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Physiological and Productivity Impact of Mechanical Wounding and Mortex Stimulation on Rubber Clones RRIM 2025 and PB 350

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ABSTRACT

The effects of mechanical wounding involving mechanical boring and tapping followed by the application of Mortex, an ethylene-based stimulant, on *Hevea* clones RRIM 2025 and PB 350 were investigated. Mortex at three different concentrations of 0.75%, 2.5% and 5% were applied at the tapping panel and the response of both clones in influencing wound-induced ethylene, latex yield, sugar, thiol and proline contents were determined. Data were analysed against control 1 (untapped and unstimulated), control 2 (tapped and unstimulated) and the application of nanosilver solution (Silversol), an ethylene inhibitor containing Ag^{+}. Latex yield of both clones appeared to be induced and dependent on ethylene stimulation. Mortex at 2.5% was suggested to be optimum for high yield stimulation in both clones. Application of 5% Mortex gave the highest yield for both clones and induced highest wound-induced ethylene release in the shaved bark of RRIM 2025. Meanwhile, 2.5% Mortex gave the highest wound ethylene evolution in shaved bark of PB 350. Wounding by tapping alone produced wound ethylene at low levels in both clones. The very low levels of accumulated wound ethylene found in bored holes at 2 cm above tapping cut was likely due to tapping activity and not influenced by Mortex application. Silversol was found to successfully inhibit ethylene action by reducing wound ethylene release in shaved bark of both clones. Reduced sucrose content in the latex with 5% Mortex was observed. In contrast, unstimulated trees and an application of Silversol appeared to reduce latex yield in both clones with a concomitant increase in sucrose content. Suppression of ethylene action by Ag^{+} also reduced thiol and inorganic phosphorus content, suggesting that Silversol was effective in overcoming the stress effects related to ethylene regulation.

Keywords: Ethylene; *Hevea brasiliensis*; latex physiology; silversol; wounding; yield productivity

INTRODUCTION

Hevea brasiliensis is a major source for natural rubber supply worldwide. The current world natural rubber production is 10.2 million tonnes and consumption is 10.3 million tonnes (Malaysian Rubber Board, 2018). Total areas planted with rubber in natural rubber producing countries (ANRPC) are 11.3 million ha, with relatively bigger proportion is contributed by smallholders. To cater for sustainable supply of natural rubber, the need for implementation of economical and practical system is crucial.

Latex harvest technology includes various tapping cut, tapping frequencies and stimulation frequencies and/or concentrations. These harvesting technologies were developed for both matured (more than 15 years) and young trees of various clones. Ethylene-based latex flow stimulants commonly used in the rubber industry containing 2-chloroethylphosphonic acid (2-CEPA) are Ethephon and Ethrel, while their counterparts that employ direct ethylene gas stimulation are REACTORRIM, RRIMFLOW and G-

FLEX. The use of these stimulants is aimed at prolonging latex flow while reducing tapping frequencies, to increase productivity and overcome shortage of skilled rubber tappers (Sivakumaran, 2002; Sivakumaran et al., 2007).

In plant system, ethylene is released upon uptake of ethephon by the stem cells (Warner and (Leopold, 1969; Yang, 1969), and translocate to the phloem (Beaudry and Kays, 1988), where laticifers are located. Stimulation with exogenous ethylene could induce endogenous (wound) ethylene biosynthesis in plant cell (Che Husin et al., 2016; Nor Mayati and Mohd Fazri, 2017). Hydrogen cyanide (HCN), a by-product of ethylene biosynthetic pathway is cytotoxic to plant as it inhibits the respiratory system (Fujita et al., 2006). Imbalance between cyanogenic and cyanide (CN)-detoxifying activities (CAS) in necrotic phloem, leads to neighbouring cells poisoning, thus spreads tissue necrosis towards the tapping cut (Chrestin et al., 2004). Common mechanical stresses or injuries to higher plants have been reported to increase wound ethylene in many plant species (Wang et al., 2002; Druege, 2006; Nor Mayati and Fazri, 2017).

Mortex is a new generation ethephon-based latex stimulant widely employed by the rubber industry (Aziz et al., 2006). Mortex containing 2-CEPA as an active ingredient was developed as a stimulant for young rubber trees. The product also contains essential nutrients for trees to cater for sustainable latex productivity. Mortex, which is fortified with crude palm oil, is believed capable of preventing the symptoms of tapping panel dryness (TPD) in stimulated *Hevea* trees. In general, ethylene-based stimulation of latex harvesting induces sucrose that serves as a precursor of rubber biosynthesis. Sucrose originates from the surrounding cells of inner bark layers and is imported into the laticiferous cells (Dusotoit-Coucaud et al., 2010). A number of studies have been carried out to investigate the effect of ethylene-based stimulants on latex yield, sucrose content and TPD (Lacote et al., 2010; Putranto et al., 2015). In this study, we presumed that Mortex stimulation and mechanical wounding by boring holes and tapping would induce wound ethylene evolution leading to enhanced latex flow in these *Hevea* clones.

The objectives of this study were to evaluate the physiological impact of mechanical wounding involving mechanical boring and tapping following the application of Mortex, and to investigate the optimum concentration of Mortex required for latex stimulation, with minimal stress on the rubber clones, RRIM 2025 and PB 350, the two high yielding clones that had been described as ethylene insensitive and sensitive clone, respectively. It was hypothesised that wound-induced ethylene can function as a stress indicator due to excessive stimulation and tapping. The data obtained will be useful for manipulation of the current and future latex harvesting technology in maximising yield production.

MATERIALS AND METHODS

Location of the trials, soil series and weather condition

The trials were conducted for two years, at Field 1 (Permatang Division) in Kota Tinggi RRIM Research Station (SPKT), Johor, Malaysia (1°46'N, 103°55'E). The soil was Bungor series, classified as fine, kaolinitic, isohyperthermic, red-yellow family of the Tipik Lutualemkuts (Malaysian Soil Taxonomy). Bungor soil family has been classified as Haplic Nitisols containing highest exchangeable aluminium (Al) and iron (Fe) values that provides a bigger sink of phosphorus (P). Bungor soil consists of fine sandy loam and sandy clay textures that has low fertility but nevertheless suitable for rubber, oil palm, fruit trees, cocoa and forest vegetation (Lau and Mahmud, 1992).

Weeds were controlled with Paraquat dichloride (Chevron) mixing with glyphosate. Fertiliser applications were carried out once a year with Muriate of Potash (MOP), containing potassium chloride and Sulphate of Potash (SOP), containing potassium sulphate. Average rainfall, recorded by the localised installed weather station was 1122 mm, temperature range at 25 to 34°C and humidity was ranged between 70 to 90%.

Sample sources and treatments

Hevea clones, RRIM 2025 and PB 350 were 8 years old after planting and at average girth of 55 cm during the open tapped. Tapping was performed once a week (d6) and subjected to ½ spiral cut on panel BO-1 (original/virgin bark 1st panel). PB 350 in Block 1 and RRIM 2025 in Block 2 were planted separately on an area of 0.028 ha containing a total of 655 trees each. The planting distance was 4 m x 4 m.

The trial experiment comprising both clones RRIM 2025 and PB 350 being treated with six treatments and with three tree replicates for each treatment, giving a total of 18 experimental units (number of trees) for each clone. Six treatments were evaluated which consisted of the following:

i) T1 for mechanically bored trunk of untapped and unstimulated trees which was control 1,
ii) T2 for mechanically bored trunk of tapped and unstimulated trees which was control 2,
iii) T3 for mechanically bored, tapped and stimulated trees (BTS) with Mortex 0.75%,
iv) T4 for BTS trees with Mortex 2.5%,
v) T5 for BTS trees with Mortex 5%, and
vi) T6 for BTS trees with 4 µL/L (Ag^+) Nanosilver Solution (Silversol) as a negative control (ethylene antagonist).

The wound ethylene gas was measured from two different tissues wounded by two different types of wounding separately. First wounding treatment was bored holes on the tree trunk. Boring was made once in every yielding period. The second type of wounding was by normal tapping (bark shaving) activity once a week.

Data for all parameters tested were compared between three different yielding periods known as low yielding period (LYP; February until May), medium yielding period (MYP; June until September) and high yielding period (HYP; October until January). The trial was carried out for a two-year period to ensure the parameter profiles were repeatable.

Boring of the tree trunk was performed at 6 cm depth x 0.8 cm diameter, using drill bit size 9 at 2 cm above the tapping cut and 150 cm above the ground on day 0 of week 1 at every yielding period (Figure 1).

Figure 1. Boring of holes on the tree trunk using drill bit at 2 cm above the tapping cut and fixed tubing for wound ethylene gas accumulation.

Ethylene stimulation, wound ethylene accumulation and measurement

Application of Mortex was on the lace for the concentrations listed above. Application was carried out once a month i.e. in the first week of every monthly cycle. Setting up of devices for wound ethylene accumulation in the bored holes and from shaved barks, and ethylene gas measurement using CI-900 Portable Ethylene Analyser (CID Bio-Science Inc. 1554 NE 3rd Ave, Camas, WA 98607, USA) were carried out as described previously (Nor Mayati and Mohd Fazri, 2017).

Ethylene concentration was displayed on the monitor of the CI-900 Portable Ethylene Analyser and calculation of the actual endogenous ethylene production rates was determined as:

i) Ethylene production rate in the bored holes using the following formula:
Er = [Ec/t]
where Er = Ethylene production rate (µL/L/h),
Ec = ethylene concentration in the ALTEF gas sampling bag read by the machine (µL/L), and,
t = incubation period (h)

ii) Wound ethylene production rate in shaved bark using the formula:
Er = [Ec x Vf / Ws] / t
where; Er = ethylene production rate (µL/kg/h),
Ec = an ethylene concentration for the sample (µL/L),
Vf = the free headspace volume of bottle/tube (L),
Ws = fresh weight of shaved bark (kg), and
t = incubation period (h)

Field latex sampling

After an hour of tapping (onset of latex flow), 25 mL latex was collected into a 30 mL plastic bottle. Ammonia (1 mL) was added to prevent coagulation and latex samples were brought back to the laboratory within 4 to 5 hours. The ammoniated latex can be stored up to a month at room temperature.

Tree productivity (g/t/t)

Tree productivity (gram/tree/tapping; g/t/t/) was calculated following the equation reported for rubber trees published earlier (Nor Mayati and Abd-Razak, 2018; Mohd Akbar, 2006).

Dry rubber content (DRC)

DRC was measured using the rapid method described by Che Husin (2006).

Latex biochemical properties

Other biochemical parameters such as sucrose, thiols and proline were determined following methods described previously (Eschbach et al., 1984; IRRDB, 1995; Jacob et al., 1988).

Bark nutrients

After analysis for wound ethylene gas, the shaved barks were analysed for the content of nitrogen (N), phosphate (P), potassium (K) and magnesium (Mg). This analysis was carried out using the services provided by GTACr (Global Testing and Consultancy for Rubber), Malaysian Rubber Board.

Statistical analysis

Experimental design was Randomised Complete Block Design (RCBD) and data collection in Split Plot using the General Linear Model (GLM) program of Statistical Analysis System (SAS®, SAS Institute Inc., Cary, NC, USA) release 9.2. For data found significantly different using the two-way analysis of variance (ANOVA), the means of treatment variables were compared by least significant difference (LSD) test at P δ 0.05. Blocking was by clones and replicate trees were random.

RESULTS

Wound ethylene evolution

Generally, the ethylene accumulated in the bored holes and from the excised (shaved) bark of both tested clones, RRIM 2025 and PB 350, was not significantly different from each other (Table 1). Extremely low levels of wound (endogenous) ethylene accumulated in bored holes of both RRIM 2025 (0.014 µL/L/h) and PB 350 (0.015 µL/L/h) were recorded (Table 1). All treatments tested did not significantly affect the low level of wound ethylene accumulated in the bored holes. However, the release of wound ethylene was significantly ($P < 0.0001$) extremely low during LYP (Feb-May) compared to MYP (Jun-Sept) and HYP (Oct-Jan) in both treated clones (Table 1).

Analysis of accumulated ethylene in the shaved bark, however, showed that Mortex at the concentrations of 5% (45.46 µL/kg/h) and 0.75% (43.79 µL/kg/h) induced significantly ($P = 0.0015$) higher wound ethylene in RRIM 2025 (Table 1). Meanwhile for clone PB 350, stimulation with low concentration of Mortex at 0.75% gave the significantly lower accumulated ethylene (37.68 µL/kg/h) in the collected shaved bark (Table 1). On the contrary, Mortex at 2.5% and 5%, and control-multiple mechanical wounding (tapping and boring holes) without stimulation induced the production of wound ethylene at a significantly higher rate in PB 350. Suppression of wound ethylene evolution was apparent for the trees treated with ethylene inhibitor 4 µL/L Silversol in both tested clones (Table 1). Wound ethylene in shaved barks of both clones was significantly reduced during LYP, similar to the trend recorded in bored holes (Table 1).

Tree productivity

Average yield between clones, RRIM 2025 (49.53 g/t/t) was significantly ($P < 0.01$) higher than PB 350 (40.44 g/t/t). Tree productivity (g/t/t) of clones RRIM 2025 and PB 350 also showed significant ($P < 0.01$) difference among treatments mean (Table 2). Bark treated with Mortex at the concentration of 5% resulted in significant ($P < 0.01$) increased latex yield in both clones compared to the unstimulated control group. Moreover, a lower concentration of Mortex at 2.5% has also significantly increased yield of PB 350. A marked decrease was observed when the trees of both clones were treated with ethylene inhibitor, Silversol (Table 2). Meanwhile, different yielding periods generally did not affect tree productivity of both clones RRIM 2025 and PB 350 (Table 2).

Dry rubber content (DRC)

Average DRC of PB 350 (34.44%) was found significantly ($P < 0.01$) higher than that of RRIM 2025 (30.16%) (Table 2). RRIM 2025 treated with Mortex at every concentration tested did not show significant increase of DRC. In contrast, DRC was significantly ($P = 0.0219$) increased with ethylene antagonist, Silversol. In contrast, Mortex concentrations at 2.5% and 5% gave an obvious reduction of DRC level in PB 350 that were not affected by Silversol. Thus, it appeared that inhibition of ethylene by nanosilver particles had a positive effect on DRC of RRIM 2025 only, albeit the negative effect on tree productivity (g/t/t) in both clones. DRC content in the latex of clone PB 350 throughout three different yielding periods was significantly (P < 0.01) higher than that of RRIM 2025 (Table 2).

Table 1. Mean ethylene evolution measured in two sources of sampling in RRIM 2025 and PB 350 stimulated with Mortex

Parameters	Mean ethylene concentration accumulated in the bored holes (µL/L/h)		Mean ethylene concentration accumulated from shaved bark (µL/kg/h)	
Clones	RRIM 2025	PB 350	RRIM 2025	PB 350
	0.014	0.015	41.08	40.78
F-test probability	0.2132		0.5897	
n	198		150	
LSD 0.05	ns		ns	
Treatments				
Control 1 (untapped and unstimulated)	0.013	0.013	nil	nil
Control 2 (tapped and unstimulated)	0.013	0.015	38.97[c]	44.07[a]
0.75% Mortex	0.015	0.015	43.79[ab]	37.68[b]
2.5% Mortex	0.016	0.014	40.69[bc]	47.73[a]
5% Mortex	0.013	0.014	45.46[a]	45.32[a]
4 $\mu L.L^{-1}$ Silversol	0.013	0.016	36.49[c]	29.13[c]
F-test probability	0.3227	0.8556	0.0015	< 0.0001
n	33	33	30	30
LSD 0.05	ns	ns	4.46	5.44
Yield Period				
HYP	0.016[a]	0.017[b]	46.79[a]	40.30[ab]
MYP	0.018[a]	0.019[a]	41.06[b]	43.60[a]
LYP	0.005[b]	0.005[c]	35.40[c]	37.51[b]
F-test probability	< 0.0001	< 0.0001	< 0.0001	0.0158
LSD 0.05	0.02	0.04	3.68	1.52

Values with different superscript letters are significant at P < 0.05. ns = parameters were not significant by ANOVA.

Latex biochemical properties

Slightly higher and significant (P = 0.0203) sucrose content was recorded in RRIM 2025 (11.15 mM/L) compared to PB 350 (10.34 mM/L) (Table 2). RRIM 2025 had shown significant reduced sucrose content after being treated with Mortex at all concentrations tested, but was not affected by treatment with Silversol. In contrast, sucrose content in PB 350 was significantly reduced to a very low level (6.42 mM/L) when treated with 2.5% Mortex. Stimulation of *Hevea* trees with an ethylene inhibitor, Silversol, however resulted in significant (P < 0.01) elevated sucrose content in PB 350 (17.22 mM/L). During the transition of yielding period, RRIM 2025 exhibited significant reduced sucrose content during LYP when the trees were experiencing wintering pattern, which was during annual dry season from February to May when the rubber trees underwent defoliation and refoliation period (Table 2).

Low levels (below 1 mM/L) and insignificant thiol content was observed in both clones RRIM 2025 and PB 350 (Table 2). Both clones are high yielding clones, prone to TPD that may occur following excessive exploitation and/or tapping activities. Ethylene sourced from Mortex did not significantly affect thiol content in latex of RRIM 2025 which was contrary to the data recorded for PB 350 (Table 2), which was observed to be significantly (P < 0.01) increased by Mortex at 2.5% (0.141 mM/L). Significantly (P = 0.0017) reduced thiol content was detected when clone PB 350 trees were treated with ethylene inhibitor Silversol (0.088 mM/L). When analysed between the transition of yieding periods, the thiol content in both clones was recorded slightly higher and significant during LYP compared to HYP and MYP (Table 2).

Significantly higher proline content (P < 0.01) was observed in PB 350 (328.31 mM/L) compared to RRIM 2025 (243.26 mM/L) (Table 2). All concentrations of Mortex applied did not significantly affect proline content in both clones. However, the inhibition effect of Silversol containing nanosize Ag^{+} had significantly (P < 0.01) reduced proline content in latex of PB 350 (210.3 mM/L) (Table 2). The significant content of proline observed climaxed during LYP for both clones at an average of 363.37 mM/L for RRIM 2025 and at average of 482.85 mM/L for PB 350 (Table 2).

Table 2. Tree productivity, sucrose, thiol and proline content in harvested latex of RRIM2025 and PB350 treated with various concentrations of Mortex and ethylene antagonist Silversol containing nanosilver particles

	Mean Tree Productivity (gram/tapping/tree; g/t/t)		Dry Rubber Content (DRC) (%)		Sucrose (mM/L)		Thiol (mM/L)		Proline (mM/L)	
Clone	RRIM 2025	PB 350	RRIM 2025	PB 350	RRIM 2025	PB 350	RRIM 2025	PB 350	RRIM 2025	PB 350
	49.53^{a}	40.44^{b}	30.16^{b}	34.44^{a}	11.15^{a}	10.34^{b}	0.12	0.11	243.26^{b}	328.31^{a}
F-test probability	< 0.0001		< 0.0001		0.0203		0.5560		< 0.0001	
LSD $_{0.05\ (n=180)}$	3.79		0.70		0.68		ns		28.97	
Treatments										
Control 2 (tapped and unstimulated)	48.94^{b}	40.33^{b}	29.82^{bc}	35.19^{ab}	12.96^{a}	9.66^{bc}	0.121	0.106^{bc}	242.33	374.13^{a}
0.75% MORTEX	48.09^{b}	39.12^{b}	30.78^{ab}	34.49^{bc}	10.91^{b}	10.39^{b}	0.114	0.103^{bc}	253.17	335.57^{a}
2.5% MORTEX	53.06^{ab}	51.09^{a}	29.69^{bc}	32.44^{d}	10.09^{b}	6.42^{d}	0.118	0.141^{a}	231.97	366.24^{a}
5% MORTEX	59.09^{a}	57.85^{a}	29.46^{c}	33.16^{cd}	8.66^{c}	8.00^{cd}	0.123	0.129^{ab}	255.32	355.31^{a}
4 µL/L Silversol	38.49^{c}	13.82^{c}	31.07^{a}	36.89^{a}	13.13^{a}	17.22^{a}	0.112	0.088^{c}	233.53	210.30^{b}
F-test probability	< 0.0001	< 0.0001	0.0219	< 0.0001	< 0.0001	< 0.0001	0.9781	0.0017	0.8005	0.0002
LSD $_{0.05\ (n=36)}$	6.21	9.92	1.16	1.91	1.20	1.77	ns	0.03	ns	77.82
Yielding Periods										
HYP	51.49	47.00^{a}	31.99^{a}	34.82^{a}	11.70^{a}	11.07^{a}	0.085^{b}	0.092^{b}	174.98^{b}	266.01^{b}
MYP	49.90	36.78^{b}	29.44^{b}	35.52^{a}	11.90^{a}	10.34^{ab}	0.110^{b}	0.103^{b}	191.43^{b}	236.07^{b}
LYP	47.70	43.57^{ab}	29.05^{b}	32.98^{b}	9.84^{b}	9.59^{b}	0.159^{a}	0.145^{a}	363.37^{a}	482.85^{a}
F-test probability	0.3127	0.1869	< 0.0001	0.0028	< 0.0001	0.1077	< 0.0001	< 0.0001	< 0.0001	< 0.0001
LSD $_{0.05\ (n=60)}$	ns	7.97	0.90	1.48	0.93	1.37	0.03	0.02	35.45	51.75

Values with different superscript letters are significant at P < 0.05. ns = parameters were not significant by ANOVA.

Bark nutrient content

In this study, it has been revealed that potassium (K) was the highest significant (P < 0.01) nutrient element found in both RRIM 2025 (0.57%) and PB 350 (0.63%) (Table 3). Highest K content was observed in PB 350 stimulated with 5% Mortex (0.70%) but the level was significantly reduced with inhibitor Silversol

(0.59%). Generally, clone PB 350 exhibited higher K content for every treatment tested and throughout every yielding period observed compared to clone RRIM 2025.

Treatments with 2.5% (0.61%) and 5% (0.62%) Mortex also accumulated significantly ($P < 0.01$) higher nitrogen (N) content in PB 350, which was inhibited by Silversol (0.50%) (Table 3). Meanwhile, phosphorus (P) content showed significant slight increase in both clones when treated with 5% Mortex. Moreover, significantly ($P < 0.01$) low magnesium (Mg) content was also observed, which was slightly increased in RRIM 2025 when treated with 5% Mortex. Rapid decrease in Mg was observed in both clones when treated with Silversol (Table 3). Therefore, N and Mg contents in both clones were significantly determined by the transition of yielding period while significant content of K was only observed in PB 350 (Table 3).

Table 3. Status of nutrient content in shaved bark of RRIM 2025 and PB 350 after stimulation with Mortex and inhibitor Silversol

Parameters	Average Nutrient Content (%)							
	N		P		K		Mg	
	RRIM 2025	PB 350	RRIM 2025	PB 350	RRIM 2025	PB 350	RRIM 2025	PB 350
Clone	0.568	0.564	0.082	0.082	0.573^{b}	0.629^{a}	0.099^{a}	0.091^{b}
P- value	0.6079		0.8957		< 0.0001		< 0.0001	
$LSD_{0.05\ (n=60)}$	ns		ns		0.019		0.003	
Treatment								
Control 2 (tapped and unstimulated)	0.549^{ab}	0.535^{b}	0.0742^{c}	0.067^{c}	0.583^{a}	0.653^{b}	0.100^{b}	0.098^{b}
0.75% Mortex	0.579^{ab}	0.545^{b}	0.078^{bc}	0.080^{b}	0.577^{a}	0.607^{c}	0.093^{c}	0.084^{c}
2.5% Mortex	0.572^{ab}	0.614^{a}	0.085^{b}	0.083^{b}	0.511^{b}	0.601^{c}	0.103^{b}	0.107^{a}
5% Mortex	0.598^{a}	0.621^{a}	0.099^{a}	0.096^{a}	0.625^{a}	0.696^{a}	0.119^{a}	0.102^{ab}
4 uL/L Silversol	0.543^{b}	0.504^{c}	0.072^{c}	0.083^{b}	0.571^{a}	0.588^{c}	0.078^{d}	0.065^{d}
F-test probability	0.1705	< 0.0001	< 0.0001	< 0.0001	0.0113	< 0.0001	< 0.0001	< 0.0001
$LSD_{0.05\ (n=12)}$	ns	0.030	0.007	0.006	0.059	0.027	0.006	0.008
Yielding Period								
LYP	0.603^{b}	0.639^{a}	0.079	0.083	0.567	0.641^{a}	0.089^{b}	0.079^{c}
MYP	0.435^{c}	0.394^{b}	0.081	0.081	0.592	0.641^{a}	0.104^{a}	0.094^{b}
HYP	0.667^{a}	0.659^{a}	0.085	0.082	0.561	0.605^{b}	0.104^{a}	0.101^{a}
F-test probability	< 0.0001	< 0.0001	0.1441	0.6265	0.3486	0.0013	< 0.0001	< 0.0001
$LSD_{0.05\ (n=20)}$	0.038	0.023	ns	ns	ns	0.021	0.004	0.006

Values in the same columns followed by different superscript letters are significantly different at $P < 0.05$ as determined by LSD test. ns = parameters were not significant by ANOVA.

DISCUSSION

The study aimed to determine the optimum concentration of Mortex stimulant used in extracting latex from *Hevea* clones, RRIM 2025 and PB 350. The optimum concentration was determined by lower capability of commercialised Mortex in triggering the stress indicated by an increase in endogenous (wound) ethylene release measured in the tested trees. At the same time, the stimulation at the particular concentration should give the highest yield in terms of tree productivity and DRC. Effects of mechanical wounding by boring holes and/or tapping with or without exogenous ethylene stimulation (Mortex) were evaluated. Endogenous ethylene was released upon boring holes above the tapping cut (remote injury) and endogenous ethylene released in healed bored holes versus endogenous ethylene released due to the activity of tapping (local and fresh injury) and upon/after *in situ* Mortex stimulation showed accurate consequences, with the latter exhibiting greater stress responses by releasing higher wound ethylene concentration. The synergy in latex cell biochemical properties such as sucrose, thiols and proline together with bark nutrient contents carried out in this study have been evaluated as supporting parameters.

It is plausible that the low level of wound ethylene in healed bored holes located at a distance of 2 cm above tapping cut is considered as endogenous ethylene produced by the trees itself. The effect of injury caused by boring was considered negligible as the boring was only carried out once a month, allowing for healing and may not contribute to the endogenous ethylene released for the following three to four weeks of sample collection. This has been supported by the findings reported for wound ethylene production in Acacia (Nor Mayati et al., 2016), and tomato (O'Donnell et al., 1996; Tatsuki and Mori, 1999) which typically occurs within minutes, hours or days after wounding, and then declines to low levels thereafter. Stimulation with Mortex did not affect measured ethylene gas in the bored holes compared to the shaved bark that was obtained from weekly tapping and with additional *in situ* Mortex stimulation. However, different yielding periods, particularly LYP, slightly contributed to the overall changes in wound ethylene profile in both samples for both clones.

The results indicated that PB 350 was positively responsive to a lower concentration of Mortex at 0.75% applied locally and produced a significantly lower wound ethylene compared to the control, 2.5% and 5% Mortex (Table 1). In contrast, RRIM 2025 released relatively significantly higher wound ethylene with 0.75% and 5% Mortex. Obviously, RRIM 2025 was more sensitive to Mortex and the fresh injury due to tapping could have contributed more stress, leading to higher wound ethylene obtained from the shaved bark samples. However, ethylene antagonist Silversol successfully reduced wound ethylene evolution in both clones.

In Malaysia, generally latex yield peaks from October to January (HYP) and the trees are considered unproductive from February to May (LYP). The transition of yielding periods from LYP to MYP to HYP throughout the year has had some prominent effects on wound ethylene release in both clones tested. Wound ethylene was at significantly lowest level during LYP in both bored holes and shaved bark. During LYP, *Hevea* trees experienced annual wintering pattern, the process where the trees experience defoliation and refoliation of the leaves during dry season starting from February until May (Chantuma et al., 2017), which also lowers the yield (Gireesh et al., 2011) and tapping rest is normally practiced. Some biological and physiological changes occur towards the end of this period when trees are in a stage of full recovery of the foliage. These include starch depletion in the trunk cambium (Chantuma et al., 2009), the demand for temporary assimilate synthesis due to hottest temperature (Silpi et al., 2007), and water stress during dry period (Kunjet et al., 2013).

Endogenous ethylene could take part in accelerating other metabolic events such as new cell division and triggering the degradation of cell walls by cellulose enzyme leading to defoliation. During this annual period, none or limited photosynthesis is occurred, and the trees are considered channelling energy for canopy replenishment known as refoliation (Priyadarshan, 2011). During this restoration event, the trees are in yield depression state and containing inadequate reserves of carbohydrate, which probably attributed to the lowest DRC and sucrose content observed.

The significantly higher sucrose level in RRIM 2025 compared to PB 350 suggested higher productivity of RRIM 2025 clone. RRIM 2025 may produce more sucrose to balance the harvested latex to ensure recovery of the trees, as rubber biosynthesis is strongly regulated by sucrose as a limiting factor in the sink regions (Tangpakdee et al., 1997). High content of sucrose in *Hevea* latex production can be an indicator of either high metabolic production of sucrose or low metabolic utilisation of sucrose (Jacob et al., 1988). The suppression of ethylene action had increased sucrose content while stimulation with Mortex decreased the content. Sucrose synthesis weakened by Ethrel application to the bark was reported earlier (Tupy and Primot, 1982). Furthermore, a reduction of the synthetic activity, followed by an increase in sucrose utilisation and an increase of latex yield, might also be due to the induced rise of pH which enhances invertase activity (Tupy and Primot, 1982; Mesquita et al., 2006). The production and commercialisation of Mortex enriched with crude palm oil is suggested to overcome the loss of nutrients during latex extraction. It is believed that the rich nutrient content of palm oil may provide topical replacement to the loss of nutrients in tapped and stimulated rubber trees. However, limited research has been carried out regarding the benefits of crude palm oil addition in Mortex on the biology and metabolism of rubber production.

At the molecular level, ethylene treatment was reported to induce higher accumulation of sugar transporter (*HbSUT1B*) in latex of clone PB 217 and stronger expression of the same transporter in inner bark tissues of clone PB 260 (Dusotoit-Coucaud et al., 2010). The higher sucrose content in latex of RRIM 2025 without ethylene stimulation and reaching the highest level with ethylene inhibitor treatment may be attributed by the higher basal expression of sugar transporter in RRIM 2025. Meanwhile, low sucrose level could be an indicator of the onset of TPD occurrence (Lacote et al., 2010).

The yield reduction during LYP could also be due to decreased total tree water conductance caused by reduced water uptake in senescencing leaves. Whole or partly leafless condition has led to a declining ability of conduits in the roots-to-stems-to-leaf to transport water (Field and Holbrook, 1989; Sethuraj et al., 1984). The steady state of soil-plant-atmosphere continuum (SPAC) is also interrupted. The flow of water may also be dependent on variable xylem sap composition which is cation composition such as K^+, Mg^+ and few other ions that depends on plant growth, nutrient uptake, seasonal variations and rehydration kinetics in the leaves (Zwieniecki et al., 2001; 2007; Espino and Schenk, 2011).

In some species, mechanical wounding-induced endogenous ethylene production has led to xylem vessel plugging as a defence and healing response (Che Husin et al., 2018; Sun et al., 2007). Latex cytoplasm of *Hevea* trees contains rubber particles that are synthesised in the highly specialised laticifer cells. Exudation of latex upon tissue injuries has been demonstrated as a defence trait in 10% of living angiosperms (Agrawal and Konno, 2009). While latex is not considered as medium for nutrient storage (Farrell et al., 1991; Hunter, 1994;), it does contain a complex mixture of terpenoids, phenolics, proteins, and alkaloids, which are involved in plant defence mechanism (Langenheim, 2003). Previous studies did not confirm latex flow at the tapping panel as a defensive mechanism, and the physiological and biochemical roles of ethylene have remained obscure (d'Auzac, 1989). In 1984, endogenous ethylene concentrations in excised bark from punctured, conventional tapped and spout wounds were examined in *Hevea* clones PB 235, RRIM 703, NAB 17, PR 261, RRIM 605 and PR 107 (Sivakumaran et al., 1984). It has been reported that wound ethylene was significantly higher in the wound-susceptible clones compared to wound-resistant clones (Sivakumaran et al., 1984).

The higher concentrations of exogenous ethylene from trees applied with Mortex at 2.5% and 5% had induced more yields concomitant with the reduced DRC. On the contrary, reduced wounding effects with Silversol contributed to highest level of DRC was observed in this study. The results could suggest that rubber biosynthesis may be activated when ethylene action at cellular level was inhibited by Ag^+ (Beyer, 1976). The result has proven that the yielding performances of the clones were dependent on ethylene regulation. However, it can also be hypothesised that latex flow increases and the duration is extended with the use of yield stimulant, which could account for the higher g/t/t. Meanwhile, the increase in extracted volume could be explained as a dilution factor that resulted in a lower DRC.

The nano size of Ag^+ contained in Silversol was also associated with increased rate of water uptake and may also participate in cation exchange processes on the negatively charged sites of xylem vessel walls to reduce vascular blockage (Veen, 1979; Che-Husin et al., 2018). The improved mobility of complex ionic Ag^+ in the water transport pathway allows effective water flow by competing with the affinity of the ionic exchange and binding site in the vessel walls (Veen and van de Geijn, 1978). The increased DRC with the application of ethylene antagonist Silversol, resulted in decreasing wound ethylene evolution together with increased sucrose and P but decreased proline, K and N contents in the ethylene-sensitive clone. These results may suggest the potential counter action of Silversol in yield inhibition and TPD occurrence in excessive stimulation application. However, further study is required.

Thiol has been described as an antioxidant that participates in reactive oxygen species (ROS)-scavenging system, thus will be able to prevent the occurrence of TPD in rubber trees (Zhang et al., 2017). Exhaustion of thiol content in harvested latex in this study was below 0.5 mM/L, thus the treated trees with Mortex at the concentrations tested could lead to the failure of the activities of ROS-scavenging enzymes (Fan and Yang, 1994; Venkatachalam et al., 2007). These results revealed that the highest wound ethylene evolved were from the trees treated with 2.5% and 5% Mortex, where wounding (tapping) and ethylene stimulation would lead to the production of ROS and trigger the disturbance of basal redox state which caused laticifer dysfunction (Zhang et al., 2017). However, supression of ethylene action by nano size silver ion (Ag^+) in Silversol that reduced wound ethylene production had also reduced thiol content in clone PB 350, suggesting the sensitivity of this clone to ethylene.

In healthy trees, the activity of tapping and exploitation with ethylene hormone accelerate bark regeneration and the TPD activated responses are directed towards healing and defence mechanism of the trees regulated by the growth related genes at the cellular level (Venkatachalam et al., 2007). Thiol also activates some key enzymes and increases regeneration of latex by increasing its metabolic activity (Zhang et al., 2017). In the stimulated trees, higher thiol content is a sign of sufficient level of metabolic activation. Meanwhile, a reduced thiol content is an indicator of high stress which leads to degradation reactions (Jetro and Simon, 2007). Accumulated proline in plant tissue was reported as a response of the trees upon physiological stress particularly related to water deficit such as drought, cold or salt (Steward, 1980; Wickremasinghe et al., 1987). The increase in proline content is an indicator of dryness (Wickremasinghe et al., 1987; Yusof et al., 1995). Incident of dryness, however was not investigated in this study. Based on the results found, the use of ethylene action inhibitor Silversol could be effective to reduce incidence of dryness upon over exploitation of ethylene sensitive clones. The positive effects of Silversol in this study can be further exploited and comprehensive research is necessary.

Nutrient contents particularly nitrogen (N), phosphorus (P), potassium (K) and magnesium (Mg) were analysed from shaved barks used for ethylene measurement in this study. According to Murbach et al. (2003), P and K elements are among the nutrients found redistributed at the highest rate in stems of rubber trees. K usually activates enzymes involved in photosynthesis and respiration by regulating osmotic potential and water loss during the opening and closing of stomata (Ahmad et al., 2012; Arquero et al., 2006). K rates also influence growth by improving plant height, stem diameter and number of leaves in rubber trees (Correia et al., 2017). Both P and Mg contents were found at extremely low levels in both RRIM 2025 and PB 350. Earlier, it has been reported that high inorganic phosphorus (P) content is an indicator of high susceptibility to TPD (Lacote et al., 2010). Meanwhile, magnesium ion assists photosynthesis and is used by plants for the metabolism of carbohydrates and in cell membrane stabilisation. In *H. brasiliensis*, Mg^{2+} was reported to affect rubber transferase activity that suggested its involvement in rubber particle stabilisation during rubber biosynthesis (Xie et al., 2015).

CONCLUSIONS

It was shown that mechanical injuries resulting from tapping and boring holes together with stimulation with exogenous ethylene using Mortex had accelerated the rise of wound ethylene production in RRIM 2025 and PB 350. Mortex at 2.5% was found to be optimum for yield stimulation in both clones. Higher Mortex concentration at 5% had triggered higher wound responses in terms of endogenous ethylene produced and higher thiol content that may trigger incidence of dryness. The treatment with ethylene inhibitor Silversol was effective in overcoming the stress effects related to ethylene regulation.

AUTHORS CONTRIBUTION

NMCH conceived and designed the work. NMCH and MFK performed the analysis. NMCH wrote the paper, and checked and approved the submission.

CONFLICT OF INTEREST

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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REFERENCES

Agrawal, A. A. and Konno, K. (2009). Latex: A model for understanding mechanisms, ecology, and evolution of plant defence against herbivory. *Annual Review Ecological Evolution System*, 40, 311-331.

Ahmad, M., Bukhsh, H. A., Ahmad, R., Iqbal, J., Maqbool, M. M. M., Ali, A., Ishaque, M., Hussain, S. and Khan, D. G. (2012). Nutritional and physiological significance of potassium application in maize hybrid crop production. *Pakistan Journal of Nutrition*, 11, 187-202.

Malaysian Rubber Board (2018). *Natural rubber statistics 2018*. [Online] Available at: http://www.lgm.gov.my/nrstat/Statistics%20Website%202018%20(Jan-Dec).pdf [Accessed 10th May 2020].

Arquero, O., Barranco, D. and Benlloch, M. (2006). Potassium starvation increases stomatal conductance in olive trees. *HortScience*, 41, 433-436.

Aziz, Y., Mohd-Said, M. A., Che-Husin, N. M. and Sarkawi, S. S. (2006). Mortex: new generation of latex stimulant for *Hevea* trees. In: *17th International Invention, Innovation, Industrial Design & Technological Exhibition, ITEX 2006, Kuala Lumpur Convention Centre (KLCC), Kuala Lumpur, 19-21 May 2006*.

Beaudry, R. M. and Kays, S. J. (1988). Application of ethylene-releasing compounds in agriculture. In: Neumann, P. M. (Ed.) *Plant growth and leaf applied chemicals.* Boca Raton, Florida, CRC Press Inc, pp. 127-156.

Beyer, E. M. (1976). A potent inhibitor of ethylene action in plants. *Plant Physiology*, 58, 268-271.

Chantuma, P., Lacointe, A., Kasemsap, P., Thanisawanyangkura, S., Gohet, E., Clement, A., Ameglio, T. and Thaler, P. (2009). Carbohydrate storage in wood and bark of rubber trees submitted to different level of C demand induced by latex tapping. *Tree Physiology*, 29, 1021-1031.

Chantuma, P., Lacote, R., Sonnarth, S. and Gohet, E. (2017). Effects of different tapping rest periods during wintering and summer months on dry rubber yield of *Hevea brasiliensis* in Thailand. *Journal of Rubber Research*, 20(4), 261-272.

Che Husin, N. M. (2006). Dry Rubber Content (DRC): The value of rubber. Advanced Course on Plantation Management 1/2006. Academy *Hevea* Malaysia, Malaysian Rubber Board.

Che Husin, N. M., Liu, J., Joyce, D. C. and Irving, D. E. (2016). Cutting wound ethylene production does not limit the vase life of *Acacia holosericea*. *Scientia Horticulturae*, 212, 35-48.

Che Husin, N. M., Joyce, D. C. and Irving, D. E. (2018). Gel xylem occlusions decrease hydraulic conductance of cut *Acacia holosericea* foliage stems. *Postharvest Biology and Technology*, 135, 27-37.

Chrestin, H., Sookmark, U., Trouslot, P., Pellegrin, F. and Nandris, D. (2004). Rubber tree (*Hevea brasiliensis*) bark necrosis syndrome III: a physiological disease linked to impaired cyanide metabolism. *Plant Discovery*, 88, 1047.

Correia, M. A. R., Maranhão, D. D. C., Flores, R. A., da Silva Júnior, S. F., de Araujo, M. A. and Leite, R. L. L. 2017. Growth, nutrition and production of dry matter of rubber tree (*Hevea brasiliensis*) in function of K fertilization. *Australian Journal of Crop Science*, 11, 95-101.

d'Auzac, J. (1989). *The hormonal stimulation of latex yield*. Boca Raton, Florida: CRC Press, Inc.

Druege, U. (2006). Ethylene and plant responses to abiotic stress. In: Khan, N. A. (Ed.) *Ethylene action in plants*. New York, Springer-Verlag Berlin Heidelberg, pp. 80-117.

Dusotoit-Coucaud, A., Kongsawadworakul, P., Maurousset, L., Viboonjun, U., Brunel, N., Pujade-Renaud, V., Chrestin, H. and Sakr, S. (2010). Ethylene stimulation of latex yield depends on the expression of a sucrose transporter (*HbSUT1B*) in rubber tree (*Hevea brasiliensis*). *Tree Physiology*, 30, 1586-1598.

Eschbach, J. M., Roussel, D., Van De Sype, H., Jacob, J. L. and D'Auzac J. (1984). Relationships between yield and clonal physiological characteristics of latex from *Hevea brasiliensis*. *Physiologie Vegetale*, 22, 295-304.

Espino, S. and Schenk, H. J. (2011). Mind the bubbles: achieving stable measurements of maximum hydraulic conductivity through woody plant samples. *Journal of Experimental Botany*, 62, 1119-1132.

Fan, X. W. and Yang, S. Q. (1994). Cause of disease and hypothesis on tapping panel dryness of *Hevea brasiliensis*. *China Journal of Tropical Crops Research*, 18, 43-48.

Farrell, B. D., Dussourd, D. E. and Mitter, C. (1991). Escalation of plant defence: do latex and resin canals spur plant diversification. *America National Journal*, 138, 881-900.

Field, C. B. and Holbrook, N. M. (1989). Catastrophic xylem failure: tree life at the brink. *Trends in Ecology and Evolution*, 4, 124-126.

Fujita, M., Fujita, Y., Noutoshi, Y., Takahashi, F., Narasuka, Y., Yamaguchi-Shinozaki, K. and Shinozaki, K. (2006). Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. *Current Opinion in Plant Biology*, 9, 436-442.

Gireesh, T., Raj, S., Mydin, K. K. and Mercykutty, V. C. (2011). Rubber yield, of certain clones of *hevea brasiliensis* and its relationship with climate variables. *National Rubber Research*, 24, 54-60.

Hunter, J. (1994). Reconsidering the functions of latex. *Trees-Structural Functional*, 9, 1-5.

IRRDB (International Rubber Research and Development Board) (1995). Manual of biochemical and physiological tests (ref 1995/3). Ref. 1995/3, January 26.

Jacob, J. L., Serres, E., Prevot, J. C., Lacrotte, R., Vidal, A., Eschbach, J. M. and D'Auzac, J. (1988). Development of the *Hevea* latex diagnostic. *Agritrop*, 12, 97.

Jetro, N. N. and Simon, G. M. (2007). Effect of 2-chloroethylphosphonic acid formulations as yield stimulants on *Hevea brasiliensis*. *African Journal of Biotechnology*, 6, 523-528.

Kunjet, S., Thaler, P., Gay, F., Chuntuma, P., Sangkhasila, K. and Kasemsap, P. (2013). Effects of drought and tapping for latex production on water relations of *Hevea brasiliensis* Trees. *Kasetsart Journal (Natural Science)*, 47, 506-515.

Lacote, R., Gabla, O., Obouayeba, S., Eschbach, J. M., Rivano, F., Dian, K. and Gohet, E. (2010). Long-term effect of ethylene stimulation on the yield of rubber trees is linked to latex cell biochemistry. *Field Crops Research*, 115, 94-98.

Langenheim, J. H. (2003). *Plant resins: chemistry, evolution, ecology, and ethnobotany*. Portland: Timber Press.

Lau, C. H. and Mahmud, A. W. (1992). Dissolution of phosphate rocks in Malaysian soils under rubber and their availability to plants. *Journal National Rubber Research*, 7, 71-83.

Mesquita, A. C., de Oliveira L. E. M., Mazzafera, P. and Delú-Filho, N. (2006). Anatomical characteristics and enzymes of the sucrose metabolism and their relationship with latex yield in the rubber tree (*Hevea brasiliensis* Muell. Arg.). *Brazilian Journal of Plant Physiology*, 18(2), 263-268.

Mohd Akbar, M. S. (2006). Harvesting the yield targeted. In: *Proceedings of Rubber Planters Conference, Malaysian Rubber Board, Kuala Lumpur, 15-17 November 2006*.

Murbach, M. R., Boatetto, A. E., Muraoka, T. and Souza, E. C. A. (2003). Nutrient cycling in a RRIM 600 clone rubber plantation. *Science Agriculture*, 60, 353-357.

Nor Mayati, C. H. and Abd-Razak, A. (2018). Preliminary results of latex yield profile and growth performance of tissue culture derived *Hevea brasiliensis* trees. *Journal of Tropical Plant Physiology*, 10, 46-55.

Nor Mayati, C. H. and Mohd Fazri, K. (2017). Wound ethylene evolution in RRIM 2025 and PB 350 during low yielding period. *Transactions of the Malaysian Society of Plant Physiology*, 25, 103-108.

Nor Mayati, C. H., Jiping, L., Daryl, C. J. and Irving, D. E. (2016). Cutting wound ethylene production does not limit the vase life of *Acacia holosericea*. *Scientia Horticulturae*, 212, 35-48.

O'Donnell, P. J., Calvert, C., Atzorn, R., Wasternack, C., Leyser, H. M. O. and Bowles, D. J. (1996). Ethylene as a signal mediating the wound response of tomato plants. *Science*, 274, 1914-1917.

Priyadarshan, P. M. (2011). *Biology of Hevea rubber*. Wallingford: CABI Publishing.

Putranto, R. A., Herlinawati, E., Rio, M., Leclercq, J., Piyatrakul, P., Gohet, E., Sanier, C., Oktavia, F., Pirrello, J., Kuswanhadi, and Montoro, P. (2015). Involvement of ethylene in the latex metabolism and tapping panel dryness of *Hevea brasiliensis*. *International Journal of Molecular Sciences*, 16, 17885-17908.

Sethuraj, M. R., Rao, G. G. and Raghavendra, A. S. (1984). The pattern of latex flow from rubber tree (*Hevea brasiliensis*) in relation to water stress. *Journal of Cellular Biochemistry*, 8B, S-236.

Silpi, U., Lacointe, A., Kasempsap, P., Thanysawanyangkura, S., Chantuma, P., Gohet, E., Musigamart, N., Clement, A., Ameglio, T. and Thaler, P. (2007). Carbohydrate reserves as a competing sink: evidence from tapping rubber trees. *Tree Physiology*, 27, 881-889.

Sivakumaran, S. (2002). Exploitation systems to maximize yield productivity and enhance profitability in rubber. In: Jacob, C. K. (Ed.) *Global competitiveness of Indian rubber plantation industry*: *Rubber Planters' Conference*. Kottayam, India, pp. 163-174.

Sivakumaran, S., Yeang, H. Y. and Ming, Y. W. (1984). Endogenous ethylene in *Hevea* bark tissues. *Journal of Rubber Research Institute of Malaysia*, 32, 155-163.

Sivakumaran, S., Ismail, T., Tham, F. K. and Tham, F. C. (2007). RRIMFLOW system of exploitation: assessment of performance on renewed bark. In: *CRRI & IRRDB International Rubber Conference 2007, Siem Reap, Cambodia, 12-13 November 2007.*

Stewart, C. R. (1980). The mechanism of abscissic acid-induced proline accumulation in barley leaves. *Plant Physiology*, 66(2), 230-233.

Sun, Q., Rost, T. L., Reid, M. S. and Matthews, M. A. 2007. Ethylene and not embolism is required for wound-induced tylose development in stems of grapevines. *Plant Physiology*, 145,1629-1636.

Tangpakdee, J., Tanaka, Y., Ogura, K., Koyama, T., Wititsuwannakul, R. and Wititsuwannakul, D. (1997). Rubber formation by fresh bottom fraction of *Hevea* latex. *Phytochemistry*, 45, 269-274.

Tatsuki, M. and Mori, H. (1999). Rapid and transient expression of 1-aminocyclopropane-1-carboxylate synthase isogenes by touch and wound stimuli in tomato. *Plant Cell Physiology*, 40, 709-715.
Tupý, J. and Primot, L. (1982). Sucrose synthetase in the latex of *Hevea brasiliensis* Muell. *Argricultural Journal of Experimental Botany*, 33, 988-995.
Veen, H. (1979). Effects of silver on ethylene synthesis and action in cut carnations. *Planta*, 145, 467-470.
Veen, H. and van de Geijn, S. C. (1978). Mobility and ionic form of silver as related to longevity of cut carnations. *Planta*, 140, 93-96.
Venkatachalam, P., Thulaseedharan, A. and Raghothama, K. (2007). Identification of expression profiles of tapping panel dryness (TPD) associated genes from the latex of rubber tree (*Hevea brasiliensis* Muell. Arg.). *Planta*, 226, 499-515.
Wang, K. L. C., Li, H. and Ecker, J. R. (2002). Ethylene biosynthesis and signaling networks. *Plant Cell*, 14, 131-151.
Warner, H. L. and Leopold, A. C. (1969). Ethylene evolution from 2-chloroethylphosphonic acid. *Plant Physiology*, 44, 156-158.
Wickremasinghe, S. I., Yapa, P. A. J. and de Soyza, T. V. W. (1987). Accumulation of free proline in bark and latex of *Hevea brasiliensis* affected by brown bast. *Journal of the Rubber Research Institute of Sri Lanka*, 67, 9-17.
Xie, W., Cornish, K., McMahan, C. M., Whalen, M. C., Shintani, D. K., Coffelt, T. A. and Mattoso, H. L.C. (2015). Magnesium affects rubber biosynthesis and particle stability in *Ficus elastica*, *Hevea brasiliensis* and *Parthenium argentatum*. Association for the Advancement of Industrial Crops Conference, 7, 292-299.
Yang, S. F. (1969). Ethylene evolution from 2-chloroethylphosphonic acid. *Plant Physiology*, 44, 1203-1204.
Yusof, F., Siti Arija, M. A., Gandimathi, H., Hamzah, Z., Sivakumaran, S. and Yeang, H. Y. (1995). Changes in some physiological latex parameters in relation to overexploitation and the onset of induced tapping panel dryness. *Journal Rubber Research*, 10, 182-198.
Zhang, Y., Leclercq, J. and Montoro, P. (2017). Reactive oxygen species in *Hevea brasiliensis* latex and relevance to tapping panel dryness. *Tree Physiology*, 37, 261-269.
Zwieniecki, M. A., Melcher, P. J. and Holbrook, N. M. (2001). Hydrogel control of xylem hydraulic resistance in plants. *Science*, 291, 1059-1062.
Zwieniecki, M. A., Brodribb, T. J. and Holbrook, N. M. (2007). Hydraulic design of leaves: insight from rehydration kinetics. *Plant, Cell and Environment*, 30, 910-921.