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PHYSIOLOGICAL PARAMETERS OF LATEX IN THE RRIM 600 AND FX 3864 RUBBER CLONES IN COLOMBIA

PARÁMETROS FISIOLÓGICOS DEL LÁTEX EN LOS CLONES RRIM 600 Y FX 3864 EN COLOMBIA

Franck Rivano¹

Juan David Hernández Arredondo²

Jorge Humberto Argüelles Cárdenas³

Maribel Tarazona Yanes⁴

David Ricardo Hernández Angarita⁵

Régis Lacote⁶

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¹ PhD in Plant Pathology, French Agricultural Research Centre for International Development (CIRAD), Montpellier, France. franckrivano@gmail.com

² Master's degree in Agricultural Sciences, Colombian Agricultural Research Corporation (AGROSAVIA), El Nus Research Center, Antioquia, Colombia. jdhernandez@agrosavia.co

³ Master's degree in Statistics, Colombian Agricultural Research Corporation (AGROSAVIA), La Libertad Research Center, Villavicencio, Colombia. jorargu@yahoo.com.mx

⁴ Bachelor's degree in Agricultural Production, Colombian Agricultural Research Corporation (AGROSAVIA), La Libertad Research Center, Villavicencio, Colombia. mtarazona@agrosavia.co

⁵ Agronomist, Colombian Agricultural Research Corporation (AGROSAVIA), La Libertad Research Center, Villavicencio, Colombia. drhernandez@agrosavia.co

⁶ PhD in Plant Ecophysiology, French Agricultural Research Centre for International Development (CIRAD), Montpellier, France. regis.lacote@cirad.fr

ABSTRACT

Contextualization: Studying the metabolic role of certain compounds in the latex produced by *Hevea brasiliensis* trees can help adjust harvesting strategies for each clone under specific environmental conditions.

Knowledge gap: In Colombia, little is known about the metabolic response of the RRIM 600 and FX 3864 clones to hormonal stimulation. This limits the development of appropriate management programs and increases the risk of overexploitation or underutilization of the clones' productive potential.

Purpose: This study aimed to characterize the metabolic status of latex from the RRIM 600 and FX 3864 clones using the Latex Diagnosis method in order to adjust harvesting practices according to the physiology of each clone.

Methodology: The study was conducted between 2021 and 2022 on 12 farms in the department of Meta, Colombia, using a randomized complete block design with two

treatments (clones) and four replications per farm. Four physiological latex parameters were evaluated using spectrophotometric analyses: sucrose content, inorganic phosphorus content, thiol content, and total solids content. Data were analyzed using a generalized linear mixed model to identify significant effects of clone, farm, and year.

Results and conclusions: RRIM 600 showed higher levels of inorganic phosphorus and total solids content, suggesting greater metabolic activity. FX 3864 had higher levels of sucrose and thiols, indicating slower metabolism, greater antioxidant capacity, and greater energy reserves. These physiological differences highlight the need to adjust stimulation intensity according to the metabolic characteristics of each clone. Latex Diagnosis is a key technical tool for improving the sustainability and efficiency of rubber cultivation in the region.

Keywords: stimulation, latex, *Hevea brasiliensis*, crop yield.

RESUMEN

Contextualización: El estudio del papel metabólico de algunos compuestos presentes en el látex producido por los árboles de *Hevea brasiliensis* permite ajustar las estrategias de explotación de cada clon a condiciones ambientales específicas.

Vacío de conocimiento: En Colombia, se desconoce la respuesta metabólica de los clones RRIM 600 y FX 3864 a la estimulación hormonal. Esta falta de conocimiento dificulta el desarrollo de programas de manejo adecuados y aumenta el riesgo de sobreexplotación o subutilización del potencial productivo de estos clones.

Propósito: El objetivo fue caracterizar el estado metabólico del látex de los clones RRIM 600 y FX 3864 mediante el método de Diagnóstico Látex, con el fin de ajustar las prácticas de explotación de acuerdo con la fisiología de cada clon.

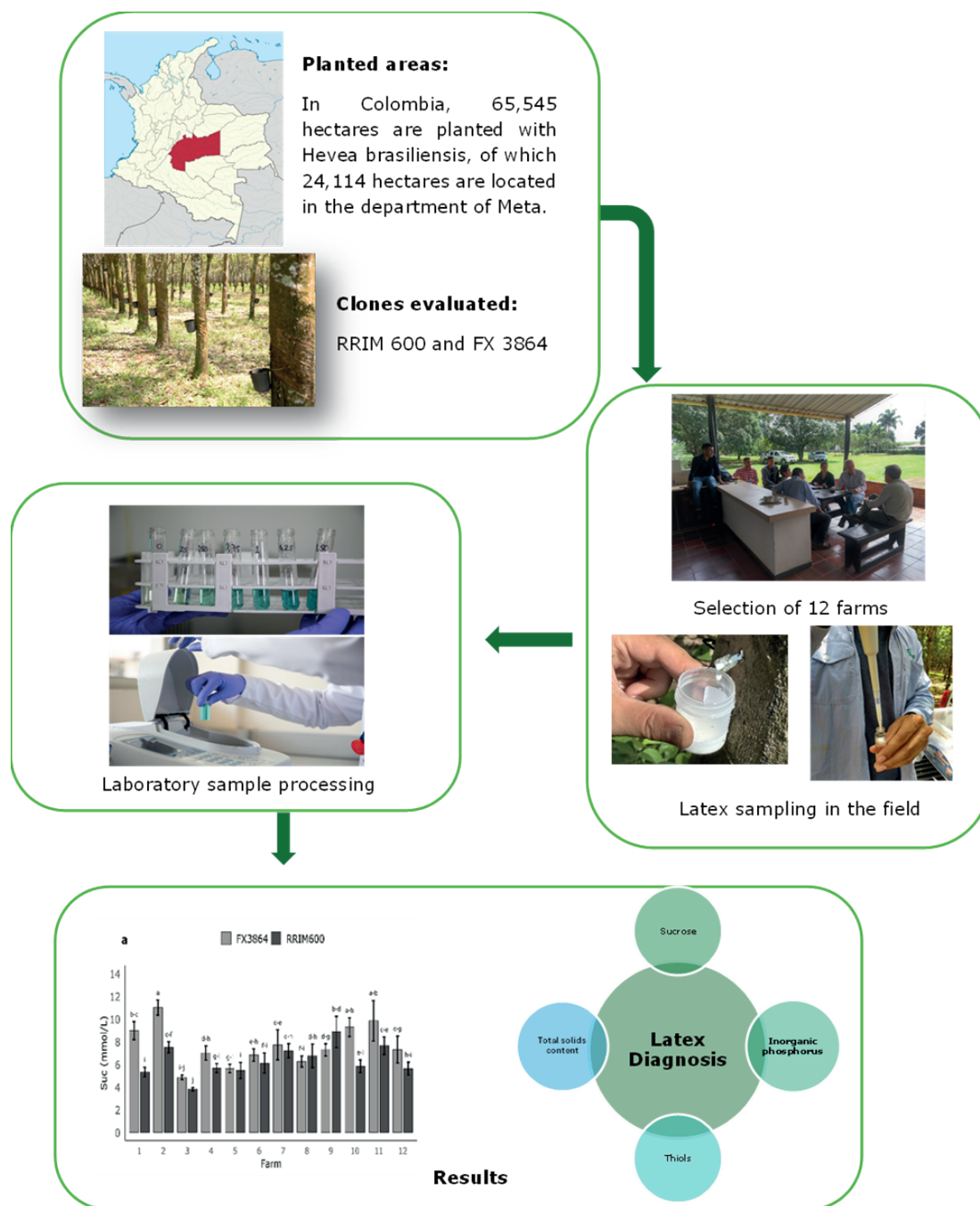
Metodología: El estudio se realizó entre el 2021 y el 2022 en 12 fincas del departamento del Meta, Colombia. Se empleó un diseño de bloques completos al azar con dos tratamientos, correspondientes a los dos clones,

y cuatro repeticiones por finca. Se evaluaron cuatro parámetros fisiológicos del látex mediante análisis espectrofotométricos: sacarosa, fósforo inorgánico, tioles y contenido de sólidos totales. Los datos se analizaron mediante un modelo lineal generalizado mixto para determinar los efectos del clon, la finca y el año.

Resultados y conclusiones: El clon RRIM 600 exhibió mayores niveles de fósforo inorgánico y contenido de sólidos totales, lo que sugiere una mayor actividad metabólica. Por su parte, FX 3864 presentó mayores niveles de sacarosa y tioles, indicativos de un metabolismo más lento, una mayor capacidad antioxidante y mayores reservas energéticas. Estas diferencias fisiológicas resaltan la necesidad de ajustar la intensidad de la estimulación a las características metabólicas de cada clon. El Diagnóstico Látex constituye una herramienta técnica clave para mejorar la sostenibilidad y la eficiencia del cultivo de caucho en la región.

Palabras clave: estimulación, látex, *Hevea brasiliensis*, rendimiento del cultivo.

GRAPHICAL ABSTRACT



Process diagram of the Latex Diagnosis method applied to the FX 3864 and RRIM 600 clones on 12 farms in the department of Meta, Colombia.

Source: Authors.

1 INTRODUCTION

Globally, natural rubber plantations cover approximately 14 million hectares, with production reaching approximately 13.7 million metric tons in 2019 (Pinizzotto, 2021). In Colombia, approximately 65,545 hectares are planted across five production zones. The Orinoquia region accounts for 33,069 hectares, equivalent to 53.75% of the national planted area, and production reached 12,102 metric tons in 2020 (Ministerio de Agricultura y Desarrollo Rural, 2021).

One viable strategy for increasing crop yield is hormonal stimulation with 2-chloroethylphosphonic acid (ethephon), which can significantly boost plantation productivity (Cruz et al., 2017; Lacote et al., 2010; Rojas Molina et al., 2017). However, excessive stimulant use must be avoided, as it can cause tapping panel dryness (TPD), or dry cutting, a condition characterized by physiological exhaustion of the trees that can lead to drying of the laticiferous system (Herlinawati et al., 2022; Jacob et al., 1989; Obouayeba et al., 2021).

Therefore, the stimulation frequency must be adjusted to improve yield without compromising tree health (Junaidi, Nuringtyas, et al., 2022; Purwaningrum et al., 2019). Latex Diagnosis (LD), a method developed by the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), makes it possible to assess tree physiological status and monitor hormonal stimulation intensity (Gohet et

al., 2005; Jacob et al., 1998; Junaidi, Clément-Vidal, et al., 2022).

The LD method is based on monitoring four latex variables: sucrose content (Suc), a precursor for rubber biosynthesis; inorganic phosphorus content (Pi), which is associated with cellular energy; thiol content (R-SH), which reflects the activity of reducing agents that protect membranes from oxidizing agents and facilitate latex drainage (Jacob et al., 1989); and total solids content (TSC), an index of the biological activity of laticiferous cells (Jacob et al., 1995). The method also makes it possible to optimize harvesting systems, including tapping frequency and stimulation, based on clonal metabolism, thereby reducing the risk of tapping panel dryness (Gohet et al., 2019; Ismawanto et al., 2024; Putranto et al., 2015; Sterling Cuéllar et al., 2020).

Reference values for the LD parameters of each clone must be determined under the edaphoclimatic conditions of each cultivation site. These values are essential to ensure that stimulation strategies are physiologically appropriate and do not compromise crop yield or tree health. Accordingly, this study aimed to characterize the physiological status of the RRIM 600 and FX 3864 clones using the Latex Diagnosis method in the Colombian Orinoquia. The RRIM 600 and FX 3864 clones were expected to exhibit significant variations in Latex Diagnosis parameters (Suc, Pi, R-SH, and TSC), reflecting differences in their metabolic processes and local harvesting

practices in the Colombian Orinoquia. These variations were expected to influence their

response to ethephon stimulation and their productive potential.

2 MATERIALS AND METHODS

The Latex Diagnosis (LD) method was validated under field conditions by AGROSAVIA at the La Libertad Research Center (Meta, Colombia), in collaboration with CIRAD. Its implementation made it possible to identify seasonal variation and establish reference values for LD parameters in the RRIM 600 and FX 3864 clones, thereby confirming its validity under local conditions.

The study was conducted in rubber plantations located between the municipalities of Puerto López and Puerto Gaitán (Meta, Colombia), established on deep, well-drained Oxisols with moderately fine textures, strongly acidic pH, high aluminum saturation, and very low fertility that is difficult to improve (Delgado Huertas et al., 2018; Instituto Geográfico Agustín Codazzi [IGAC], 2004). The region has a unimodal rainfall pattern, with a rainy season extending from April to mid-December, followed by a dry season from mid-December through March (Gobernación del Departamento del Meta & IGAC, 2004; Gutiérrez-Vanegas et al., 2020).

Fieldwork began in 2021 and focused on the FX 3864 and RRIM 600 clones. Twelve farms were selected, each with more than 20 hectares planted with rubber, including homogeneous plots that had been actively tapped for 3–8 years and received ethephon stimulation (2.5% 2-chloroethylphosphonic acid).

A half-spiral tapping system (S/2) was used at frequencies of d3, d4, or d5, depending on the farm. Tapping was performed six days per week, and ethephon stimulation (2.5%) was applied 8–12 times per year. The trunk was divided into two tapping panels (BO-1 or BO-2), following the international notation described by Vijayakumar et al. (2009) (Annex 1).

Latex samples were collected every two months during the peak production season (June–November) in 2021 and 2022. For each sampling event, latex was collected from 10 trees, yielding 3 mL per sample. Two 1-mL aliquots were separated: one for biochemical analysis of sucrose, inorganic phosphorus (Pi), and thiols, and the other for total solids content (TSC).

Latex samples collected in the field were processed in the laboratory at AGROSAVIA's La Libertad Research Center, following the method developed by CIRAD (International Rubber Research and Development Board, 1995; Jacob et al., 1988; Nguyen et al., 2017; Purwaningrum et al., 2019). During collection, the samples were preserved in a solution containing EDTA (ethylenediaminetetraacetic acid) and 2.5% TCA (trichloroacetic acid). In the laboratory, the coagulum was macerated with a glass rod to extract the serum, which was filtered through Whatman No. 40 filter paper.

For the analysis of R-SH, Pi, and Suc, colorimetric methods with calibration curves were used. Absorbance was measured using a GENESYS 10S UV-Vis spectrophotometer (Thermo Scientific) at wavelengths of 412 nm for thiols, 410 nm for phosphorus, and 627 nm for sucrose.

For thiol determination, 1 mL of Tris aminomethane, 1.5 mL of sample, and 0.05 mL of Ellman's reagent (DTNB) were mixed, vortexed, and allowed to react for 15 min until a yellow color developed. Inorganic phosphorus was determined by mixing 0.5 mL of sample, 1 mL of 2.5% TCA, and 3 mL of molybdate–vanadate reagent and allowing the mixture to react for 10 min until a yellow color developed.

Sucrose content was determined by mixing 0.1 mL of sample, 0.4 mL of 2.5% TCA, and 3 mL of anthrone reagent. After vortexing, the samples were heated in a water bath at 90 °C for 10 min until a dark green-to-blue color developed, after which absorbance was measured. TSC was determined by weighing 1 mL of fresh latex in preweighed glass vials, drying the samples at 70 °C for 24 h (with drying time adjusted according to vial diameter), and calculating the percentage of solids based on the difference between dry and fresh weights.

Data Analysis and Processing

On each farm, homogeneous blocks of each clone were selected based on genetic material, planting year, and agronomic management. Within these blocks, four tasks (replicates) were selected for latex sampling. In each task, 10 trees located along a diagonal were randomly selected. The experimental design used across the 12 selected farms was a randomized complete block design with two treatments (clones) and four replicates (tasks) per clone.

Combined analyses of variance were performed for repeated observations over time (2021 and 2022) to determine the effects of clone, farm, and evaluation year on the LD variables sucrose content (Suc), inorganic phosphorus content (Pi), thiol content (R-SH), and total solids content (TSC). These analyses were complemented by Tukey's multiple-comparison tests ($\alpha = .05$) using a generalized linear mixed model fitted with the GLIMMIX procedure in SAS Enterprise Guide 8.3 (version 8.3.0.103). The study included 12 farms, two clones, and two evaluation years; the blocks consisted of four tasks per clone within each farm.

The statistical model was expressed as follows:

$$Y_{ijkl} = \mu + F_i + B_j(F_i) + C_k + (F * C)_{ik} + (T)_l + (F * T)_{il} + (C * T)_{kl} + (F * C * T)_{ikl} + (F * B * C)_{ijk} \quad (1)$$

In this model, Y_{ijkl} denotes the observed value of the response variable, μ denotes the grand mean, F_i represents the effect of the i th farm or location, $B_j(F_i)$ represents

the random effect of the block (task) within the farm, C_k represents the effect of the k th clone, $(F * C)_{ik}$ represents the farm $\times$ clone interaction, $(T)_l$ represents the effect of the

lth time point, $(F \times T)_{il}$ represents the farm $\times$ time interaction, $(C \times T)_{kl}$ represents the clone $\times$ time interaction, $(F \times C \times T)_{ikl}$ represents the farm $\times$ clone $\times$ time interaction, and $(F \times B \times C)_{ijk}$ represents the experimental error.

The model-selection criteria were the Akaike information criterion (AIC), corrected Akaike information criterion (AICc), and Bayesian information criterion (BIC) (Co-

rra Morales & Salazar Uribe, 2016). No transformations were applied to the data; only least-squares means were estimated. Tukey's multiple-comparison tests were performed at $\alpha = .05$.

Graphs were generated using R (version 4.4.2; R Core Team, 2024) within the RStudio interface (Posit Team, 2024) to display the interactions between the factors and response variables.

3 RESULTS AND DISCUSSION

The farm $\times$ clone $\times$ year and clone $\times$ year interactions were significant ($p < .05$) for Pi and TSC. The farm $\times$ year and farm $\times$ clone

interactions were significant ($p < .05$) for all variables (Table 1).

Table 1.
Analysis of Variance for Latex Diagnosis Variables

Source of variation	Suc (mmol/L)		R-SH (mmol/L)		Pi (mmol/L)		TSC (%)	
	F value	Pr > F	F value	Pr > F	F value	Pr > F	F value	Pr > F
Clone	33.86	**	67.64	**	91.72	**	20.31	**
Farm	11.83	**	8.07	ns	14.04	**	8.05	**
Farm $\times$ clone	4.28	**	11.03	**	6.38	**	5.71	**
Year	27.68	**	0.55	ns	3.64	ns	0.19	ns
Clone $\times$ year	2.69	ns	0.98	ns	5.29	*	12.75	**
Farm $\times$ year	2.44	**	2.23	*	17.95	**	3.17	**
Farm $\times$ clone $\times$ year	1.24	ns	1.36	ns	2.30	*	3.28	**

Note. ** = significant at $p < .01$; * = significant at $p < .05$; ns = not significant.
Suc = sucrose content; Pi = inorganic phosphorus content; R-SH = thiol content;
TSC = total solids content.

Source: Authors.

As shown by Tukey's multiple-comparison tests ($\alpha = .05$) in Table 2, FX 3864 had higher estimated mean values than RRIM 600

for Suc and R-SH. Conversely, the RRIM 600 clone had higher mean values for Pi and TSC.

Table 2.

Comparison of Least-Squares-Adjusted Means Between Clones for Latex Diagnosis Variables

Clone	Suc (mmol/L)	R-SH (mmol/L)	Pi (mmol/L)	TSC (%)
FX 3864	7.64 a	0.26 a	9.70 a	46.55 a
RRIM 600	6.34 b	0.22 b	13.49 b	48.81 b

Note. Means sharing at least one letter within a column do not differ significantly according to Tukey's test ($\alpha = .05$).

Suc = sucrose content; Pi = inorganic phosphorus content; R-SH = thiol content; TSC = total solids content.

Source: Authors.

Figure 1 shows the farm $\times$ clone $\times$ year interaction, which had a significant effect on total solids content (TSC) ($p < .01$), revealing a combined response to clone, sampling year, and farm management. The RRIM 600 clone exhibited the highest TSC values in the first year, primarily on farms 8, 12, and 6 (53.7–55.6%), whereas the FX 3864 clone exhibited the lowest values during the first and second years on farms 11 and 12, respectively ($\leq 40\%$) (Figure 1a).

Variations in TSC are attributed to differences in tapping frequency and ethephon use, which prolong latex flow and increase latex volume but gradually reduce solids concentration (Hernández-Arredondo et al., 2023; Putranto et al., 2015). The progressive decrease in solids concentration during latex flow may explain the lower TSC values observed in certain clones and years, as these lower values are associated with the depletion of sucrose reserves (Junaidi et al., 2019).

Significant variations in inorganic phosphorus (Pi) content in response to the farm $\times$ clone $\times$ year interaction demonstrate the sensitivity of phosphate metabolism to genetic and environmental factors. The higher values observed in RRIM 600 on farms 1, 6, 7, and 11 contrast with the lowest values recorded in FX 3864 on farm 5 (Figure 1b). This suggests that RRIM 600 has higher metabolic activity without compromising latex regeneration capacity, as the thiol concentration observed under the study conditions did not pose a significant risk to membrane integrity.

These results are consistent with those reported by Purwaningrum et al. (2019) and Yan et al. (2023), who identified Pi as a key regulator of energy status and biosynthetic activity in the laticifers of *Hevea brasiliensis* and reported a positive correlation between Pi and sucrose assimilation and latex flow dynamics.

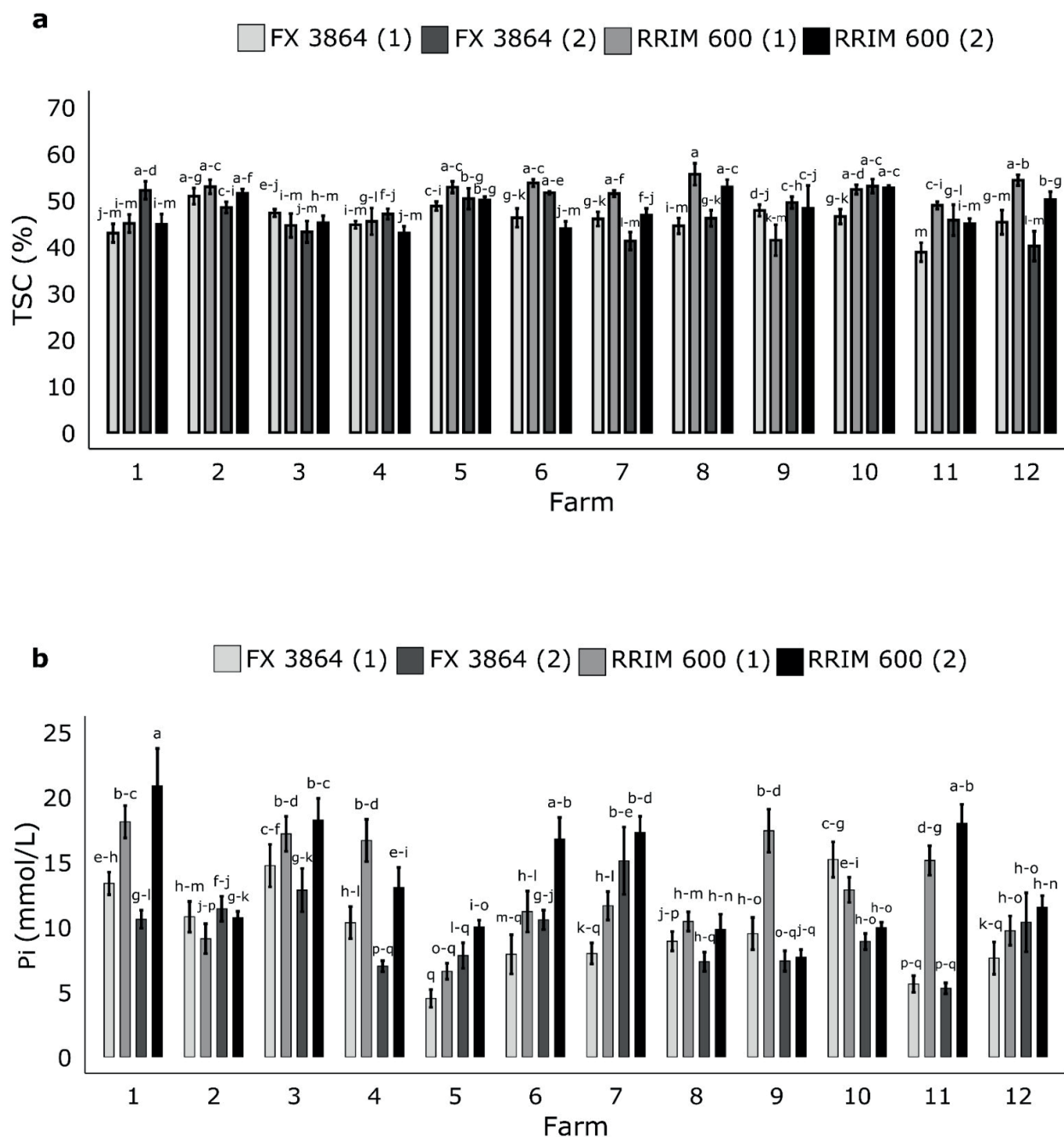
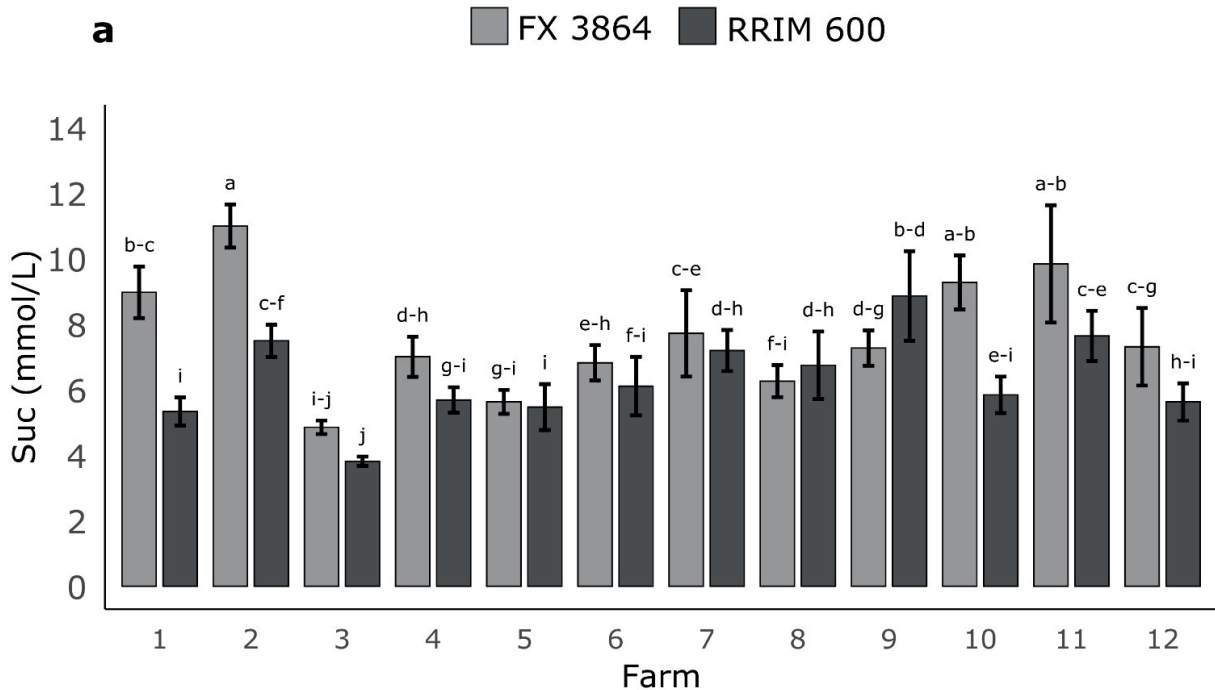


Figure 1. Farm $\times$ clone $\times$ year interaction for variables related to Latex Diagnosis, based on least-squares-adjusted means. (a) TSC = total solids content; (b) Pi = inorganic phosphorus content.

Source: Authors.

The significant farm $\times$ clone interaction showed that clone performance varied with local management and environmental conditions. FX 3864 exhibited higher and more stable Suc concentrations, particularly on farms 2, 10, and 11 (Figure 2a), accompanied by stable thiol levels (Figure 2b) and a more tightly regulated inorganic phosphorus (Pi) balance, indicating sustained metabolic activity and efficient latex regeneration (Jacob et al., 1989).

In contrast, RRIM 600 displayed greater variability in sucrose concentrations, with peaks on farms 9, 11, and 2, along with lower thiol content and higher, more variable Pi levels. These patterns are consistent with the management effects reported by Chantuma et al. (2006) and Sainoi et al. (2017), who found that increased tapping or stimulation reduced sucrose and thiol levels while increasing Pi, suggesting a combined influence of clone and local management on latex metabolism.



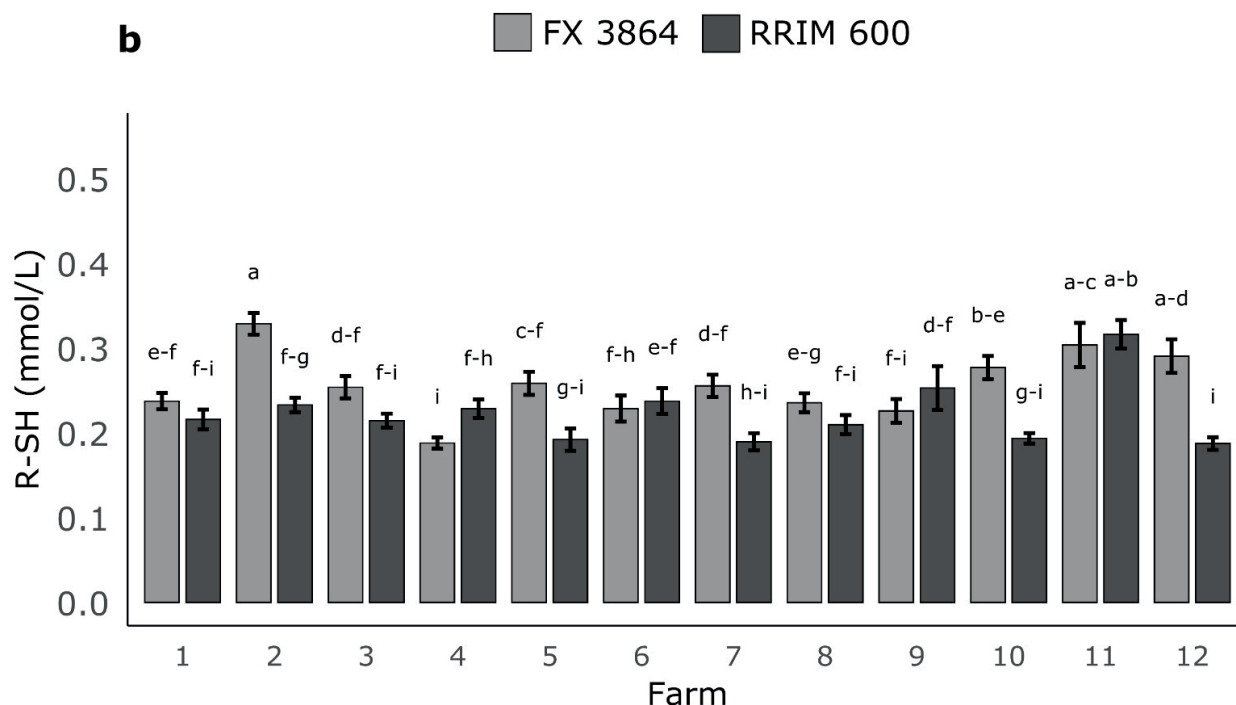


Figure 2. Farm $\times$ clone interaction for variables related to Latex Diagnosis, based on least-squares-adjusted means. (a) Suc = sucrose content; (b) R-SH = thiol content.

Source: Authors.

The analysis of the Latex Diagnosis parameters revealed significant differences between the RRIM 600 and FX 3864 clones. FX 3864 showed higher levels of sucrose and thiols, indicating greater energy reserves and protection against tapping panel dryness (TPD), and was classified as a clone with medium-to-low metabolic activity and high sugar availability (class C4), according to Gohet et al. (2005). In contrast, RRIM 600 had higher concentrations of inorganic phosphorus and total solids, which are associated with more active metabolism (Pethin et al., 2015).

The farm $\times$ clone interactions confirmed that physiological responses are strongly influenced by local management conditions,

such as tapping frequency and tapping duration (Gohet et al., 2013; Lacote et al., 2004). FX 3864 maintained higher sucrose levels on most farms, reaching up to 10.85 mmol/L, a value comparable to that reported in Brazil (Moraes et al., 2011), while RRIM 600 showed levels similar to those observed in Guatemala (Sosof et al., 2011).

Both clones exhibited values above the threshold associated with overexploitation (< 5 mmol/L), suggesting that the stimulation practices were appropriate for the site conditions (d'Auzac & Jacob, 1989). However, applying the same stimulation program to both clones could limit the productive potential of FX 3864, which might benefit from management better tailored to its physiological

profile. Nevertheless, environmental factors such as water stress during the dry season

can restrict its productivity (Gohet et al., 2015; Krishan, 2015; Yang et al., 2024).

4 CONCLUSIONS

Overall, the results have significant practical implications for rubber production systems in the Orinoquia region. The Latex Diagnosis parameters revealed significant differences between the clones, indicating that each requires a stimulation intensity suited to its metabolism. FX 3864, with its greater stability in sucrose and thiol concentrations, can therefore sustain more intensive management regimes with a lower risk of tapping panel dryness and greater resilience to environmental stress.

In contrast, RRIM 600, with its more active metabolism and higher levels of inorganic phosphorus and total solids, could achieve high yields under less frequent and more strictly controlled management. Tailoring tapping and stimulation practices to the physiological profile of each clone would optimize production efficiency, extend the productive life of the trees, and strengthen the economic sustainability of rubber plantations in the Orinoquia region.

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USE OF ARTIFICIAL INTELLIGENCE

Not applicable.

CONFLICT OF INTEREST

All authors made substantial contributions to this study and approved the

manuscript for publication. They declare that they have no conflicts of interest.

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Conflicto de intereses

Los autores declaran no tener ningún conflicto de intereses.



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