Physiology of the Rubber Tree Linked to Yield: A Case Study; Impact of Reduced Tapping Frequency on Potential Latex Yield of Clone RRIM 600

Thongchai Sainoi

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Plant Science

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This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

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I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

....Signature
(Mr. Thongchai Sainoi)
Candidate

ชื่อวิทยานิพนธ์ ความสัมพันธ์ของสรีรวิทยาต่อผลผลิตของยางพารา กรณีศึกษาผลกระทบของการลดความถี่การกรีดต่อศักยภาพการให้ผลผลิตของยางพาราพันธุ์ RRIM 600

ผู้เขียน นายธงชัย ไทรน้อย

สาขาวิชา พืชศาสตร์

ปีการศึกษา 2559

บทคัดย่อ

เกษตรกรสวนยางพาราต้องเผชิญกับสภาวะผันผวนของราคายางพาราและปัญหาการขาดแคลนแรงงานกรีดยาง การใช้ระบบกรีดที่มีความถี่ต่ำอาจเป็นทางเลือกหนึ่งในการแก้ไขปัญหาเหล่านั้นได้ เพราะเป็นระบบกรีดที่สามารถเพิ่มผลผลิตต่อวันกรีด ดังนั้น จึงได้มีการศึกษาการใช้ระบบกรีดแบบ d3 (กรีดทุกสามวัน) ร่วมกับการกรีดแบบครึ่งลำต้น (S/2) และแบบหนึ่งในสามของลำต้น (S/3) ร่วมกับการกระตุ้นด้วยเอทธิฟอนในพื้นที่ภาคใต้ของประเทศไทย ทำการทดลองที่สถานีวิจัยเทพา อำเภอเทพา จังหวัดสงขลา โดยแบ่งเป็น 3 การทดลอง สำหรับการทดลองที่ 1 ใช้ยางพาราพันธุ์ RRIM 600 อายุ 8 ปีวางแผนการทดลองแบบ Randomized Complete Block Design ประกอบด้วย 5 สิ่งทดลอง คือ สิ่งทดลองที่ 1: กรีด 1 ใน 3 ของลำต้น กรีด 2 วัน เว้น 1 วัน (S/3 d1 2d/3; ระบบกรีดที่เกษตรกรใช้), สิ่งทดลองที่ 2: กรีดครึ่งลำต้น กรีดวันเว้นวัน (S/2 d2; ระบบกรีดที่สถาบันวิจัยยางแนะนำ), สิ่งทดลองที่ 3: กรีดครึ่งลำต้น กรีด 1 วัน เว้น 2 วัน ใช้สารเคมีเร่งน้ำยางเอทธิฟอน 2.5% จำนวน 8 ครั้งต่อปี (S/2 d3 ET 2.5% Pa1(1) 8/y (m)), สิ่งทดลองที่ 4: กรีด 1 ใน 3 ของลำต้น กรีดวันเว้นวัน ใช้สารเคมีเร่งน้ำยางเอทธิฟอน 2.5% จำนวน 4 ครั้งต่อปี (S/3 d2 ET 2.5% Pa1(1) 4/y (m); ระบบกรีดที่สถาบันวิจัยยางแนะนำ) และสิ่งทดลองที่ 5: กรีด 1 ใน 3 ของลำต้น กรีด 1 วัน เว้น 2 วัน ใช้สารเคมีเร่งน้ำยางเอทธิฟอน 2.5% จำนวน 12 ครั้งต่อปี (S/3 d3 ET 2.5% Pa1(1) 12/y (m)) จำนวน 3 ซ้ำ (แปลงย่อย) ใช้ต้นยางพาราจำนวน 10 ต้นต่อหนึ่งสิ่งทดลองของแต่ละแปลงย่อย ผลการทดลองพบว่า การใช้ระบบกรีดแบบ S/2 d3 ใช้สารเคมีเร่งน้ำยางเอทธิฟอนจำนวน 8 ครั้งต่อปี มีประสิทธิภาพในการให้ผลผลิตต่อครั้งกรีดสูงซึ่งนำไปสู่การชดเชยผลผลิตสะสม (กก./ต้น) ได้ อย่างไรก็ตาม การให้ผลผลิตสูงส่งผลให้เกิดการลดลงของปริมาณซูโครสและปริมาณไธออล นอกจากนี้ การใช้ระบบกรีดที่มีความถี่ต่ำมีความสิ้นเปลืองเปลือกน้อยและไม่มีผลกระทบต่อการเจริญเติบโตทางลำต้น มีการเกิดอาการหน้ายางแห้งน้อย สำหรับการทดลองที่ 2 ใช้ยางพาราพันธุ์ RRIM 600 อายุ 9 ปีวางแผนการทดลองแบบ One Tree Plot Design ประกอบด้วย 5 สิ่งทดลอง คือ สิ่งทดลองที่ 1: กรีด 1 ใน 3 ของลำต้น กรีด 2 วัน เว้น 1 วัน (S/3 d1

2d/3; ระบบกรีดที่เกษตรกรใช้), สิ่งทดลองที่ 2: กรีดครึ่งลำต้น กรีดวันเว้นวัน (S/2 d2; ระบบกรีดที่สถาบันวิจัยยางแนะนำ), สิ่งทดลองที่ 3: กรีดครึ่งลำต้น กรีด 1 วัน เว้น 2 วัน ใช้สารเคมีเร่งน้ำยางเอทธิฟอน 2.5% จำนวน 8 ครั้งต่อปี (S/2 d3 ET 2.5% Pa1(1) 8/y (m)), สิ่งทดลองที่ 4: กรีด 1 ใน 3 ของลำต้น กรีดวันเว้นวัน ใช้สารเคมีเร่งน้ำยางเอทธิฟอน 2.5% จำนวน 4 ครั้งต่อปี (S/3 d2 ET 2.5% Pa1(1) 4/y (m); ระบบกรีดที่สถาบันวิจัยยางแนะนำ) และสิ่งทดลองที่ 5: กรีด 1 ใน 3 ของลำต้น กรีด 1 วัน เว้น 2 วัน ใช้สารเคมีเร่งน้ำยางเอทธิฟอน 2.5% จำนวน 12 ครั้งต่อปี (S/3 d3 ET 2.5% Pa1(1) 12/y (m)) จำนวน 3 ซ้ำๆ ละ 1 ต้น ต่อหนึ่งสิ่งทดลอง ผลการทดลองพบว่า การตอบสนองต่อการกระตุ้นขึ้นอยู่กับสถานะทางสรีรวิทยาของต้นยางพาราตามการแปรปรวนของฤดูกาลในช่วงการให้ผลผลิตสูงและการให้ผลผลิตต่ำของต้นยางพารา อย่างไรก็ตาม ผลผลิตของยางพารามีปริมาณมากในช่วงการให้ผลผลิตสูงซึ่งมากกว่าช่วงการให้ผลผลิตต่ำของยางพารา แต่การตอบสนองต่อการกระตุ้นของค่าการไหลของน้ำยาง ดัชนีการจับตัวของน้ำยางและปริมาณซูโครสมีค่าสูงในช่วงการให้ผลผลิตต่ำของยางพารา สำหรับงานทดลองที่ 3 ใช้ยางพาราพันธุ์ RRIM 600 อายุ 10 ปีวางแผนการทดลองแบบ One Tree Plot Design ประกอบด้วย 6 สิ่งทดลอง คือ สิ่งทดลองที่ 1: กรีด 1 ใน 3 ของลำต้น กรีด 2 วัน เว้น 1 วัน (S/3 d1 2d/3; ระบบกรีดที่เกษตรกรใช้), สิ่งทดลองที่ 2: กรีดครึ่งลำต้น กรีดวันเว้นวัน (S/2 d2; ระบบกรีดที่สถาบันวิจัยยางแนะนำ), สิ่งทดลองที่ 3: กรีดครึ่งลำต้น กรีด 1 วัน เว้น 2 วัน ใช้สารเคมีเร่งน้ำยางเอทธิฟอน 2.5% จำนวน 8 ครั้งต่อปี (S/2 d3 ET 2.5% Pa1(1) 8/y (m)), สิ่งทดลองที่ 4: กรีด 1 ใน 3 ของลำต้น กรีดวันเว้นวัน ใช้สารเคมีเร่งน้ำยางเอทธิฟอน 2.5% จำนวน 4 ครั้งต่อปี (S/3 d2 ET 2.5% Pa1(1) 4/y (m); ระบบกรีดที่สถาบันวิจัยยางแนะนำ), สิ่งทดลองที่ 5: กรีด 1 ใน 3 ของลำต้น กรีด 1 วัน เว้น 2 วัน ใช้สารเคมีเร่งน้ำยางเอทธิฟอน 2.5% จำนวน 12 ครั้งต่อปี (S/3 d3 ET 2.5% Pa1(1) 12/y (m)) และ สิ่งทดลองที่ 6: ต้นยางพาราที่ยังไม่เปิดกรีด จำนวน 6 ซ้ำๆ ละ 1 ต้น ผลการทดลองพบว่า การใช้ระบบกรีดแบบความถี่ต่ำมีการสะสมปริมาณคาร์โบไฮเดรตในเนื้อไม้และในเปลือกน้อยกว่าการใช้ระบบกรีดแบบที่เกษตรกรใช้ แต่มีการสะสมมากบริเวณใต้รอยกรีดในเนื้อไม้ จากผลการทดลองแสดงให้เห็นว่า จากการเปลี่ยนแปลงของภูมิอากาศทางภาคใต้ของประเทศไทย การใช้ระบบกรีดแบบ S/2 d3 กระตุ้นด้วยเอทธิฟอน 8 ครั้งต่อปี มีศักยภาพในการเพิ่มผลผลิตยางพาราในแต่ละครั้งกรีด ดังนั้น มีความเป็นไปได้ที่ชาวสวนยางพาราจะประยุกต์ใช้ระบบกรีดดังกล่าวภายใต้สภาวะความผันผวนของราคายางพาราและการขาดแคลนแรงงานกรีด

Thesis title Physiology of the Rubber Tree Linked to Yield: A Case Study; Impact of Reduced Tapping Frequency on Potential Latex Yield of Clone RRIM 600

Author Thongchai Sainoi

Major Program Plant Science

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ABSTRACT

Rubber smallholders face both rubber price fluctuation and labour shortage. Low frequency tapping (LFT) systems may be interesting in such context as they can increase yield per tapping day. Then, LFT systems (tapping every 3 days, d3) were tested in S/2 and S/3 compensated by different levels of ethylene (ET) stimulation in southern Thailand. An experiment was established at the Thepa Research Station, Songkhla province. There were three parts of investigation. The first study was used 8-year-old trees of RRIM600 clone, it was designed as a randomized complete block design with five treatments (T1: S/3 d1 2d/3 (usual system for Thai smallholders), T2: S/2 d2 (standard for RRIT), T3: S/2 d3 ET 2.5% Pa1(1) 8/y (m), T4: S/3 d2 ET 2.5% Pa1(1) 4/y (m) (standard for RRIT) and T5: S/3 d3 ET 2.5% Pa1(1) 12/y (m)) with three replications (elementary plot). There were ten trees per treatment in each elementary plot. The results showed that the so-called LFT system (S/2 d3) with stimulation 8 times per year efficiently gave the yield per tapping able to compensate the cumulative yield in kg/t comparing with the conventional tapping system (S/3 2d/3). The higher yield per tapping affected latex biochemistry was leading to decrease in sucrose (Suc) and reduced thiol (RSH) contents. Although LFT system with stimulation expressed less bark consumption with no prominent effect on girth increment, it still slightly increased of TPD (tapping panel dryness). The second study was used 9-year-old trees of RRIM600 clone, it was designed as one tree plot design with five treatments (T1: S/3 d1 2d/3 (usual system for Thai smallholders), T2: S/2 d2 (standard for RRIT), T3: S/2 d3 ET 2.5% Pa1(1) 8/y (m), T4: S/3 d2 ET 2.5% Pa1(1) 4/y (m) (standard for RRIT) and T5: S/3 d3 ET 2.5% Pa1(1) 12/y (m)) with three trees per treatment. The results showed that the response to stimulation was dependent on the physiological status of the tree according to the seasonal variation of the tree metabolism in both periods (high yield and low yield period). However, the yield was always higher in the high yield period than in the low yield period, even the relative higher response to stimulation on initial flow rate (IFR), plugging index (PI) and sucrose content (Suc) were found in the low yield period. The third study was used 10-year-old trees of RRIM600 clone, it was designed as one tree plot design with six treatments (T1: S/3 d1 2d/3 (usual system for Thai smallholders), T2: S/2 d2 (standard for RRIT), T3: S/2 d3 ET 2.5% Pa1(1) 8/y (m), T4: S/3 d2 ET 2.5% Pa1(1) 4/y (m) (standard for RRIT), T5: S/3 d3 ET 2.5% Pa1(1) 12/y (m) and T6: untapped trees) with six trees per treatment. It was found that the expression of potential yield under the LFT system was slightly leading to a relatively lower storage of the TNC (total nonstructural carbohydrate) content in wood and bark comparing with the conventional tapping system, but it was still accumulated in the drainage area of wood. In overview of three experiments, LFT system (S/2 d3) with stimulation 8 times per year could efficiently give the yield per tapping leading to compensate the cumulative yield in kg/t for the clone RRIM 600. It is remarkable, under the climate variability of southern Thailand, it is possible of using ethylene stimulation to increase the potential yield of the rubber tree at each tapping under LFT system. This suggests that rubber smallholders in southern Thailand possibly apply LFT systems with stimulation under rubber price fluctuation and tapping labour shortage.

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Thongchai Sainoi

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LIST OF ABBREVIATIONS

AY	= average latex yield
d	= daily tapping
ET	= ethephon
HFT	= high frequency tapping system
HY	= high yield
IFR	= initial flow rate
LD	= latex diagnosis
LFT	= low frequency tapping system
LY	= low yield
Pi	= inorganic phosphorus content
PI	= plugging index
RSH	= reduced thiol content
S	= spiral cut
Suc	= sucrose content
TNC	= total nonstructural carbohydrate
TPD	= tapping panel dryness
TSC	= total solid content

CHAPTER 1
INTRODUCTION

Rubber tree (*Hevea brasiliensis*) is an important economic crop in the world that relates to natural rubber. It is extensively planted in the Southeast Asia. In Thailand, 85% of the total rubber areas are owned by smallholders (Chantuma *et al.*, 2015). This could be a cause of diversification of tapping systems in each region (Chambon *et al.*, 2014). Under situation of climatic variability and rubber price fluctuation, rubber smallholders mainly use a high frequency tapping systems (HFT), such as daily tapping (d1) during three days followed by one day tapping rest (3d/4) (Chantuma *et al.*, 2011). These systems could compensate the reduction of the number of tapping days leading to loss of revenue, due to the rainy days without any possibility to work in field. However, HFT may cause tapping panel dryness (TPD) high, bark consumption and girth increment of rubber trees leading to reduction of economic life span. In addition, there is another problem of labour shortage rubber plantations with an increase of the labour-cost affecting rubber smallholders' management. Therefore, choosing appropriate tapping systems is still essential for getting a good tree productivity, land productivity and labour productivity.

It was suggested that low frequency tapping systems (LFT) will be the solution to solve those problems (Soumahin *et al.*, 2009; Kudaligama *et al.*, 2010; Prasanna *et al.*, 2010; Soumahin *et al.*, 2010). LFT systems are characterized by the reduction of tapping frequency possibly resulting to unable compensation of the cumulative latex production. Researchers have been trying to enhance latex yield under this tapping system by using ethylene stimulation. An ethylene generator, 2-chloroethylphosphonic acid (ethephon), is applied to the tapping panel, which enhance latex production because it increases the duration of latex flow after tapping with the reduction of latex coagulation by activating latex cell metabolism (Jacob *et al.*, 1989; d'Auzac *et al.*, 1997). Then use of ethylene stimulation results to enhance land and labor productivity (Sivakumaran and Chong, 1994; Sivakumaran, 2002; Jetro and Simon, 2007; Lacote *et al.*, 2010; Njukeng *et al.*, 2011; Traore *et al.*, 2011). In southern Thailand, rubber smallholders do not adopt the LFT, although there are recommendations to use the LFT system with ethylene stimulation by Rubber Research Institute of Thailand (RRIT). It is difficult to convince the rubber smallholder for changing the tapping system practice. Hence, it needed to assess the efficiency of LFT systems with ethylene stimulation to be a guideline that the smallholder may adopt regarding the scarcity of rubber price and labour shortage.

LITERALS REVIEW

Generality

Rubber tree (*Hevea brasiliensis*) is a native species of the Amazon rainforests, a member of family Euphorbiaceae which is primary commercial source of natural latex. It is a tropical tree crop and mainly grown for the industrial production of latex in the world (Purseglove, 1987). Rubber trees are fast growing trees and exceed to 25 meter or more high in plantation. The economic life span of rubber trees is around 20-25 years or more depending on the tapping panel management of rubber smallholders. Generally, rubber trees grow without tapped during the first seven years and following by 13-18 productive years (Allen, 2004).

The optimum conditions biophysical of rubber trees are including: the mean annual temperature are 23-35°C ranges, annual rainfalls are 1500-3000 millimeter ranges and soil pH are 4.5-6. Besides, growth and latex production of rubber trees are influenced by soil type. High soil pH values may cause the latex coagulation and reduces the time of latex flow (Verheye, 2010). Akpan *et al.* (2007) found that the rubber tree growing in acidic soil (pH 4.37-4.54) showed low latex production. Nevertheless, these are preliminary factors of smallholder’s management because the unsuitable of environments will affect rubber physiology.

Climate change

Climate change is the climatic variability and change due to human activity (directly or indirectly) and nature in the same time (Boonpragob, 2011). However, this phenomenon was the most influencing on the plant, because plants were differently sensitive responded to climate factors depending on physiological process of plants (Jintrawet, 2011). Besides, there have been observing the effect on rubber trees. The trend of latex production expressed to decrease under climatic variation for example when increasing rainfalls leaded to reduction of the number of tapping days. Moreover, rainfalls, temperatures and sunshine hours are the major climatic factors affecting physiological development and latex potential of rubber trees (Raj *et al*., 2011). It was supported by Chantuma *et al*. (2012) that the climatic variability was shown not only by decreasing the number of cold days but also increasing the high temperature days. It results in shorter winter season, but a longer summer season. The change of climatic pattern may induce high amount of rainfall causing flood or hot dry drought in each year. Hence, the productions have been changed from time to time because it was affected by the climate variable and unexpected seasonal factors of drought and heavy rain.

Besides, physiological processes of rubber trees are directly affected by temperature, 18-24 °C temperatures are suitable for latex flow and 27-33 °C temperatures are optimum for photosynthesis, meanwhile over 38 °C temperature trended to reduce photosynthesis of rubber trees (Rao *et al*., 1998; Kositsup *et al*., 2007). Satheesh and Jacob (2011) and Gohet *et al.* (2015) reported that various agro-climatic region affected the latex production differently. Obviously, the changes of both maximum temperature and minimum temperature have a strong influence by reducing the latex production (Gohet *et al.,* 2015). Nevertheless, some researchers reported that the rubber tree could acclimate to difference agro-climatic conditions; under colder and warmer climates (Alam *et al.*, 2005). This depends on physiological efficiency of various clones, for instance the PB 235 clone could maintain photosynthetic performance leading to better growth than RRII 105 clone in the colder climate condition. Therefore, the climatic change is the importantly factor for the growth and development of rubber trees.

Tapping systems

The rubber tree is one of the major economic crops advanced source of natural rubber because it has good yield and excellent physical properties of latex production (Zhu and Zhang, 2009). Thus, life span of the rubber tree is influenced by the management of rubber plantation. One of them is the tapping system involving harvest processing of latex production. Certainly, tapping systems are variously used in the rubber production country. Therefore, the appropriate tapping systems were defined by clones and environment in rubber plantations. In Thailand, the tapping systems have been divided into 2 types (Somboonsuke *et al*., 2011); the first one is recommended by Rubber Research Institute of Thailand (RRIT) and the second one is implemented by rubber smallholders in each area.

The tapping process consists of shaving the cut, according to certain rules including the establishment of slope of tapping cut (Obouayeba *et al*., 2011). The tapping cut of rubber trees can make through to variable lengths for example full spiral (S), the cut is made on the whole circumference of the tree, half spiral (S/2), the cut is made on the half of the circumference and third spiral (S/3), the cut is made on the third of the circumference of the tree. The vegetative growth producing wood biomass and the latex production requiring large amounts of energy and photosynthesis are also in competition. Therefore, the good growth is one of index to determine a good balance between physiological profile and good health of rubber trees (Obouayeba *et al*., 2012a). Decreasing of girth increment was reported by Silpi *et al*. (2006) after resumption of tapping. It led to a decrease in growth rate within two weeks. These evidenced that the tapping showed impacts on growth and it was much stronger in the second year of tapping than in the first year, whereas latex production increased significantly between the first and the second year (Silpi *et al*., 2006).

Laticiferous vessel

Basically, latex cells are located in every part of rubber trees (roots, trunk, branch and leaves) at any age. However, the trunk of rubber trees is the part expressing the highest latex extraction production and easier to management (Obouayeba *et al*., 2012b). Latex is the production from laticiferous vessels of rubber trees. It is a specialized fluid cytoplasm produced in the specialized cells calling laticiferous vessel in phloem. Nevertheless, it contains the articulated branched laticiferous vessel and extracted using multi-annual tapping systems (Gidrol *et al*., 1988; Thomas *et al*., 1995; Vinod *et al*., 2000; Pickard, 2008; Zhu and Zhang, 2009; Lacote *et al*., 2010).

In addition, the number of laticiferous vessel is one important factor influencing latex production of rubber trees. It is found in the secondary phloem and arranged in the inner bark of the rubber tree trunk (Hao and Wu, 2000; Bingzhong and Jilin, 2004; Dusotoit-Coucaud *et al*., 2009). Mesquita *et al*. (2006) found that the number of laticiferious vessels, laticiferious rings per millimeter and laticiferous diameters of RRIM 600 clone were higher than the GT 1 and Fx 2261 clone in the South east of Brazil. At the same time, the latex flow and the rate of latex regeneration are determined the quantities of latex production after tapping (Njukeng *et al*., 2011). Latex flow is easily inhibited by latex physiological or environmental processes leading to coagulation. Latex regeneration is completed within 3 days or 72 hours for latex production efficiency (Mesquita *et al*., 2006; Mingwu and Liuhong, 2006). Riches and Gooding (1952) found that the latex flow was possibly continued for as long as 2-3 hours, the exuded of latex was very viscous and high rubber content (50-60%). In addition, the exudation rate of latex was not constant because the flow rate of latex was rapid at first after tapping. High turgor pressure discharges latex from the laticiferous vessels after that it gradually slowed less steady rate due to loss of turgor pressure. It resulted to retard and cease finally by the mechanism of laticiferous vessel plugging. Moreover, the latex vessel plugging rate was increased by shorting cut and decreased by ethephon stimulation. So, it maybe involves the damage of lutoids after tapping (the sharing effect) leading to the latex coagulation on cut end of laticiferous vessels at the tapping panel (Yeang, 2005). However, the index to measuring laticiferous vessel plugging rate is called the “Plugging Index” estimating the average plugging rate over the total flow (Milford *et al.*, 1969; Paardekooper and Samosorn, 1969). Plugging index had relation to many other clonal characters. Although it was negatively correlated with yield and incidence of tapping panel dryness but it was positively correlated with girth increment, dry rubber content and response to yield stimulation (Waidyanatha and Pathiratnb, 1971). Hence, the plugging index could be preliminary index to evaluate the efficiency of any tapping system.

Low frequency tapping system

Low frequency tapping (LFT) system is leading to reduce number of tapping days and to increase the delay between two tapping. However, less of number tapping days may cause to loss of the latex production. Therefore, those are compensated by using ethylene stimulation (Silva *et al*., 2010). Diarrassouba *et al*. (2012) mentioned that one of major difficulties in rubber plantation management was to tailor the harvesting technology which can gave appropriate productions without uncompromising the growth, physiological and health of the rubber tree (Chrestin *et al.*, 1985). So that, he adapting these tapping with stimulation can avoid tapping panel dryness. At the same time, the less number of tapping days per year was advantaged to increasing economic life span of the rubber tree with reduced number of tapping and could also increase tapper's income (Rodrigo, 2007). Bark consumption was reduced significantly by using LFT systems resulting in a longer life span of the tapping panel on virgin bark and reduced replanting needs (Kudaligama *et al*., 2010). Karunaichamy *et al*. (2012a) reported that increasing of labour productivity by adopting LFT systems with stimulation are one of the method to reduce the cost of the latex production and could to overcome labour shortage.

Mainly, the reducing of tapping frequency was concentrated to resolve the harvest of the latex production. Rodrigo (2007) mentioned that LFT systems are widely used with fourth daily tapping (d4) and sixth daily tapping (d6) in India, while third daily tapping (d3) and fifth daily tapping (d5) are practiced in China, but third daily tapping (d3) is basically in Sri Lanka. In Africa, fourth daily tapping (d4), fifth daily tapping (d5) and sixth daily tapping (d6) give latex production inferior than third daily tapping (d3) but with no negative impacts on the physiological of rubber trees (Soumahin *et al*., 2012). In Sri Lanka, third daily tapping (d3) increased up to 36 years the life span of the trees (Nugawela *et al*., 2000). Soumahin *et al*. (2009) found that LFT system was possible to remedy labour shortage, cost and compensate production by stimulation. It reduced by up to 33% of labour requirement. Obouayeba *et al*. (2010) reported that reduction the tapping frequencies were compensated by stimulations to sustain the yield in south-eastern Côte d'Ivoire. These system could be alternatives the traditional system to reduce the need for labour.

At the same time, long interval frequency of tapping days could increase the advantage to rubber trees. The highest latex production of RRIM 600, PB 260, IAN 873 and GT 1 clones always shown with fourth daily tapping (d4) in Columbia (Mendez *et al*., 2009). Besides, Obouayeba *et al*. (2009) reported that half spiral downward cut at fourth daily tapping (S/2 d4) with stimulation gave the improving of carbohydrates supply and enhanced the maintaining of sucrose availability. Interval frequency of third daily tapping (d3) and fourth daily tapping (d4) with ethephon stimulation provided the highest latex production per year in RRIM 600 and PR 261 clones (Silva *et al*., 2010). Similarly, Karunaichamy *et al*. (2012b) suggested that adopting frequency of tapping day at third daily tapping (d3) and fourth daily tapping (d4) can reduce the need for labour by 32 % and 48 %, respectively when compared to alternate daily tapping (d2). There have investigated half spiral downward cut at fourth daily tapping (S/2 d4) system, it reduced both the cost by 19% and labour requirement by 50%, increased both the profitability by 22 % and tapper's income by 26 % (Rodrigo *et al*., 2011). However, LFT systems were variously used by several countries. The interval at third daily tapping (d3) and fourth daily tapping (d4) were commonly used to study with half spiral downward cut (S/2).

Ethephon stimulation

Ethephon (2-chloroethyl phosphonic acid) is an ethylene releaser which a well know plant growth regulator used to increasing yield, promoting fruit maturity, improving color and advancing harvest timings (Bharadwaj *et al*., 1988; Chrestin *et al*., 1997). In rubber trees, Audley *et al*. (1976) reported that it is used to prolong the latex flow after stimulation applied on the panel. However, the ethylene releases in the bark, induces delaying plugging and increasing turgor pressure in laticiferous vessels. Moreover, it is advantageous to reducing tapping frequency that results in increasing land and labour productivity (Wenxian *et al*., 1986; Lacote *et al*., 2010). The stimulation could increase the latex yield by 1.5-2.0 folds and improved the supply of carbon source for rubber biosynthesis (Zhu and Zhang, 2009).

The tapping system with stimulants was widely used in the rubber growing industry because the stimulation influenced cumulative yield and latex cell biochemistry (Yeang, 2005). Although, increasing the frequency of ethephon stimulation leading to increase of latex flow duration (Jacob *et al.,* 1989; d'Auzac *et al.,* 1997; Obouayeba *et al*., 2009), however, it depends on physiological character of each clone (Jacob *et al.,* 1989; d'Auzac *et al.,* 1997). Lacote *et al*. (2010) reported that the ethephon stimulation on latex production increasing was significantly expressed in clones with high sucrose content and low inorganic phosphorus content. In addition, Traore *et al*. (2011) studied the stimulation frequency on GT1 clone and found that half spiral downward cut at fourth daily tapping (S/2 d4) stimulated with ethephon concentration 2.5% at 4 times per year gave high yield without causing prejudices on the growth, physiological and sensitivity of tapping panel dryness. However, both of the ethylene stimulation frequency and ethylene concentration should be appropriated in each clone, age and tapping systems (Jacob *et al.,* 1989; d'Auzac *et al.,* 1997; Gohet *et al.,* 2003; Lacote *et al.,* 2010; Njukeng *et al*., 2011) to reduce the risk of physiological process.

Latex diagnosis

Latex diagnosis technique involves biochemical analysis of some parameters using to detect the effect on tapping systems on the latex characteristics of rubber trees. Latex sucrose content, latex inorganic phosphorus content, latex reduced thiols content and total solid content parameters were used in latex diagnosis under regional scale or large estates and companies, using methods developed by CIRAD and CNRA (Jacob *et al*., 1988) adapted in 1995 by IRRDB (IRRDB, 1995; Gohet *et al.,* 2008). Latex sucrose content is preliminary molecules of the latex synthesis in the cytoplasm (Jacob *et al.,* 1989; Dusotoit-Coucaud *et al.,* 2010), reflects to balance between sucrose consumption by uptake and utilization. It is *in situ* essential energy for latex regeneration and latex flow. In addition, sucrose contents are related with latex metabolism to quantity of latex production, in particular, high sucrose content may indicate a good loading of the laticiferous vessel. Nevertheless, high sugar content may also indicate low metabolic utilization of sucrose content and result in finally low productivity. Moreover, the relation of tapping to sucrose content was observed. Latex sucrose content was drained below and above the tapping cut as in consequence of latex regeneration, however, high frequency tapping systems leading to decrease in latex sucrose content (Tupy, 1973; 1985; Moraes *et al.,* 1978; Lacote *et al.,* 2004; 2010). The latex inorganic phosphorus content is an indicator of the latex metabolic activity in the laticiferious vessels. It is an essential component for energy metabolism and also important for both phospholipid membrane and protein in laticiferious vessels with express to role in maintaining the stability of rubber particle. Therefore, high inorganic phosphorus contents are benefit to latex production (Jacob *et al.,* 1989; She *et al.,* 2013). In addition, the stimulation can increase inorganic phosphorus content because it increases the metabolism activity of laticiferous vessels (Chantuma *et al.,* 2006; Jetro and Simon, 2007; Lacote *et al.,* 2010). But high inorganic phosphorus content can be a sign of an over metabolic activity when related to low sucrose content. The latex reduced thiol content is able to neutralize various forms of toxic oxygen and protect elements in the laticiferous system as a scavenger (Jacob *et al.,* 1989; d'Auzac *et al.,* 1997). There are potential activators of main enzymes in metabolism processes and also is antioxidant of lutoid membrane (Jacob *et al.,* 1989; d'Auzac *et al.,* 1997). Besides, latex reduced thiol content is related with tapping panel dryness when low thiol content is indicating the weakness the ability of non-enzymatic protection system (Chrestin *et al.,* 1985; Coupé *et al.,* 1989). The total solid content is an indicator the viscosity of latex characteristic (Chrestin *et al.,* 1985; Coupé *et al.,* 1989; Jetro and Simon, 2007).

Carbohydrate

Carbohydrates are elemental source to reserve energy stored in the vegetative organs of plants. More commonly they refer to total nonstructural carbohydrate (TNC) (Smith *et al.,* 1964; Fick and Nolte, 1986) comprising two components; total starch and total sugar. Therefore, it uses a measure the reserve material of plant. In other words, reserves of available carbohydrates are largely accumulated in parenchyma due to survival (Kramer and Kozlowski, 1979). Basically, plants are accumulated carbohydrates during periods of excess production and showed depleted carbohydrates when the rate of utilization exceeds the rate of production. They were presenting an important role in metabolism, growth, defense, cold hardiness and postponement or prevention of plant mortality (Kozlowsky, 1992). Carbohydrates are used to measure of carbon shortage or surplus balance for growth (Körner, 2003; Rosas *et al.,* 2013). Carbohydrate reserves are depleted from twigs, stem and roots during the most rapid growth period in a pattern that varies with species growth characteristic. In addition, seasonal carbohydrate cycle is particularly well determined in many deciduous trees of the temperate zone. Total carbohydrate contents of stems and branches reach a maximum in the autumn about the time of leaf fall, begin to decrease in late winter and decrease rapidly in early spring, as a results, carbohydrates are being depleted to accelerated respiration and used in growth of new tissue (Kramer and Kozlowski, 1979).

However, there are few reports about the TNC in rubber trees. Researchers have studied on carbohydrate contents in the trunk of the rubber tree. Silpi *et al.* (2007) studied carbohydrates reserve about TNC in rubber trees and found that starch contents were decreased in stem wood of untapped trees between bud break to re-foliation periods and TNC concentration was increased during the vegetative season. Moreover, starch contents were disturbed by tapping cut leading to increase of starch contents in tapped trees and even more enhanced by ethephon stimulation. It was confirmed by Chantuma *et al.* (2007). Tapping cut affects the level and distribution pattern of carbohydrate in the trunk. In addition, TNC in the bark and wood of rubber trees have been studied. It was found that tapped trees stored TNC more than untapped trees. Although starch contents were lower in bark than in wood, but it was contrary for soluble sugar (Chantuma *et al.*, 2009). However, TNC in wood varied throughout in the harvest season and observed the same trend between tree ages of rubber trees (Chiarawipa and Prommee, 2013).

Tapping panel dryness

Latex production can be decreased due to symptoms appearing in/on the bark of the rubber tree. High frequency tapping systems might directly affect the development of oxidative stress leading to tapping panel dryness (TPD) (Bealing and Chua, 1972; de Fay, 1988; de Fay and Jacob, 1989). Meanwhile, it also resulted in the stress-hormones level (ethylene and abscisic acid) and decrease of the growth hormones level (auxin and gibberellic acid) in bark during oxidative stress (Krishnakumar *et al*., 2012). TPD is a serious problem to natural latex production and clearly expressed from excised lacticifer cell which is not exuded of latex. Several studies explain that TPD is not caused by pathogenic agent (Nandris *et al*., 2004). Once TPD occurs in rubber trees, the tapping panel is partly or entirely dried. They are conduced to 15-20% loss of the annual latex production. In addition, the clonal differences to susceptibility of TPD are involved in reduction of latex production; for instance, the high latex production clones are more susceptible to TPD than the low latex production clones (Senevirathna *et al*., 2007). Okoma *et al.* (2011) found that IRCA 41, PB 217, AF 261 and RRIM 712 clones are little sensitive; IRCA 130, AVROS 2037, IRCA 209 and GT 1 clones are fairly sensitive whereas RRIC 100, IRCA 230, PB 254, PB 260 and PB 235 clones appear enough susceptible to TPD. Especially PB235 clone is reputed to be a high latex production due to active metabolism and susceptible to TPD (Obouayeba *et al*., 2009).

Actually, characteristic of TPD symptoms are clearly expresses in the bark of rubber trees trunk. The macroscopic symptoms are expressed brown spots in the bark, bark thickening, bark cracking and bark deformations (Bhatia *et al.*, 1994; Krishnakumar *et al.*, 2003; de Fay *et al*., 2010; Venkatachalam *et al.*, 2010; Okoma *et al.*, 2011; Narayanan and Mydid, 2012). It is not only now received that TPD is a physiological disorder but also it has basically been divided two forms of TPD occur: on the one hand, it is reversible tapping cut dryness without any visible sign of bark necrosis, and on the other hand, it is an irreversible bark necrosis that complete stoppage of latex flow due to degeneration followed by the death of the laticiferous vessels with severely necrosis of bark at tapping panel. Furthermore, membrane destabilization leading to lutoid bursting has also been associated with the occurrence of an uncompensated oxidative stress in the bark of TPD affected trees (Venkatachalam *et al*, 2009; de Fay, 2011). Some researchers have concentrated on sieve tube characteristics. It has been observed that the sieve tube characteristics of healthy trees showed better functions than that of the unaffected area of TPD trees (Gopal and Thomas, 2012). It indicated that the sieve tube may be damage and collapse in TPD trees. Actually, loss of functional sieve tubes by collapse is a normal seasonal phenomenon happening when sieve tubes are ageing (de Fay *et al*., 2010).

Besides, there has one disease that calls trunk phloem necrosis (TPN). TPN is a physiological disease of rubber tree which is an irreversible disease. It spreads to tapping panel causing necrosis of the internal phloem and leading to damage of laticiferous vessels so stopping latex production (Pellegrin *et al*., 2007; Venkatachalam *et al*, 2009). TPN was sometimes observed on untapped or newly opened trees, it has been suspected to be supported by other factors than over exploitation (de Fay, 2011).

Nandris *et al*. (2004) suggested that TPN was supported by some environmental factors such as high soil compaction. The first stage is begins in the internal bark of trunk that is difficult to detect by macroscopic. The investigation of phloem necrosis by transmission electron microscopy revealed that cell wall, the middle lamella, plasmodesmata and cell membranes that are plasmalemma were disorganized (Nicole *et al*., 1991; Wongcharoen *et al*., 2011).

The occurrence of TPD which used tapping systems in rubber tree was studied. Senevirathna *et al*. (2007) reported that LFT systems are more suitable to minimize the incidence of TPD. There tested LFT system under drier climatic conditions in Sri Lanka with no indication on incidence of TPD (Kudaligama *et al*., 2010). The TPD is strongly correlated with sucrose decrease in the drained area (Jacob *et al.*, 1989). The used of LFT system allows a good carbohydrate supply and enhances the sustaining of sucrose available in the drained area (Obouayeba, *et al*., 2009). In addition, Silva *et al*. (2010) expressed that the half spiral downward cut (S/2) at third daily tapping (d3) and fourth daily tapping (d4) with ethephon concentration 2.5% provided lower TPD incidence compared with ethephon concentration 5% in the RRIM 600 and PR 261 clones. However, Traore *et al*., (2011) reported that the susceptibility to TPD increased with the number of annual stimulation because stimulated treatments presented the lowest thiol contents.

OBJECTIVE

Rubber smallholders in southern Thailand are using different tapping systems. The recommendation to change tapping frequency was not adopted by the rubber smallholder. Hence, the aim of this study is to show the importance of LFT systems with stimulation to sustain latex production. Furthermore, there was evident of climatic variability affective to rubber yield. Hence, it needs to investigate LFT tapping system to enhance latex yield potential.

The objectives of this study are as followings:

1. To assess the effect of reduced tapping frequency with stimulation on the physiological response and latex yield potential during seasonal variation,

2. To studies the response of latex cells metabolism to stimulation and their capability to sustain good yield,
3. To evaluate the reduction of tapping frequency with stimulation on total nonstructural carbohydrates content of rubber tree and latex yield potential, maybe considered as an indicator of the rubber tree potential yield

EXPECTED OUTPUT/OUTCOME

The LFT should be assessed and a potentially way to increase yield at each tapping should be found out to sustain land productivity without trespassing the rubber tree potential yield. Smallholders will be able to assess the benefit of LFT systems with ethylene stimulation with the objective to increase the income at each tapping. Then, it will be applied in southern Thailand to sustain rubber productions regarding the tapping labour shortage, scarcity of rubber price and climatic variability.

SCOPE OF RESEARCH

How to apply LFT systems with ethylene stimulation in southern Thailand? There were three parts of research including

1) the effect of reduced tapping frequency with stimulations on agronomical and physiological responses during seasonal variation
2) the changes of latex physiological parameters after stimulation
3) the evaluation of total nonstructural carbohydrate content under reduced tapping frequency systems of rubber trees

CHAPTER 2
RESEARCH METHODOLOGY

Materials and Methods

Plant materials

The experimentation were conducted at Thepa Research Station ($6^o48'0.7''$ North $100^o56'37.2''$ East, altitude 33 meter above from mean sea level) Thepa district, Songkhla province in southern Thailand. The climate is humid tropics dividing into two seasons comprised of dry and rainy season (Sternstein, 1962; TMD, 2013). Dry season is between mid-February to mid-May period and rainy season is between mid-May to mid-February period, characterized by temperature amplitude 22-33 °C; mean relative humidity is 79 %. Annual rainfall is up to 2,000 millimetres or more. The soil texture in this site was sandy loam with pH 5.5 (Coated, isohyperthermic, Typic Quartzipsamments) (Sainoi and Sdoodee, 2012).

The experiment was focusing on the RRIM 600 clone which is the most widely planted in Thailand (Chantuma *et al.*, 2011). The tree was planted in 2005 at 7×3 meters spacing that total area was 2 hectares (394 trees per hectare). An experiment was started in July 2013 until March 2016. The tree was selected before opening for homogenous girth, tapping started at 1.50 meters from the ground on panel BO-1. Climatic data was monthly recorded by Thepha Rainfall Station including total rainfall.

Methodologies

Experiment I: The effect of reduced tapping frequency with stimulations on agronomical and physiological responses during seasonal variation

This experiment was primarily assessing the effected of reduced tapping to latex production, girth increment, bark consumption, latex diagnosis and tapping panel dryness. The experiment was used 8-year-old trees and arranged as randomized complete block design, with five treatments (Table 1) each comprising three replications (elementary plot). There were ten trees per treatment in each elementary plot. The five treatments were established which modified from Vijayakumar *et al.* (2009) and Sopchoke (2010) as presented following;

Table 1 Treatments (tapping system) of the experiment

Treatments	**Tapping system and Description**	**TI***
T1	S/3 d1 2d/3 (usual system for Thai smallholders) 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 (standard for RRIT) 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) (standard for RRIT) 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 (%)

Tapping intensity can be calculated from various components of the tapping notation to provide a parameter for comparison and evaluation (Vijayakumar *et al.*, 2009) that explained using the relative intensity. The relative intensity is expressed in percentage of the standard system.

To calculate the relative intensity, multiply four times the ratio of the length of tapping cut (expressed in fraction) and the tapping interval with 100.

Example: S/2 d2 $= 4 \times \frac{1}{2} \times \frac{1}{2} \times 100 = 100\%$

The panel management schedule was set following the tapping systems (Figure 1). Tapping sequences were presented in Table 2 following the tapping frequency in each treatment. There was tapping rest period (stop tapping) during the defoliation to refoliation period in each year.

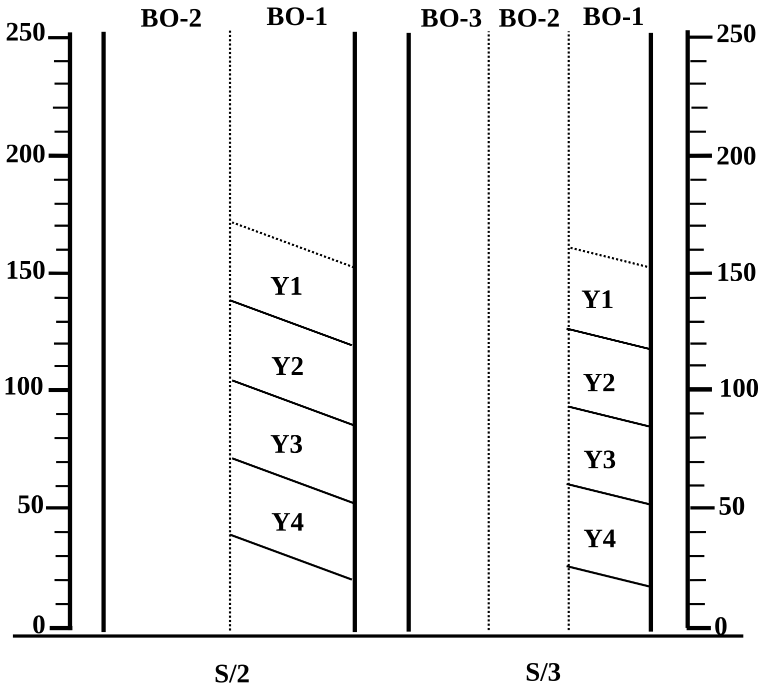


Figure 1 Panel management of half spiral cut (S/2) and third spiral cut (S/3)

Table 2 Tapping sequences of each tapping system

Treatments		Tapping sequence												
	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
T1		✓	✓		✓	✓		✓	✓		✓	✓		✓
T2		✓		✓		✓		✓		✓		✓		✓
T3	ET 8/y	✓			✓			✓			✓			✓
T4	ET 4/y	✓		✓		✓		✓		✓		✓		✓
T5	ET 12/y	✓			✓			✓			✓			✓

Note: ET is Ethephon stimulation

The ethylene stimulation agenda of the T3, T4 and T5 were treated following the number of application. The stimulation of this experiment was concentrated in the low yield period and high yield period. The T3 stimulated eight rounds per year distributed in May, June, August, September, October, November, December and March. The T4 stimulated four rounds per year in May, October, December and March. The T5 was monthly stimulated (Table 3).

Table 3 Ethylene stimulation (ethephon) agenda of the T3, T4 and T5

Treatments	Monthly											
	May	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**
T3 : 8/y	ET	ET		ET	ET	ET	ET	ET			ET	
T4 : 4/y	ET					ET		ET			ET	
T5 : 12/y	ET	ET	ET	ET	ET	ET	ET	ET	ET	ET	ET	ET

Note: ET is Ethephon stimulation

Parameters

Latex production

Latex yield was assessed by coagulate rubber. It was measured by weighing of cumulative coagulate rubber of each tree every month, then it was air-dried until constant weight determined as dry rubber. The latex production was calculated by subtracting 15% of the moisture content from the total air-dried weight and expressed in cumulative of kilogram per tree (kg/t) and average of gram per tree per tapping (g/t/t). The formulas were calculated following:

$$\text{Latex production (kg/t)} = \frac{\text{Annual total of cumulative yield}}{\text{Number of tree tapped}} \times 0.85$$

$$\text{Latex production (g/t/t)} = \frac{\text{Total of yield in each tree}}{\text{Number of tapping}} \times 0.85$$

Girth measurement

The girth increment was measured every year in March at 1.70 cm above the ground level and expressed in cm.

Bark consumption

Bark consumption was measured on the tapped panel every year from of the beginning to the end of the tapping period and expressed in cm.

Latex biochemistry or latex diagnosis (LD)

The latex diagnosis was assessed every month. Sampling was applied on trunk under the tapping cut (downward tapping) of rubber tree, the latex was collected in the morning. Iron punch equipment was punched in the bark until reaching the wood of rubber tree. The position was 5 cm below at the middle of the cut, polyethylene tube was inserted inside the hold in order to collect the latex. The first 2 drops of latex were discarded because its unstable and contaminated latex, following 7-10 drops were collected in sampling tube. The procedure of latex sampling was pooled latex sampling (Jacob *et al.*, 1989). The total solid content (%), sucrose content (mM), inorganic phosphorus content (mM) and reduced thiol contents (mM) were evaluated according to method developed by CIRAD and CNRA adapted in 1995 by IRRDB (IRRDB, 1995) and updated for Thailand by Gohet and Chantuma (1999).

Total solid content (TSC)

TSC calculation is as following:

$$TSC = [(Wr - Wo)/(Wf - Wo)] \times 100$$

Wf = weight of pill + 1 ml fresh latex (g)

Wr = weight of pill + dried latex (g)

Wo = weight of empty pill (g)

Sucrose content (Suc)

Suc calculation is as following:

$$[Suc]mM = OD \times K_{Suc} \times 10$$

OD = the reading value absorbance in 627 nm at spectrophotometer

K = coefficient of the standard curve

Inorganic phosphorus content (Pi)

Pi calculation is as following:

$$[Pi]mM = OD \times K_{Pi} \times 10$$

OD = the reading value absorbance in 410 nm at spectrophotometer

K = coefficient of the standard curve

Reduced thiol content (RSH)

RSH calculation is as following:

$$[RSH]mM = OD \times K_{RSH} \times 10$$

OD = the reading value absorbance in 412 nm at spectrophotometer

K = coefficient of the standard curve

Tapping panel dryness (TPD)

Tapping panel dryness was evaluated by counting the trees which was bark dryness. It expressed as the percentages in each treatment. Assessment of tapping panel dryness was noted on a scale from 0 to 6 according to the importance of the syndrome by visual estimation (Van de Sype, 1984). On each tapping tree, the notation followed to the code below:

Class 0: healthy tree, easy flow on the length of the cut

Class 1: 10 % - 20 % of the cut length is dries (difficult flow)

Class 2: 21 % - 40 % of the cut length is dries (less than the half cut)

Class 3: 41 % - 60 % of the cut length is dries (half cut)

Class 4: 61 % - 80 % of the cut length is dries (more than half cut)

Class 5: 81 % - 100 % of the cut length is dries (almost full dry)

Class 6: 100 % of the cut length is dry; no latex, tapping will be stopped

The calculation of tapping panel dryness is followed:

Number of trees:

n0 = number of healthy trees in class 0

n1 = number of trees in class 1

n2 = number of trees in class 2

n3 = number of trees in class 3

n4 = number of trees in class 4

n5 = number of trees in class 5

n6 = number of trees in class 6

Total number of trees (Tn) = n0 + n1 + n2 + n3 + n4 + n5 + n6

Percentage of the total tapping panel dryness is followed:

$$TPD = \frac{(0.1\times n1)+(0.3\times n2)+(0.5\times n3)+(0.7\times n4)+(0.9\times n5)+n6}{Tn}$$

Experiment II: The changes of latex physiological parameters after stimulation

The response of the trees to ethylene stimulation (ethephon) was studied in each tapping day before and after stimulation. The experiment was 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 yield at each tapping is usually high and full canopy stage (Sopharat *et al.,* 2015).

The experiment was used 9-year-old trees and arranged as one tree plot design. There were five treatments (T1: S/3 d1 2d/3 (usual system for Thai smallholders), T2: S/2 d2 (standard for RRIT), T3: S/2 d3 ET 2.5% Pa1(1) 8/y (m), T4: S/3 d2 ET 2.5% Pa1(1) 4/y (m) (standard for RRIT) and T5: S/3 d3 ET 2.5% Pa1(1) 12/y (m)) comprising three trees per treatment. The tapping agenda is presented in Table 4.

Table 4 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
1st tapping Tapping (-2)	2nd tapping Tapping (-1)	ET	1st tapping Tapping (+1)	2nd tapping Tapping (+2)	3rd tapping 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

Parameters

Average latex yield (AY)

Latex yield (g/t/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 into grams of dry rubber per tree and per tapping.

Initial flow rate (IFR)

Initial flow rate was collected in the first 5 minutes in each tree and calculated using the formula:

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

The volume was expressed in milliliter per minute (ml/min).

Plugging index (PI)

Plugging index was determined by initial flow rate and total of latex. It was calculated following the method of Milford *et al.* (1969) using the formula:

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

Sucrose content (Suc)

The main latex biochemical parameters, i.e. sucrose (Suc) content, was measured in a latex sample of 1 milliliter in the first five 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 (mM) of latex and updated for Thailand by Gohet and Chantuma (1999).

Experiment III: The evaluation of total nonstructural carbohydrate content under reduced tapping frequency of rubber trees

The experiment was evaluating the dynamics of total nonstructural carbohydrate content on the panel of rubber tree using reduced tapping system under two periods including the low yield period (LY) and the high yield period (HY). LY was in May 2015 when tapping was started. HY was in October 2015, in the rainy season, when yield at each tapping is usually high and full canopy stage (Sopharat *et al.*, 2015). The experiment was used 9-year-old trees and arranged as one tree plot design. There were six treatments (T1: S/3 d1 2d/3 (usual system for Thai smallholders), T2: S/2 d2 (standard for RRIT), T3: S/2 d3 ET 2.5% Pa1(1) 8/y (m), T4: S/3 d2 ET 2.5% Pa1(1) 4/y (m) (standard for RRIT), T5: S/3 d3 ET 2.5% Pa1(1) 12/y (m) and T6: untapped trees) and all treatments comprising six trees per treatment. The first of three trees in each treatment was sampling to collect in the low yield period and the last of three trees in each treatment was sampling to collect in the high yield period.

The position of puncture was markedly made at the tapping panel downward cut because the cumulative effect of tapping resulted in a shortage of starch within superficial wood layers behind the tapping panel, whereas starch accumulated above the tapping cut (Gohet, 1996 refer by Chantuma *et al*., 2007). There have three positions for wood and bark collection, the first position puncture was below approximately 10 centimeter from tapping cut panel. The distance between first, second and third position puncture was 10 centimeter below as presented in Figure 2.

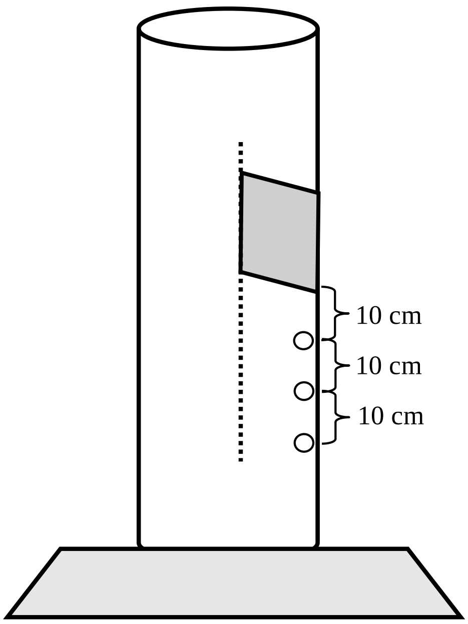


Figure 2 The sampling design of position punctured on the tapping panel

The sampling was collected by using increment borer equipment (5 mm diameter) to extract that punched 5 centimeter depth at the trunk of the rubber tree. Samplings were separated between bark and wood (Figure 3) and stored on ice, inside paper bags to analyze TNC.

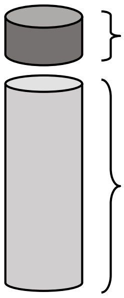


Figure 3 The sampling of bark and wood

Parameter

Total nonstructural carbohydrate

All samples (wood and bark) were dried weigh for 72 hour at 70°C by oven. Afterwards the dried samples were grind to a fine powder. The TNC was measured by the Manual Clegg Anthrone method as described by Osborne and Voogt (1978) with weighting 0.1 g of samplings powder inside the tube and poured 1.0 ml of distilled water following 1.3 ml of 52% perchloric acid to extract the starch (Brooks *et al*., 1986; Chen *et al*., 2012). Sample was stirred for approximately 5 minutes on a magnetic plate agitator and waited for 10 minutes to continue the reaction of solution. The solution was filtered and adjusted to 50 ml with distilled water. After that 1.0 ml of solution was poured in the glass tube in 5 ml of 0.1% anthrone and vibrated with vortex mixture approximately 5 minutes. The tube was brought to the water bath, immerged approximately 15 minute when water temperature was stabilised at 80°C. After cooling at room temperature, the absorbance at 630 nm was recorded in each sampling tube and compared the total nonstructural carbohydrate value with glucose standard curve. The volume was expressed in as milligram per gram dry weight (mg/g DW).

Statistical analysis

The data were tested using analysis of variance (ANOVA) in the DSAASTAT v 1.1 package (Onofri, 2007). The differences among mean were separated using Duncan's multiple rang test (DMRT) at *P*-value ≤0.05 and highly significant at *P*-value ≤0.01.

CHAPTER 3
RESULTS

Weather condition

The weather condition data was recorded in this experiment (June 2013 – March 2016) as shown in Figure 4. In 2013, there was rainfall in every month from June to December 2013. The highest of the rainfall was recorded in December (243 mm) and it caused flooding in the trial. In 2014, there was rain during summer, particularly in March, April and May with more rainfall in May. After that there was rainfall to the end of year and the highest of the rainfall was shown in December (503.3 mm). In 2015, there was rainfall in dry season (April - May) until the end of year with the highest rainfall in November (306.2 mm). In 2016, there was slightly rainfall at the beginning of year (January). However, there was partial rainfall in dry season and it accumulated to get the high rainfall in the end of year, especially in September to November in each year.

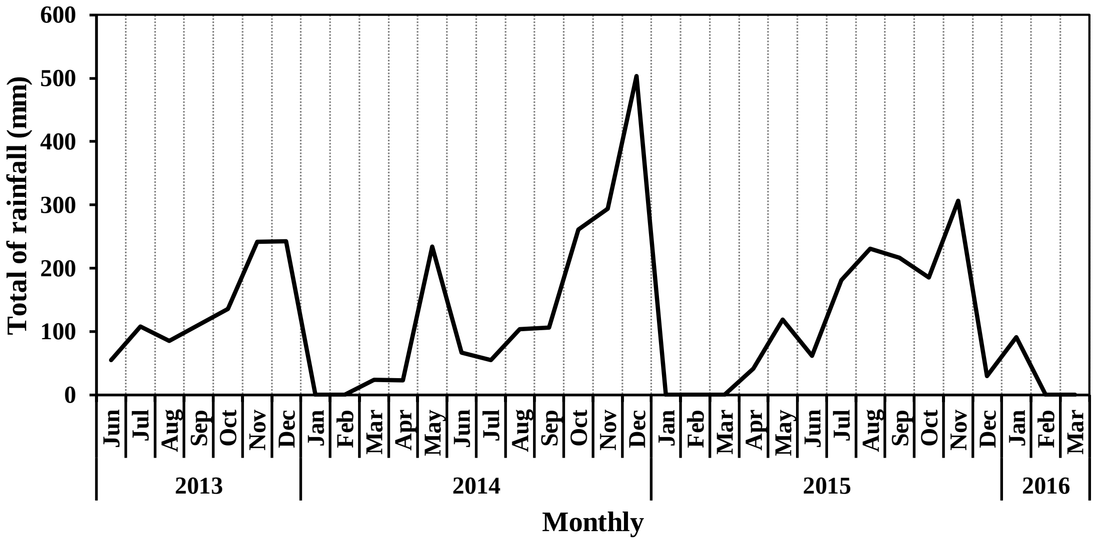


Figure 4 Total of rainfall (mm) among June 2013 to March 2016 of experiment

The effect of reduced tapping frequency with stimulations on agronomical and physiological responses during seasonal variation

Latex production

After 3 years of tapping, an average latex cup lump yield (g/t/t) was shown in Table 5. There was a significant difference among the 5 treatments. The highest of the yield per tree and per tapping was found for T3 with 78.32 g/t/t and reached over 68% comparing with T1 (conventional tapping system), which gave the lowest of the yield with 46.57 g/t/t. Besides, the low frequency tapping systems (S/2 d3 and S/3 d3) compensated by different level of ethylene stimulation showed higher yield than the other treatments. For the d2 tapping frequencies, yield of T2 with a longer cut length (S/2) was not significantly different from T4 which a shorter cut length (S/3) and ethylene stimulation. For the d3 tapping frequencies, T3 with a longer cut length (S/2) and stimulation gave higher yield than that of T5 with a shorter cut length (S/3) and ethylene stimulation.

There was a significant difference among the 5 treatments (Table 5) for average cumulative latex cup lump (kg/t). T1 gave the highest cumulative yield (7.2 kg/t), in contrast, the lowest was found in T5 (6.5 kg/t) or decreased 9.5% comparing with T1. However, the cumulative yield of the treatments T2, T3 and T4 were not significantly different from T1 but they showed lower values by decreasing 1% in T2 and T3 and 4% in T4. For the d2 tapping frequencies, the cumulative yield of T2 (S/2, no stimulation) expressed not significantly different from T4 (S/3 with stimulation). For the d3 tapping frequencies, T3 (S/2 with stimulation) gave significantly higher of cumulative yield than T5 (S/3 with stimulation).

Table 5 Average latex cup lump (g/t/t) and average cumulative latex cup lump (kg/t) among the 5 treatments during 3 years tapping (August 2013 – March 2016).

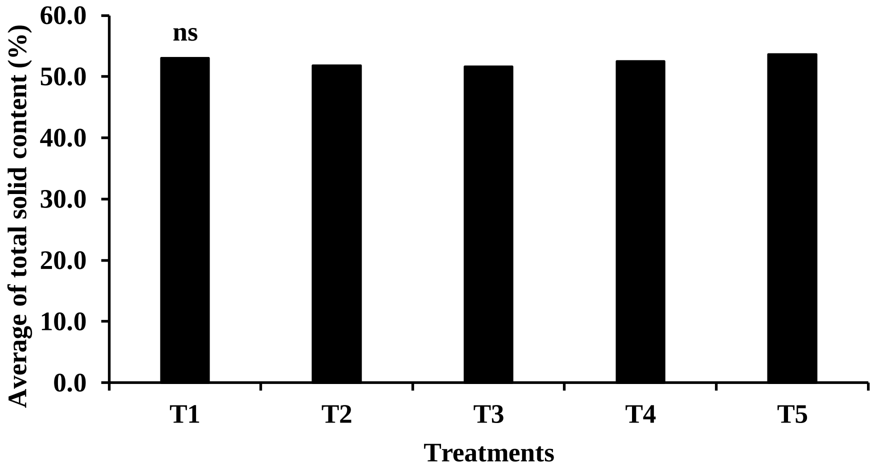
Treatments	**Average latex cup lump**		**Average cumulative latex cup lump**	
	g/t/t	**%**	**kg/t**	**%**
T1: S/3 d1 2d/3	46.57d	100	7.2a	100
T2: S/2 d2	62.88c	135.0	7.1a	99.1
T3: S/2 d3 ET 2.5% Pa1(1) 8/y (m)	78.32a	168.2	7.1a	99.4
T4: S/3 d2 ET 2.5% Pa1(1) 4/y (m)	61.22c	131.5	6.9ab	96.5
T5: S/3 d3 ET 2.5% Pa1(1) 12/y (m)	71.31b	153.1	6.5b	90.5
F-test	**		**	
C.V. (%)	15.05		16.83	

Note: Values with the different letters in the same column indicate significant difference with $P \leq 0.01$ by DMRT

Latex biochemistry

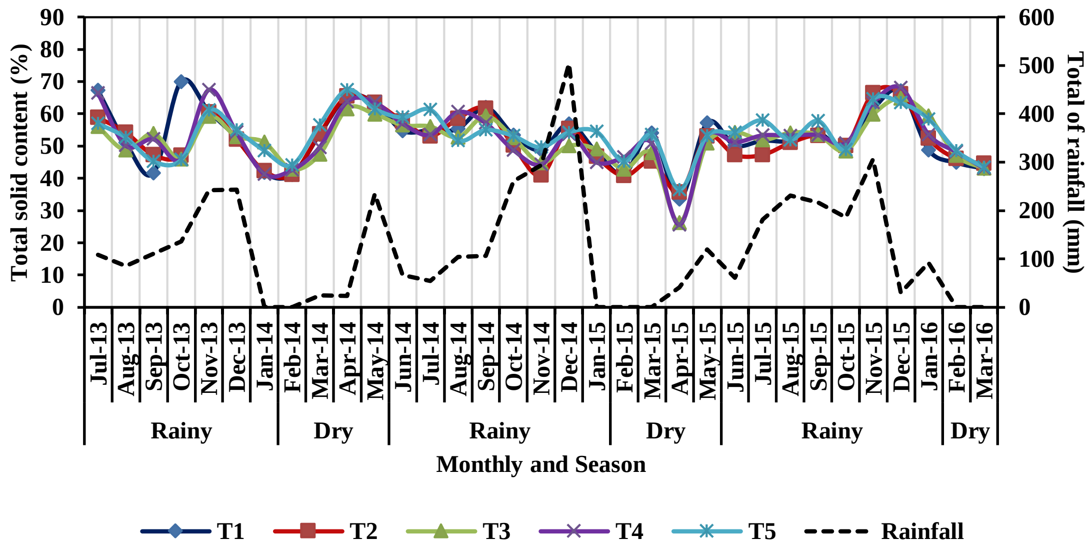
Figure 5 showed an average of total solid content (TSC). There was no significantly different among the 5 treatments. TSC was over 50% of the 5 treatments, it varied from 51.6–53.6%. The highest TSC was found in T5, while the lowest was found in T3.

The TSC was varying in each month of each year as shown in Figure 6. The 5 treatments showed same trend. Trend of the TSC decreased when the rainfall decreased in January 2014, April 2015 and February 2016. The TSC was decreased when the dry season was starting. In the rainy season, the TSC seems to be related to the rainfall.



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)

Figure 5 Average of total solid content (%) of the 5 treatments in 3-year tapping (July 2013 – March 2016); ns = non-significant difference.

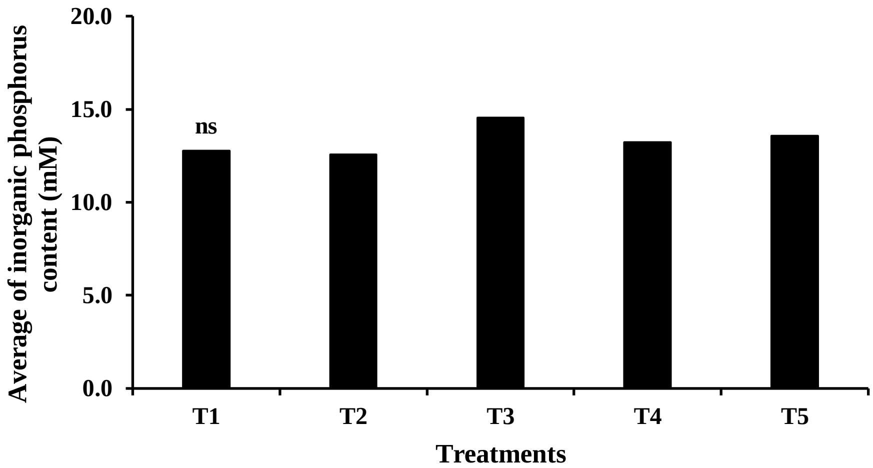


T1: S/3 d1 2d/3, T2: S/2 d2, T3: S/2 d3 ET 2.5% Pa1(1) 8/y (m), T4: S/3 d2 ET 2.5% Pa1(1) 4/y (m), T5: S/3 d3 ET 2.5% Pa1(1) 12/y (m)

Figure 6 Variation of total solid content (%) of the 5 treatments and total of rainfall (mm) in each month (July 2013 – March 2016)

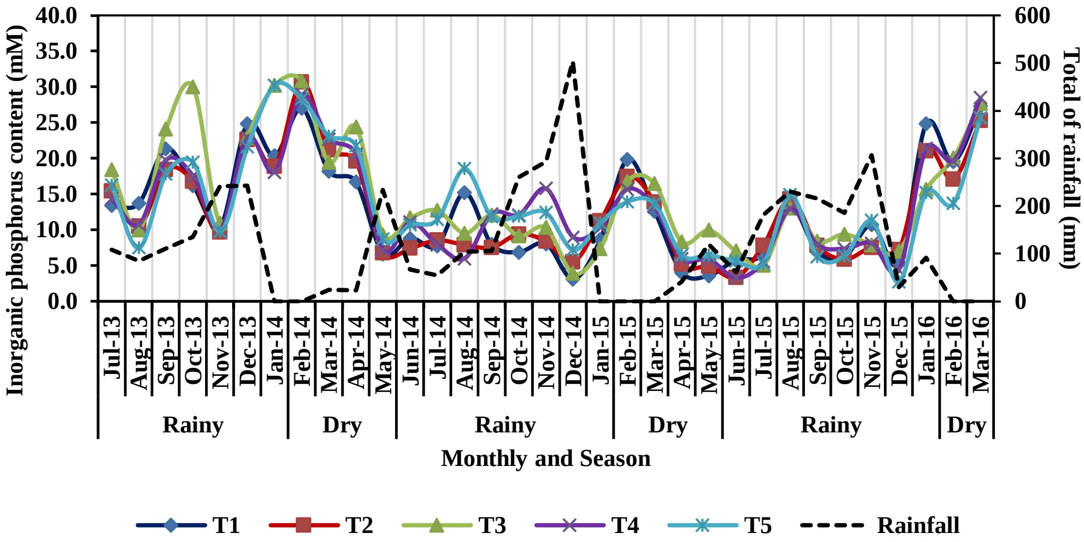
Average of inorganic phosphorus content (Pi) was not significantly different among the 5 treatments (Figure 7). However, T3 had the highest Pi (14.55 mM), whereas the lowest value was found for T2 (12.58 mM). Treatments reducing tapping frequency (d3) showed higher Pi than the other treatments (2d/3 and d2) which had higher tapping frequency.

Pi varied in each month of each year. The Pi of the 5 treatments had the same trend (Figure 8). Besides, the Pi was related to rainfall intensity. It showed high value when starting in dry season. Then it increased when the rainfall decreased as showed in January 2014, February 2015 and January 2016.



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)

Figure 7 Average of inorganic phosphorus content (mM) of the 5 treatments in 3 year tapping (July 2013 – March 2016); ns = non-significant difference.

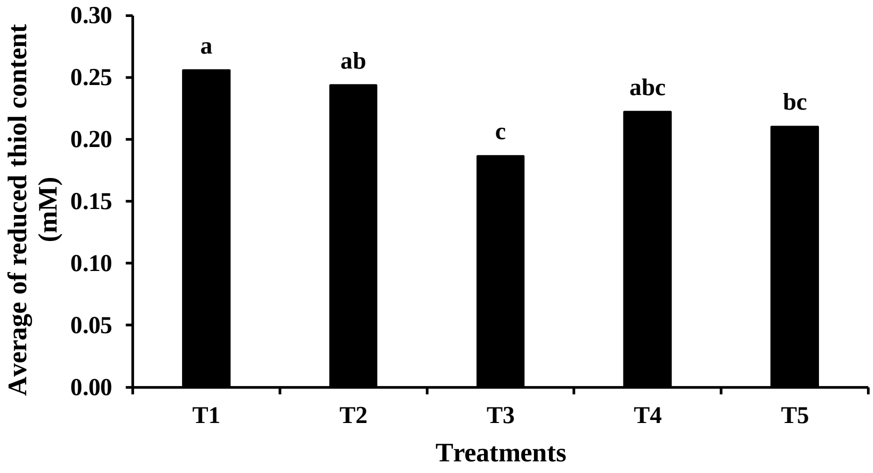


T1: S/3 d1 2d/3, T2: S/2 d2, T3: S/2 d3 ET 2.5% Pa1(1) 8/y (m), T4: S/3 d2 ET 2.5% Pa1(1) 4/y (m), T5: S/3 d3 ET 2.5% Pa1(1) 12/y (m)

Figure 8 Variation of inorganic phosphorus content (mM) of the 5 treatments and total of rainfall (mm) in each month (July 2013 – March 2016)

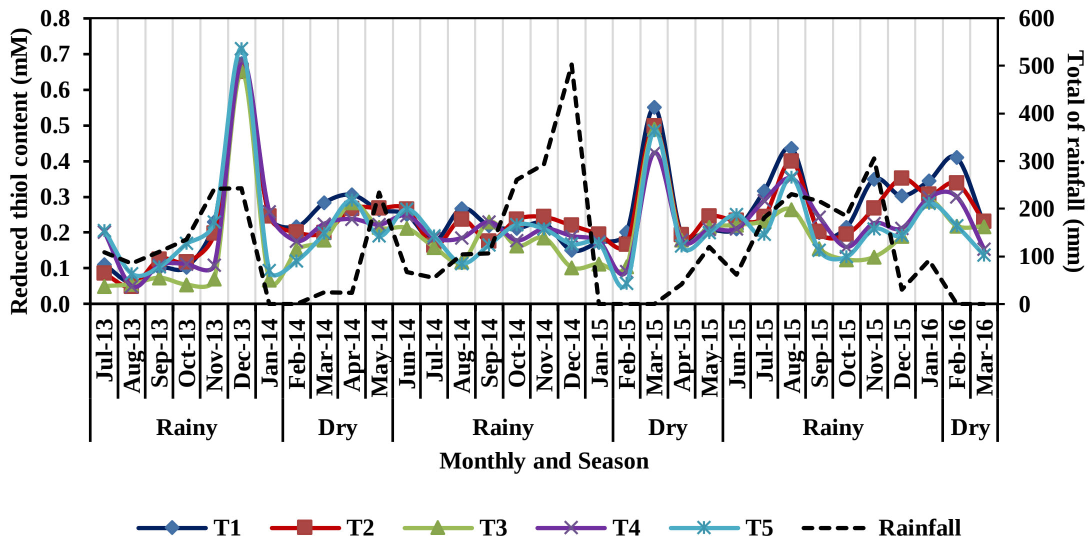
Average of reduced thiol content (RSH) was shown in the Figure 9. The RSH of the 5 treatments were significantly different. The highest RSH was found in T1 (0.26 mM), whereas T3 showed the lowest value (0.19 mM). However, the RSH was lower for the d3 tapping frequency systems than the d2 tapping frequency systems. Besides, treatments with stimulation (T3, T4 and T5) showed lower RSH than the treatments without stimulation (T1 and T2).

The RSH varied in each month of each year in Figure 10. RSH of the 5 treatments was the same direction. The T1 and T2 showed higher RSH than that of the stimulation treatments (T3, T4 and T5) in each month. RSH increased in dry season. RSH increased after the rainfall increased which is shown in December 2013, March, August and November 2015 and February 2016.



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)

Figure 9 Average of reduced thiol content (mM) of the 5 treatments in 3 year tapping (July 2013 – March 2016); different letters in each bar graph indicate significant difference at $P \leq 0.05$ by DMRT

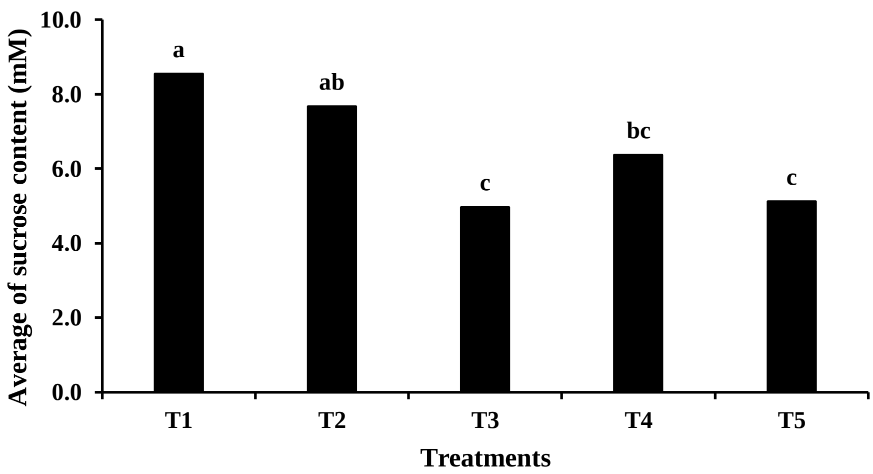


T1: S/3 d1 2d/3, T2: S/2 d2, T3: S/2 d3 ET 2.5% Pa1(1) 8/y (m), T4: S/3 d2 ET 2.5% Pa1(1) 4/y (m), T5: S/3 d3 ET 2.5% Pa1(1) 12/y (m)

Figure 10 Variation of reduced thiol content (mM) of the 5 treatments and total of rainfall (mm) in each month (July 2013 – March 2016)

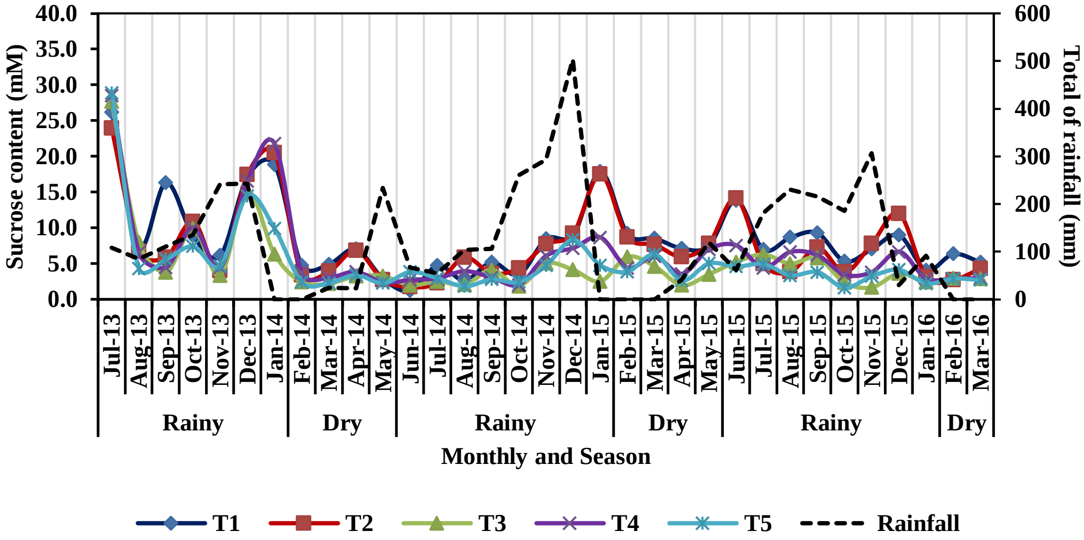
Figure 11 showed an average sucrose content (Suc). There were significant differences among the 5 treatments. The Suc of T1 was the highest value (8.54 mM) and the lowest was found in T3 (4.96 mM). The treatments with ethylene stimulation showed lower the Suc than that of the non-stimulated treatments. Besides, the Suc under d3 tapping frequencies showed lower content than the higher tapping frequency (2d/3, d2).

The variation of Suc in each month of each year was shown in Figure 12. The Suc of the 5 treatments had the same trend and was related with the rainfall. The Suc was increased when the rainfall increased like in January 2014, January, June and December 2015. However, the non-stimulated treatments (T1 and T2) had clearly shown higher Suc than the stimulated treatments (T3, T4 and T5).



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)

Figure 11 Average of sucrose content (mM) of 5 treatments in 3 year tapping (July 2013 – March 2016); different letters in each bar graph indicate significant difference at $P \leq 0.05$ by DMRT



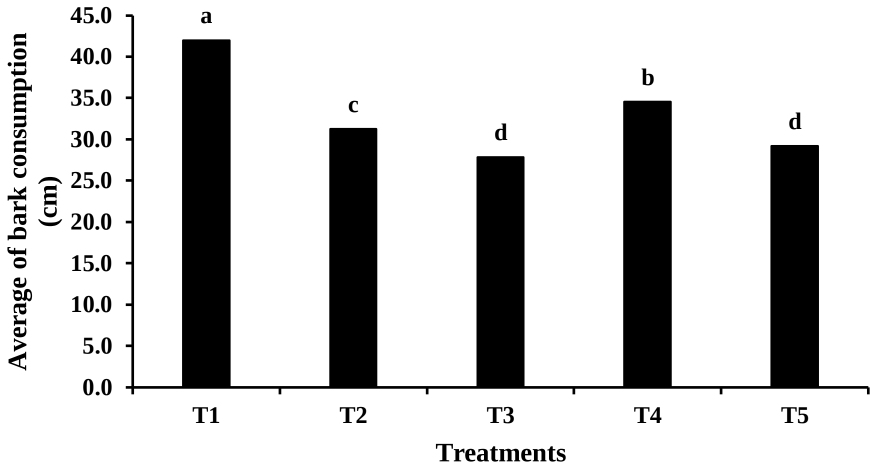
T1: S/3 d1 2d/3, T2: S/2 d2, T3: S/2 d3 ET 2.5% Pa1(1) 8/y (m), T4: S/3 d2 ET 2.5% Pa1(1) 4/y (m), T5: S/3 d3 ET 2.5% Pa1(1) 12/y (m)

Figure 12 Variation of sucrose content (mM) of the 5 treatments and total of rainfall (mm) in each month (July 2013 – March 2016)

Bark consumption and girth increment

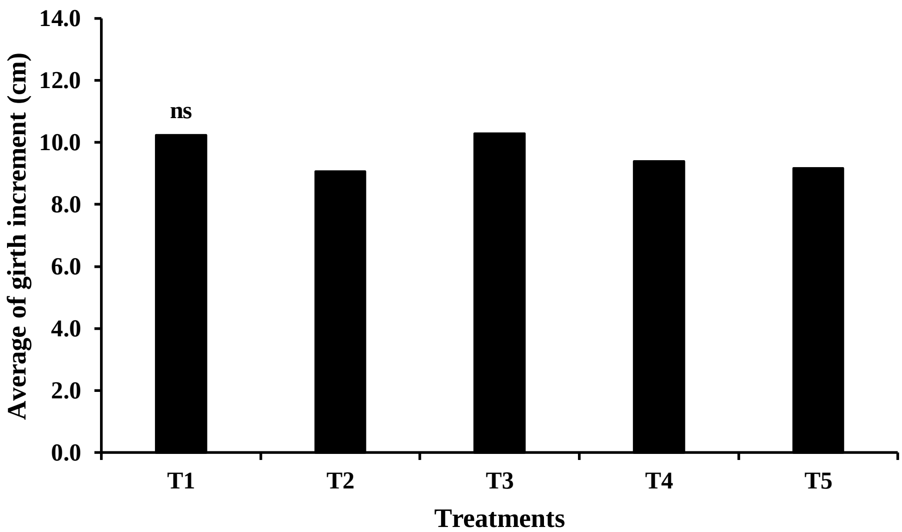
Average of bark consumption was significantly different among the 5 treatments. During 3-year tapping, T1 showed the highest bark consumption (42.0 cm). The d3 tapping frequency systems showed the lowest of bark consumption compared with the other treatments (Figure 13).

Figure 14 shows an average of girth increment, there was no significant differences among the 5 treatments. The girth increment of T3 was the highest (10.3 cm) and the lowest was shown with T2 (9.1 cm). For the d2 tapping frequencies, T4 (S/3 with stimulation) had a girth increment higher than T2 (S/2). For the d3 tapping frequencies, T3 (S/2 with ethylene stimulation) had higher girth increment than that of T5 (S/3 with stimulation). It seems logical, lower the tapping frequency lower the bark consumption. But there was no effect of treatments on girth increment, as the yield was not different too. Only T5 showed the lowest yield and low girth increment.



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)

Figure 13 The bark consumption (cm) of the 5 treatments in 3-year tapping (August 2013 – March 2016); different letters in each bar graph indicate significant difference at $P \leq 0.05$ by DMRT



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)

Figure 14 The girth increment (cm) of the 5 treatments in 3 year tapping (August 2013 – March 2016); ns = non-significant difference

Tapping panel dryness

Tapping panel dryness (TPD) was only found in the treatments with ethylene stimulation (Table 6). The rate of TPD was related with the number of ethylene stimulation. T3 and T5 showed higher TPD than that of T4.

Table 6 Tapping panel dryness (%) among the 5 treatments in 3 year tapping (August 2013 – March 2016).

Treatments	**Tapping panel dryness (%)**
T1: S/3 d1 2d/3	0.00
T2: S/2 d2	0.00
T3: S/2 d3 ET 2.5% Pa1(1) 8/y (m)	5.00
T4: S/3 d2 ET 2.5% Pa1(1) 4/y (m)	3.00
T5: S/3 d3 ET 2.5% Pa1(1) 12/y (m)	5.33

The changes of latex physiology parameters after stimulation

The difference of tapping systems on latex parameters

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 (low yield period and high yield period) of study are shown in Table 7. IFR was significantly different in each tapping day in the both low yield period and high yield period of the 5 treatments excepted 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 other treatments in the high yield period. The IFR increased on the first tapping day after stimulation only, of T3 and T5 in the low yield period. Conversely, IFR of the ethylene stimulation treatments rapidly increased on the first tapping day after stimulation in the high yield period. Under alternated tapping frequencies, IFR of T2 was higher than T4 in the both periods.

The PI was significantly different on each tapping day of the both periods among the 5 treatments except the 2 tapping days before stimulation in the low yield period (Table 7). The 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 for T2 in the high yield period. The stimulated treatments (T3, T4 and T5) showed a lower PI than that of the non-stimulated treatments (T1 and T2) and it rapidly decreased on the first tapping day after stimulation in the two periods.

The 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 7). The 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, while T3 showed the highest. However, AY of the T2 and T4 did not show significant difference on each tapping day. Besides, AY of the T3 and T5 immediately increased on the first tapping day after ethylene stimulation. In the high yield period, AY of the T4 was the highest in the 2 tapping days before stimulation and the first tapping day after stimulation, while the T3 was the highest on the second and the third tapping days after stimulation. In addition, the AY in the 3 tapping days after stimulation of the T2, T3, T4 and T5 was higher than that of the T1.

Regarding the sucrose content of the latex cells, Suc showed significant difference in each tapping day in both periods among the 5 treatments (Table 7). The trend of the Suc change was ambiguous. In the both periods, Suc of T1 was the highest in the 2 tapping days before stimulation. The Suc decreased in the all treatments except the T3, on the first tapping day after stimulation. In addition, Suc of the 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 the Suc during the high yield period was somewhat less than in the low yielding period.

Table 7 Initial flow rate (IFR) (ml/min); plugging index (PI); average latex yield (AY) (g/t/t) and sucrose content (Suc) (mM) in each tapping day among the 5 treatments in the low yield period and the high yield period

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)

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)**
	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}
IFR	**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}
	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}
PI	**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}
	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}
AY	**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}
	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}
Suc	**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

Effect of seasonal variation on latex physiology

Table 8 shows the comparison of IFR and PI between both periods in each tapping day of the 5 treatments. IFR in each tapping day of T1, T2 and T4 in the low yield period was lower than that of the high yield period, but IFR of 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 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 8 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 9 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 the T3 did not show any significant different 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.

Table 9 Average latex yield (g/t/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/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

Changes of latex physiology after stimulation

Table 10 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, T1 and T2 did not receive any stimulation. For the response to ethylene stimulation in both periods, IFR of 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 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 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.

Table 10 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 ($P \leq 0.01$)

The response of PI to ethylene stimulation on the 3 tapping days after stimulation of the 5 treatments is shown in Table 11. 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 different 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 in the 3 tapping days after stimulation. While, 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.

Table 11 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 ($P \leq 0.01$)

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 12. 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 difference 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 high yield period.

Table 12 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/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 13 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 yid period. Besides, the response to ethylene stimulation showed that Suc of T3 in the low yield period showed significant difference among the taping 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 the Suc in the high yield period showed no significant difference except on the first tapping day after stimulation.

Table 13 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 ($P \leq 0.01$)

The evaluation of total nonstructural carbohydrate content on reduced tapping frequency of rubber trees

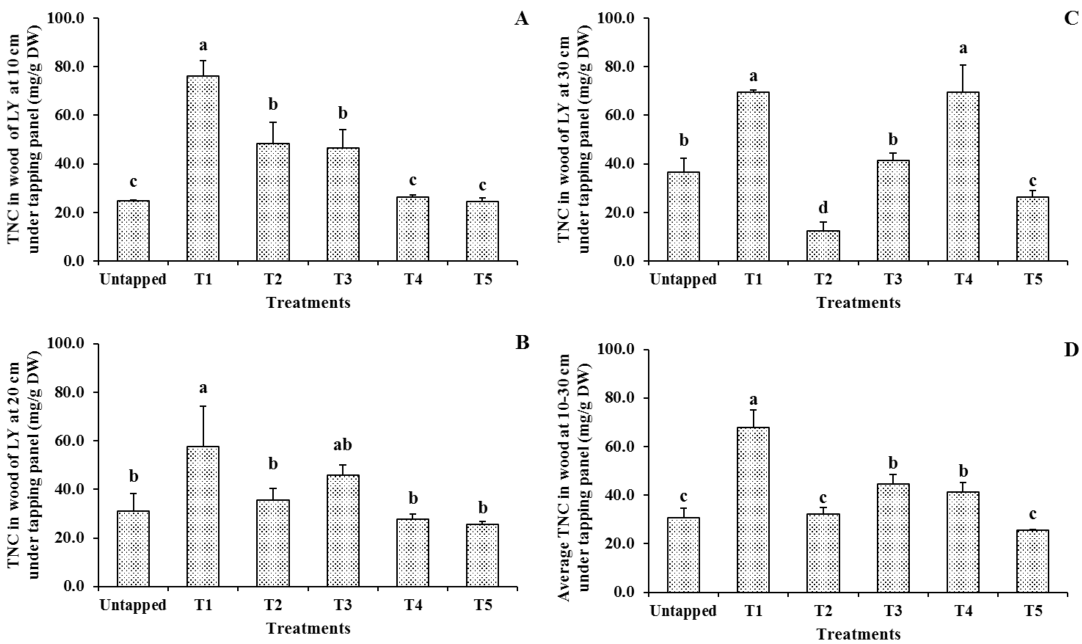
Effect of different tapping systems on TNC in wood for the low yield period

In the low yield period, total nonstructural carbohydrate content (TNC) in wood (mg/g DW) among the 6 treatments on each distance showed significant difference (Figure 15). At the 10 cm distance under tapping panel (Figure 15A), T1 (farmers tapping system) had the highest TNC content in wood, while the lowest was found for T5. The d2 tapping frequencies, T2 (S/2, no stimulation) showed higher TNC content than T4 (S/3 with stimulation). The d3 tapping frequencies, T3 (S/2 with stimulation) had higher TNC content than T5 (S/3 with stimulation). However, the TNC content of T4 and T5 showed similar and no significant difference with the untapped tree.

At the 20 cm distance under tapping panel (Figure 15B), T1 showed the highest TNC content but it did not show significant difference with T3, the lowest was seen for T5. The d2 tapping frequencies, T2 (S/2) had higher TNC content than T4 (S/3) with stimulation. For the d3 tapping frequencies, T3 (S/2) with stimulation gave higher TNC content than T5 (S/3) with stimulation. In addition, all of treatments did not show a significant difference with the untapped tree excepting T1.

At the 30 cm distance under tapping panel (Figure 15C), TNC content was the highest in T4 but it did not show significant difference with T1 and TNC was the lowest in T2. For the d2 tapping frequencies, T2 had lower TNC content than T4. For the d3 tapping frequencies, T3 gave higher TNC content than T5. The TNC content of the untapped trees and T3 had similar value, significantly higher than that of T2 and T5.

An average of TNC content on the 10 - 30 cm distance under tapping panel is shown in Figure 15D. T1 gave the highest TNC content, the lowest was found in T5. For the d2 tapping frequencies, T2 had lower TNC content than T4. For the d3 tapping frequencies, T3 showed higher TNC content than T5. Besides, TNC of T3 and T4 was not significantly different but showed higher TNC than the untapped tree, while T2 and T5 showed similar.



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), T6: Untapped

Figure 15 TNC in wood (mg/g DW) of low yield period (LY) at 10 cm (A); at 20 cm (B); at 30 cm (C) and average at 10-30 cm (D) under tapping panel among the 6 treatments in May 2015; different letters in each bar graph indicate significant difference at $P \leq 0.05$ by DMRT

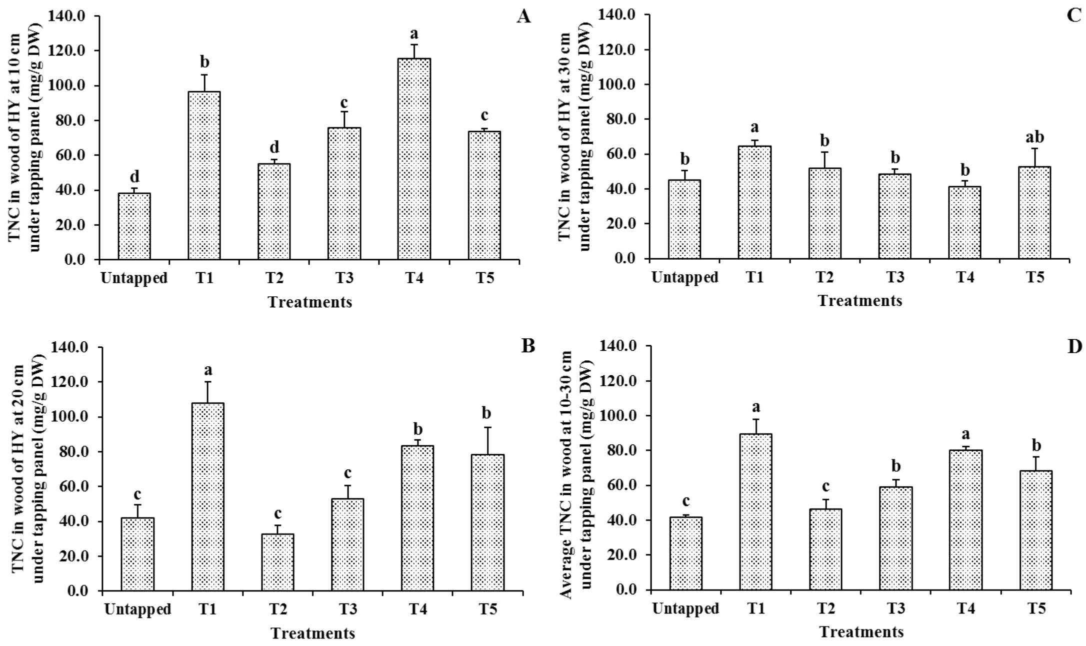
Effect of different tapping systems on TNC in wood for the high yield period

In the high yield period, total nonstructural carbohydrate content (TNC) in wood (mg/g DW) among the 6 treatments on each distance showed significant difference (Figure 16). At the 10 cm distance under tapping panel (Figure 16A), T4 showed the highest TNC content, while the untapped tree was the lowest TNC content. For the d2 tapping frequencies, T2 with S/2 cut length showed lower TNC content than T4 with S/3 cut length with stimulation. The difference is made by the length of the cut and ethylene stimulation application. For the d3 tapping frequencies, T3 with S/2 cut length and with ethylene stimulation had higher TNC content than T5 with a shorter cut length (S/3) still with stimulation but it did not show significant difference. Besides, the untapped tree had not significant difference for TNC content with T2.

At the 20 cm distance under tapping panel (Figure 16B), T1 showed the highest TNC content. The lowest TNC was found for T2. For the d2 tapping frequencies, T2 (S/2) had lower TNC content than T4 (S/3, with stimulation). For the d3 tapping frequencies, T3 had lower TNC content than T5. The TNC content of T2 and T3 were not a significantly different with the untapped tree.

At the 30 cm distance under tapping panel (Figure 16C), the TNC content for T1 was the highest but did not show significant difference with T5. The lowest TNC was found in T4. For the d2 tapping frequencies, T2 had higher TNC content than T4. For the d3 tapping frequencies, T3 had lower TNC content than T5 but it did not show significant difference. However, the TNC content of the untapped trees was not significantly different with T2, T3, T4 and T5.

An average of TNC content on the 10 - 30 cm distance under tapping panel is shown in Figure 16D. T1 gave the highest TNC content but it did not show a significant difference with T4. The lowest TNC was found for the untapped tree. For the d2 tapping frequencies, T2 had lower TNC content than T4. For the d3 tapping frequencies, T3 had lower TNC content than T5.



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), T6: Untapped

Figure 16 TNC in wood (mg/g DW) of high yield period (HY) at 10 cm (A); at 20 cm (B); at 30 cm (C) and average at 10-30 cm (D) under tapping panel among the 6 treatments in October 2015; different letters in each bar graph indicate significant difference at $P \leq 0.05$ by DMRT

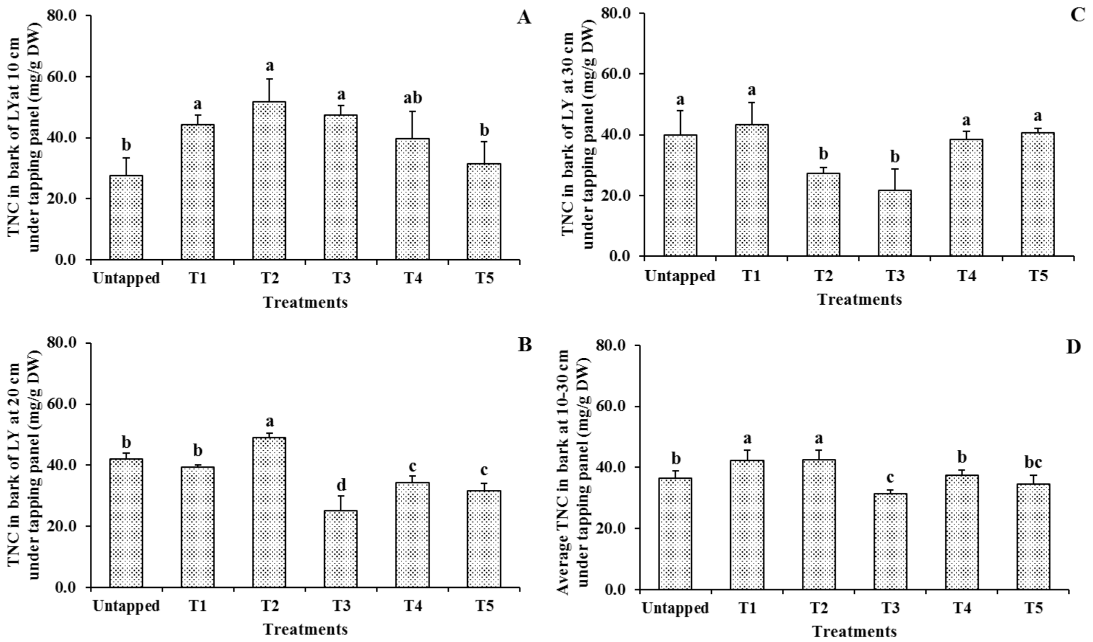
Effect of different tapping systems on TNC in bark for the low yield period

In low yield period, total nonstructural carbohydrate content (TNC) in bark (mg/g DW) among the 6 treatments on each distance were significantly different as shown in Figure 17. At the 10 cm distance under tapping panel (Figure 17A), T2 showed the highest TNC content, while the untapped tree had the lowest TNC content. For the d2 tapping frequencies, T2 with longer cut length showed higher TNC content in bark than T4 with shorter cut length but with ethylene stimulation. For the d3 tapping frequencies, T3 with S/2 cut length with stimulation gave higher TNC content than T5 with a shorter cut length (S/3) with more intensive stimulation (12/y). Besides, the untapped tree was not shown significant differences with T4 and T5.

At the 20 cm distance under tapping panel (Figure 17B), T2 showed the highest TNC content. The lowest was found for T3. For the d2 tapping frequencies, T2 (S/2) showed higher TNC content than T4 (S/3 with stimulation). For the d3 tapping frequencies, T3 (S/2 with stimulation) gave lower TNC content than T5 (S/3 with stimulation). The TNC contents of the untapped tree and T1 were similar and higher than TNC of stimulated treatments (T3, T4 and T5).

At the 30 cm distance under tapping panel (Figure 17C), the TNC content was the highest in T1 and the lowest was found in T3. For the d2 tapping frequencies, T2 (S/2) had lower TNC content than T4 (S/3 with stimulation). For the d3 tapping frequencies, T3 (S/2 with stimulation) had lower TNC content than T5 (S/3 with stimulation). However, the TNC content of the untapped trees had not significant difference with T1, T4 and T5. Besides, the longer cut length treatments (T2 and T3) showed lower TNC than that of the other treatments.

An average of TNC content on the 10 - 30 cm distance under tapping panel is shown in Figure 17D. T2 gave the highest TNC content but it did not had significant difference with T1. The lowest TNC was found for T3. For the d2 tapping frequencies, T2 had higher TNC content than T4. For the d3 tapping frequencies, T3 had lower TNC content than T5. The d3 tapping systems showed lower TNC content than the d2 and 2d/3 frequency and then than the untapped trees 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), T6: Untapped

Figure 17 TNC in bark (mg/g DW) of low yield period (LY) at 10 cm (A); at 20 cm (B); at 30 cm (C) and average at 10-30 cm (D) under tapping panel among the 6 treatments in May 2015; different letters in each bar graph indicate significant difference at $P \leq 0.05$ by DMRT

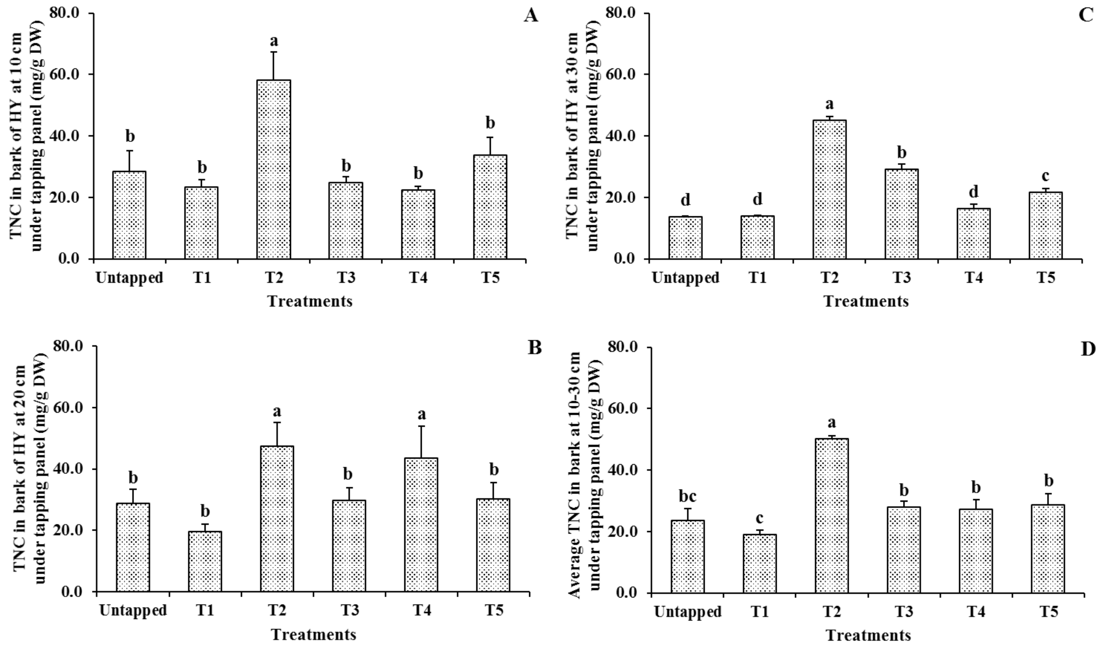
Effect of different tapping systems on TNC in bark for the high yield period

In high yield period, total nonstructural carbohydrate content (TNC) in bark (mg/g DW) among the 6 treatments on each distance were significantly different as shown in Figure 18. At the 10 cm distance under tapping panel (Figure 18A), the TNC content was the highest in T2, the lowest was found in T4. So, for the d2 tapping frequencies, T2 with longer cut length had higher TNC content than T4 with a shorter cut length and with ethylene stimulation. For the d3 tapping frequencies, T3 with S/2 cut length with ethylene stimulation had lower TNC content than T5 with a shorter cut length (S/3) with ethylene stimulation. However, the TNC content of the untapped tree did not show significant difference with the remaining treatments except T2.

At the 20 cm distance under tapping panel (Figure 18B), T2 had the highest TNC content. The farmer tapping system (S/3 2d/3) had the lowest value. For the d2 tapping frequencies, T2 had higher TNC content than T4. For the d3 tapping frequencies, T3 had lower TNC content than T5 similar the untapped tree.

At the 30 cm distance under tapping panel (Figure 18C), the highest TNC was found for T2 and T1 had the lowest value. For the d2 tapping frequencies, T2 (S/2) had higher TNC content than T4 (S/3 with stimulation). For the d3 tapping frequencies, T3 (S/2 with stimulation) had higher TNC content than T5 (S/3 with stimulation). The TNC content of the untapped tree and T1 were did not show significantly differences from T4.

An average of TNC content on the 10 - 30 cm distance under tapping panel is shown in Figure 18D. T2 showed the highest TNC content and the lowest was found in T1. For the d2 tapping frequencies, T2 had higher TNC content than T4. For the d3 tapping frequencies, T3 had lower TNC content than T5. The stimulation treatments showed similar TNC content with the untapped tree.



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), T6: Untapped

Figure 18 TNC in bark (mg/g DW) of high yield period (HY) at 10 cm (A); at 20 cm (B); at 30 cm (C) and average at 10-30 cm (D) under tapping panel among the 6 treatments in October 2015; different letters in each bar graph indicate significant difference at $P \leq 0.05$ by DMRT

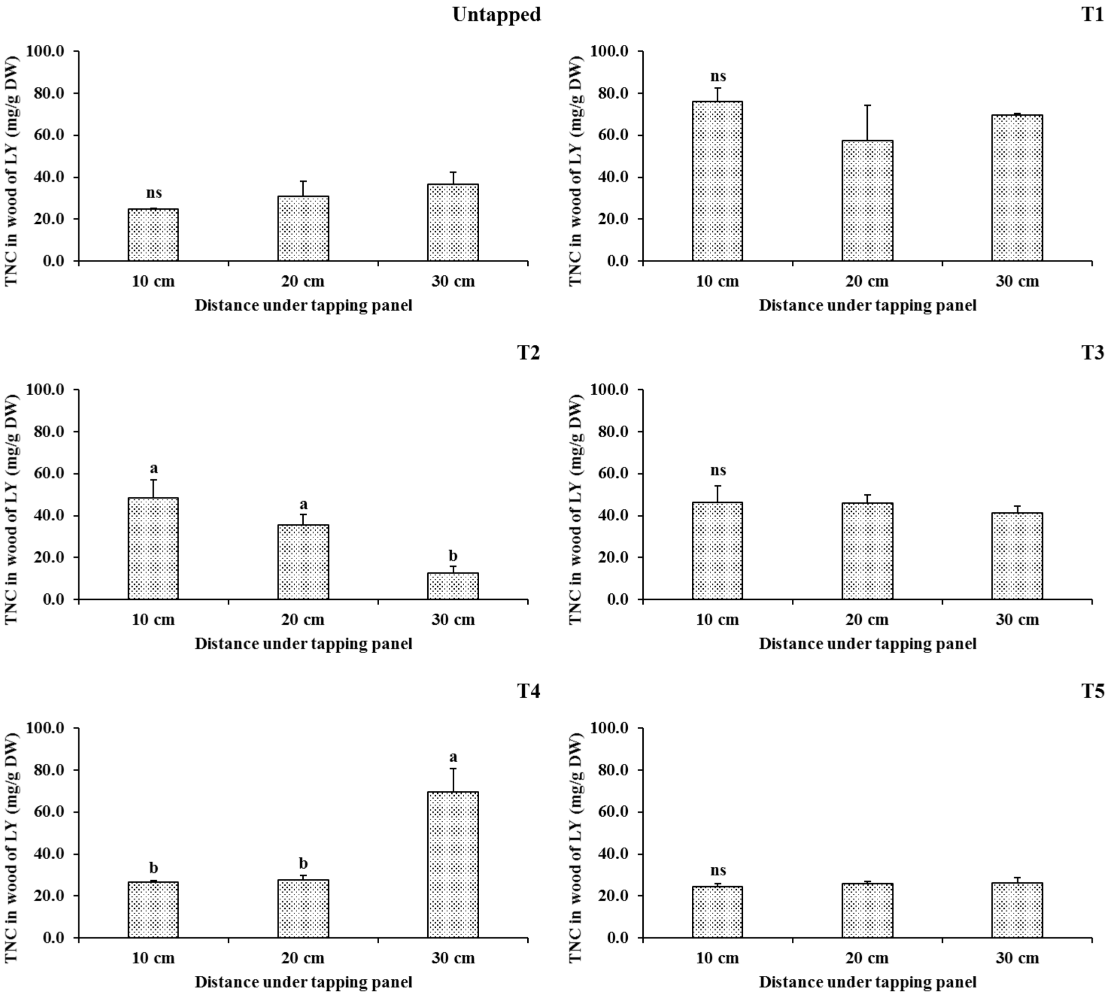
Comparison among the distance of TNC in wood

The low yield period

The comparison among the distance of TNC content in wood of low yield period on each treatment is shown in the Figure 19. There were significant differences among the distance under tapping panel for T2 and T4. The TNC content in wood of the untapped tree, T4 and T5 were high at 30 cm distance, middle at 20 cm distance and low at 10 cm distance under tapping panel, however, T4 showed a very high TNC content at 30 cm distance under tapping panel. The TNC content in wood of T1 was high at 10 cm distance, low at 20 cm distance and middle at 30 cm distance under tapping panel. The TNC contents in wood of T2 and T3 were high at 10 cm distance, middle at 20 cm distance and low at 30 cm distance under tapping panel, especially for T2.

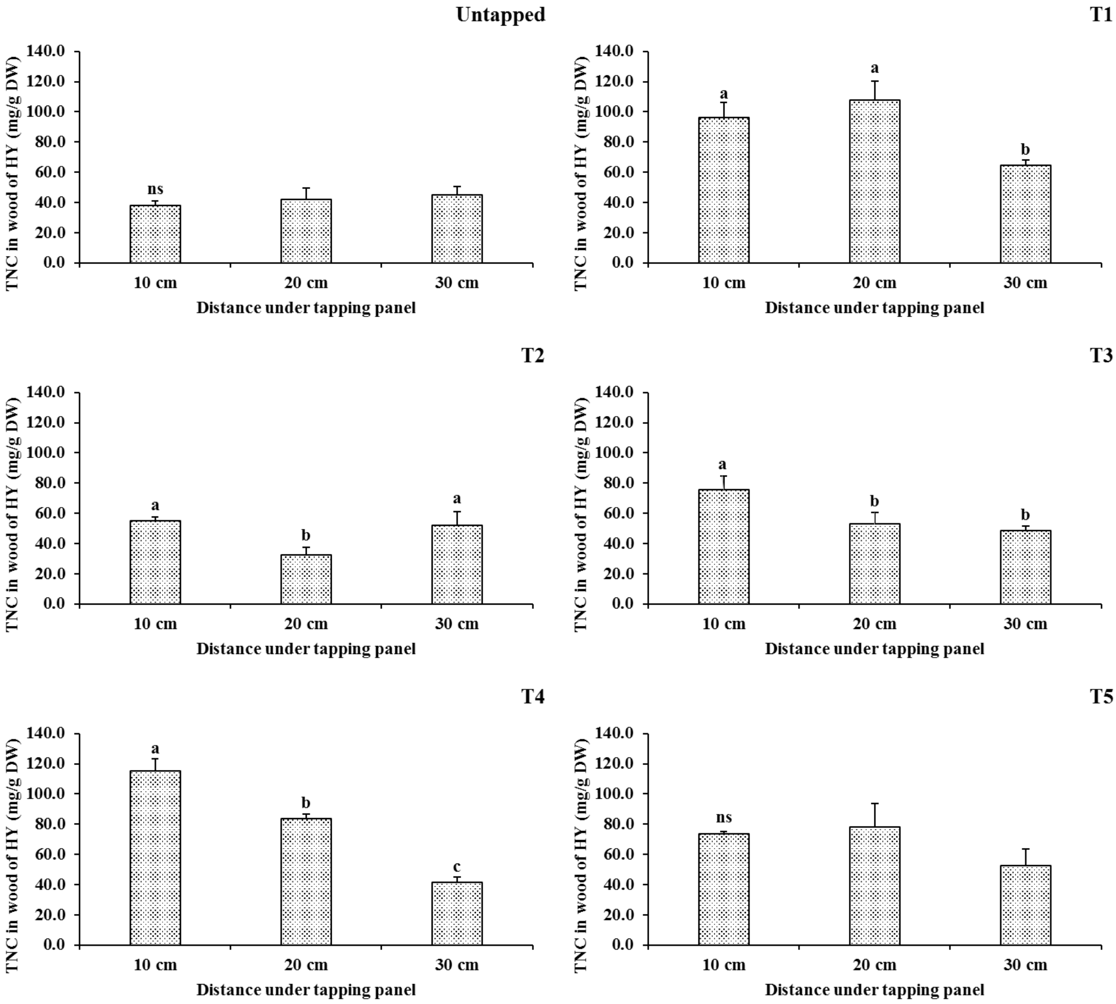
The high yield period

The comparison among the distance of TNC content in wood of high yield period on each treatment is shown in the Figure 20. There were significant difference among the distance under tapping panel for T1, T2, T3 and T4. The TNC content in wood of the untapped tree was high at 30 cm distance, middle at 20 cm distance and low at 10 cm distance under tapping panel. The TNC content in wood of T1 and T5 were high at 20 cm distance, middle at 10 cm distance and very low at 30 cm distance under tapping panel. In T2, the TNC content in wood was high at 10 cm distance with a similar value at 30 cm distance and it was low at 20 cm distance under tapping panel. The TNC content in wood of T3 and T4 were high at 10 cm distance, middle at 20 cm distance and low at 30 cm distance under tapping panel, especially for T4.



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), T6: Untapped

Figure 19 TNC in wood (mg/g DW) of low yield period (LY) in each treatment in May 2015 among the distance under tapping panel; different letters in each bar graph indicate significant difference at $P \leq 0.05$ by DMRT, ns = not significant



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), T6: Untapped

Figure 20 TNC in wood (mg/g DW) of high yield period (HY) in each treatment in October 2015 among the distance under tapping panel; different letters in each bar graph indicate significant difference at $P \leq 0.05$ by DMRT, ns = not significant

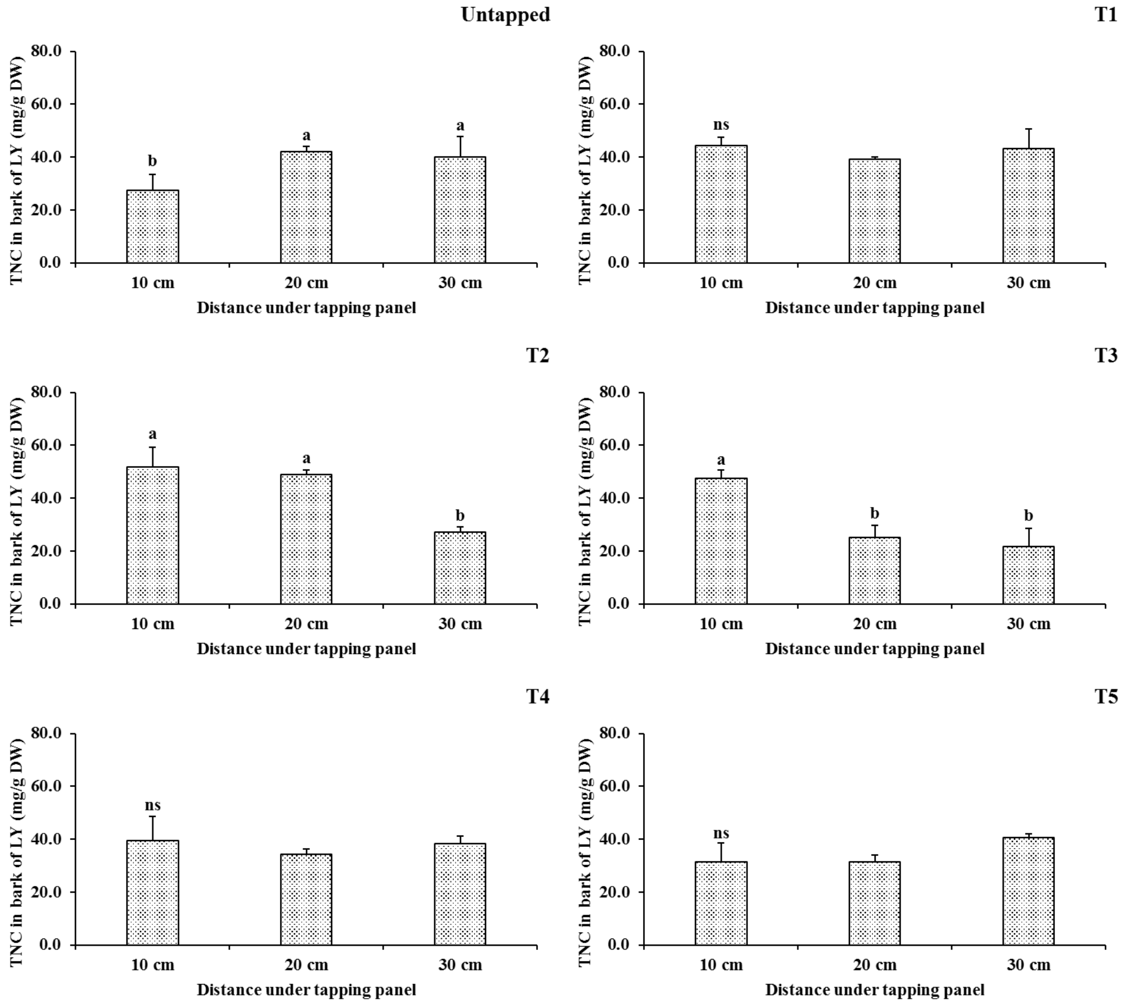
Comparison among the distance of TNC in bark

The low yield period

The comparison among the distance of TNC content in bark of low yield period on each treatment is shown in the Figure 21. There were significant difference among the distance under tapping panel for the untapped tree, T2 and T3. The TNC content in bark of the untapped tree was high at 20 cm distance, similar at 30 cm distance and very low at 10 cm distance under tapping panel. The TNC content in bark of T1 and T4 were high at 10 cm distance, low at 20 cm distance under tapping panel and without any difference at 30 cm distance. The TNC content in bark of T2 and T3 were high at 10 cm distance, middle at 20 cm distance and low at 30 cm distance under tapping panel. The TNC content in bark of T5 was high at 30 cm distance, middle at 20 cm distance and low at 10 cm distance under tapping panel.

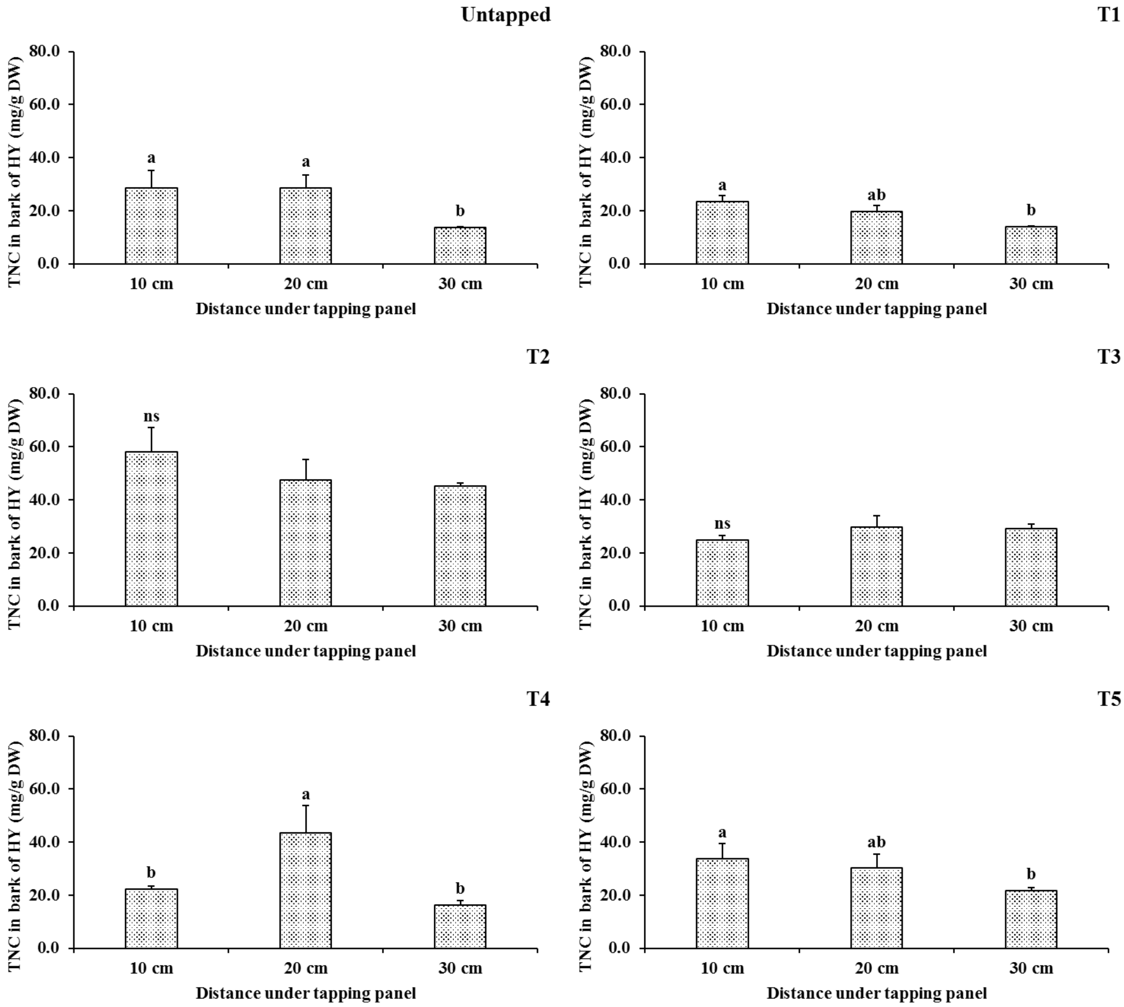
The high yield period

The comparison among the distance of TNC content in bark of high yield period on each treatment is shown in the Figure 22. There were significant difference among the distance under tapping panel for the untapped tree, T1, T4 and T5. The TNC content in bark of the untapped tree was high at 20 cm distance and low at 30 cm distance under tapping panel. The TNC content in bark of T1, T2 and T5 were high at 10 cm distance, middle at 20 cm distance and low at 30 cm distance under tapping panel. The TNC content in bark of T3 was high at 20 cm distance, nearby the value at 30 cm distance and it was low at 10 cm distance under tapping panel. The TNC content in bark of T4 was high at 20 cm distance, middle at 10 cm distance and low at 30 cm distance under tapping panel.



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), T6: Untapped

Figure 21 TNC in bark (mg/g DW) of low yield period (LY) in each treatment in May 2015 among the distance under tapping panel; different letters in each bar graph indicate significant difference at $P \leq 0.05$ by DMRT, ns = not significant



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), T6: Untapped

Figure 22 TNC in bark (mg/g DW) of high yield period (HY) in each treatment in October 2015 among the distance under tapping panel; different letters in each bar graph indicate significant difference at $P \leq 0.05$ by DMRT, ns = not significant

The difference of season on TNC content

In the wood

Table 14 shows the comparison of TNC content in wood between the low yield period and high yield period on each distance under tapping panel of the 6 treatments. At 10 cm distance under tapping panel, all treatments showed significant difference between both periods except for T2. The TNC content in wood was higher in the high yield period than the low yield period on each treatment. At 20 cm distance under tapping panel, only T1, T4 and T5 had significant differences between both periods. The TNC content in wood was higher in the high yield period than the low yield period for each treatment except for T2. At 30cm distance under tapping panel, all treatments were significantly different except for the untapped tree and T1 between both periods. The TNC content in wood was higher in the high yield period than in the low yield period for each treatment except for T1 and T4. At average 10-30 cm distance under tapping panel on each treatment, all treatments had significant difference between both periods. The TNC content in wood was higher in the high yield period than the low yield period on each treatment.

In the bark

Table 14 shows the comparison of TNC content in bark between the low yield period and high yield period on each distance under tapping panel of the 6 treatments. At 10 cm distance under tapping panel, the 6 treatments had significant differences between both periods. The TNC content in bark was higher in the low yield period than in the high yield for T1, T3 and T4. At 20 cm distance under tapping panel, only the untapped tree and T1 had significant difference between both periods. The TNC content in bark was higher in the low yield period than in the high yield period for each treatment except for T3 and T4. At 30 cm distance under tapping panel, was all treatments had significant difference between both periods except for T3. The TNC content in bark was higher in the low yield period than in the high yield period for each treatment except for T2 and T3. At average 10-30 cm distance under tapping panel, all treatments had significant differences between both periods except for T3 and T5. The TNC content in bark was higher in the low yield period than the high yield period on each treatment except for T2.

Table 14 TNC in wood and bark (mg/g DW) among the low yield (LY) and high yield (HY) periods in each the distance under tapping panel of the 6 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), T6: Untapped

T[a]	**PR[b]**	**TNC in wood (mg/g DW)**				**TNC in Bark (mg/g DW)**			
		10 cm	**20 cm**	**30 cm**	**Average 10-30 cm**	**10 cm**	**20 cm**	**30 cm**	**Average 10-30 cm**
Untapped	**LY**	24.78b	31.00	36.60	30.79b	27.56	42.04a	39.99a	36.53a
	HY	38.09a	41.89	45.07	41.68a	28.44	28.68b	13.69b	23.60b
T1	**LY**	75.98b	57.47b	69.59	67.68b	44.27a	39.26a	43.24a	42.25a
	HY	96.27a	107.93a	64.33	89.51a	23.42b	19.69b	13.91b	19.01b
T2	**LY**	48.30	35.45	12.45b	32.06b	51.77	48.93	27.17b	42.63b
	HY	54.81	32.66	51.83a	46.43a	58.10	47.49	45.12a	50.24a
T3	**LY**	46.42b	45.97	41.35b	44.58b	47.38a	25.05	21.75	31.39
	HY	75.65a	53.01	48.40a	59.02a	24.83b	29.83	29.07	27.91
T4	**LY**	26.43b	27.68b	69.61a	41.24b	39.62a	34.30	38.45a	37.46a
	HY	115.32a	83.40a	41.23b	79.98a	22.34b	43.44	16.28b	27.35b
T5	**LY**	24.49b	25.68b	26.21b	25.46b	31.48	31.55	40.72a	34.58
	HY	73.52a	78.35a	52.50a	68.12a	33.85	30.31	21.73b	28.63

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

CHAPTER 4
DISCUSSION

Weather condition

It was found that the weather condition during the experiment periods exhibited high rainfall at the end of year, Particularly in November and December causing flooding in the experimental plot. Normally, the rainy season starts from mid-May to mid-February periods in the South of Thailand. In addition, the heavy rainfall was found in the high yield period of the rubber tree, affecting tapping days. This led to decrease the yield during that period. However, the rainfall decreased in January and February before defoliation. It was found that there was intermittent rainfall in the dry season, especially in March and April. Hence, under the weather condition in southern Thailand, timing to harvest the latex yield was directly affected by rainfall. Raj *et al*. (2011) reported that rainfalls and temperatures are still the major climatic factors to physiology development and latex potential of rubber trees. The change of climatic pattern may be induce high amount of rainfall that leads to flood and/or hot dry drought in same each year. Hence, the productions have been changed from time to time because it was affected by the climate variable and unexpected seasonal factors of drought and heavy rain (Chantuma *et al.,* 2012).

The effect of reduced tapping frequency with stimulations on agronomical and physiological responses during seasonal variation

The yield per tree and per tapping of low frequency tapping systems (LFT) (S/2 d3 and S/3 d3) with stimulation was significant higher than that of the traditional tapping system (S/3 2d/3), commonly used in southern Thailand and other tapping systems (S/2 d2 and S/3 d2 with stimulation). LFT systems combining reduction of tapping frequency with ethephon stimulation increase the duration of latex flow after tapping, with the reduction of latex coagulation, and activate the latex cell metabolism (Jacob *et al.,* 1989; d'Auzac *et al.,* 1997) leading to export more latex at each tapping. Moreover, it was found that it was possible to compensate the reduction of tapping frequency by using suitable ethylene stimulation. The cumulative yield was nearly the same over 3 years of tapping among the treatments, only the treatment using S/3 d3 with stimulation showed the lowest significant yield compared with S/3 2d/3 though it gave higher yield per tapping but the reduction of the cut length and tapping frequency, while using intensive of stimulation induced a lower cumulative yield. On the other hand, LFT system S/2 d3 with stimulation showed no significant difference with the traditional tapping system (S/3 2d/3) and other tapping systems (S/2 d2 and S/3 d2 with stimulation). This indicated that the reduction of tapping frequency with suitable stimulation could compensate the cumulative yield per tree with higher yield per tapping even under prolonged rainy season in southern Thailand. These results are also supported by Njukeng and Gobina (2007) and Rodrigo *et al.* (2011). They mentioned that LFT system must be applied with stimulation to increase potential yield at each tapping. Besides, the reduction tapping frequency d3, could delay to recover complete latex cell regeneration which is longer than the higher tapping frequency d2 (Jacob *et al.,* 1989; d'Auzac *et al.,* 1997, Obouayeba *et al.,* 2011, Diarrassouba *et al.,* 2012).

The latex parameters consist of total solid content (TSC), inorganic phosphorus content (Pi), reduced thiol content (RSH) and sucrose content (Suc). TSC did not show significant difference among the treatments, so there was no effect of tapping cut length and tapping frequency on the TSC. The TSC was varying in each month of each year due to the difference of rainfall. Trend of the TSC was decreasing immediately after rainfall decreased, and re-increasing in dry season. The decreasing of TSC relates with the yield and tapping day increasing before defoliation of rubber trees. The highest of Pi content was found in the LFT systems d3 with ethylene stimulation, mainly with the longest cut S/2. This reflects a good metabolic activity of the yield (Jacob *et al.,* 1988; 1989; Gohet, *et al.,* 2003; Lacote *et al.,* 2010). This is also the case with the S/2 d3 with ethylene stimulation giving one of the highest yields. These implied that the increase of latex yield by increasing metabolic activity with ethylene stimulation, leads to high Pi content and will deplete the Suc content involved in the latex regeneration after each tapping to maintain a high yield. The Pi in each month was varying following the rainfall pattern of each year. The increase of Pi relates with the decrease of rainfall and corresponds to high metabolism in the latex vessel. The d3 tapping frequencies systems with stimulation showed a lower RSH content in latex than the d2 tapping frequencies. However, long cut length at d3 frequency with stimulation still induced lower RSH content than other treatments. The colloidal stability of the latex was more preserved in the short cut length than the long cut length (Obouayeba *et al.,* 2011). Moreover, the effect of stimulation is well known on the use of RSH as scavengers to protect the stability of the membranes of the vacuo-lysosomal system in the latex cells (Jacob *et al.,* 1989; d'Auzac *et al.,* 1997). Besides, the RSH was varying in months of each year. It increased after high rainfall. A decrease of Suc content was clearly found in the LFT systems d3 with stimulation and it is related to the increase of yield per tapping and per tree, as the cumulative yield was balanced with all treatments with higher frequency of tapping but without ethylene stimulation. A lower of Suc content indicates a higher Suc consumption due to a more activated metabolism of the latex cells under ethylene stimulation in d3 tapping frequency: higher the volume of latex exported at each tapping, higher the need to regenerate the latex cell content by using more Suc (Jacob *et al.,* 1989; Lacote *et al.,* 2004) leading to a depletion of sucrose in latex (Gohet *et al.,* 2003; Obouayeba *et al.,* 2009; Rodrigo *et al.,* 2011). Suc in each month was varying, increasing when the rainfall were high especially in the end of year. Our results showed that the sugar loading capacity of the latex cells is one of the main factors that enable a significant increase in latex yield after ethylene stimulation (Gohet *et al.,* 2001; 2003).

LFT systems showed a markedly decrease of bark consumption comparing with the traditional tapping system in Thailand S/3 2d/3 and other tapping systems (S/2 d2). Less bark consumption lead to increase economic life span of rubber trees because it may increase replanting cycle up from 30 years to 36 years (Nugawela *et al.,* 2000; Rodrigo 2007). Besides, the commencement of tapping in renewed bark could be delayed, this may increase the additional time for bark regeneration (Rodrigo *et al.,* 2011).

Girth increment did not show any differences between treatments. A slight difference occurred although Obouayeba *et al.* (2011) showed that the reduction of tapping cut length enhanced a better vegetative radial growth. Nugawela *et al.* (2000) found that low frequency tapping system with stimulation did not show negative effect on the growth of rubber trees. In our experiment, combining length of the tapping cut (S/2) with a lower tapping frequency (d3) than the farmer system (2d/3) with the use of ethylene stimulation increased significantly the yield at each tapping while not reducing the girth increment of the trees.

The rate of tapping panel dryness (TPD) was found only with stimulation tapping systems. It related with stimulation, while Obouayeba *et al.* (2009) reported that the rate of TPD related to the intensity of tapping. So it seems that the incidence of TPD under d3 tapping frequency systems depends on the ethylene stimulation rate, even that rate of TPD in our experimental condition was still quite low.

The changes of latex physiological parameters after stimulation

The purpose of this study was to analyze the responses of yield and the latex cells parameters linked with yield potential to the ethylene stimulation application. 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 have impacted IFR. The latex flow characteristics confirmed 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. This could be linked to the length of the cut, with the effect of the sharing effect after tapping with short cut. IFR gradually increased during succeeding tapping days in all treatments due to the stress of successive 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 was high for the traditional tapping systems or non-stimulated treatments. However, they were low for the ethylene application treatments. Actually, the response of PI during succeeding tapping days decreased, resulting in prolonging 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 relative higher decrease of PI in the low yield period may be due to the effect of ethylene stimulation after initiating tapping period, as the flow duration at this time was short; the relative impact of ethylene application was relatively higher than in high yield period. Indeed, 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. The sudden decrease of PI resulted in high yield per tapping because of the delay of plugging of latex vessel (Jetro and Simon, 2007).

Average latex yield (AY) in g/t/t was the highest for the treatments with d3 tapping frequency with stimulation in the low yield period in comparison the others treatments with higher tapping frequency. There was a quick effect of the reduction of tapping frequency, at this period, on the yield at each tapping. While, AY of d2 and d3 treatments were higher than the traditional tapping system in the high yielding period. In our study, AY increased during succeeding tapping days for 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. 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). So, in the high yield period, AY is higher than in low yield period even before as after any ethylene stimulation, it seems logical that due to the highest yield observed in high period before ethylene stimulation, the response to stimulation was lower than in low yield period where a very low AY was observed before ethylene stimulation. The half spiral cut downward with every third day tapping with stimulation 8 times per year (T3) showed superior AY per tapping in comparison with third spiral cut downward at third daily tapping with stimulation 12 times per year (T5). This is related to the study of Traore *et al.* (2011) concerning the frequency of stimulation: higher the frequency of stimulation, lower the response to ethylene stimulation at each tapping.

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. Then, utilization of Suc 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 content was due to the metabolism dedicated more to rubber biosynthesis creating a sink for sucrose content to be connected with the corresponding latex cell metabolism (Gohet *et al.,* 2003; Jetro and Simon, 2007; Lacote *et al.,* 2010).

The evaluation of total nonstructural carbohydrate content on reduced tapping frequency of rubber trees

The total nonstructural carbohydrate (TNC) content showed different trends for each tapping system under tapping panel in both wood and bark, with two periods (the low yield period and the high yield period).

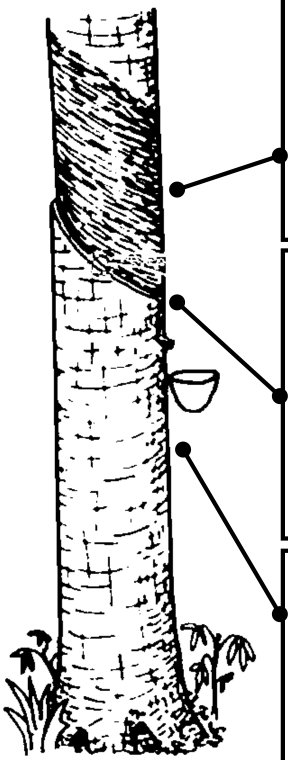
The difference of tapping systems in the both periods had an impact on the TNC content in both wood and bark. In wood, the average of TNC content for the untapped tree treatment showed lower reserve than that of the tapped tree treatments in both periods. The TNC concentration of the tapped trees was significantly higher than in the untapped tree (Silpi *et al.,* 2007; Chantuma *et al.,* 2009), particularly starch because it was the major component in wood (Ketkakomol *et al.,* 2014) that can be accumulated near the tapping cut. Moreover, the TNC content under tapping panel of the d2 and d3 tapping frequency treatments (with and without stimulation) in both periods were lower than the S/3 2d3, widely used in southern Thailand. It expressed that the difference of tapping frequency could affect the TNC content in the drainage area. The tapping systems, giving the higher latex yield per tapping may be induce a depletion of the TNC content in the drainage area that produced the latex exported after tapping. However, this result contrasted with Chantuma *at al.,* (2009), reported that the very specific double cut alternate tapping (DCA) system, which provides the higher latex yield, could also lead to high TNC content more than the traditional tapping system due to the use of 2 tapping cut. In addition, when using ethylene stimulation, higher TNC content under tapping panel was seen in comparison to the S/2 d2 without stimulation in both periods. It clearly expressed that ethylene stimulation could benefit to enhance the carbohydrate mobilization in the tree (Eklund and Little, 1998) and also increases latex metabolism (d' Auzac *et al.,* 1997), while the TNC content was well balanced in trunk of tree. For the d2 tapping frequency treatments, it was seen that the TNC content was more balanced in the drainage area for the S/3 with stimulation than for S/2 treatments in both periods. For the d3 tapping frequency treatments, the TNC content in the drainage area of the S/2 with 8 stimulations per year was higher than the S/3 with monthly stimulated in the low yield period but it was contrasted with the high yield period. After the re-foliation stage, the TNC content was decreased in the trunk wood because it was a net mobilization of demand to growth of new shoot with increased respiration of the tree (Silpi *et al.,* 2007). For the high yielding period, the latex vessel was more activated. The treatment giving high yield per tapping led to loss of the TNC reserve in wood because it was depleted to compensate the yield in each tapping (d' Auzac *et al.,* 1997). But in comparison to the untapped trees, it was seen that the TNC content was more balanced in the drainage area for tapped trees. In bark, the average of TNC content of the untapped tree treatment was also lower than the tapped tree treatments in the both periods, however, it involved with latex regeneration which occurred in bark resulting to high storage in the tapped trees (Chantuma *et al.,* 2009) because tapping induces to additional sucrose sink in bark (Jacob *et al.,* 1998) led to also metabolically actives pool of TNC in the tree (Zhang *et al.,* 2014) except in the d3 tapping frequencies treatments during the low yield period. It indicated that reduced tapping frequency with stimulation induced to consume the TNC content in bark after the re-foliation stage because carbohydrate in bark is the local reserve compartment which directly involved in latex regeneration (Chantuma *et al.,* 2007). In addition, the traditional tapping system had higher TNC content than the stimulation treatments in the low yield period; however, it showed contrast in the high yield period. In this case it seems that ethylene was affected the reserve of carbohydrate in bark, and play a role in the TNC content to compensate the balance of latex yield and growth of new shoot after the re-foliation stage (Silpi *et al.,* 2007). For the d2 tapping frequencies, TNC content of the S/2 was higher than the S/3 with stimulation in both periods. Carbohydrate in bark was utilized in the latex regeneration process of the high latex yield treatment. In addition, for the d3 tapping frequencies it was found that the S/2 with stimulation 8 times per year showed TNC lower than that of the S/3 with monthly stimulated in both periods. The longer cut length may be induced to depletion of the TNC content because provided highly latex yield (Chantuma *et al.,* 2009).

The expression of TNC content in wood and bark were different among the distance under tapping panel in both periods. In wood, the TNC content of the S/2 d2 and the LFT system (S/2 d3) stimulated 8 times per year were the highest at 10 cm distance under tapping panel, near tapping cut, in both periods. The higher storage carbohydrate near the tapping cut could benefit to latex yield per tapping by balancing the latex regeneration in the latex vessels. Latex regeneration requires carbohydrate as a source of metabolic energy (Wycherley, 1976; Tupy, 1985), so, it led to high yield in longer cut length. Besides, the TNC content of the untapped tree was the highest at 30 cm distance under 1.50 m from the ground, in both periods. It seemed that the TNC content of the untapped tree treatment had an ascending gradient from 10 to 30 cm distance below in vertical at 1.50 m from the ground. These supported by Chantuma *et al.* (2007) about the accumulation of starch as it was higher in the lower part than the top part of the trunk tree. However, TNC content under tapping panel of the traditional tapping system was decreasing from the low yield period to the high yield period, while for the S/3 treatments with ethylene stimulation TNC contents were increased from the low yield period to the high yield period. It seemed that tapping had still an impact on carbohydrate in the trunk of the vertical distance under tapping panel (Silpi *et al.*, 2007). In bark, the TNC content of the treatments without stimulation was high at 10 cm distance under tapping in both periods. Chantuma *et al.* (2009) reported that the area closer the tapping cut showed more storage capacity for the carbohydrate in bark. It seemed that ethylene induced carbohydrate consumption in bark. The TNC content of the untapped tree was high at 20 cm distance under 1.50 m from the ground in both periods. However, the TNC content of both the S/2 d3 and S/3 d2 with stimulation were decreased from the low yield period to the high yield period. Change of carbohydrate in bark concerned with the metabolism in the latex vessel which used in latex regeneration, moreover, the carbohydrate is also impacted by tapping in the trunk under tapping panel (Silpi *et al.*, 2007).

The difference of season between low yield period and high yield period, on TNC content in wood under tapping panel of all treatments showed higher significant differences in the high yield period than that of the low yield period. Under the difference of season, the storage of TNC content was related with the climate, being the lowest after the re-foliation stage in dry season. Würth *et al.*, (2005), noticed that growth and carbohydrate demand were limited by drought. In addition, Silpi *et al.*, (2007) reported that TNC concentration expressed the highest at leaf-fall stage after that it dropped at re-foliation stage. So, the difference of TNC content was influenced by phenological bud-break which was associated to a decrease of TNC concentrations because wood tissues provide the carbon to support shoot growth (Rosas *et al.*, 2013). However, the average of TNC content in bark of all treatments in the low yield period showed storage higher than that of the high yield period. TNC in bark acts as a local buffer (Chantuma *et al.*, 2009) and it seems that it is in opposite state than the TNC content in wood. The storage of carbohydrate in bark related to latex regeneration process because it was the higher after just growths new shoot (leaves and flowers). After that in the high yield period or high metabolism of rubber trees, low TNC content was shown in bark. It means that carbohydrates were more utilized in the latex vessel (Chantuma *et al.*, 2007).

General discussion

The reduction of tapping frequency was accessed in the Southern Thailand. Low frequency tapping (LFT) systems were tested with ethylene stimulation and found that S/2 d3 ET 2.5% Pa 1(1) 8/y (m) could show the suitable results as explained with following the diagram:



Effect of LFT tapping system with stimulation

- Highest yield per tree and per tapping
- Compensated cumulative latex yield per tree per year
- Less bark consumption per year
- Not any effected on girth increment
- Activate the metabolism at each tapping (lower Suc and RSH)
- Slight effect on TPD

Response of latex physiology after stimulation

- Increased the initial flow rate (IFR)
- Reduced the plugging index (PI)
- Gave a higher the average latex yield (AY)
- Increased the sucrose content (Suc)

Evaluation of TNC content under tapping systems

- Stored in wood more than bark
- In wood, more storage in the high yield period due to the tapping system allowing higher g/t/t
- In bark, more storage in the low yield period

Latex harvest system can be improved by reducing the tapping frequency. The advantage of LFT system could solve the problem of HFT system. The LFT is made to lengthen the economic life span of the rubber tree. Therefore, that in the future, LFT system could be apply for sustainability under rubber price fluctuation and labour shortage.

CHAPTER 5
CONCLUSION

Under the weather condition in southern Thailand, timing to latex yield harvesting was directly affected by rainfall.

The effect of reduced tapping frequency with stimulations on agronomical and physiological responses during seasonal variation

Over 3 tapping years, LFT system (S/2 d3) with stimulation 8 times per year gave the highest yield per tapping and cumulative yield in kg/t was not significantly different from the traditional tapping system (S/3 2d/3). Latex biochemistry of LFT systems with stimulation showed no significant difference in both total solid content and inorganic phosphorus content, but they showed lower reduced thiols content and sucrose content than the other treatments as a sign of an activated metabolism but still under controlled. The variations of latex biochemistry of all treatments were related with the rainfall. Bark consumption was less because of reduced tapping frequency. The use of ethylene stimulation with lower tapping frequencies did not show any effect on girth. However, tapping panel dryness was slightly increased by the use of ethylene stimulation. Therefore, ethylene stimulation must be used with care to prevent any latex cells dysfunctioning.

The changes of latex physiological parameters after stimulation

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 LFT 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 dependent 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 as a logical artefact. 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 the most changes in sucrose content.

The evaluation of total nonstructural carbohydrate content on reduced tapping of rubber trees

The total nonstructural carbohydrate (TNC) content was significantly different among the tapping systems under tapping panel in both wood and bark, with two periods (the low yield period and the high yield period). In wood of both periods, TNC content was higher in tapped trees than untapped trees. LFT system (S/2 d3) stimulation 8 times per year showed less significant reserved TNC content under tapping panel than the traditional tapping system (S/3 2d3) but it is still higher than S/2 d2. In the bark for both periods, TNC content of untapped trees was similar with stimulation treatments. S/2 d2 provided the highest TNC content under tapping panel. LFT system showed lower storage than traditional tapping system in the low yield period only. However, LFT system was accumulated TNC content nearly tapping cut (10 cm distance under tapping panel) nearly the same with the traditional tapping system in wood but different in bark. TNC content of all treatments in high yield period still showed more storage than low yield period in wood; however, it contrasted in bark as it was explained by different of behavior of TNC in the two compartments.

In overview conclusion of this experiment, LFT system (S/2 d3) stimulation 8 times per year could efficiently give the yield per tapping leading to compensate the cumulative yield in kg/t for clone RRIM 600. It is remarkable, that in Thailand, in a heavy and long rainy area, it is possible to use ethylene stimulation to increase the potential yield of the trees at each tapping. This suggests that the rubber smallholder in southern Thailand could apply low frequency tapping systems with stimulation.

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APPENDICES

Appendix A

Table 1 Latex physiological parameters of clone RRIM 600 were analyzed during 4 years (1999-2003) (Chantuma *et al.*, 2003)

Parameters	**Low**	**Medium**	**High**	**Unit**
TSC	<42.05	42.05 – 45.21	>45.21	%
Suc	<2.44	2.44 – 11.73	>11.73	mM
Pi	<13.44	13.44 – 29.12	>29.12	mM
RSH	<0.20	0.20 – 0.57	>0.57	mM

Note: TSC = total solid content, Suc = sucrose content, Pi = inorganic phosphorus content, RSH = reduced thiol content

Appendix B

Figure 1 Rubber plantation in the experiment

Figure 2 Tapping system of T1: S/3 d1 2d/3

Figure 3 Tapping system of T2: S/2 d2

Figure 4 Tapping system of T3: S/2 d3 ET 2.5% Pa1(1) 8/y (m)

Figure 5 Tapping system of T4: S/3 d2 ET 2.5% Pa1(1) 4/y (m)

Figure 6 Tapping system of T5: S/3 d3 ET 2.5% Pa1(1) 12/y (m)

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Publication

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Sainoi, T., Sdoodee, S., Lacote, R. and Gohet, E. 2017. Low frequency tapping systems applied to young-tapped trees of *Hevea brasiliensis* (Willd. ex A. Juss) Müll. Arg. in Southern Thailand. Agr. Nat. Resour. 51: impress.

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