



**Implications of Agroecological Changes in Rubber-based
Intercropping System on the Sustainability of *Hevea* Rubber
Production**

Zar Ni Zaw

**A Thesis Submitted in Fulfillment of the Requirements for the
Degree of Doctor of Philosophy in Tropical Agricultural Resource
Management**

Prince of Songkla University

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Thesis Title Implications of Agroecological Changes in Rubber-based Intercropping System on the Sustainability of *Hevea* Rubber Production

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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.

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Major Program	Tropical Agricultural Resource Management
Academic Year	2022

ABSTRACT

Hevea rubber cultivations supply to meet the requirement of the world consumption and generate major incomes for millions of rubber smallholders. However, its conventional cultivation practices like monocropping, replanting, and long-term utilization of chemical fertilizer have accumulated negative impacts substantially on the environmental and socio-economic concerns of rubber growing regions. Thus, to reduce those impacts and develop sustainability in the natural rubber production, the rubber-based intercropping system, which improves the agroecology and livelihoods of smallholders, became the most recommended option for the smallholders. However, some combinations of the rubber-based intercropping were observed with adverse effects on the growth and yield of the crops. Thus, this thesis research studied the different rubber-based intercropping practices in terms of agroecology and tree physiology, and their implications for ensuring the sustainability of natural rubber production integrated with intercropping systems. Two experimental studies were set up in Songkhla province, southern Thailand. The first experiment was a case study to investigate the changes in agroecosystem components of a rubber-based intercropping farm and their interactions under integrated fertilizations mixed with organic soil amendments. The second experiment aimed to study the seasonal changes in leaf area index (LAI) and soil moisture content (SMC) under rubber-based intercropping farms, and their interrelations with the latex biochemical compositions, yield, and technological properties. The first experiment was conducted at a rubber-salacca intercropping farm and identified the consequences of the integrated fertilization combined with two organic soil amendments: humic acid (HSA); chitosan (CSA) compared to conventional chemical fertilization. The CSA application increased

soil organic matter by 80%. In the 21-40 cm soil depth, the rubber roots treated with HSA and the salacca palm roots treated with CSA showed greater fine root length density. Under CSA, the physiological status of the rubber trees showed less stress. The treatments of HSA and CSA showed 145% and 72%, respectively, higher total production of salacca palm than that of the chemical fertilization. Improvements in the soil fertility, the root's function, the crops' yields, and the tree's physiological status were consequences as complementarity in the system under the integrated fertilizations. The second experiment selected three rubber-based intercropping farms: rubber-bamboo (RB); rubber-melinjo (RM); rubber-coffee (RC), and one rubber monocropping farm (RR). Among the rubber-based intercropping farms, the mean relative humidity of RB and RM throughout the study period were higher than that of RR by 14% and 18%, respectively, whereas RC had a mere 6% higher than RR. However, regarding the mean temperature, RB and RM maintained only 4% less than RR, while RC had the same mean temperature as RR. Over the study period, RB, RM and RC exhibited significantly higher LAI values at 1.2, 1.05 and 0.99, respectively, while RR had a low LAI of 0.79. Increasing SMC trends by soil depths were pronounced in all rubber-based intercropping farms. RB and RM expressed less physiological stress and delivered latex yield on average 40% higher than RR. With higher molecular weight distributions, their rheological properties were comparable to those of RR. However, the latex in RB and RM significantly increased the Mg contents to 660 and 742 ppm, respectively, in S2. Their dry rubbers contained ash contents of more than 0.6% in S3. This research would contribute to the sustainability of natural rubber production integrated with rubber-based intercropping ensuring the complementarity benefits in the farm ecosystem leading to the superiority of *Hevea* rubber's technological properties.

ACKNOWLEDGEMENTS

I would like to express my deepest thanks to my supervisor Assistant Professor Dr. Rawee Chiarawipa, Chairman of the Tropical Agricultural Resource Management Program, for his continuous support of my study and guidance in my experimental research and thesis. I would like to express special gratitude to Professor Dr. Sayan Sdoodee for his valuable advice on my experiments and writing the thesis dissertation and manuscript publications.

I also really appreciate Professor Dr. Buncha Somboonsuke, Assistant Professor Dr. Vichot Jongrungrot, and Dr. Narumon Preuksa for their participation in the committee of the thesis examination and their comments and suggestions on my thesis dissertation. Special thanks are given to the external examiner, Dr. Supat Isarangkool Na Ayutthaya from Khon Kaen University who chaired the thesis exam.

I acknowledge the farmers for allowing me to conduct my experiments and for their participation. Songkhla Rubber Regulatory Center, Rubber Authority of Thailand (Southern Region), and Sino-Thai International Rubber College are appreciated for their laboratory testing services.

I am grateful to Graduate School, Prince of Songkla University for providing the Thailand Education Hub scholarship award and research grant to me for my study. I am thankful to the helpful staff and teachers from the Tropical Agricultural Resource Management Program, and Natural Resource Faculty for their great care and support during my study. I also thank my friends for their kindly assistance in my experimental and laboratory works.

Finally, I am sincerely grateful to my family for their encouragement and motivation to accomplish my study journey.

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LIST OF ABBREVIATIONS AND SYMBOLS

- ANRPC = Association of Natural Rubber Producing Countries
- CIRAD = French Agricultural Research and Cooperation Organization
- CSA = Chitosan soil amendment
- DRC = Dry rubber content
- EDTA = Ethylene-diamine-tetra acetic acid
- FAO = Food and Agricultural Organization
- FRD = Fine root diameter
- FRLD = Fine root length density
- g mol^{-1} = gram per mole
- $\text{g tree}^{-1} \text{ tap}^{-1}$ = Gram per tree per tapping (daily dry rubber yield per tree)
- GLA = Gap Light Analyzer
- GPC = Gel permeation chromatography
- HSA = Humic acid soil amendment
- IPP = Isopentenyl pyrophosphate
- ISO = International Organization for Standardization
- K = Potassium
- LAI = Leaf area index
- Mg = Magnesium
- MV = Mooney viscosity
- Mw = Weight average molecular weight
- MwD = Molecular weight distribution
- N = Nitrogen
- P = Phosphorous
- P_0 = Initial plasticity
- P_i = Inorganic phosphorous
- ppm = parts per million
- PRI = Plasticity retention index

RB = Rubber-bamboo intercropping farm

RC = Rubber-coffee intercropping farm

RH = Relative humidity

RM = Rubber-melinjo intercropping farm

RR = Rubber monocropping farm

RRIM = Rubber Research Institute of Malaysia

R-SH = Reduced thiols

S = Season

SEC = Size-exclusion chromatography

SMC = Soil moisture content

SOM = Soil organic matter

Suc = Sucrose

T = Treatment

TCA = Trichloroacetic acid

THF = Tetrahydrofuran

TSC = Total solid content

y = Year

LIST OF PUBLICATIONS

- Zaw, Z.N., Chiarawipa, R., Pechkeo, S. and Saelim, S. 2022. Complementarity in rubber-salacca intercropping system under integrated fertilization mixed with organic soil amendments. *Pertanika Journal of Tropical Agricultural Science*, 45(1): 153-170. <https://doi.org/10.47836/pjtas.45.1.09>
- Zaw, Z.N., Chiarawipa, R. and Sdoodee, S. 2022. *Hevea* rubber physiological status and relationships under different rubber-based intercropping systems. *Songklanakarin Journal of Science and Technology*, 44(1): 6-12. <https://doi.org/10.14456/sjst-psu.2022.2>

CHAPTER I
GENERAL INTRODUCTION

General Introduction

1.1. Rational

Hevea rubber sourced from *Hevea brasiliensis* rubber tree is an indispensable commodity for manufacturing a wide range of rubber-based products due to its irreplaceable outstanding properties. *Hevea* rubber tree has been planted conventionally as monocropping with a mass-production-based objective, mainly in South East Asian countries, India, Sri Lanka and China, where over 90% of the world's total natural rubber production exists (ANRPC, 2021). As most of them are developing countries, over 85% of their natural rubber production is supplied by small farmers who mainly depend on rubber farms for daily incomes (Fox and Castella, 2013).

Although these rubber monocropping supplies to meet the requirement of the world consumption and generates major incomes for the rubber smallholders as benefits, there have been apparent negative impacts on the agroecosystem, including environmental and socio-economic concerns. Extensively expansion, and conventional cultivation practices like monocropping, replanting, and long-term utilization of chemical fertilizer practices of the rubber monocropping have degraded the environment and natural ecosystem with adverse consequences notable deforestation, greenhouse gas emission, soil erosions, soil nutrient depletion, agricultural pollution, changing local climate, and losses of natural resources, carbon stocks and biodiversity (Zhang et al., 2007; Ziegler et al., 2009; Umami et al., 2019; Vrignon-Brenas et al., 2019). Besides, due to the large participation of smallholders by monocropping in rubber-growing countries, socio-economic issues associated with weakening of rubber price, low income and narrowing income sources of farmers have been generated resulting in unstable employment, shortage of workers and high cost of production (Fu et al., 2010; Fox and Castella, 2013; Xu et al., 2014).

With the instability of rubber prices in the last two decades, some rubber farmers started converting to rubber-based intercropping systems from the conventional monocropping practices to widen the on-farm income sources and increase land productivity (Hougni et al., 2018; Romyen et al., 2018). It has been reported in many studies that rubber-based intercropping delivered ecological and economic benefits

such as improvements in soil and microclimate conditions and land productivity, reduction in carbon emission and biodiversity loss, and increased incomes and resilient level of farmers (Werner et al., 2006, Zhang et al., 2007, Elmholt et al., 2008, Guardiola-Claramonte et al., 2008, Tan et al., 2011, Chen et al., 2019).

With realizing these scenarios, some concerned governmental institutes, international organizations, and international natural rubber buyers have committed to sourcing raw natural rubber produced in sustainable ways without degrading the environment and ecosystem. Then, in order to reduce the impacts and develop sustainability in the natural rubber production, rubber-based intercropping and agroforestry became the most recommended options in smallholders' rubber production.

Although the system could improve the agroecosystems and livelihoods of the rubber smallholders, some combinations of rubber-based intercropping were observed with adverse effects on the growth and yield of the crops because of high competition between the two crops for resource uptakes and improper farming practices (Newman, 1985; Langenberger et al., 2017). As the nature of intercropping has greater diversification and high complexity, it needs to integrate different kinds of technical management such as selecting compatible associated crops, planting timing and spacing, integrated fertilization, controlled pruning, integrated disease control, and harvesting, etc. (Guo et al., 2006). For instance, integrated nutrient management utilizing farm organic wastes together with reduced chemical fertilizers could be considered in rubber-based intercropping to reduce fertilization costs by improving or rehabilitating soil properties. Thus, rather than the resource partition, facilitative complements among the agroecological components are fundamentally essential in rubber-based intercropping systems in order to achieve ecological and economic benefits (Bybee-Finley and Matthew, 2018).

In addition, with the changes in microclimate conditions, the physiological response of rubber trees under the rubber-based intercropping varied from that of monocropping. Since rubber latex exuded from rubber trees is a secondary metabolite biosynthesized from the tree's defense mechanism in the laticiferous cells responding physiologically to human interventions (latex harvesting) and abiotic

stresses (Jacob et al., 1989), these agroecology changes under the rubber-based intercropping would have great influences the latex biochemical composition and isoprene biosynthesis, leading to variations in yield potential and inherent technological properties of *Hevea* rubber (Van Gils, 1951; d'Auzac et al., 1997; Roux et al., 2000). Thus, it needs to extend the realization of the implications of these ecophysiological changes on the production and quality properties of *Hevea* rubber to ensure sustainable natural rubber production integrated with the rubber-based intercropping farms.

1.2. Literal review

1.2.1. *Hevea* rubber

Rubber is an elastomer material composed of polymers of organic or inorganic compounds. It is obtained originally from rubber-bearing plants as a natural biosynthetic polymer called natural rubber while synthetic rubbers are produced by man-made polymerizations. There are some numbers of rubber-yielding plants under the Euphorbiaceae, Moraceae, Apocynaceae, and Asteraceae families (George and Panikkar, 2000) which contain laticiferous cells in most parts of the plants to produce latex.

Among the latex-yielding plants, *Hevea brasiliensis* is well-known and the only species cultivated commercially as the major source of natural rubber among the species of the genus *Hevea*, belonging to the family Euphorbiaceae. *Hevea brasiliensis* is a perennial tree and indigenous to the Amazon rainforest as native forest trees growing together with the other nine species of the genus *Hevea* (Wycherley, 1992; Kaliane et al., 2020). *Hevea* latex is a milky cytoplasm in which water, proteins, sucrose, lipids, inorganic ions, alkaloids, and enzymes exist together with rubber particles as secondary metabolites that are synthesized from plant defensive function (Samanani, 2006; Konno, 2011). Its bark responsively exudes a considerable amount of rubber containing latex when being wounded or tapped. Since the latex is exploitable regularly for many years from the bark, it is cultivated primarily as the major source of natural rubber. Compared to the other sources, *Hevea brasiliensis* expresses the highest level of isoprene biosynthesis in laticiferous cells that contributes to its unique features in elasticity, durability, flexibility, adhesive strength and thermal resilience (Malmonge

et al., 2009; Honorato et al., 2016). Due to these superior qualities over the other rubbers, *Hevea* rubber became an indispensable source for rubber-based products and its consumption has gradually increased with technological advancements and the global population growth. Supplying to the high demand by extensive participation of smallholders, it is also an economically substantial source of daily income and stable employment for millions of smallholders in the major rubber-producing countries.

1.2.2. Historical developments of *Hevea* rubber cultivation

Until the 1830s, the applications of rubber and its demand were not promising yet considerably as rubber manufacture technology was undeveloped. At that time, for producing rubber products, raw rubbers were sourced from some species of *Hevea*, *Ficus elastica*, and *Castilla elastica* which grew naturally in wild forests. During 1838 and 1844, the historical discoveries of vulcanization by Charles Goodyear from the United States and subsequently followed by Thomas Hancock from the United Kingdom started the remarkable milestone that accelerated the development of rubber technology and inventions of new rubber-based products (Duerden, 1986). Vulcanization is a curing process that cross-links the rubber polymer chains by mixing with sulphur at a high temperature to transform the greater mechanical properties which possess high resistance to cracking and melting due to low and high temperatures. Based on these findings, many new rubber products had been invented and the new inventions of the pneumatic tire in 1845 by Robert William Thomson and in 1888 by John Boyd Dunlop accelerated the demand for raw rubber around the late 19th century (Tompkins, 1981). Thus, European and American rubber manufacturers started to consider sustainable adequate supply sources of raw rubber.

Then, in the 1870s, the British attempted the introduction of wild rubber from the Amazon forest to its colonies countries in South East Asia for cultivation. The milestone attempt of Sir Henry Wickham was successfully accomplished in June 1876 by carrying about 70,000 *Hevea brasiliensis* seeds from the Santarem area of Brazil, the upper part of the Amazon forest, to the Kew Royal Botanical Gardens where the seeds were germinated, in London. From these seeds, about 2,000 survival seedlings were despatched to Ceylon (Sri Lanka) and planted at the Botanical Gardens of Peradeniya in August 1876 and subsequently at the Henarathgoda Botanical Gardens

(Dean, 1987; Loadman, 2005). These gardens distributed the rubber seeds and seedlings for experimental planting to the British colonized countries of Malaya (Malaysia and Singapore), India and Burma (Myanmar), and also Netherland East Indies (Indonesia) as well (Loadman, 2005). Although the first experimental tapping was started at the Henerathgoda Botanical Garden in 1881 by Dr. Henry Trimen, the innovation of the tapping (harvesting) method was devised in 1889 by Sir Henry Ridley, a British botanist and the director of the Royal Botanical Gardens, based on his experimental testing, was a great contribution to realize that rubber could be harvested commercially for many years (Wycherley, 1959).

In the 1890s, due to the outbreak of coffee leaf rust disease in Ceylon and Malaya, *Hevea* rubber was started to plant by coffee growers for alternative sources of income (Rodrigo et al., 2005; Thomas and Panikkar, 2000). In the meantime, the development of the pneumatic-tire-used motorcar industry induced the soaring rubber price and demand. These stimulated the establishment of commercial rubber plantations and estates in South East Asia countries in the early 1900s. Results of continuous research in the early 1900s by Sir Henry Ridley and his team on tapping standards, spacing of planting, fertilization, identification and controls of diseases, coagulation of latex, and inventions of processing equipment and utensils technically contributed to the development of plantations ensuring in mass production with an efficient yield (Eaton, 1935; Wycherley, 1959).

Then, the areas of rubber cultivations expanded gradually to around 3,600,000 hectares until World War II in the Southeast Asian countries notably Malaya, the Netherland East Indies, Ceylon, India, Burma and other Indo-China countries, of which, over 50% of the area were under estate plantations (Baulkwill, 1989). During the War, most of the plantation and production of rubber was suspended and after that in the 1950s, yields of the most early planted rubber trees dropped due to the end of the productive lifespan of those rubber trees (Baulkwill, 1989). Thus, the major rubber producers such as Malaya, Ceylon and India implemented replanting programs that replaced exhausted old trees with high-yield clonal trees during the 1950s and 1960s (Commonwealth Secretariat, 1973). As the result, it was found that the yields of rubber in these countries increased apparently from the 1970s onwards.

During World War II, since most rubber-growing areas in Asia were under the control of Japan, the major rubber buyers from the United States started to depend on synthetic rubber production (Gropman, 1996). In the 1950s, synthetic rubbers were marketed with technical specifications based on the general technological properties required by rubber goods manufacturers. Thus, research institutes in natural rubber-producing countries conducted extensive research and development on types of raw rubber and their processing technologies to regain the market share taken by synthetic rubber. In 1965, the Rubber Research Institute of Malaysia officially introduced Standard Malaysian Rubber defined by the Standard Malaysian Rubber Scheme into the market to compete with synthetic rubber. As it is traded based on the technical specifications, it is also called Technically Specified Rubber (TSR) (Commonwealth Secretariat, 1973). Due to the development of this scheme, other natural rubber-producing countries like India, Sri Lanka, Indonesia, and Thailand started similar schemes titled with their countries' names in order to promote their produced natural rubbers in the global market (Graham, 1969). The development of TSRs, in which quality parameters are specified, accelerated a higher demand for natural rubber from tire manufacturers, which consume about 70% of the total natural rubber production. Then the rubber cultivations were gradually saturated in the traditional rubber growing areas during the 1980s and 1990s.

During the 2000s, since China became the world's biggest rubber consumer in the mid-2000s, driven mainly by the rapid growth of the country's tyre and automobile industries, rubber cultivation drastically expanded to the new marginal areas near the Chinese borders in the mainland southeast Asia, notably north-eastern Myanmar, Laos, north-eastern Thailand, northern Cambodia, and northern Vietnam, and in south-western China (Viswanathan, 2009; Ahrends et al., 2015; ERIA, 2016). It was estimated that over 1.5 million hectares were transformed into rubber cultivated land in the area between 2000 and 2010 (Li and Fox, 2012; Langenberger et al., 2017).

1.2.3. Agroclimatic conditions of *Hevea* rubber distribution regions

The origin habitat of *Hevea* rubber is the Amazon rainforest basin situated between 5° latitudes south and north of the equator at an altitude of less than 200 m. The climate in that area is predominantly wet equatorial type in which an

average temperature of 25°C to 28°C and an annual rainfall of 2,500 to 3,500 mm are evenly distributed throughout the year without a remarkable dry period (Webster and Paardekooper, 1989; Satyamurty et al., 2010).

Like it well develops naturally in its origin Amazon forest, it expresses optimum performances in the tropical rainforest regions, where the area between the latitudes of 10°S and 8°N. Most commercial plantings were in those regions, particularly in Indonesia, Malaysia, southern Thailand, southern Myanmar, south-western India, Sri Lanka and central and western Africa, where the climate conditions are similar to its habitat area (Vijayakumar et al., 2000). The climatic conditions in the regions are characterized by annual precipitation of over 2,000 mm evenly distributing 125 to 150 annual rainy days, average temperatures ranging between 25°C and 28°C, average annual relative humidity of over 80%, and sunshine period of about 2,000 hours per annual comprised of 6 hours of the average daily sunshine period (Webster and Paardekooper, 1989). Since the cultivated rubber expresses optimum performances such as plant growth, rubber yield, and quality, in these regions, most areas had been saturated by commercial rubber plantings and are considered the traditional rubber growing areas. However, the evergreen tree *Hevea brasiliensis* in its native Amazon changes to a deciduous tree that imparts a regular annual leaf fall entirely or partially from its canopy for a short period due to marked drier weather in the non-native areas where commercial cultivations developed (Chen and Cao, 2014; Li et al., 2016).

Its cultivations also expand to the other parts of the tropic areas including the humid areas of the tropical savanna climate in which north-eastern India, Bangladesh, southern China and the mainland of Southeast Asia consist of north-eastern Thailand, north-eastern Myanmar, northern Cambodia, and Vietnam exist as the marginal or non-traditional rubber growing areas (Fox and Castella, 2013). The areas receive an average annual rainfall of less than 1500 mm and a high range of mean temperature variation between 14°C and 38°C with a long dry period of about 5 to 6 months a year. Although *Hevea brasiliensis* is adaptable in the marginal area, high variation of the climatic conditions limits the performance of the trees. With a deficit content of soil moisture due to low rainfall and a longer marked dry season, the period of immaturity to meet the standard tappable growth takes longer, and the average rubber

yield is lower than that experienced in the traditional areas. In the area where the temperature is less than 20°C in most periods of the year, the tree is not well developed. The high diurnal temperature in a longer dry season shortens the latex flowing period resulting in less yield of rubber (Huang and Zheng, 1983).

1.2.4. Impacts of the monocrop rubber cultivation

Hevea brasiliensis has been cultivated conventionally as monocropping with a mass-production-based objective to supply raw natural rubber commodity for manufacturing a wide range of rubber-based products. Although these intensive rubber cultivations supplied to meet the requirement of the world consumption and meanwhile generated major incomes for the rubber smallholders as benefits, there have been apparent negative impacts of these intensively monocropping to agroecosystems including environmental and socio-economic concerns.

The expansion of rubber monocropping has degraded the environment and natural ecosystem with adverse consequences, notably deforestation, soil erosions, changing local climate, losses of natural resources and biodiversity, and higher emission rate of carbon and greenhouse gas (Zhang et al., 2007; Ziegler et al., 2009). Since about 70% of the new expansion during the 2000s in mainland southeast Asia replaced natural forests in the area (Ziegler et al., 2009; Fox and Castella, 2013) and rapidly transformed the landscape of the area into a large area of rubber cultivated land. It resulted in siltation in the flow of streams due to a higher amount of run-off water leading to less soil organic matter and moisture content and a drier climate throughout catchment areas (Guardiola-Claramonte et al., 2008). Consequently, changes in microclimate and loss of biodiversity in the area have resulted (Aratrakorn et al., 2006). In addition, substantial low levels of soil carbon content and high level of greenhouse gas emission were investigated under rubber monocropping during the land clearing and immature stage, and its steady stage could be reached after 20 years of rubber planting (Werner et al., 2006; Zhang et al., 2007).

Due to gradually increased market demands and related government replanting programs, most rubber planting areas in major rubber-producing countries are under second or third replanting cycles of rubber monocropping. Nowadays, the annual replanting areas in the major rubber-producing countries: Indonesia, Thailand,

Vietnam, and Malaysia, represented 50%, 34%, 24%, and 17%, respectively, of the yearly total planted areas in 2021 (ANRPC, 2021). These replanting practices apparently degraded the soil structures and nutrients. Although the mature stage of the first replanting cycle shows above and average organic matter according to soil fertility standards, the third replanting cycle exhibits significant reductions in organic matter and major nutrient contents in the soil (Karthikakuttyamma, 1997). Besides the low level of soil fertility, the degrading of soil structure, properties, and functions follow inefficient water and nutrient cycles resulting in lower productivity in the traditional rubber growing areas (Werner et al., 2006; Zhang et al., 2007; Kotowska et al., 2015).

Besides, due to the large participation of smallholders by monocropping in rubber-growing countries and their high dependency on rubber income, socio-economic issues associated with the weakening of rubber price and high rate of worker wages have been generated (Fu et al., 2010; Fox and Castella, 2013; Xu et al., 2014). Since most rubber smallholders depend only on rubber production for their daily income, weaker rubber prices since 2011 caused low income, unstable employment, and a shortage of workers. Then, farmers could not follow proper agricultural practices resulting in uneconomic production and inferior quality of produced rubber. These impacts caused higher production costs and lesser farmgate prices and adversely affected the livelihood of the rubber farmer. In some cases, some rubber smallholders could not survive under the prolonged weakened price; thereby, they abandoned rubber productive lands and looked for alternative incomes (Simien and Penot, 2017).

1.2.5. Development of rubber-based intercropping

Since *Hevea brasiliensis* is originally a forest tree and naturally growing together with other trees in its origin, the Amazon basin, its nature is basically adaptable along with other plants (Wycherley, 1992; Budiman and Penot, 1997). When it was first introduced into Sri Lanka in the late 1870s, it was mentioned to be planted as an intercrop in perennial plantations such as tea and cocoa before its commercial cultivation started in the East Asian countries (Rodrigo et al., 2005). It was documented that smallholders started rubber planting in Indonesia in 1918 with the slashed-and-burned system in a forest without proper management practices, called jungle rubber agroforestry (Budiman and Penot, 1997; Joshi et al., 2002).

However, at the beginning of natural rubber cultivation development, the majority of rubber production was supplied from commercial estates under monocropping plantations in which only leguminous cover crops were recommended to be planted between the rubber rows to control soil erosion and soil moisture, and reduce the cost of fertilizer (Baulkwill, 1989) without considering intercropping and other productions from the land because of higher market demand of natural rubber due to automobile industry booming.

Production of the rubber was highly concentrated in a large-scale monocropping plantation at that time. Munro et al. (1981) reported that 60% of the world's natural rubber production was supplied from the monocropping rubber plantations, and the rest comprised the wild sources and smallholders in 1914. Later, the structural diversification of the holding size of rubber planting had been wider with the participation of smallholders because of simple agricultural and processing methods and higher prices in the market. Consequently, before the Second World War, rubber production from smallholders reached 50% of the world supply (Byerlee, 2014). However, despite increasing the production of smallholders, their development was not improved. Thus, after the war, China, India, Malaysia, Sri Lanka, Thailand and Indonesia started implementing smallholder development programs and regulations in which high-yield planting materials, technical support, and subsidies were provided (Budiman and Penot, 1997; Fox and Castella, 2013). As a result of these programs, smallholders could supply a higher share of rubber production in the major rubber-producing countries as crucial stakeholders in the industry.

However, the programs targeted only to increase the production amount of rubber from their countries; thus, smallholders were driven into monocropping (Budiman and Penot, 1997). Since most smallholders have depended only on rubber monocropping, the source of income generation is narrow; consequently, the farmers are hard to survive, especially when rubber price declines.

Then, some concerned governments promoted intercropping programs in rubber planting to maximize the incomes of the farmers. India Rubber Board initiated an intercrop promotion scheme in 1957 in the Kerala area with concerns about food security, income generation and employment creation (Siju et al., 2012). In China,

rubber-based intercropping was strongly encouraged during the 1970s and 1980s in order to generate additional incomes for farmers and also half the impact of typhoon damage to rubber trees (Zaizhi, 2000). In Sri Lanka, rubber-based intercropping could be firstly recommended for smallholders in 1979 (Chandrasekera, 1979). With the encouragement of the research institute, in the early 2000s, 50% of the smallholders were planting intercrops in the rubber rows before the mature period of rubber in Sri Lanka (Rodrigo et al., 2001). In Malaysia, the beginning of rubber-based intercropping started during the Japanese occupation around the 1940s by planting food crops in the rubber field by smallholders and planters to supply the shortage of food (RRIM, 2009). The Indonesian Jungle rubber agroforestry has been practiced traditionally since the beginning of the rubber planting around the 1920s and covered over 2.5 million hectares of area in 1997 (Budiman and Penot, 1997). It was reported that there were some on-farm activities of the combination of rubber with fruit crops and livestock observed in Thailand in the 1980s (Somboonsuke and Wettayaprasit, 2013).

Around the 2010s, to address the issues resulting from the conventional rubber production practices, the key players of the natural rubber industry, consisting of tire and rubber goods manufacturers, rubber traders, processors and industrial institutes and organizations, started a commitment to aligning the industry towards the industrial sustainability by sourcing natural rubber produced in sustainable ways to ensure socioeconomic and environmental safeguards. With this paradigm shift to the sustainable natural rubber production in the industry, rubber-based intercropping and agroforestry systems have been recommended and promoted to improve the smallholders' livelihood and agroecosystem of rubber planting areas to rehabilitate the degradations resulting from the long-term monocropping practices.

1.2.6. Ecological benefits of rubber-based intercropping

Generally, yields and growth rates of both crops in the rubber-based intercropping system improve with sufficient light distribution, reduction in weed, and more utilization of resources when there is a complementarity effect in the system (Mousavi and Eskandari, 2011). Rodrigo et al (2005) observed that the immature period of rubber in the intercropping system was less than that of the monocropping because of a greater girth incremental rate and higher stands of productive trees. With improved

girth and height, and higher stands per hectare of mature rubber trees, it could be expected that not only higher yield could be harvested but also a larger volume of rubber timber per hectare could be achieved at the end of the rubber economic lifespan, leading to a sustainable higher income to the farmers.

Improved growth of the crops in the rubber-based intercropping is associated with a higher photosynthetic rate, low-temperature stress, efficient water usage, and high relative humidity in the understory environment of the farm. In most rubber-based intercropping, leaf area of the farms improves showing higher leaf area index which represents the amount of one-sided leaf area coverage on one unit of specific farm area (ratio of the areas between leaf and ground) (Erasmus et al., 2021). Its variation is related to tree's biophysical functions and is measured as a key indicator of the growth and productivity of forest or agricultural land at spatial scales (Nathalie, 2003; Cotter et al., 2017). The multi-layer arrangements of the crops under the intercropping enhance efficient light distribution through the canopies and allows greater light energy capture of understory plants, thus improving the photosynthetic rate in the system (Powels, 1984). It creates an improved microclimate environment in the system to adapt to the extreme climate changes. The canopy shade of rubber trees lessened the pressure on coffee plants and incidences of Cercosporiosis at the coffee leaves, and the coffee grains were larger with high organoleptic quality although the coffee yield under the rubber trees was lesser than that of the sole coffee planting under the full sun (Araujo et al., 2016). The better microclimate conditions under the system increase ecosystem diversities as a result. It was observed that soil's properties and structure under the system became rehabilitated by a higher organic matter and residues (Chen et al., 2019; Carson et al., 2014). Chen et al (2019) also observed that greater root proliferation in rubber-based intercropping significantly improves hydraulic conductivity, infiltration and moisture holding capacity of capillary porosity in the average soil depth. Since soil water content and plant water use efficiency are mutually related with plant's growth and productivity, the water cycle in the system became efficient ensuring the healthy physiological status of the crops (Guardiola-Claramonte et al., 2008; Tan et al., 2011). Sufficient intakes of soil moisture by plant impart the translocation of nutrient and mineral assimilates. Improvement in root distribution mainly contributed to soil aggregation and stabilization that induce soil microbial

activities (Elmholt et al., 2008). Due to the improved soil structure and properties, soil microbial diversity increased efficient nutrient uptakes and reduced soil pathogens.

1.2.7. Constraints in smallholders' rubber-based intercropping

Although there are many studies and observations that rubber-based intercropping yielded advantages with respect to the livelihoods of rubber smallholders and agroecology on the farms, the farmers have been encountering some obstacles in practicing the system in on-ground situations.

Some combinations of rubber intercropping cause low yield of intercrops and adverse effects on the growth and yield of rubber (Liu et al., 2020). It was observed on the ground that most farmers started intercropping on their rubber farms when the rubber trees reached the mature stage and selected perennial shade-tolerant crops such as coffee, tea, cacao, ginger, salacca, and bamboo for the long-term incomes from the farm (Jongrungrat and Thungwa, 2014). Thus, in some cases, if the intercrops are planted in high-density or the rubber trees' canopy is too dense, intense competition happens in both above- and below-ground interactions. There are also some reports that the yield of coffee under mature rubber trees was not comparable to that of coffee monoculture under the full sun (Wintgens, 2009; Araujo et al., 2016). The associated crops like coffee and cocoa have a similar root system to rubber root's development, and it could be greater competition in water and nutrient uptakes in high-density planting (Newman, 1985; Huang et al., 2020). A study suggested wider spacing of rubber rows for coffee plants intercropped in mature rubber farms since pioneer rubber roots affected the coffee root distribution (Chiarawipa et al., 2021). Root harvested crops like cassava and sweet potato could interfere with the development of rubber roots and residues from the harvested roots, particularly from Cassava which is under the same family of rubber (*Euphorbiaceae*), induced root disease pathogen of rubber roots in the soil (Blencowe, 1989; Liu et al., 2020). Thus, in the combination of rubber trees with root crops, the intercrops were recommended to be planted two meters away from the rubber trees and need to be confined to prevent from the roots invading (Somboonsuke and Wettayaprasit, 2013; Langenberger et al., 2017).

As a nature of the deciduous tree, the rubber tree typically occurs defoliation for around one to two months in most rubber growing regions during the

dry season when the water deficit is severe. Thus, during that time, rubber farms could not provide canopy shades for the understory environment (Premakumari and Saraswathyamma, 2000). Such changes in light intensity influence the above- and below-ground water availabilities in the system, and affect the vegetative growth and yields of the associated crop (Galhidy et al., 2005).

Like other intercropping systems, rubber-based intercropping has greater diversification and high complexity, thus effective management systems such as integrated farming and good agricultural practices are required to achieve both ecological and economic benefits sustainably. Intercropping system needs harmonious integration of different kinds of farm resources and technical management such as selecting cultivars, planting spacing, upkeeping, pruning, fertilization, disease control and harvesting, etc. (Guo et al., 2006; Bybee-Finley and Matthew, 2018). Improper combination of associated crops, incorrect planting spacing and time, and uneven fertilizer application are the main reason for the failure of the rubber-based intercropping system in terms of farmers' agricultural practices (Romyen et al, 2017; Liu et al., 2020).

1.2.8. Latex biochemical composition

Since the rubber tree basically transforms sucrose as a raw material into natural rubber, cis-polyisoprene, by consuming natural resources like sunlight, nutrients, and water from its environment, the production and composition of the latex are strongly linked to the physiological responses of the rubber tree to the agroecosystem changes (d'Auzac et al., 1997; Roux e al., 2000). Besides the rubber molecules and water, biochemical contents are also contained in *Hevea* latex. The biochemical contents of latex are indicators of the physiological status of the rubber tree's latex metabolism. Among the biochemical composition, contents of sucrose (Suc), inorganic phosphorous (Pi) and reduced thiols (R-SH) and magnesium are mainly assessed to evaluate the two primary factors – latex flow and regeneration – that limit production capacity in relation to tree physiology. These are basic parameters of physiological diagnosis of the latex production capacity (Jacob et al., 1989; Obouayeba et al., 2011).

Sucrose is the raw material of isoprene synthesis and initiates energy generation for the synthesis. As the isoprene biosynthesis transforms the sucrose produced from photosynthesis into rubber molecules in the laticiferous cells, low sucrose content in the latex associated with high latex yield reflects the active metabolism of the biosynthesis process. However, insufficient sucrose obtained due to less photosynthesis efficiency also causes the low content of sucrose in the latex. Thus, exhausted or over-exploited rubber trees are normally associated with too low sugar content in the latex. Conversely, high sucrose content in the latex indicates low metabolism in consuming sucrose for the rubber molecules or sufficient sugar loading in the laticiferous system (Jacob et al., 1989; Dounghmusik and Sdoodee, 2012). Inorganic phosphorus plays an important role in latex regeneration as an essential element for the production of nucleic acids required in isoprene metabolism. It shows the level of laticifer biosynthetic activity or metabolic utilization of sucrose. Thus, low Pi content in the latex indicates a poor metabolism associated with low latex production. It also means the tree has no energy for latex metabolism (Atsin et al., 2016). Reduced thiols are antioxidants that reduce the oxidative stresses of tapping and yield stimulation, and protect the membrane of latex organelles. Low R-SH shows the poor physiological condition of the laticiferous system or excessive exploitation of latex from the tree (Purwaningrum et al., 2019). This can also cause destabilization of organelles, particularly lutoids, in the latex, resulting in faster coagulation and latex destabilization after tapping. In general, a rubber tree under a highly active metabolic status is associated with greater production together with high Pi and R-SH (Sulochanamma and Thomas, 2000).

1.2.9. Technological properties of *Hevea* rubber

Rubber from *Hevea brasiliensis* has unique features, that other sources of elastomer are incomparable, like elasticity (resilience), durability, flexibility, shock and vibration tolerances, and adhesive strength, thus being an indispensable commodity in the global market for manufacturing various rubber products. These features are because of *Hevea* rubber's technological properties which possess outstanding molecular structure, rheological properties, mechanical properties, and processability (Malmonge et al., 2009; Honorato et al., 2016).

The technological properties of natural rubber are generally specified based on the basic properties mainly non-isoprene contents and rheological properties. Generally, the non-isoprene contents in dry rubber include volatile matter, dirt content, ash content, and nitrogen (N) content. Like that, magnesium (Mg) content is one of the major parameters in rubber latex as non-isoprene content. At the same time, plasticity (P_0), plasticity retention index (PRI), and Mooney viscosity (MV) are tested for rheological properties of the dry raw rubber.

Most of these parameters are influenced by the molecular weight structure of *Hevea* rubber (Kovuttikulrangsie and Sakdapipanich, 2005). Thus, the isoprene biosynthesis process in the tree, which varies with the tree's physiological responses to its environmental changes, has strong relations with the inherent technological properties of *Hevea* rubber (d'Auzac et al., 1997; Roux et al., 2000). For instance, Roux et al (2000) observed some significant correlations, particularly between the Pi and plasticity retention index, and between the TSC and the Mooney viscosity. High values of Pi were observed associated with high rainfall causing in high production. Since high Pi represents a high rate of metabolism, after the synthesis, the production of the macromolecular chain could not be protected completely leading to being highly sensitive to thermo-oxidative degradation. As a result, rheological properties – PRI and the Mooney viscosity – were poor normally in high metabolism clones and high production periods.

1.3. Objectives

The overall objective of the thesis research was to investigate the seasonal variations of rubber-based intercropping practices in agroecology and tree physiology, and their implications in order to ensure the sustainability of natural rubber production integrated with intercropping systems.

The thesis research was composed of two experimental studies. The first study was a case study conducted to investigate the changes in agroecosystem components of a rubber-based intercropping farm and their interactions under integrated fertilizations mixed with organic soil amendments.

The second experiment was laid out to study the ecological changes mainly in leaf area and soil water content of different rubber-based intercropping farms,

and their interrelations with the latex biochemical compositions, yield, and technological properties of *Hevea* rubber.

CHAPTER II

EXPERIMENT I

Complementarity in rubber-salacca intercropping system under integrated fertilization mixed with organic soil amendments

2.1. Introduction

Natural rubber (*Hevea brasiliensis*) has been planted traditionally in Southern Thailand since rubber planting industry began in Thailand over last one hundred years ago. The country's rubber planting concentrated in the area which has over 60% of the country's agricultural land and accounts for about 85% of the total rubber planting area (NSO, 2013; Penot, 2017; Chaiya and Ferdoushi, 2019). Most rubber farms in the area are currently in second or third replanting cycle of rubber monocropping (Panklang et al., 2022). This replanting practice of the same perennial monocrop has depleted substantially soil fertility (Karthikakuttyamma et al., 2000; Panklang et al., 2021). Besides the low level of soil nutrients, degrading of soil structure, properties and functions were also investigated as negative impacts, thus lower yields in the long-term conventional rubber monocropping (Zhang et al., 2007; Warren-Thomas et al., 2015).

According to the Agricultural Census report for Southern Thailand in 2013, about 67% of the rubber area in the region was under intensive application of the inorganic fertilizer (NSO, 2013) to meet targeted immature period and economic yield. Over usage of inorganic fertilizer accumulated adverse effects on soil such as acidification of soil, pollution of soil water, leaching and shortage of soil organic matter resulting in degradation of soil fertility, structure, aggregation and also soil microbial diversity (Verma et al., 2012). In addition, its long-term application retards the development of root distribution and nutrient-uptake function of roots (Mahajan et al., 2008).

Over the last decade, small rubber farmers in the area have faced the problem of low income resulting from the prolonged poor rubber price since 2011, leading to inadequate fertilization in the farms. Thus, some rubber farmers started converting their farms from monocropping to intercropping to increase the on-farm income and land productivity (Hougni et al., 2018; Romyen et al., 2018). In the area, most rubber-based intercropping farms were converted from mature monocropping rubber farms. And most of these intercropping farms selected perennial cash crops like bamboo, coffee, cacao, ginger, and salacca as the associated crops in anticipation of long-term economic benefits (Jongungrot et al., 2014). However, some combinations

of rubber-based intercropping encountered adverse effects on the growth and yield of the crops due to severe competition in root interactions and resource uptakes (Langenberger et al., 2017). Therefore, in permanent rubber-based intercropping, facilitative complements among the above- and under-ground components in the system rather than the resource competition are an important consideration to ensure the ecological advantages together with healthy physiological status of the crops and vegetative growth, and sustainable crop yields for long-term economic benefits (Bybee-Finley and Matthew, 2018).

Available farm wastes like animal manures, green manures, crop residues, compost, etc. have been utilized as organic fertilizer and/or soil amendment in vegetable and horticulture farms resulting in beneficial effects, however, rubber farmers have been still applying inorganic fertilizer in the region to meet targeted immature period and economical yield. For these reasons, as the principle of integrated nutrient management, harmonious utilization of on-farm nutrient sources such as organic manure, green manure and farm wastes mixed with inorganic fertilizers as integrated fertilization could be considered in the rubber-based intercropping system to increase land productivity and cost-saving of fertilization through improvement or rehabilitation of soil properties (Liu et al., 2009).

One of the integrated usages of available farm wastes, humic acid extracted from vermicompost has been being applied widely as an organic soil amendment. As its main feature, it can attract insoluble minerals and nutrients in the soil, thus, higher soil nutrient content. In humic acid-treated soil, better root performances were found with improved soil physical properties such as soil porosity, water holding capacity, hydraulic conductivity and infiltration (Vista, 2015).

Likewise, chitin and chitosan processed from chitin-containing wastes from fishery industry, which is available in the area, have been widely applied in agriculture. It was reported that chitosan-treated plants were improved in pathogen resistance because of the chitosan's antimicrobial properties (Sharp, 2013).

Although the sources for these organic soil amendments could be accessed and processed easily in the area, their usages have not been found yet in the rubber farms and rubber-based intercropping as well. Since scientific studies related to

the applications of organic soil amendments in rubber-based intercropping systems are also limited, it needs to explore the effects of these organic soil amendment applications on a rubber-based intercropping system. Thus, an on-farm experiment was conducted at a mature rubber farm intercropped with a perennial crop that has a different rooting system with rubber to investigate the consequences of the interactions among the agroecosystem components under the application of different organic soil amendments combined with mixed organic and inorganic fertilization compared to that of chemical fertilization conventionally applied by farmers in the area.

2.2. Materials and methodology

A rubber-based intercropping farm associated with salacca palm (*Salacca zalacca*) situated at geographical coordinates of 6°59'46.9"N, 100°34'58.6"E in Na Mom district, Songkhla province, southern Thailand was selected for the on-farm experimental study. The area is characterized by an annual rainfall of about 2,000 mm distributed from June to December. In general, monthly rainfall between June and September is less than 200 mm and between October and November is around 300 mm. The rain peaks in December with about 500 mm.



Figure 2.1. The rubber-salacca intercropping farm

The farm replanted RRIM 600 rubber clones as a monocrop replanting in 2002 in 6 m x 3 m spacing on flat land. The rubber trees started harvesting in 2008 implementing with S/3 2d3 tapping system (one-third spiral of tapping cut length and

two-day tapping in three days of harvesting frequency). The farm intercropped salacca palm as an associated crop in 2008 in the interrow space of rubber trees with space as same as the rubber planting. When the experiment was conducted, the heights of the rubber tree were around 18 m, and the stem girths were around 79 cm at the height of 170 cm from the ground, on average. The salacca palm growths were uniform and their canopies were at an average height and width of 3.6 m and 4.5 m, respectively.

The experiment was structured in a randomized complete block design in which three fertilization treatments with three replications were comprised. Each replication consisted of one row of ten rubber trees and adjacent two rows of the salacca palms. The treatments were set up to evaluate the applications of two different organic soil amendments mixed with organic and chemical fertilization against the controlled application of conventional chemical fertilizer practiced by the farmer. The treatments are depicted in Table 2.1.

Table 2.1. Summary of the three treatments of fertilizations

Treatments	Chemical fertilizer		Organic fertilizer		Organic soil amendment	
	Types	Application rate	Types	Application rate	Types	Application rate
T1	Compound fertilizer (30-5-18)	1 kg tree ⁻¹ y ⁻¹ (3 times)	-	-	-	-
T2	Compound fertilizer (30-5-18)	0.5 kg tree ⁻¹ y ⁻¹ (3 times)	Composted cow manure	10 kg (3 times)	Humic acid	100 mL 20 L ⁻¹ water (3 times)
T3	Compound fertilizer (30-5-18)	0.5 kg tree ⁻¹ y ⁻¹ (3 times)	Composted cow manure	10 kg (3 times)	Chitosan	100 mL 20 L ⁻¹ water (3 times)

In the control treatment (T1), chemical compound fertilizer (30-5-18) was broadcasted at the application rate of 1 kg per rubber tree per year between the rows of the rubber trees and the salacca palm in March, July, and November 2016. Under the other treatments (T2 and T3), 0.5 kg of the chemical fertilizer was mixed with 10 kg of organic fertilizer made of composted cow manure and was applied between the rubber and the salacca rows from April. Then, humic acid soil amendment

(HSA) solution prepared by mixing 100 mL of vermicompost-derived humic acid (pH 6.5, 5% humic acid, 50% organic matter, 5% total nitrogen, 2.5% total potassium, 0.06% total phosphorus, 0.25% calcium) in 20 L of water was sprayed on the soil between the rubber trees and the palms in T2 from May. Likewise, with the same application rate as the HSA treatment, 100 mL of the chitosan (pH 5.5-6, 6.5% organic carbon, 0.05% nitrogen, 0.01% phosphorus oxide, 0.01% potassium) mixed with 20 L of water was applied as the chitosan soil amendment (CSA) in T3 from May. All these fertilizations were applied three times with a third-monthly interval during the study period.

To compare the soil organic matters of each plot before and after the treatments, soils sampled from two levels of soil depths at 0-20 cm and 21-40 cm below the ground of each treatment plot were tested using Walkley-Black's titration method (FAO, 2020) in February and December 2016.

Changes in leaf area index (LAI) at the farm were monitored monthly by the hemispherical photography method from June to December 2016. The hemispherical photos were taken vertically upward from 1.5 m above the ground at three different points in the interrow between the rubber trees and the salacca palms at every treatment plot by using Nikon Coolpix 8400 camera with a fish-eye lens (Chen et al. 1997). The Gap Light Analyzer (GLA) software version 2.0 was used to analyse the fish-eye captured images.

Monthly changes in fine root traits such as root diameter, root length and root length density of both crops were monitored in two layers of soil depths (0-20 cm and 21-40 cm) by using the PSU minirhizotron root scanner through 100 mm in diameter with 1 m long of two acrylic access tubes per treatment plot installed with 45° angle of slop in the soil (Saelim et al., 2019; Vameralli et al. 2011) between the rubber tree and the salacca palm at 1.5 m far from each. Two months after installing the acrylic tubes, the root images were scanned every month from June to December 2016. The scanned images were analyzed by the Rootfly software (version 2.0.2).

To analyse the latex production, latex samples were collected from each plot once a month during the study period. Dry rubber weight per tree per tap (g/t/t)

was measured from the collected latex samples by coagulating them with formic acid, and then oven-dried at 70 °C for 16 h following ISO 126:2005. Productions of the salacca palms in yield per cluster, and total yield per palm were measured collectively at the end of the study period from randomly selected seven palms from each plot.

Latex samples were taken monthly from selected rubber trees of each treatment plot to analyse the biochemical composition of latex: sucrose content (Suc); inorganic phosphorus content (Pi); reduced thiols (R-SH) in the latex following the latex micro-diagnosis method of the CIRAD (Chantuma et al., 2011).

Data collected from different samplings were analysed separately using a one-way analysis of variance (ANOVA) at $p \leq 0.05$ with R software (version 3.6.2). Duncan's multiple range tests were performed at $p \leq 0.05$ to compare the data pairs, and Pearson's linear correlation (r) at $p \leq 0.05$ was applied in correlation analysis.

2.3. Results

2.3.1. Comparisons of soil organic matter

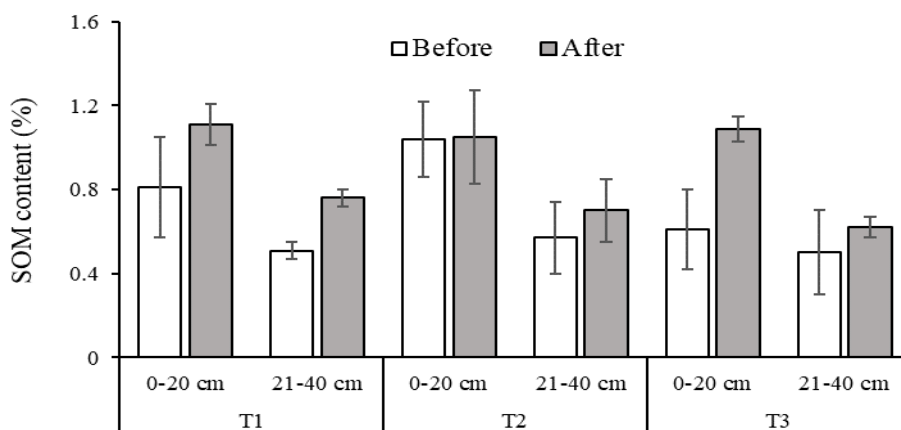


Figure 2.2. Comparison of soil organic matter (SOM) among the treatments before and after the experiment

The higher content of organic matter was found in the topsoil layer (0-20 cm in depth) while the deeper soil layers had relatively lower organic matter content, under all treatments after the experiment (Figure 2.1). Despite all treatments increasing

the soil organic matter in all layers of soil depth, only the top layers under T1 and T3 showed remarkably higher contents of the soil organic matter. T3 increased the soil organic matter in the topsoil layer by 80% followed by T1 with an increase of 38% after the experiment.

2.3.2. Changes in leaf area index (LAI) of the farm

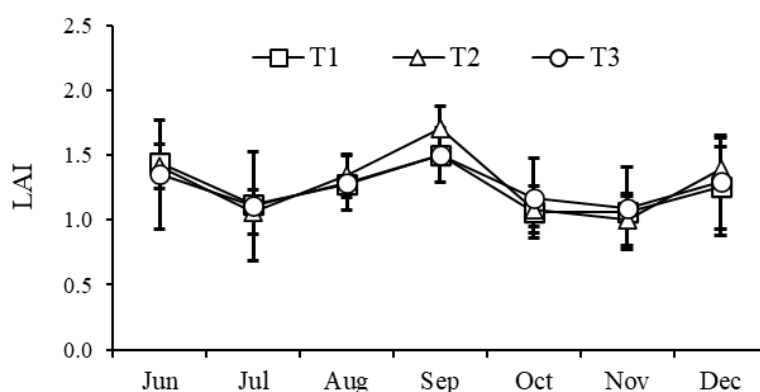


Figure 2.3. Changes in leaf area index (LAI) of the farm under the tree treatments (from June to December 2016)

There were no significant differences in changes in the LAIs under all treatments during the study (Figure 2.2). Although these changes followed a similar trend, the trend varied monthly. The LAIs of the farm started an upward trend in July with just over 1.10 and reached their maximum values ranging between 1.5 and 1.7 in September. Then the LAIs decreased to their lowest values between 1.00 and 1.20 in October and November, respectively. However, the leaf development of the farm increased again to the LAI values ranging between 1.29 and 1.39 in December.

2.3.3. Changes in fine root traits of the rubber trees

The fine roots' diameters of rubber trees under T1 were found as the largest over those of the other treatments from June to September in both soil layers of 0-20 cm and 21-40 cm soil depths, respectively (Figure 1.3 A1 and A2). In the soil depth of 21-40 cm, the average monthly root diameter under T1 was longer than that of T2 and T3 by 27% and 28%, respectively, during that period (Figure 2.3 A2).

In terms of changes in FRLD (Figure 2.3 B1), all treatments resulted in a stable trend ranged between 0.34 and 0.70 cm cm^{-2} in the topsoil layer during the study period. In the soil depth of 21-40 cm (Figure 2.3 B2), the rubber trees under T2 were observed with the highest FRLD at over 1.44 cm cm^{-2} between July and October. After October, however, it decreased slightly with the densities of 1.46 and 1.09 cm cm^{-2} in November and December, respectively.

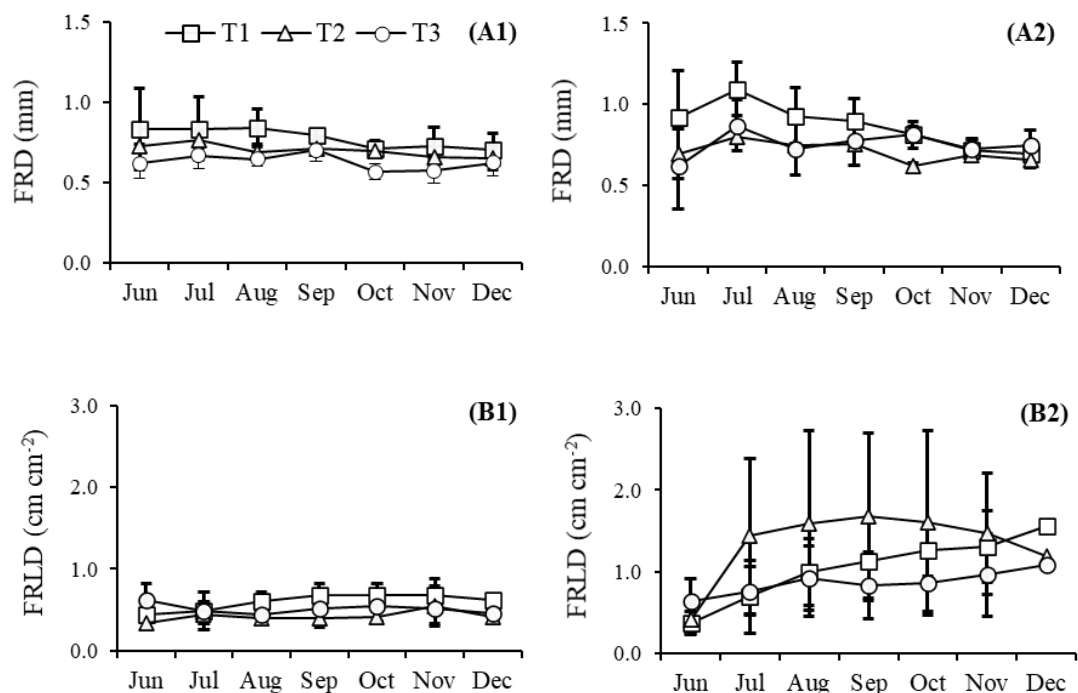


Figure 2.4. Monthly changes in fine root traits of the rubber tree: fine root diameter (FRD) at the soil depths of (A1) 0-20 cm and (A2) 21-40 cm; fine root length density (FRLD) at the soil depth of (B1) 0-20 cm and (B2) 21-40 cm (from June to December 2016)

2.3.4. Changes in fine root traits of the salacca palm

The fine roots of the salacca palm in the soil depth of 0-20 cm (Figure 2.4 A1) under T1 showed the largest diameter sizes ranged between 0.82 to 1.23 cm while the other treatments resulted in smaller sizes of the FRDs range d between 0.67 and 0.95 cm. In the soil depth of 21-40 cm, the sizes of FRD under T1 also higher than those under other treatments in July, August, September, and October (Figure 2.4 A2).

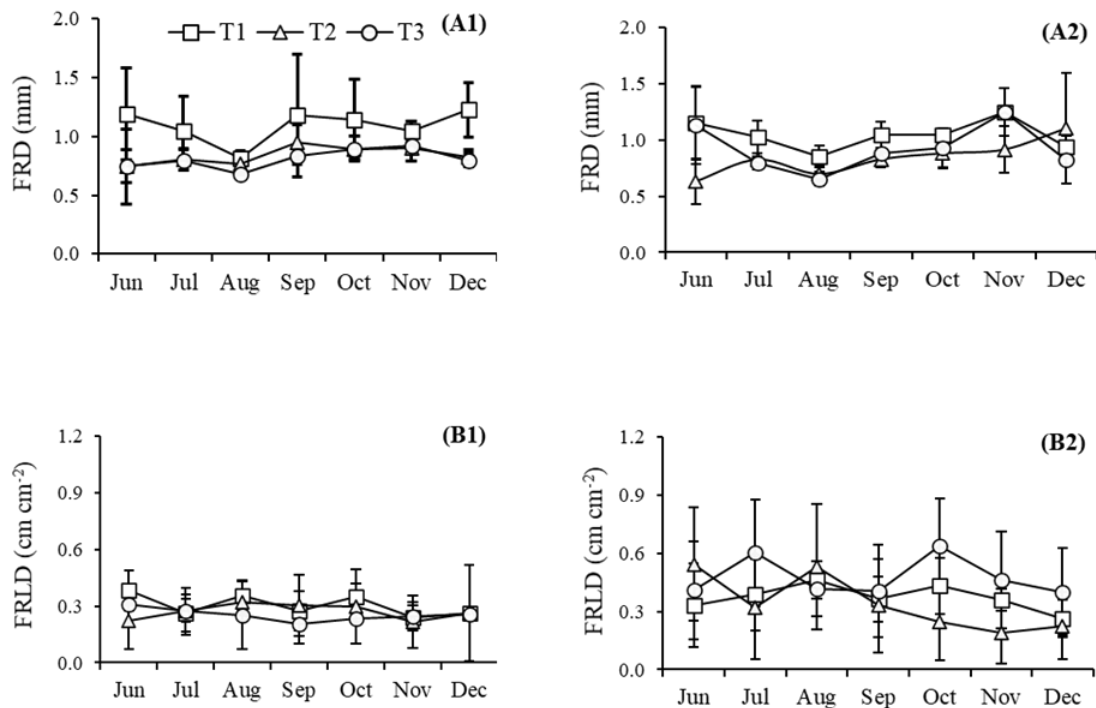


Figure 2.5. Monthly changes in fine root traits of the salacca palm: fine root diameter (FRD) at the soil depths of (A1) 0-20 cm and (A2) 21-40 cm; fine root length density (FRLD) at the soil depth of (B1) 0-20 cm and (B2) 21-40 cm (from June to December 2016)

Monthly changes of the FRLD of the salacca palm (Figure 2.4 B1) in the soil depth of 0-20 cm were stable between 0.20 and 0.38 cm cm^{-2} and did not show a significant difference during the study period. However, in the soil depth of 21-40 cm, T3 resulted in the highest FRLD in July, October, November, and December with 0.60, 0.64, 0.46, and 0.40 cm cm^{-2} , respectively (Figure 2.4 B2).

2.3.5. Changes in latex production

Although there were no significant differences in the latex productions among the treatments, the latex productions varied with different seasons (Figure 2.5). At the beginning of the rainy season, the productions under all treatments dropped their yields from about 60 $\text{g tap}^{-1} \text{tree}^{-1}$ in June to less than 40 $\text{g tap}^{-1} \text{tree}^{-1}$ in July. Then, the productions increased to the highest level between 73 and 80 $\text{g tap}^{-1} \text{tree}^{-1}$ in September.

However, all treatments showed low yields of around 30 g tap⁻¹ tree⁻¹ in November. In December, the productions under T1, T2, and T3 surged back respectively with 80, 65, and 50 g tap⁻¹ tree⁻¹. The result of Pearson's linear correlation ($r = + 0.6024$) at $p \leq 0.05$ confirmed a positive correlation between the monthly changes of the LAIs and the latex production under all treatments (Figure 2.6).

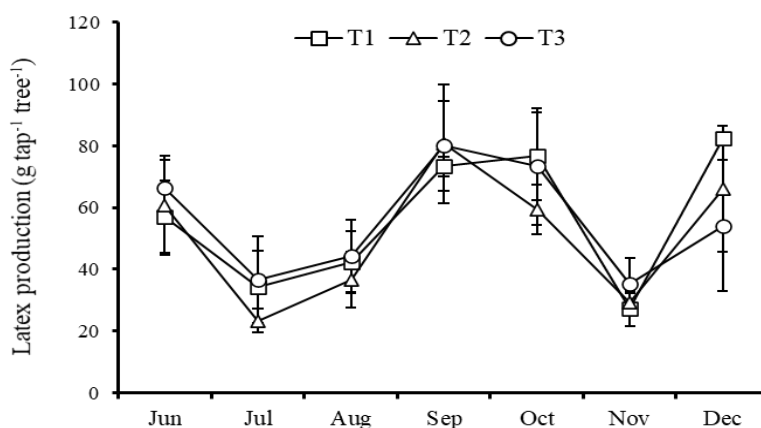


Figure 2.6. Monthly changes in average daily production of latex (g tap⁻¹ tree⁻¹) under the treatments (from June to December 2016)

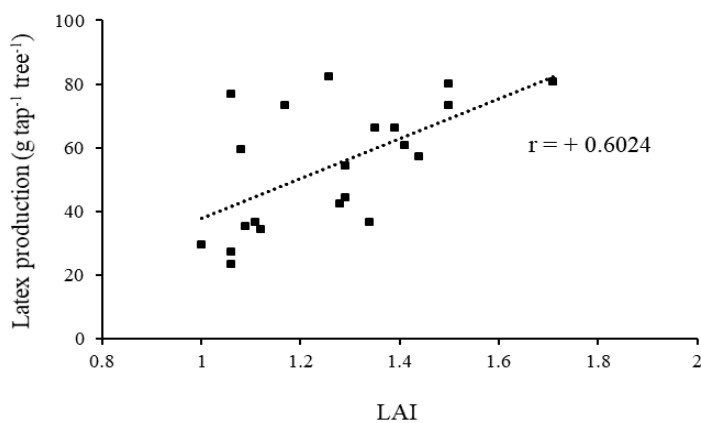


Figure 2.7. Relationship between the changes of LAI and latex productions

2.3.6. Changes in latex biochemical composition

Suc contents of all treatments decreased gradually between July and October, except that of T2 showed a peak at 13.66 mM in August (Figure 1.7 A). The Suc contents of T1 and T2 reached their minimum levels of 1.79 and 2.43 mM,

respectively, in November. However, T3 showed an upward trend in November after its lowest level of 4.65 mM in October. In December, the Suc content under T3 reached 9.77 mM as the highest level in that month, followed by that of T2 and T1 with 6.76 and 3.53 mM, respectively.

Pi content under T2 decreased from 21.33 mM in June to 10.52 mM in July (Figure 1.7 B). The contents under T1 and T3, however, were stable between 10.54 and 12.61 mM from June to September. Between September and November, the Pi contents of all treatments increased, and that of T3 was the highest with 30.59 mM followed by that of T2 and T1, respectively, in November. Then, the Pi contents under all treatments, however, decreased again in December.

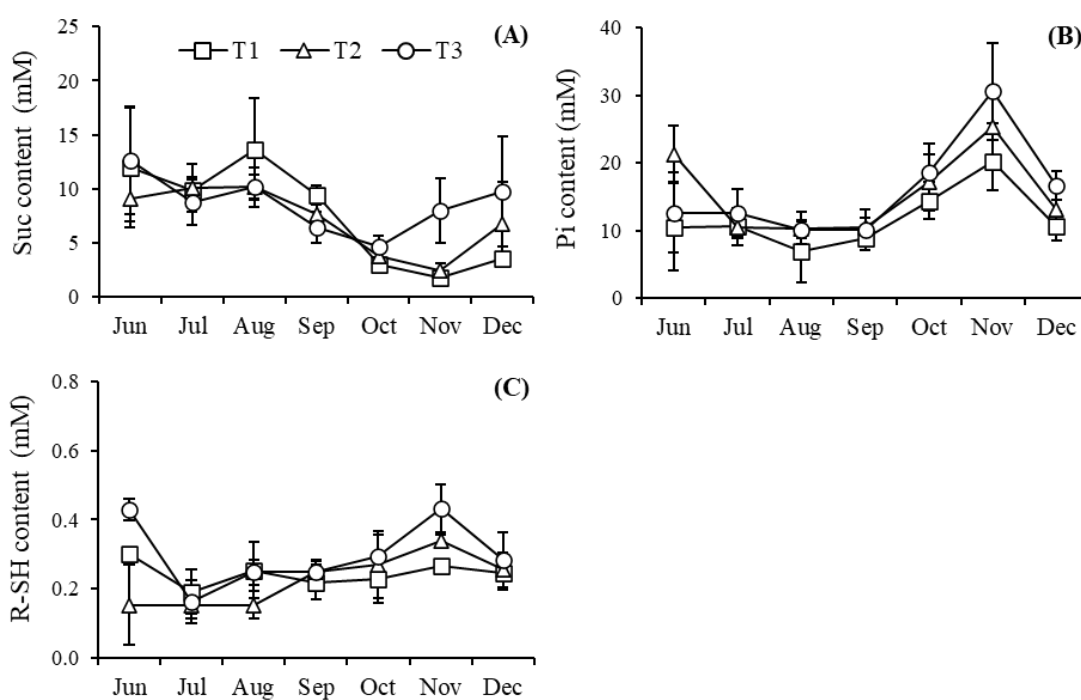


Figure 2.8. Monthly changes in biochemical composition (A) sucrose – Suc content; (B) inorganic phosphorus – Pi content; (C) thiols – R-SH content of latex under the treatments (from June to December 2016)

R-SH levels of the treatments were different in June as that of T3 was at 0.43 mM as the highest, followed by T1 and T2 with 0.30 and 0.15 mM, respectively (Figure 2.7 C). After July, however, all treatments increased slightly until November,

and the R-SH level under T3 was the highest in November. Then in December, the R-SH level of all treatments declined under 0.30 mM.

2.3.7. Salacca palm production

Table 2.2. Production of the salacca palms among the treatments

Treatment	Yields of the salacca palm	
	kg cluster ⁻¹	kg palm ⁻¹
T1	0.77 ± 0.05 c	2.50 ± 0.89 c
T2	1.60 ± 0.09 a	6.13 ± 1.10 a
T3	1.33 ± 0.21 ab	4.38 ± 1.50 b

The salacca productions were significantly different among the treatments in terms of yield per cluster and total yield per palm (Table 2.2) as T2 delivered the highest weight with 1.60 kg cluster⁻¹ followed by T3 with 1.33 kg cluster⁻¹ while that of T1 was the lowest at 0.77 kg cluster⁻¹. Likewise, the total yields (kg palm⁻¹) of T2 and T3 were 145% and 72%, respectively higher than that of T1.

2.4. Discussion

2.4.1. Soil fertility improvement

The study observed that the plot amended with the CSA had a maximum level of the SOM content in the topsoil layer. The result was likely due to enzymatic soil microbial activities improved by the CSA, enhancing the decomposition process of organic materials in the topsoil layer (Kong et al., 2010). Besides, the soil microbial population increased and decomposed themselves, resulting in a higher level of organic matter in the soil. The higher content of SOM is an indicator of healthy soil with efficient infiltration and water-holding capacity, thus higher nutrient availability (Chen et al., 2017; Nannipieri et al., 2017).

2.4.2. Development of the fine root traits

It was noticed that the FRD of both crops under T1 showed a larger size in both soil layers in general. It signaled high limitation in the movements of water and nutrients from the soil to the roots resulting in low vegetative growth and productivity

(Comas et al., 2013). Conversely, roots with smaller diameters have greater hydraulic conductivity and tolerant to drought conditions (Henry et al., 2012). The small diameters of the fine roots under T2 and T3 reflected the better performance of the root functions because of the higher availability of nutrients and water in the soil under the organic soil amendment application (du Jardin, 2015).

In all treatments, the FRLD of rubber trees in the soil depth of 21-40 cm showed upward trends once the rainy season began, but it did not change a significant difference in the soil depth of 0-20 cm. These indications mean that the development of rubber fine roots in the soil depth of 21-40 cm was more responsive to the rainfall than that of the topsoil layer. A study conducted in the same province by Saelim et al. (2019) also found that the fine roots of the 16-year-old rubber, particularly in the soil depth 20-30 cm developed at a higher rate in the rainy season. The result was consistent with the finding of Maeght et al. (2015) in north-eastern Thailand that the rubber fine roots within the soil depth of 2 m exhibited higher root emergences during the rainy season. Among the treatments, the rubber trees treated with the HAS showed higher FRLD in the soil depth of 21-40 cm from July to October. Wasson et al. (2012) remarked that a root system that has a greater FRLD in deeper soil could uptake water and nutrients at high efficiency. Cahyo et al. (2014) reported that root growth and performance were more obvious than other vegetative parts under the HSA. It could serve as an auxin and promote cell enlargement by stimulating the cell wall loosening leading greater vegetative growth (Muscolo et al., 1999). However, it was noticed that the FRLDs of the salacca palm were higher under the CSA in the soil depth of 21-40 cm. CSA could enhance cation properties and water holding capacity in the soil, thereby more significant development of fine roots resulting in better nutrient uptakes and improved crop yield (Sharp, 2013).

2.4.3. The vegetative growth and production of the crops

The study observed that there was a positive relationship between the LAIs and latex production under all treatments. The latex productions under all treatments were at maximum levels in September, while leaves in the rubber canopy reached the ultimate growth stage. Since the planted cultivar, RRIM 600 clone, is susceptible to *Phytophthora* leaf fall disease (Krishnan et al., 2019), which occurs

typically during the rainy season, the rubber trees in the farm were attacked by the disease, thus less values of LAI in November. In the meantime, it was observed that the latex yields under all treatments dropped from their maximum yields. Leaf area is a functional part of a tree's photosynthesis and determines photosynthetic efficiency, which reflects sucrose synthesis (Campbell and Norman, 1989; Lambers et al., 2008). Since natural rubber is a photosynthesis product of *Hevea brasiliensis* through sucrose synthesis in non-photosynthesis laticiferous tissue, the leaf area of rubber tree influences latex yield and dry mass production of rubber (Righi and Bernardes, 2008; Zhu et al., 2018).

Regarding the salacca production, the treatments of the HSA delivered significantly higher yields compared to that of the other treatments. It was contributed by the beneficial effects of organic soil amendment and fertilization that enhances the chemical properties and nutrients in the soil essentially required for plant's vegetative growth (Angelova et al., 2013). The organic fertilizer and organic soil amendments could promote inorganic fertilization effectiveness, thereby more extended availability of nutrients in the soil (Wu et al., 2020). It could improve the soil's physical properties such as cation exchange capacity and water holding capacity (Tejeda and Gonzaler, 2009), enhancing root proliferation and the root system's nutrient uptake functions, resulting in higher crop yield (Sharp, 2013; Khan et al., 2017).

In addition, it was noticed that yields per cluster in all treatments were apparently higher than the average yield of around 0.6 kg per cluster of conventional salacca-fruit intercropping (Sumantra and Martiningsih, 2018). In rubber-based intercropping, the canopy of mature rubber tree reduces extreme temperature and intense irradiance, improving the adaptability of understorey plants especially shade-required species like salacca palm (Montagnini, 2011). Along with the favorable weather conditions, the co-existence of the different canopy architectures like the combination of rubber trees and salacca palms, enhancing light interception and distribution in the farm contributes to a greater photosynthetic rate resulting in yield improvement of the crops (Sumantra et al., 2012; Xianhai et al., 2012; Tang et al., 2019).

2.4.4. Less physiological stress of the rubber tree

It is noticed that all treatments showed higher Suc content, lower Pi content, and lower yields at the beginning of the rainy season after the dry season. It reflected low metabolic utilization or insufficient conversion of sucrose into cis-isoprene rubber molecules in the latex resulting in higher Suc content remaining and fewer rubber particles in the latex (Jacob et al., 1989). Then, in September and October, the yields of all treatments were at a high level with an elevation of the Pi contents. It indicated the high metabolism of the laticiferous contributed by the regular tapping activity (Jacob et al., 1989). However, in November, the Suc contents under T1 and T2 declined to the lowest level, and their productions also plunged to less than 30 g tap⁻¹ tree⁻¹ at that month, reflecting that the rubber trees were exhausted with the shortage of sucrose supply due to the occurrences of the abnormal leaf fall disease. The abnormal leaf fall disease destructed the photosynthesis functions, thereby reducing the Suc's sufficient supply, resulting in the yield drop. However, the Suc content, the Pi content, and the R-SH content under T3 were at a high level, and the yield in T3 remained over 30 g tap⁻¹ tree⁻¹ and was not as low as that of the others. These indications reflected less physiological stress of the laticiferous system (d'Auzac et al., 1997) and the lesser effect of the *Phytophthora* leaf disease attack under T3 compared to those of the other treatments. It was likely to be the CSA's antimicrobial effect since its application enhances the plant's immune system leading to restrain and slow down the growth of the pathogen (Sunpapao and Pornsuriya, 2014).

2.5. Conclusions

The study observed that both HSA and CSA treatments improved the fine root trait developments of the crops, particularly in the soil depths of 21-40 cm. The fine rubber roots were responsive under the HSA, while the fine root growths of the salacca showed more significance under the CSA. It was found that a positive correlation between the average yields of rubber and the LAI in the farm. The study highlighted that the advantages of CSA on rubber trees that its application improved the tree physiological status. Thus, the latex biochemical composition levels and the daily yield were maintained under the CSA application during the intensive latex

harvest practices and the phytophthora leaf disease attack. A significant increase in soil organic matter under the CSA treatment was also advantageous.

The higher yields per cluster of salacca trees in all treatments compared to other conventional salacca farms indicated the beneficial effect of the rubber-salacca combination. In addition, the significantly higher yields of salacca under the HAS and CSA further approved the effect of the integrated fertilizations.

The study highlighted the complementarity effect resulting from harmonious interactions between the integrated fertilization and agroecosystem components of the rubber-salacca intercropping. Therefore, it is suggested that the mixed organic-inorganic fertilization with organic soil amendments could be utilized in rubber-based intercropping as effectively integrated fertilization to reduce the usage of chemical fertilizer without affecting the crop yields.

CHAPTER III

EXPERIMENT II

Variation in latex production and technological properties of *Hevea* rubber in relation to seasonal ecophysiological changes under different rubber-based intercropping practices

3.1. Introduction

Natural rubber, a biosynthetic polymer, commercially sourced from *Hevea* rubber tree (*Hevea brasiliensis*), is a strategically indispensable commodity for manufacturing a wide range of rubber-based products due to its unique features mainly elasticity, durability, flexibility, adhesive strength and thermal resilience. These features are the contributions of *Hevea* rubber's inherent technological properties, possessing outstanding rheological and mechanical properties, and processability (Malmonge et al., 2009; Rippel and Galembeck, 2009).

Unlike other crops, *Hevea* rubber tree has been primarily harvested for latex, a milky cytoplasm in which rubber particles are the main constituent existing together with water, proteins, sucrose, lipids, inorganic ions, and enzymes, which is basically a secondary metabolite synthesized through the isoprene biosynthesis pathway in the plant defensive mechanism, originated from sucrose by plant photosynthesis (d'Auzac and Jacob, 1989; Konno, 2011; Zhu et al., 2018).

Since leaf area, representing the above-ground biomass production of farming land, is functionally associated with photosynthesis capacity (Weraduwege et al., 2015), the variation in leaf area of a rubber farm strongly influences the isoprene biosynthesis of the rubber trees (Zhu et al., 2018). In addition, leaf area development on rubber farm improves the understory microclimate condition, governing the evapotranspiration of the farm linked with below-ground water availability (Ayutthaya et al., 2011; Giambelluca et al., 2016). However, the leaf area of *Hevea* rubber tree typically reduces in the dry season when the soil water deficit is severe, and the tree limits the water and nutrients translocations due to its deciduous nature (Premakumari and Saraswathyamma, 2000), affecting the isoprene biosynthesis process and latex biochemical composition (Jacob et al., 1989). It has been reported that normal yield depression and high variation in technological properties were observed in the deciduous period (Moreno et al., 2005; Giraldo-Vasquez and Velasquez-Restrepo, 2017). Conversely, when the soil water availability is sufficient, that improves the tree's physiological status with efficient utilization of sucrose and nutrients in the latex metabolism, resulting in higher yield potential (Roux et al., 2000). Thus, the seasonal changes in leaf area and soil water content of the *Hevea* rubber farm as the above- and

below-ground factors greatly implicate the physiological status and yield potential of the rubber tree (Sumit et al., 2013; Forrester, 2015).

In addition, the *Hevea* rubber molecular characteristics, the core determinants of the technological properties (Kovuttikulrangsie and Sakdapipanich, 2005), are related to the sucrose and nutrient supplies to the isoprene biosynthesis pathway (d'Auzac et al., 1997), and these supplies depend on the water availability (Roux et al., 2000; Liu, 2016). As the irreplaceable natural features, the molecular characteristics of *Hevea* rubber are far superior to that of other rubber-producing plants and synthetic polymers (Swanson and Buchanan, 1979; Malmonge et al., 2009).

The combination of its irreplaceable features and technological advances has led to the widespread development of rubber-based products and increasing market demand in line with the world's growing population. Due to the strong market demand, *Hevea* rubber planting has extensively grown by massively monocropping in tropical regions especially in developing countries, of which the majority are in the Southeast Asian countries, and has become a smallholder crop that generates major incomes for millions of farmers (Langenberger et al., 2017).

However, the extensive monocropping and replanting of the same perennial crop over a long period have accumulated significant adverse impacts in terms of ecological and socio-economic concerns (Xu et al., 2014; Warren-Thomas et al., 2015). The issues have aggravated in the last ten years with the price volatility and, affected the major incomes of rubber farmers.

Hence, some farmers have started rubber-based intercropping, focusing mainly on widening the income sources from the rubber farm rather than environmental benefits (Hougni et al., 2018; Romyen et al., 2018). Many studies confirmed that rubber-based intercropping or agroforestry enhanced not only the economic benefits but also the agroecosystem sustainability with improvements in microclimate and soil conditions of the farms, thus tree's vegetative developments and physiological status as well (Werner et al., 2006; Jongrungrat et al., 2014; Langerberger et al., 2017; Chen et al., 2019).

Thus, it is necessary to understand the implications of these ecophysiological changes on the isoprene biosynthesis and inherent technological properties of *Hevea* rubber to ensure the sustainable valued chain of the natural rubber production integrated with the rubber-based intercropping practices. The study aimed to investigate the seasonal changes in leaf area coverage and soil water content of different rubber-based intercropping farms compared with those of rubber monocropping, and their interrelations with the latex biochemical compositions, latex yield, and technological properties of *Hevea* rubber.

3.2. Materials and methodology

3.2.1. Study location and planting materials

The study selected three rubber-based intercropping farms: rubber-bamboo (*Gigantochloa nigrociliata*) (RB); rubber-melinjo (*Gnetum gnemon*) (RM); rubber-coffee (*Coffea canephora*) (RC) and one conventional rubber monocropping farm (RR) located at 6° 59' N, 100° 08' E in Khao Phra village, Rattaphum district, Songkhla province, one of the major traditional rubber growing areas in Southern Thailand. Generally, in the area, the rain distributes between May and December as the rainy season with an annual rainfall of about 2,000 mm. On average, around 200 mm monthly rainfall precipitates from May to August, followed by high monthly rainfall of around 500 mm from September to December. The dry season is usually from January to April (Climatological Group, 2015). During that period, between February and March, rubber trees normally shed senescent leaves due to their deciduous process.

The soil type of the study area belongs to the Tha Sae series, with a feature of good drainage and average permeability, and it is mainly recommended for planting rubber, horticulture and upland crops (Land Development Department, 2003).

All selected farms planted RRIM 600 rubber clone in monocropping in 2008 with the spacing of 6 m x 3 m on flat land and began the latex harvesting by implementing a tapping system of S/3 2d3 (two days of tapping frequency in three days with one-third spiral length of tapping cut) (Vijayakumar et al., 2009) since 2014. The intercropping farms started planting the associated crops in the interrow space of the

rubber trees in 2014. All selected farms applied compound chemical fertilizer (30-5-18) at a rate of 0.50 to 0.75 kg per rubber tree in May 2020 before the rainy season.

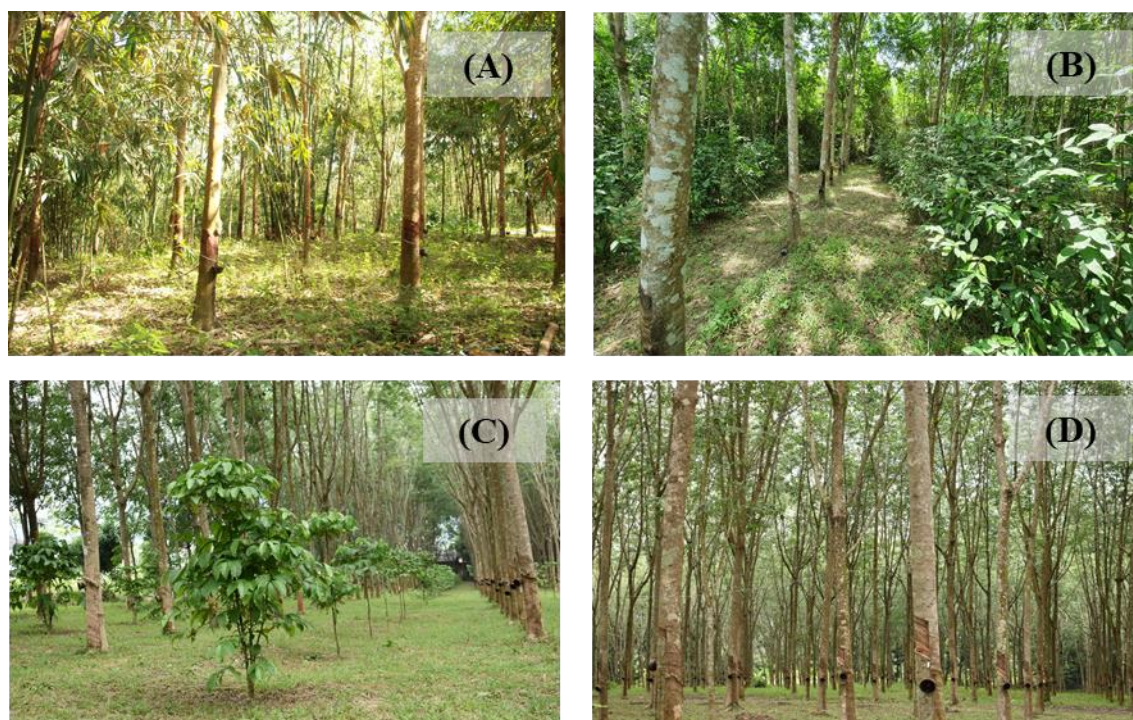


Figure 3.1. The selected farms: (A) rubber-bamboo intercropping; (B) rubber-melinjo intercropping; (C) rubber-coffee intercropping; (D) rubber monocropping

3.2.2. Weather data

A mini weather station (WatchDog 2700 Weather Station, Spectrum Technologies, USA) was set up in the village to monitor weather conditions such as temperature (°C), relative humidity (%RH), total rainfall (mm) and rainy days in the study area. To measure specifically the temperature and humidity of each farm, a data logger – CEM DT-172 (Shenzhen Everbest Machinery, China) was equipped in the middle of each farm.

3.2.3. Soil moisture content

Soil moisture content (SMC) of each farm at the soil depths of 0-10 cm, 11-20 cm, 21-30 cm, 31-40 cm and 41-60 cm, respectively, were measured every season in volume percent using the PR2/6 profile probe (Delta-T Devices, UK) through six access tubes installed at a 1.5-meter distance far from the rubber row in each farm.

3.2.4. Leaf area index

Leaf area index (LAI) of each farm was measured seasonally with the hemispherical photography method by taking fisheye photos at the height of 1.5 meters from the ground with a vertically upward position adjusted on the north pole compass (Bianchi et al., 2017) using Nikon Coolpix 8400 camera (Nikon, Japan) from randomly selected five different points in the interrow space of the farm. Then, the fisheye photos were processed using the Gap Light Analyser software version 2.0 (Simon Fraser University, Canada) for the LAI analysis.

3.2.5. Latex biochemical diagnosis

Latex micro diagnosis was carried out with three replicates of sampling every four months at each farm. The latex samples were taken from randomly selected ten trees in each replicated plot in the early morning before the normal tapping was carried out, by puncturing the bark at around 5 cm below the tapping cut and then, after discarding the first two to three latex drops, the flowing out latex (around ten drops) was collected and kept under 4 °C to ensure no more extended metabolism. 1 ml of the sample latex was added into 9 ml of trichloroacetic acid – TCA solution (TCA 2.5% w/v mixed with ethylene-diamine-tetra acetic acid – EDTA 0.01% w/v) to separate the serum from the sample for the analysis of biochemical composition: contents of sucrose (Suc); inorganic phosphorus (Pi); reduced thiols (R-SH), following the latex diagnosis method of CIRAD (Jacob et al., 1985), compiled from the anthrone method (Ashwell, 1957), the molybdate ammonium method (Tausky et al., 1953), and the dithiobis nitrobenzoic method (Boyne and Ellman, 1972).

3.2.6. Latex production analysis

Latex production in dry rubber weight per tree per tapping ($\text{g tree}^{-1} \text{ tap}^{-1}$) of each farm was calculated by measuring the latex weight and average dry rubber content of randomly selected ten trees by three replications. From each sample, firstly, 10 g of latex was taken to determine the total solids content (TSC) through oven drying at $70 \text{ }^\circ\text{C} \pm 5 \text{ }^\circ\text{C}$ for 16 hours in accordance with the ISO 124:2014. For determination of the dry rubber content (DRC), 10 g of sample latex from each was coagulated with

a 2% acetic acid solution, and then the coagulum was pressed followed by drying at 70 °C \pm 5° C for 16 hours in an oven, following the ISO 126:2005.

3.2.7. Technological properties analysis

For determination of the technological properties, the fresh latices were sampled from each replicated plot and kept under 4°C. Then each sample was split into two parts for measuring dry rubber properties and molecular characteristics.

The latex from the former part was coagulated with formic acid (3%) and the coagulum was sheeted, followed by oven-drying at 70°C for 24 hours. The dry rubber sheets were then measured the dry rubber properties notably ash content (%), nitrogen (N) content (%), initial plasticity (P_0), plasticity retention index (PRI) and Mooney viscosity (MV) according to the RRIM test methods for Technically Specified Rubber (RRIM, 2018). Magnesium (Mg) content (ppm) was determined using the serum 10 mL obtained from the coagulation, following the method of ISO 11852:2017.

Molecular weight characteristics of rubber particles namely weight average molecular weight (Mw) and molecular weight distribution (MwD) were investigated from the latter part using the gel permeation chromatography (GPC) technique at the rubber quality testing laboratory of Sino-Thai International Rubber College. After the sample latex was precipitated in cold methanol and oven-dried at 70 °C for 24 hr, the dry rubber of 0.015 g was dissolved in 10 mL of tetrahydrofuran (THF) eluent. Then the rubber solution was filtered using a syringe filter with a pore size of 0.45 μ m. The sample was injected into the two Agilent GPC/SEC columns (PLgel 10 μ m MIXED-B, 7.5 x 300 mm) to process in a refractive index detector, the Agilent PL-GPC 220 integrated GPC/SEC System (Agilent Technologies, USA) at a constant flow rate of 1 mL min⁻¹ at 40 °C. The Agilent GPC/SEC software was performed for the calibration curve and the determinations of the average molecular weight and the molecular weight distribution (polydispersity index) (Agilent Technologies, 2015).

3.2.8. Data collection and analysis

The data collections were carried out from September 2020 until August 2021, in which three seasonal periods were split based on the climatic pattern of the area: Season 1 (S1) (September to December 2020), Season 2 (S2) (January to April

2021) and Season 3 (S3) (May to August 2021). The overall significance data were analyzed with a two-way analysis of variance (ANOVA). In comparing the significant data among the farms, Duncan's multiple range test was performed at $p < 0.05$. The significant correlations among the data were identified using Pearson's linear correlation analysis. IBM SPSS Statistics (Version 23.0) analytics software was performed for the data analysis processes.

3.3. Results

3.3.1. Agroclimatic condition

During the study period, from September 2020 to August 2021, the area experienced 178 raining days with a total rainfall of 2,561 mm, of which 45.67%, 10.42%, and 43.91% were distributed in S1, S2, and S3, respectively (Figure 3.1 A). S1 was the highest rainfall period with 80 raining days, distributed with an average monthly rainfall of 292 mm and received a peak of 425 mm in October. During S2, a dry period, there were only 31 rainy days with an average monthly rainfall of 66 mm. Then, the rainy season began in S3 and received an average monthly rainfall of 240 mm with 67 raining days in total.

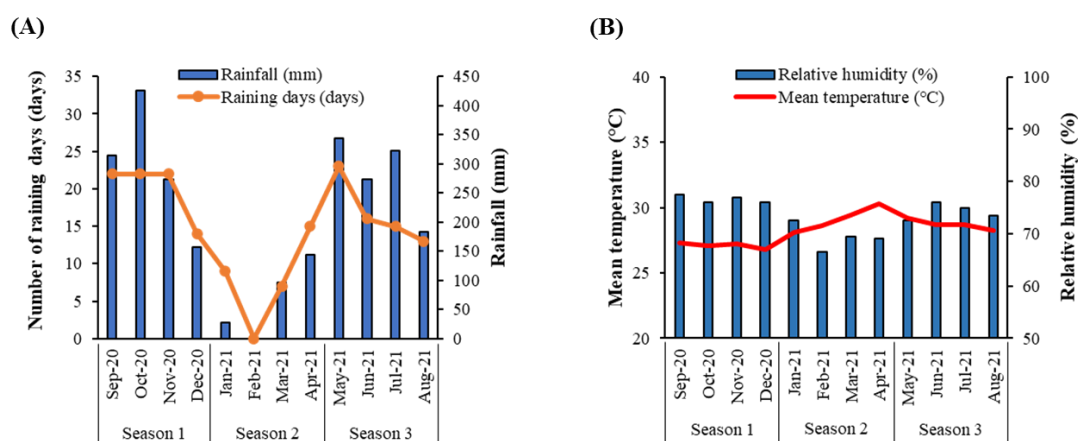


Figure 3.2. Monthly weather conditions of the study area from September 2020 to August 2021: (A) monthly rainfall and raining days; (B) monthly relative humidity and mean temperature.

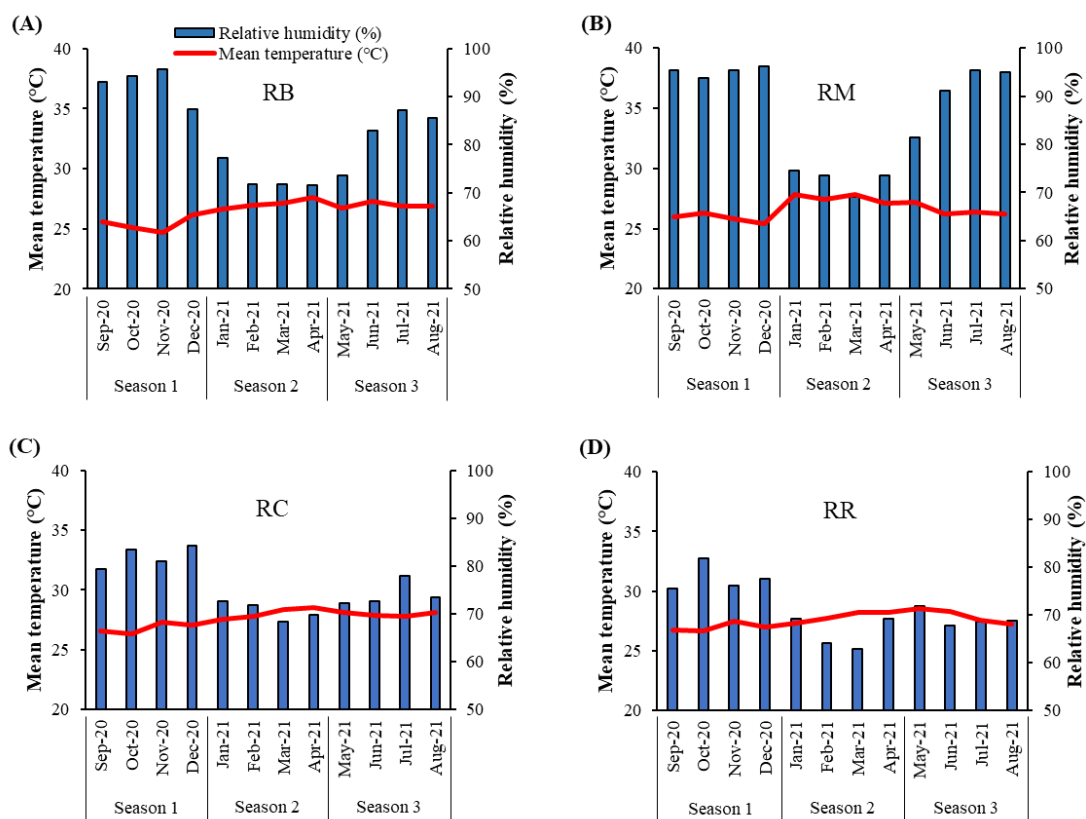


Figure 3.3. Monthly relative humidity and mean temperature in the studied farms: (A) Rubber-bamboo intercropping – RB; (B) Rubber-melinjo intercropping – RM; (C) Rubber-coffee intercropping – RC; (D) Rubber-monocropping – RR, from September 2020 to August 2021.

In the area, the mean temperature and relative humidity during S1 were 27 °C and 77%, respectively. However, the mean temperature increased to 30 °C with a minimum relative humidity of 67% in S2. Then, in S3, the mean temperature slightly dropped to 29 °C, while the relative humidity rose to 74% (Figure 3.1 B).

Among the rubber-based intercropping farms, the mean relative humidity of RB and RM over the study period were higher than that of RR by 14% and 18%, respectively, whereas RC had a mere 6% higher than RR (Figure 3.2). However, regarding the mean temperature during the study, RB and RM maintained only 4% less than RR, while RC had the same mean temperature as RR. During S1, the relative humidity in RB and RM were at the highest levels with 93% and 95%, respectively, followed by RC with 82%, while RR was the lowest at 78%. Regarding the mean

temperature of each farm in that season, RB and RM were at 25 °C and 26 °C, while RC and RR were at 27 °C on average, respectively.

During the dry season, S2, the temperature peaked in all studied farms, with the highest in RR and RC at 28 °C, and RB and RM had 27 °C and 27.5 °C, respectively. The relative humidity in all farms decreased to their minimums during that season. It was observed that the intercropping farms, RB, RM, and RC, maintained their RHs between 73% and 70%, respectively, while the monocropping RR dropped apparently to 66%. In S3, the average temperatures in all fields did not change significantly and remained the same as those in S2.

However, it was observed that the relative humidity in all intercropping farms, RB, RM and RC, surged apparently to 82%, 90% and 74%, respectively, in S3, while that of RR could merely increase to 69%, which was the lowest among the farms.

3.3.2. Leaf area index

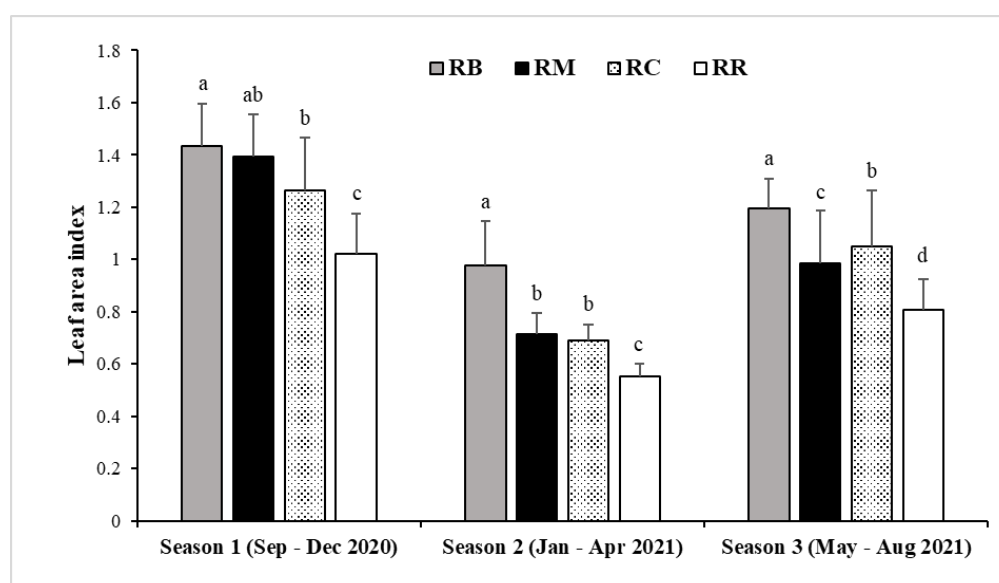


Figure 3.4. Leaf area index of the studied farms in season 1, season 2 and season 3.

Different letters above the bars in each season indicate significantly different at $p < 0.05$, tested by Duncan's multiple range test.

All intercropping farms exhibited significantly higher LAI values on average over the study period at 1.2, 1.05 and 0.99, respectively, while RR had a low

LAI value of 0.79. The LAI values of all farms in S1 were the highest compared to those of the other seasons. Compared to S1, the LAI values in S2 decreased by around 45% to 49% in RM, RC and RR, respectively, but only 32% in RB. After the dry season, during S3, all intercropping farms increased the leaf area and their LAI values were above one. However, the LAI value of the monocropping farm was only 0.8, which was 32% to 30% less than that of the intercropping farms (Figure 3.3).

3.3.3. Soil moisture content

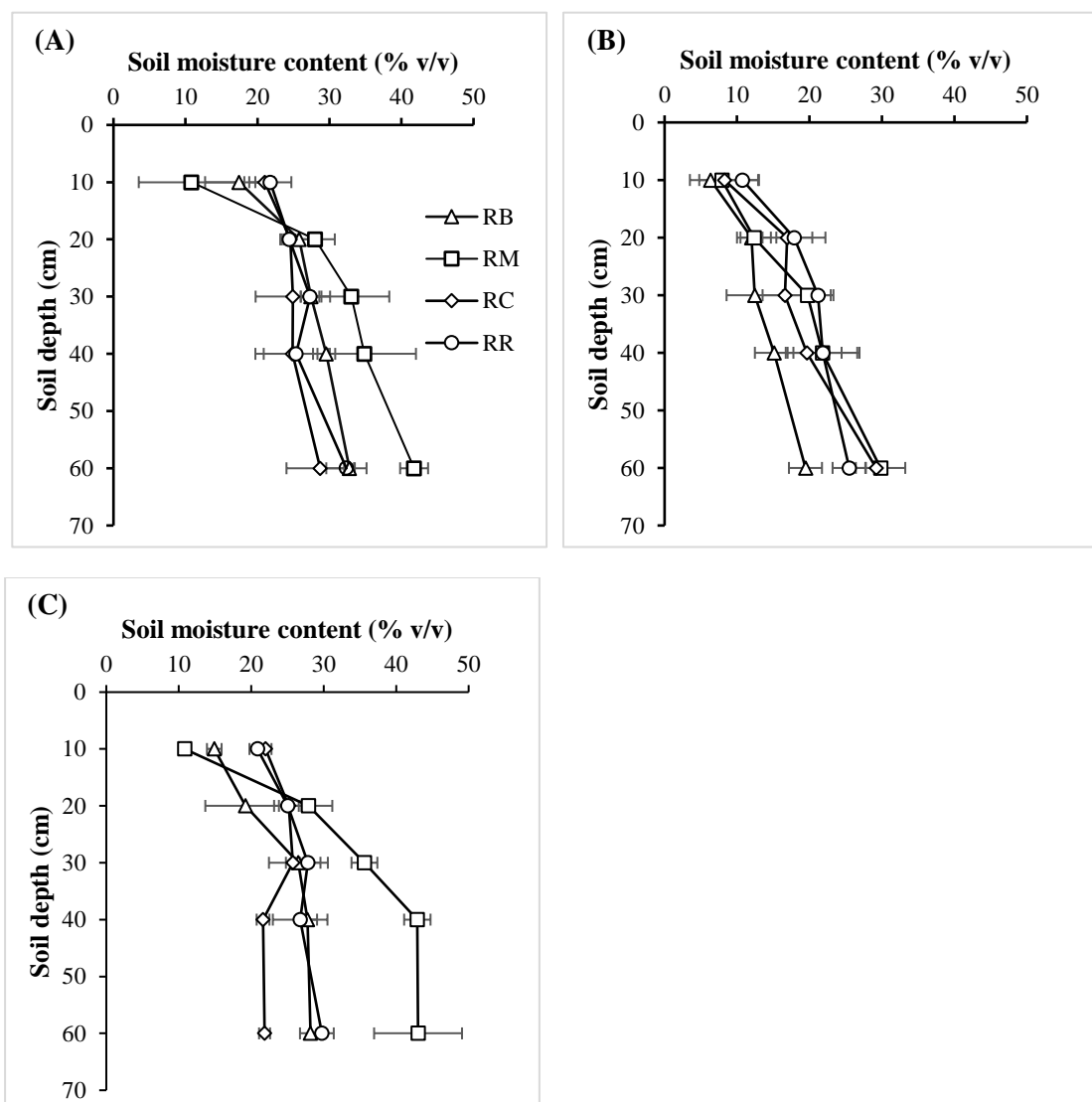


Figure 3.5. Dynamics of soil moisture content by soil depths in (A) season 1 (Sep – Dec 2020), (B) season 2 (Jan – Apr 2021), and (C) season 3 (May – Aug 2021) in the studied farms.

Increasing trends of the SMC by the soil depths were observed distinctly in all intercropping farms, but not significantly in the monocropping farm (Figure 3.4). However, the SMCs in the topsoil layer of RR were significantly higher than in the other farms. During S2, all farms decreased their SMCs apparently (Figure 3.4 B), in which RB farm showed the lowest SMC with an average value of 13.06%, whereas the SMCs in RM, RC and RR were 18.3%, 18.1% and 19.4%, respectively. Among the studied farms, RM had the highest SMC on average in all seasons, more evident in S1 and S3.

3.3.4. Latex biochemical composition

Table 3.1. Latex biochemical contents in the studied farms by the seasons

Latex biochemical contents	RB	RM	RC	RR	p-value
Season 1 (Sep – Dec 2020)					
Suc	1.93 b	2.07 b	3.71 a	4.87 a	0.004
Pi	16.08 b	31.49 a	12.22 c	12.16 c	<0.001
R-SH	0.76 a	0.80 a	0.67 b	0.62 b	0.002
Season 2 (Jan – Apr 2021)					
Suc	0.85	1.20	1.33	1.36	0.32
Pi	14.35	14.54	10.05	10.67	0.403
R-SH	0.57	0.65	0.53	0.48	0.146
Season 3 (May – Aug 2021)					
Suc	9.33 a	3.77 b	6.76 a	2.45 b	0.002
Pi	20.67	17.64	23.86	18.59	0.154
R-SH	0.59	0.57	0.56	0.57	0.959

The values represent the means of the tests and the different letters in each row (parameter) are significantly different at $p < 0.05$, processed by Duncan's multiple range test. ns = non significance. RB = rubber-bamboo intercropping; RM = rubber-melinjo intercropping; RC = rubber-coffee intercropping; RR = rubber monocropping.

In S1, the latex from RB and RM contained the lowest Suc, while RC and RR had the highest Suc content in the latex. Pi and R-SH in the latex of RM showed the highest followed by RB, whereas those of RC and RR were at the lowest levels, in S1 and S2 (Table 3.1). All latex biochemical compositions in the latex received from all farms dropped to their minimum values in S2. There were no statistically differences in Suc and Pi among the farms.

The Suc contents in S3 from all studied farms were higher than in S1 and S2. Among the intercropping farms, the sucrose content of RM was the lowest. However, the monocropping farm had the least Suc among the farms in S3, but higher than that of S2.

The Pi content increased to a high level in all farms in the S3. The study found there were no significant differences in Pi between the farms. Although the Pi content in the latex from the RR expressed higher in the S3 than in S1 and S2, it was at a lower level compared to that of the other farms in that season.

It was observed that the R-SHs of all intercropping farms were higher than that of the monocropping by 8% to 29% in S1, and 10% to 35% in S2, respectively. In S3, however, the R-SHs were not statistically different between the farms.

3.3.5. Latex production parameters

In comparing the latex production (daily yield) on average over the study period, RB and RM were the highest with 35 g tree⁻¹ tap⁻¹, followed by RC with 28 g tree⁻¹ tap⁻¹, whereas RR resulted in the lowest yield of 25 g tree⁻¹ tap⁻¹. It was noticed that in all farms, the seasonal trends of latex production were inversely related to the changes in TSC and DRC of the latex.

The latex productions of all farms in S2 were markedly lower than in the other seasons (Table 3.2). Conversely, their TSCs and DRCs expressed at higher levels in S2. In S3, although the productions increased back to over 35 g tree⁻¹ tap⁻¹ in RB and RM, the yields in RC and RR did not exceed 30 g tree⁻¹ tap⁻¹.

In S1 and S3, TSCs ranged between 31% and 42%, and DRC values were from 28% to 40%, respectively, in all farms. However, in S2, except RM, all farms

increased TSC and DRC to over 45% and 43%, respectively. TSC and DRC in RM were the lowest values of 41% and 38%, respectively, in that season.

Table 3.2. Latex production parameters of the studied farms by the seasons

Latex production parameters	RB	RM	RC	RR	p-value
Season 1 (Sep – Dec 2020)					
Daily yield (g tree ⁻¹ tap ⁻¹)	39.97 a	35.85 c	38.06 b	30.69 d	<0.001
TSC (%)	38.68 b	31.66 c	41.65 a	41.17 a	<0.001
DRC (%)	36.14 b	28.93 c	40.02 a	39.44 b	<0.001
Season 2 (Jan – Apr 2021)					
Daily yield (g tree ⁻¹ tap ⁻¹)	29.51 a	21.32 b	16.23 c	15.63 c	<0.001
TSC (%)	47.42 a	40.86 d	45.86 c	46.52 b	<0.001
DRC (%)	44.50 a	37.54 d	43.19 c	43.96 b	<0.001
Season 3 (May – Aug 2021)					
Daily yield (g tree ⁻¹ tap ⁻¹)	35.38 b	47.82 a	29.09 c	28.47 c	<0.001
TSC (%)	39.73 b	41.14 a	38.42 d	39.21 c	<0.001
DRC (%)	37.34 b	38.78 a	36.32 d	36.69 c	<0.001

The values represent the means of the tests and the different letters in each row (parameter) are significantly different at $p < 0.05$, processed by Duncan's multiple range test. RB = rubber-bamboo intercropping; RM = rubber-melinjo intercropping; RC = rubber-coffee intercropping; RR = rubber monocropping

3.3.6. Technological properties of *Hevea* rubber

3.6.1. Non-isoprene contents

In S1, the Mg contents in the latex of all farms were found at a normal level and ranged between 330 and 405 ppm. However, it was noticed that all farms

increased the latex Mg contents in S2. In that season, the latex in RB and RM remarkably increased the Mg contents to 660 and 742 ppm, respectively, while RC and RR had slight increases to a mere around 413 ppm. In S3, the Mg contents in the latex of RB and RC were over 600 ppm, while that of RM and RR contained 454 and 412 ppm, respectively (Table 3.3).

In comparing the ash contents, in S1, dry rubber from RC and RR had high contents with 0.44% and 0.42%, while RB and RM were at low levels with 0.31% and 0.32%, respectively. Compared between the seasons, the ash contents in S1 were lower than that of S2 and S3. In S2, RM showed the lowest ash content among the farms. However, in S3, the ash contents in RB, RM, and RR were notably high and more than 0.60%, while that of RC was the lowest at 0.47%.

In S1 and S2, the N contents in the dry rubbers from RB and RM were observed at high levels ranging between 0.34% and 0.39%, whereas that of RC and RR were stable at around 0.3%. Then, in S3, except RB, all farms increased the N content to around 0.38%.

3.6.2. Rheological properties

In S1, dry rubbers from all farms had P_0 ranging between 36 and 38, and their PRIs were over 95. MV of RB was the lowest with 59.65 ML (1+4) 100 °C among the farms in that season. It was noticed that during S2, although the PRI values decreased, the values of P_0 and MV became higher than those of S1, in all rubbers from the studied farms. Among the farms, RM exhibited the highest P_0 and MV at 62 and 90.7 ML (1+4) 100 °C, respectively, with the lowest PRI of 75 in that season. In S3, RB and RM had high P_0 at 39.75 and 43.75, and MV at 65.05 and 65.95 ML (1+4) 100 °C, respectively, while RC and RR were lower in P_0 and MV. In the meantime, the lowest PRI value of 92.4, was observed in the rubber of RR, whereas the intercropping farms, RB, RM, and RC, had the high PRI values of 93.67, 96.73, and 98.07, respectively.

Table 3.3. Seasonal variations in non-isoprene contents and rheological properties of rubber from the studied farms

Technological properties	RB	RM	RC	RR	p-value
Season 1 (Sep – Dec 2020)					
<i>Non-isoprene contents</i>					
Mg content (ppm)	333.67 b	404.33 a	380.67 ab	367.00 ab	0.093
Ash content (%)	0.31 d	0.32 c	0.44 a	0.42 b	<0.001
N content (%)	0.37 b	0.39 a	0.32 c	0.29 d	<0.001
<i>Rheological properties</i>					
P ₀	36.44 b	37.84 a	38.27 a	36.17 b	<0.001
PRI	95.97 c	98.13 a	98.63 a	97.11 b	0.001
Mooney viscosity	59.65 d	68.87 a	67.03 b	61.45 c	<0.001
Season 2 (Jan – Apr 2021)					
<i>Non-isoprene contents</i>					
Mg content (ppm)	660.07 b	741.65 a	412.68 c	413.38 c	<0.001
Ash content (%)	0.61 a	0.39 d	0.463 c	0.57 b	<0.001
N content (%)	0.38 a	0.34 b	0.3 c	0.32 b	<0.001
<i>Rheological properties</i>					
P ₀	44.67 c	62.00 a	47.67 b	49.00 b	<0.001
PRI	90.3 b	75 c	88.83 b	91.5 a	<0.001
Mooney viscosity	70.87 d	90.7 a	73.43 c	76.93 b	<0.001
Season 3 (May – Aug 2021)					
<i>Non-isoprene contents</i>					
Mg content (ppm)	617.46 a	454.10 b	603.77 a	411.81 c	<0.001
Ash content (%)	0.65 a	0.63 a	0.47 b	0.66 a	<0.001
N content (%)	0.35 b	0.37 a	0.38 a	0.38 a	0.004
<i>Rheological properties</i>					
P ₀	39.67 b	44.00 a	34.50 c	33.33 c	<0.001
PRI	93.67 b	96.73 a	98.07 a	92.43 b	<0.001
Mooney viscosity	64.97 b	65.83 a	54.3 c	51.20 d	<0.001

The values represent the means of the tests and the different letters in each row (parameter) are significantly different at $p < 0.05$, processed by Duncan's multiple range test. RB = rubber-bamboo intercropping; RM = rubber-melinjo intercropping; RC = rubber-coffee intercropping; RR = rubber monocropping

3.6.3. Molecular characteristics

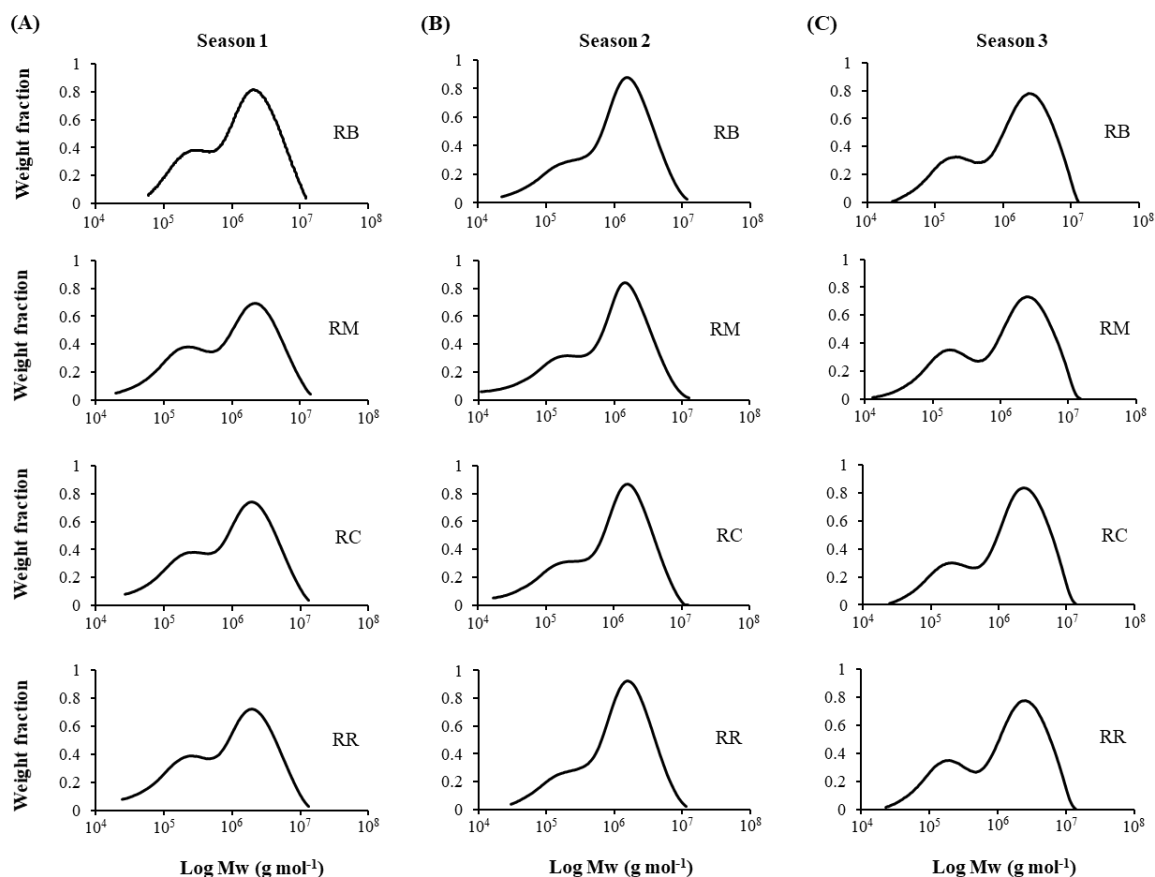


Figure 3.6. Molecular structure of isoprene in the rubbers from the studied farms in three seasons: (A) season 1 (Sep – Dec 2020); (B) season 2 (Jan – Apr 2021); (C) season 3 (May – Aug 2021).

All rubbers showed bimodal molecular weight distribution distinctly in S1 and S3 (Figure 3.5 A and Figure 3.5 C). However, they expressed relatively weaker bimodal distribution in S2 (Figure 3.5 B). In particular, the molecular weight distribution of RR was the narrowest in that season. It appeared to have a more extended deviation from the bimodal distribution with a shorter peak in the low molecular weight region and a higher peak in the high molecular weight region compared to those of the others.

In S1, Mw of rubber from RB was the highest with 21.02×10^5 g mol⁻¹. However, its MwD was at a minimum of 4.60, while that of RM had the highest MwD of 7.54, followed by RR and RC with 6.18 and 5.91, respectively (Table 3.4).

Table 3.4. Molecular weight averages and distributions by the seasons

	RB	RM	RC	RR	p-value
Season 1 (Sep – Dec 2020)					
Mw (g mol ⁻¹)	21.02 x 10 ⁵ a	19.43 x 10 ⁵ b	18.50 x 10 ⁵ c	18.12 x 10 ⁵ c	<0.001
MwD	4.60 c	7.54 a	5.93 b	6.19 b	0.001
Season 2 (Jan – Apr 2021)					
Mw (g mol ⁻¹)	15.37 x 10 ⁵	15.87 x 10 ⁵	16.12 x 10 ⁵	17.5 x 10 ⁵	0.101
MwD	4.41 c	6.71 a	5.65 b	3.94 c	<0.001
Season 3 (May – Aug 2021)					
Mw (g mol ⁻¹)	22.00 x 10 ⁵ b	22.12 x 10 ⁵ b	23.21 x 10 ⁵ a	21.88 x 10 ⁵ b	<0.001
MwD	6.18 ab	6.76 a	5.48 b	5.91 ab	0.043

The values represent the means of the tests and the different letters in each row (parameter) are significantly different at $p < 0.05$, processed by Duncan's multiple range test.

In S2, Mw of RB, RM and RC dropped markedly by 27%, 18% and 13%, but slightly decreased by 3% in RR. Compared to S1, all farms delivered lesser values of MwD in S2, in which RM was the highest at 6.71, followed by RC and RB with 5.68 and 4.41, respectively and RR showed the lowest value of 3.94.

Increases in Mw of rubbers from all farms were observed in S3, and RC was the highest Mw among the farms. RC had a maximum in Mn also, compared to that of the other farms. In terms of MwD, however, the rubber from RC was the lowest at 5.48, whereas that of RM delivered the highest at 6.76.

3.3.7. Interrelationships of the ecophysiological changes to latex production and technological properties of *Hevea* rubber

Regarding the agroecological interrelations, the variations in LAI (Figure 3.6 A) and SMC (Figure 3.6 B) under the different farms had the same relationships to the field temperature and the relative humidity. The correlation analysis indicated that the LAI had a greater influence than the SMC on the relative humidity with r-value of + 0.875 (Figure 3.6 A1) and the temperature with r-value of + 0.865 (Figure 3.6 A2), respectively.

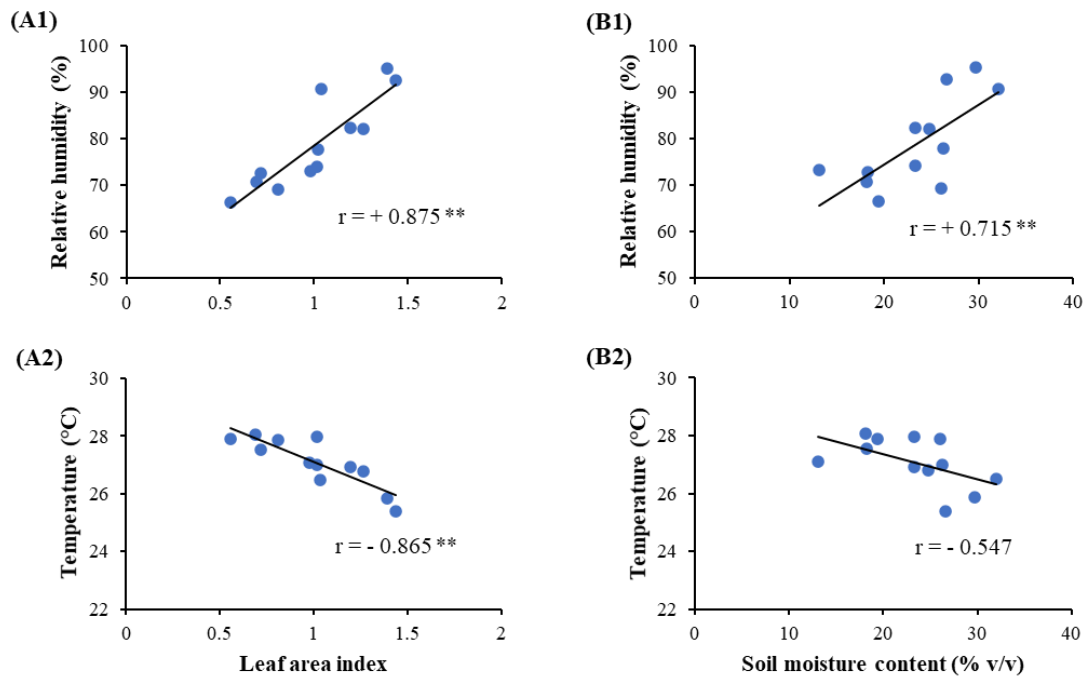


Figure 3.7. Relations of relative humidity and temperature with changes in (A) leaf area index and (B) soil moisture content. (r) represents the Pearson linear correlation coefficient. (**) indicates the significant correlation at $p < 0.01$.

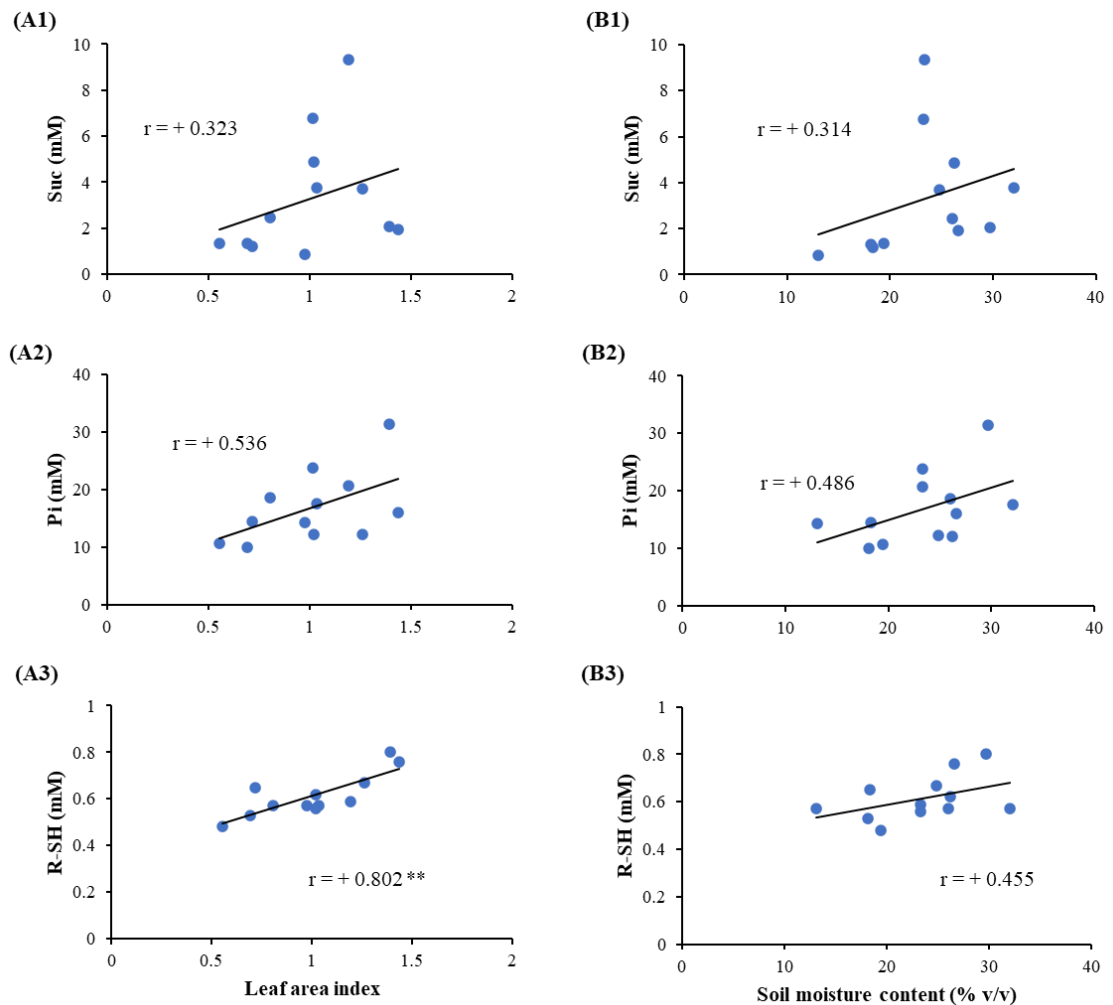


Figure 3.8. Relations of biochemical contents: sucrose – Suc; inorganic phosphorous – Pi; reduced thiols – R-SH with changes in (A) leaf area index and (B) soil moisture content. (r) represents the Pearson linear correlation coefficient. (**) indicates the significant correlation at $p < 0.01$.

In correlations to the variations in biochemical compositions (Suc, Pi, and R-SH), although both LAI (Figure 3.7 A) and SMC (Figure 3.7 B) showed positive associations with them, the variation in R-SH was highly associated with the LAI changes with the r-value of + 0.802 (Figure 2.7 A3) rather than the other relations.

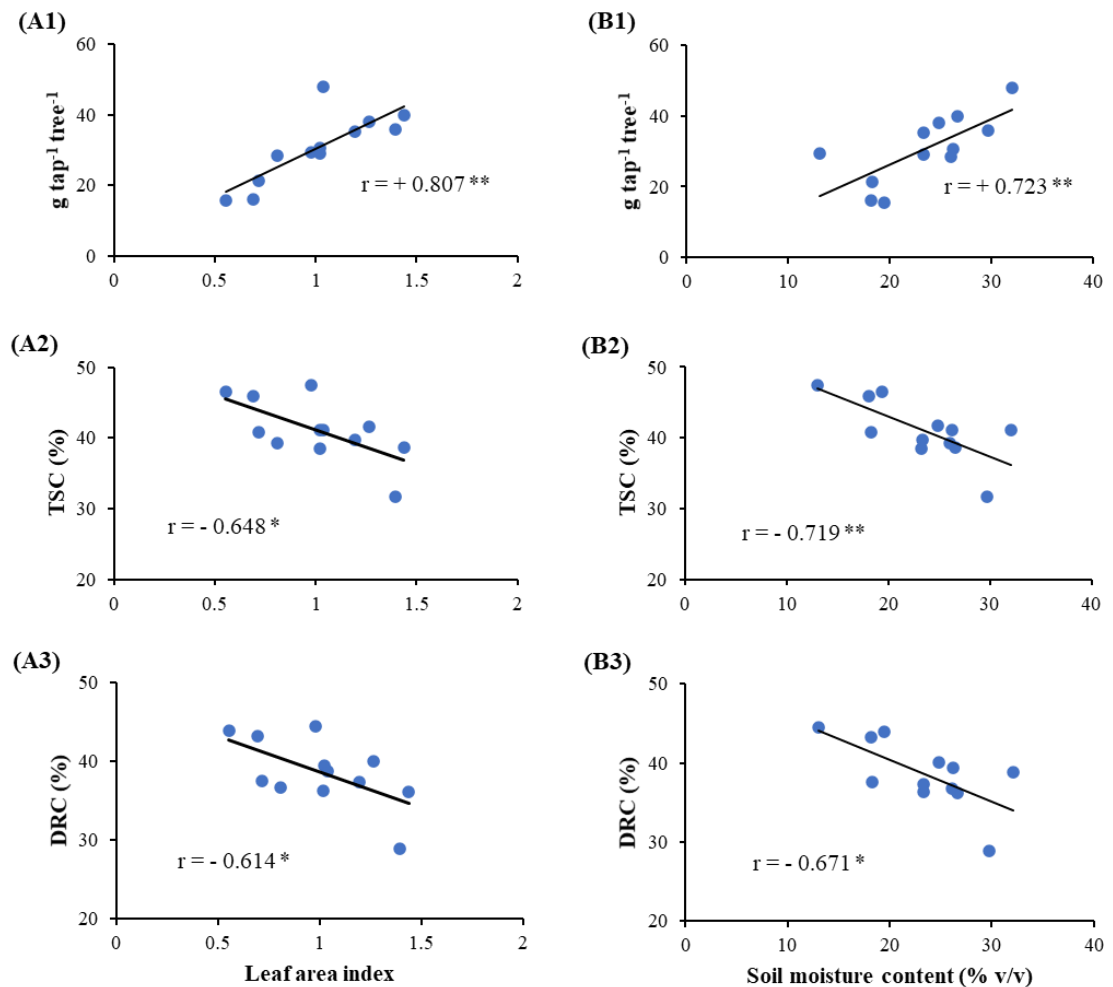


Figure 3.9. Relations of production parameters: dry rubber weight per tree per tapping – g tree⁻¹ tap⁻¹; total solids content – TSC; dry rubber content – DRC with changes in (A) leaf area index and (B) soil moisture content. (r) represents the Pearson linear correlation coefficient. (*) indicates the significant correlation at $p < 0.05$ and (**) indicates the significant correlation at $p < 0.01$.

The variations in latex production parameters were highly correlated to the changes in the LAI and SMC (Figure 3.8 A and Figure 3.8 B). It was found that the DRC and TSC had negative correlations with the LAI and SMC, while the daily productivity (g tree⁻¹ tap⁻¹) had positive relations.

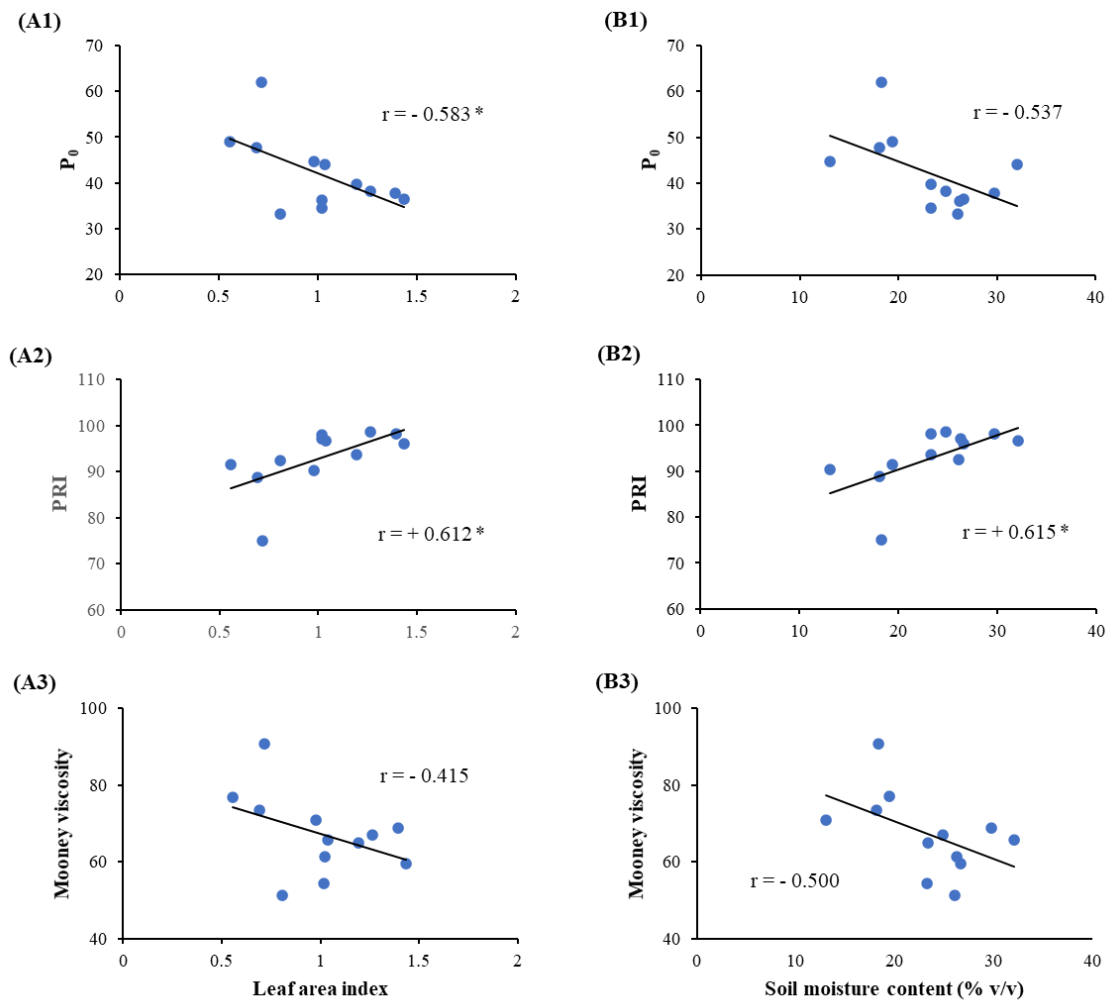


Figure 3.10. Relations of rheological parameters: initial plasticity – P_0 ; plasticity retention index – PRI; Mooney viscosity with changes in (A) leaf area index and (B) soil moisture content. (r) represents the Pearson linear correlation coefficient. (*) indicates the significant correlation at $p < 0.05$.

Regarding the implications to the rheological parameters, both LAI and SMC showed positive relations with PRI but negative to P_0 and MV (Figure 3.9 A and Figure 3.9 B). It was observed that variations in PRI were strongly related to the LAI and SMC. P_0 showed significant relation with LAI rather than SMC.

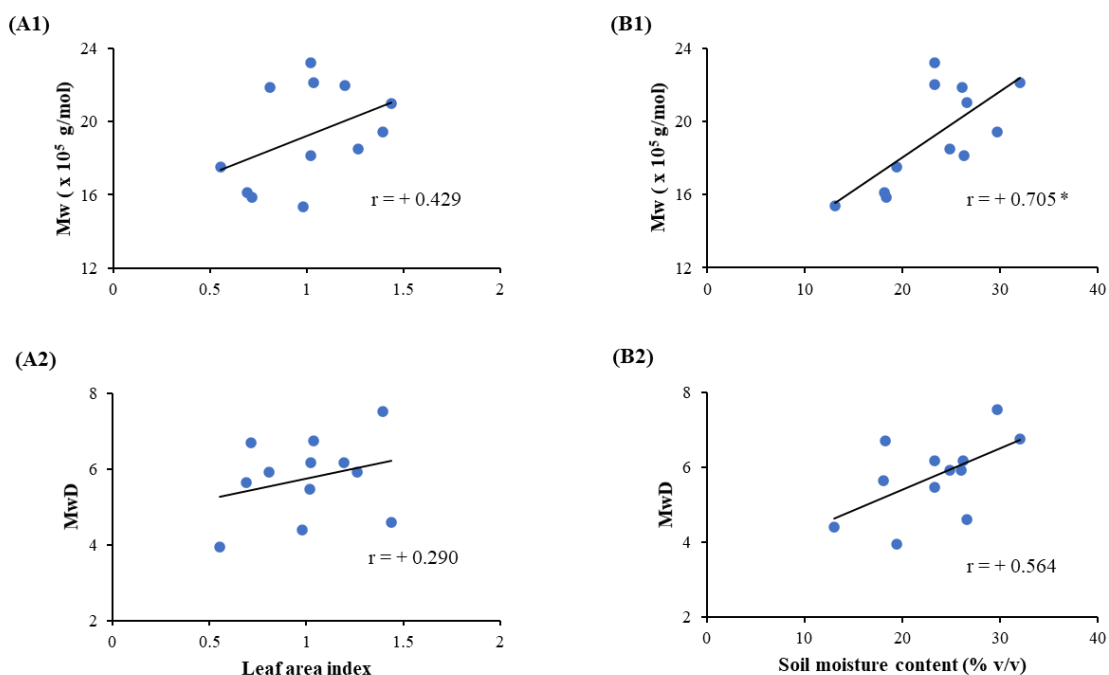


Figure 3.11. Relations of molecular weight structure: weight average molecular weight – Mw; molecular weight distribution – MwD with changes in (A) leaf area index and (B) soil moisture content. (r) represents the Pearson linear correlation coefficient. (*) indicates the significant correlation at $p < 0.05$.

In terms of molecular weight structure, the SMC exhibited greater relations with Mw and MwD with r-values of + 0.705 (Figure 3.10 B1) and + 0.564 (Figure 3.10 B3), respectively. The study found that Mn had no association with the LAI and SMC.

It was observed that the changes in Mg content and the biochemical composition influenced some critical parameters of technological properties and molecular weight structure. The variation in the Mg content in latex affected the changes of PRI with a negative relation at r-value of 0.617 (Figure 3.11 A). Similarly, a strong correlation with r-value of + 0.742 was found between the Pi and the N content (Figure 3.11 B). The increase in molecular weight distribution was associated with Pi and R-SH variations in the latex positively, and these relations had r-values of + 0.533 and + 0.445, respectively (Figure 3.11 C and Figure 3.11 F). The changes in the Suc content had high relation with Mw at r-value of +0.653 (Figure 3.11 E). Like that, variation in Pi influenced Mw with r-value of +0.561 (Figure 3.11 D).

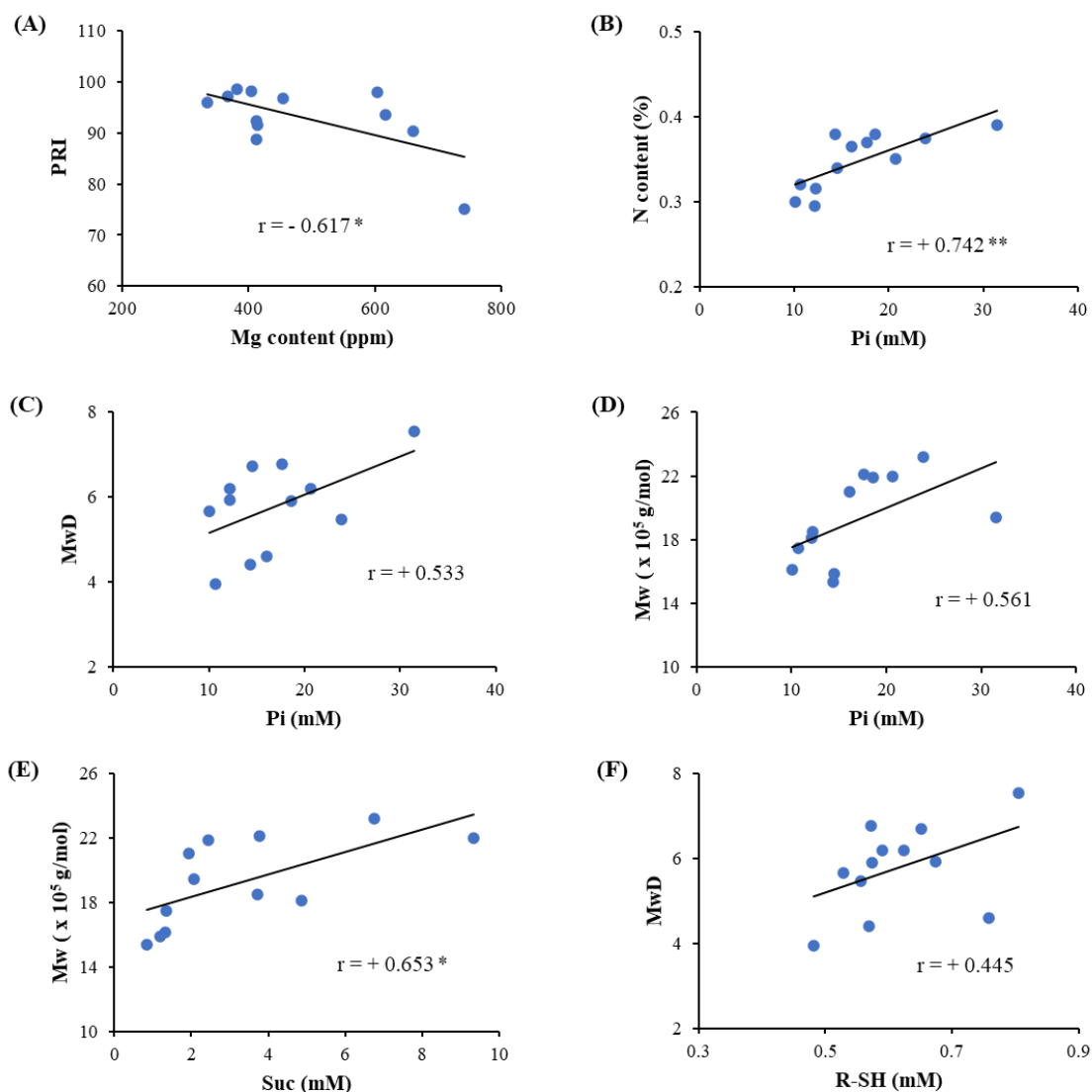


Figure 3.12. Relations between latex biochemical compositions and Hevea rubber technological properties: (A) magnesium – Mg content and plasticity retention index – PRI; (B) inorganic phosphorous – Pi content and nitrogen – N content; (C) inorganic phosphorous – Pi and molecular weight distribution – MwD; (D) inorganic phosphorous – Pi content and weight average molecular weight – Mw; (E) sucrose – Suc content and weight average molecular weight – Mw; (F) reduced thiols – R-SH content and molecular weight distribution – MwD. (r) represents the Pearson linear correlation coefficient. (*) indicates the significant correlation at $p < 0.05$ and (**) indicates the significant correlation at $p < 0.01$.

3.4. Discussion

3.4.1. Seasonal changes in agro-ecophysiological conditions

Throughout the study period, all intercropping farms could maintain lower temperatures with higher relative humidity in their farms in comparing with the surrounding area. In contrast, the monocropping farm had insignificant differences from the surrounding area. It was noticed that although the temperature difference was not markedly significant between the intercropping farms and the monocropping farm, the relative humidity in the latter was considerably lower than in the former in all seasons, reflecting that rather than the lesser temperature, the higher relative humidity in the farm was the significant effect of the rubber-based intercropping system.

Higher LAI values of the intercropping farms than the monocropping farm in all seasons indicated the significant coverage of the above-ground vegetation in these intercropping systems. The significant correlation results of the LAI associated with relative humidity and temperature approved that the better growth of the above-ground vegetation resulting from the rubber-based intercropping system improved the understorey environment of the farm by reducing the extreme weather conditions, especially in the dry season (Rappaport and Montagnini, 2014).

Higher SMC on average in the monocropping farm than in the intercrop farms expressed higher soil water consumption under these intercropping systems, indicating some degree of competition in soil water uptake between the rubber trees and the associated crops.

The increased SMC with the soil depth under the intercropping plots implied that the soil water competition was generally intense in the topsoil layers. It is likely because the root zones of the associated crops could not reach the relatively deeper soil layer where the rubber roots could access. Under tree-crop combination, the vertical root distribution of the associated crop, which planted after the primary tree, was generally highly concentrated in the topsoil layer, thereby the significant amount of soil water consumption in the shallow soil layer rather than the deeper layer (van Noordwijk et al., 1996; Schroth, 1999).

However, interestingly, the SMC trends by the soil depths exhibited the different degrees of soil water competition in the different associated crop combinations. In addition, with the significant associations of LAI and SMC with RH, the study exposed the different soil water utilization related to the above- and below-ground interactions that influenced the microclimate condition under the different rubber-based intercropping practices.

In the rubber-bamboo combination (RB), it was observed that the least SMCs were experienced in most soil depths, and more pronounced in the dry season, whereas on the above-ground, the LAI and RH were maintained at a high level in all seasons. It indicated the existence of a beneficial ecosystem in which the vegetative development was processed through efficient soil water uptakes and improved canopy transpiration. Bamboo species generally uptake more soil water with a high water storage mechanism to facilitate the leaf transpiration process, indicating high water use efficiency, especially when the above-ground water availability is constrained (Mei et al., 2016; Zhang et al., 2019). It implies the improvements in the canopy transpiration process with high water use efficiency, as the complementarity interactions among the above- and below-ground components (Forrester, 2015). Marshal et al. (2020) observed that the fibrous root system of bamboo species improved the water-stable aggregates and infiltration capacity of the soil, ensuring rainwater conservation and the soil hydraulic conductivity to access the soil water resulting in less vulnerability to the drought condition.

In the rubber-melinjo intercropping (RM), the SMCs were at the minimum level in the topsoil layer, albeit significantly higher in the subsoil layer, in all seasons. Meanwhile, its above-ground understorey environment maintained the improved ecosystem with relatively high LAI and RH in all seasons, as the rubber-bamboo combination did. Although the melinjo is originally a deep-rooted small- to medium-sized perennial tree with a height of around 15 meters (Orwa et al., 2009), in the rubber-melinjo combination, its height was maintained at about 1.5 to 2 meters for leaf harvesting by regular pruning under the rubber tree. Due to the regular leaf pruning throughout the year, the plant's assimilates were translocated for the above-ground shoot biomass rather than the root system, resulting in the reduction of root length

density and less root distribution in the deeper soil layer (Schroth and Zech, 1995; van Noordwijk and Purnomosidhi, 1995). In addition, due to the late planting of melinjo as an intercrop after the rubber tree's roots had occupied the most soil area, the successive crop had poor root traits and could not penetrate into deeper soil (Bauhus and Messier, 1999). Thereby, the melinjo's root proliferation was likely more pronounced in the topsoil layer leading to less competition for soil water uptake with the rubber tree's root system in the deeper soil layer (Bouttier et al., 2014). The study noted that the pruning practice of the associated crop in this combination delivered less competition not only in the above-ground resources but also in the soil water and nutrients in the below-ground environment through the spatial separation of vertical root distribution.

However, among the intercropping farms, the rubber-coffee combination (RC) had less RH in the understory environment. It was also noticed that the SMC dynamic by depth was not evident as in the other intercropping farms. Since the stomatal conductance of coffee species was highly sensitive to the internal water deficit (Damatta and Ramalho, 2006), the underlying reason could be related to the less transpiration of the coffee plant due to inadequate water uptake by the root system. It was reported that the root system of coffee plants, intercropped with matured rubber trees under the conventional spacing, was restricted by the pioneer invasion of the rubber roots resulting in less accessibility to soil water and nutrients from most soil depths (Defrenet et al., 2016; Langenberger et al., 2017; Chiarawipa et al., 2021). Thus, it was noted that the rubber-coffee combination in this study could not utilize the soil water resource efficiently for the ecological complementarity as the other intercropping farms could.

Regarding the biochemical composition, the intercropping farms: RB and RM expressed lower Suc and higher Pi and R-SH in the latex than those of RC and the monocropping farm, RR, in S1. These indications of RB and RM depicted more efficient utilization of sucrose for the polyisoprene biosynthesis in the tree defense mechanism with lesser physiological stress than the RC and RR (d'Auzac et al., 1997).

In the dry season, all biochemical contents markedly decreased. As the nature of a deciduous tree, during the dry season when the water deficit is severe, *Hevea* rubber trees typically undertake the abscission process by reducing completely or

partially the leaf area of the tree (Webster and Paardekooper, 1989). With the depressive effect of the defoliation, photosynthesis functions are restricted, resulting in less sugar supply. Thereby, the tree mainly utilizes the preserved carbohydrates from the sink primarily for the new vegetative cycle, which started with young leaf shooting together with flowering, rather than the secondary metabolic functions (Jacob et al., 1989). His team also reported that since the lack of latex biosynthesis metabolism in the laticiferous system, less content of Pi was produced in the latex and also accompanied by the low R-SH content, which expresses the inactive enzymatic functions in laticiferous system. The results of low Suc content and high Pi and R-SH in the latex of RB and RM reflect that the rubber trees in these intercropping plots could effectively partition the assimilate translocations. Thus, refoliation could take place faster, thereby the earlier resuming of the photosynthesis process, resulting in higher biochemical composition in the new season, representing healthy physiological status with greater Suc supply and latex biosynthetic capacity.

In addition, the high R-SH content in the latex of all intercropping farms and its significant association with LAI approved that the increased leaf area coverage of the rubber-based intercropping farm reduced not only the environmental stresses of the tree (Rappaport and Montagnini, 2014) but also the oxidative stress in the lactiferous cell, caused by the regular latex harvesting, and these favored the latex regeneration metabolic activity with high yield potential (d'Auzac et al., 1997).

3.4.2. Variations in latex production

The study results approved that the latex yields in the intercropping farms were higher than that of the monocropping plot in all seasons. The significant positive relations of the latex production with LAI and SMC also confirmed that complementary interactions of the above and below-ground components under the rubber-based intercropping systems lead to higher latex yields.

Since plant leaf is the essential vegetative part of the photosynthesis process (Weraduwege et al., 2015), the variations in leaf area influenced the dry mass production and latex yield of rubber tree (Zhu et al., 2018). Likewise, soil water content has a significant effect on latex production since sufficient water content in the laticifers enhanced the latex flow with a longer flowing time resulting in higher production

(Pakianathan et al., 1989). These were obviously reflected in the yield result of RB and RM in the dry season because their daily yields did not drop as that of RR and RC which had low LAI and soil water uptake. In addition, in that season, Pi and R-SH in the latex of RB and RM were observed to be higher than those of RC and RR. That expressed stable metabolism in latex regeneration of the laticiferous system with less oxidative stress (d'Auzac et al., 1997), imparted by the optimum soil water status and LAI of the intercropping farms even in the dry season.

3.4.3. Variations in technological properties of *Hevea* rubber

3.4.3.1. Non-isoprene contents

Although the latex from all farms in S1 delivered the Mg contents that ranged between 300 and 410 ppm, the intercropping farms, RB and RM, in the dry season, expressed remarkably high Mg contents that were over the normal level of around 500 ppm, generally contained in fresh latex in that season (Puangmanee et al., 2014). However, the Mg increases in RC and RR in that season were below the normal level.

Since magnesium is an essential nutrient in chlorophyll synthesis associated with the photosynthesis process and plant growth, its concentration in plant leaves could be more significant in trees with healthy physiological status (Hauer-Jakli and Trankneret, 2019; Yang et al., 2019). However, during the onset of the deciduous process, with a reduction in leaf area, restrictions in photosynthesis capacity limit the utilization of primary metabolites, including magnesium. This induces the translocation of Mg, which has high mobility (Gerendas and Fuhrs, 2013), into the laticiferous mechanism, resulting in an increased accumulation of Mg content in the latex. However, it should be noted that the high Mg content affects the latex mechanical stability because the Mg²⁺ ions, released from lutoids, attract the rubber particles in the field latex colloidal leading to fast coagulation (Yip et al., 1990; Fong, 1992).

The ash contents in the dry rubber of all farms in S1 were stable and met the technically specified standard, not exceeding 0.6% (ISO, 2020). However, it was noticed that in S2, except RM, the other farms increased the ash content. Ash content in dry rubber represents the residues of non-volatile minerals in raw rubber, so its

variations could depend on tree mineral uptakes and soil water status (Yip, 1990). It has been reported that higher ash content was observed in the dry season in some clones in relation to tree physiological status (Roux et al., 2000). However, the higher ash content in S3 could be related to the fertilizer application that was carried out before the onset of the rainy season, and the mineral uptake of the root system.

The study results showed that throughout the seasons, the N contents in the dry rubber from all farms were not exceeding 0.4%, which was below the technically specified limit of 0.6% (ISO, 2020). It was noted that the dry rubber N contents of RB and RM were statistically greater than that of RC and RB in S1 and S2, while they were not significantly different in S3. Nitrogen is an essential plant nutrient in amino acid production for protein biosynthesis mainly associated with the primary functions of plant development (Wang et al., 2014). Regarding the technological properties of raw rubber, the N content is considered the protein residues in the dry rubber. It has been reported that an active supply of nitrogen could reconstitute sufficiently the protein lost in the harvested latex because the nitrogen in the laticiferous enhances protein biosynthesis, inciting Pi content with latex metabolism (Jacob et al., 1989; Othman et al., 1993). The study result of the significant correlation between the dry rubber N content and the Pi was consistent with the above statement. Conversely, less translocation of nitrogen amid intense soil water deficit affects photosynthesis capacity, metabolic pathways and crop yields (Clark et al., 1990). Moreno et al. (2005) also observed that most *Hevea* rubber clones expressed less N content in the dry rubber during the deciduous and flowering periods. According to the above reports, thus, the optimal N contents in the dry rubber of RB and RM in the dry season reflected the active latex metabolism contributed by the greater LAI and soil water uptake under the rubber-based intercropping ecosystem. However, it should be noted that its exceeded content over the limit affects the efficiency of successive processes, mainly maturation rate and vulcanization properties (Zhong et al., 2009).

3.4.3.2. Rheological properties

In terms of the rheological properties, all test results of P_0 , PRI and MV were higher than the minimum acceptable level recommended for most technically specified rubber (ISO, 2020). The study observed that the seasonal variations of P_0 and

MV were inversely associated with that of PRI, and these variations were mainly influenced by the latex biochemical compositions related to the soil water availability and LAI. P_0 and MV are generally tested to evaluate the visco-elastic behavior of natural rubber, which is the core property of *Hevea* rubber regarded as the processability of dry rubber (Yip, 1990; Malmonge et al., 2009). PRI represents the thermal-oxidative resistance of natural rubber, an important feature of the processability (Zhong et al., 2009).

It was noticed that the increases of P_0 and MV values in S2 identically followed the changes of DRC and TSC. It was reported that P_0 and MV values were associated with DRC and relatively increased during the onset of the deciduous period (Roux et al., 2000). The study noticed that the negative correlation between PRI and Mg content was more obvious in the dry season. It could be because photosynthesis depression in the dry season enhances the latex Mg content together with the presence of reactive oxygen species in the laticiferous cells leading to oxidative degradation in the rubber molecules (Hauer-Jakli and Trankneret, 2019). The study observed noticeably the adverse effects of the excessively high Mg content on the rheological results of RM in the dry season, with the significant excessive values of P_0 and MV and too low PRI, indicating the inferior processability of harder rubber. This implies intrinsically high oxidative degradable rubber with undesirable visco-elastic nature. Harder rubber which has high Mooney viscosity of over 60 ML (1+4) 100 °C requires a larger power consumption in the mastication process (Babu et al., 2000). However, the other intercropping farms did not express the unsatisfied results in the rheological properties, although their Mg contents were relatively high in S2 and S3.

3.4.3.3. Molecular characteristics

In the dry season, the rubbers from all farms exhibited narrower MwD than in other seasons, and some extent of deviations from the normal bimodal distribution, reflecting the less comprise of the low molecular weight fraction in the macrostructure of the rubber chain. The deviation degree was more obvious in the rubber from the monocropping farm. It implies less existence of gel content associated with the crosslinks to non-rubber constituents in the isoprene chain (Monadjemi et al., 2016; Thuong et al., 2018).

Although *Hevea* rubber is structured of cis-1,4 isoprene chain, the chain terminals have natural linkages of non-rubber constituents in the structure, which can contribute to the mechanical properties of rubber, particularly the improvement of the tensile strength and stability of unvulcanized rubber, called the green strength (Thuong et al., 2018). Better green strength ensures greater cohesiveness, firmness, and resistance to tearing and fracture of unvulcanized rubber, resulting in the flawless dimensional stability of vulcanized rubber (Hamed, 1981). The *Hevea* rubber, bearing distinctly inherent bimodal distribution in molecular structure, typically has greater mechanical properties with the green strength due to the complex distribution of low and high molecular weight chains and the gel formation in the isoprene chain (Kawahara et al., 2002). That is one of the outstanding features of *Hevea* rubber quality that synthetic rubbers cannot replace. However, the cross-linkages in the chain are dynamic with the storage time, and the prolonged storage imparts the raw rubber hardening with the increases in initial plasticity and Mooney viscosity (Amnuaypornsi et al., 2002; Zhong et al., 2009).

Increased Suc content in latex with a high Mw at a positive correlation depicts inefficient secondary metabolism. It could happen typically in the leaf fall season, resulting in less sucrose utilization for isoprene biosynthesis: thus, high Suc content in the latex, and higher Mw which is associated with narrow MwD. Kekwick (1989) reported that unstimulated rubber trees that had a narrow MwD contained a lesser proportion of low molecular weight fractions, with increased high molecular weight fractions, i.e., high molecular weight, at the compensation of the low portion, compared to the stimulated trees.

The highest MwD exhibited in the rubber from RM and the results of its positive associations with Pi and R-SH highlighted the significant implications of latex biochemical composition in the isoprene chain. The sufficient soil water supply enhanced the nutrient translocations into the laticiferous cells, imparting better latex biochemical compositions with increased Pi and R-SH contents. They improved not only the latex metabolism but also induced protein biosynthesis, thus higher latex yield with greater isoprene molecular structure (Coup and Chrestin, 1989; Roux et al., 2000).

However, the excessively increased latex Mg content in the dry season affected the molecular characteristics. *Hevea* rubber, polyisoprene, is the product of the natural polymerization of isopentenyl pyrophosphate (IPP), catalyzed by various enzyme reactions regulated by magnesium ions as an activator of the isoprene transferase enzymes along the rubber biosynthesis pathway (d'Auzac et al., 1997; Cornish and Xie, 2012). Despite the magnesium being functionally essential at an optimal level for rubber biosynthesis, the excessive content inhibited the IPP isomerase activities leading to a reduction in the molecular weight (Xie et al., 2013; Cherian et al., 2019).

3.5. Conclusions

All intercropping farms were observed to improve the understorey environment with higher relative humidity and lower temperature than those of the monocropping farm. Over the study period, RB, RM and RC exhibited significantly higher LAI values at 1.2, 1.05 and 0.99, respectively, while RR had a low LAI of 0.79. Increasing SMCs by soil depths were pronounced in all rubber-based intercropping farms. RB and RM expressed less physiological stresses and delivered latex yield on average 40% higher than RR. With higher molecular weight distributions, their rheological properties were comparable to those of RR. However, the latex Mg content of RB and RM significantly increased to 660 and 742 ppm, respectively, in the season 2. Their dry rubbers contained ash contents of more than 0.6% in the season 3.

This study revealed noteworthy the variations in technological properties and production of *Hevea* rubber associated with the interrelations of seasonal ecophysiological changes in the rubber-based intercropping farms. It would contribute to the effective development of sustainable natural rubber production integrated with rubber-based intercropping practices ensuring the superiority of the inherent technological properties of *Hevea* rubber.

CHAPTER IV

CONCLUDING REMARKS

4. Concluding Remarks

The first experiment highlighted the improvements in the soil fertility, the root's function, the crops' productions, and the tree physiological status as the complementarity interactions of the rubber-based intercropping system contributed by beneficial effects of the integrated fertilization. The study also suggests that the mixed organic-inorganic fertilization combined with organic soil amendments could be used as effectively integrated fertilization to improve soil fertility of the rubber replanting area and rubber-based intercropping farms.

The second experiment confirmed the improvements in ecophysiological components in rubber-based intercropping system compared to conventional rubber monocropping system. These improvements were more significant in the rubber-bamboo and rubber-melinjo combinations among the farms due to the greater above-ground vegetative growth and efficient soil water uptake observed in these farms. With these improvements, rubber trees in the above intercropping farms efficiently performed the latex metabolism with less physiological stress and ensured the optimal latex yields along the seasons. Thus, to ensure the ecological and economic benefits of the rubber-based intercropping farm, it is suggested that the selection of associated crops and the farming system, including planting timing and harvesting practices of the intercrops, are crucially important to achieve the facilitative interrelations of the above- and below-ground components, by efficiently sharing and consuming the natural resources.

As an overall result of this thesis research, both experimental studies confirm that complementarity interactions in the rubber-based intercropping system improve the agroecology of the farms with the tree's physiological conditions. The studies exposed the facilitative interactions of the above- and below-ground components associated with soil water utilization and leaf area development of crops, mutually depending on the improvement of microclimate conditions in the rubber-based intercropping farms. These ecological improvements enhanced the physiological status and latex metabolism of rubber trees, ensuring optimal latex yields along the seasons. The studies point out that in order to achieve complementary interactions with ecological and economic benefits by efficiently sharing and consuming the natural

resources with the facilitative interrelations of the above- and below-ground components, it is suggested that besides selecting the compatible associated crops, the integrated nutrient management and farming systems, including planting space and time, and harvesting practices, are also crucially important.

Both studies also approved that farmers' conventional practices like replanting the same perennial monocrops and long-term utilization of chemical fertilizers degraded the soil fertility and soil water functions affecting the vegetative developments and physiological status of the crops.

The study on the technological properties revealed the greater molecular weight structure of *Hevea* rubber sourced from the rubber-based intercropping compared to that of the monocropping farm. Consequently, the high molecular weight distribution imparted superiority in technological properties, mainly rheological properties, green strength, and processability. However, the study discovered that the rubbers obtained from the intercropping farm were higher in magnesium and ash contents, exceeding the recommended limits.

In conclusion, the findings from the thesis research would contribute to the sustainable valued chain of *Hevea* rubber production integrated with the rubber-based intercropping system ensuring the complementarity benefits in the farm ecosystem leading to the superiority technological properties of *Hevea* rubber.

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APPENDICES

Paper I

Zaw, Z.N., Chiarawipa, R., Pechkeo, S., Saelim, S. 2022. Complementarity in rubber-salacca intercropping system under integrated fertilization mixed with organic soil amendments. *Tropical Agricultural Science*, 45(1).
<https://doi.org/10.47836/pjts.45.1.09>



Complementarity in Rubber-Salacca Intercropping System Under Integrated Fertilization Mixed with Organic Soil Amendments

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ABSTRACT

The replanting practice of rubber monocropping in Southern Thailand has depleted soil fertility. Most rubber planted areas in the region were under intensive chemical fertilization resulting in less soil organic matters and root proliferation. With the instability of rubber prices, some rubber farmers converted from monocropping into intercropping. Integrated fertilization in which mixed organic-inorganic fertilizers are combined with organic soil amendments could be considered in a rubber-based intercropping system to increase land productivity with cost-saving fertilization by rehabilitating soil properties. A study was conducted at a rubber-salacca intercropping farm comprised of 14-year-old mature rubber trees associated with eight-year-old salacca palms to identify the consequences of the integrated fertilization combined with two organic soil amendments: humic acid (HSA); chitosan (CSA). Changes in soil organic matter (SOM), leaf area index (LAI), fine root traits, tree physiological status, and crop productions under the two integrated fertilization were compared against the controlled application of conventional chemical fertilizer. The

CSA application increased the SOM in the topsoil layer by 80%. In the 21-40 cm soil depth, the rubber roots treated with HSA and the salacca palm roots treated with CSA showed greater fine root length density (FRLD). Under CSA, the physiological status of the rubber trees showed less stress. The treatments of HSA and CSA showed 145% and 72%, respectively, higher in total production of salacca palm than that of the

ARTICLE INFO

Article history:

Received: 14 July 2021

Accepted: 22 November 2021

Published: 24 January 2022

DOI: <https://doi.org/10.47836/pjas.45.1.09>

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ISSN: 1511-3701
e-ISSN: 2231-8542

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chemical fertilization. Improvements in the soil fertility, the root's function, the crops' yields, and the tree physiological status were consequences as complementarity in the system under the integrated fertilizations.

Keywords: Chitosan, *Hevea brasiliensis*, humic acid, intercropping, integrated fertilization, soil amendment

INTRODUCTION

Most natural rubber (*Hevea brasiliensis*) growing areas in Southern Thailand are currently in the second or third replanting cycle of rubber monocropping. This replanting practice of the same perennial monocrop has depleted soil fertility substantially (Umami et al., 2019; Vignon-Brenas et al., 2019). Besides, about 67% of the region's rubber growing area was under intensive application of chemical fertilizer (National Statistical Office, 2013) to meet targeted immature period and economic yield. Its long-term application accumulated adverse effects on soil structure, such as soil acidification, soil water pollution, and soil organic matter shortage — consequently, root functions like less root proliferation and nutrient-uptake activities.

In the last two decades, due to the instability of rubber prices, some rubber farmers in the area started converting into intercropping to widen the on-farm income sources and increase land productivity (Hougni et al., 2018; Romyen et al., 2018). In the area, most rubber-based intercropping farms were transformed from mature monocropping rubber farms and mostly

intercropped with perennial cash crops like bamboo, coffee, cacao, ginger, and salacca anticipation long-term economic benefits (Jongrungrat et al., 2014). However, some combinations of rubber-based intercropping experienced adverse effects on the growth and yield of the crops due to some competitions in root interactions and nutrient uptakes (Langenberger et al., 2017). Thus, in these types of permanent rubber-based intercropping, complementarity interactions in the system are the main consideration in which the crops and other components are facilitative complements each other to achieve ecological benefits together with healthy physiological status of the crops and vegetative growth, ensuring sustainable crop yields for long-term economic benefits (Bybee-Finley & Matthew, 2018).

Since rubber tree transforms sucrose into natural rubber, cis polyisoprene, as a product of the tree's defense mechanism in response to human interventions (latex harvesting) and environmental conditions, the healthy physiological status of the tree plays a crucial role in natural rubber production (Adou et al., 2017; Obouayeba et al., 2011). Biochemical compositions, mainly sucrose (Suc), inorganic phosphorus (Pi), and reduced thiols (R-SH) contents, are analyzed to evaluate the physiological status and yield potential of rubber trees (Christophe et al., 2018). As the sucrose are transformed into rubber molecules in the laticiferous system, high Suc content in the rubber latex indicates less sucrose utilization in the defense mechanism. Overexploitation in latex harvesting significantly reduces

the Suc content in the latex, reflecting the high stress of the physiological status of the tree (Doungmusik & Sdoodee, 2012). The Pi represents the main constituent of the energy metabolism in the laticiferous system and exhibits the level of sucrose utilization and intensity of biosynthetic activity. Atsin et al. (2016) reported that Pi content was positively associated with the active metabolism; thus, a higher Pi indicated a significant yield potential under healthy rubber trees. The reduced thiols are important antioxidants to protect the laticiferous cells in the defense mechanism and reduce oxidative stresses mainly caused by latex harvesting (Purwaningrum et al., 2019). Low R-SH content in the latex indicates high physiological stress of the laticiferous system.

According to the principle of integrated nutrient management, harmonious utilization of farm nutrient sources such as organic manure and farm wastes, mixed with inorganic fertilizers could be considered an integrated fertilizer (Food and Agriculture Organization of the United Nations [FAO], 2016) in the rubber-based intercropping system to increase land productivity with cost-saving fertilization through improvement or rehabilitation of soil properties.

One of the integrated usages of available farm wastes, humic acid extracted from vermicompost of biodegradable farm wastes like animal manures, green manures, and crop residues, has been widely applied as an organic soil amendment (Selladurai & Purakayastha, 2016). It enhances microbial

activities and a population that transform insoluble mineral nutrients into available nutrient form for plant in the soil, thus higher soil nutrient content (Li et al., 2019). In humic acid-treated soil, pH buffering capacity, organic matter, and cation exchange capacity were improved with more significant soil physical properties resulting in enhanced root performances like fine root proliferation and nutrient uptake (Buyukkeskin et al., 2015; Cahyo et al., 2014). It was reported that the growth rates of nursery and immature rubber plants were enhanced by reducing chemical fertilizer usages and supplementing a humic acid application (Dharmakeerthi et al., 2013). Likewise, chitin and chitosan processed from chitin-containing wastes from the fishery industry, available in the area, have been widely applied as a natural plant elicitor. Chitosan-treated plants improved pathogen resistance because of their antimicrobial properties and defense mechanism (Sharp, 2013). With improved plant metabolism, vegetative growth of plant and crop yield were significant under chitosan application in combination with chemical fertilizer (Y. C. Chen et al., 2016).

Although the sources for these organic soil amendments are available in the area, their usages have not been found yet in the rubber farms and rubber-based intercropping. Furthermore, studies related to the integrated fertilizations in rubber-based intercropping systems are also limited in the scientific literature. Thus, an experiment was conducted at a mature rubber-intercrop farm to investigate

the consequences of the agroecosystem components' interactions under integrated fertilizer applications combined with different organic soil amendments compared to conventional chemical fertilization.

MATERIALS AND METHODS

A mature rubber farm intercropped with salacca palm (*Salacca zalacca*) situated at 6°59'46.9"N, 100°34'58.6"E in Na Mom district, Songkhla province, Southern Thailand, was selected for the experimental study. The area receives an annual rainfall of about 2,000 mm distributed from June to December. In general, monthly rainfall precipitates less than 200 mm from June to September, around 300 mm in October and November, and peaks in December with about 500 mm.

The farm was started as a monocrop rubber replanting with RRIM 600 cultivar planted in a spacing of 6 m x 3 m on flat land in 2002. The rubber trees have been harvested, applying a tapping system of

one-third spiral of tapping cut length and two-day tapping in three days since 2008. The heights of the rubber tree were around 18 m, and the stem girths were average at 79 cm at the height of 170 cm from the ground. The associated plant, salacca palm, was intercropped in 2008 between the rubber rows with the same spacing as the rubber planting. As a result, the palm's growths were uniform, with the average height and width of their canopies of 3.6 m and 4.5 m, respectively.

The experiment was designed in a randomized complete block design comprised of three fertilization treatments with three replications. Each replicated plot covered one row of ten rubber trees and adjacent two rows of the salacca palms. The treatments were formulated to compare the applications of two different organic soil amendments combined with mixed organic-inorganic fertilizer against the controlled application of conventional chemical fertilizer (Table 1).

Table 1
Summary of the three treatments of fertilizations

Treatments	Chemical fertilizer		Organic fertilizer		Organic soil amendment	
	Types	Application rate	Types	Application rate	Types	Application rate
T1	Compound fertilizer (30-5-18)	1 kg tree ⁻¹ y ⁻¹ (3 times)	-	-	-	-
T2	Compound fertilizer (30-5-18)	0.5 kg tree ⁻¹ y ⁻¹ (3 times)	Composted cow manure	10 kg (3 times)	Humic acid	100 mL 20 L ⁻¹ water (3 times)
T3	Compound fertilizer (30-5-18)	0.5 kg tree ⁻¹ y ⁻¹ (3 times)	Composted cow manure	10 kg (3 times)	Chitosan	100 mL 20 L ⁻¹ water (3 times)

In the control treatment (T1), chemical compound fertilizer 30-5-18 nitrogen-phosphorus-potassium (N-P-K) was broadcasted at a rate of one kilogram per rubber tree per year between the rows of the rubber trees and the salacca palms in March, July, and November 2016. In the other treatments (T2 and T3), the chemical fertilizer mixed at a rate of 0.5 kg per rubber tree with 10 kilograms of composted cow manure was applied from April. Then, humic-acid soil amendment (HSA) prepared by mixing 100 mL of vermicompost-derived humic acid (pH 6.5, 5% humic acid, 50% organic matter, 5% total nitrogen, 2.5% total potassium, 0.06% total phosphorus, 0.25% calcium) with 20 L of water and sprayed on the soil between the rubber trees and the palms in T2 from May (Ruangkhanab & Lim, 2005). Then, with the same application rate as the HSA treatment, 100 mL of the chitosan (pH 5.5–6, 6.5% organic carbon, 0.05% nitrogen, 0.01% phosphorus oxide, 0.01% potassium) mixed with 20 L of water was applied as the chitosan soil amendment (CSA) in T3 from May. All these fertilizations were applied three times with a third-monthly interval during the study period.

Soil organic matters (SOM) from soil depths of 0–20 cm and 21–40 cm of each plot were determined using Walkley-Black's titration method (FAO, 2020) in February 2016 and February 2017 to compare the SOM contents before and after treatments.

Changes in leaf area index (LAI) at the farm were monitored monthly by the hemispherical photography method from

June to December 2016. The hemispherical photos were taken vertically upward from 1.2 m above the ground at three different points in the inter-row between the rubber trees and the salacca palms at every treatment plot by using Nikon Coolpix 8400 camera (Nikon, Japan) with a fish-eye lens (Bianchi et al., 2017). The Gap Light Analyzer (GLA) software version 2.0 was used to analyze the fish-eye captured images.

Changes in fine root traits, notably fine root diameter (FRD) and fine root length density (FRLD) of both crops, were monitored in two layers of soil depths (0–20 cm and 21–40 cm) by using the Prince of Songkla University (PSU) minirhizotron root scanner through 10 cm in diameter with 100 cm long of two acrylic access tubes per treatment plot installed with 45° angle of slope in the soil (Saelim et al., 2019; Vamerali et al. 2011) between the rubber tree and the palm. Two months after installing the acrylic tubes, the root images were scanned monthly from June to December 2016. The scanned images were analyzed using the Rootfly software (version 2.0.2).

Latex samples were collected monthly from each plot to analyze the latex production expressed in dry rubber weight per tapping per tree (g tap⁻¹ tree⁻¹). The collected samples were coagulated using formic acid and then dried at 70 °C for 16 h to calculate the dry weight of rubber content in the latex as recommended by ISO 126:2005. Productions of the salacca palms in yield per cluster and total yield per palm were recorded collectively at the end of the study period from randomly selected seven palms from each plot.

The biochemical parameters of latex, namely sucrose (Suc) content, inorganic phosphorus (Pi) content, and reduced thiols (R-SH) content, were measured monthly from latex samples taken from selected rubber trees of each treatment plot by following the latex micro-diagnosis method of the French Agricultural Research Centre for International Development (CIRAD). (Chantuma et al., 2011).

Data collected were analyzed with the R software (version 3.6.2) using a one-way analysis of variance (ANOVA). In addition, Duncan's multiple range tests were performed at $p \leq 0.05$ to compare the data pairs, and Pearson's linear correlation (r) at $p \leq 0.05$ was applied in correlation analysis.

RESULTS

Comparisons of SOM

The higher content of SOM was found in the topsoil layer (0–20 cm depth). In comparison, the deeper soil layers had relatively lower organic matter content under all treatments after the experiment (Figure 1). Although all treatments increased the SOM in all layers of soil depth, the top layers under T1 and T3 showed remarkably higher soil organic matter contents. T3 increased the SOM in the topsoil layer by 80%, followed by T1, with an increase of 38% after the experiment.

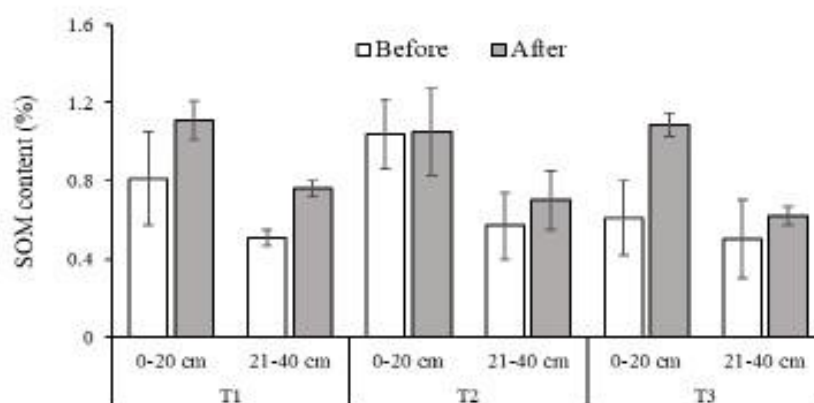


Figure 1. Comparison of soil organic matter (SOM) among the treatments before and after the experiment

LAI of the Farm

Although there were no significant differences in the LAIs among the treatments during the study, the changes followed a similar trend (Figure 2). The LAIs of the

farm started increasing in July with just over 1.10 and reached their maximum values ranging between 1.50 and 1.71 in September. Then they decreased to their lowest values between 1.00 and 1.20 in October and

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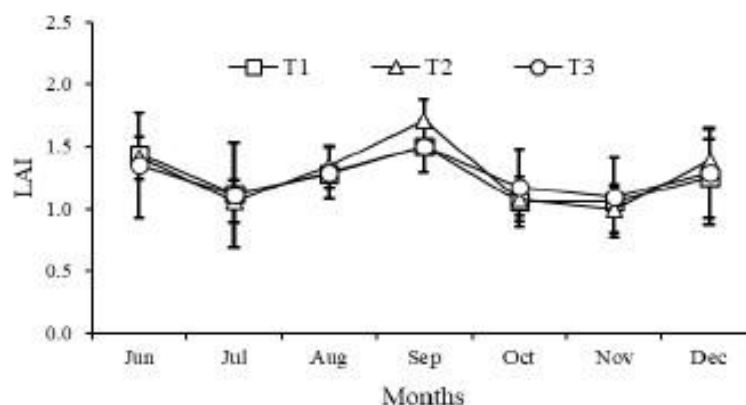


Figure 2. Changes in leaf area index (LAI) of the farm under the three treatments (from June to December 2016)

November, respectively. However, the LAI values of the farm increased back in the range of 1.29 and 1.39 in December.

Fine Root Traits of the Rubber Tree

FRDs of the rubber trees under T1 were found as the largest over those of the other treatments from June to September in both soil layers (0–20 cm and 21–40 cm) (Figure 3). In the soil depth of 21–40 cm, the average size of the FRD under T1 was higher than that of T2 and T3 by 27% and 28%, respectively (Figure 3B).

In terms of changes in FRLD (Figure 3 C), all treatments resulted in a stable trend ranging between 0.34 and 0.70 cm cm^{-2} in the topsoil layer during the study period. In the soil depth of 21–40 cm (Figure 3 D), the rubber trees under T2 were observed with the highest FRLD at over 1.44 cm cm^{-2} between July and October. After October, however, it decreased slightly with the densities of 1.46 and 1.09 cm cm^{-2} in November and December, respectively.

Fine Root Traits of the Salacca Palm

The fine roots of the salacca palm in the soil depth of 0–20 cm (Figure 4A) under T1 showed the largest diameter sizes ranged between 0.82 to 1.23 cm, while the other treatments resulted in smaller sizes of the FRDs ranging between 0.67 and 0.95 cm. In the soil depth of 21–40 cm, the sizes of FRD under T1 were also larger than those under other treatments in July, August, September, and October (Figure 4 B).

Monthly changes of the FRLD of the salacca palm (Figure 4 C) in the soil depth of 0–20 cm were stable between 0.20 and 0.38 cm cm^{-2} and did not show a significant difference during the study period. However, in 21–40 cm soil depth, T3 resulted in the highest FRLD in July, October, November, and December with 0.60, 0.64, 0.46, and 0.40 cm cm^{-2} , respectively (Figure 4 D).

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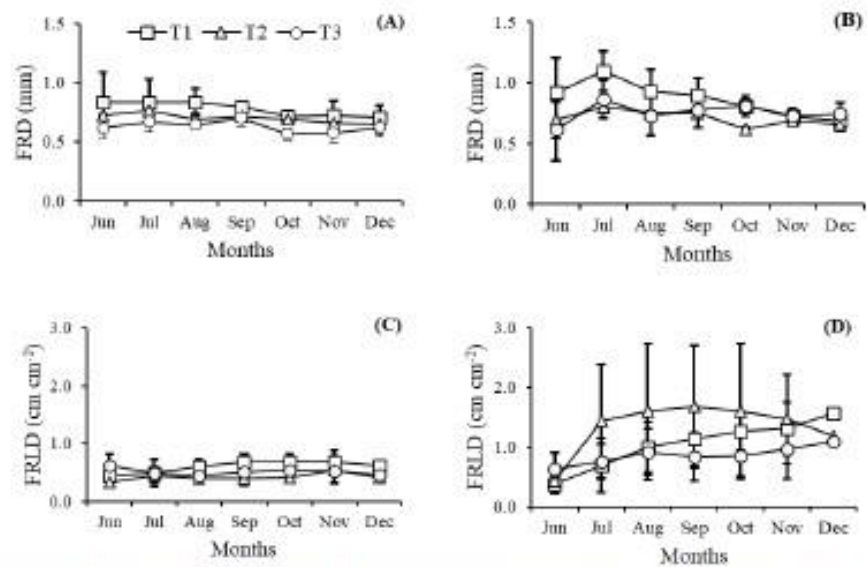


Figure 3. Monthly changes in fine root traits of the rubber tree: fine root diameter (FRD) at the soil depths of (A) 0-20 cm and (B) 21-40 cm; fine root length density (FRLD) at the soil depth of (C) 0-20 cm and (D) 21-40 cm (from June to December 2016)

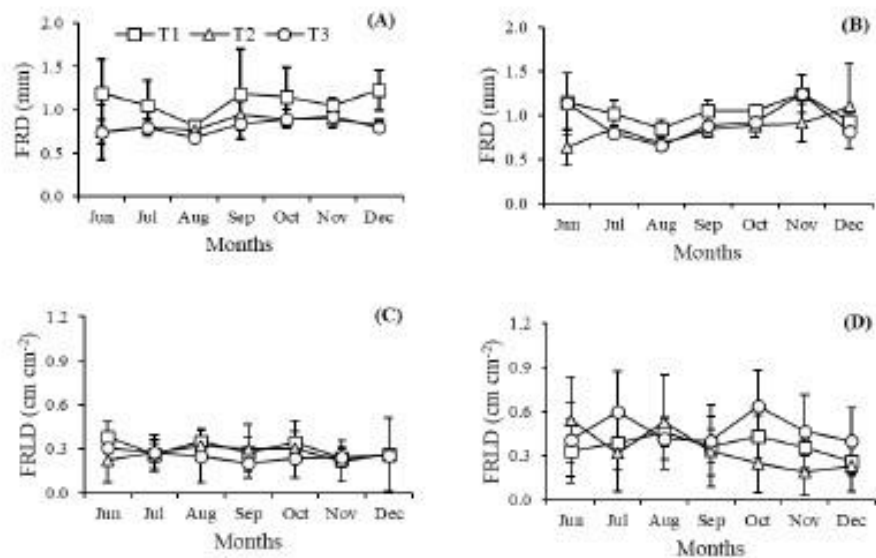


Figure 4. Monthly changes in fine root traits of the salacca plum: fine root diameter (FRD) at the soil depths of (A) 0-20 cm and (B) 21-40 cm; fine root length density (FRLD) at the soil depth of (C) 0-20 cm and (D) 21-40 cm (from June to December 2016)

Latex Production

Although there were no significant differences among the latex productions under the different treatments, the latex productions varied with different seasons (Figure 5). At the beginning of the rainy season, the productions under all treatments dropped their yields from about 60 g tap⁻¹ tree⁻¹ in June to less than 40 g tap⁻¹ tree⁻¹ in July. Then, the production increased to the highest level between 73 and 80 g tap⁻¹

tree⁻¹ in September. However, all treatments showed less production with around 30 g tap⁻¹ tree⁻¹ in November. Finally, in December, the productions under T1, T2, and T3 surged back, respectively, with 80, 65, and 50 g tap⁻¹ tree⁻¹. The result of Pearson's linear correlation ($r = +0.6024$) at $p \leq 0.05$ confirmed a positive correlation between the monthly changes of the LAIs and the latex production under all treatments (Figure 6).

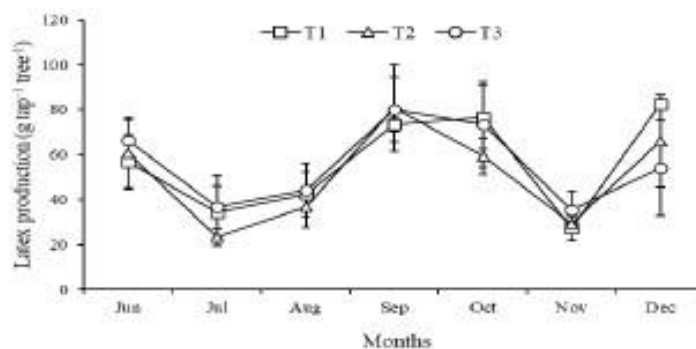


Figure 5. Monthly changes in average daily production of latex (g tap⁻¹ tree⁻¹) under the treatments (from June to December 2016)

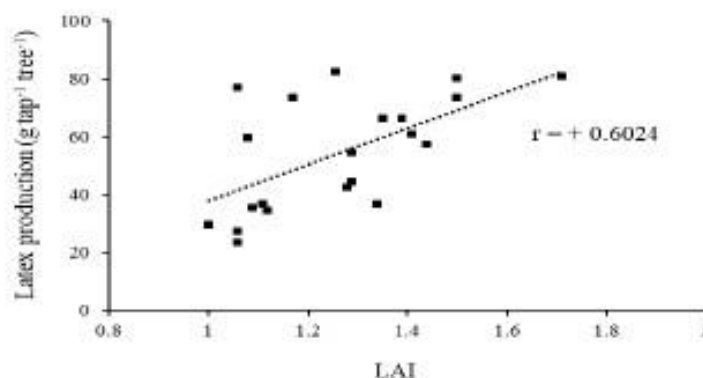


Figure 6. Relationship between the changes of LAI and latex productions

Latex Biochemical Composition

Suc contents of all treatments decreased gradually between July and October, except that of T2 showed a peak at 13.66 mM in August (Figure 7 A). The Suc contents of T1 and T2 reached their minimum levels of 1.79 and 2.43 mM, respectively, in November. However, T3 showed an upward trend in November after its lowest level of 4.65 mM in October. In December, the Suc content under T3 reached 9.77 mM as the highest level in that month, followed by T2 and T1 with 6.76 and 3.53 mM, respectively.

Pi content under T2 decreased from 21.33 mM in June to 10.52 mM in July (Figure 7 B). The contents under T1 and T3, however, were stable between 10.54 and

12.61 mM from June to September. Between September and November, the Pi contents of all treatments increased, and that of T3 was the highest with 30.59 mM followed by that of T2 and T1, respectively, in November. Then, the Pi contents under all treatments decreased again in December.

R-SH levels of the treatments were different in June as that of T3 was at 0.43 mM as the highest, followed by T1 and T2 with 0.30 mM and 0.15 mM, respectively (Figure 7C). After July, however, all treatments increased slightly until November, and the R-SH level under T3 was the highest in November. Then in December, the R-SH level of all treatments declined under 0.30 mM.

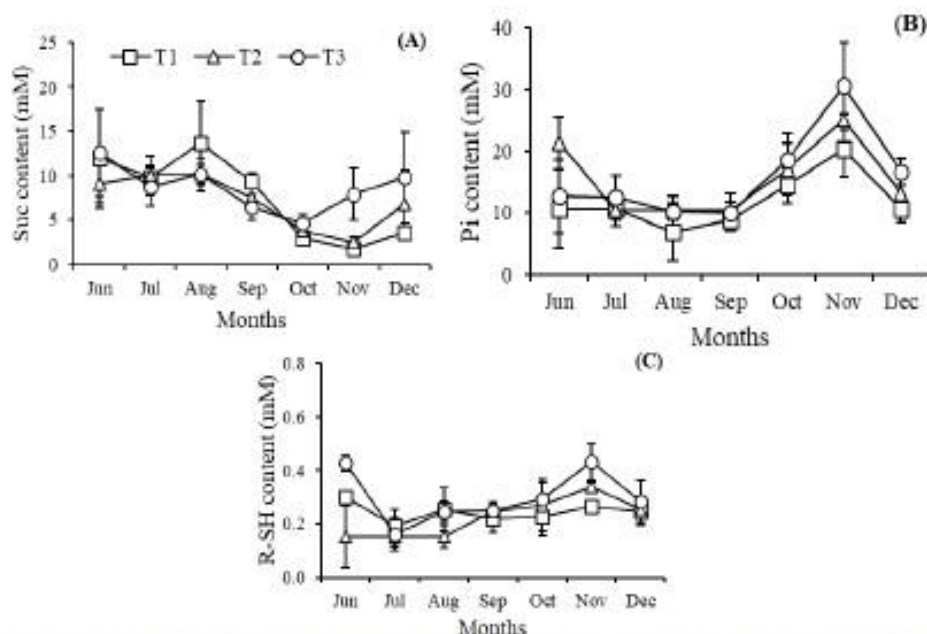


Figure 7. Monthly changes in biochemical composition (A) sucrose – Suc content; (B) inorganic phosphorus – Pi content; (C) reduced thiols – R-SH content of latex under the treatments (from June to December 2016)

Salacca Palm Production

The salacca productions were significantly different among the treatments in yield per cluster, and total yield per palm (Table 2) as T2 delivered the highest weight with 1.60 kg

cluster⁻¹ followed by T3 with 1.33 kg cluster⁻¹ while that of T1 was the lowest at 0.77 kg cluster⁻¹. Likewise, the total yields (kg palm⁻¹) of T2 and T3 were 145% and 72%, respectively, higher than T1.

Table 2

Production of the salacca palms among the treatments

Treatment	Yields of the salacca palm	
	kg cluster ⁻¹	kg palm ⁻¹
T1	0.77 ± 0.05 ^c	2.50 ± 0.89 ^c
T2	1.60 ± 0.09 ^a	6.13 ± 1.10 ^a
T3	1.33 ± 0.21 ^{ab}	4.38 ± 1.50 ^b

Note. Different lower-case letters in the same column are significantly different at $p \leq 0.05$ by Duncan's multiple range test

DISCUSSION

Soil Fertility Improvement

The study observed that the plot amended with CSA had a maximum level of SOM content in the topsoil layer. The result was likely due to the enzymatic soil microbial activities improved by CSA, enhancing the decomposition process of organic materials in the topsoil layer (Sawaguchi et al., 2015). Besides, the soil microbial population increased and decomposed themselves, resulting in a higher level of organic matter in the soil. The higher content of SOM is an indicator of healthy soil with efficient infiltration and water-holding capacity, thus higher nutrient availability (C. Chen et al., 2017; Nannipieri et al., 2017).

Development of the Fine Root Traits

It was noticed that the FRD of both crops

under T1 showed a larger size in both soil layers in general. It signaled high limitation in the movements of water and nutrients from the soil to the roots resulting in low vegetative growth and productivity (Comas et al., 2013). Conversely, roots with smaller diameters have greater hydraulic conductivity and tolerate drought conditions (Henry et al., 2012). The small diameters of the fine roots under T2 and T3 reflected the better performance of the root functions because of the higher availability of nutrients and water in the soil under the organic soil amendment application (du Jardin, 2015).

In all treatments, the FRLD of rubber trees in the soil depth of 21–40 cm showed upward trends once the rainy season began but in the soil depth of 0–20 cm. It indicated that the development of rubber fine roots in the soil depth of 21–40 cm was more

responsive to the rainfall than the topsoil layer. A study conducted in the same province by Saelim et al. (2019) also found that the fine roots of the 16-year-old rubber, particularly in the soil depth 20–30 cm developed at a higher rate in the rainy season. The result was consistent with Maeght et al.'s (2015) finding in north-eastern Thailand that the fine rubber roots within the soil depth of 2 m exhibited higher root emergences during the rainy season. Among the treatments, the rubber trees treated with the HSA showed higher FRLD in 21–40 cm soil depth from July to October. Wasson et al. (2012) remarked that a root system with greater FRLD in deeper soil could uptake water and nutrients at high efficiency. Cahyo et al. (2014) reported that root growth and performance were more obvious than other vegetative parts under the HSA. It could serve as auxin and promote cell enlargement by stimulating the cell wall loosening leading to greater vegetative growth (Jindo et al., 2012). However, it was noticed that the FRLDs of the salacca palm were higher under the CSA in the soil depth of 21–40 cm. CSA could enhance cation properties and water holding capacity in the soil, thereby more significant development of fine roots resulting in better nutrient uptakes and improved crop yield (Sharp, 2013).

The Vegetative Growth and Production of the Crops

The study confirmed a positive relationship between the LAIs and latex production under all treatments. At the beginning of the rainy

season, in July and August, latex harvest (tapping) activities could not be carried out regularly due to the disturbance of uneven raining patterns resulted in yield drops in all treatments. The latex productions under all treatments were at maximum levels in September, while leaves in the rubber canopy reached the ultimate growth stage. Since the planted cultivar, RRIM 600 clone, is susceptible to phytophthora leaf fall disease (Krishnan et al., 2019), which occurs typically during the rainy season, the rubber trees in the farm were attacked by the disease, thus fewer values of LAI in November. In the meantime, it was observed that the latex yields under all treatments dropped from their maximum yields. Leaf area is a functional part of a tree's photosynthesis and determines photosynthetic efficiency, reflecting sucrose synthesis (Weraduwage et al., 2015). Since natural rubber is a photosynthesis product of *H. brasiliensis* through sucrose synthesis in non-photosynthesis laticiferous tissue, the leaf area of the rubber tree influences latex yield and dry mass production of rubber (Zhu et al., 2018).

Regarding salacca production, the treatments of the integrated fertilizations delivered significantly higher yields compared to that of the chemical fertilization. It was contributed by the beneficial effects of the integrated fertilization that organic fertilizer and organic soil amendments could promote inorganic fertilization effectiveness, thereby more extended availability of nutrients in the soil (Wu et al., 2020). In addition, it could improve the soil's physical

properties such as cation exchange capacity and water holding capacity, enhancing root proliferation and the root system's nutrient uptake functions, resulting in higher crop yield (Sharp, 2013).

In addition, it was noticed that yields per cluster in all treatments were apparently higher than the average yield of around 0.6 kg per cluster of conventional salacca-fruit intercropping (Sumantra & Martiningsih, 2018). In rubber-based intercropping, the canopy of mature rubber trees reduces extreme temperature and intense irradiance, improving the adaptability of understory plants especially shade-required species like salacca palm (Montagnini, 2011; Rappaport & Montagnini, 2014). Along with the favorable weather conditions, the co-existence of the different canopy architectures, like the combination of rubber trees and salacca palms, enhancing light interception and distribution in the farm contributes to a greater photosynthetic rate resulting in yield improvement of the crops (Sumantra et al., 2012; Tang et al., 2019; Xianhai et al., 2012).

Less Physiological Stress of the Rubber Tree

It was observed that all treatments showed higher Suc content, lower Pi content, and lower yields at the beginning of the rainy season after the dry season. It reflected low metabolic utilization or insufficient conversion of sucrose into cis-isoprene rubber molecules in the latex resulting in higher Suc content remaining and fewer rubber particles in the latex (Purwaningrum

et al., 2015). Then, in September and October, the yields of all treatments were at a high level with an elevation of the Pi contents. It indicated the high metabolism of the laticiferous contributed by the regular tapping activity (Atsin et al., 2016). However, in November, the Suc contents under T1 and T2 declined to the lowest level, and their productions also plunged to less than 30 g tap⁻¹ tree⁻¹ at that month, reflecting that the rubber trees were exhausted with the shortage of sucrose supply because of the effects of the high-frequency latex harvest practice (overexploitation) and the occurrences of the abnormal leaf fall disease. A study by Obouayeba et al. (2011) indicated that low sucrose content less than around 3-4 mM associated with yield drops reflected the initial symptom of the tree stress with physiological disorders in the laticiferous system leading to tapping panel dryness. The intensity of physiological stress could vary between rubber clones due to their different sugar loading capacities (Gohet et al., 2015). In addition, the abnormal leaf fall disease destructed the photosynthesis functions, thereby reducing the Suc's sufficient supply, resulting in the yield drop. However, the Suc content, the Pi content, and the R-SH content under T3 was at a high level, and the yield in T3 remained over 30 g tap⁻¹ tree⁻¹ and was not as low as that of the others. These physiological responses reflected less physiological stress of the laticiferous system (Sainoi et al., 2017) and the lesser effect of the phytophthora attack under T3 compared to those of the other treatments. It was likely to be the CSA's antimicrobial

effect since its application restrained and slowed down the growth of the pathogen by enhancing the response of the plant's immune system (Sunpapao & Pornsuriya, 2014).

CONCLUSION

The study observed that both HSA and CSA treatments improved the fine root trait developments of the crops, particularly in the soil depths of 21–40 cm. The fine rubber roots were responsive under the HSA, while the fine root growths of the salacca showed more significance under the CSA. It was found that a positive correlation between the average yields of rubber and the LAI in the farm. The study highlighted that the advantages of CSA on rubber trees that its application improved the tree physiological status. Thus, the latex biochemical composition levels and the daily yield were maintained under the CSA application during the intensive latex harvest practices and the phytophthora leaf disease attack. A significant increase in soil organic matter under the CSA treatment was also advantageous.

The higher yields per cluster of salacca trees in all treatments compared to other conventional salacca farms indicated the beneficial effect of the rubber-salacca combination. In addition, the significantly higher yields of salacca under the HSA and CSA further approved the effect of the integrated fertilizations.

The study highlighted the complementarity effect resulting from harmonious interactions between the

integrated fertilization and agroecosystem components of the rubber-salacca intercropping. Therefore, it is suggested that the mixed organic-inorganic fertilization with organic soil amendments could be utilized in rubber-based intercropping as effectively integrated fertilization to reduce the usage of chemical fertilizer without affecting the crop yields.

ACKNOWLEDGEMENTS

The study was funded by the Natural Rubber Innovation Research Institute and Thailand's Education Hub for ASEAN Countries (THE-AC), Graduate School, Prince of Songkla University scholarship under project number NAT581136S. The authors thank the farm's owner for allowing this experimental study.

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Paper II

Zaw, Z.N., Chiarawipa, R., Sdoodee, S. 2022. *Hevea* rubber physiological status and relationships under different rubber-based intercropping systems. *Songklanakarin Journal of Science & Technology*, 44(1): 6-12

Original Article

Hevea rubber physiological status and relationships under different rubber-based intercropping systems*

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Received: 30 March 2021; Revised: 9 June 2021; Accepted: 15 June 2021

Abstract

The study aimed to preliminarily explore the relationship of the rubber tree's physiological status and technological properties with response to different agroecosystem components, especially leaf area index (LAI) and soil moisture contents (SMC) under three types of rubber-based intercropping, notably rubber-bamboo (RB), rubber-coffee (RC) and rubber-melinjo (RM) compared to a rubber monocrop (R). RB and RM significantly showed the highest LAI values of around 1.4, while that of R had the smallest value (about 30% lesser than the highest values). RM, RB dramatically increased the SMC with the soil depths. The rubber-based intercropping farms indicated better biochemical composition in the latex, showing efficient metabolism of the latex biosynthesis. Technological properties of the raw rubber from the rubber-based intercropping farms expressed the premium results in both non-rubber components and rheological properties with higher molecular weights. The correlation analysis revealed some significant relations among the LAI, SMC, biochemical composition and technological properties.

Keywords: rubber-based intercropping, leaf area index, soil moisture content, physiological status, technological properties

1. Introduction

Hevea rubber, cis-1, 4-polyisoprene, is a secondary metabolite of *Hevea brasiliensis* biosynthesized from sucrose in the laticiferous system for physiological defense mechanism (Jacob *et al.*, 1989). Since the sucrose is the resultant of photosynthesis by consuming natural resources such as sunlight, nutrients and water from the environment, the technological properties of natural rubber are strongly linked to the rubber tree's physiological responses to its environment (d'Auzac *et al.*, 1997; Roux *et al.*, 2000; Van Gils, 1951).

Conventionally, natural rubber has been sourced from *Hevea* rubber monocrop cultivation, which generates major incomes of the rubber smallholders, for the requirement

of the word rubber consumption. However, commercial rubber monocropping has degraded the environment and natural ecosystem with adverse consequences such as deforestation, agricultural pollutions, changing local climate, and losses of natural resources (Zhang, Yang, & Du, 2007; Ziegler, Fox, & Jianchu, 2009). Moreover, due to the extensive involvement of smallholders as the major rubber producers, socio-economic issues like low income, high production cost, and shortage of workers have been raised associated with instability of rubber price (Fox & Castella, 2013).

Many researches highlighted that the agroecosystem of rubber growing area and socio-economic factors were improved under rubber-based intercropping compared to the rubber monocropping (Chen, Liu, Wu, Jiang, & Zhu, 2019; Elmholt, Schjonning, Munkholm, & Deboz, 2008; Guardiola-Claramonte *et al.*, 2008; Werner *et al.*, 2006; Zhang *et al.*, 2007). Thus, rubber-based intercropping became a recommended practice for smallholders to reduce the impacts and develop sustainable natural rubber production of the smallholders (Langerberger, Cadish, Martin, Min, & Waibel, 2017; Polthanee, Promkhambut, & Khamla, 2016; Rodrigo, Stirling, Naranpanawa, & Herath, 2001). However, studies

*Peer-reviewed paper selected from The 1st International Conference on Sustainable Agriculture and Aquaculture (ICSAA-2021)

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related to the technological properties of raw rubber sourced from the rubber-based intercropping systems are limited.

In this context, it needs to extend the realization on variation in biochemical composition of latex under the rubber-based intercropping and its relations to the technological properties of raw natural rubber. Therefore, a preliminary study was conducted to investigate changes in the rubber tree's physiological status and the technological properties of raw rubber related to agroecosystem components, especially leaf area index (LAI) and soil moisture contents (SMC), in the rubber-based intercropping systems.

2. Materials and Methods

Three farms of rubber-based intercropping: rubber-bamboo (*Gigantochloa nigrociliata*) (RB), rubber-coffee (*Coffea canephora*) (RC) and rubber-melino (*Gnemon gnemon*) (RM), and one monocrop rubber farm (R) were selected for the study structured in complete randomized design at Khao Phra village located at Khao Phra village located at 6° 59' N, 100° 08' E, in Ratanaphum district, Songkhla province in Southern Thailand. The soil in the area is under the Tha Sae Series which is suitable for *Hevea* rubber, horticulture, and upland crops and vegetables, and generally it is well-drained with moderate permeability (Land Development Department, 2003). Specifically, it is found that the soil in the RC, R, and RB are medium to coarse textured with sandy loam, loamy sand and sandy clay loam, while that of RM is fine to medium textured consists of silt loam, clay and silty clay loam.

Rubber trees in the farms were RRIM 600, aged around ten years and planted in 6 m x 3 m spacing on flat land. The trees were tapped in S/3 2d3 tapping system (one-third spiral cut of tapping length with two-day tappings in three days) (Vijayakumar *et al.*, 2009) and their tapping had been implemented for four years. Farms were converted into intercropping in the first year of tapping, and the associated crop was planted between the rubber rows.

Weather data, such as temperature, relative humidity, rainfall, and raining days in the area were monitored by a mini weather station installed in the area. Leaf area index (LAI) of the farms were measured using the hemispherical photography method (Chen, Rich, Gower, Norman, & Plummer, 1997). Fisheye photos were captured by Nikon Coolpix 8400 camera from five different points in each farm. The captured images were analyzed using the GAP Light Analyzer software version 2.0. Soil moisture content (SMC) of the experimental farms were measured by PR2/6 profile probe (Delta-T Devices, Cambridge, UK) at the soil depths of 0-10 cm, 11-20 cm, 21-30 cm and 31-40 cm, respectively from two locations in each farm through three access tubes installed at each location.

Latex samples were collected from ten trees of each plot with three replications from each farm for measuring biochemical composition: sucrose content (Suc), inorganic phosphorus content (Pi), reduced thiols (R-SH) and dry rubber content (DRC). The samples were analyzed using the latex diagnosis method of CIRAD (Gohet & Channama, 1999).

From each farm, field latex from ten trees was collected with three replications to investigate the technological properties: ash content, nitrogen (N) content,

initial plasticity (Pi), plasticity retention index (PRI), and Mooney viscosity (MV). The test methods followed the RRIM test methods (Tong, 1992). Molecular weights (Mw) of the rubber from the samples were tested using the Gel Permeation Chromatography method with Agilent GPC/SEC software (Agilent Technologies, 2015). Field latex of each farm was also sampled from ten trees by three replications to analyse the average yields (gram per tapping per tree - g/t) (Malaysian Rubber Board [MRB], 2009).

All data collections and measurements were taken between May and August of 2020. The data were analyzed in ANOVA and compared by Duncan's multiple range test (DMRT) at $P \leq 0.05$. Multiple correlation analysis was applied to investigate relationships among the studied data.

3. Results and Discussion

3.1 Weather condition

Generally, there are two seasons comprised of the rainy season and the dry season in the area. The average maximum and minimum temperatures are typically 30 °C and 37 °C, respectively. The monsoon rainy season starts from May to January with 2,200 mm of average annual rainfall. The dry season lasts three to four months from January to April with around 6 to 7 hours of average sunshine period per day while the other months have around 4 to 5 hours per day (Thai Meteorological Department, 2019). Table 1 describes monthly weather conditions (from May to August 2020) analyzed from the records of the mini weather station where the experiments were conducted. The total rainfall in the farms' area during the study period was over 900 mm which was accounted for around 40% of the average total annual rainfall. The mean temperatures were relatively lower in June, July and August, compared to that of May. The maximum and minimum relative humidity increased over 95% and 50%, respectively in those three months also.

3.2 Soil moisture content

In the soil depth of 0-10 cm, SMCs of R and RC were 21.6% and 23.1%, respectively, and higher than that of RB and RM (Table 2). However, it was noticed that RM showed the highest SOM significantly in the deeper soil layers. It was also observed that SMC of RM and RB increased dramatically with the soil depths. RC could show the highest SMC only in the soil depth of 0-10 cm, but in the other soil depths, its SMCs were at the lowest level compared to that of the other experimental farms. Chen, Liu, Wu, Jiang, and Zhu (2019) reported that rubber-intercropping significantly improved SMCs because of its significant moisture-holding capacity of capillary porosity in the average soil depth due to its better root distribution. The higher SMC in the average deep soil could be the complementary result of a rubber-based intercropping system as the crops shared nutrients, soil water and also the space in the soil (Langenberger *et al.*, 2017; Wu, Liu, & Chen, 2016) that enhanced the root proliferation which improved soil porosity, hydraulic conductivity, water infiltration and soil water holding capacity (Chen, Liu, Jiang, & Wu, 2017; Elmholt *et al.*, 2008).

Table 1. Monthly weather condition in the study area (May to August 2020)

Month	Mean temperature (°C)	Maximum relative humidity (%)	Minimum relative humidity (%)	Rainfall (mm)	Raining days
May	29.4	80	42	304.7	21
June	28.5	96	53	208.9	18
July	28.2	97	51	319.9	22
August	28.3	96	51	83.7	13

Table 2. Soil moisture content (SMC) among the farms

Studied farms	Soil moisture content (%)			
	0-10 cm	11-20 cm	21-30 cm	31-40 cm
Monocrop rubber	21.62 ab	24.09 b	27.88 b	26.12 b
Rubber-coffee	23.06 a	20.73 c	21.58 c	23.66 c
Rubber-bamboo	15.27 b	21.27 c	26.57 b	26.62 b
Rubber-melinjoo	12.39 c	30.95 a	34.53 a	40.26 a

Means with different letters in each column are significantly different at $p \leq 0.05$ and ranked by the DMRT.

Table 3. Leaf area index (LAI) among the farms

Studied farms	Leaf area index (LAI)
Monocrop rubber	1.02 c
Rubber-coffee	1.26 b
Rubber-bamboo	1.44 a
Rubber-melinjoo	1.39 ab

Means with different letters in the column are significantly different at $p \leq 0.05$ and ranked by the DMRT.

3.3 Leaf area index

Table 3 represents the LAIs among the farms. RB and RM significantly showed the highest LAI values of around 1.4, followed by RC with 1.26, while that of R was the smallest value, which was about 30% lesser than the highest value. The results of LAIs confirmed that the above-ground vegetative growth of RB, RM and RC were greater than that of the monocrop rubber farm. High LAI values in a multi-story rubber intercropping allowed efficient light distribution through the canopies of the crops in the system, thus a greater light energy capture, ensuring in improved photosynthesis function (Chow, Qian, Goodchild, & Anderson, 1988; Vandermeer, 1992).

3.4 Latex biochemical composition and yield

Table 4 depicts the physiological status expressed in biochemical composition, namely Suc, Pi and R-SH contents, DRC of the latex and yield sourced from the farms. It was found that Suc content of R was the highest at 4.87 mM, followed by that of RC with 3.70 mM, while RB and RM showed around 2 mM as the lowest content. RM significantly showed the highest values of Pi with 31.49 mM that was around 96% higher than that of RB. Pi's lowest values were observed in R and RC with about 12 mM, which was 163 and 34% lesser than that of RM and RB, respectively. R-SH contents of RM, RB and RC were 0.80 mM, 0.76 mM, and 0.67 mM, respectively, and higher than that of R, which was the minimum level at 0.62 mM. DRC of RC and R were the highest at 40.02 and 39.44%, respectively, followed by RB

with 36.14%, while RM showed the lowest DRC of 28.93%. Average yields of a tree from the farms were ranged between 30 and 40 g/t. Although yield analysis data did not prove statistical differences among the farms significantly, it was found that R delivered the least yield among the others.

Sucrose is the raw material of rubber synthesis and initiates the generation of energy for synthesis. High content of Suc indicates either the efficient supply of laticiferous rings or low metabolic utilization of sucrose for the rubber synthesis (d'Auzac *et al.*, 1997). Since Pi represents the level of laticifer biosynthetic activity or metabolic utilization of sucrose (Jacob *et al.*, 1989), rubber trees in RM and RB with higher values of Pi and lower values of Suc were under the healthy physiological status with efficient utilization of sucrose for latex production. R-SH are antioxidants that reduce the oxidative stresses resulted from latex harvesting. Thus, the high content of R-SH in RM and RB signaled better physiological status of rubber trees. In contrast, the lowest R-SH value in R depicted the laticiferous system's poor physiological condition or overexploitation of latex from the rubber tree (d'Auzac *et al.*, 1997). High DRC with less yield resulted in the farm R, also reflected the less efficient metabolism of latex biosynthesis. DRC represents the laticifers' synthesis activity and regeneration capacity, and the standard DRC from fresh latex ranges between 30 and 35% by weight of latex (Sarith Kumara, 2003). A distinct low level of DRC signals a deficient metabolism or incomplete regeneration process. Likewise, a significant high DRC increases latex viscosity and limits the latex flow, resulting in low latex production (Van Gels, 1951). When the water availability is limited for the laticiferous cells, latex production typically reaches the lowest associated with the high DRC of the latex (Pakianathan, Boatman, & Taysum, 1966). Vijayakumar, Chandrashekar, and Philip (2000) reported that a high turgor pressure increased the latex flow rate leading to a higher latex volume with a slight decrease in DRC.

3.5 Technological properties

In comparing the non-rubber components, namely nitrogen (N) content and ash content, of the raw rubbers from

Table 4. Latex biochemical composition (Suc, Pi, R-SH, DRC) and yield among the farms

Studied farms	Suc (mM)	Pi (mM)	R-SH (mM)	DRC (%)	Yield (g/tt)
Monocrop rubber	4.87 a	12.16 c	0.62 d	39.44 a	30.69
Rubber-coffee	3.70 b	12.22 c	0.67 c	40.02 a	38.06
Rubber-bamboo	1.93 c	16.09 b	0.76 b	36.14 b	39.97
Rubber-melinjo	2.08 c	31.49 a	0.80 a	28.93 c	35.85

Means with different letters in each column are significantly different at $p \leq 0.05$ and ranked by the DMRT.

the farms, R and RC had the lowest contents in N around 0.3%, but the highest ash contents with 0.42 and 0.43%, respectively, compared to the others (Table 5). RM showed the highest content of N at 0.39%. The lowest levels of the ash content were found in the raw rubbers of RM and RB with 0.32 and 0.31%, respectively. N content in the raw natural rubber represents a residue of protein content that affects the curing properties and efficiency in vulcanization processes (Sadeesh Babu, Gopalakrishnan, & Jacob, 2000). Results of the nitrogen content ranged between 0.3 and 0.4%, which did not exceed the maximum level of 0.6% recommended for all grades of the technically specified rubber (Sadeesh *et al.*, 2000; MRB, 2009). The ash content represents the presence of nonvolatile mineral oxide (Giraldo-Vasquez & Velasquez-Restrepo, 2017), and the results' values were less than the standard permissible amount 0.6% (MRB, 2009).

Regarding the rheological properties, RM delivered better results in P_0 of 38, PRI of 98, and MV of 69, compared to the others. RB showed high values of P_0 and PRI with 36 and 96, respectively. However, its Mooney viscosity was the lowest at 60 among that of the farms. RC had good results in P_0 and PRI and averaged in MV, whereas R resulted in low values of P_0 , PRI, and MV at 36, 97, and 61, respectively. All results of the P_0 and PRI showed significantly higher levels as they were higher than the standard minimum level recommended for most technically specified rubber (MRB, 2009). The MV of the raw rubber from RB and R had the lowest values showing around 60. These values are in the range of the optimal feature of the MV required by most rubber manufacturer which is around 60 to 65 ML (1+4) 100 °C because harder rubber (over 70 in MV) required larger power consumption for mastication (Sadeesh *et al.*, 2000).

In comparing the average molecular weights (Mw) of the raw rubbers from the farms, RM and RB delivered higher Mw of 19.45×10^3 and 20.99×10^3 g/mol, respectively, compared to the others, R and RC, which had 18.08×10^3 g/mol and 18.48×10^3 g/mol, respectively. Mw of raw natural rubber is a major determinant of the raw rubber's processibility. It widely ranges typically between 10^4 and 10^5 g/mol according to not only cultivars and the tree's age but also the soil condition and seasonal variation of the rubber farm (Kovutikulrangsi & Sakdapipanch, 2005).

3.6 Relationships among parameters

It was found that the LAI was negatively correlated to the Suc content but positively to the RSH content, significantly. Since low content of Suc associated with high RSH content reflects efficient utilization of sucrose reserved by photosynthesis in rubber biosynthesis and less stress of physiological condition ensuring in improved latex production (d'Auzac *et al.*, 1997; Tupy, 1989). Meanwhile, the SMC

expressed a positive relation with the Pi content but a negative association with the DRC. Roux *et al.* (2000) observed that water availability was a significant determinant for latex synthesis and rubber tree metabolism activities. Pi represents a rubber tree's metabolism, and a rubber cultivar that has a high content of Pi is regarded as high metabolism clone that delivered a high volume of latex but a slight decrease in DRC (Jacob *et al.*, 1989).

The LAI also affected the non-rubber components as it increased with a decrease of the ash content and an increase of the N content. These relations replicated the findings of Moreno, Ferreira, Goncalves, and Mattoso (2005) that a minimum level of ash content was observed during the rubber tree's leaf area developed its maximum. They also found that the N content was falling when the leaf defoliation period. High N content in natural rubber was associated with increased protein biosynthesis (Othman, Hepburn, & Hasma, 1993), showing high photosynthesis efficiency which could be improved by leaf area development (Moreno *et al.*, 2005).

SMC did not show a significant correlation to the non-rubber components in the study. Regarding the associations with the rheological properties, however, it expressed a significant association with the MV, whereas the LAI values were directly proportional to the Mw. Although the study did not show an apparent association between the MV and Mw, some studies reported that maximum leaf area and sufficient moisture availability favored low evapotranspiration (Jacob *et al.*, 1989; Roux *et al.*, 2000), leading to more significant isoprene biosynthesis resulting in higher Mw. A study by Kovutikulrangsi and Sakdapipanch (2005) observed the latex from matured rubber trees delivered high Mw compared to the young rubber trees.

There were some associations among the technological properties as P_0 , PRI and MV had positive correlations themselves. These relations replicated an observation of Roux *et al.* (2000) that seasonal variations of P_0 , PRI and MV followed a similar trend. The Mw showed negative correlations with the ash content and the PRI values, but it was positively related to the N content. Since the Mw was positively associated with the LAI, it showed similarly as the LAI's relations with the ash content and N content. Roux *et al.* (2000) also reported that high production of the macromolecular chain of a high metabolism clone could not be protected completely, leading to high sensitivity to thermo-oxidative degradation resulting in lower PRI value.

There were some associations between the biochemical compositions and the technological properties since the ash content increased with the Suc content and decreased with the N content and the Mw. Ash contents were at a high level associated with high DRC when water availability was limited, causing less translocation of inorganic elements from the absorbed nutrients (Giraldo-

Table 5. Technological properties (Ash, N, P_i, PRI, MV, Mw) of raw rubber sourced from the farms

Studied farms	Ash (%)	N (%)	P _i	PRI	MV (mL (1+4) 100 °C)	Mw (g/mol)
Monocrop rubber	0.42 a	0.30 c	36 b	97 b	61 c	18.08 x 10 ³ b
Rubber-coffee	0.43 a	0.32 c	38 a	99 a	67 b	18.48 x 10 ³ b
Rubber-bamboo	0.31 b	0.37 b	36 b	96 b	60 c	20.99 x 10 ³ a
Rubber-melinjo	0.32 b	0.39 a	38 ab	98 a	69 a	19.45 x 10 ³ a

Means with different letters in each column are significantly different at $p \leq 0.05$ and ranked by the DMRT.

Table 6. Pearson correlation coefficients within the study parameters: LAI, SMC, latex biochemical compositions (Suc, Pi, R-SH) and technological properties (Ash, N, P_i, PRI, MV, Mw)

	LAI	SMC	DRC	Suc	Pi	R-SH	Ash	N	P _i	PRI	MV	Mw
LAI	1.00											
SMC	0.17	1.00										
DRC	-0.60	-0.81	1.00									
Suc	-0.97	-0.25	0.73	1.00								
Pi	0.55	0.88	-0.99	-0.66	1.00							
R-SH	0.90	0.52	-0.89	-0.96	0.84	1.00						
Ash	-0.79	-0.23	0.76	0.90	-0.66	-0.89	1.00					
N	0.88	0.52	-0.90	-0.95	0.86	0.99	-0.91	1.00				
P _i	0.28	0.58	-0.29	-0.16	0.40	0.28	0.20	0.23	1.00			
PRI	-0.21	0.53	-0.02	0.31	0.16	-0.15	0.58	-0.19	0.88	1.00		
MV	0.21	0.81	-0.50	-0.16	0.61	0.35	0.10	0.32	0.94	0.87	1.00	
Mw	0.84	-0.18	-0.42	-0.88	0.30	0.73	-0.89	0.74	-0.28	-0.70	-0.33	1.00

The correlation coefficients in bold are significantly different at $p \leq 0.05$ (Pearson linear coefficient, $|r| \geq 0.7$).

Vasquez & Velasquez-Restrepo, 2017). These conditions could delay metabolic utilization of sucrose, leading to high sucrose content in the latex so that lower Mw resulted. On the other hand, the N content showed a significant positive association with Pi and R-SH contents. Pi is essential for the formation of nucleic acid, which induces protein biosynthesis (Coupe & Chrestin, 1989). High N content was followed by high Pi and R-SH values that improve the physiological status of laticiferous system and stability of organelles, particularly hutoids in latex (d'Auzac *et al.*, 1997).

4. Conclusions

The study comparing the LAIs confirmed the higher remarkable growth of the above-ground vegetative parts in the rubber-based intercropping system than that of the monocrop rubber farm. The rubber-based intercropping farms conducted in the study expressed a higher content of the soil moisture, one of the below-ground components of the agroecosystem, in the soil depth of 11-40 cm. And it was also worth noting that the intercropping farms, RM and RB, dramatically increased the SMC with the soil depths. Regarding the rubber trees' physiological status in the farms, the rubber-based intercropping farms indicated better biochemical composition in the latex, particularly in Suc, Pi, R-SH, showing efficient metabolism of the latex biosynthesis. Technological properties of the raw rubber sourced from the rubber-based intercropping farms also reflected the premium results in both non-rubber components and rheological properties with higher molecular weights. The correlation analysis found some significant relations notably between the LAI and technological properties of N content, ash content and Mw, and between the SMC and MV. Likewise, the associations of the physiological changes to the technological properties were observed

particularly between the biochemical composition (Suc, Pi and R-SH) and the non-rubber components (N content, ash content) and Mw. In conclusion, this preliminary study could highlight some changes in the rubber-based intercropping system in terms of mainly LAI, SMC and physiological status, and their relations to the technological properties of the raw natural rubber sourced from the system.

Acknowledgements

The authors wish to acknowledge Thailand's Education Hub for ASEAN Countries (THE-AC) scholarship, Graduate School, Prince of Songkla University, and the faculty of Natural Resources, Prince of Songkla University for supporting facilities to conduct the study. The study was financially supported by the Office of the National Digital Economy and Society Commission under the Digital Economy and Society Development Funds 2020 (Project no. ONDE 1-025/63).

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INFOGRAPHIC OF THE THESIS RESEARCH

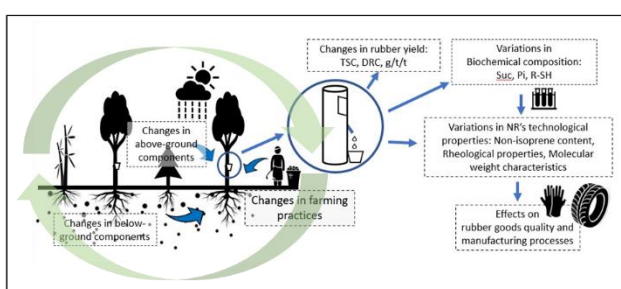
Implications of Agroecological Changes in Rubber-based Intercropping System on the Sustainability of *Hevea* Rubber Production

Rational:

Rubber-based intercropping has been recommended for ecological and economic benefits. However, **uncompetitive growth and yields of the crops under some combinations and conventional farming practices** are experienced on the ground. And it still needs to understand the **implications of these ecophysiological changes** in the system on the production and technological properties of *Hevea* rubber.

Hypothesis:

- **Complementarity in the system** – With **compatible associated crops & Integrated farming practices** – facilitative interactions among the above- and below-ground components ensuring ecological and economic improvements in the long term
- **These ecophysiological changes in the agroecosystem** – affect the **production and technological properties of *Hevea* rubber**



Conceptual framework of the research

Objectives:

Overall objective: to ensure the sustainability in natural rubber production integrated with intercropping systems concerning agroecological changes

Experiment I – to investigate the changes in agroecosystem of a rubber-based intercropping farm and their interactions under integrated fertilizations mixed with organic soil amendments

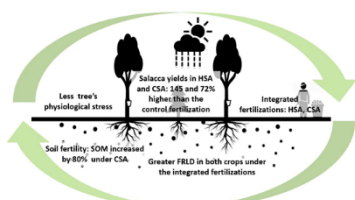
Experiment II – to study the seasonal changes in leaf area coverage and soil water content under rubber-based intercropping farms, and their interrelations with the latex biochemical compositions, yield and technological properties of *Hevea* rubber.

Experiment I: “Complementarity in rubber-salacca intercropping system under integrated fertilization mixed with organic soil amendments”

Methodology:

Treatments: Integrated fertilizations combined with two organic soil amendments: humic acid (HSA); chitosan (CSA) compared to conventional chemical fertilization
Study parameters: soil organic matter (SOM), root traits, physiological status of the crops, crops' productions

Results:

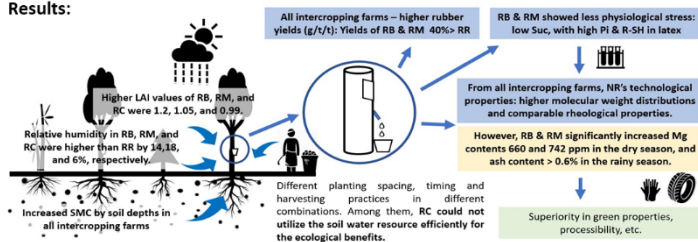


Experiment II: “Variation in latex production technological properties of *Hevea* rubber in relation to seasonal ecophysiological changes under different rubber-based intercropping practices”

Methodology:

Treatments: Three rubber-based intercropping farms: rubber-bamboo (RB); rubber-melino (RM); rubber-coffee (RC), and one rubber monocropping farm (RR) were selected.
Study parameters: Relative humidity, temperature, leaf area index (LAI), soil moisture content (SMC), latex biochemical composition, latex production, technological properties (non-isoprene contents, rheological properties, molecular weight characteristics) of *Hevea* rubber

Results:



Concluding Remarks of the thesis research's findings:

Both experiments confirm that complementarity interactions in the rubber-based intercropping system improve the farm's agroecology and tree's physiological conditions, ensuring optimal latex yields along the seasons. Selecting the compatible associated crops, and implementing integrated nutrient management and farming systems: planting space & timing, and harvesting practices – are crucially important.

Greater molecular weight structures of *Hevea* rubber – sourced from the rubber-based intercropping – imparted superiority in technological properties. However, Mg and ash contents in rubber from the intercropping farms – excessively higher in the dry season.

This thesis research would contribute to the sustainable valued chain of *Hevea* rubber production integrated with the rubber-based intercropping system ensuring the complementarity benefits in the farm ecosystem leading to the superiority technological properties of *Hevea* rubber.

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Educational Attainment

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Bachelor of Science. (Mathematics)	Dagon University	2000
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Scholarship Awards during Enrolment

- The 2018-2021 scholarship awards for the degree of Doctor of Philosophy in Tropical Agricultural Resource Management from Thailand's Education Hub for Southern Region of ASEAN Countries (THE-AC)
- Thesis research grant in 2019 for the degree of Doctor of Philosophy in Tropical Agricultural Resource Management from the Graduate School, Prince of Songkla University
-

List of Publications and Proceedings

- Zaw, Z. N. and Myint, H. 2016. Common agricultural practices and constraints of natural rubber industry in Myanmar. *Songklanakarin Journal of Plant Science*, 3(3): 1-8
- Zaw, Z.N., Sdoodee, S. and Lacote, R. 2016. Preliminary study on low intensity tapping system with rainguard in a high rainfall area in Myanmar. *In: Proceedings of CRRI and IRRDB International Rubber Conference 2016, Siem Reap, Cambodia. 21st November 2016. Cambodia Rubber Research Institute and International Rubber Research and Development Board. pp. 230-240. <https://agritrop.cirad.fr/582504>*
- Zaw, Z.N., Sdoodee, S. and Lacote, R. 2017. Performances of low frequency rubber tapping system with rainguard in high rainfall area in Myanmar. *Australian Journal of Crop Science*, 11(1): 1451-1456. <https://doi.org/10.21475/ajce.17.11.pne593>
- Zaw, Z.N., Chiarawipa, R., Pechkeo, S. and Saelim, S. 2022. Complementarity in rubber-salacca intercropping system under integrated fertilization mixed with organic soil amendments. *Pertanika Journal of Tropical Agricultural Science*, 45(1): 153-170. <https://doi.org/10.47836/pjtas.45.1.09>
- Zaw, Z.N., Chiarawipa, R. and Sdoodee, S. 2022. *Hevea* rubber physiological status and relationships under different rubber-based intercropping systems. *Songklanakarin Journal of Science and Technology*, 44(1): 6-12. <https://doi.org/10.14456/sjst-psu.2022.2>