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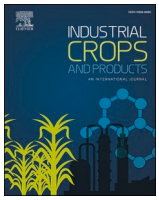

Reduction of tapping frequency associated with stimulation of two *Hevea brasiliensis* clones in Thai non-traditional rubber area.1. Effect on production, biochemical composition and particle size of latex

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A R T I C L E I N F O

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A B S T R A C T

This study (Part 1) is the first of a two-part series investigating how reduced tapping frequency, combined with hormonal stimulation, impacts latex yield, as well as biochemical composition and structure (particle size) of latex in two *Hevea brasiliensis* clones (RRIM600 and RRIT251) over 4 years of tapping. The trial was set in Udon Thani, Thailand, a non-traditional area for rubber cultivation. Results show that the S/2 d3 ET 2.5 % Pa 0.7 tapping system significantly increased latex yield per tapping without compromising cumulative production per tree compared to the conventional S/2 d2 system. As compared to RRIM600, clone RRIT251 exhibited superior yield potential, supported by higher biochemical indicators such as sucrose, inorganic phosphorus, and reduced thiols, reflecting robust metabolic activity and antioxidant capacity in latex cells. Biochemical characterization revealed clonal differences in latex composition: RRIM600 displayed larger rubber particles and higher neutral lipid content, while RRIT251 showed higher levels of phospholipids and proteins. Despite these differences, the tapping system had minimal impact on latex biochemical properties, highlighting the role of hormonal stimulation in maintaining rubber biosynthesis. These findings emphasize the importance of clone-specific strategies in optimizing yield and resource efficiency. Furthermore, this study provides a basis for the subsequent analysis of how tapping systems and clonal variations influence the properties of latex-derived materials, including latex films and technically specified rubber samples (TSR5 and TSR10), as detailed in Part 2 of this study (Liengprayoon *et al.*). This research contributes to developing sustainable latex tapping practices tailored to specific clones and tapping systems.

Abbreviations: CI, Confidence Interval; DRC, Dry Rubber Content; EMM, Estimated Marginal Mean; KDa, kilo Dalton; LD, Latex Diagnosis; LRP, Large Rubber Particles; MW, Molecular Weight; NR, Natural Rubber; SDS-PAGE, Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis; SRP, Small Rubber Particles; TLC, Thin Layer Chromatography; TSC, Total Solid Content; NL, Neutral lipids; GL, Glycolipids; PL, Phospholipids.

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1. Introduction

Natural rubber (NR), a biopolymer extracted from the latex of *Hevea brasiliensis*, is a critical raw material widely used in sectors ranging from automotive to healthcare. Latex, the cytoplasm of laticiferous cells, typically consists (% w/w fresh latex) of approximately 60 % water, 35 % cis-1,4-polyisoprene and 5 % non-isoprene components, including lipids, proteins, carbohydrates and minerals (Bottier, 2020). Latex is harvested by tapping, a controlled wounding technique that severs laticiferous vessels. This process is repeated over multi-year cycles that can last 15–30 years (Paardekooper, 1989).

Global NR production reached 14.5 million tons in 2023, with Thailand contributing 37 % of this total (IRSG, 2023). The crop occupies 14.6 million hectares worldwide, with average yields of 1 ton of dry NR per hectare annually, rising to 1.34 tons per hectare in Thailand. However, NR production systems are facing structural changes. Expansion into climatically marginal zone, driven by land and labor constraints in traditional areas, raises new challenges regarding environmental suitability and productivity. Simultaneously, labor shortages and persistently low NR prices have spurred interest in less labor-intensive tapping systems.

Reducing tapping frequency, coupled with hormonal stimulation (*e.g.* ethephon), is a promising solution to enhance latex yield per tapping and overall labor productivity (Eschbach and Banchi, 1985; Sivakumaran and Chong, 1994; Lukman, 1995; Thanh et al., 1996; Vijayakumar et al., 2000, 2001). Ethylene-based stimulation enhances latex yield per tapping, primarily by increasing the duration of latex flow and activating laticifer metabolism (Lustinec and Resing, 1965; Jacob et al., 1989; D'Auzac et al., 1997; Lacote et al., 2010). However, the response to both reduced tapping frequency and stimulation varies among genotypes and may be affected by environmental conditions (Gohet et al., 1995, 2003). In Thailand, common tapping systems include S/3 d1, S/3 d1 2d/3, and S/2 d2 (Vijayakumar et al., 2009; Chambon et al., 2014). Systems with lower frequency (*e.g.* S/2 d3) remain uncommon (Chambon et al., 2014), and their impact on both yield and latex quality in marginal climates is insufficiently studied. Recent work in China demonstrated that extending the tapping interval from 4 to 6 days, with appropriate stimulation, led to higher labor productivity and improved NR quality (Zhao et al., 2025). While promising, such findings may not be directly applicable to other environmental or production contexts, especially in marginal climates.

Despite growing interest in labor-saving tapping systems, relatively few studies have investigated how tapping frequency and ethylene stimulation might affect the biochemical composition and physical properties of fresh latex, both of which are critical determinants of NR quality. Indicators such as fresh latex pH, sugar content, protein and lipid composition, as well as rubber particle size play central roles in latex coagulation and consequently on the mechanical and processing performance of dry rubber (Wititsuwannakul et al., 2008; Ng et al., 2022; Noinart et al., 2022b; Baudoin et al., 2025b). While several studies have documented genotypic differences in these traits (Gohet et al., 1996, 2001, 2003; Xin et al., 2021; Baudoin et al., 2025a), the influence of tapping systems and marginal climatic conditions on latex composition and structure remains largely underexplored.

This study aims to address these gaps by evaluating the combined effects of climatic marginality and reduced tapping frequency on latex productivity and quality. The trial was conducted in Udon Thani province, northeastern Thailand, a non-traditional rubber-growing zone with low, irregular rainfall and strong temperature variation (Gohet et al., 2015), using two contrasting *Hevea brasiliensis* clones (RRIM600 and RRIT251). Over this 4-year period, we compared two tapping systems, T1: S/2 d2 (tapping every other day, no stimulation) and T2: S/2 d3 (tapping every three days, with ethephon stimulation), by assessing:

- Fresh latex: production, biochemical composition (sucrose, inorganic phosphorus, thiol contents; lipid and protein contents and profiles; total solid and dry rubber contents), pH and particle size.
- Dry NR samples (latex films and Technically Specified Rubber): biochemical composition (lipids, proteins) and properties (plasticity, storage hardening, mesostructure, gel content, viscosity).

Given the scope of the data set, results are presented in two parts. Part 1 (this article) focuses on fresh latex composition and structure. Part 2 will address dry NR characteristics (Liengprayoon *et al.*). The study is guided by the following hypotheses:

- Productivity is constrained by marginal climatic conditions, but reducing tapping frequency, if combined with well-managed stimulation, does not significantly reduce cumulative yield;
- Ethylene stimulation activates laticifer metabolism, potentially altering the biochemical composition and particle size of latex;
- Clone-specific responses are expected in both productivity and latex quality traits.

2. Material and methods

2.1. Geographical location and meteorological conditions during the study

The trial was conducted for 4 years, from June 2020 to October 2023, at the STEP (Sumirubber Thai Eastern Plantation) plantation, Udon Thani province, Thailand (17.40713° North, 102.619° East West, altitude 200 m above sea level). During this 4-year period, the climate was characterized by an average temperature of 26.9°C with thermal amplitude of 20.8–34.0°C, an average humidity amplitude of 53–83 % and an average annual rainfall of 1025 mm. The rainfalls were not homogeneously distributed along the year with long dry periods, and according to Gohet *et al.*, an average annual rainfall lower than 1500 mm is considered as a marginal dry climate for rubber cultivation (Gohet et al., 2015). Low rainfall and large average temperature fluctuations classify the Udon Thani region as non-traditional for rubber cultivation.

2.2. Agronomical field trial

Trees with a homogenous girth were selected for the trial. Tapping began in 2018 at 1.40 m from the ground on half a spiral downward (S/2), on the first tapping panel (BO-1). The experiment was set up in May 2020 with a randomized complete block design, with two tapping systems and two clones RRIM600 and RRIT251 whose clonal conformity was checked using polymorphic microsatellite markers (Le Guen et al., 2011). Each treatment comprises 3 and 2 replications for RRIT251 and RRIM600, respectively, and each replicate comprises 25 homogeneous trees. The project started with 3 replications for RRIM600 but for one of them the genotype was later shown to be non-conform. Consequently, this replication was removed from the trial.

The 2 tapping systems applied on the two clones were:

- T1: S/2 d2 7d/7 9 m/12 no hormonal stimulation (control), *i.e.* trees tapped on half a spiral downward, tapping at alternate daily tapping (once in two days), 6 days on 7, 9 months on 12, without stimulation. From then on, this treatment will be named T1 S/2 d2.
- T2: S/2 d3 7d/7 9 m/12 with hormonal stimulation, *i.e.* trees tapped on half a spiral downward, tapping at third daily tapping (once in three days), 7 days on 7, 9 months on 12, with application of stimulation. From then on, this treatment will be named T2 S/2 d3 Stim.

According to Vijayakumar *et al.* the tapping intensity of T1 S/2 d2 is 100 % and the one of T2 S/2 d3 Stim is 67 % (Vijayakumar et al., 2003). The S/2 d2 latex harvesting system was selected as the reference, since it serves as the standard for calculating tapping intensity (100 %) and is widely adopted by smallholders in Thailand. This system is never combined with hormonal stimulation, which may induce adverse physiological effects (Jacob et al., 1989). In our study, we did not aim to

evaluate the effects of hormonal stimulation independently at each tapping frequency. Instead, we compared two tapping systems to explore the potential for reducing tapping frequency and, consequently, saving labor in rubber plantations in Southeast Asia. The S/2 d3 system, representing a lower tapping intensity (67 % of S/2 d2), was introduced as a preliminary step toward developing labor-saving latex harvesting practices well-suited to our trial objectives.

Stimulation was applied on panel above the tapping cut on 1 cm large with a brush and using 0.7 g of a ready to use Ethephon at 2.5 % concentration. Stimulation was made 24 h before the first next tapping. Stimulant was applied at an annual frequency depending on clone characteristics (Gohet et al., 2003). Both studied clones (RRIM600 and RRIT251) received the same treatment, *i.e.* 5 stimulations per year at 2.5 % Ethephon, with 0.7 g of stimulant applied on tapping panel on 1 cm band: ET 2.5 % Pa 0.7(1) 5/y (June, July, August, September, October).

2.3. Latex collection and sampling periods

Trees were tapped around 4 AM by one single tapper.

For determination of sucrose, thiol and inorganic phosphorus contents (see paragraph 2.7) measurements were made from a pooled latex sample of 10 trees belonging to the 25 trees in each replicate. Four samplings were performed every year in June, August, September and October leading to a total of 40 samples over the 4-year tapping period. Just after tapping, an approximate volume of 6 mL of latex was collected (20 drops/tree, 10 trees/treatment/replication) by discarding the first 2 drops of latex. Lattices were kept on ice until further use.

For measurement of other indicators (pH, total solid and dry rubber contents, lipid and protein contents, particle size) and preparation of dry rubber sample (Part 2 of this study, (Liengprayoon *et al.*)), lattices were let to flow for about 4 h in clean cups. Lattices from the 25 trees of each replicate were then collected, polled together and filtered through a stainless-steel sieve of 2 mm mesh size. Lattices were kept in glass bottles on ice until further use. Three samplings were performed every year in June, August and October leading to a total of 12 sampling campaigns.

2.4. Latex yield

The latex yield of tapped trees has been pooled and recorded from each elementary plot by weighing the cumulative coagulated rubber every 10 days in a month over the 4-year tapping period. For each period, the whole production was creped and dried to get the yield in dry rubber per elementary plot. Latex yield was expressed in grams per tree (g tree^{-1}) and in grams per tree per tapping (g tree^{-1} tapping^{-1}) averaged over the 4-year tapping period.

2.5. Total solid content (TSC) and dry rubber content (DRC) of latex

Empty aluminum cup was weighed (W_0, precision 0.1 mg) and 2 mL of fresh latex were poured into the aluminum cup, then the weight (W_1) of aluminum cup containing latex was recorded. Cup containing latex was put in a hot air ventilated oven at 105 ± 5°C for 2 h. The aluminum cups containing dry latex were cooled down in desiccator, weighed (W_2) and put back for 15 min at 105 ± 5°C. The last two steps are repeated until constant weight (less than 0.5 mg difference between two consecutive weighings). The total solid content (TSC) is defined as: TSC (%) = $(W_2\text{-}W_0)/(W_1\text{-}W_0) \times 100$. For each sample, three replications were done. The result of two replicate determinations shall not differ by more than 0.2 % (w/w).

Standard ISO126:2005 was followed for DRC measurement with the following adaptations: the sample is made of 2 g + /- 0.2 g (W_0, precision 0.1 mg) of fresh latex instead of 10 mL of concentrated latex. The volume and concentration of added acid are 15 mL of 2 % acetic acid instead of 25–35 mL of 5 % acetic acid. The acidified rolled rubber is dried at 70°C until disappearance of white spot and constant weight (W_1). The DRC is given by the following formula: DRC (%) = $W_1/W_0 \times 100$ (W_0 weight in g of initial latex sample, W_1 weight in g of dry sheet). Three repetitions were done for one sample. Each of them must not be more different than 0.1 % from average.

2.6. Particle size distribution

The particle size distribution of latex was analyzed using a Malvern particle size analyzer (Mastersizer-3000) based on laser light scattering. 100 µL of fresh latex were dispersed in 450 mL distilled water, with continuous stirring, until the signal intensity reached 15–20 % before being subjected to measurement of the volume-weighted mean diameter D[4,3]. The ratio of large rubber particles (LRP) and small rubber particles (SRP) was determined in % volume density.

2.7. Biochemical parameters of the latex cells (latex diagnosis)

The latex biochemical parameters, sucrose content (Suc), inorganic phosphorus content (Pi) and reduced thiols content (RSH) were evaluated according to the method developed by CIRAD and adopted in 1995 by IRRDB (Jacob et al., 1988, IRRDB, 1995). Sucrose, inorganic phosphorus and thiols contents were measured using the methods of Ashwell (1957), Taussky and Shorr (1953) and Boyne and Ellman (1972), respectively. These biochemical indicators were expressed in millimoles per liter of latex (mmol l^{-1}) averaged over the 4-year tapping period.

2.8. Lipids

2.8.1. Extraction of lipids from fresh latex

Collected fresh latex was extracted after filtration using the method previously developed (Liengprayoon et al., 2008). In brief, 10 mL of latex was diluted at 1:1 (v/v) with distilled water and added dropwise for 4 min into stirred extraction solvent (chloroform: methanol; 2:1, v/v). Coagulum was removed by filtration and the extract was transferred to separating funnel. Water soluble content was removed using 1/5 vol of 0.9 % NaCl as described by Folch et al. (1957). After decantation, lipid-containing bottom phase was collected and evaporated. The obtained dry lipid extract was weighed. The extraction yield was calculated versus the dry rubber weight.

2.8.2. Lipid profiles by thin layer chromatography (TLC)

The lipid extracts were diluted in chloroform proportionally to the lipid content in rubber in order to visually illustrate on the TLC the individual lipid content in rubber (concentration range 5–10 mg/mL) A volume of 8 µL of each solution was deposited on silica gel 60 G TLC plates (10 × 20 cm silica gel 60 coated plate; 0.25 mm thick, Merck, Darmstadt, Germany). The migration of lipids was performed in different mobile phases suitable for NL, GL and PL before detecting with specific reagents and/or heating conditions as previously described (Liengprayoon et al., 2008).

2.8.3. Lipid class separation by solid-phase extraction

Total lipid extract was separated into 3 lipid classes *i.e.* NL, GL and PL by solid-phase extraction (SPE) as previously described (Wadeesirisak et al., 2017). An SPE cartridge was activated before use by rinsing successively with 2.7 mL of methanol and 2.7 mL of chloroform. Thirty milligrams of lipids dissolved in 0.5 mL of chloroform was loaded into the activated SPE cartridge. NL, GL, and PL were eluted successively with 8.1 mL of chloroform, 8.1 mL of a mixture of acetone/methanol (9:1; v/v) and 8.1 mL of methanol, respectively. The flow rate was controlled approximately at 3 mL/min using a vacuum pump. Each eluted lipid class was collected, evaporated and weighed. Purity of lipid classes were verified by TLC as described previously.

2.8.4. Quantification of free fatty acids

Free fatty acids (FFAs) in the lipid samples were quantified using the

method described by Van Autryve et al. (1991). The principle of this method is based on the specific complexation of FFAs with rhodamine 6 G. The absorbance of the complex was measured at a wavelength of 513 nm.

2.9. Proteins

2.9.1. Total nitrogen content

The nitrogen content of latex was measured on dry latex films. Films were prepared from a volume of 250 mL of latex that was poured on a $25 \times 33.5 \times 2\ cm^3$ tray. The tray was placed in a ventilated oven at 70°C for approximately 18–20 h. Regular visual verifications were made every 5 h and the film considered dry by the disappearance of white spot.

The nitrogen content of latex films was determined with a CHN Determinator (LECO CHN628) on a 100 mg sub-sample. The protein content is given by multiplying the nitrogen content by a factor of 6.25.

2.9.2. Extraction of proteins from fresh latex

Fresh latex was mixed with extraction buffer (100 mM Tris, 100 mM EDTA, 10 % glycerol, 2 % Triton X-100, 20 mM DTT and 2 mM PMSF, pH 8.0) at a ratio of 1:2 w/w latex/buffer. This mix was agitated on a rotating machine at 4°C for 40 min. The solution was then centrifuged at 20,000 g for 35 min at 4°C. The rubber phase was moved aside with a spatula to easily collect an intermediate phase containing proteins. The solution was centrifuged twice at 24,320 g for 35 min at 4°C to remove remaining rubber particles. Such obtained protein extract was mixed with Laemmli 2X buffer (125 mM Tris, 20 % w/v glycerol, 4 % w/w SDS, 0.01 % bromophenol blue, pH 6.8) at a ratio of 1:1 v/v protein extract/buffer.

2.9.3. SDS-PAGE electrophoresis of protein extracts

Sodium dodecyl sulfate–polyacrylamide gel (SDS-PAGE) electrophoresis was run in a Bio-Rad apparatus (Mini-Protean Tetra Cell). 14 % acrylamide/bis-acrylamide gels were prepared with a comb of 10 wells per gel (volume capacity = 44 mL/well). Before loading the protein extracts in the SDS-PAGE gel, 2-beta-mercaptoethanol was added to the protein extracts at a final concentration of 4 % v/v, and extracts were then heated at 95°C for 10 min. The protein extracts were finally added with glycerol at a final concentration of 10 % v/v. Each well was loaded with 22.9 is µL protein extract and gels were run under a constant voltage of 200 V. The staining of the gels was performed for 2 h in staining solution (40 % ethanol, 10 % acetic acid, 0.1 % Coomassie Blue R-250) and complete destaining was achieved within 2–3 h in 10 % acetic acid solution.

Gels were scanned on a GS-900 calibrated densitometer (Bio-Rad) and analyzed using Phoretix 1D software (version 1.0.628, TotalLab Ltd. Newcastle Upon Tyne, UK) in automatic detection mode. Lanes of similar width were drawn on the gels. The molecular weights of protein bands were assigned after a calibration procedure using a protein standard containing 10 proteins of various molecular weights: 250, 150, 100, 75, 50, 37, 25, 20, 15 and 10 kDa (Precision Plus Protein #1610363, Bio- Rad). The density (% of total lane density) of three area: area of high molecular weights (from 250 to 33 kDa), area of medium molecular weights (from 33 to 16 kDa) and area of low molecular weights (from 16 to 10 kDa). In addition, the density of the two main bands was measured. These 2 bands located at about 21–23 and 13–15 kDa contain the proteins SRPP1 (Small Rubber Particle Protein, theoretical MW: 22.4 kDa) and REF1 (Rubber Elongation Factor, theoretical MW: 14.7 kDa), respectively (Bottier et al., 2019).

2.10. Statistical analysis

All analyses were conducted at a significance level of $\alpha = 0.05$.

2.10.1. Statistical analysis of production and LD data

All agronomic data were carried out in R software (version 4.5.0, 2025). Descriptive statistics analysis was first computed to summarize each agronomic response variable (Y). Model assumptions were checked graphically with QQ-plots (normality) and residual-versus-fitted plots (homoscedasticity). Variables that clearly violated either assumption were log-transformed; estimates and 95 % confidence intervals (CI) were subsequently back-transformed to the original scale.

Because yearly measurements were repeated on the same experimental units (clone × treatment × replicate: $n = 16$ for RRIM600; $n = 24$ for RRIT251), we fitted a linear mixed-effects model:

$$g(Y_{ijklm}) = \mu + (clone_i \times tapping_j \times year_k) + (1|pe_l) + (1|rep:clone_m) + \varepsilon_{ijklm}$$

Where g(.) is either the identity link for untransformed variables or the natural logarithm for transformed variables. µ is the overall intercept; the term "$clone_i \times tapping_j \times year_k$" represents all fixed effects and their interactions; year was treated as a categorical factor. Random intercepts were included for each experimental unit "pe" and for replication blocks nested within clone "rep:clone". Residual error ε_{ijklm} were assumed $N(0, \sigma^2)$. Models were fitted with lmer function in the lmer4 package (Bates et al. 2015) using Restricted Maximum Likelihood (REML). Denominator degrees of freedom were approximated by Satterthwaite's method (package lmerTest (Kuznetsova et al. 2017)) to control Type-I error in small, unbalanced samples.

Estimated marginal means (EMMs) and their 95 % CIs were obtained with "emmeans" (Lenth, 2025). For log-transformed outcomes, EMMs are presented as geometric means. Pairwise contrasts among EMMs were adjusted with the Šidák correction for multiple testing, and the results are displayed with compact-letter displays generated by cld function in multcomp R package.

2.10.2. Statistical analysis of biochemical, pH and particle size data

Data of latex biochemical composition (lipids, protein, TSC, DRC), pH and particle size were analyzed using JMP® 12.2. A mean comparison was carried out between treatments (RRIT251/T1 S/2 d2, RRIT251/T2 S/2 d3 Stim, RRIM600/T1 S/2 d2, RRIM600/T2 S/2 d3 Stim) using the Tukey-Kramer HSD (honestly significant difference) test.

2.10.3. Statistical effects of clone, tapping system and their interaction on measured indicators

A multiple linear model was applied using JMP® 12.2 to evaluate the effects of the two studied parameters (*i.e.* clone and tapping system) and their interaction on measured indicators. The effects were considered to be very significant when $P < 0.0001$, significant when $P < 0.05$ or not significant when $P > 0.05$. To ensure temporal consistency in this analysis, LD data were from LD measurement made on the day of latex sampling while the latex production data was the cumulated yield of the month of the latex sampling.

3. Results and discussion

3.1. Reducing tapping frequency combined with stimulation sustains the yield over 4 years

Reduction of the tapping frequency in d3 combined to stimulation has a significant positive effect on the yield per tree and per tapping for the two clones over the 4-year period of tapping (Fig. 1). This effect has more than compensated for the production per tree (kg $tree^{-1}$) which became higher (not significantly) for the d3 tapping frequency and for both clones. Clone RRIM600 showed the highest response to hormonal stimulation in gram per tree (g $tree^{-1}$) and gram per tree per tapping (g $tree^{-1}$ $tapping^{-1}$) to compensate well for the cumulated yield (kg $tree^{-1}$). RRIT251 showed the lowest at it was the highest producing clone without hormonal stimulation. However, RRIT251 showed a higher capacity than RRIM600 to produce for both tapping systems.

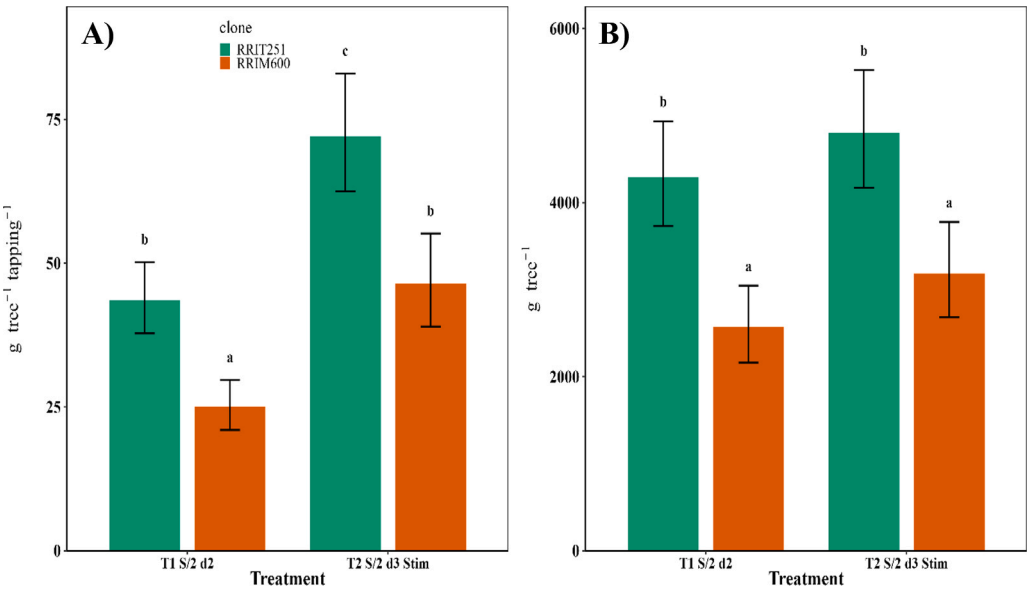

Fig. 1. Estimated marginal means (EMMs) of latex yield, expressed in A) grams per tree per tapping (g tree^{-1} tapping^{-1}) and in B) grams per tree per year (g tree^{-1}), over 4-year period according to tapping system and clone. EMMs are presented on the response scale with 95 % confidence intervals. Different letters indicate statistically significant differences between tapping systems within each clone ($\alpha = 0.05$).

Obviously, the production per tree of the two clones is much lower than that usually measured in traditional climatic zones in Thailand (Sainoi et al., 2017a, 2017b; Chotiphan et al., 2019; Chotiphan et al., 2023). Both the annual number of tappings and the gram per tree and per tapping are higher in traditional area. It is remarkable to be able to compensate the reduction of the number tapping days without a loss of yield when using a tailored hormonal stimulation to clones (Gohet et al., 1991, 2003) in such marginal area.

While ethephon stimulation is known to enhance latex yield by promoting ethylene-induced latex flow, it has also been associated with an increased risk of tapping panel dryness (TPD), particularly under conditions of high tapping intensity, excessive stimulation frequency, or in genetically sensitive clones (Herlinawati et al., 2022; Aji et al., 2025). In the present study, ethephon was applied at moderate frequency and dosage, and no visible signs of TPD were observed throughout the 4-year trial. This outcome supports previous findings indicating that when stimulation is applied under controlled conditions – especially in combination with reduced tapping frequency – the risk of TPD can be minimized (Jacob et al., 1989; Lacote et al., 2010).

3.2. *Reducing tapping frequency combined with stimulation does not significantly affect the metabolic activity*

Latex diagnosis (LD) is widely used to optimize rubber production through a management system based on the physiological aspects of latex tapping (Eschbach and Banchi, 1985; Jacob et al., 1989; Junaidi et al., 2022). Sucrose (Suc), inorganic phosphorus (Pi) and reduced thiols (RSH) contents allow the monitoring of the carbohydrate availability, metabolic activity, and antioxidant status, respectively (Eschbach and Banchi, 1985; D'Auzac et al., 1997). Over the 4-year

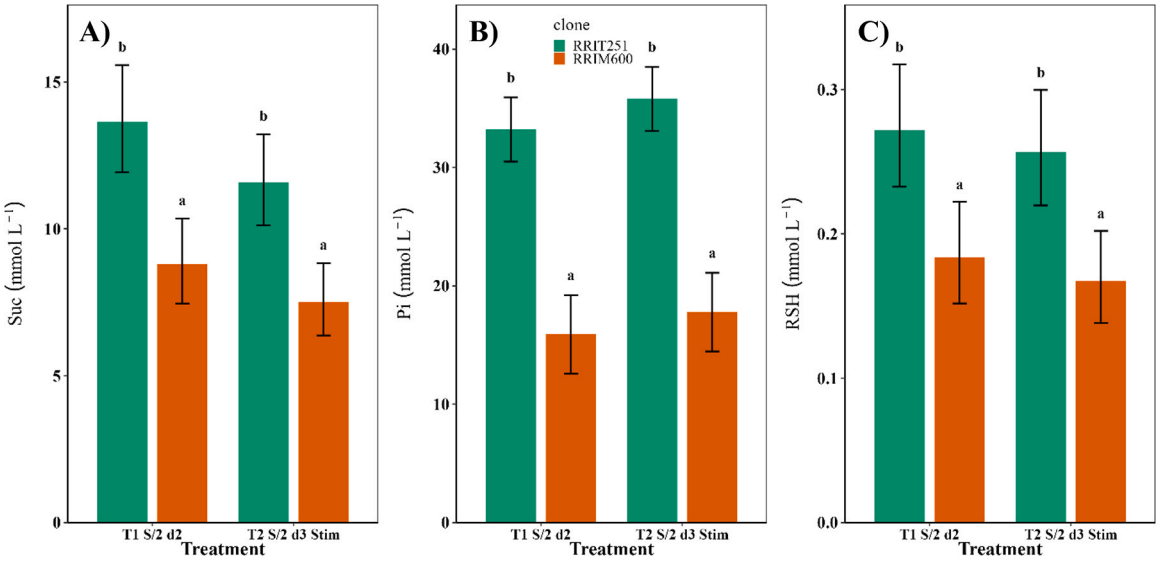

Fig. 2. Estimated marginal means of A) sucrose (Suc), B) inorganic phosphorus (Pi), and C) reduced thiol (RSH) contents (mmol L^{-1}) over a four-year period by tapping system and clone (response scale, 95 % CI); different letters indicate statistically significant differences between tapping systems within each clone ($\alpha = 0.05$).

tapping period, no significant difference has been observed in Suc, Pi and RSH contents between the 2 tapping systems T1 S/2 d2 and T2 S/2 d3 Stim (Fig. 2). Treatments with higher yield (g tree^{-1} tapping^{-1}), as it was in d3 tapping frequency, showed slightly lower Suc and RSH contents, but not significantly. Considering the reduction of the tapping frequency in d3, hormonal stimulation sustained the metabolic pathway (Pi content) to rubber biosynthesis to make it possible to produce more rubber at each tapping. So that, Suc is used to sustain such synthesis (Lacote et al., 2010), and scavengers (RSH) are also used more to control the reactive oxygen species to prevent an early coagulation (Chrestin et al., 1984). LD parameters were therefore explaining the difference in yield (g tree^{-1} tapping^{-1}), leading to even a better cumulated yield (kg tree^{-1}) when reducing the tapping frequency and using stimulation (Lacote et al., 2010). The Pi content was the indicator of an activated metabolism of the latex cells in S/2 d3 tapping frequency under hormonal stimulation. Hormonal stimulation made it possible to sustain the latex cell metabolism, sugar allocation, metabolic activity (Pi) without a detrimental effect on scavengers when reducing the tapping frequency. This is observed for the two clones. Clone RRIT251 showed, for each tapping system, the highest Suc, Pi and RSH contents. This can be related to the highest yield in g tree^{-1} tapping^{-1} and to cumulated yield in kg tree^{-1}. Clone RRIT251 showed in our trial conditions a higher yield potential than clone RRIM600. Over the medium-term duration of the trial (4 years) conducted in a marginal area, no limitations were observed regarding the use of hormonal stimulation on either yield potential or biochemical indicators of latex cell functioning, consistent with findings from traditional rubber-growing regions (Lacote et al., 2010). However, longer-term studies should be considered to account for the potential impacts of climate change in this region.

3.3. *Reducing tapping frequency combined with stimulation does not affect the size distribution of fresh latex nor the volume density of small and large rubber particle fractions*

Some representative particle size distributions of fresh latex dispersions from both clones and both tapping systems are shown in Fig. 3. In agreement with literature (Wood and Cornish, 2000; Singh et al., 2003; Wisunthorn et al., 2008), all distributions are bimodal highlighting the presence of two populations: small rubber particles (SRP) and large rubber particles (LRP) (Payungwong et al., 2024). For both tapping systems, the bimodality is more marked for RRIM600 than for RRIT251, as observed by other authors (Lehman et al., 2024). The d3 tapping system seems to slightly expand this characteristic.

The size and % volume density of SRP and LRP are reported in Table 1. For both clones, no significant difference in rubber particle size and % of LRP and SRP in fresh latex dispersion was observed between T1 S/2 d2 and T2 S/2 d3 Stim. Although not significantly different, the SRP fraction is reduced under T2 S/2 d3 Stim. This observation is inconsistent with the study of Wang *et al.* who observed an accumulation of SRP under ethylene stimulation (Wang et al., 2015).

Table 1
Size of fresh latex dispersions and % volume density of LRP and SRP fractions (± standard error).

Clone	RRIM600		RRIT251	
Tapping system	**T1 S/2 d2**	**T2 S/2 d3 Stim**	**T1 S/2 d2**	**T2 S/2 d3 Stim**
Particle size D[4,3] (µm)	0.527 ± 0.010^{a}	0.535 ± 0.013^{a}	0.474 ± 0.006^{b}	0.480 ± 0.005^{b}
LRP fraction (% volume density)	0.983 ± 0.002^{a}	0.986 ± 0.002^{a}	0.977 ± 0.001^{b}	0.981 ± 0.001^{a,b}
SRP fraction (% volume density)	0.017 ± 0.002^{b}	0.014 ± 0.002^{b}	0.023 ± 0.001^{a}	0.019 ± 0.001^{a,b}

Note: Values with different letters in the same line indicate significant difference at $P \leq 0.05$.

In contrast to tapping systems, significant differences were found between clones. The rubber particle sizes of clone RRIM600, ranging from 0.527 µm to 0.535 µm, are significantly larger than the ones of clone RRIT251, ranging from 0.474 µm to 0.480 µm. In addition, for T1 S/2 d2, the proportion of LRP/SRP is significantly higher/lower in clone RRIM600 compared to clone RRIT251. This observation is valid for T2 S/2 d3 Stim although values are not significantly different between clones.

3.4. *Reducing tapping frequency combined with stimulation does not affect the biochemical composition of fresh latex*

Table 2 gathers, for both clones and both tapping systems, several indicators of fresh latex including pH as well as dry rubber (DRC), total solid (TSC), total lipid, phospholipid (PL), glycolipid (GL), neutral lipid (NL) and protein contents. Lipids and proteins were extracted from fresh latex and their thin layer chromatography (TLC) and SDS-PAGE electrophoresis profiles are shown in Figs. S1 and S2, respectively (Supplementary material). SDS-PAGE of protein extracts from fresh latex were analyzed and the density of area 250–33 kDa, 33–16 kDa and 16–10 kDa, as well as the density of both bands containing SRPP1 and REF1 proteins, are listed in Table 2.

For both clones, reducing the tapping frequency from d2 (T1 S/2 d2) to d3 (T2 S/2 d3 Stim) has no significant impact on all measured indicators. The pH ranges from 6.58 (RRIM600/ T2 S/2 d3 Stim) to 6.68 RRIT251/ T2 S/2 d3 Stim). Tupy reported values ranging from 6.71 to 7.04 for GT1 clone (Tupy, 1988). For RRIT251, although not significantly different, treatment T2 S/2 d3 Stim results in lower DRC than T1 S/2 d2 due to ethylene stimulation which favors water circulation between the laticifers (Tungngoen et al., 2011; An et al., 2015b), resulting in latex dilution (An et al., 2015a) and consequently in lower TSC and DRC. Surprisingly, this tendency is not observed for RRIM600 whose DRC and TSC values are similar for T1 S/2 d2 and T2. Both lipid and protein content are not affected by the tapping system. In agreement with this observation, it was previously reported that ethylene

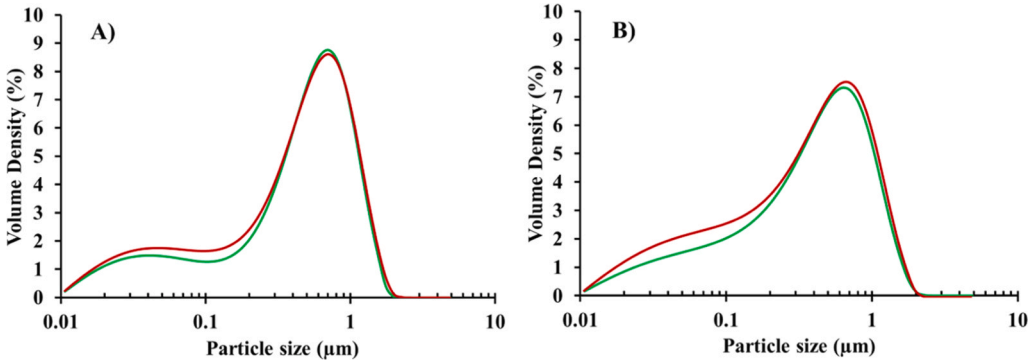

Fig. 3. Particle size distribution of RRIM600 (A) and RRIT251 (B) for both tapping systems T1 S/2 d2 (red) and T2 S/2 d3 Stim (green).

Table 2
Properties (pH, DRC, TSC) and biochemical composition of fresh latex (total lipids, phospholipids, glycolipids, neutral lipids, free fatty acids, proteins and indicators from SDS-PAGE (area 250–33 kDa, area 33–16 kDa, area 16–10 kDa, band containing SRPP1 and REF1 proteins) (± standard error).

Clone	RRIM600		RRIT251	
Tapping system	**T1 S/2 d2**	**T2 S/2 d3 Stim**	**T1 S/2 d2**	**T2 S/2 d3 Stim**
pH	6.61 ± 0.02^{b}	6.58 ± 0.02^{b}	6.63 ± 0.02^{a,b}	6.68 ± 0.01^{a}
DRC (%)	34.1 ± 0.7^{a}	34.0 ± 0.6^{a}	36.9 ± 0.8^{a}	35.1 ± 0.8^{a}
TSC (%)	37.8 ± 0.7^{a}	37.8 ± 0.6^{a}	40.5 ± 0.7^{a}	38.6 ± 0.7^{a}
Total lipids (% w/w fresh latex)	1.28 ± 0.04^{a}	1.29 ± 0.03^{a}	1.18 ± 0.02^{a}	1.22 ± 0.02^{a}
Phospholipids (% w/w fresh latex)	0.22 ± 0.01^{b}	0.26 ± 0.01^{a,b}	0.28 ± 0.01^{a}	0.27 ± 0.01^{a}
Glycolipids (% w/w fresh latex)	0.29 ± 0.01^{a}	0.27 ± 0.01^{a}	0.30 ± 0.01^{a}	0.30 ± 0.01^{a}
Neutral lipids (% w/w fresh latex)	0.77 ± 0.03^{a}	0.75 ± 0.02^{a}	0.61 ± 0.01^{b}	0.61 ± 0.02^{b}
Free fatty acids (% w/w fresh latex)	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}
Proteins (% w/w fresh latex)	1.50 ± 0.02^{b}	1.56 ± 0.02^{b}	1.75 ± 0.02^{a}	1.80 ± 0.02^{a}
Area 250–33 kDa in SDS-PAGE (% total density)	45.2 ± 0.2^{a}	45.6 ± 0.2^{a}	45.3 ± 0.2^{a}	45.6 ± 0.2^{a}
Area 33–16 kDa in SDS-PAGE (% total density)	32.4 ± 0.4^{a}	32.6 ± 0.4^{a}	32.4 ± 0.3^{a}	32.3 ± 0.3^{a}
Area 16–10 kDa in SDS-PAGE (% total density)	22.4 ± 0.3^{a}	21.8 ± 0.3^{a}	22.3 ± 0.3^{a}	22.1 ± 0.3^{a}
Band containing SRPP1 in SDS-PAGE (% total density)	10.0 ± 0.3^{a}	9.9 ± 0.2^{a}	9.5 ± 0.3^{a}	9.4 ± 0.3^{a}
Band containing REF1 in SDS-PAGE (% total density)	14.7 ± 0.4^{a}	14.4 ± 0.3^{a}	14.3 ± 0.3^{a}	14.1 ± 0.3^{a}

Note: Values with different letters in the same line indicate significant difference at $P \leq 0.05$.

stimulation did not result in an increase of soluble protein level in latex cytosol (Tupy, 1988).

In contrast, the clone has significant effects on several indicators including pH, phospholipid, neutral lipid and protein contents. Although the difference is not significant, RRIT251 has higher DRC and TSC than RRIM600. The clonal effect on DRC and TSC was mentioned by other studies (Le Roux et al., 2000; Rodrigo et al., 2011; Silva et al., 2021; Noinart et al., 2022a; Keereerak et al., 2024). Yip reported DRC values ranging from 25 % to 45 % depending on the clone (Yip, 1990). Among them, RRIM600 appears within class I with DRC of 34–38 %, in agreement with our results.

The total lipid contents of RRIM600 for T1 S/2 d2 and T2 S/2 d3 Stim (1.28 and 1.29 % w/w fresh latex, respectively) were similar to the one found for this clone tapped in S/2 2d3 (Liengprayoon et al., 2013). This indicator is slightly lower for RRIT251, but no significant difference was observed. From the TLC profiles of NL, GL and PL (Fig. S1), the distribution of lipid species within total lipids is not affected by the tapping system neither **(**Fig. S1**)**. For RRIM600, the distribution of PL, GL and NL within total lipids agrees with previous studies (Liengprayoon et al., 2013; Wadeesirisak et al., 2017). Nevertheless, when compared between clones, RRIM600 has significantly higher NL content than RRIT251 (0.75–0.77 % *vs* 0.61 % w/w fresh latex), possibly explained by different number of detected esterified lipid species of RRIM600 compared to RRIT251 (Fig. S1A**)**. For PL content, while no significant difference between clones was observed for T2 S/2 d3 Stim (0.26 *vs* 0.27 % w/w fresh latex), a significant difference between clones appeared for T1 S/2 d2 where PL content in RRIM600 was significantly lower than in RRIT251 (0.22 *vs* 0.28 % w/w fresh latex). Likely, the lower PL content could be due to both major phospholipids PC and PI that are less prominent in RRIM600 compared to RRIT251 **(**Fig. S1C**)**. Though GL was previously studied in 3 different *Hevea* clones (RRIM600, PB235 and BPM24) and also reported to be clonal dependent (Liengprayoon et al., 2011), no significant effect of clone on GL content was observed in this study nor major difference in GL species **(**Fig. S1B**)**.

As mentioned above, the clone impacts the protein content: RRIT251 latex is significantly richer in proteins than RRIM600. This clonal effect on nitrogen content was previously reported by others (Yip, 1990; Moreno et al., 2007; He et al., 2022; Lehman et al., 2024). Representative SDS-PAGE gels of the protein extracts are shown in Fig. S2A and display the similar pattern as previously reported (Liengprayoon et al., 2021). All profiles show numerous protein bands whose molecular weight (MW) varies from 250 to 10 kDa. Some slight differences are observed between treatments, in agreement with Wang *et al.* who reported that some proteins were activated while others were inhibited under ethylene-induced stimulation (Wang et al., 2015). For instance, for RRIT251/T1 S/2 d2, a rather intense protein band located at about 37 kDa disappears for RRIT251/T2 S/2 d3 Stim. For RRIM600/T2 S/2 d3 Stim, a protein band visible at about 35 kDa is not present for RRIM600/T1 S/2 d2. Each SDS-PAGE lane was divided into three zones (250–33 kDa, 33–16 kDa and 16–10 kDa) (Fig. S2B) and two specific bands located at about 23 and 14 kDa known to contain SRPP1 and REF1 proteins, respectively (Fig. S2C). The density of those three zones and two bands are reported in Table 2. Although the stimulation might affect specific bands, it does not significantly impact the density of the three above-defined zones nor the ones of SRPP1 and REF1 bands. A band per band analysis might have revealed significant differences between clones or tapping system but this analysis was not conducted at this stage.

Table 3 summarizes the effects of clone and tapping system, on biochemical, physical, and structural indicators of fresh latex. We also tested the interaction effect of clone × tapping system but as no effect was observed, the effect of interaction is not reported in Table 3. As detailed above, the impact of the clone on these indicators is much more pronounced than the one of tapping system. Clone has a strong influence on most indicators: it has a very significant effect on particle size, contents of inorganic phosphorus, thiols, neutral lipids and proteins and a significant one on pH, DRC, TSC, LRP/SRP fractions as well as contents of sucrose, total lipids, phospholipids and glycolipids. In contrast, tapping system only has a very significant impact on latex production and significant impact on sucrose and protein contents.

4. Conclusion

This study carried out in a non-traditional area for rubber cultivation (Udon Thani, Thailand) demonstrates that reducing the tapping frequency from every two days (d2) to every three days (d3) combined with hormonal stimulation significantly enhanced latex yield per tapping without compromising cumulative production per tree for both RRIM600 and RRIT251 clones. RRIT251 exhibited superior yield potential, with consistently higher latex diagnosis indicators such as sucrose, inorganic phosphorus, and reduced thiols, which supported its robust metabolic activity and antioxidant capacity. The latex particle size distribution confirmed the clonal differences. RRIM600 displays larger rubber particles and higher neutral lipid content, while RRIT251 has higher phospholipid and protein levels.

In contrast to the clone, the biochemical composition of latex remains largely unaffected by the tapping system, indicating that hormonal stimulation, tailored to the two clones, effectively sustains the physiological functions necessary for rubber biosynthesis. These findings underscore the importance of tailored stimulation protocols and clone selection in optimizing latex yield while maintaining resource efficiency.

The characterization of biochemical indicators of fresh latex was required to apprehend the second step of this study where various sample types were manufactured from fresh latex, *i.e.* latex films and

Table 3
Statistical effects (highlighted in red when very significant, yellow when significant or notified by NS for not significant) of clone (RRIT251, RRIM600) and tapping system (T1 S/2 d2, T2 S/2 d3 Stim) on latex production, properties (pH, DRC, TSC), particle size of rubber particles and biochemical indicators of latex.

Indicator	**Mean from all data**	Standard error	Number of observations	**Clone effect**	RRIM600 > RRIT251	RRIT251 > RRIM600	**Tapping system effect**	T1 S/2 d2 > T2 S/2 d3 Stim	T2 S/2 d3 Stim > T1 S/2 d2
Production (g/t/t)	51.0	2.7	120	<0.0001		X	<0.0001		X
pH	6.62	0.01	120	0.0005		X	NS		
DRC (%)	35.2	0.4	120	0.0150		X	NS		
TSC (%)	38.8	0.4	120	0.0245		X	NS		
Particle size D [4,3] (mm)	0.499	0.005	89	<0.0001	X		NS		
LRP fraction (% volume density)	0.982	0.001	90	0.0003	X		NS		
SRP fraction (% volume density)	0.018	0.001	90	0.0003		X	NS		
Sucrose (mM)	11.7	0.5	115	0.0014		X	0.0041	X	
Inorganic phosphorus (mM)	29.2	1.3	116	<0.0001		X	NS		
Thiols (mM)	0.25	0.01	116	<0.0001		X	NS		
Total lipids (% w/w fresh latex)	1.24	0.01	120	0.0048	X		NS		
Phospholipids (% w/w fresh latex)	0.27	0.01	120	0.0048		X	NS		
Glycolipids (% w/w fresh latex)	0.29	0.01	120	0.0482		X	NS		
Neutral lipids (% w/w fresh latex)	0.67	0.01	120	<0.0001	X		NS		
Free fatty acids (% w/w fresh latex)	0.040	0.001	120	NS			NS		
Proteins (% w/w fresh latex)	1.68	0.01	111	<0.0001		X	0.0024		X
Area 250-33 kDa on SDS-PAGE gel (% total density)	45.4	0.1	120	NS			NS		
Area 33-16 kDa on SDS-PAGE gel (% total density)	32.4	0.2	120	NS			NS		
Area 16-10 kDa on SDS-PAGE gel (% total density)	22.2	0.2	120	NS			NS		
Band of assumed SRPP1 protein on SDS-PAGE gel (% total density)	9.7	0.1	120	NS			NS		
Band of assumed REF1 protein on SDS-PAGE gel (% total density)	14.4	0.1	120	NS			NS		

technically specified rubber samples (TSR5 and TSR10). The impact of tapping system and clone on the biochemical composition and properties (plasticity, accelerated storage hardening, viscosity, molar mass distribution of polyisoprene chains) of dry samples is described in Part 2 of this study (Liengprayoon *et al.*).

CRediT authorship contribution statement

Coffi Belmys Cakpo: Software, Formal analysis. **Frédéric Gay:** Writing – review & editing, Methodology, Conceptualization. **Hathainat Kum-ourm:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Vincent Ferrer:** Writing – review & editing, Methodology. **Siriluck Liengprayoon:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Christine Char:** Formal analysis. **Ai Matsuura:** Resources, Project administration, Funding acquisition. **Noriyuki Onozuka:** Supervision, Resources, Project administration, Methodology, Funding acquisition. **Yumi Sakaguchi-Kitaura:** Project administration. **Laurent Vaysse:** Writing – review & editing, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Yukino Miyagi-Inoue:** Validation, Project administration. **Lucksanaporn Tarachiwin:** Resources, Project administration, Funding acquisition, Conceptualization. **Régis Lacote:** Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Bottier Celine:** Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jatuporn Chaiyut:**

Methodology, Formal analysis. **Saowalak Jantarasunthorn:** Methodology, Formal analysis. **Lerksamran Tucksin:** Methodology, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2025.121758.

Data availability

The data that has been used is confidential.

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