

Latex quality and yield parameters of *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. clone PB 260 for different tapping and stimulant application frequencies

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ABSTRACT

Tapping and stimulant applications are an attempt to increase latex yield. A combination of stimulants with low intensity tapping systems was expected to reduce tapping costs. In addition, it was necessary to regulate the stimulant application frequency based on type and potential clone production. The objective was to determine the effect on physiological parameters and latex yield of *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. clone PB 260 for different tapping and stimulant application frequencies. The study design was split block in randomized complete block plots with three replicates and two factors. The main plot was the tapping system consisting of four levels: quarter-spiral downward tapping (S/4 d3), quarter-spiral upward tapping (S/4U d3), half-spiral downward tapping (S/2 d3), and half-spiral upward tapping (S/2U d3). The subplots were four stimulant application frequencies consisting of ethephon 2.5% applied once every 15 d (ET 15d) and gas stimulant (equal to $\pm 100\%$ ethylene) applied once every 9 d (ETG 9d), once every 18 d (ETG 18d), and once every 27 d (ETG 27d). The highest sucrose levels (3.62 mM) were obtained in S/2 d3 and ET/15d combination. The highest inorganic P content (27.68 mM) was in S/2 d3 and ETG/18d. The highest yield (2418.53 kg ha⁻¹ yr⁻¹) was in S/4d3 and ETG/18d. The tapping system S/4d3 and ETG/18d combination was able to increase production by 102.26% compared with the conventional tapping system (S/2 d3 and ET/15d). After the 1-yr trial, all treatments showed 0.47 to 0.54 mM thiol content, which indicated that plants did not experience over-exploitation stress.

Key words: Ethephon, ethylene gas, inorganic phosphorus, rubber tree, sucrose, thiol.

INTRODUCTION

Latex exploitation intensity is determined by slice length, tapping frequency, and stimulant application. These three factors react to rubber tree (*Hevea brasiliensis* [Willd. ex A. Juss.] Müll. Arg.) clones, plant age, and seasonal variations; each clone has a different exploitation system (Boerhendhy and Amypalupy, 2011). Stimulants are commonly used to extend the latex flow period and increase yield and labor efficiency (Tistama, 2013). According to Krishnakumar et al. (2011), stimulant application can be an alternative to reduce tapping costs caused by higher labor costs and the lack of skilled workers. Herlinawati and Kuswanhadi (2013) reported that stimulant application at certain frequencies and concentrations can increase yield and maximize profits. Fahmi et al. (2015) stated that stimulant application was basically intended to reduce the tapping cost using a low intensity tapping system.

Active ethephon-based stimulants are hydrolyzed into ethylene, hydrochloric acid, and phosphoric acid in the rubber tree. Ethylene in the rubber tree tends to stimulate proton H⁺/sucrose pumps that activate sugar transport to latex vessel

cells and then activate ATP-ase and P-ase proton pumps, which cause acidification of the luteoid serum and cytosolic basification (d'Auzac and Jacob, 1984). Lacote et al. (2010) reported that ethephon use can increase latex yield without disrupting plant physiological properties. However, effectiveness of the active ethephon ingredient has an indirect effect on latex yield, which can be seen within 5 to 6 h after its application, and yield increases only reach less than 50% (Karyudi et al., 2006). The directly applied gas stimulant is absorbed in the latex vessels and absorbs water from the surrounding cells. This absorption increases turgor pressure and is accompanied by an increased latex flow rate (Jacob et al., 1989). Kuswanhadi et al. (2009) explain that the use of ethylene gas stimulants can increase productivity from 75% to 100% on average compared with conventional tapping systems combined with ethephon stimulants.

Stimulant application affects latex cell metabolism as indicated by various changes in the physiological parameters, including sucrose, inorganic P, thiol, and dry rubber content (DRC) levels (Obouayeba et al., 2009). Sucrose is a raw material that synthesizes *cis*-polyisoprene, which is needed by latex cells for latex regeneration (Priyadarshan, 2017). Inorganic phosphate levels show the intensity of metabolic activity in latex vessels (Lacote et al., 2010). Thiol levels are parameters related to antioxidants that reflect the plant's ability (active oxygen species) to prevent cell damage by free radicals (Jacob et al., 1995). Conte and Carroll (2013) state that thiols protect subcellular particle membranes; they are also anti-senescent agents.

Currently, the most widely planted clone in North Sumatra is clone PB 260. This clone is characterized by high production potential, resistance to tapping panel dryness (KAS), and strength against wind disturbances. It is also one of the latex-producing clones that were recommended for their superiority between 2010 and 2015 (Siregar et al., 2008). Until now, clone PB 260 has been used to rejuvenate and expand rubber plantations. Its yield is initially high and then continues to increase and it is resistant to leaf disease; moreover, tapping could occur after 5 yr (Sumarmadji and Atminingsih, 2013).

The exploitation system does not usually consider concentration, stimulant application frequency, and clone properties. If this occurs over a long period of time, it triggers physiological stress on the plant due to each clone's different responses to stimulants, which can be seen from their physiological conditions. Physiological stress caused by complex interactions between clone sensitivity and exploitation intensity influences production more than the imbalance of environmental factors (Rodrigo et al., 2006). Using stimulants is one way to increase latex yield. It is also necessary to regulate stimulant application frequency based on the type and potential of clone production (Sumarmadji, 2011).

Therefore, the objective of the present study was to determine the effect on the physiological parameters and latex yield of the *Hevea brasiliensis* clone PB 260 for different tapping and stimulant application frequencies.

MATERIALS AND METHODS

Trial location and plant material

The field trial was carried out at two locations, Sungei Putih Research Center, Indonesian Rubber Research Institute (3°25'33.8" N, 98°52'04.8" E; 25 m a.s.l.) and Sei Putih Estate, PT. Perkebunan Nusantara III (3°24'35.1" N, 98°52'58.5" E). Both sites were located in the Deli Serdang Regency, North Sumatra Province, Indonesia. The soil type of the sites is Ultisol.

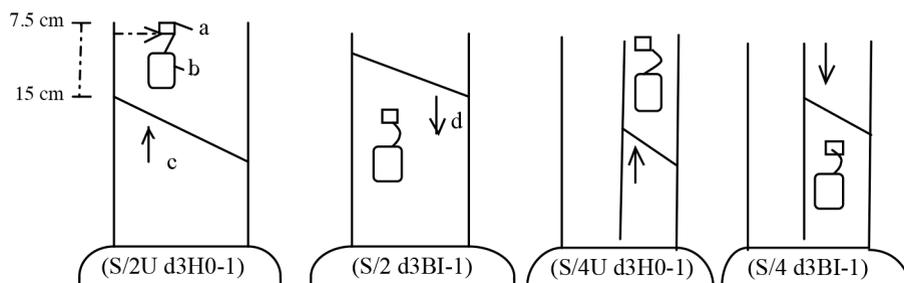
Clone PB 260 (15 yr old) was used as plant material in this study, and planting space was 2.5 m × 5 m. Prior to the treatment, randomized sampling was performed to select 75 plants with a 65-70 cm girth.

Experimental design

The study design was split block in randomized complete block plots with three replicates and two factors. The main plot was the tapping system that consisted of four levels: quarter-spiral downward tapping (S/4 d3), quarter-spiral upward tapping (S/4U d3), half-spiral downward tapping (S/2 d3), and half-spiral upward tapping (S/2U d3) (Figure 1).

The subplots were stimulant applications of liquid ethephon (2-chloro acid ethyl phosphate) 2.5% and gas stimulant (equal to ± 100% ethylene). The subplot stimulant application frequency treatments consisted of four levels: ethephon 2.5% applied once every 15 d (ET 15d or 24 treatments in 1 yr) and gas stimulant applied once every 9 d (ETG 9d or 41 treatments in 1 yr), once every 18 d (ETG 18d or 20 treatments in 1 yr), and once every 27 d (ETG 27d or 16 treatments in 1 yr).

Figure 1. Layout of gas applicator position of each tapping system.



Head applicator (a), gas sac (b), upward tapping (c), downward tapping (d).

S/2U: half-spiral upward tapping, S/2: half-spiral downward tapping, S/4U: quarter-spiral upward tapping, S/4: quarter-spiral downward tapping, d3: once every 3 d, H0-1: upper virgin bark, BI-1: lower renewable bark.

Stimulant application

The liquid stimulant was applied on the virgin basal bark (B0-1) by the groove application method (Ga), while the bark application method (Ba) was used on the virgin upper bark (H0-1). The liquid stimulant concentration was 2.5% with an application frequency of once every 15 d according to Junaidi et al. (2010).

The gas stimulant was also applied on B0-1 and H0-1 panels. Gas applicators were installed 15-20 cm above and 6.0-7.5 cm to the left of the tapping cut (Figure 1). The tapping activity was conducted at 3-d intervals (d3) and gas refilling was carried out in accordance with the treatments.

Yield and latex parameter measurements

Fresh latex was collected on every tapping day. The yield (dry rubber yield) calculation was fresh yield multiplied by dry rubber content (DRC). Yield per tree per tapping ($\text{g t}^{-1} \text{t}^{-1}$) was total dry yield divided by the number of tapped trees and number of tapping days.

Latex parameters were observed at the Sungei Putih Research Center Laboratory. Measurements included sucrose, inorganic P, and thiol contents. Trichloroacetic acid (TCA) serum was used for this analysis. Latex samples were collected from 135 trees of 450 trees ha^{-1} . The serum was made from 1 mL latex and 9 mL TCA prior to the analysis. All latex parameters were measured with a Beckman DU 650 spectrophotometer (Beckman Coulter, Brea, California, USA).

Sucrose content

Latex samples were approximately 150 μL and TCA 2.5% was added to complete a total volume of 500 μL . Then 3 mL anthrone reagent was added and vortexed; it was heated by soaking in boiling water for 15 min and cooled. The next stage was absorbance at λ 627 nm measured by the anthrone method. Sucrose dehydration in concentrated sulfuric acid (70% H_2SO_4) and heating provide furfural derivatives that react with anthrone to produce a blue color. Absorbance was measured at λ 627 nm according to the Dische's anthrone method (Dische, 1962).

Inorganic P and thiol

Inorganic P (Pi) was measured by the binding principle with ammonium molybdate and reduced by FeSO_4 in an acid reaction to obtain a blue color. Absorbance measurements were taken at λ 750 nm by the Taussky and Shorr (1953) methods.

To determine thiol, latex samples were approximately 1.5 mL to which TCA 2.5% was added for a total volume of 1.5 mL; then 10 mM DTNB 75 μL plus 1.5 mL buffer Tris 0.5 M were added and vortexed. The solution was kept at room temperature for 30 min. Absorbance was read at λ 412 nm with a spectrophotometer (Beckman) or measured from TCA serum based on the reaction principle with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) to form yellow TNB absorbed at λ 421 nm according to the McMullen method (McMullen, 1960).

Data analysis

Data analysis was performed with the Statistical Analysis System (SAS) software version 9.1 (SAS Institute, Cary, North Carolina, USA). Duncan's multiple range test was applied to compare treatments at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Agro-climatic conditions

Agro-climatic conditions can affect the physiology, growth, and yield of rubber trees (Okoma et al, 2011; Purwaningrum et al., 2016a). A yield increase due to stimulant use is strongly influenced by the environmental conditions of the study site. Rainfall data during the study showed monthly variations. Wet months were from September to November, while dry months were from January to March; the other months were in the intermediate category (Figure 2). Oktavia and Lasminingsih (2010) state that latex yield fluctuations are notably affected by tree leaf conditions, which are closely related to monthly rainfall patterns.

Climatic factors greatly affect rubber tree phenology, including leaf-fall, new leaf formation, flowering, and fruit formation (Sayurandi et al., 2017). In North Sumatra, leaf-fall occurs from January to March during the dry period. New leaves are formed in April. In the wet months, when leaves are fully formed, production is generally high if there is no rain. This pattern also affects monthly production distribution. The highest production is generally obtained from September to December, while the lowest from January to April (Junaidi et al., 2015).

Latex diagnosis and latex sucrose concentration

Latex diagnosis determines the tree's physiological variables by the applied exploitation system (Traoré et al., 2011), including sucrose, Pi, and thiol latex. D'Auzac and Jacob (1984) and Gohet et al. (2008) indicated that stimulants affect latex cell metabolism, which are indicated by changes in latex physiological parameters, including sucrose, Pi, and thiol levels.

Sucrose is a raw material for *cis*-polyisoprene synthesis, which is needed by latex cells for latex regeneration (Jacob et al., 1989). The tapping and stimulant frequency application combination significantly affected the latex sucrose levels of clone PB 260 (Table 1).

The highest sucrose levels (3.62 mM) were obtained in the treatment S/2d3 tapping system and ET 15d stimulant application frequency combination. The lowest sucrose level (1.49 mM) was in the S/4U d3 tapping system and ETG 9d gas stimulant application frequency combination. This is because active ethephon-based stimulants are hydrolyzed first into ethylene gas, hydrochloric acid, and new phosphoric acid, and then hydrolyze sucrose into latex, thus leading to higher sucrose levels.

Figure 2. Monthly rainfall variation at the research site.

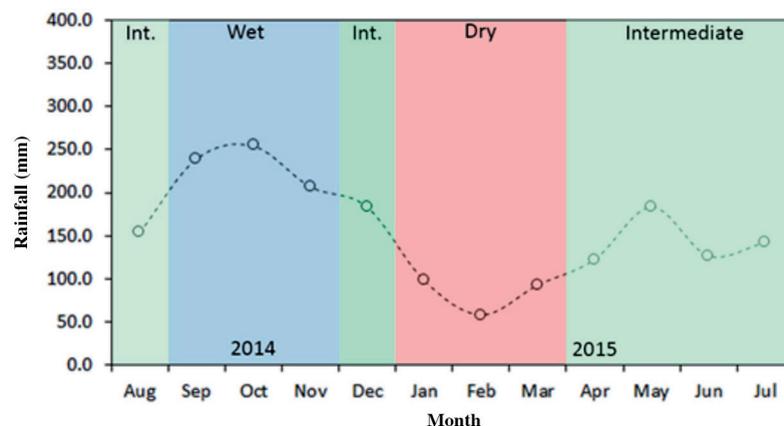


Table 1. Latex sucrose content of 15-yr-old rubber tree clone PB 260.

Treatment Tapping length	Stimulant frequency				Mean
	ET 15d	ETG 9d	ETG 18d	ETG 27d	
mM					
S/2 d3	3.62a	3.11c	2.62d	2.40e	2.69a
S/2U d3	1.66gh	2.38e	2.13f	2.26ef	2.11c
S/4 d3	1.88g	2.81d	3.33b	2.10f	2.53ab
S/4U d3	2.18ef	1.75g	1.49h	3.59a	2.25c
Mean	2.34c	2.51ab	2.39c	2.59a	

Values in the same column and treatment group followed by different letters are significantly different according to Duncan's test ($P = 0.05$).

ET 15d: Ethephon once every 15 d; ETG 9d: ethylene gas once every 9 d; ETG 18d: ethylene gas once every 18 d; ETG 27d: ethylene gas once every 27 d; S/2 d3: half-spiral downward tapping once every 3 d; S/2U d3: half-spiral upward tapping once every 3 d; S/4 d3: quarter-spiral downward tapping once every 3 d; S/4U d3: quarter-spiral upward tapping once every 3 d.

The direct application of gas stimulants to rubber trees as ethylene gas without going through the hydrolysis process accelerates the assimilate distribution into the latex vessel tissue. The sucrose content then decreases with gas stimulant use because of increased metabolic activity as indicated by increased Pi (Table 2). Another result of increased Pi is increased sucrose consumption in the latex regeneration process; lower sucrose levels are therefore obtained (Lacote et al., 2013). Doungmusik and Sdoodee (2012) state that the effect of gas stimulants on latex regeneration is reflected in the parameters of sucrose and Pi levels in which the sucrose concentration decreases due to increased metabolic activity as indicated by increases in the Pi concentration.

Latex inorganic P concentration

Inorganic P is an indicator of metabolic activity; in this case, it shows the plant's ability to convert raw materials (sucrose) into rubber particles (Jacob et al., 1995). According to Sumarmadji and Tistama (2004), the optimal Pi concentration range is from 10 to 30 mM. The combination of tapping system and stimulant application frequency has a significant effect on the latex Pi levels of clone PB 260 (Table 2). The highest Pi concentration (27.68 mM) was obtained in the S/2U d3 tapping system and ETG 18d gas stimulant application frequency combination, and the lowest Pi content (21.09 mM) in the S/2U d3 tapping system and ET 15d stimulant application frequency combination. These values concur with Anggraini et al. (2017) who show high Pi concentration in clone PB 260. This is because directly applying gas stimulants to rubber trees as ethylene gas without going through the hydrolysis process activates sugar transport into latex vessel cells and increases latex Pi levels through cell physiology by maintaining high turgor pressure (Siregar and Suhendry, 2013).

Table 2. Latex inorganic P content of 15-yr-old rubber tree clone PB 260.

Treatment Tapping length	Stimulant frequency				Mean
	ET 15d	ETG 9d	ETG 18d	ETG 27d	
mM					
S/2 d3	21.35de	21.88de	23.68cde	23.53cde	22.61c
S/2U d3	21.08e	26.88ab	27.68a	24.00cd	25.09ab
S/4 d3	25.22abc	24.78bc	27.47ab	23.85cd	25.33ab
S/4U d3	23.73cde	26.30abc	26.83ab	25.00abc	25.47a
Mean	17.58c	24.96ab	26.42a	23.96ab	

Values in the same column and treatment group followed by different letters are significantly different according to Duncan's test ($P = 0.05$).

ET 15d: Ethephon once every 15 d; ETG 9d: ethylene gas once every 9 d; ETG 18d: ethylene gas once every 18 d; ETG 27d: ethylene gas once every 27 d; S/2 d3: half-spiral downward tapping once every 3 d; S/2U d3: half-spiral upward tapping once every 3 d; S/4 d3: quarter-spiral downward tapping once every 3 d; S/4U d3: quarter-spiral upward tapping once every 3 d.

Latex thiol concentration

In the latex diagnosis, sucrose and Pi level measurements are more effective when combined with thiol level measurements because it reflects the ability of latex vessels to deal with aging mechanisms (Jacob et al., 1992). Thiol availability in latex is important for plants because it functions as an enzyme activator and is associated with luteal membrane stability to prolong latex flow (Conte and Carroll, 2013).

Thiol works as an antioxidant to suppress oxidative stress due to active cell metabolism in cells; thiol status shows the plant's response to exploitation stress. Thiol levels are inversely proportional to exploitation intensity; when exploitation intensity is higher, the thiol level is lower (Jacob et al., 1995). Thiol has the ability to protect subcellular organelles and capture toxic oxygen molecules. These molecules cause latex vessel cell fatigue, which triggers tapping panel dryness.

The combination of tapping system and stimulant application frequency had nonsignificant effect on latex thiol levels (Table 3). However, the combination is still at a safe level and does not cause stress because the thiol level is still in the 0.4 to 0.5 mM range. According to Sumarmadji and Junaidi (2008), the optimal thiol level ranges from 0.4 to 0.9 mM in clone PB 260. Increasing the exploitation intensity affects increased thiol level, but in the case of overexploitation, thiol levels decrease (Gao et al., 2006).

Latex yield

Latex formation begins with the conversion of photosynthesis results, that is, assimilates as sucrose are converted into glucose. Saccharides as sucrose are inside the latex vessels. These compounds are precursors to form rubber particles (*cis*-1,4 polyisoprene) (Boureau, 2013). The amount of sucrose measured in latex is the difference between the sucrose influx and sucrose used in the latex metabolism (Gohet et al., 2003).

The combination of tapping systems and stimulant application frequencies has a significant effect on the latex yield of clone PB 260 (Table 4). The highest latex yield was obtained in the S/4 d3 tapping system and ETG/18d gas stimulant

Table 3. Latex thiol content of 15-yr-old rubber tree clone PB 260.

Treatment Tapping length	Stimulant frequency				Mean
	ET 15d	ETG 9d	ETG 18d	ETG 27d	
	mM				
S/2 d3	0.48	0.53	0.46	0.46	0.48
S/2U d3	0.54	0.46	0.45	0.50	0.49
S/4 d3	0.48	0.48	0.54	0.46	0.49
S/4U d3	0.47	0.44	0.45	0.50	0.47
Mean	0.49	0.48	0.48	0.48	

ET 15d: Ethephon once every 15 d; ETG 9d: ethylene gas once every 9 d; ETG 18d: ethylene gas once every 18 d; ETG 27d: ethylene gas once every 27 d; S/2 d3: half-spiral downward tapping once every 3 d; S/2U d3: half-spiral upward tapping once every 3 d; S/4 d3: quarter-spiral downward tapping once every 3 d; S/4U d3: quarter-spiral upward tapping once every 3 d.

Table 4. Latex yield of 15-yr-old rubber tree clone PB 260.

Treatment Tapping length	Stimulant frequency				Mean
	ET 15d	ETG 9d	ETG 18d	ETG 27d	
	kg ha ⁻¹ yr ⁻¹				
S/2 d3	1195.75k	1229.62j	2271.61b	2134.97d	1707.99a
S/2U d3	1267.03i	1116.83m	2080.70e	2126.09d	1647.66ab
S/4 d3	1250.61i	1189.84l	2418.53a	1598.99g	1614.49ab
S/4U d3	1295.33h	1211.30k	2202.68c	2059.81f	1692.28ab
Mean	1243.18c	1186.90c	2243.38a	1979.97ab	

Values in the same column and treatment group followed by different letters are significantly different according to Duncan's test (P = 0.05).

ET 15d: Ethephon once every 15 d; ETG 9d: ethylene gas once every 9 d; ETG 18d: ethylene gas once every 18 d; ETG 27d: ethylene gas once every 27 d; S/2 d3: half-spiral downward tapping once every 3 d; S/2U d3: half-spiral upward tapping once every 3 d; S/4 d3: quarter-spiral downward tapping once every 3 d; S/4U d3: quarter-spiral upward tapping once every 3 d.

frequency (2418.53 kg ha⁻¹ yr⁻¹) combination, and the lowest latex production was found in the S/2Ud3 tapping system and ETG 9d (1116.83 kg ha⁻¹ yr⁻¹) combination. Purwaningrum et al. (2015; 2016a; 2016b) show that the highest latex yield occurs in the S/4 d3 tapping system and gas stimulant application (ETG). Shortening the length of the tapping slices from S/2 to S/4 increases latex yield because the latex flow pressure is higher with short slices (S/4); therefore, the latex flow velocity increases compared with long slices (S/2) (Sumarmadji et al., 2008).

In addition, short slice tapping provides benefits; for example, when the panel is moved, there is no full spiral slice and the relationship between the upper and lower latex vessels is unbroken; moreover, there is more efficient bark consumption, shorter tapping time, higher dry rubber content, less latex flow deviation, and relatively better tapping quality (Sumarmadji, 2011; Traoré et al., 2011). Ethylene gas in the latex vessels also absorbs water from the cells around it. This absorption increases turgor pressure, which is accompanied by an increased latex flow rate. This condition is associated with an increase in luteoid stability, which delays the latex vessel blocking process, so that latex drainage is extended and latex yield increases.

CONCLUSIONS

Stimulant application must be based on physiological conditions (inorganic P and sucrose levels) and plant health. The limiting factors for increased yield are inorganic P (Pi) and sucrose levels. The highest concentration of sucrose was obtained in the half-spiral downward tapping (S/2 d3) system with stimulant application frequency consisting of liquid ethephon applied once every 15 d (ET 15d). The highest Pi content occurred in the combination of the S/2 d3 tapping system and gas stimulant applied once every 18 d (ETG 18d). The highest latex yield was in the quarter-spiral downward tapping (S/4 d3) system and ETG 18d stimulant frequency combination. The S/4 d3 tapping system combined with the ETG 18d stimulant frequency was able to increase latex yield by 102.26% compared with the conventional tapping system (S/2 d3 and ET 15d). After the 1-yr trial, all treatments showed a thiol concentration (0.40 to 0.50 mM) that indicated that plants had not experienced over-exploitation stress.

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