quad P (g.a^{-1.s^{-1}}) = -0.3158 \cdot iP^2 + 18.201 \cdot iP - 196.23 \quad ; \quad R^2 = 0.9548$$

6. DISCUSSION

Rubber production increased with the annual number of exogenous hormone stimulation up to 6 and 26 annual stimulations respectively for AMC and MMC except in SMC where production was statistically identical regardless of the frequency of annual stimulation applied. These results illustrate the more or less important effect of exogenous hormonal stimulation in the productivity of Hevea brasiliensis clones according to the class of metabolic activity. In fact, the stimulating products commonly used have as their active ingredient 2-chloroethylphosphonic acid or Ethephon, which generates ethylene in laticifers, specialised cells that elaborate latex. The presence of ethylene in these tissues allows an extension of the latex's flow time and, consequently, an important rubber yield [22,23]. The activation of the metabolism, which explains the increase in production, takes place up to a certain limit of the intralaticiferous sugar of each metabolic class, as has been shown in some studies [24,22,6,25]. In the case of this study, this limit was reached with 6 annual stimulations (6y) for clones with active metabolism and 26 annual stimulations (26y) for clones with slow metabolism. Furthermore, the drop in production beyond 6 and 26 stimulations in these two groups of clones, would be a sign of a dysfunction of the laticiferous system resulting from overstimulation according to the work of [22] and [6]. Furthermore, due to the increase in the annual frequency of hormonal stimulation, a decrease in the sugar content of latex and even starch in the bark appears [26]. This persistent decrease would seem to indicate an acceleration of the cellular activity of the laticifers in situ leading to a subsequent drop in rubber production [2]. Thus, these various stimulations beyond which there was a drop in production could constitute the bearable limit of the number of stimuli at the level of these 2 groups of clones.

The absence of difference between the production level of unstimulated and stimulated trees in clones with moderate metabolism highlights the less depressive action of the stimulation intensities in this group. This could be explained by the high intrinsic energy level favouring good regeneration of the RSH, leading to a weak action of toxic compounds (AOS) following the intensification of stimulation in this group [27,22]. The average RSH content obtained in this study for this group of clones illustrates this well. In fact, thiol groups (RSH) are a set of major antioxidant molecules which make it possible to fight against the physiological mechanisms releasing oxygen in toxic form (AOS) when laticigene activity is increased by ethylenic stimulation [28,29]. Their regeneration requires biochemical energy in the form of ATP and also depends to a certain extent on the sugar available in situ, which is the source of molecular carbon and numerous reactions. This energy having been moderately sufficient given the level of RSH concentration obtained in this study, made it possible to reach the production potential. Thus, increasing the stimulation frequencies would only be too much and even harmful on the parameters likely to favour future good rubber production at the level of this group of clones [22,6,25]. It should also be noted that the degree of sensitivity was lower despite the high stimulation intensities in this study for this group of clones. These results are similar to the work of [30]. They noted that cells subjected to environmental stresses, such as the increase in ethylenic treatments at the laticifer level, require more hydrocarbon feed to combat such situations.

Evaluation of the effect of the frequency of annual hormonal stimulation on the growth of Hevea brasiliensis trees by class of metabolic activity of the clones showed a decrease in the increase in circumference as a function of the intensity of stimulation (0 to 78 y). This highlights the depressive impact of the stimulant on the bark of Hevea brasiliensis trees. The ratio between the percentages due to bleeding and stimulation (% Tapping/ % Stim), all classes below 1, shows this in this study. This ratio would indicate that the effect of intensification by stimulation on growth is more important than the effect of bleeding alone. Indeed, the activation of the laticigene function by hormonal stimulation leads to an increase in energy requirements and in hydrocarbon assimilates important for rubber production. It thus appears a decrease in vegetative growth linked to an intense solicitation
of the laticiferous system during latex production [6,3,9]. This decrease in growth was variable according to the class of metabolic activity of the clones in this study. Hence the appearance of complexity in the phenomenon of antagonism between growth and production metabolisms long evoked by several authors [31,6]. This would be linked to the energy cost necessary for the production of latex, which is different for each metabolic class [6,25]. Thus, unlike the other classes, the decrease in growth was accentuated beyond 4 stimuli per year (4y) in clones with slow metabolism. This difference could be explained by the ability of slow metabolizing clones to mobilize sugars within their laticigenic tissues but also by the availability of apoplastic sugars. This would have enabled them to minimise the overall energy costs linked to latex production, thus leading to a small deviation of photosynthetic assimilates towards the synthesis of secondary biomass during the first stimulation frequencies per year (0 and 4y) at the level of this group of clones. The similarity between the growth of unstimulated and stimulated trees 4 times per year (4y) would thus reflect a weak antagonism between growth and production.

Overall, the results show strong significant correlations between rubber production, circumference increase, sucrose and inorganic phosphorus content of latex across all classes of metabolic activity of the clones. This would indicate a close relationship between, on the one hand, the regeneration of latex within the laticiferous system and the ease and quantity of latex flow and the availability of water, on the other hand [32,22,33,5]. Furthermore, the behaviour of slow metabolizing clones illustrates this relationship well. Indeed, the slightest decrease in growth in response to the increase in production linked to intensification by stimulation could result in a greater accumulation of hydrocarbon compounds in the storage tissues available for the maintenance of the vegetative growth metabolism. This would appear to reflect a good state of equilibrium between laticigenic and vegetative growth metabolism. Therefore, this appears to be a good indicator of low competition between growth and production in *Hevea brasiliensis*.

7. CONCLUSION

The exogenous hormonal stimulation of rubber production influences the vegetative growth of *Hevea brasiliensis*. In order to achieve a sustainable good rubber harvest of *Hevea brasiliensis*, the effect of exogenous hormonal stimulation on the metabolic growth-production partition of the clones according to the class of metabolic activity was evaluated for 9 years in the south-western Ivory Coast. The results showed that the ratio describing the effect of stimulation is less than 1, whatever the class of metabolic activity of the clone. Moreover, it affects more clones with active and moderate metabolism by reducing the average annual increase in circumference by 56.04 and 61.69% respectively. The optimal annual stimulation frequencies for clones with active and slow metabolisms ensuring better production are 6 and 26 applications respectively. This shows the strong variability of production linked to the intensification of stimulation according to the class of metabolic activity of the clones. Strong correlations of polynomial function (R² > 0.5) appear between agronomic and physiological parameters. This relationship seems very clearly dependent on the content of latex thiol groups (R-SH) and the sensitivity to dry notching of the clone. This reflects a good state of equilibrium between laticigene metabolism and vegetative growth metabolism. The slow metabolizing clones are exemplary given the high R-SH content and the low level of sensitivity to dry notching compared to the other groups. Therefore, the stimulation intervals [0.6] : [0.8] and [0.26] appear to be good indicators of low competition between growth and production in *Hevea brasiliensis*.

ACKNOWLEDGEMENTS

We would like to thank the Southwestern Agricultural Civil Society (SCASO) for allowing us to carry out our work on their plantations and all the people for their valuable contributions to this work. We would also like to thank all the anonymous readers of our manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Thomas V, Premakumari D, Reghu CP, Panikkar AON, Saraswathy ACK. Anatomical and histochemical aspects of bark regeneration in *Hevea brasiliensis*. Ann. Bot Comp. 1995;75:421-426.

2. Jacob JL, Prévôt JC, Lacrotte R, Eschbach JM. Latex diagnosis. Plantations, Research, Développement, 1995;2:34-37.
3. 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.

4. Eschbach JM, Roussel D, Van De Sype H, Jacob JL, d’Auzac J. Relationships between yield and clonal physiological characteristics of latex from Hevea brasiliensis. Physiol Vég. 1984;22:295-304.

5. Moraes VHF, Moraes LAC. Effect of rubber tree (Hevea spp.) budded crowns on the content of magnesium and latex regeneration of the clone Fx 3899,” Agrotropica. 1997:9:59–66.

6. Gohet E. Latex production by latex Hevea brasiliensis. Relationship with growth. Influence of different factors: Clonal origin, hormonal stimulation, hydrocarbon reserves. PhD thesis, University of Montpellier II, France. 1996;343.

7. Wycherley PR. Tapping and partition, J Rub Res Inst Malay. 1976;21(4):169-194.

8. Sethuraj M. Characteristic of latex from between yield and clonal physiological characteristics of Hevea brasiliensis clone PB 217 and PB 235. In: Recueil de fiches de clones (internal document). 1993;5.

9. Obouayeba S, Boa D, Kéli JZ. Adequacy between quantity of stimulating paste and rubber production in Hevea brasiliensis in the south-east of Ivory Coast. Tropicultura. 1996;14(2):54-58.

10. Welsh R. Metabolism of adenyl nucleotides in Hevea brasiliensis latex. Effect of ethylene. Thesis University of Montpellier II, Faculty of Sciences and Techniques of Languedoc, France. 1998;154.

11. Soumahin E, Obouayeba S, Anno AP. Low tapping frequency with hormonal stimulation on Hevea brasiliensis clone PB 217 reduces tapping manpower requirement. Journ Anim Plant Sci. 2009;2(3):109–117.

12. Soumahin EF. Optimisation of rubber farming systems by reducing tapping intensities. Single PhD thesis, UFR Biosciences, UniversityCocody, Côte d’Ivoire. 2010;189.

13. Obouayeba S, Soumahin EF, Okoma KM, N’Guessan AEB, Lacote R, Coulibaly LF, Ake S. Relationship between the tapping cut length and the parameters of vegetative growth and rubber yield of Hevea brasiliensis, clones GT 1 and PB 235 in south-eastern Côte d’Ivoire. Journ Crops Sci. 2011;2(2):27-44.

14. Jacob JL, Prévôt JC. Bark dryness: Histological, cytological and biochemical Aspect. IRRDB. Workshop on tree dryness, Penang, Malaysia. 1989;37–54.

15. Gohet E, Lacrotte R, Obouayeba S, Commère J. Tapping systems recommended in West Africa. Proc Rubb Grow Conf., Rubb. Res. Inst. Malaysia, ed., Kuala Lumpur; 1991.

16. Brou YT. Climate, socio-economic changes and landscapes in Ivory Coast. Memory of synthesis of scientific activities. University of Science and Technology of Lille. 2005;332.

17. Ministry of planning and development. Monographic and economic studies of the districts of Cote d’Ivoire, Bas-Sassandra. 2015;70.

18. Jacob JL, Serrès E, Prévôt JC, Lacrotte R, Clément-Vidal A, Eschbach JM, Auzac J. Development of latex diagnosis. Agritrop. 1988;12:97-118.

19. Obouayeba S, Boa D, Gohet E, Dian K, Ouattara N, Kéli J. Dynamics of vegetative growth of Hevea brasiliensis in the determination of tapping norms. J Rubb Res. 2000;3(1):53-62.

20. Anonymous. Clones cards, 4 11 et 12 : GT 1, PB 217 et PB 235. In : Recueil de fiches de clones (internal document). 1993;5.

21. Lacrotte R. Study of the relationship between the sugar content of latex and production. Approach to the mechanisms of sucrose loading of Hevea brasiliensis. Ph.D thesis, University of Sciences and Techniques of Languedoc, Montpellier II. 1991;266.

22. Dick A, Traore MS, Elabo A, Soumahin E, Evelyne G, Atsin O, et al. Effect of different annual frequencies of ethylene stimulation on agrophysiological parameters and sensibility to the dry notch of Hevea brasiliensis in south-eastern Côte d’Ivoire: Case of clones PB 235 and PB 260 of the rapid metabolic activity class. Journal Biological Chemical Sciences. 2014;9(3):956-974.

23. Buttery BR, Boatman SG. Effects of tapping, wounding and growth regulators on turgor pressure in Hevea brasiliensis. J Exp Bot. 1967;18:644-659.
25. Traore MS. Effect of different annual frequencies of ethylenic stimulation on the agrophysiological parameters of clones of Hevea brasiliensis, PB 235, PB 260, GT 1 et PB 217 in south-eastern Côte d’Ivoire. Doctoral Thesis, University Cocody-Abidjan, Ivory Coast. 2015;161.

26. Lacote R, Gabla O, Obouayeba S, Eschbach JM, Rivano F, Dian K, Gohet E. Long-term effect of ethylene stimulation on the yield of rubber trees is linked to latex cell biochemistry. Field Crop Res. 2010;115:94–98.

27. Prévôt JC, Jacob JNL, Lacrotte R, Vidal A, Serrès E, Eschbach JM, Gigault J. 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 Yangqing and Lhao Canwen Eds, South China Academy of Tropical Crops of functioning (Hainan). 1986;36-157.

28. Chrestin H. Stimulation in the ethrel of the rubber tree: How far not go too far. Caouth Plast. 647/648. 1985;75–78.

29. D’Auzac J. Toxic oxygen: A defence against pathogens. Plantations Recherche, Développement. 1996;3:153-163.

30. Truernit E, Schmidt J, Egyle P, Saver N. The sink specific and stress regulated Arabidopsis STP4 gene. Enhanced expression of a gene encoding a monosaccharid transporter by wounding elicitors and pathogen challenge. Plant Cell. 1996;8:2169-2172.

31. Templeton JK. Partition of assimilates. J Rubb Res Inst Malaya. 1969;21:259–273.

32. Patric JW. Sieve element unloading: cellular pathway, mecanism and control. Physiol Plant. 1990;78:298-308.

33. Serrès E, Lacrotte R, Prévôt JC, Clément A, Commère J, Jacob JL. Metabolic aspects of latex regeneration in situ for three Hevea clones. Rubb Ind J. 1994;7:79-84.