

Stimulation affecting latex physiology and yield under low frequency tapping of rubber (*Hevea brasiliensis*) clone RRIM 600 in southern Thailand

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Abstract

Tapping and ethylene stimulation induces the dynamic change of latex cell metabolism. Those changes under the implement of low frequency tapping systems with ethylene stimulation were investigated during the both periods (low yield and the high yield) of the year of production. The experiment was established at Thepa Research Station, Songkhla province by using 9-year-old rubber trees (clone RRIM 600). An experiment was arranged as One Tree Plot design, there were five treatments following T1: S/3 d1 2d/3, T2: S/2 d2, T3: S/2 d3 ET 2.5% Pa1(1) 8/y (m), T4: S/3 d2 ET 2.5% Pa1(1) 4/y (m) and T5: S/3 d3 ET 2.5% Pa1(1) 12/y (m). There were three replicates in each treatment. It was found that ethylene stimulation affected the responses of initial flow rate (IFR), plugging index (PI), average latex yield (AY) and sucrose content (Suc). There was a significant difference among the treatments in the both periods. After ethylene stimulation, IFR of the T3 and T5 were superior in the low yield period and showed inferior in the high yield period compared with the T1. PI rapidly decreased in the ethylene application treatments in the both periods. AY of the T3 was the highest in the low yield period and AY of the ethylene stimulation treatments was higher than non-stimulated treatments in the high yield period. Suc of the T3 only increased on the first tapping day after stimulation in the both periods. Expression to the ethylene stimulation in the low yield period was higher than the high yield period. It was remarkable that the stimulation was effectively expressed during a full canopy stage. With the positive impact of stimulation on latex physiological parameters, latex yield under low frequency tapping could be compensated by ethylene application.

Keywords: *Hevea brasiliensis*, Initial flow rate, Plugging index, Latex yield, Sucrose content, Stimulation.

Abbreviations: S_spiral cut; d_daily tapping; IFR_initial flow rate; PI_plugging index; AY_average latex yield; Suc_sucrose content; LY_low yield period; HY_high yield period

Introduction

Rubber tree (*Hevea brasiliensis*) is an important global economic crop yielding natural rubber. It is extensively planted in the Southeast Asia. In Thailand, RRIM 600 clone is grown on 75% of the rubber plantation in the region (Nakkanong et al., 2008; Pethin et al., 2015), especially in the South of Thailand. The yield is based on latex biosynthesis of rubber trees. Latex is extracted by using a multi-annual tapping system. Linked to the yield per tapping (Lacote et al., 2013), physiological parameters known as Latex Diagnosis or LD (Jacob et al., 1989) are indicators of the potential yield. It is well known that LD parameters can be evaluated under different latex harvesting systems; including tapping frequency and the use of ethylene stimulation (2-chloroethylphosphonic acid) (Jacob et al., 1989; d'Auzac et al., 1997; Soumahin et al., 2009; Kudaligama et al., 2010; Lacote et al., 2010; Njukeng et al., 2011; Traore et al., 2011). It has been reported that the yield at each tapping depends on both internal factors (clone and tree age) and external factors (tapping systems, stimulation, the depth, slope and length of

tapping cut) (Njukeng et al., 2011; Traore et al., 2011; Gunasekera et al., 2013).

The latex physiological processes involved in latex flow, such as initial flow rate, plugging index, and dry rubber content is closely linked to yield (Gunasekera et al., 2002). Buttery and Boatman (1964, 1966) showed that the flow rate is slow down rapidly due to loss in turgor until the flow ceases eventually by the mechanism of latex vessel plugging (Milford et al., 1969). In addition, the diameter and density of latex vessel can assist with obtaining increased yield (Mesquita et al., 2006). Biochemical parameters of latex itself also relate to the yield (Jacob et al., 1989; Gunasekera et al., 2013; Pethin et al., 2015), especially sucrose because latex biosynthesis takes place within the latex vessels using sucrose (Tupy, 1973, 1985; Dusotoit-Coucaud et al., 2009). Latex exudation after tapping causes rapidly initial latex-flow. Latex flow duration is normally extended by ethylene stimulation (Yeang, 2005) because of physiological change in the drainage area of the cut after tapping (Gunasekera et al., 2013). The measurement of the latex vessel plugging rate is

commonly used as the plugging index (Yeang, 2005; Gunasekera et al., 2013). Plugging index is related to many other clonal characters. It is negatively correlated with yield and incidence of dryness and positively correlated with girth, dry rubber content of latex and magnitude of the response to yield stimulation (Waidyanatha and Pathiratne, 1971). It is well known that the low frequency tapping system with stimulation could reduce the risk on rubber production (Soumahin et al., 2009, 2010; Prasanna et al., 2010) and it has been applied to test the efficiency in the South of Thailand. The objective of this investigation was to assess the responses of yield and the changes of biochemical parameters to ethylene stimulation under low frequency tapping comparing with the other tapping intensities.

Results

The difference of tapping systems on latex physiology

The changes of initial flow rate (IFR), plugging index (PI), average latex yield (AY) and sucrose content (Suc) according to the different tapping systems and tapping days before and after stimulation in the two periods of study are shown in Table 3. IFR was significantly different each tapping day in both the low yield period and the high yield period of the 5 treatments except on the second tapping day before stimulation in the low yield period. The response of IFR gradually increased during succeeding tapping days. IFR of T3 and T5 with reduced tapping frequency were higher than the other treatments on each tapping day in the low yield period; conversely, these treatments showed lower IFR than that of the other treatments in the high yield period. IFR increased on the first tapping day after stimulation only, of T3 and T5 in the low yield period. Conversely, IFR of ethylene stimulation treatments rapidly increased on the first tapping day after stimulation in the high yield period. Under alternating tapping frequencies, IFR of T2 was higher than T4 in the both periods.

PI was significantly different on each tapping day of both low yield period and high yield period among the 5 treatments, except the 2 tapping days before stimulation in the low yield period (Table 3). PI responded to tapping and gradually decreased during succeeding tapping days. The highest PI in each tapping day was found for T1 in the low yield period and T2 in the high yield period. Stimulation treatments (T3, T4 and T5) showed lower PI than that of the non-stimulated treatments (T1 and T2) and it rapidly decreased on the first tapping day after stimulation in both the low yield period and the high yield period.

AY showed significant difference each tapping day in the both periods of the 5 treatments except on the second tapping day before ethylene stimulation (Table 3). AY tended to increase during succeeding tapping days. AY of T3, T4 and T5 were higher than that of T1 and T2 for the 3 tapping days after stimulation in the low yield period, with T3 showed the highest. However, AY of T2 and T4 did not show significant difference on each tapping day. Besides, AY of T3 and T5 immediately increased on the first tapping day after ethylene stimulation. In the high yield period, AY of T4 showed the highest in the 2 tapping days before stimulation and the first tapping day after stimulation, while T3 expressed the highest on the second and the third tapping days after stimulation. In addition, AY in the 3 tapping days after stimulation of T2, T3, T4 and T5 expressed higher than that of the T1.

Regarding the sucrose content of the latex cells, Suc showed a significant difference in each tapping day in both the low yield period and the high yield periods among the 5

treatments (Table 3). The trend of Suc change was ambiguous. In both periods, Suc of T1 was the highest in the 2 tapping days before stimulation. Suc decreased in all treatments except T3, on the first tapping day after stimulation. In addition, Suc of T4 and T5 recovered and it was higher than the other treatments on the second and the third tapping days after ethylene stimulation. However, the response to ethylene stimulation of Suc during the high yield period was somewhat less than in the low yielding period.

The variation of season on latex physiology

Table 4 shows the comparison of IFR and PI between both periods on each tapping day of the 5 treatments. IFR on each tapping day of the T1, T2 and T4 in the low yield period was lower than that of the high yield period, but IFR of the T3 and T5 in the low yield period was higher than the high yield period in the 3 tapping days after stimulation. PI in the 2 tapping days before stimulation in the low yield period was significantly higher than the high yield period of the 5 treatments except the T2. In the same manner for the 3 tapping days after stimulation, PI of the 5 treatments in the low yield period were significantly higher than that of the high yield period.

Table 5 shows the comparison of AY and Suc between both periods on each tapping day of the 5 treatments. AY on each tapping day in the high yield period was higher than that of the low yield period. On 2 tapping days before stimulation, AY of the 5 treatments in the high yield period was significantly higher than in the low yield period except T5 on the first tapping day before stimulation. On the 3 tapping days after stimulation, AY of T1, T2, T4 and T5 in the high yield period was significantly higher than in the low yield period. But AY of T3 did not show any significant difference in the both periods. Suc of the 5 treatments in the low yield period were higher than that of the high yield period except Suc in T2.

Percentage changed of latex physiology after stimulation

Table 6 shows the response of IFR to ethylene stimulation on the 3 tapping days after stimulation of the 5 treatments. In both periods, IFR of T1 did not show significant differences on the 3 tapping days except on the second tapping day in the high yield period. IFR of T2 showed a significant difference on the 3 tapping days. However, the T1 and T2 did not receive any stimulation. For the response to ethylene stimulation in both periods, IFR of the T3 showed a significant difference on the 3 tapping days after stimulation except on the second tapping day after stimulation in the high yield period. IFR of the T4 showed no significant difference on the 3 tapping days after stimulation except on the first tapping day after stimulation in the high yield period. IFR of the T5 showed a significant difference on the 3 tapping days after stimulation except on the first tapping day after stimulation in the low yield period.

The response of PI to ethylene stimulation on the 3 tapping days after stimulation of the 5 treatments is illustrated in Table 7. In both periods, PI of T1 did not show a significant difference on the 3 tapping days except on the second tapping day in the low yield period and on the third tapping day in the high yield period. PI of T2 did not show significant differences on the 3 tapping days except on the first and the third tapping days in the high yield period. However, T1 and T2 did not receive any stimulation. Besides, the response to ethylene stimulation in the both periods showed that PI of T3 was significantly different on the 3 tapping days after

Table 1. Treatments (tapping system) of the experiment.

Treatments	Tapping system and Description	TI*
T1	S/3 d1 2d/3 Third spiral cut downward at daily tapping, two days in tapping followed by one day of tapping rest in three days	89
T2	S/2 d2 Half spiral cut downward at alternate daily tapping	100
T3	S/2 d3 ET 2.5% Pa1(1) 8/y (m) Half spiral cut downward at third daily tapping, stimulated with ethephon of 2.5% active ingredient with 1 gram of stimulant applied on panel on 1 centimeter band, 8 applications per years	67
T4	S/3 d2 ET 2.5% Pa1(1) 4/y (m) Third spiral cut downward at alternate daily tapping, stimulated with ethephon of 2.5% active ingredient with 1 gram of stimulant applied on panel on 1 centimeter band, 4 applications per years	67
T5	S/3 d3 ET 2.5% Pa1(1) 12/y (m) Third spiral cut downward at third daily tapping, stimulated with ethephon of 2.5% active ingredient with 1 gram of stimulant applied on panel on 1 centimeter band, 12 applications per years	44

Note: *TI is tapping intensity

Table 2. Schedule of tapping and latex sampling.

Before stimulation ^[a]		Stimulation day ^[c]			After stimulation ^[b]	
Day 1	Day 2	Day 3		Day 4	Day 5	Day 6
1 st tapping	2 nd tapping	ET		1 st tapping	2 nd tapping	3 rd tapping
Tapping (-2)	Tapping (-1)			Tapping (+1)	Tapping (+2)	Tapping (+3)

Note: ^[a] Before ethylene stimulation was tapping day without ethylene stimulation; ^[b] After ethylene stimulation was tapping day with ethylene stimulation; ^[c] Stimulation day was day that ethylene stimulation (no tapping). ET = ethephon stimulation.

Table 3. Initial flow rate (IFR) (ml/min); plugging index (PI); average latex yield (AY) (g/t) and sucrose content (Suc) (mM) in each tapping day among the 5 treatments (T1: S/3 d1 2d/3; T2: S/2 d2; T3: S/2 d3 ET 2.5% Pa1(1) 8/y (m); T4: S/3 d2 ET 2.5% Pa1(1) 4/y (m); T5: S/3 d3 ET 2.5% Pa1(1) 12/y (m)) in the low yield period and the high yield period.

P ^[a]	T ^[b]	Low yield period					High yield period				
		TP ^[c] (-2)	TP (-1)	TP (+1)	TP (+2)	TP (+3)	TP (-2)	TP (-1)	TP (+1)	TP (+2)	TP (+3)
IFR	T1	0.51 ^b	1.20	1.11 ^b	1.29 ^b	2.07 ^a	1.71 ^{ab}	1.86 ^a	1.81 ^c	2.34 ^b	2.34 ^{ab}
	T2	0.57 ^b	0.80	1.13 ^b	1.30 ^b	1.99 ^a	1.91 ^{ab}	1.91 ^a	3.00 ^a	3.67 ^a	3.11 ^a
	T3	0.78 ^{ab}	0.88	1.78 ^a	2.05 ^a	2.39 ^a	1.34 ^b	0.97 ^b	1.51 ^d	1.51 ^c	1.56 ^b
	T4	0.59 ^b	0.73	0.88 ^b	0.85 ^b	1.00 ^b	2.17 ^a	1.59 ^a	2.12 ^b	1.14 ^c	1.43 ^b
	T5	0.92 ^a	0.82	1.27 ^{ab}	2.27 ^a	2.29 ^a	0.53 ^c	0.64 ^b	1.22 ^d	1.38 ^c	1.84 ^b
PI	T1	7.37	7.50	6.85 ^a	4.71 ^a	5.13 ^a	5.05 ^a	2.58 ^a	2.51 ^a	2.09 ^a	1.90 ^a
	T2	6.21	5.95	4.43 ^b	3.54 ^b	3.72 ^b	5.39 ^a	2.96 ^a	2.69 ^a	2.45 ^a	1.93 ^a
	T3	5.46	4.98	1.64 ^c	1.35 ^c	1.45 ^c	2.18 ^b	1.57 ^b	1.18 ^b	0.87 ^b	0.90 ^b
	T4	7.83	6.47	2.59 ^c	1.76 ^c	1.46 ^c	1.73 ^b	1.31 ^b	1.20 ^b	0.86 ^b	0.93 ^b
	T5	5.13	6.72	2.17 ^c	2.16 ^c	2.11 ^c	2.86 ^b	1.56 ^b	1.58 ^b	0.93 ^b	1.10 ^b
AY	T1	3.39 ^b	7.22	8.06 ^c	13.10 ^d	18.25 ^d	16.14 ^c	36.61 ^b	34.52 ^c	53.22 ^c	55.56 ^c
	T2	3.86 ^b	6.43	11.81 ^c	17.47 ^{cd}	24.60 ^{cd}	18.04 ^c	35.19 ^b	55.41 ^b	70.81 ^{ab}	69.40 ^{ab}
	T3	6.92 ^a	8.84	50.91 ^a	68.39 ^a	71.11 ^a	26.37 ^b	30.75 ^b	59.09 ^{ab}	78.29 ^a	73.33 ^a
	T4	3.98 ^b	5.50	16.97 ^{bc}	24.92 ^c	33.94 ^c	47.32 ^a	56.20 ^a	66.56 ^a	57.97 ^{bc}	59.78 ^{bc}
	T5	7.68 ^a	5.97	28.40 ^b	45.69 ^b	47.86 ^b	7.99 ^d	20.36 ^c	40.17 ^c	74.18 ^a	73.04 ^a
Suc	T1	9.30 ^a	7.81 ^a	3.88 ^a	3.76 ^b	2.28 ^c	5.91 ^a	3.71 ^a	1.72 ^c	2.78 ^{bc}	2.52 ^{bc}
	T2	7.01 ^{ab}	3.35 ^c	2.03 ^b	1.31 ^c	1.67 ^c	3.12 ^{bc}	3.64 ^a	2.96 ^a	2.23 ^c	3.50 ^a
	T3	-	3.09 ^c	4.48 ^a	3.30 ^{bc}	3.96 ^b	1.57 ^d	1.63 ^d	1.77 ^c	2.83 ^{bc}	1.89 ^c
	T4	5.48 ^b	3.50 ^{bc}	1.78 ^b	6.77 ^a	7.37 ^a	2.78 ^c	2.38 ^c	1.34 ^c	4.28 ^a	2.81 ^b
	T5	5.05 ^b	4.51 ^b	3.53 ^a	7.91 ^a	4.93 ^b	4.02 ^b	3.01 ^b	2.34 ^b	3.09 ^b	2.72 ^b

Note: Means with different letters in the same column indicate significant difference at $P \leq 0.05$ by DMRT. ^[a]P = Parameters; ^[b]T = Treatments; ^[c]TP = Tapping; TP (-2, -1) = tapping days before ethylene stimulation; TP (+1, +2, +3) = tapping days after ethylene stimulation.

Table 4. Initial flow rate (ml/min) and plugging index in each tapping day between the low yield period (LY) and the high yield period (HY) of the 5 treatments (T1: S/3 d1 2d/3; T2: S/2 d2; T3: S/2 d3 ET 2.5% Pa1(1) 8/y (m); T4: S/3 d2 ET 2.5% Pa1(1) 4/y (m); T5: S/3 d3 ET 2.5% Pa1(1) 12/y (m)).

T ^[a]	PR ^[b]	IFR: Initial flow rate (ml/min)					PI: Plugging index				
		TP ^[c] (-2)	TP (-1)	TP (+1)	TP (+2)	TP (+3)	TP (-2)	TP (-1)	TP (+1)	TP (+2)	TP (+3)
T1	LY	0.51 ^b	1.20	1.11 ^b	1.29 ^b	2.07	7.37 ^a	7.50 ^a	6.85 ^a	4.71 ^a	5.13 ^a
	HY	1.71 ^a	1.86	1.81 ^a	2.34 ^a	2.34	5.05 ^b	2.58 ^b	2.51 ^b	2.09 ^b	1.90 ^b
T2	LY	0.57 ^b	0.80 ^b	1.13 ^b	1.30 ^b	1.99	6.21	5.95	4.43 ^a	3.54 ^a	3.72 ^a
	HY	1.91 ^a	1.91 ^a	3.00 ^a	3.67 ^a	3.11	5.39	2.96	2.69 ^b	2.45 ^b	1.93 ^b
T3	LY	0.78	0.88	1.78	2.05	2.39 ^a	5.46 ^a	4.98 ^a	1.64 ^a	1.35 ^a	1.45 ^a
	HY	1.34	0.97	1.51	1.51	1.56 ^b	2.18 ^b	1.57 ^b	1.18 ^b	0.87 ^b	0.90 ^b
T4	LY	0.59 ^b	0.73 ^b	0.87 ^b	0.85	1.00	7.83 ^a	6.47 ^a	2.59 ^a	1.76 ^a	1.46
	HY	2.17 ^a	1.59 ^a	2.12 ^a	1.14	1.43	1.73 ^b	1.31 ^b	1.20 ^b	0.82 ^b	0.93
T5	LY	0.92	0.82 ^a	1.27	2.27 ^a	2.29	5.13 ^a	6.72 ^a	2.17 ^a	2.16 ^a	2.11 ^a
	HY	0.53	0.64 ^b	1.22	1.38 ^b	1.84	2.86 ^b	1.56 ^b	1.58 ^b	0.93 ^b	1.10 ^b

Note: Means with different letters in the same column indicate significant difference at $P \leq 0.05$ by DMRT. ^[a]T = Treatments; ^[b]PR = Periods; ^[c]TP = Tapping; TP (-2, -1) = tapping days before ethylene stimulation; TP (+1, +2, +3) = tapping days after ethylene stimulation.

Table 5. Average latex yield (g/t) and sucrose content (mM) in each tapping day between the low yield period (LY) and the high yield period (HY) of the 5 treatments (T1: S/3 d1 2d/3; T2: S/2 d2; T3: S/2 d3 ET 2.5% Pa1(1) 8/y (m); T4: S/3 d2 ET 2.5% Pa1(1) 4/y (m); T5: S/3 d3 ET 2.5% Pa1(1) 12/y (m)).

T ^[a]	PR ^[b]	AY: Average latex yield (g/t)					Suc: Sucrose content (mM)				
		TP ^[c] (-2)	TP (-1)	TP (+1)	TP (+2)	TP (+3)	TP (-2)	TP (-1)	TP (+1)	TP (+2)	TP (+3)
T1	LY	3.39 ^b	7.22 ^b	8.06 ^b	13.10 ^b	18.25 ^b	9.30 ^a	7.81 ^a	3.88 ^a	3.76 ^a	2.28
	HY	16.14 ^a	36.61 ^a	34.52 ^a	53.22 ^a	55.56 ^a	5.91 ^b	3.71 ^b	1.72 ^b	2.78 ^b	2.52
T2	LY	3.86 ^b	6.43 ^b	11.81 ^b	17.47 ^b	24.60 ^b	7.01 ^a	3.35	2.03 ^b	1.31 ^b	1.67 ^b
	HY	18.04 ^a	35.19 ^a	55.41 ^a	70.81 ^a	69.40 ^a	3.12 ^b	3.64	2.96 ^a	2.23 ^a	3.50 ^a
T3	LY	6.92 ^b	8.84 ^b	50.91	68.39	71.11	-	3.09 ^a	4.48 ^a	3.30	3.96 ^a
	HY	26.37 ^a	30.75 ^a	59.09	78.29	73.33	1.57	1.63 ^b	1.77 ^b	2.83	1.89 ^b
T4	LY	3.98 ^b	5.50 ^b	16.97 ^b	24.92 ^b	33.94 ^b	5.48 ^a	3.50	1.78	6.77 ^a	7.37 ^a
	HY	47.32 ^a	56.20 ^a	66.56 ^a	57.97 ^a	59.78 ^a	2.78 ^b	2.38	1.34	4.28 ^b	2.81 ^b
T5	LY	7.68	5.97 ^b	28.40	45.69 ^b	47.86 ^b	5.05	4.51 ^a	3.53 ^a	7.91 ^a	4.93 ^a
	HY	7.99	20.36 ^a	40.17	74.18 ^a	73.04 ^a	4.02	3.01 ^b	2.34 ^b	3.09 ^b	2.72 ^b

Note: Means with different letters in the same column indicate significant difference at $P \leq 0.05$ by DMRT. ^[a]T = Treatments; ^[b]PR = Periods; ^[c]TP = Tapping; TP (-2, -1) = tapping days before ethylene stimulation; TP (+1, +2, +3) = tapping days after ethylene stimulation.

Table 6. Response of IFR to ethylene stimulation in percentage of the difference in delta (% Δ) before and after ethylene application in the low yield period and the high yield period among the 5 treatments (T1: S/3 d1 2d/3; T2: S/2 d2; T3: S/2 d3 ET 2.5% Pa1(1) 8/y (m); T4: S/3 d2 ET 2.5% Pa1(1) 4/y (m); T5: S/3 d3 ET 2.5% Pa1(1) 12/y (m)).

Treatments	IFR: Initial flow rate (ml/min)					
	Low yield period			High yield period		
	% Δ TP ^[a] (+1)	% Δ TP (+2)	% Δ TP (+3)	% Δ TP (+1)	% Δ TP (+2)	% Δ TP (+3)
T1	-8.0 ^{ns}	7.7 ^{ns}	72.0 ^{ns}	-2.6 ^{ns}	25.9 ^{**}	25.8 ^{ns}
T2	42.0 ^{**}	62.6 ^{**}	150.0 [*]	56.9 ^{**}	92.0 ^{**}	62.5 [*]
T3	103.1 [*]	133.3 [*]	173.0 ^{**}	56.0 [*]	55.7 ^{ns}	61.4 [*]
T4	20.3 ^{ns}	17.1 ^{ns}	37.0 ^{ns}	33.8 [*]	-28.0 ^{ns}	-9.8 ^{ns}
T5	54.8 ^{ns}	176.9 ^{**}	179.5 ^{**}	91.1 ^{**}	116.0 [*]	187.8 [*]

Note: ^[a]TP = Tapping; Data of tapping day after stimulation (TP (+1), TP (+2) and TP (+3)) were compared with tapping day before stimulation (TP (-1)) of the 5 treatments. ns = not significant, * = significant ($P \leq 0.05$), ** = highly significant ($P \leq 0.01$).

Table 7. Response of PI to ethylene stimulation in percentage of the difference in delta (% Δ) before and after ethylene application in the low yield period and the high yield period among the 5 treatments (T1: S/3 d1 2d/3; T2: S/2 d2; T3: S/2 d3 ET 2.5% Pa1(1) 8/y (m); T4: S/3 d2 ET 2.5% Pa1(1) 4/y (m); T5: S/3 d3 ET 2.5% Pa1(1) 12/y (m)).

Treatments	PI: Plugging index					
	Low yield period			High yield period		
	% Δ TP ^[a] (+1)	% Δ TP (+2)	% Δ TP (+3)	% Δ TP (+1)	% Δ TP (+2)	% Δ TP (+3)
T1	-8.6 ^{ns}	-37.2 [*]	-31.6 ^{ns}	-2.9 ^{ns}	-19.2 ^{ns}	-26.6 [*]
T2	-25.6 ^{ns}	-40.5 ^{ns}	-37.5 ^{ns}	-8.8 ^{**}	-17.2 ^{ns}	-34.8 ^{**}
T3	-67.0 ^{**}	-72.9 ^{**}	-70.9 ^{**}	-24.8 [*]	-44.7 ^{**}	-42.4 ^{**}
T4	-60.0 ^{**}	-72.7 ^{**}	-77.5 ^{**}	-8.2 ^{ns}	-37.8 [*]	-28.7 ^{ns}
T5	-67.7 [*]	-68.0 [*]	-68.6 [*]	-1.2 ^{ns}	-40.5 [*]	-29.9 ^{ns}

Note: ^[a]TP = Tapping; Data of tapping day after stimulation (TP (+1), TP (+2) and TP (+3)) were compared with tapping day before stimulation (TP (-1)) of 5 treatments. ns = not significant, * = significant ($P \leq 0.05$), ** = highly significant ($P \leq 0.01$).

Table 8. Response of AY to ethylene stimulation in percentage of the difference in delta (% Δ) before and after ethylene application in the low yield period and the high yield period among the 5 treatments (T1: S/3 d1 2d/3; T2: S/2 d2; T3: S/2 d3 ET 2.5% Pa1(1) 8/y (m); T4: S/3 d2 ET 2.5% Pa1(1) 4/y (m); T5: S/3 d3 ET 2.5% Pa1(1) 12/y (m)).

Treatments	AY: Average latex yield (g/t)					
	Low yield period			High yield period		
	% Δ TP ^[a] (+1)	% Δ TP (+2)	% Δ TP (+3)	% Δ TP (+1)	% Δ TP (+2)	% Δ TP (+3)
T1	11.7 ^{ns}	81.5 ^{ns}	152.9 ^{**}	-5.7 ^{ns}	45.3 ^{ns}	51.7 [*]
T2	83.6 [*]	171.6 ^{**}	282.5 ^{**}	57.5 ^{**}	101.2 ^{**}	97.2 ^{**}
T3	476.0 ^{**}	673.8 ^{**}	704.5 ^{**}	92.1 [*]	154.6 ^{**}	138.4 ^{**}
T4	208.5 ^{**}	353.1 ^{**}	517.0 ^{**}	18.4 ^{**}	3.2 ^{ns}	6.4 ^{ns}
T5	375.7 [*]	665.3 ^{**}	701.7 ^{**}	97.4 ^{**}	264.4 ^{**}	258.8 ^{**}

Note: ^[a]TP = Tapping; Data of tapping day after stimulation (TP (+1), TP (+2) and TP (+3)) were compared with tapping day before stimulation (TP (-1)) of 5 treatments. ns = not significant, * = significant (P \leq 0.05), ** = highly significant (P \leq 0.01).

Table 9. Response of Suc to ethylene stimulation in percentage of the difference in delta (% Δ) before and after ethylene application in the low yield period and the high yield period among the 5 treatments (T1: S/3 d1 2d/3; T2: S/2 d2; T3: S/2 d3 ET 2.5% Pa1(1) 8/y (m); T4: S/3 d2 ET 2.5% Pa1(1) 4/y (m); T5: S/3 d3 ET 2.5% Pa1(1) 12/y (m)).

Treatments	Suc: Sucrose content (mM)					
	Low yield period			High yield period		
	% Δ TP ^[a] (+1)	% Δ TP (+2)	% Δ TP (+3)	% Δ TP (+1)	% Δ TP (+2)	% Δ TP (+3)
T1	-50.4 ^{**}	-51.9 ^{**}	-70.8 ^{**}	-53.5 ^{**}	-25.0 [*]	-32.1 ^{**}
T2	-39.6 ^{**}	-60.9 ^{**}	-50.3 ^{**}	-18.8 [*]	-38.7 ^{**}	-3.8 ^{ns}
T3	45.2 ^{**}	7.0 ^{ns}	28.2 ^{**}	8.5 ^{ns}	73.5 [*]	16.1 ^{ns}
T4	-49.0 [*]	93.6 ^{**}	110.7 [*]	-43.6 ^{**}	79.8 ^{**}	18.1 [*]
T5	-21.8 [*]	75.5 ^{**}	9.4 ^{ns}	-22.3 ^{**}	2.4 ^{ns}	-9.9 ^{ns}

Note: ^[a]TP = Tapping; Data of tapping day after stimulation (TP (+1), TP (+2) and TP (+3)) were compared with tapping day before stimulation (TP (-1)) of 5 treatments. ns = not significant, * = significant (P \leq 0.05), ** = highly significant (P \leq 0.01).

stimulation. However, PI of T4 and T5 showed significant differences on the 3 tapping days after stimulation except on the first and the third tapping days after stimulation in the high yield period. The response of AY to ethylene stimulation on the 3 tapping days after stimulation in both periods for the 5 treatments is shown in Table 8. In both periods, AY of T1 did not show a significant difference in the 2 tapping days after stimulation except on the third tapping day. But AY of T2 was significant difference on the 3 tapping days after stimulation. However, T1 and T2 did not receive any stimulation. The response to ethylene stimulation in both periods, showed that AY of T3 and T5 had similarly significant differences on the 3 tapping days after stimulation. But T4 showed the only significant difference on the 3 tapping days after stimulation in the low yield period and on the first tapping day after stimulation in the high yield period. Table 9 shows the response of Suc to ethylene stimulation on the 3 tapping days after stimulation of the 5 treatments. In both periods, Suc of T1 was significantly different on the 3 tapping days after stimulation. In the same manner, Suc of T2 was significantly different on the 3 tapping days after stimulation except on the third tapping day after stimulation in the high yield period. Besides, the response to ethylene stimulation showed that Suc of T3 in the low yield period showed significant difference among the tapping days except on the second tapping day after stimulation. In contrast, Suc of T3 in the high yield period was not significantly different except for the second tapping day after stimulation. Suc of T4 in both periods was significantly different on the 3 tapping days after stimulation. Suc of T5 in the low yield period showed significant difference except on the third tapping day after stimulation, while Suc in the high yield period showed no significant difference except on the first tapping day after stimulation.

Discussion

The purpose of this study was to analyze the responses of yield and the changing parameters linked to yield potential to

the ethylene stimulation. Initial flow rate (IFR) between tapping systems on each tapping day was high in the reduced tapping frequency (d3) treatments in the low yield period, but it was low in the high yield period in comparison with the traditional tapping system widely used in Thailand S/3 2d/3. It seems that the differences in the periods in each season has impacted IFR. The latex flow characteristics conformed to the results of Sreelatha et al. (2007). IFR of the d3 treatments (T3 and T5) were nearly the same though the cut length and stimulation frequency were different. Under alternate (d2) treatments, IFR of the d2 treatment without ethylene stimulation was higher than that of the d2 with stimulation treatment in both periods with a shorter cut length. IFR gradually increased during succeeding tapping days in all treatments due to the stress of tappings inducing flow and regeneration processes involving water exchanges in latex vessels (Jacob et al., 1988; d'Auzac et al., 1997). The change of IFR in both periods resulted in significant differences on each tapping day. IFR rapidly increased after ethylene stimulation because ethylene induced high turgor pressure in the latex vessel. It increased the latex flow (Jacob et al., 1988; d'Auzac et al., 1997). In the low yield period, IFR of the 5 treatments were lower than that of the high yield period (Sreelatha et al., 2007), therefore, low IFR and high PI were concomitantly shown lower in the low yield period. Plugging index (PI) among the tapping systems in both periods were high for the traditional tapping systems or non-stimulated treatments. However, they were low in the ethylene application treatments. Actually, the response of PI during succeeding tapping days decreased, resulting in the prolonged latex flow per tapping. Besides, PI suddenly decreased after ethylene stimulation in the low yield period. It gradually decreased after stimulation in the high yield period. It was clear that stimulation reduced the plugging of latex flow and resulted in increasing the latex yield after tapping (Gunasekera et al., 2013). The sudden decrease of PI resulted in high yield per tapping because of the delay of plugging of latex vessel (Jetro and Simon, 2007). In addition, ethylene

stimulation was relatively more effective in the low yield period; starting tapping combined with high temperature and new canopy (Sreelatha et al., 2007), than the high yield period in which latex flow before stimulation was initially longer than the low yield period. Average latex yield (AY) was the highest in the d3 treatments with stimulation in the low yield period. While, AY of d2 and d3 treatments were higher than the traditional tapping system in the high yielding period. In our study, the AY increased during succeeding tapping days of the 5 treatments. For the treatments with ethylene stimulation, AY rapidly increased after stimulation in both periods confirmed by Jetro and Simon (2007) regarding the response of the latex yield after stimulation on each tapping day. The half spiral cut downward with every third day tapping with stimulation 8 times per year showed superior AY per tapping in comparison with third spiral cut downward at third daily tapping with stimulation 12 times per year. This is related to the study of Traore et al. (2011) concerning the frequency of stimulation: the higher the frequency of stimulation, the lower the response at each tapping. This could impact the laticiferous system because of overstimulation. AY in the high yield period of the 5 treatments were significantly higher than in the low yield period. As observed by Sreelatha et al. (2007). Priyadarshan (2003) reported low yield during May to September and high yield during October to January in any given year. In addition, high temperature, defoliation and refoliation were also impacted on latex yield (Rao and Vijayakumar, 1992). Lower temperature (23-26 °C) lead to high yields because they prolong the latex flow (Shuogang and Yagang, 1990). The best response to ethylene stimulation was found in the low yield period because of the relatively higher effect on the metabolism of the rubber trees at this stage. Sucrose content (Suc) before ethylene stimulation was higher in the traditional tapping systems than in other tapping systems. But after stimulation on the first tapping day, Suc of half spiral cut downward with d3 frequency only increased and was the highest in both periods as a result of ethylene on the processes of sucrose transportation in the latex cells (Jacob et al., 1989; d'Auzac et al., 1997; Lacote et al., 2010; Dusotoit-Coucaud et al., 2009). The third spiral cut downward (S/3) treatments recovered more Suc than the other treatments because ethylene application inducing sucrose loading in latex cells and utilization resulted in increased rubber biosynthesis and yield. However, Suc content between both periods showed that Suc in the low yield period was higher than in the high yield period. Sreelatha et al. (2007) reported that an activator of sucrose synthase was high in the low yielding season, and resulted in an increase of sucrose available for both physiological processes in the trees: completed foliage, and yield due to the restarting of tapping. In high yield period the lower Suc metabolism dedicated more to rubber biosynthesis creating a sink for sucrose content has to be connected with the corresponding (Jetro and Simon, 2007). The result was could show the effectiveness of stimulation in the low yield period.

Materials and Methods

Experimental site and plant material

These experiment were carried out at the Thepa Research Station (6°48'0.7" N 100°56'37.2" E, altitude 33 m above sea level) Thepa district, Songkhla province in southern Thailand. The climate is tropical with two seasons, a dry season and a rainy season (Sternstein, 1962; TMD, 2013). The dry season is from mid-February to mid-May, and the

rainy season is from mid-May to mid-February. RRIM 600 clones were used in the experiment. Rubber trees were planted (7×3 m spacing) in 2005, and they were selected before tapping by homogenous girth at 1.70 m from the ground. The tapping was opened at 1.50 m from the ground on panel BO-1. The experiments were started in August 2013 and the study was focused on the period between May and October 2014.

Experimental treatments and design

The experiment was arranged as One Tree Plot design (Sdoodee et al., 2012) with five treatments comprising three trees per treatment. Protocol of the study treatments is presented in Table 1 according to Vijayakumar *et al.* (2009) international notation.

The response of the trees to the ethylene stimulation (ethephon) was studied in each tapping day before and after stimulation. The tapping agenda is presented in Table 2. The experiments were made in two periods, low yield period (LY) and high yield period (HY). LY was in May when tapping was started. HY was in October, in the rainy season, when the yield at each tapping is usually high.

Data collection and analysis

Latex yield (gram per tree per tapping: g/t) was calculated from each tree by weighting the latex yield at each tapping. Total solid content was measured from a bulk sample taken in each treatment in order to convert fresh weights in grams of dry rubber per tree and per tapping. Initial flow rate and plugging index were calculated according to the methods of Milford et al. (1969). The initial flow rate was collected in the first 5 minutes, expressed in milliliters per minute (ml/min) and calculated following the formula:

$$\text{Initial flow rate} = \frac{\text{volume of latex first five minutes}}{5}$$

Plugging index was calculated following the formula:

$$\text{Plugging index} = \left(\frac{\text{Initial flow rate}}{\text{Volume of latex}} \right) \times 100$$

The main latex biochemical parameters, i.e. sucrose (Suc) content, was measured in a latex sample of 1 milliliter in the first 5 minutes of the flow after tapping, from each tree taken in each treatment, using methods developed by CIRAD (Jacob et al., 1989) adapted in 1995 by IRRDB (1995). Sucrose content was expressed in millimoles of latex (mM) and updated for Thailand by Gohet and Chantuma (1999). The data was statistical analyzed by DSAASTAT v 1.1 package software (Onofri, 2007).

Conclusion

Ethylene application has affected all the parameters linked to potential yield of the trees. The initial flow rate (IFR), plugging index (PI), average latex yield (AY) and sucrose content (Suc) of rubber clone RRIM 600 under low frequency tapping systems were significantly affected. There was significant difference among the tapping systems during the low yield and the high yield periods. It showed that the response to stimulation was dependant on the physiological status of the trees according to the seasonal variation of the tree metabolism in the low and high yield periods. The yield was always higher in the high yield period than in the low yield period, even the relative higher response to stimulation on IFR, PI and Suc were seen in the low yield period. But a more sustainable positive effect of stimulation is expected in the yield period corresponding to metabolism dedicated more

to rubber biosynthesis creating a sink for sucrose. In any case, the treatments in d3 tapping frequency with stimulation showed drastic changes in sucrose content. It is remarkable, that in Thailand, even in a heavy rainy area, it is possible to use ethylene stimulation to increase the potential yield of the trees at each tapping.

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