Can fertilization be a driver of rubber plantation intensification?

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Abstract

Improving the productivity of existing rubber plantations is necessary to cope with the growing demand for natural rubber (NR) while limiting the environmental and social impacts linked to the expansion of rubber cultivation at the expense of natural ecosystems and food crops. The effect of fertilization on NR yield is still unclear and is poorly documented particularly in Thailand, the world leader in NR production. Hence, the main objective of this study was to re-assess the possible effects of fertilization on the yield of intensively managed rubber plantations in Thailand. Our main hypothesis was that the effect of fertilization would be higher with intensive latex harvesting practices (high tapping frequency with ethylene stimulation). To test this hypothesis, we set-up a split-plot experiment with four fertilization doses (T1, no fertilization, T2, T3, T4, respectively low, medium and high doses of NPK fertilizer) and two tapping systems (S/2 d2 with and without ethylene stimulation). Here, we present the results of the first three years on dry rubber yield, latex metabolism assessed with the latex diagnosis method, and latex and rubber properties related to their technological properties. Our results showed a positive effect of fertilization on yield from the first year, but the effect was only statistically significant ($p<0.05$) in the third year. The maximum effect of fertilization (+13%) compared to the control treatment (T1) was obtained with the highest dose of fertilizer (T4). Cumulatively over the 3-year period, the increase in yield was +5% with the T2 dose, and +8% with the T3 and T4 dose. Contrary to our main assumption, we observed no interaction between fertilization and ethylene stimulation. Latex diagnosis revealed that the effect of fertilization on yield was linked with a direct effect on latex metabolism mainly through an increase in inorganic phosphorus content (Pi). Finally, we observed no detrimental effect of the fertilizer treatments on latex and rubber quality. We can thus conclude that increasing latex yield through fertilization combined with intensive tapping does not involve any identified risks for the quality of rubber. Taken together, our results demonstrate that fertilization can help increase the yield of rubber plantations where intensive latex harvesting is practised. However, in a context of low NR prices for rubber farmers, the economic return of a +8% increase in yield is questionable. Our trial is continuing to assess the long term effects of the fertilization on yield as well as on the growth and nutritional status of the trees, and the nutrient balance of the plantation.

Keywords: Natural rubber, fertilization, ethylene stimulation, yield, latex metabolism, latex properties,

1. Introduction

Natural rubber (NR) extracted from rubber trees (*Hevea brasiliensis*) plays a crucial role in the socio-economic development of many tropical countries. NR is an indispensable raw material for several manufacturing industries, particularly the tyre sector which absorbs 70% of the total supply. During the last 20 years, high demand on the world market has led to the expansion of rubber plantations, particularly in South East Asia (Fox and Castella, 2013). Expansion raises questions about their environmental impacts as it triggers forest clearance (Guardiola-Claramonte et al., 2010; Hauser et al.; 2015; Hughes, 2017; Warren-Thomas et al., 2018) and about the socio-economic impact when rubber trees are planted on agricultural land where they compete with food crops (Fox and Castella, 2013; Chambon et al., 2016). One way to limit such impacts is to increase productivity per planted area by intensifying agricultural practices.

In most crops, intensification relies mainly on nutrient management and the use of fertilizers (Cassman, 1999; Witt et al., 2006). However, rubber tree is a crop where the harvested component, latex, is not a vegetative or reproductive organ whose biomass is directly linked to primary production. Natural rubber (NR) obtained from the latex of the trees is mainly comprised of cis, 1-4, polyisoprene, a secondary metabolite very rich in carbon (see review by Vaysse et al., 2012). Latex does not naturally exude from the trees and only tapped trees need to regenerate latex through a tapping-induced metabolism. Hence, improving tapping techniques has been the main driver of rubber plantation intensification along with the release of modern high-yielding clones. In particular, stimulation of latex metabolism with ethylene-based stimulant has made it possible to maintain or even increase the yield while reducing tapping frequency (Eschbach and Banchi, 1985; Gohet et al., 1996; Thanh et al., 1996; Zhu and Zhang, 2009; Lacote et al., 2010). Lacote et al. (2010) found the maximum increase in the yield of stimulated trees ranged from +30% to +78%, compared to that of unstimulated trees, depending on the clone. Conversely, the effect of fertilization on latex yield are still unclear, and this research topic has been almost ignored for the last 40 years, as most references in the literature date from the 1970s in Malaysia (Sivanadyan et al., 1972; Pushparajah, 1973; Yogaratnam and Weerasuriya 1984) or in Côte d'Ivoire (Compagnon, 1973; Du Plessix et al., 1973). Since the 1980s, the maximum reported increase in yield due to fertilization has been 26% (Onuwaje 1983) but most other reports showed no effects below 20% or no effects at all (Murbach et al., 1999; Virgens Filho et al 2001; Gohet et al. 2013).

For several authors, a mature rubber plantation is a forest-like ecosystem, which is ″self-sustainable" or in a "steady-state" with respect to the nutrient requirements of the trees (Krishnakumar et Potty, 1992; Sivanadiyan et al., 1995; further developed by George and Joseph, 2011). Hence, the management of fertilization of a rubber plantation is different than that of annual crops. In particular, high nutrient returns to the soil through leaf fall, self-pruning and fine root turnover need to be taken into account (Samarappuli, 2000). The recycling of these nutrients is hypothesized to be enough to support the yield and the functioning of the trees thanks to the low nutrient exports through latex harvesting, which is rather low compared to the tree biomass (4-5 kg/tree/year) and contains only small amounts of mineral nutrients (Bolton, 1964; Samarappuli, 2000; Murbach et al., 2003). According to these authors, annual exports of nitrogen range from 5 to 12 $kg.ha^{-1}.year^{-1}$ while recycling of N through litter falls amount to more than 20 $kg.ha^{-1}.year^{-1}$ (Murbach et al., 2003). These figures suggest that rubber tree only needs a small amount of fertilizer to support latex production and to enable a satisfactory girth increment during the mature stage. Based on experiments by Sivanadyan (1983), Watson (1989) reported that fertilization only needed to start four years after the beginning of tapping, and that N application would be sufficient if the plantation was well managed during the immature phase (e.g. with both leguminous cover and regular fertilizer applications). If required P, K and Mg fertilizers could be applied at 3 to 5-year intervals.

Fertilization can also change the mineral composition of the latex with possible detrimental effects on quality. For instance, divalent cations such as Mg^{2+} are known to be detrimental to latex stability, and industrial production of latex concentrate generally includes Mg^{2+} precipitation through phosphate addition prior to centrifugation. Collier and Lowe (1969) observed that nitrogen fertilization increased the Mg^{2+} content of field latex while decreasing dry matter of the latex and increasing nitrogen content of air-dried sheet rubber. The higher Mg^{2+} content reduced the mechanical stability of concentrated latex. Conversely, applying potassium or phosphate fertilizers may counteract the effect of Mg^{2+} on latex. A long-term experiment carried out by the Rubber Research Institute of Malaysia (cited in Watson, 1989) revealed a positive effect of K fertilizers on latex stability. The decrease in Mg content after K fertilizer is applied leads to antagonism of root uptake between the two cations. A study by Philpott and Westgarth (1953) demonstrated the beneficial role of combined K and P fertilizers in latex stability, which reduced the Mg/P ratio in the latex.

However, in Thailand, the world's biggest producer of NR, the amount of fertilizer recommended by R&D institutions is high and, in practice, farmers sometimes apply even more than the recommended rate of NPK fertilizers in tapped plantations (Chambon et al, 2018). A recent socio-economic survey showed that farmers believe such practices are beneficial (Chambon et al., 2018). However, this assumption is not supported by field data and such practices may not only be a waste of money for the farmers, but a source of environmental pollution if the nutrients are not effectively taken up by the trees. Such contradictions may arise from the indirect effects of NPK fertilization on latex yield through the improvement of the nutrient status of the whole tree, resulting in long-term benefits. As farmers' benefits rely on good management to maintain a balance between rubber production and plant growth, it is also important to understand how regular fertilization affects tree growth. Another possible explanation for these contradictions? is that Thai rubber farmers have adapted their fertilization practices to the very intensive tapping frequencies they use (Chambon et al., 2014). According to Gohet et al. (2013), potential gain from fertilization can only be achieved through the intensification of tapping practices. As tapping drives the demand for latex regeneration, it is not surprising that no effect of fertilization can be demonstrated if the same tapping system is applied in fertilized and unfertilized plots. However, interactions between latex harvesting technologies and fertilization of tapped rubber trees are poorly scientifically documented, particularly the use of ethylene stimulation to enhance latex yield, although, Sivanadyan et al. (1972) reported a positive interaction between fertilization and ethylene stimulation, which was partly confirmed by Gohet et al (2013).

In this context, the main objective of our work was to re-assess the possible effects of fertilizers combined with ethylene stimulation on latex yield in rubber plantations in Thailand. Our starting hypothesis was that there is a positive interaction between fertilization and stimulation, as the higher metabolic demand induced by stimulation would require additional nutrient resources. To this end, we analysed yield data from a three-year experiment with four fertilizer treatments applied to stimulated and unstimulated trees. To better understand the effect of fertilization and stimulation on yield, we collected data on the latex metabolism using the latex diagnosis approach (Jacob et al., 1989, d'Auzac et al. 1997). The underlying assumption was that fertilization would have an effect on latex metabolism before having a statistically noticeable effect on yield. We also measured certain latex properties to assess possible negative effects of fertilization on yield and on the quality of the latex.

2. Material and methods

2.1. Site description

The three-year experiment was carried out from May 2014 to April 2017 in a traditional rubber growing area at the Sithiporn Kridakorn Research Station (latitude 10°59'13.35"N, longitude 99°29'22.41"E) of Kasetsart University, Prachuap Khirikhan province, Thailand (Fig.1). The research station is located at the limit between two main climatic regions of Thailand: one with an equatorial climate, which extends southward from Chumphon province to the border with Malaysia, and the other with a tropical monsoon climate with long rainy season, which covers several provinces scattered in the northern, central and the east-central parts of the country. Weather data collected at the station from 2000 to 2010 showed average annual rainfall of 1985 mm (s.e. 383 mm) with 106 rainy days (s.e. 11 days) (Table 1). The rainiest months are October and November with more than 250 mm on average. The driest month is January with 64 mm on average. These climatic conditions are perfect for rubber tree cultivation with high latex yield potential (Gohet et al. 2015). Total rainfall in the two first years of the experiment (2014-15 and 2015-16) was respectively 20% and 27% lower than in the 2000-2010 reference period, and 22% higher in the third year (Table 1). The number of rainy days was higher than the reference period in all three years with respectively, 112, 109 and 129 days of rain. The soil of the experimental plot, classified as Rhodic Kandiudults, is deep with a sandy-loam texture. The topsoil (0-30 cm) has the following average characteristics: 7.7% clay; 71.4% sand; 20.9% loam; pH4.9 (1:1 water); 0.25% C and 0.032% N (Elemental Analysis); CEC 2.1 meq/100g (pH7 ammonium-acetate); available P 12 ppm and available K 32 ppm (ICP-AES).

Figure 1 and Table 1 to be inserted here

2.2. Experimental design

The experimental plot was a ca. 9 ha rubber plantation set up in 2007 using the *Hevea brasiliensis* RRIM 600 clone, which has medium to high metabolic activity and is used in 90% of rubber plantations in Thailand (Delarue and Chambon, 2012). The plot previously contained a coconut plantation. From planting to the beginning of the experiment in May 2014, the plantation was regularly weeded but not fertilized. The trees were planted with a spacing of 8 m between tree lines and 2.5 m between trees resulting in an average density of 500 $trees.ha^{-1}$. Tapping the tree bark to harvest latex started at the beginning of the experiment in May 2014. Only the trees with a trunk girth of 47 cm measured 100 cm above the ground were opened on this occasion. The first tapping cut was made 150 cm from the ground by removing a thin layer of bark along a downward half spiral on the tree trunk. The trees were then tapped every two days without any tapping rest in a week until the trees completely shed their leaves in February 2015. Tapping stopped during the refoliation period and resumed in May 2015 for another 10-month period using the same tapping system. This tapping system is noted S/2 d2 7d7 10m(MAY-FEB)/12 with respect to the revised international notation for latex harvesting technology (Vijayakumar et al., 2009). We did not use rain guards to make it possible to tap the trees on rainy days as it is not a common practice in Thailand. Hence, a substantial number of tapping days were loss every year because 90% of the rainy days occurred during the tapping period from May to February. In the end, trees were tapped 106, 115 and 106 days in the first, second and third year, respectively, out of the 150 tapping days expected with the d2 tapping frequency (i.e. 23% to 30% loss of tapping days).

The trial was set up as a split plot design in randomized complete blocks with four fertilizer treatments in the main plots, two tapping treatments in the subplots and four replications (blocks). Elementary plots contained 108 trees and covered an area of 2,160 m². Each plot was separated from adjacent plots or plantation edges by two lines of tree (16 m) or six trees (15 m) (Fig. 1). The four fertilization treatments were (T1) no fertilization, (T2) 75/45/100 $g.tree^{-1}.year^{-1}$ of N,P (as P_2O_5) and K (as K_2O) in one application in June in each year, (T3) 180/80/170 $g.tree^{-1}.year^{-1}$ of N,P and K in two applications in June and October in each year, and (T4), 306/136/289 $g.tree^{-1}.year^{-1}$ of N,P and K also in two applications (Table 2). Treatment T2 corresponded to the fertilization programme recommended by CIRAD in rubber estates (Gohet et al., 2013), while T3 corresponded to the current recommendation in Thailand (Chambon et al., 2018). Treatment T4 was based on the highest rate of fertilization found in a survey of fertilization practices used by rubber growers in Thailand (Chambon et al., 2018). The two tapping treatments differed in stimulation, no stimulation (NS) or four stimulations per year, i.e. in June, July, August and September (ST). Stimulation was done by applying ca. 0.8 $gram.tree^{-1}$ of a ready to use paste containing 2.5% of ethephon on 1 cm of the tapping panel located above the tapping cut. Stimulation is not usually applied when trees are tapped every two days (d2) but only when the tapping frequency is reduced (d3 and more). Our stimulated treatments thus represented a very intensive system in which we expected a clearer effect of fertilization according to the hypothesis that the potential gain from fertilization can only be achieved through intensified tapping.

The experiment included three tapping seasons hereafter referred to as "years" (first year or year 1 = May 2014-April 2015; second year or year 2 = May 2015-April 2016; third year or year 3 = May 2016-April 2017). In May 2015 and May 2016, trees that had reached 47cm of trunk girth measured 100 cm above the ground in the previous cropping seasons were opened. On average, 57%, 66% and 70% of the 54 trees in each subplot were tapped in 2014-15, 2015-16 and 2016-17, respectively. There was no statistical difference in the number of tapped trees between blocks and treatments (Table 1). We did not observed any significant rate of tapping panel dryness as well.

2.3. Measurements and data processing

2.3.1. Latex and rubber yield

On each tapping day, tapping started at 8 P.M. and latex was collected separately from each subplot between 5 and 8 A.M. The fresh latex was weighed immediately, and a 200 mL sub-sample was mixed with 400 mL of water to determine the dry rubber content (DRC) using the Metrolac method (Smith, 1947). DRC measurements with Metrolac were calibrated against the standard NF ISO126: 2005 twice a year (see 'latex and rubber quality' sub-section). This calibration indicated that the Metrolac method underestimated DRC by 17%. Rubber yield per subplot was obtained by multiplying fresh latex weight by calibrated DRC values. We then calculated the rubber yield per tree in $g.tree^{-1}$ by dividing the rubber yield per subplot by the number of tapped trees in the subplot. In May at the end of each tapping season, the number of tapped trees was updated to take the newly opened trees and the trees affected by tapping panel dryness into account. At the same time, we measured the trunk girth of all the trees in the experimental plots 1.7 m above the ground.

2.3.2. Physiological status of the latex cells (latex diagnosis).

Latex diagnosis (LD) is a method used to assess the impact of tapping on the physiological status of the latex cells (Jacob et al., 1989, D'Auzac et al. 1997). According to Jacob et al. (1989), the biochemical ability of latex cells to produce rubber is based on their sucrose (SUC), inorganic phosphorus (Pi) and thiol (RSH) contents. SUC reflects the balance between sucrose consumption by the latex cells for latex biosynthesis, and the transfer of sucrose from the apoplast to the latex cells. Pi indicates the intensity of metabolic activity in the latex cells and RSH indicates the efficiency of scavengers in counteracting oxidative stress. We applied the LD method as developed by CIRAD (Jacob et al., 1988, 1995). The latex diagnosis was performed each year in September or October during the most regular and high yielding period. In each subplot, a composite sample was obtained from ten randomly selected tapped trees and ten drops of latex were collected from each tree. The LD parameters were measured using the Ashwell anthrone method (1957) for SUC, the Taussky and Shorr method (1953) for Pi, and the Boyne and Ellman method (1972) for RSH. Sucrose, thiol and inorganic phosphorus contents were expressed in millimoles per litre of latex ($mmol.1^{-1}$).

2.3.3. Latex and rubber quality

Two sampling campaigns were carried out each year in October and June to collect latex and dry rubber samples for quality analysis. The campaigns were systematically planned around 15 days after fertilizer application. As it was not possible to obtain representative unsmoked sheet due to drying problems following the first sampling campaign in October 2014, supplementary sampling was conducted in January 2015. Several indicators of the technological properties of either latex or rubber were measured: dry rubber content (DRC), mechanical stability (MST) of the fresh latex; initial plasticity (P_0), the plasticity retention index (PRI) and its component P_{30} (SMR bulletin N°7 1992 - part B.8), and the ash content of rubber processed as unsmoked sheet (USS). Mineral content (N, P, K, Ca and Mg) was also analyzed in both latex films and rubber USS. Figure 2 summarizes the processing of the latex samples collected in each plot and the methods used to measure those indicators. Latex samples were collected between 4 am and 6 am in half the 32 subplots on a given tapping day, and in the other half on the following tapping day (normally 48 h later, if no rain was falling). However, MST was measured in all the subplots on the two collection days. We used the average of those two measurements as the MST value for one subplot. The standard NF ISO35:2006 method was used with the following adaptations: the sample was made of 80 mL of fresh latex instead of 80 mL of concentrated latex. The latex was not heated, and all measurements were made within two hours after collection. The rest of the latex sample was divided into three sub-samples used separately for fresh latex and rubber analysis, and DRC measurement. As mentioned above, the standard NF ISO126: 2005 was applied for the DRC with the following adaptations: the sample comprised 2 mL of fresh latex instead of 10 mL of concentrated latex. The volume and concentration of added acid were 15 mL of 2% acetic acid instead of 25-35 mL of 5% acetic acid. Mineral and ash contents of the latex were measured on latex films. The latex films were made as follows: within the two hours after latex collection, 50 mL of latex were spread on a flat square recipient (22.5 x 22.5 x 3.75 cm) and were placed in a ventilated oven at 70 °C until no more white spots were visible on the surface of the sample (approx. duration 24 h). The resulting latex films were rolled and stored in plastic bags containing desiccant (5 g silica gel) prior to analysis. The mineral content expressed versus dry film weight were converted into a fresh latex reference by multiplying by the estimated total solid content of latex (TSC). The TSC was estimated by dividing measured DRC by 0.9. This numerical conversion value of the DRC/TSC ratio is regularly used in the field. It was checked experimentally in two consecutive sampling campaigns by measuring both DRC and TSC on the whole set of samples (2 x 32), and the average ratio obtained was 0.90 (SD 0.03). Unsmoked sheets (USS) were made following the recommendations of the Rubber Research Institute of Thailand. First, latex was coagulated by mixing 3 L of latex with 2 L of water and 300 mL of formic acid solution 1.56% (94% diluted 60x). After 45 min, the coagulum was manually pressed, then passed 1-2 time(s) through a crusher (final thickness 10 mm), 3-4 times through a flat hand mangle and twice through a ribbed hand mangle (final thickness 2-3 mm). Finally, the USS were hung up outside in the shade to dry for one to two weeks (turned daily). The nitrogen content of both latex films and USS rubber was determined with a CHN Determinator (LECO CHN628) on a 100 mg sub-sample. Total Mg, Ca, P and K content of both latex films and USS rubber were determined with a ICP-AES (ICP Agilent 720 AES) on ashes from a 2 g sub-sample placed in a furnace at 500 °C for two hours.

Figure 2 to be inserted here

2.4. Statistical analysis

A three-way ANOVA was performed on data collected each year to test the effect of blocks (4 modalities, n=8), fertilization (4 modalities, n= 8) and stimulation treatments (2 modalities, n=16), and their respective interactions on rubber yield (in g.tree^{-1}.year^{-1}), LD parameters (SUC, Pi, RSH), latex and rubber quality parameters of the two sampling campaigns. Additionally, an ANOVA was performed on cumulative rubber yield (g.tree^{-1}) at the end of the third cropping season. Tukey's HSD test was used to compare the means at the 5% probability level. The relationship between LD parameters and yield was tested every year with a multiple linear regression. Statistical analyses were performed with Xlstat software (2018.6 version, Addinsoft, Paris, France).

3. Results and Discussion

Our study is the first assessment of the interactions between latex harvesting technologies (stimulation) and fertilization on yield of tapped rubber trees in Thailand, the world's largest producer of natural rubber. We found only one publication in English reporting on a fertilizer trial in a mature rubber plantation in Thailand (Mak et al., 2008). However, this study only compared different formulae of fertilizers applied at the same dose. We also investigated the influence of stimulation and fertilization on latex physiology and latex quality parameters for the first time. Indeed, the literature on the effect of fertilization on natural rubber production is scarce and not very recent (Gohet et al., 2013). According to Gohet et al. (2013) and Bolton (1964), one reason for the lack of studies on this topic is the technical constraints to the implementation of fertilizer trials. This kind of trial requires a large homogeneous plantation area covering several hectares, which is difficult to find in rubber smallholdings.

The average yield of our experimental plot (all treatments) was high with ca. 5.8 kg. tree^{-1}. year^{-1} in the first year and more than 7.2 kg. tree^{-1}. year^{-1} in the second and third year (Table 3). Annual rubber yield per tree recorded for the control treatment without fertilization nor stimulation (T1NS) ranged between 5.3 and 6.5 kg.tree^{-1}.year^{-1}, which is in the upper range of yield data reported in rubber production trials in Thailand using the same tapping system on the clone RRIM600 (Mak et al., 2008; Chantuma et al., 2006, 2011, 2017; Sdoodee et al., 2012; Sainoi et al., 2017). In general, the good performances of the plantation was explained by the high tapping frequency (d2) despite the loss of tapping days. Yet, the number of tapping days was similar to that used by rubber smallholders in Thailand (Chambon et al., 2014). Our results suggest that the low soil fertility (low organic matter and low N, P and K contents) was not particularly limiting. The depth of the soil and the absence of any obstacle to root growth may have compensated for the low mineral content. Roots were observed at a depth of more than 2 m (data not shown), in agreement with the results of Maeght et al. (2015). Moreover, the absence of significant tapping panel dryness (TPD) on tapped trees at the end of the third year suggests that the trees were not over-exploited. This observation was confirmed by the results of the latex diagnosis (see sub-section 3.2). The good performances of the unfertilized and unstimulated plots (T1NS, control treatment), along with the absence of major block effects on yield (Table 3), suggest that our experimental field was well managed and was located in a favourable environment. We can therefore go further with the analysis of the treatment effects on rubber yield (section 3.1), latex metabolism indicators (section 3.2) and latex properties (section 3.3).

3.1. Effects of stimulation and fertilization on rubber yield (Table 3)

Our results show a positive effect of fertilization on yield from the first year, but the effect was only statistically significant ($p=0.023$) in the third year. This year, we observed that yield increased with the dose of fertilizers: +8% than the yield of T1 for T2, +10% for T3 and +13% for T4 in the third year. After three years, the cumulative yield of T3 and T4 treatments was 8% higher than T1. This effect was only significant at the 10% level ($p=0.071$). Stimulation by applying ethephon on the tapping cut four time a year had a strongly significant positive effect ($p<0.0001$) on rubber yield the three years of the experiment. The strongest effect of stimulation was observed in the third year with a 20% increase in yield, whereas in the first two years, the effect was similar (+12-13%). After three years of tapping, the cumulative yield of stimulated trees was 15% higher than that of unstimulated trees. It is noteworthy that the yield of stimulated and fertilized treatments (ST or T2, T3, T4) increased the third year compared to the second year, while it decreased for control treatments (NS or T1). We did not find significant interactions between fertilization and stimulation treatments.

The maximum effect of fertilization on rubber yield (+13%) we observed was similar to that reported by Gohet et al. (2013) and Murbach et al. (1999), two of the most recent works on the effect of fertilization on modern high-yielding varieties with intensive tapping systems. Yield increases of +20% and more were obtained in works conducted before the 1990s in less intensive rubber cultivation systems (Sivanadyan et al. 1972; Pushparajah, 1973; Yogaratnam et al., 1984; Onuwaje et al., 1983). However, in our specific conditions, the response to fertilization may have been restricted because the yield of the unfertilized control was already high, despite the apparent low soil fertility. Increasing yield through external inputs is of course harder when the base line yield is already high. In other words, there were probably no major nutrient limitations in the rubber plot before the beginning of the experiment. Nevertheless, the response of rubber yield to fertilizer addition was quite rapid. We observed a positive but not significant effect from the first year that became significant at $p<0.05$ in the third year. The effect on cumulative yield over the three years of the experiment was significant at the 10% probability threshold. According to Bolton (1964), we could have expected a slower response of yield to fertilizer. Bolton argued that additional fertilizer would first affect tree growth before increasing yield because of the low level of nutrients exported by latex harvesting compared to the total amount of nutrients required for tree growth. However, we observed no effect of the fertilization treatments on trunk radial growth (Table 3).

As expected, stimulation with an ethylene based product had the strongest effect on yield. Unlike fertilization, stimulation significantly increased rubber yield since the first year of the experiment. The effect of stimulation we observed with 4 ethephon applications per year (+15% in cumulated yield over the three-year experiment) was much lower than the effects reported by Lacote et al. (2010) that ranged from +29% to +85%. This result can be explained by the more intensive tapping frequency in our study (d2, meaning tapping every other day) than in the study of Lacote et al. (2010), which was with a d4 (tapping once in 4 days). Although stimulation is not recommended with d2 tapping frequency, we choose to include a stimulated d2 tapping system in our experiment to test the hypothesis that fertilization can have a bigger effect on yield when trees are stimulated because of their increased nutrient requirements, or that only an increase in tapping intensity can exploit the higher yield potential enabled by fertilization (Sivanadyan et al. 1972, Gohet et al. 2013). Our results do not support this hypothesis as we did not find any significant interaction between the stimulation and the fertilization treatments. In other words, yield response to fertilizer application was similar with and without stimulation. In particular, we observed that fertilization contributed, as stimulation did, to maintain or even increase rubber yield the third year while yield of the control (unfertilized) treatment decreased compared to the second year.

Altogether, these results demonstrate that fertilization, independently of the use of ethylene stimulation, can increase the yield of rubber plantation under high tapping frequency system (tapping once every 2 or 3 days) such as those used by smallholders in Thailand. As ethylene stimulation is not recommended with these tapping systems, improving the nutrition of the trees can thereby be a main driver of rubber plantation intensification. However, the maximum effect, and the most significant, of fertilization in our experiment was obtain with the highest dose of fertilizer (T4 treatment, Eq. to 306/136/289 g.tree^{-1}.y^{-1}, Table 2). This treatment corresponds to the highest dose of inorganic fertilizers reported by rubber smallholders in Thailand (Chambon et al. 2018). This dose is 1.7 times higher than the standard fertilizer recommendation in Thailand and 4 times higher than the CIRAD recommendation for industrial estates. Chambon et al. (2018) calculated that this dose of fertilizer with a 10% increase in yield would only be profitable if the price of rubber is more than USD4.kg^{-1}, while the price had rarely exceeded USD 2.kg^{-1} over the last 10 years. Additionally, the environmental impacts, i.e. the risks of nutrient leaching to groundwater and N_20 emissions to the atmosphere, with such a high fertilization rate must be high (Zhou et al., 2016).

In the next sub-section, we analysed the effect of fertilization and stimulation on the indicators of latex metabolism we measured. We collected these data in order to better understand the effect of fertilization on rubber yield. The underlying assumption was that fertilization would have an effect on latex metabolism before having a statistically noticeable effect on yield in relation to better latex regeneration.

Table 3 to be inserted here

3.2. Effects of stimulation and fertilization on latex metabolism

Latex diagnosis (LD) is a method used to assess the impact of tapping on the physiological status of the latex cells (Jacob et al., 1989, D'Auzac et al. 1997). Both fertilization and stimulation treatments had significant effects on the LD indicators, but the effects were different among the years and among the indicators. Inorganic phosphorus content (Pi) was the indicator most affected by the treatments. Pi increased with both stimulation and fertilization (Table 4). These effects were statistically significant in both the second and third year but not in the first year. Fertilization had a stronger effect on Pi than stimulation with an average increase of 16% increase with high levels of fertilization (T3 and T4) compared to control plots (T1) in 2015-2016, and of about 30% in 2016-2017. Low fertilization (T2) had little or no effect. There was also a slight interaction between stimulation and fertilization treatments in the second and third year (significant in the third year). Without stimulation, Pi increased with the dose of fertilizers but, with stimulation, only T3 had a significantly higher Pi than control (T1). This means that the effect of fertilization on Pi was stronger in non-stimulated trees. The sucrose content of the latex (SUC) decreased with stimulation the three years and with fertilization on years 2 and 3 (Table 4). These effects were only significant for stimulation on year 1 and 2. Indeed, we observed a decrease in the effect of stimulation on SUC over time (-38% in 2014-2015, -18% in 2015-2016 and -12% in 2016-2017). There was no consistent effect of either stimulation or fertilization on thiol content (RSH). Stimulation had a strong negative effect on RSH in the first tapping season (-24%), but no effect in the second and third years. Fertilization had a positive effect on RSH in general but with no clear significant trend linked with the dose of fertilizer. The high variability of this parameter (up to 21% within the same treatment) partly explains the lack of statistical significance. For a better understanding of the effect of latex diagnosis parameters on yield, we performed multiple linear regressions between the three latex diagnosis parameters and the latex yield expressed in g.tree-1.year-1 in each year. The results in Table 5 show that the p-value of the model decreased from the first to the third cropping season. This means that the fitness of the multiple linear regression to predict yield improved year after year. In 2014-2015, the three latex parameters explained only 30% of the yield variation whereas they explained nearly 60% in 2016-2017. SUC had a significant effect on yield in the second and the third year (p-value<0.05). Pi had a significant effect on yield only in the third year but this effect was stronger than for SUC.

Our starting hypothesis was that stimulation and fertilization would have a positive effect on latex yield through improved latex regeneration. The latex diagnosis (LD) indicators confirmed this hypothesis. Taken together, the results of the LD showed that latex metabolism was well activated after the first year of tapping and was well balanced in the two following years. In agreement with Jacob et al. (1989), the increase in sucrose content (SUC) along with a decrease in inorganic phosphorus (Pi) and thiol (RSH) content we observed in the third year suggest that the risk of over-exploitation of the trees was low. Similarly, the negative correlation between SUC and yield, and the positive correlation between Pi and yield revealed by the multiple linear regression between LD indicators and rubber yield are consistent with the results of previous studies showing that SUC decreases and Pi increases with an increase in tapping intensity (Tupy, 1985; Gohet et al., 1996; Jacob et al., 1998). In particular, these correlations and their underlying mechanisms have been well documented in the case of increases in yield obtained by ethylene stimulation (Amalou et al. 1992; Dusotoit-Coucaud et al., 2009; Lacote et al., 2010).

In addition, our results provide original insights into the combined effect of stimulation and fertilization on latex metabolism and yield. The main effect of fertilization on latex metabolism was on Pi. The maximum effect of fertilization on Pi was even stronger than that of stimulation after the first year. In the third year, both treatments had a highly significant positive impact on Pi with +33.5% for T4 compared to T1, and +24.7% for stimulated trees compared to unstimulated ones. The multiple linear regression between yield and LD indicators showed that Pi became the main explanatory factor in the third year. Positive correlation between rubber yield and Pi is well-known (Amalou et al., 1992). The metabolism of rubber particles involves active biochemical mechanisms which require a lot of energy and then fast cycling of ATP and NADPH, releasing free Pi into the cell cytoplasm. The positive effect of stimulation on Pi is in line with the results of Lacote et al. (2010). Gohet et al. (2013) also reported a +20% significant increase in Pi in response to a combination of intensive stimulation and a high dose of fertilizer. However, the effect of fertilization on Pi in our study was stronger and was significant in both the stimulated and unstimulated treatments. Moreover, the effect of fertilization on Pi was stronger on unstimulated trees.

As expected, stimulation had a significant negative effect on SUC (Lacote et al., 2010). The decrease in SUC content in stimulated trees can be explained by activation of rubber biosynthesis, which consumes more sucrose (Tupy and Primot,1976). Surprisingly, the positive effect of fertilization on yield did not result in a significant reduction in SUC with the dose of fertiliser. However, this result does not mean that fertilization had no effect on SUC. On the contrary, as a decrease in SUC was expected, this could mean that fertilization enhanced the supply of SUC to latex cells to cope with the increased latex productivity. The positive, but not significant, effect of fertilization on SUC observed in the first year is consistent with this hypothesis. This hypothesis is also in line with the results of Gohet et al. (2013) who observed a significant increase in SUC in response to high dose of fertilizer combined with intensive stimulation. In their study, SUC was low (<5 mMol) even in the control treatment. In our study, SUC levels were rather high in all treatments particularly in the third year (Chantuma et al., 2006). Thus, another possible explanation for the absence of response of SUC to fertilization is that sucrose was not limiting for rubber production in our experimental conditions. This would explain the high yields obtained with the control treatment right from the onset of the experiment. This would also explain the increase in SUC in the third year in all treatments and the decline in the effect of stimulation on SUC over time (-38% in 2014-2015 with $p<0.0001$, -18% in 2015-2016 with $p=0.001$ and -12.3% in 2016-2017 with $p>0.05$).

Our analysis demonstrated that fertilization had a positive impact on latex metabolism by 1) increasing inorganic phosphorus (Pi) content and hence the energy available to regenerate rubber particles, 2) supporting the supply of sucrose (SUC) in the latex despite the increased output of latex. These results suggest that the effect of fertilization on yield was directly related to the effect of fertilization on latex metabolism, likely through an effect on the enzymatic activity of the latex cells. Our analysis showed that SUC and Pi were the LD parameters the most correlated with yield and that they are good predictors of the latex yield in response to fertilization.

Next, we presented the results on the effect of the treatments on latex and rubber properties. As we observed significant effects on LD indicators, we could wonder whether these properties, which are linked to the quality of latex and rubber, were also affected by the experimental treatments.

Table 4 and 5 to be inserted within this sub-section

3.4. Effect of stimulation and fertilization on latex and rubber properties.

We quantified six indicators of the technological properties of latex and rubber: the dry rubber content (DRC) and the mechanical stability (MST) of the fresh latex; the initial plasticity (P_0), the plasticity retention index (PRI) and its component P_{30}, and the ash content of rubber processed as unsmoked sheet (USS) (Table 6). Simultaneously, we analysed the mineral content (N, P, K, Ca, Mg) of fresh latex and dry rubber (Table 7).

Over the three-year period, we observed a decrease in MST (from 61 to 50 s.), PRI (from 104 to 91) and rubber USS ash content (from 0.33 to 0.27%) and an increase in P_0 (from 34 to 42). We found no significant effects of fertilization on the technological properties of the latex and USS rubber. Stimulation had a significant negative effect on DRC (p=0.001, ST/NS=-3.50%) and a significant positive effect on PRI (p=0.004, ST/NS=+3.10%) in the third year only. Although not significant, stimulation and fertilization tended to destabilize the latex in the two first cropping years (-5.9% and -12.4% MST with stimulation; -12.6% and -15.6% MST for T4 treatment).

Over the three-year period, Mg and N contents in both fresh latex and USS rubber tended to increase whereas P, K and Ca contents decreased. The only significant effect of fertilization on mineral contents was on the N content of fresh latex in the third year (+6.3% on average, p=0.046). The significance of this difference was not conserved in USS rubber, even though USS rubber from T4 contained 4.5% more nitrogen than USS rubber from T1. The P content of latex also tended to increase with fertilization during the third cropping year, but the effect was not statistically significant (T4/T1 +13.9%, p=0.122). Several effects of stimulation on mineral contents were observed. N, P, K and Mg contents of USS rubber from trees growing in the stimulated plot were higher, and this effect became significant in the third cropping year. Logically, ash content of USS rubber from stimulated plots was up to 5% higher than that of non-stimulated ones (Table 6).

To our knowledge, this study is the first to provide a full set of data on latex and rubber properties, including mineral contents and several indicators of technological properties such as MST or PRI, in response to stimulation and fertilization treatments. The results revealed no significant effects of fertilization on the technological properties of either fresh latex or USS rubber. However, although not significant, the reduced stability of the latex in the fertilized treatments compared to controls observed in the first two years is in accordance with the findings of Collier and Lowe (1969). The only significant effect of fertilization on latex mineral content and properties, an increase in N content, was observed the third year. Although not significant, latex P content showed the same tendency. These results are in agreement with data on rubber yield and latex metabolism and support the conclusion that fertilization significantly modified the latex properties three years after the beginning of the experiment. Longer-run observations will be necessary to confirm this conclusion.

Significant effects of stimulation on latex properties also appeared in the third year. Increased mineral contents (except Ca) with stimulation are consistent with reports in the literature (D'Auzac et al., 1989). It is noteworthy that stimulation affected the mineral contents of USS rubber and not those of fresh latex. This can be attributed to a concomitant decrease in dry rubber content (DRC), which is a well-documented effect of stimulation (Coupé et Chrestin, 1989). This phenomenon was particularly clear in the October measurements, which were preceded by four applications of stimulant (data not shown). USS rubber is indeed the dry matter of latex from which the hydrophilic matter is removed during USS processing (from 8% to 10% of total dry matter). In fact, our measurements showed that significant amounts of minerals were lost during this process. Vaysse et al. (2017) calculated that about 33% of the nitrogen contained in latex was lost during USS processing. Losses of K (ca. 90% of initial content in latex), Mg (ca. 80%) and P (ca. 60%) were even greater. Only Ca content was preserved. This finding highlights the complex relationships between the mineral composition of fresh latex and dry rubber, and their possible effects on rubber quality. However, in our study, the observed differences in the mineral content of USS rubber had no direct effect on the technological properties of USS rubber. In particular, the increase in ash content of USS rubber induced by stimulation led to a content that was less than half the maximum accepted by the Technically Specified Rubber standard (0.6% for TSR5 grade, ISO 2000:2003, TSR standard is used as reference as no ash content standard is available for sheet rubber). Similarly, stimulation had significant effects on P_0 and PRI but technologically speaking, the averaged differences (less than 2 points "P_0" and less than 3 points "PRI" respectively, see supplementary Table 4) are not significant (Bateman and Sekhar, 1966).

Finally, the absence of an effect of both fertilization and stimulation treatments on the technological properties of NR is a positive result. Indeed, it proved that, while having a positive effect on production, none of the treatment had a detrimental effect on quality.

Table 6 and 7 to be inserted here

4. Conclusion and perspectives

Our study is the first experimental work to investigate the effects of increased doses of mineral fertilizers combined with stimulation of trees on the yield, latex metabolism and technological properties of latex and dry rubber in a rubber plantation in Thailand. Our results show a positive and significant effect of fertilization on yield three years after the beginning of the experiment. The effect of fertilization was similar with and without ethylene stimulation. The hypothesis that the effect of fertilization on latex yield would only be higher under more intensive tapping was thus not confirmed. In practice, the effect of fertilization and stimulation were additive. We also demonstrate that the effect of fertilization on yield was linked to a direct effect on latex metabolism mainly through an increase in inorganic phosphorus content (Pi). We observed no significant effect of the fertilizer treatments on latex and rubber properties. Therefore, increasing latex yield by fertilization combined with intensive tapping did not have any identifiable risks on the technological properties of rubber. Thus, our study suggest that fertilization can be an efficient driver of the intensification of rubber plantation. In order to validate this result on the long-term, our study is continuing. In addition to direct effects on yield and the latex regeneration processes, we are analysing the effect of fertilization on the growth and the nutritional status of the trees. We are also collecting data in order to quantify the actual mineral balance of the different fertilization treatments and to assess their respective environmental impacts through nutrient leaching or N_2O emissions. Similarly, we are studying the feasibility of recycling the water used for USS processing which contains large amounts of minerals. Finally, the profitability of the fertilization practices must be assessed with respect to the expected effect on yield and the price of natural rubber. Altogether, these new data will contribute to a multi-criteria evaluation of fertilization practices in rubber plantation that can help farmers to decide whether or not using fertilizers in their rubber plantations.

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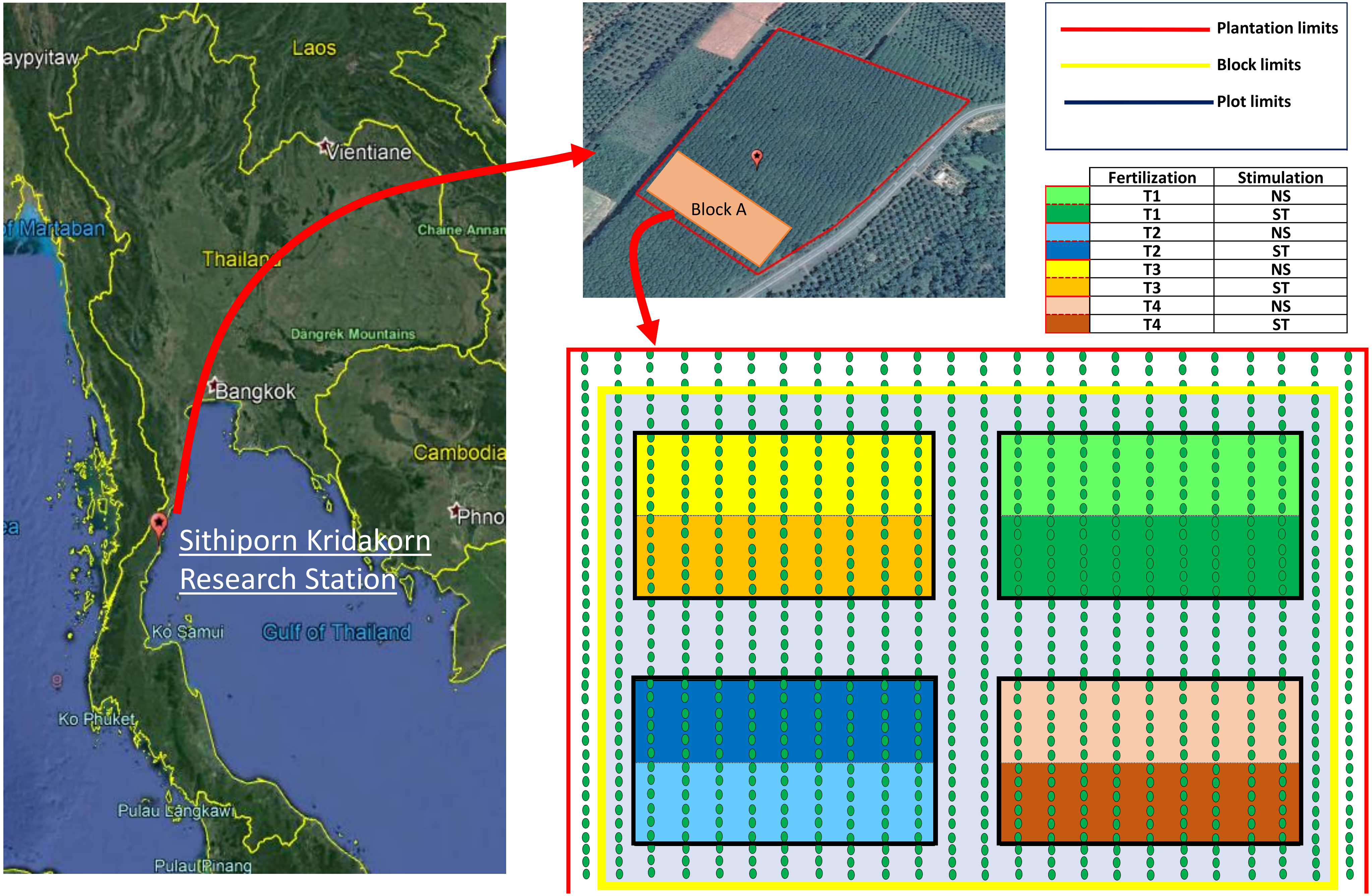
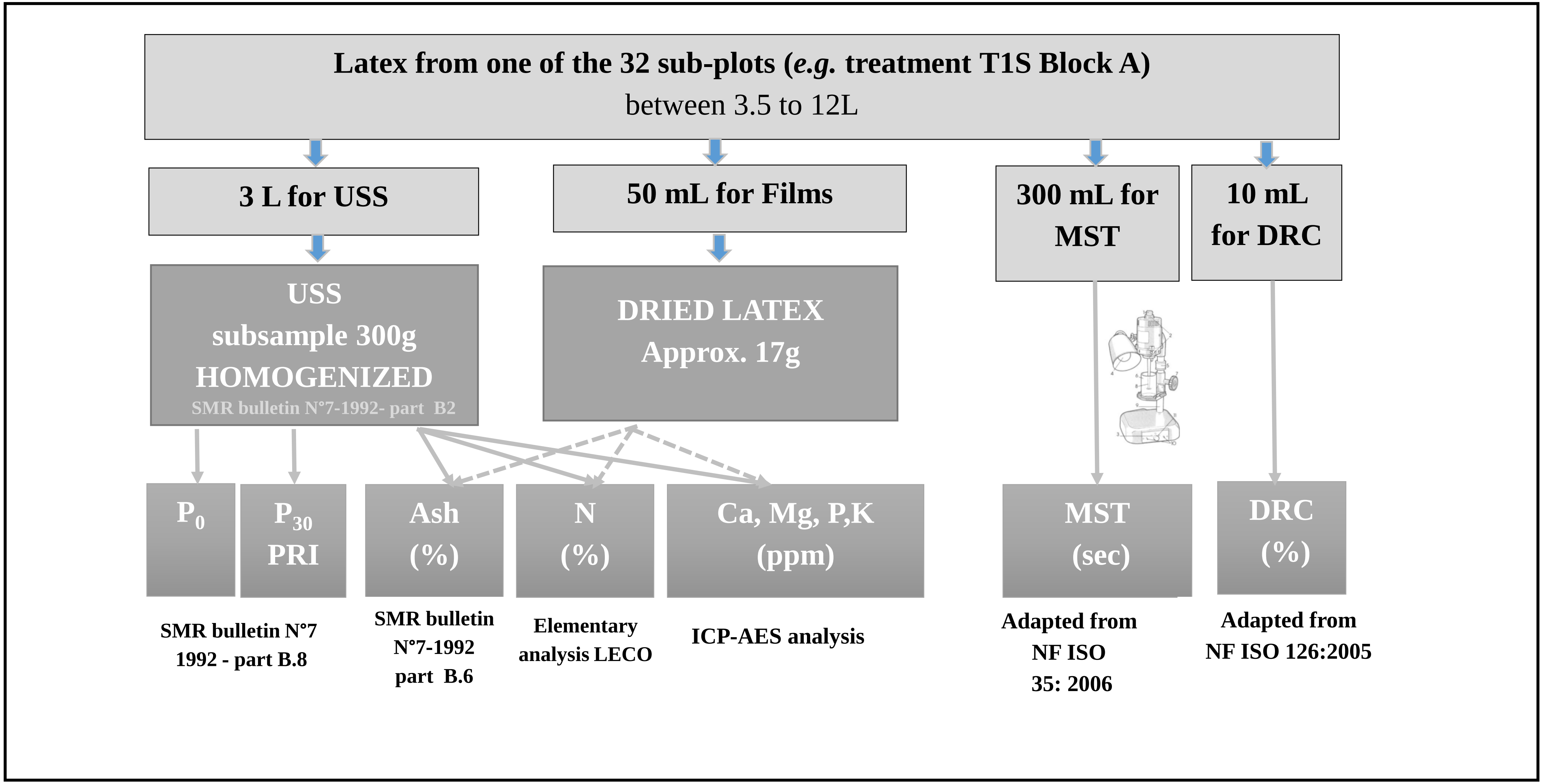


Laos
Vientiane
Dângrêk Mountains
Bangkok
Cambodia
Sithiporn Kridakorn Research Station
Ko Samui
Gulf of Thailand
Ko Phuket
Pulau Langkawi
Pulau Pinang
Block A
Plantation limits
Block limits
Plot limits
Fertilization Stimulation
T1 NS
T1 ST
T2 NS
T2 ST
T3 NS
T3 ST
T4 NS
T4 ST



Latex from one of the 32 sub-plots (e.g. treatment T1S Block A)
between 3.5 to 12L
3 L for USS
50 mL for Films
300 mL for MST
10 mL for DRC
USS
subsample 300g
HOMOGENIZED
SMR bulletin N°7-1992- part B2
DRIED LATEX
Approx. 17g
P_0
P_{30}
PRI
Ash
(%)
N
(%)
Ca, Mg, P,K
(ppm)
MST
(sec)
DRC
(%)
SMR bulletin N°7
1992 - part B.8
SMR bulletin
N°7-1992
part B.6
Elementary
analysis LECO
ICP-AES analysis
Adapted from
NF ISO
35: 2006
Adapted from
NF ISO 126:2005

Table 1. Some features of the three tapping seasons: cumulative rainfall and rainy days, and mean temperature compared to the 2000-2010 statistics; number of tapping days, average number of tapped trees and trees affected by tapping panel dryness per subplot (n=16). Numbers in brackets are standard errors of the mean.

Year	Rainfall (mm)	Rainy days (n°)	Temperature (°C)	Tapped trees (n°)	Tapping days (n°)	TPD trees (n°)
Reference period (2000-2010)	1985 (383)	106 (11)	26.6 (1.3)	NA	NA	NA
2014-2015	1598	112	27.3	31 (2.7)	106	0
2015-2016	1452	109	27.4	36 (2.7)	115	0
2016-2017	2428	129	26.7	38 (3.1)	106	1 (1.5)

Table 2. Details of fertilization treatments

Treatment code	NPK Fertilization		Comments
	Early rainy season	Late rainy season	
T1	0	0	Control
T2	500g/tree 15/9/20 NPK	0	Eq. to 75/45/100 $g.tree^{-1}.y^{-1}$
T3	500g/tree 21/7/14 NPK	500g/tree 15/9/20 NPK	Eq. to 180/80/170 $g.tree^{-1}.y^{-1}$
T4	850g/tree 21/7/14 NPK	850g/tree 15/9/20 NPK	Eq. to 306/136/289 $g.tree^{-1}.y^{-1}$

Table 3. Average annual and cumulative dry rubber yield (g.tree^{-1}), and average trunk girth (cm) at the end of the third year in each treatment (fertilization x stimulation). Letters in brackets show significant differences among the calculated means (Tukey's test, $p<0.05$). The rows "Stimulation effect", "Fertilization effect", "Block effect" and "Fert.xStim effect" give the results of the ANOVA of the main treatment, sub-treatment, block and interaction effect. p-values in bold are statistically significant effects. Percentages represent the ratio of the yield of a given treatment to the control (ST to NS for stimulation treatment, T2, T3 or T4 to T1 for fertilization treatment).

Treatment	Rubber yield 2014-15 (g.tree^{-1}.year^{-1})	Rubber yield 2015-16 (g.tree^{-1}.year^{-1})	Rubber yield 2016-17 (g.tree^{-1}.year^{-1})	Cumulative Rubber yield 2014-17 (g.tree^{-1})	Trunk girth April 2017 (cm)
All means	5794	7238	7239	20272	53.5
Stimulation effect	**p<0.0001**	**p<0.0001**	**p<0.0001**	**p<0.0001**	p=0.661
NS	5447 (b)	6830 (b)	6573 (b)	18850 (b)	53.6
ST	6142 (a)	7647 (a)	7905 (a)	21693 (a)	53.3
ST/NS	**+13%**	**+12%**	**+20%**	**+15%**	0%
Fertilization effect	p=0.131	p=0.297	**p=0.023**	p=0.071	p=0.868
T1	5552	6973	6731 (b)	19257	53.3
T2	5779	7239	7240 (ab)	20258	53.4
T3	5957	7369	7394 (ab)	20720	53.3
T4	5890	7372	7590 (a)	20851	53.9
T2/T1	+4%	+4%	+8%	+5%	0%
T3/T1	+7%	+6%	+10%	+8%	0%
T4/T1	+6%	+6%	**+13%**	+8%	+1%
Block effect	p=0.234	p=0.821	p=0.912	p=0.988	p=0.458
Fert x Stim effect	p=0.934	p=0.903	p=0.788	p=0.948	p=0.447

Table 4. Average annual values of sucrose, inorganic phosphorus (Pi) and thiol contents of the latex in each treatment (fertilization x stimulation). Letters in brackets show significant differences among the calculated means (Tukey's test, $p<0.05$). The rows "Stimulation effect", "Fertilization effect", "Block effect" and "Fert.xStim effect" give the results of ANOVA of the main treatment, sub-treatment, block and interaction effect. p-values in bold are statistically significant effects. Percentages represent the ratio of the yield of a given treatment to the control (ST to NS for stimulation treatment, T2, T3 or T4 to T1 for fertilization treatment).

Treatment	Sucrose content (mM)			Pi content (mM)			Thiol contents (mM)		
	2014-15	2015-16	2016-17	2014-15	2015-16	2016-17	2014-15	2015-16	2016-17
All mean	9.7	9.6	13.3	14.1	16.4	12.0	0.21	0.36	0.21
Stimulation effect	**p<0.0001**	**P=0.001**	p=0.066	p=0.070	**p=0.008**	**p<0.0001**	**p<0.0001**	p=0.738	p=0.609
NS	11.9 (a)	10.5 (a)	14.2	13.5	15.5 (b)	10.7 (b)	0.24 (a)	0.37	0.21
ST	7.4 (b)	8.6 (b)	12.4	14.7	17.3 (a)	13.3 (a)	0.18 (b)	0.36	0.21
ST/NS	**-38%**	**-18%**	-12.3%	**9%**	**11%**	**24.7%**	**-24%**	-1%	-2.5%
Fertilization effect	p=0.253	p=0.182	p=0.543	p=0.929	**P=0.008**	**p<0.0001**	p=0.350	**p=0.018**	p=0.187
T1	8.9	10.3	14.5	13.8	15.2 (b)	10.3 (b)	0.21	0.35 (b)	0.20
T2	9.3	9.5	12.6	14.2	15.3 (b)	11.0 (b)	0.20	0.39 (a)	0.22
T3	9.9	9.6	13.0	14.3	17.8 (a)	13.2 (a)	0.22	0.37 (ab)	0.21
T4	10.6	9.0	13.0	14.0	17.4 (ab)	13.7 (a)	0.23	0.35 (b)	0.23
T2/T1	4%	-7%	-13%	3%	0%	7.1%	0%	**13%**	12%
T3/T1	10%	-7%	-10%	4%	**17%**	**29%**	5%	6%	6%
T4/T1	18%	-12%	-10%	1%	15%	**33%**	9%	2%	16%
Block effect	p=0.614	p=0.789	p=0.983	p=0.285	p=0.595	p=0.628	p=0.692	p=0.751	p=0.803
Fert.xStim. effect	p=0.679	p=0.203	p=0.871	p=0.765	p=0.657	**p=0.025**	p=0.521	p=0.777	p=0.279

Table 5. Parameters of the multiple linear regressions between latex yield (in g.tree^{-1}.year^{-1}) and the three biochemical indicators of the latex diagnosis (sucrose content (SUC), thiol contents (THI) and inorganic phosphorus content (Pi)) for each year. p-values in bold highlight significant effects of the model or variables.

	2014-15	2015-16	2016-17
p value model	**0.016**	**0.004**	**< 0.0001**
R^2 model	0.304	0.378	0.595
p value SUC	0.534	**0.006**	**0.001**
p value RSH	0.223	0.416	0.180
p value Pi	0.775	0.052	**< 0.0001**
Coefficient norm. SUC	-0.187	-0.484	-0.462
Coefficient norm. RSH	-0.368	0.134	-0.169
Coefficient norm. Pi	0.048	0.321	0.617

Table 6. Technological properties of fresh latex and USS rubber**.** Main results of the ANOVA**.** Columns "Stimulation effect", "Fertilization effect", "Block effect" and "Fert.xStim effect" give the results of ANOVA of the main treatment, sub-treatment, block and interaction effect. p-values in bold are statistically significant. Percentages represent the ratio of the yield of a given treatment to the control (ST to NS for stimulation treatment, T2, T3 or T4 to T1 for fertilization treatment). Data per treatment are presented in Supplementary Tables S1 to S4

Treatment	Year	All mean	Stimulation effect (p-value)	ST/NS	Fertilization effect (p-value)	T2/T1	T3/T1	T4/T1	Block effect (p-value)	Fert.xStim. effect (p-value)
MST Latex (sec)	**2014-15**	61	0.369	-5.9%	0.303	1.6%	-10.0%	-12.6%	0.645	0.637
	2015-16	58	0.059	-12.4%	0.322	-4.4%	-1.4%	-15.6%	0.535	0.889
	2016-17	50	0.167	3.2%	0.568	3.7%	2.9%	5.7%	0.089	0.301
DRC latex (%)	**2014-15**	33.3	0.662	-0.9%	0.730	-1.1%	-2.9%	-2.3%	0.581	0.992
	2015-16	32.0	0.669	-1.0%	0.877	-2.6%	-1.2%	-0.5%	0.932	0.806
	2016-17	37.9	**0.001**	-3.5%	0.346	0.4%	-1.0%	-2.1%	**0.035**	0.479
P_0 USS	**2014-15**	34	0.257	1.6%	0.925	1.3%	0.7%	0.7%	0.759	0.839
	2015-16	38	0.429	-1.0%	0.398	-0.1%	-2.7%	-1.8%	**0.026**	0.489
	2016-17	42	**0.047**	-2.7%	0.268	-1.6%	-2.9%	-3.4%	**0.020**	0.300
P_{30} USS	**2014-15**	36	**0.023**	2.5%	0.002	3.8%	6.0%	5.8%	**0.002**	0.396
	2015-16	37	0.814	-0.2%	0.126	2.0%	-0.9%	-1.3%	**0.009**	0.560
	2016-17	38	0.859	0.3%	0.849	-0.3%	-1.8%	-1.6%	0.193	0.973
PRI USS	**2014-15**	104	0.545	0.8%	0.050	2.4%	5.1%	5.1%	0.106	0.787
	2015-16	98	0.349	0.9%	0.350	2.0%	1.9%	0.6%	0.118	0.096
	2016-17	91	**0.004**	3.1%	0.664	1.4%	1.2%	1.7%	0.140	0.086
Ash USS (%)	**2014-15**	0.33	0.347	-2.4%	**0.016**	-0.4%	-5.1%	-10.9%	**<0.0001**	0.551
	2015-16	0.27	0.077	5.1%	0.948	-1.1%	1.1%	-0.2%	0.840	0.909
	2016-17	0.27	0.034	5.7%	0.346	1.4%	5.5%	5.0%	0.484	0.452

Table 7. Nutrient contents of fresh latex and USS Rubber. Main results of the ANOVA test. See table 6 for details. Data per treatment are given in Supplementary Tables S1 to S4.

Treatment	**Year**	All means	Stimulation effect (p-value)	S/NS	Fertilization effect (p-value)	T2/T1	T3/T1	T4/T1	Block effect (p-value)	Fert.xStim. effect (p-value)
N latex (ppm)	**2014-15**	2251	0.142	5.00%	0.754	4.30%	1.10%	4.00%	0.110	0.890
	2015-16	2483	0.469	1.10%	0.269	-3.40%	-0.40%	0.20%	0.892	0.475
	2016-17	2649	0.699	0.70%	**0.046**	5.10%	6.30%	7.50%	0.751	0.715
P latex (ppm)	**2014-15**	633	**0.049**	4.90%	0.838	-1.80%	1.30%	-0.60%	**0.042**	0.817
	2015-16	489	0.221	4.90%	0.592	-4.40%	3.00%	0.50%	0.187	0.676
	2016-17	580	0.057	8.10%	0.122	9.00%	11.80%	13.90%	0.951	0.251
K latex (ppm)	**2014-15**	1716	0.732	-0.80%	0.877	-1.90%	-2.50%	-1.30%	0.964	0.641
	2015-16	1528	0.676	-1.20%	0.127	-2.80%	6.20%	4.50%	0.255	0.841
	2016-17	1483	0.570	3.40%	0.745	4.10%	7.70%	7.90%	0.453	0.458
Ca latex (ppm)	**2014-15**	20	0.902	-1.10%	0.802	-11.00%	-4.80%	-8.90%	0.800	0.119
	2015-16	11	0.733	2.90%	0.863	-9.30%	-6.00%	-3.30%	0.848	0.819
	2016-17	12	0.865	-1.00%	0.448	3.00%	-0.90%	-9.10%	**<0.0001**	0.401
Mg latex (ppm)	**2014-15**	325	**0.040**	11.00%	0.191	-13.20%	-4.20%	-10.10%	**0.013**	0.905
	2015-16	406	0.384	6.80%	0.972	3.20%	4.70%	1.20%	0.824	0.985
	2016-17	553	0.121	8.80%	0.363	6.50%	-6.00%	-4.40%	**0.023**	0.631
N USS (ppm)	**2014-15**	4597	**0.025**	8.70%	0.079	-0.80%	-1.90%	10.20%	0.280	0.0656
	2015-16	5148	0.244	2.80%	0.984	0.10%	1.10%	0.70%	0.946	0.953
	2016-17	4598	**0.005**	5.00%	0.272	1.20%	2.70%	4.50%	0.491	0.969
P USS (ppm)	**2014-15**	692	0.083	4.80%	0.522	3.10%	5.20%	0.90%	0.970	0.939
	2015-16	625	0.099	5.20%	0.876	1.30%	3.40%	2.50%	0.493	0.524
	2016-17	610	**0.004**	7.40%	0.133	0.50%	4.50%	7.20%	0.279	0.574
K USS (ppm)	**2014-15**	682	0.471	3.60%	0.378	9.70%	7.70%	12.80%	**0.013**	0.283
	2015-16	423	0.797	1.00%	0.240	4.60%	0.20%	10.60%	0.110	0.617
	2016-17	455	**0.010**	8.90%	0.084	-2.10%	3.00%	9.30%	**<0.0001**	0.290
Ca USS (ppm)	**2014-15**	30	0.380	10.50%	0.620	-12.70%	2.40%	-6.40%	0.174	0.129
	2015-16	38	0.574	-4.70%	0.992	-4.50%	-1.50%	-4.30%	**<0.0001**	0.449
	2016-17	32	0.318	12.60%	0.985	-3.40%	-6.30%	-2.70%	0.590	0.215
Mg USS (ppm)	**2014-15**	196	0.105	7.90%	0.977	1.90%	2.60%	0.60%	**0.026**	0.529
	2015-16	206	0.286	3.70%	0.608	-0.90%	-4.20%	2.10%	0.172	0.813
	2016-17	232	**0.004**	10.70%	0.302	-2.20%	-1.90%	6.00%	**0.021**	0.346