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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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/4 U d3), half-spiral downward tapping (S/2 d3), and half-spiral upward tapping (S/2 U 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 ± 100% ethylene) applied once every 9 d (ETO 9d), once every 18 d (ETO 18d), and once every 27 d (ETO 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 ETO/18d. The highest yield (2418.53 kg ha⁻¹ yr⁻¹) was in S/4d3 and ETO/18d. The tapping system S/4d3 and ETO/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 (Boerendy and Ampalapuy, 2011). Stimulants are commonly used to extend the latex flow period and increase yield and labor efficiency (Tistarna, 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. Herinawati and Kuswanadi (2013) reported that stimulant application at certain frequencies and concentrations can increase yield and maximize profits. Falmi 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-proton pumps, which cause acidification of the lutoid serum and cytosolic basification (d'Azurac 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 stimulus is absorbed in the latex vessels and it absorbs water from the surrounding cells. This absorption increases turgor pressure and is accompanied by an increased latex flow rate (Jacob et al., 1995). Kusvanjadi 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 (Priyadarshana, 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-senescence 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 (Rodriguez 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 brasiiliensis 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, Sungai Puth Research Center, Indonesian Rubber Research Institute (3°25’33.8”N, 98°52’04.8”E; 25 m a.s.l.) and Sei Puth 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-clooro 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).
**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 days 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-day 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 (g/t) 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 BeckmanDU 630 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 boiling water for 15 min and cooled. The next stage was absorbance at λ 627 nm measured by the anthrone method. Sucrose hydration in concentrated sulfuric acid (70% H₂SO₄) 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 FeSO₄ 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 (Junaedi 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’Azucar 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.

Succrose 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 FB 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 | mM  |
|-----------|----------------|---------------------|-----|
|           |                | ET 15d | ETG 9d | ETG 18d | ETG 27d | Mean |
| S/2 d3    | 3.62a          | 3.11c  | 2.62d  | 2.40c   | 2.69a   |
| S/2U d3   | 1.66gh         | 2.58e  | 2.15f  | 2.26ef  | 2.11e   |
| 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 Sodoodee (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 Sumarmadj 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 Sulhendry, 2013).

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

| Treatment | Tapping length | Stimulant frequency | mM  |
|-----------|----------------|---------------------|-----|
|           |                | ET 15d | ETG 9d | ETG 18d | ETG 27d | Mean |
| S/2 d3    | 21.35de        | 21.88de | 23.68cde | 23.53cde | 22.61c  |
| S/2U d3   | 21.08e         | 26.88ab | 27.98a  | 24.00cd  | 25.05ab |
| S/4 d3    | 25.22ab        | 24.78bc | 27.47ab | 23.85cd  | 25.33ab |
| S/4U d3   | 23.73bc        | 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 Pb 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 lutoid 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 Sumarmadj and Junaidi (2008), the optimal thiol level ranges from 0.4 to 0.5 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 | mM |
|---------------------------|---------------------|----|
| S/2 d3                    | ET 15d              | 0.48 |
|                           | ETG 9d              | 0.54 |
|                           | ETG 18d             | 0.46 |
|                           | ETG 27d             | 0.46 |
| S/2U d3                   | ET 15d              | 0.48 |
|                           | ETG 9d              | 0.48 |
|                           | ETG 18d             | 0.45 |
|                           | ETG 27d             | 0.50 |
| S/4 d3                    | ET 15d              | 0.47 |
|                           | ETG 9d              | 0.44 |
|                           | ETG 18d             | 0.45 |
|                           | ETG 27d             | 0.50 |
| S/4U d3                   | ET 15d              | 0.49 |
|                           | ETG 9d              | 0.48 |
|                           | ETG 18d             | 0.48 |
|                           | ETG 27d             | 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 | kg ha⁻¹ yr⁻¹ |
|---------------------------|---------------------|-------------|
| S/2 d3                    | ET 15d              | 1287.62 |
|                           | ETG 9d              | 1216.83 |
|                           | ETG 18d             | 2271.61 |
|                           | ETG 27d             | 1213.97 |
|                           | Mean                | 1707.99 |
| S/2U d3                   | ET 15d              | 1350.61 |
|                           | ETG 9d              | 1189.84 |
|                           | ETG 18d             | 2418.53 |
|                           | ETG 27d             | 1598.99 |
|                           | Mean                | 1614.49 |
| S/4 d3                    | ET 15d              | 1295.33 |
|                           | ETG 9d              | 1211.30 |
|                           | ETG 18d             | 2202.68 |
|                           | ETG 27d             | 2099.81 |
|                           | Mean                | 1692.29 |
| S/4U d3                   | ET 15d              | 1243.18 |
|                           | ETG 9d              | 1186.90 |
|                           | ETG 18d             | 2243.38 |
|                           | Mean                | 1979.97 |

Values in the same columns and treatment group followed by different letters are significantly different according to Duncan’s test (P ≤ 0.05).
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. Purwaringrum 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) (Sumarmadjı 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 (Sumarmadjı, 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 lutoid 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 (Pf) 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 (ETG 15d). The highest Pf 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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