MJFAS
Malaysian Journal of Fundamental and Applied Sciences

RESEARCH ARTICLE

Implications of Seasonal Ecophysiological Changes under Rubber-Based Intercropping Practices on Latex Production and Technological Properties of *Hevea* Rubber

Zar Ni Zaw, Rawee Chiarawipa*, Sayan Sdoodee

Agricultural Innovation and Management Division, Faculty of Natural Resources, Prince of Songkla University, Songkhla, 90110, Thailand

Abstract Rubber-based intercropping is a recommended practice due to its ecological and economic benefits. Understanding the implications of ecophysiological changes in intercropping farms on the production and technological properties of *Hevea* rubber is still necessary. This study investigated the effects of seasonal changes in the leaf area index (LAI) and soil moisture content (SMC) of rubber-based intercropping farms (RBIFs) on the latex biochemical composition, yield, and technological properties of *Hevea* rubber. Three RBIFs: rubber-bamboo (RB); rubber-melinjo (RM); rubber-coffee (RC), and one rubber monocropping farm (RR) were selected in a village in southern Thailand. Data were collected from September to December 2020 (S1), January to April 2021 (S2), and May to August 2021 (S3). Over the study period, RB, RM, and RC exhibited significantly high LAI values of 1.2, 1.05, and 0.99, respectively, whereas RR had a low LAI of 0.79. The increasing SMC with soil depths was pronounced in all RBIFs. RB and RM expressed less physiological stress and delivered latex yield, which was on average 40% higher than that of 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 mg/kg, respectively, in S2. Their dry rubbers had an ash content of more than 0.6% in S3.

Keywords: *Hevea brasiliensis*, leaf area index, latex biochemical composition, latex Mg content, molecular weight characteristics, rheological properties, soil moisture content.

Introduction

Natural rubber, a biosynthetic polymer, commercially sourced from monocrop cultivation of *Hevea* rubber trees (*Hevea brasiliensis*), is a strategically indispensable commodity for manufacturing a wide range of rubber-based products because of its unique features mainly elasticity, durability, flexibility, adhesive strength, and thermal resilience. These features are due to the inherent technological properties of *Hevea* rubber, including outstanding rheological and mechanical properties, and processability [1-2]. Unlike other crops, the *Hevea* rubber tree is primarily harvested for latex which is a secondary metabolite synthesized via the isoprene biosynthesis pathway in the plant defense mechanism. *Hevea* latex primarily contains rubber particles along with water, protein, sucrose, lipids, inorganic ions, and enzymes. These constituents are related to the physiological response of trees to the environment, and any changes in them affect the technological properties [3].

As leaf area, that represents the above-ground biomass production of farming land, is functionally associated with photosynthetic capacity, the variations in leaf area of a rubber farm highly influence the isoprene biosynthesis of rubber trees [4]. In addition, leaf area development on rubber farms improves understory microclimate conditions, governing the evapotranspiration of the farm linked with below-ground water availability [5]. However, the leaf area of the *Hevea* rubber tree typically decreases during the dry season when the soil water deficit is severe, and the tree limits water and nutrients translocations

***For correspondence:**
rawee.c@psu.ac.th

Received: 14 Aug. 2024
Accepted: 13 May 2025

owing to its deciduous nature [6], affecting the isoprene biosynthesis process and latex biochemical composition [7]. 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 rubber trees. Isoprene biosynthesis influences the molecular weight characteristics, which are the core determinants of the technological properties, of *Hevea* rubber. Thus, these molecular weight characteristics are highly associated with the supply of sucrose and nutrients for the isoprene biosynthesis pathway and water availability [8-9].

Previous studies have recommended implementing rubber-based intercropping and agroforestry systems not only for the economic benefits of small farmers by widening farm income sources but also for agroecological sustainability with improvements in the microclimate and soil conditions of the farms [10-13]. However, understanding the implications of these ecophysiological changes on the isoprene biosynthesis and the inherent technological properties of *Hevea* rubber is important to ensure the sustainable value chain of natural rubber production integrated with rubber-based intercropping practices. Exploring the changes in the physiological status and technological properties of *Hevea* rubber associated with rubber-based intercropping was previously conducted as a preliminary study [14]. The study noted significant differences between rubber-based intercropping and rubber monocropping farms in soil moisture content, leaf area index and rubber tree's physiological status, affecting the technological properties of *Hevea* rubber. However, since the results were based on the preliminary data collected only during the rainy season, it was necessary to extend the study to understand seasonal variations in these parameters and their implications. Thus, as a continuation, the current study investigated the seasonal changes in leaf-area coverage and soil-water status of different rubber-based intercropping farms (RBIFs) compared with those of rubber monocropping farms, and their effects on the latex biochemical composition, latex yield, and technological properties of *Hevea* rubber.

Materials and Methods

Study Location and Planting Materials

The study selected three rubber-based intercropping farms (RBIFs): rubber-bamboo (*Gigantochloa nigrociliata*) (RB); rubber-melinjo (*Gnetum gnemon*) (RM); rubber-coffee (*Coffea canephora*) (RC) and one conventional rubber monocropping farm (RR), which were adjacently located at 6° 59' N, 100° 08' E in Khao Phra village, Rattaphum district, Songkhla province, southern Thailand. Soil types of the study area belong to the Tha Sae series with a feature of good drainage, aeration and water retention, and rich nutrients and are recommended for planting rubber, horticulture and upland rice [15]. The study was an on-farm experiment, and the farms were selected primarily based on geographical proximity to ensure homogeneity in soil type and environmental conditions. Therefore, as a limitation of the study, it was unable to include additional farms located in other different areas.

All selected farms planted the RRIM 600 rubber clone in monocropping in 2008 at a spacing of 6 m × 3 m on flat land and began 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) in 2014. RBIFs started planting the associated crops in the interrow space of the rubber trees in 2015. All selected farms applied compound chemical fertilizer, N-P-K (30-5-18), at a rate of 0.50 to 0.75 kg per rubber tree in May 2020, during the onset of the rainy season.

Microclimate Data and Soil Moisture Content

To measure the temperature and relative humidity (RH) of each farm, a data logger (CEM DT-172; Shenzhen Everbest Machinery, China) was installed in the middle of each farm. SMC of each farm at soil depths of 0-10, 11-20, 21-30, 31-40, and 41-60 cm, respectively, was measured every season in volume percent using the PR2/6 profile probe (Delta-T Devices, Cambridge, UK) through six access tubes installed at a distance of 1.5 m away from the rubber row at each farm.

Leaf Area Index

LAI of each farm was measured seasonally through the hemispherical photography by taking fisheye photos at a height of 1.5 m from the ground in a vertically upward position adjusted on the north pole compass [16] using a Nikon Coolpix 8400 camera (Nikon, Japan) from five randomly selected points in the interrow space of the farm. The fisheye photos were processed using the Gap Light Analyzer software (version 2.0; Simon Fraser University, Canada) for LAI analysis.

Latex Biochemical Diagnosis

Latex samples were taken every four months from three replicates of sampling. The samples were collected from ten randomly selected trees in each replicated plot in the early morning before normal tapping was carried out, by puncturing the bark at around 5 cm below the tapping cut. After discarding the first two to three latex drops, the flowing-out latex (around ten drops) was collected and kept at 4°C to ensure no extended metabolism. Subsequently, 1 mL of the latex sample was added to 9 mL of trichloroacetic acid solution (TCA 2.5% w/v mixed with ethylenediaminetetraacetic acid [EDTA] 0.01% w/v) to separate the serum from the sample for the analysis of biochemical composition: sucrose (Suc), inorganic phosphorus (Pi), and reduced thiols (R-SH), following the latex diagnosis method [17].

Latex Production

Latex samples were collected in triplicate at each farm to determine latex production. Latex production was expressed as dry rubber weight in gram per tree per taping (g/t/t) and calculated by measuring the latex weight and average dry rubber content (DRC) of randomly selected ten trees for each replication. To determine the DRC, 10 g of each latex sample was coagulated with 2% acetic acid solution, and then the coagulum was pressed, followed by oven-drying at 70°C for 16 h, according to the International Organization for Standardization method (ISO 126:2005).

Technological Properties

Fresh lattices were sampled every season from three replications of each farm to determine the technological properties and kept at 4°C. Each sample was then split into two parts to measure the 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 16 h. After drying, the rubber sheets were measured for the dry rubber properties, namely the ash content (%), nitrogen (N) content (%), initial plasticity (P_0), plasticity retention index (PRI), and Mooney viscosity (MV), according to the Rubber Research Institute of Malaysia (RRIM) test methods for Technically Specified Rubber [18]. Magnesium (Mg) content (mg/kg) was determined using 10 mL serum obtained from the coagulation, according to 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). Agilent GPC/SEC software was used for the calibration curve and determining the Mw and MwD [19].

Data Collection and Analysis

Data collections were carried out from September 2020 to August 2021; three seasonal periods were specified based on the climatic pattern of the area: Season 1 (S1) (from September to December 2020), Season 2 (S2) (from January to April 2021), and Season 3 (S3) (from May to August 2021). Data were analyzed using a one-way analysis of variance. To compare the significant data among the farms, Duncan's multiple range test was performed at P<0.05, using the analytics software.

Results and Discussion

Seasonal Changes in Ecophysiological Conditions

Among the RBIFs, the mean RHs of RB and RM during the study period were higher than that of RR by 14 and 18%, respectively, whereas that of RC was only 6% higher than that of RR (Figure 1). However, the mean temperature of RB and RM was only 4% less than that of RR, and RC had the same mean temperature as RR. During S2, the temperature peaked in all studied farms, with the highest in RR and RC at 28°C and in RB and RM at 27 and 27.5°C, respectively. Evidently, the RBIFs, RB, RM, and RC, maintained their RHs between 73 and 70%, however, the RH in monocropping RR dropped to 66%. In S3, the average temperatures in all fields did not apparently change and remained the same as that in S2. However, the RH in all RBIFs (RB, RM and RC) increased to 82, 90, and 74%, respectively, in S3, whereas that of RR merely increased to 69%, which was the lowest among the farms. RB, RM and RC exhibited significantly higher LAI values on average over the study period at 1.2, 1.05, and 0.99, respectively, however, RR had a low LAI value of 0.79. Compared to those in S1, the LAI values in S2 decreased by around 45 to 49% in RM, RC, and RR, but only by 32% in RB. During S3, all RBIFs showed increased leaf area, and their LAI values were above one. However, the LAI value of RR was only 0.8, which was 32 to 30% less than that of the RBIFs (Figure 2). Increasing trends of SMC with soil depths were pronounced in all RBIFs (Figure 3). However, the SMC in the topsoil layer of RR was significantly higher than that in the other farms. During S2, all farms decreased their SMCs (Figure 3B); the RB farm showed the lowest SMC with an average value of 13.1%, and RM, RC, and RR had SMC of 18.3, 18.1, and 19.4%, respectively. Among the studied farms, RM had the highest SMC, on average, in all seasons, which was more evident in S1 and S3.

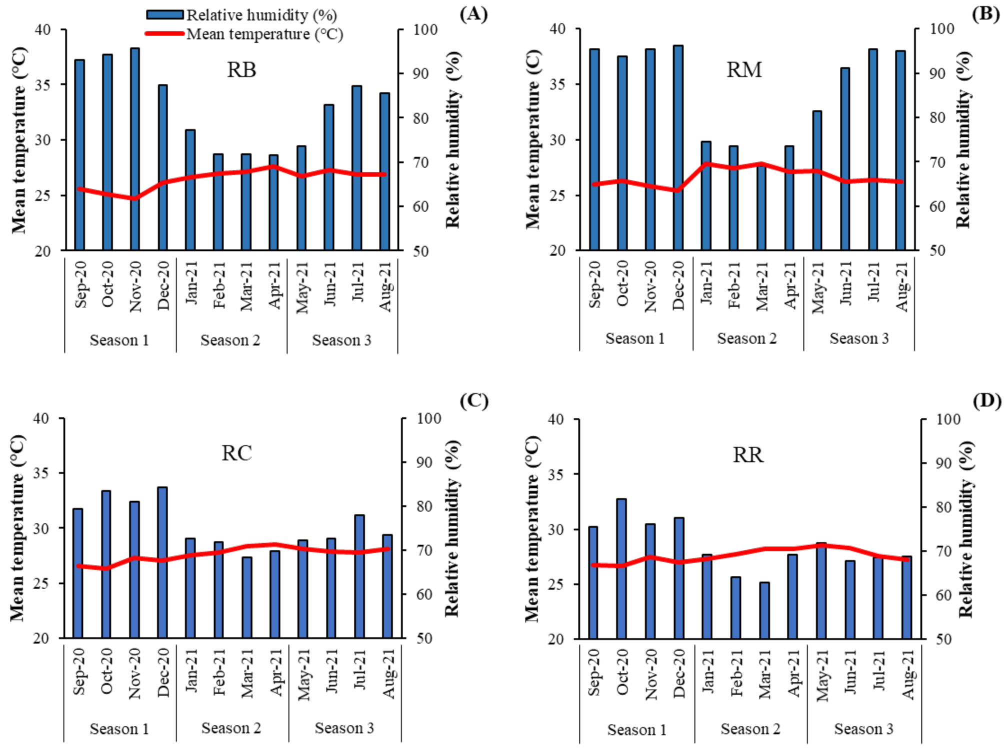

Figure 1. Monthly relative humidity and mean temperature in the studied farms: (A) rubber-bamboo intercropping (RB); (B) rubber-melinjo intercropping (RM); (C) rubber-coffee intercropping (RC); and (D) rubber-monocropping (RR), from September 2020 to August 2021

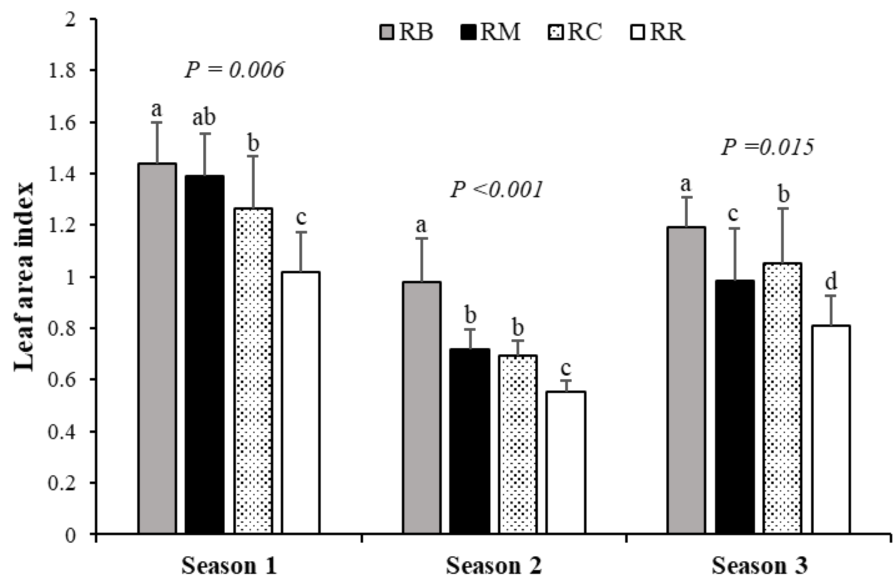

Figure 2. Leaf area index of the studied farms: rubber-bamboo intercropping (RB); rubber-melinjo intercropping (RM); rubber-coffee intercropping (RC); rubber-monocropping (RR), in season 1, season 2 and season 3. Different letters above the bars in each season indicate significantly different at P<0.05, tested by Duncan's multiple range test

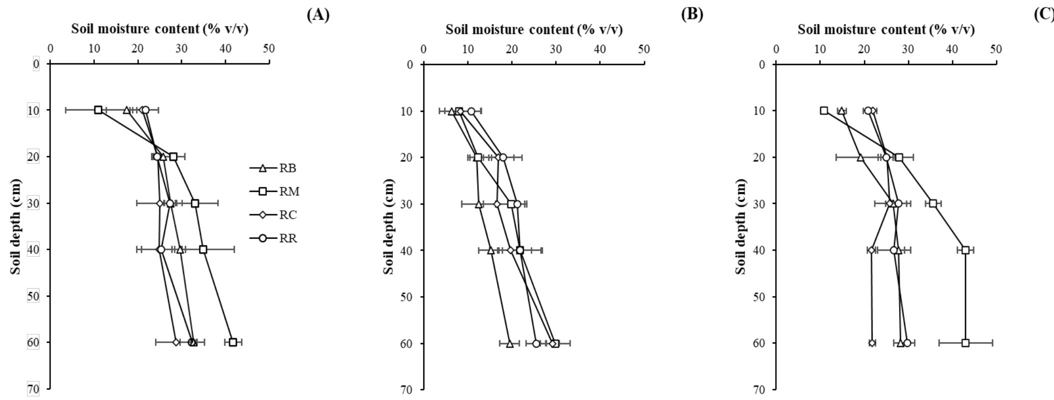

Figure 3. Dynamics of soil moisture content by soil depths in (A) season 1, (B) season 2, and (C) season 3 in the studied farms: rubber-bamboo intercropping (RB); rubber-melinjo intercropping (RM); rubber-coffee intercropping (RC); rubber-monocropping (RR)

Higher LAI values of the RBIFs than those of the monocropping farm in all seasons imply significant coverage of the above-ground vegetation in these intercropping systems. These results indicated that better growth of the above-ground vegetation resulting from the rubber-based intercropping system improved the understory environment of the farm by reducing the extreme weather conditions [6]. On average, higher SMC in the RR than in the RBIFs showed 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 soil depth under the RBIFs implied that soil water competition was generally intense in the topsoil layers. This is likely because the vertical root distribution of the associated crop, which was planted after the primary tree, was generally highly concentrated in the topsoil layer, thereby causing a significant amount of soil water consumption in the shallow soil layer rather than in the deeper layer [20]. However, the SMC trends by soil depth exhibited different degrees of soil water competition for the different associated crop combinations. They are associated with the above- and below-ground interactions that influence the microclimate conditions of farms. In RB, low SMC was observed at most soil depths, and was more pronounced in the dry season; however, on the above-ground, the LAI and RH were maintained at a high level in all seasons. This indicates the existence of a beneficial ecosystem in which vegetative development is processed through efficient soil water uptake and improved canopy transpiration. Bamboo species generally take up more soil water with a high-water-storage mechanism to facilitate the leaf transpiration process, indicating high water-use efficiency [21].

In RM intercropping, SMC was at the minimum level in the topsoil layer but significantly higher in the subsoil layer, in all seasons. Meanwhile, the above-ground understory environment maintained an improved ecosystem with relatively high LAI and RH in all seasons. Although the melinjo is originally a deep-rooted small- to medium-sized perennial tree with a height of around 15 m [22], in the RM combination, its height was maintained at about 1.5 to 2 m for leaf harvesting by regular pruning under the rubber tree. Because of regular leaf pruning throughout the year, the plant assimilates were translocated to the above-ground shoot biomass rather than the root system, thereby reducing root length density and root distribution in the deeper soil layer [23]. Therefore, melinjo root proliferation was likely more pronounced in the topsoil layer, leading to less competition for soil water uptake with the root system of rubber trees in the deeper soil layer [24-25]. 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 SMC dynamics by depth under RC were not evident as in the other RBIFs. Moreover, there was less RH in the understory environment. As the stomatal conductance of coffee species is highly sensitive to internal water deficit [26], the underlying reason could be related to the less transpiration of the coffee plant due to inadequate water uptake by the root system. Reportedly, the root system of coffee plants, intercropped with mature rubber trees under conventional spacing, is restricted by the pioneer invasion of the rubber roots, resulting in less accessibility to soil water and nutrients from most soil depths [12,27]. Thus, the RC combination in this study could not efficiently utilize the soil water resources for ecological complementarity as the other RBIFs could.

Variations in the Latex Biochemical Contents and Production of *Hevea* Rubber

In S1, the latex from RB and RM contained the lowest Suc, whereas that from RC and RR had the highest Suc content, significantly. Pi and R-SH in the latex of RM were the highest, followed by RB, whereas those of RC and RR were the lowest in S1 and S2 (Table 1). All latex biochemical compositions in the latex from all farms decreased to their minimum values in S2. Although there were no statistical differences in Suc and Pi among the farms, RB and RM farms showed lower Suc and higher Pi values than RC and RR farms. R-SHs of RB and RM were significantly higher than that of RC and RR, in S1. RB and RM showed 22 and 29% higher R-SH content than RR, respectively. However, in S2 and S3, the R-SHs were not statistically different between the farms. Comparing the latex production (daily yield) on average over the study period, RB and RM had the highest yield at 35 g/t/t, followed by RC at 28 g/t/t; however, RR had the lowest yield at 25 g/t/t. Latex productions in all farms in S2 were considerably lower than that in the other seasons (Table 2). Conversely, their DRCs were high in S2. In S3, although the production increased to over 35 g/t/t in RB and RM, it did not exceed 30 g/t/t in RC and RR. In S1 and S3, the DRC values ranged between 28 and 40% in all farms. In S2, except for RM, all farms increased the DRC to over 43%. However, the DRC in the latex from RM was the lowest (38%) in that season.

Regarding the biochemical composition, RB and RM showed lower Suc and higher Pi and R-SH in the latex than RC and the monocropping farm, RR, in S1. This indicates that RB and RM more efficiently utilized sucrose for polyisoprene biosynthesis in the tree defense mechanism with less physiological stress than RC and RR [28]. In the dry season, all biochemical contents were considerably decreased in the latex from all farms. Being a deciduous tree, during the dry season, when the water deficit is severe, *Hevea* rubber trees typically undertake the abscission process by completely or partially reducing the leaf area of the tree. With the depressive effect of defoliation, photosynthetic functions are restricted, resulting in less sugar supply. Therefore, the tree primarily utilizes the preserved carbohydrates from the sink for the new vegetative cycle rather than secondary metabolic functions [29]. During that period, because of the lack of latex biosynthesis metabolism in the laticiferous system, low Pi content was produced in the latex and accompanied by a low R-SH content, showing inactive enzymatic functions in the laticiferous system [30]. The low Suc content and high Pi and R-SH in the latex of RB and RM indicated that the rubber trees in these intercropping farms could effectively partition the assimilate translocations.

The results showed that the latex yields in the RBIFs were higher than that of the monocropping plot in all seasons. Because plant leaves are the essential vegetative part of the photosynthesis process, variations in leaf area influence the dry mass production and latex yield of rubber trees [4]. Similarly, soil water content has a significant effect on latex production because sufficient water content in the laticifers enhances latex flow with a longer flowing time, resulting in higher production [31]. These were reflected in the yield results of RB and RM in the dry season because their daily yields did not drop as much as those of RR and RC, which had low LAI and soil water uptake. In addition, during that season, Pi and R-SH in the latex of RB and RM were higher than those of RC and RR. This expressed stable metabolism in latex regeneration of the laticiferous system with less oxidative stress, imparted by the optimum soil water status and LAI of the intercropping farms even during the dry season.

Table 1. Latex biochemical contents in the studied farms by the seasons

Latex biochemical contents	RB	RM	RC	RR
Season 1				
Suc (mM)	1.93b	2.07b	3.71a	4.87a
Pi (mM)	16.08b	31.49a	12.22c	12.16c
R-SH (mM)	0.76a	0.80a	0.67b	0.62b
Season 2				
Suc (mM)	0.85	1.20	1.33	1.36
Pi (mM)	14.35	14.54	10.05	10.67
R-SH (mM)	0.57	0.65	0.53	0.48
Season 3				
Suc (mM)	9.33a	3.77b	6.76a	2.45b
Pi (mM)	20.67	17.64	23.86	18.59
R-SH (mM)	0.59	0.57	0.56	0.57

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. Suc = sucrose content; Pi = inorganic phosphorus; R-SH = reduced thiols; RB = rubber-bamboo intercropping; RM = rubber-melinjo intercropping; RC = rubber-coffee intercropping; RR = rubber monocropping.

Table 2. Latex production parameters of the studied farms by the seasons

Latex production parameters	RB	RM	RC	RR
Season 1				
Daily yield (g/t/t)	39.97a	35.85c	38.06b	30.69d
DRC (%)	36.14b	28.93c	40.02a	39.44b
Season 2				
Daily yield (g/t/t)	29.51a	21.32b	16.23c	15.63c
DRC (%)	44.50a	37.54d	43.19c	43.96b
Season 3				
Daily yield (g/t/t)	35.38b	47.82a	29.09c	28.47c
DRC (%)	37.34b	38.78a	36.32d	36.69c

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. DRC = dry rubber content; RB = rubber-bamboo intercropping; RM = rubber-melinjo intercropping; RC = rubber-coffee intercropping; RR = rubber monocropping.

Variations in the Technological Properties of *Hevea* Rubber

All farms had increased latex Mg content in S2. In that season, the latex Mg content in RB and RM remarkably increased to 660 and 742 mg/kg, respectively, whereas that in RC and RR slightly increased to approximately 413 mg/kg. In S3, the Mg content in the latex of RB and RC was over 600 mg/kg, significantly, whereas that in RM and RR was 454 and 412 mg/kg, respectively (Table 3). In S1, dry rubber from RC and RR had significant high ash contents of 0.44 and 0.42%, respectively; however, RB and RM had low ash contents of 0.31 and 0.32%, respectively. Compared between the seasons, the ash contents in S1 were lower than those in S2 and S3. In S2, RM had the lowest ash content among the farms, significantly. However, in S3, the ash contents in RB, RM, and RR were remarkably high (>0.60%), whereas that in RC was the lowest at 0.47%. In S1 and S2, the N content in the dry rubbers from RB and RM was high, ranging between 0.34 and 0.39%, whereas that of RC and RR was stable at approximately 0.3%. During S2, although the PRI values decreased, the values of P_0 and Mooney viscosity (MV) were higher than those of S1 for all rubbers from the studied farms (Table 3). 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 39.67 and 44, and MV at 64.97 and 65.83 ML (1+4) 100°C, respectively; however, RC and RR had low P_0 and MV. The lowest PRI value (92.43) was observed in the rubber of RR, and RBIFs had higher PRI values ranging from 93 to 98.

Table 3. Seasonal variations in non-isoprene contents and rheological properties of rubber from the studied farms

Technological properties	RB	RM	RC	RR
Season 1				
1. Non-isoprene contents				
Mg content (ppm)	333.67	404.33	380.67	367.00
Ash content (%)	0.31d	0.32c	0.44a	0.42b
Nitrogen content (%)	0.37b	0.39a	0.32c	0.29d
2. Rheological properties				
P_0	36.44b	37.84a	38.27a	36.17b
PRI	95.97c	98.13a	98.63a	97.11b
Mooney viscosity (ML (1+4) 100°C)	59.65d	68.87a	67.03b	61.45c
Season 2				
1. Non-isoprene contents				
Mg content (ppm)	660.07b	741.65a	412.68c	413.38c
Ash content (%)	0.61a	0.39d	0.463c	0.57b
Nitrogen content (%)	0.38a	0.34b	0.3c	0.32b
2. Rheological properties				
P_0	44.67c	62.00a	47.67b	49.00b
PRI	90.30b	75.00c	88.83b	91.5a
Mooney viscosity (ML (1+4) 100°C)	70.87d	90.70a	73.43c	76.93b
Season 3				
1. Non-isoprene contents				
Mg content (ppm)	617.46a	454.10b	603.77a	411.81c
Ash content (%)	0.65a	0.63a	0.47b	0.66a
Nitrogen content (%)	0.35b	0.37a	0.38a	0.38a

Technological properties	RB	RM	RC	RR
Season 3				
2. Rheological properties				
P_0	39.67b	44.00a	34.50c	33.33c
PRI	93.67b	96.73a	98.07a	92.43b
Mooney viscosity (ML (1+4) 100°C)	64.97b	65.83a	54.30c	51.20d

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. P_0 = initial plasticity; PRI = plasticity retention index; RB = rubber-bamboo intercropping; RM = rubber-melinjo intercropping; RC = rubber-coffee intercropping; RR = rubber monocropping.

All rubbers showed distinctly bimodal MwD in S1 and S3 (Figure 4A and 4C). However, they showed a relatively weak bimodal distribution in S2 (Figure 4B). In particular, the MwD of RR was the narrowest during that season. It likely had 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, the Mw of rubber from RB was the highest at 21.02 x 10^5 g/mol and the MwD of RB was at a minimum of 4.60; however, that of RM had the highest MwD of 7.54, followed by that of RR and RC with 6.18 and 5.91, respectively (Table 4). In S2, the Mw of RB, RM and RC considerably decreased by 27, 18, and 13%, respectively; however, that of RR decreased slightly by 3%. Compared with those in S1, the rubbers from all farms showed lower values of MwD in S2, in which RM was the highest at 6.71 and RR had the lowest value of 3.94. The Mw of rubbers from all farms increased in S3, and RC had the highest Mw among the farms significantly. However, the rubber from RC had the lowest MwD at 5.48, whereas that from RM had the highest MwD at 6.76.

Although the latex from all farms in S1 delivered Mg content ranging between 300 and 410 mg/kg, the RB and RM in the dry season, showed remarkably high Mg contents that were above the normal level of around 500 ppm, generally contained in fresh latex in that season [32]. However, the Mg contents in the latex of RC and RR during that season were below the normal level. As Mg is an essential nutrient for chlorophyll synthesis associated with photosynthesis process and plant growth, its concentration in plant leaves can be high in trees with a healthy physiological status [33]. However, during the onset of the deciduous period, with a reduction in leaf area, restrictions in photosynthetic capacity limit the utilization of primary metabolites, including magnesium. This induces the translocation of Mg, which has high mobility [34], into the laticiferous mechanism, resulting in increased accumulation of Mg in the latex. This high Mg content in the latex could be related to the high soil Mg content. High soil Mg, especially in severe water deficit conditions, could impart increased Mg levels in plant tissues and its incorporation into secondary metabolites [35]. However, it should be noticed that high Mg content affects the latex mechanical stability because the Mg^{2+} ions released from lutoids attract the rubber particles in the field latex colloidal, leading to fast coagulation [36]. Ash content in the dry rubber of all farms in S1 was stable and met the specification of technically specified rubber (TSR) recommended by ISO 2000:2020, not exceeding 0.6%. However, in S2, RB increased the ash content in the rubber. Ash content in dry rubber represents the residues of non-volatile minerals in the raw rubber; therefore, its variations can depend on the tree mineral uptake and soil water status [37]. Thus, in S3, with sufficient soil water due to rain and a high mineral supply from fertilizer application, RB and RM had high ash content in the dry rubber.

The results showed that throughout the seasons, N content in the dry rubber from all farms did not exceed 0.4%, which was below the technically specified limit of 0.6%, recommended by ISO 2000:2020. The dry rubber N contents of RB and RM were greater than those of RC in S1 and S2. Nitrogen is an essential plant nutrient for amino acid production for protein biosynthesis and is mainly associated with the primary functions of plant development [38]. Regarding the technological properties of raw rubber, the N content is considered to be the protein residue in dry rubber. An active supply of N can sufficiently reconstitute the protein lost in the harvested latex because N in the laticiferous enhances protein biosynthesis, inciting Pi content with latex metabolism [1]. Conversely, less translocation of nitrogen amid intense soil water deficit affects the photosynthetic capacity, metabolic pathways, and crop yields [39]. There was also observed that most *Hevea* rubber clones expressed less N content in the dry rubber during deciduous and flowering periods [37]. According to the above mentioned studies, the optimal N content in the dry rubber of RB and RM in the dry season reflects the active latex metabolism contributed by the greater LAI and soil water uptake in the rubber-based intercropping ecosystem. However, exceeding this limit affects the efficiency of successive processes, primarily the maturation rate and vulcanization properties of rubber [40].

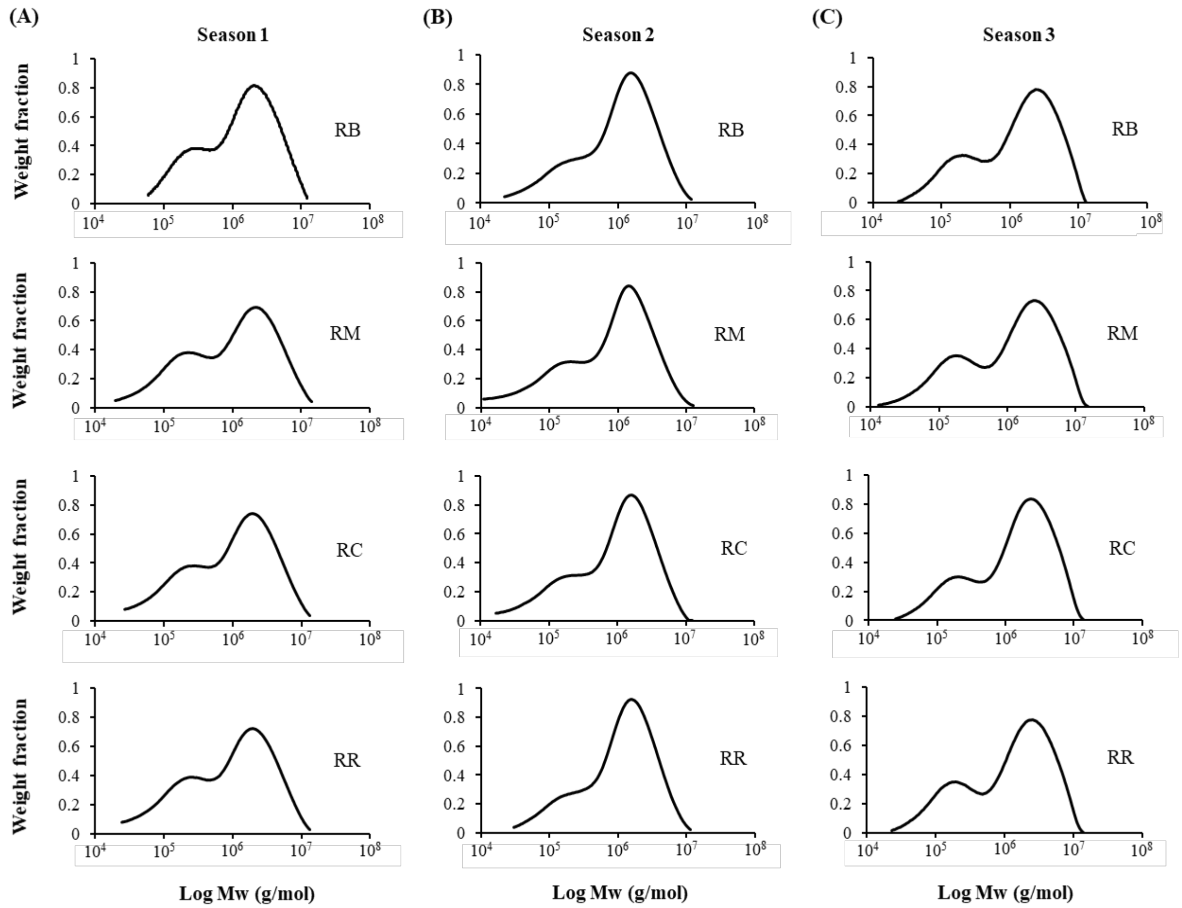

Figure 4. Molecular weight distribution of isoprene in the rubbers from the studied farms: rubber-bamboo intercropping (RB); rubber-melinjo intercropping (RM); rubber-coffee intercropping (RC); rubber-monocropping (RR), in (A) season 1, (B) season 2, and (C) season 3

Table 4. Molecular weight averages and distributions by the seasons

Seasons	RB	RM	RC	RR
Season 1				
Mw (g/mol)	21.02 x 10^5a	19.43 x 10^5b	18.50 x 10^5c	18.12 x 10^5c
MwD	4.60c	7.54a	5.93b	6.19b
Season 2				
Mw (g/mol)	15.37 x 10^5	15.87 x 10^5	16.12 x 10^5	17.5 x 10^5
MwD	4.41c	6.71a	5.65b	3.94c
Season 3				
Mw (g/mol)	22.00 x 10^5b	22.12 x 10^5b	23.21 x 10^5a	21.88 x 10^5b
MwD	6.18ab	6.76a	5.48b	5.91ab

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. Mw = molecular weight average; MwD = molecular weight distribution; RB = rubber-bamboo intercropping; RM = rubber-melinjo intercropping; RC = rubber-coffee intercropping; RR = rubber monocropping.

In terms of rheological properties, P_0, PRI, and MV were higher than the minimum acceptable levels recommended in the specification of TSR by ISO 2000:2020. P_0 and MV are generally tested to evaluate the visco-elastic behaviour of natural rubber, which is the core property of *Hevea* rubber considered as the processability of dry rubber [1]. PRI represents the thermal-oxidative resistance of natural rubber, an important feature of processability [40]. Increases in P_0 and MV values in S2 identically followed the changes in DRC, similar to the results reported that P_0 and MV values are associated with DRC and increase during the onset of the deciduous period [3]. This study observed significantly increased Mg content and relatively low PRI in the rubbers from RBIFs during the dry season. This may be because the decrease in photosynthetic capacity during the dry season enhances the latex Mg content together with the presence of reactive oxygen species in the laticiferous cells [33], leading to the oxidative degradation in the rubber molecules. We also observed the adverse effects of the excessively high Mg content on the rheological results of RM in the dry season, with significantly high values of P_0 and MV and extremely low PRI, indicating the inferior processability of hard rubber. This implies an intrinsically high oxidative degradable rubber with an undesirable visco-elastic nature. Hard rubber which has a high MV of over 60 ML (1+4) 100 °C requires large power consumption in the mastication process [41]. However, the other RBIFs did not exhibit unsatisfactory rheological properties, although their Mg contents were relatively high in S2 and S3.

The rubbers from all farms exhibited narrower MwD in the dry season than in other seasons, with some deviations from the normal bimodal distribution, revealing low composition of the low-molecular-weight fraction in the macrostructure of the rubber chain. The degree of deviation was more evident in the rubber from the monocropping farm. This implies that there is less gel content associated with crosslinks to non-rubber constituents in the isoprene chain [42]. Although *Hevea* rubber is structured as a 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 in the tensile strength and stability of unvulcanized rubber, called the green strength [42]. *Hevea* rubber, bearing a distinctly inherent bimodal distribution in its molecular structure, typically has superior mechanical properties with green strength owing to the complex distribution of low- and high-molecular-weight chains and gel formation in the isoprene chain [43]. This is one of the outstanding properties of *Hevea* rubber that synthetic rubbers cannot replace. High MwD and high contents of Pi and R-SH in the rubber from RM highlighted the significant implications of the soil water content and latex biochemical composition on the isoprene chain. Sufficient soil water supply enhanced nutrient translocation into laticiferous cells, imparting better latex biochemical compositions with increased Pi and R-SH contents. They not only improved the latex metabolism but also induced protein biosynthesis, resulting in high latex yield with better isoprene molecular structure [3,44].

Conclusions

All intercropping farms improved the understory environment with high RH and low temperature. These improvements were more pronounced in the RB and RM combinations because of the greater above-ground vegetative growth and efficient soil water uptake observed in these farms. With these improvements, rubber trees in these intercropping farms efficiently performed latex metabolism with less physiological stress and ensured optimal latex yields throughout the seasons. Thus, to ensure the ecological and economic benefits of rubber-based intercropping, the selection of associated crops and the farming system, including planting spacing and timing, and harvesting practices of the intercrops, are crucial to achieve the facilitative interrelations of the above- and below-ground components. Regarding the technological properties of rubbers from the intercropping farms, with high MwD, their rheological properties were comparable to those of rubber from the monocropping farm. However, Mg and ash contents were higher in the rubber of the intercropping farms than in the monocropping farm and exceeded the standard recommended limits, particularly during the dry season. These findings suggest the need to be aware of the non-isoprene content in *Hevea* rubber, sourced from RBIFs.

The findings of this study will contribute to the effective development of a sustainable value chain of natural rubber production integrated with intercropping practices, ensuring complementarity benefits in the farm ecosystem and the superiority of *Hevea* rubber's inherent technological properties.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

Acknowledgement

This research was supported by National Science, Research and Innovation Fund (NSRF) and Prince of Songkla University (Ref. No. NAT6701061S). The authors would like to thank the rubber quality testing laboratories from the Songkhla Rubber Regulatory Center, Rubber Authority of Thailand (Southern Region), and Sino-Thai International Rubber College for their testing services. The authors are grateful to Thailand’s Education Hub for ASEAN Countries (THE-AC) scholarship, Graduate School, Prince of Songkla University for providing research facilities for this study.

References

[1] Malmonge, J. A., Camillo, E. C., Moreno, R. M. B., Mattaso, L. H. C., & McMahon, C. M. (2009). Comparative study on the technological properties of latex and natural rubber from *Hancornia speciosa* Gomes and *Hevea brasiliensis*. *Journal of Applied Polymer Science, 111*, 2986–2991. http://doi.org/10.1002/app.29316

[2] Honorato, L., Dias, M. L., Azuma, C., & Nunes, R. C. R. (2016). Rheological properties and curing features of natural rubber compositions filled with fluoromica ME100. *Polimeros, 26*. http://doi.org/10.1590/0104-1428.2352

[3] Roux, Y. L., Ehabe, E., Saint-Beuve, J., Nkengafac, J., Nkeng, J., Ngolemasango, F., & Gobina, S. (2000). Seasonal and clonal variation in the latex and raw rubber of *Hevea brasiliensis*. *Journal of Rubber Research, 3*, 142–156.

[4] Zhu, J., Qi, J., Fang, Y., Xiao, X., Li, J., Lan, J., & Tang, C. (2018). Characterization of sugar contents and sucrose metabolizing enzymes in developing leaves of *Hevea brasiliensis*. *Frontiers in Plant Science, 9*, 58. http://doi.org/10.3389/fpls.2018.00058

[5] Andriyana, Y., Thaler, P., Chiarawipa, R., & Sopharat, J. (2020). On-farm effect of bamboo intercropping on soil water content and root distribution in rubber tree plantation. *Forests, Trees and Livelihoods, 29*(4), 205–221.

[6] Rappaport, D., & Montagnini, F. (2014). Tree species growth under a rubber (*Hevea brasiliensis*) plantation: Native restoration via enrichment planting in southern Bahia, Brazil. *New Forests, 45*, 715–732. http://doi.org/10.1007/s11056-014-9433-9

[7] Giraldo-Vasquez, D. H., & Velasquez-Restrepo, S. M. (2017). Variation of technological properties of field natural rubber lattices from *Hevea brasiliensis* clones and natural rubber-based compounds. *DYNA, 84*, 80–87. http://doi.org/10.15446/dyna.v84n203.65689

[8] Carr, M. K. V. (2012). The water relations of rubber (*Hevea brasiliensis*): A review. *Experimental Agriculture, 48*, 176–193. http://doi.org/10.1017/S0014479711000901

[9] Wei, Y., Zhang, H., & Wu, L. (2017). A review on characterization of molecular structure of natural rubber. *MOJ Polymer Science, 1*, 197–199. http://doi.org/10.15406/mojps.2017.01.00032

[10] Zhang, M., Yang, X. D., & Du, J. (2007). Soil organic carbon in pure rubber and tea-rubber plantations in South-western China. *Journal of Tropical Ecology, 48*, 201–207.

[11] Chen, C., Liu, W., Jiang, X., & Wu, J. (2017). Effect of rubber-based agroforestry systems on soil aggregation and associated soil organic carbon: Implication for land use. *Geoderma, 299*, 13–24. http://doi.org/10.1016/j.geoderma.2017.03.021

[12] Langenberger, G., Cadish, G., Martin, K., Min, S., & Waibel, H. (2017). Rubber intercropping: A viable concept for the 21st century? *Agroforestry Systems, 91*, 577–596. http://doi.org/10.1007/s10457-016-9961-8

[13] Chen, C., Liu, W., Wu, J., Jiang, X., & Zhu, X. (2019). Can intercropping with the cash crop help improve the soil physico-chemical properties of rubber plantations? *Geoderma, 335*, 149–160. http://doi.org/10.1016/j.geoderma.2018.08.023

[14] Zaw, Z. N., Chiarawipa, R., & Sdoodee, S. (2022). *Hevea* rubber physiological status and relationships under different rubber-based intercropping systems. *Songklanakarin Journal of Science and Technology, 44*, 6–12. http://doi.org/10.14456/sjst-psu.2022.2

[15] Land Development Department. (2003). *Characterization of established soil series in the Peninsular and Southeast Coast Region of Thailand reclassified according to soil taxonomy 2003*. Land Development Department.

[16] Bianchi, S., Cahalan, C., Hale, S., & Gibbons, J. M. (2017). Rapid assessment of forest canopy and light regime using smartphone hemispherical photography. *Ecology and Evolution, 7*, 10556–10566. http://doi.org/10.1002/ece3.3567

[17] Obouayeba, S., Soumahin, E. F., Regis, L., Essehi, J. L., Gohet, E., & Obouayeba, A. P. (2021). Improvement of productivity of the moderate metabolism clone GT 1 of *Hevea brasiliensis* Muell. Arg. by early upward tapping in Cote d'Ivoire. *American Journal of BioScience, 9*, 25–33. http://doi.org/10.11648/j.ajbio.20210901.14

[18] Rubber Research Institute of Malaysia. (2018). *RRIM test methods for Standard Malaysia Rubbers: SMR Bulletin no. 7*. Malaysian Rubber Board.

[19] Agilent Technologies. (2015). *An Introduction to Gel Permeation Chromatography and Size Exclusion Chromatography*. Agilent Technologies.

[20] Schenk, H. J. (2008). Soil depth, plant rooting strategies and species' niches. *New Phytologist, 178*, 223–225. http://doi.org/10.1111/j.1469-8137.2008.02427.x

[21] Mei, T., Fang, D., Roll, A., Niu, F., Hendrayanto, & Holscher, D. (2016). Water use patterns of four tropical bamboo species assessed with sap flux measurements. *Frontiers in Plant Science, 6*. http://doi.org/10.3389/fpls.2015.01202

[22] Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., & Anthony, S. (2009). *Gnetum gnemon*. *Agroforest tree database: A tree reference and selection guide version 4.0*. World Agroforestry.

[23] van Noordwijk, M., & Purnomosidhi, P. (1995). Root architecture in relation to tree-soil-crop interactions and shoot pruning in agroforestry. In F. L. Sinclair (Ed.), *Agroforestry: Science, Policy and Practice* (pp. 161–173). Springer Science.

[24] Bouttier, L., Paquette, A., Messier, C., Rivest, D., Olivier, A., & Cogliastro, A. (2014). Vertical root separation and light interception in a temperate tree-based intercropping system of Eastern Canada. *Agroforestry Systems, 88*, 693–706. http://doi.org/10.1007/s10457-014-9721-6

[25] Bayala, J., & Prieto, I. (2020). Water acquisition, sharing and redistribution by roots: Applications to agroforestry systems. *Plant Soil, 453*, 17–28. http://doi.org/10.1007/s11104-019-04173-z

[26] Avila, R. T., Cardoso, A. A., de Almeida, W. L., Costa, L. C., Machado, K. L. G., Barbosa, M. L., … DaMatta,

F. M. (2020). Coffee plants respond to drought and elevated [CO2] through changes in stomatal function, plant hydraulic conductance, and aquaporin expression. *Environmental and Experimental Botany, 177*, 104148. http://doi.org/10.1016/j.envexpbot.2020.104148

[27] Chiarawipa, R., Suteekanjanothai, P., & Somboonsuke, B. (2021). Adaptive ecophysiological characteristics of leaves and root distribution of Robusta coffee saplings as affected by age of rubber trees under an intercropping system. *Journal of Agricultural Science and Technology, 23*(2), 387–402.

[28] Atsin, G. J. O., Soumahin, E. F., Kouakou, T. H., Elabo, A. E. A., Okoma, K. M., & Obouayeba, S. (2016). Agronomic potential of some rubber tree clones (*Hevea brasiliensis*) of the fast metabolic activity class in the absence of hormonal stimulation in southwestern of Cote d'Ivoire. *American Journal of Experimental Agriculture, 13*(4), 1–13. http://doi.org/10.9734/AJEA/2016/26930

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

[30] Sreelatha, S., Simon, S. P., Kurup, G. M., & Vijayakumar, K. R. (2007). Biochemical mechanisms associated with low yield during stress in *Hevea* clone RRII 105. *Journal of Rubber Research, 10*, 107–115.

[31] Sainoi, T., Sdoodee, S., Lacote, R., Gohet, E., & Chantuma, P. (2017). Stimulation affecting latex physiology and yield under low frequency tapping of rubber (*Hevea brasiliensis*) clone RRIM 600 in southern Thailand. *Australian Journal of Crop Science, 11*, 220–227. http://doi.org/10.21475/ajcs.17.11.02.p305

[32] Puangmanee, S., Taweepreda, W., & Sdoodee, S. (2014). Effect of tapping method on natural rubber latex consistency. In *Proceedings of the Sixth International Conference on Science, Technology and Innovation for Sustainable Well-Being* (pp. 158–162).

[33] Hauer-Jakli, M., & Trankneret, M. (2019). Critical leaf magnesium thresholds and the impact of magnesium on plant growth and photo-oxidative defense: A system review and meta-analysis from 70 years of research. *Frontiers in Plant Science, 10*, 766. http://doi.org/10.3389/fpls.2019.00766

[34] Gerendas, J., & Fuhrs, H. (2013). The significance of magnesium for crop quality. *Plant Soil, 368*, 101–128. http://doi.org/10.1007/s11104-012-1555-2

[35] Ahmed, N., Zhang, B., Bozdar, B., Chachar, S., Rai, M., Li, J., Li, Y., Hayat, F., Chachar, Z., & Tu, P. (2023). The power of magnesium: Unlocking the potential for increased yield, quality, and stress tolerance of horticultural crops. *Frontiers in Plant Science, 14*. http://doi.org/10.3389/fols.2023.1285512

[36] Attanayake, A. P., Karunanayake, L., & Nilmini, A. H. R. L. (2018). Effect of ethephon stimulation on natural rubber latex properties; new insight into ethephon stimulation. *Journal of the National Science Foundation of Sri Lanka, 46*(2), 179–185. http://doi.org/10.4038/jnsfsr.v46i2.8418

[37] Moreno, R. M. B., Ferreria, M., Foncalves, P. D. A., & Mattoso, L. H. C. (2005). Technological properties of latex and natural rubber of *Hevea brasiliensis* clones. *Scientia Agricola, 62*, 122–126. http://doi.org/10.1590/S0103-90162005000200005

[38] Wang, M., Shem, Q. R., Xu, G. H., & Guo, A. W. (2014). New insight into the strategy for nitrogen metabolism in plant cells. *International Review of Cell Molecular Biology, 310*, 1–37. http://doi.org/10.1016/B978-0-12-800180-6.00001-3

[39] Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., & Suzuki, A. (2010). Nitrogen uptake, assimilation and remobilization in plants: Challenges for sustainable and productive agriculture. *Annals of Botany, 105*, 1141–1157. http://doi.org/10.1093/aob/mcq028

[40] Zhong, J. P., Li, C. P., Li, S. D., Kong, L. X., Yang, L., Liao, A. Q., & She, X. D. (2009). Study on the properties of natural rubber during maturation. *Journal of Polymer Materials, 26*, 351–360.

[41] Babu, P. S. S., Gopalakrishnan, K. S., & Jacob, J. (2000). Technically specified rubber. In P. J. George & C. J. Kuruvilla (Eds.), *Natural Rubber: Agromanagement and Crop Processing* (pp. 434–478). Rubber Research Institute of India.

[42] Thuong, N. T., Nghia, P. T., & Kawahara, S. (2018). Factors influencing green strength of commercial natural rubber. *Journal of Green Processing and Synthesis, 7*, 399–403. http://doi.org/10.1515/gps-2018-0019

[43] Kawahara, S., Yoshinobu, I., Sakdapipanich, J. T., Tanaka, Y., & Eng, A. H. (2002). Effect of gel on the green strength of natural rubber. *Rubber Chemistry and Technology, 75*, 739. http://doi.org/10.5254/1.3544999

[44] Cherian, S., Ryu, S. B., & Cornish, K. (2019). Natural rubber biosynthesis in plants, the rubber transferase complex, and metabolic engineering progress and prospects. *Plant Biotechnology Journal, 17*, 2041–2061. http://doi.org/10.1111/pbi.13181