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Influence of Leaf Density on Latex Production of *Hevea brasilensis* (Muell. Arg.) in Industrial Plantation of Kango in the Northwest of Gabon

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Abstract

Four clones of *Hevea brasilensis* Mull. Arg. were evaluated in the industrial plantation of Ekouk-Kango in Gabon. This study was conducted to determine the influence of leaf density on latex production. The clones GT1, PB235, PB260 and RRIM600 aged 25 to 27 years were selected by block following three replicates comprising 25 trees in different plots. The leaf densities were determined visually according to a rating scale between 0 and 4. The clones were bled on day 5, 1⁄2 spiral in descending bleeding and 1⁄4 spiral in ascending bleeding, and the stimulation was done with ether. The collection of coagulated latex was done daily and weighed in the early afternoon. The results showed that the decrease in leaf density leads to a drop in latex production of more than 10.17%. The month of July was characterized by a rating scale (leaf density) between 4 and 3 for clones PB235 and PB260 and between 3 and 2 for clones GT1 and RRIM600. The months of August and September were marked by a considerable drop in leaf density with rating scales between 3 and 2 for clones PB235 and PB260 and between 2 and 1 for clones GT1 and RRIM600. This decrease in leaf density (between 52.87% and 73.26%), followed by a leaf emission (Stage A) of the order of 17.33% to 58.66 led to a drop in latex production estimated at between 10.17% and 30.41%. In view of these results, the recommended stimulation would be a single for the months of August and September, since these two months are characterized by a more extensive wintering period at the clone level.

Keywords

Hevea brasilensis, Clone, Leaf Density, Latex Production, Wintering

1. Introduction

Hevea (*Hevea brasiliensis* Muell. Arg.) is the world's main source of natural rubber, a strategic raw material for the tire and manufacturing industry [1]. Faced with fluctuating world prices and rising demand, optimizing latex yields has become a major challenge for African producer countries [2]. Gabon, through its policy of economic diversification, has massively revived its rubber sector by relying on large agro-industrial complexes. The production of latex by the tree depends on a complex physiological balance, strongly influenced by environmental factors and the structure of the canopy [3]-[5]. Among these factors, leaf density plays a decisive role [6]. The leaves are the main site of photosynthesis, a process that provides sucrose, an essential precursor for the regeneration of latex in laticifer vessels after bleeding [5]. A change in leaf mass, whether related to the natural defoliation-refoliation cycle or to parasitic attacks, can therefore radically alter the tree's metabolism and productivity [6]. If the scientific literature has widely documented the impact of climate and clones on yield, the specific effect of canopy density remains poorly quantified in the particular pedoclimatic conditions of Central Africa. The agro-industrial farm of Kango, located in northwestern Gabon, offers an ideal study environment due to its hot and humid equatorial climate, but is subject to marked seasonal variations that influence tree phenology. The main objective of this study is to assess the influence of leaf density on the production of latex of *Hevea brasiliensis* in the industrial plantation of Kango to provide decision-making indicators to optimize bleeding management and anticipate production drops. Specifically, this work aims to characterize the variations in leaf density according to the exploited rubber clones, to measure the evolution of cup-bottom latex mass production over the defoliation-refoliation period, and correlate the leaf density index with wet coagulated cup-bottom mass yield.

2. Materials and Methods

2.1. Materials

2.1.1. Experimental Site

The study was conducted at the Ekouk-Kango industrial plantation in Gabon from July to September 2014. The site is located in the North East at 0.688˚South latitude, 10.23.401˚East longitude and an altitude ranging from 50 to 200 m (**Figure 1**). The average annual temperature is 23˚C for a rainfall of 1,900 mm (*Source:* SIAT Kango, 2014).

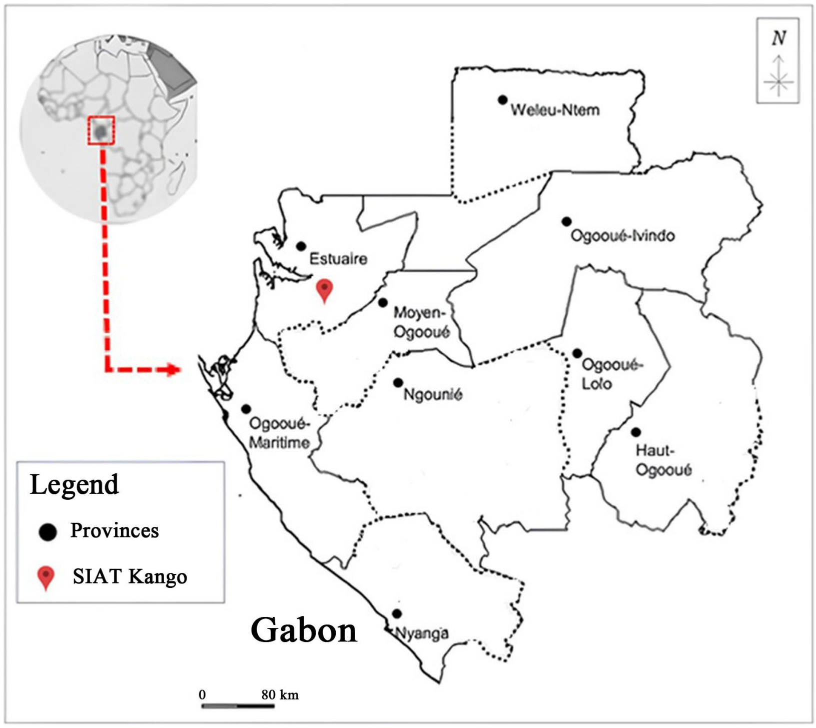

Figure 1. Localization of the experimental site (SIAT Kango).

2.1.2. Plant Material

The plant material consists mainly of clones of *Hevea brasiliensis* (Muell. Arg) GT1, PB235, PB260 and RRIM600; aged 25 to 27 years selected by block in differ (**Figure 2**).

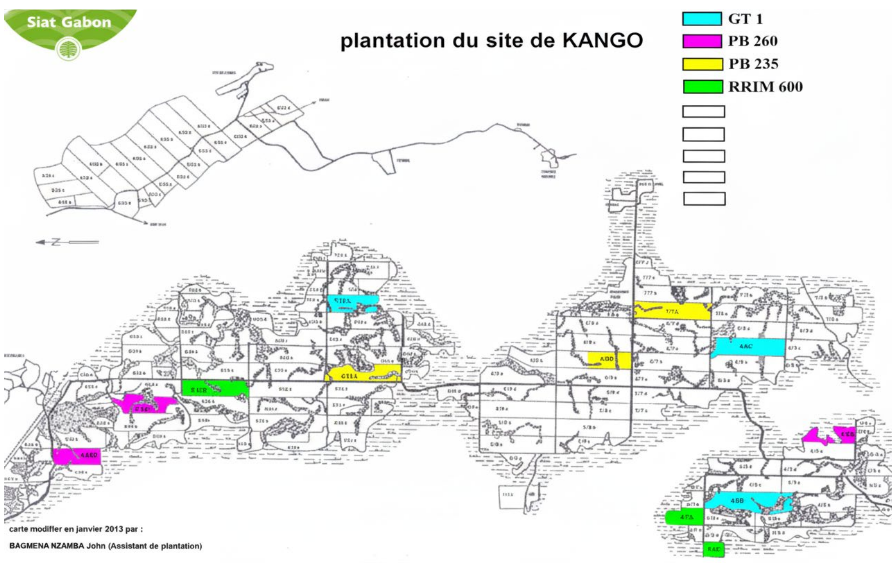

Figure 2. Distribution of block files (*Source:* SIAT Kango, 2013).

Table 1 below presents the list of all studied clones, their origin and a brief description of their characteristics.

Table 1. Origin and description of experimental rubber clones.

Clone	Origin	Description
GT1 (*Gondang Tapen 1*)	Indonesia, selected around 1930	Known for its good adaptation, used as a reference in many studies, very abundant nectar glands (3 to 6 glands) good grafting success rate, presents mainly vegetative shoots before 3 - 4 years.
PB235 (*Prang Besar* 235)	Malaysia	known for its very strong vigor, allowing early bleeding (6 months to 1 year before GT1), fast and high production, but has a marked sensitivity to dry notch and wind.
PB260 (*Prang Besar* 260)	Malaysia	Mainly recognized as a fast metabolic type clone, characterized by a high production of latex from the opening of the bleed. Sensitive to overuse, subject to bark bursting disease in certain humid regions, resistance to breakage in the wind.
RRIM600 (*Rubber Research Institute of Malaysia* 600)	Derived from the TJIR1 x PB86 cross and created in 1937 in Malaysia	Very vigorous in the immature phase, very high production with a rapid increase, very active metabolism, showing a weak response to stimulation, very sensitive to dry notch, a syndrome causing the flow of latex to stop, very sensitive to wind damage.

Source: CIRAD Agripog.

2.2. Methods

2.2.1. Experimental Design

The structure of the experiment was based on four clones (GT1, RRIM 600, PB 235 and PB 260) from three different blocks (repetitions) according to the planting dispositions (Figure 2). Five trees within five different lines were randomly selected to get 25 trees per clone and per block to make a good mixed to better integrate the environmental parameters while avoiding those of the border. The same trees were monitor each period.

2.2.2. Determination of Leaf Density

The methodological principle of leaf density monitoring results from the work of [7] and [8] carried out at the SIAT Mitzic site on artificial defoliation. The densities were taken every 15 days referring to each period. Leaf density represents a visual assessment of the percentage of leaves on the tree relative to what it should have been. If only a few leaves remain on the tree, the density is considered to be zero. Leaf density values range from 0% (no leaves at all) to 100% (maximum leaves at the most mature stage on all branches) and are noted as 5 in 5. To better appreciate the evolution of the defoliation-refoliation phenomenon, the different foliar density percentages were grouped by class. This scale ranges from 0 to 4 and is as follows: 0 = no leaves; 1 = 0% to 25% leaves; 2 = 26% to 50% leaves; 3 = 51% to 75% leaves and 4 = 76% to 100% leaves.

2.2.3. Foliar Emission Stage

For a stage to be considered present, at least three twigs had to bear leaves from that stage. The different stages according to [9] are defined as follows: Stage A: bud break (9 days), the leaves are performed in the terminal bud; Stage B: growth (11 days), rapid elongation of the internodes separating the scales and leaves appears reddish-purple; Stage C: Foliar maturation (10 days), the leaf blades undergo rapid growth and the leaves appear light green; in Stage D: Dormancy (12 days), the blade becomes stiff and begins to recover, and the leaves appear dark green.

2.2.4. Bleeding and Latex Production

All clones are bled according to specific bleeding systems; half-spiral for descending bleeding and quarter-spiral for ascending bleeding. The stimulation is done with Ethrel (Ethéphon active substance) at 2.5% for the descending bleeding (clone PB 235) and 5% for the ascending bleeding (clones GT 1, RRIM 600 and PB 260) according to the frequency of 16 stimulations per year (16Y). At the level of each block, 25 trees from the clone were bled on D5. The collection of wet coagulated latex (wet coagulated cup bottom mass) was done daily and was deposited on the racks and weighed in the early afternoon. The data collection periods were distributed as recorded in **Table 2**.

Table 2. Distribution of data collection periods in 2014 at Siat Kango site (P: period).

Periods	P1	P2	P3	P4	P5	P6
Days/Months	From 06/07 to 22/07	From 22/07 to 06/08	From 06/08 to 22/08	From 22/08 to 06/09	From 06/09 to 22/09	From 22/09 to 06/10

2.2.5. Data Analysis

The Production Management Information System (IMIS PRO), a calculator (spreadsheet type) optimized on the characteristics of an agricultural holding was used and made it possible to quickly calculate production balances by blocks and plots. The average values per block (from the 25 threes) for the six periods were used for the analysis of variance following the Tukey test at $p < 0.05$ using JMP and Minitab14 softwares. The raw values of the six periods with three repetitions per period were used for the Pearson correlation test at $p < 0.05$.

3. Results

3.1. Evolution of Rainfall during the Defoliation/Refoliation Period

The evolution of rainfall following the months related to defoliation/refoliation varies from one year to another during the last five years (**Figure 3**). With the exception of 2013, when no rainfall was observed during the months of July, August and September, the other years were marked by significant rainfall in September (corresponding to the start of refoliation), reaching 306.3 mm for 2014.

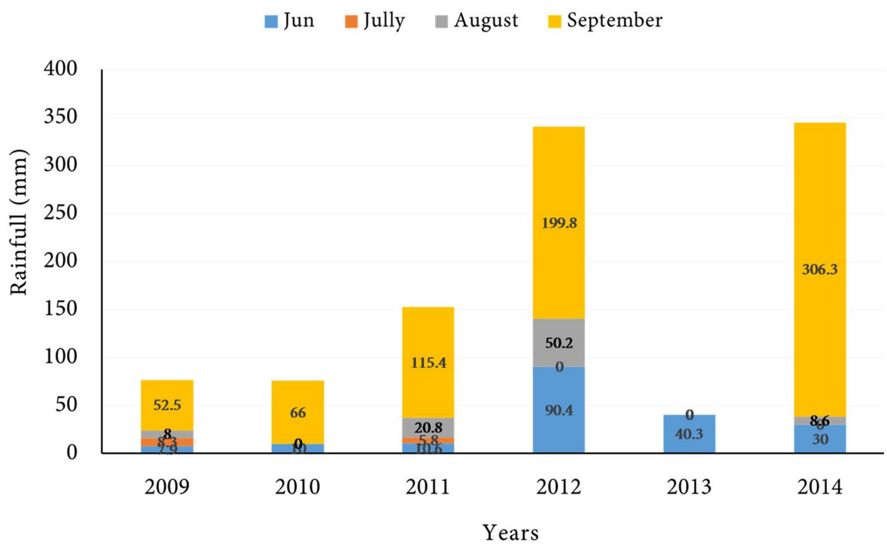

Figure 3. Evolution of rainfall over the last five years during the defoliation/refoliation months.

3.2. Evolution of Leaf Density and Production of Clone GT1

Table 3 shows the evolution of leaf density, latex wet coagulated cup-bottom mass as well as leaf emission periods (Stage A) of clone GT1.

Table 3. Variation in leaf density (LD) and latex wet coagulated cup-bottom mass (CB) per block file (BF) of the GT1 clone.

Periods	LD (%)	CB/BF (Kg)	Rating scale	Stage A (%)
P1	60.80 ± 0.53^{a}	9.28 ± 0.25^{a}	3	0
P2	42.00 ± 5.01^{b}	9.17 ± 0.34^{a}	2	0
P3	29.53 ± 1.81^{b}	8.50 ± 0.34^{b}	2	0
P4	23.60 ± 0.69^{c}	8.25 ± 0.60^{b}	1	0
P5	16.26 ± 3.48^{c}	8.22 ± 0.54^{bc}	1	37.33
P6	23.40 ± 1.91^{c}	7.72 ± 0.17^{c}	1	58.66

The averages followed by the same letter on the columns are not significantly different according to the Tukey-Kramer test at 5%.

The study period, which extends from early July to early October, was marked by three rating scales: scale 3 corresponds to the beginning of the experiment with a leaf density of 60.80%; scale 2 is characterized by a leaf density between 26 and 50% (P2 and P3). and the rating scale 1, which corresponds to a leaf density between 1 and 25% (P4, P5, and P6). Between periods 1 and 5, we observed a decrease in leaf density of 73.26% on average over the three (3) File-blocks monitored. On the other hand, between periods 5 and 6, we observe an increase in leaf density of 29.38%, corresponding to a period of marked leaf emission (58.66%), located at the end of September following a very clear defoliation that occurred at the end of August. Since refoliation took place during the rainy season, young foliage was very heavily affected by an ascomyconic melanin fungus called *Colletotrichum gloeosporioides*, causing the absence of refoliation on some trees at this time. The production of wet coagulated cup-bottom latex mass decreased by 16.81% (9.28 kg to 7.72 kg) during the study period for the 25 cup bottoms of the File-blocs.

A Pearson correlation between leaf density and wet coagulated cup-bottom latex mass was determined and presented in Figure 4.

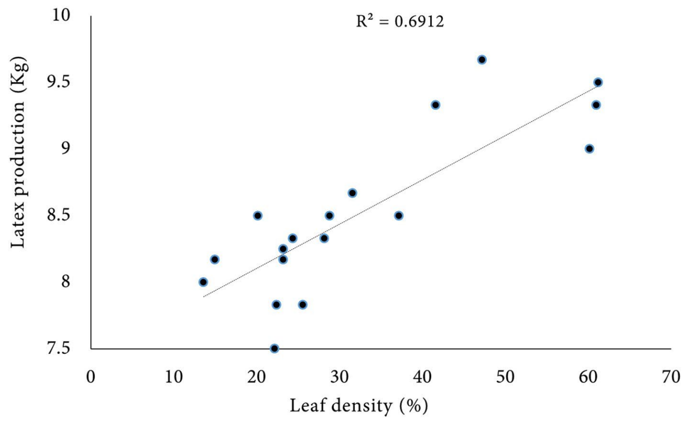

Figure 4. Correlation between leaf density and wet coagulated cup-bottom latex mass of the clone GT1.

The results from the statistical analyses for clone GT1 gave a Pearson correlation between leaf density and cup base per block file equal to 0.83 (r = 0.83) for a value of $p < 0.0001$ (Figure 3). From this analysis, taking into account the value of r^2 (0.6889), it appears that 68.89% of production is influenced by leaf density.

3.3. Evolution of Leaf Density and Production of Clone PB235

The evolution of leaf density, production and leaf emission periods (Stage A) of clone PB 235 are presented in Table 4.

Table 4. Variation in leaf density (LD) and wet coagulated cup-bottom latex mass (CB) per block file (BF) of the PB 235 clone.

Periods	LD (%)	CB/BF (Kg)	Rating scale	Stage A (%)
P1	80.07 ± 1.63^{a}	8.78 ± 0.69^{a}	4	10.66
P2	70.07 ± 4.82^{b}	8.86 ± 0.13^{a}	3	0
P3	61.20 ± 3.67^{b}	8.00 ± 0.60^{ab}	3	0
P4	53.40 ± 2.12^{b}	6.72 ± 0.58^{b}	3	0
P5	40.93 ± 1.86^{c}	6.08 ± 0.58^{c}	2	2.66
P6	37.73 ± 0.42^{c}	6.11 ± 0.68^{c}	2	17.33

The averages followed by the same letter on the columns are not significantly different according to the Tukey-Kramer test at 5%.

The leaf density of PB235 clone during the experimental period is characterized by three main rating scales. The first scale of 4 for a leaf density of 80.07% (period 1), the second scale of 3 observed in periods 2, 3 and 4 for a leaf density ranging from 70,7% to 53.40%, and finally the third scale characterized by a leaf density of 40.93% for period 5 and 37.73% for period 6. The clone PB235 started with 10,66% leaf emission at period 1 and defoliated then after from period 2 to priod4, and finally issued many young leaves at the end of September. The trees in rather poor condition on the foliar level were able to reconstitute a correct canopy (+17%) more in line with the usual appearance of this clone. The PB235 clone recorded a decrease in leaf density of 63.12% during the study period from July (80.07%) to period 6, corresponding to the end of September or the beginning of October (37.73%). Production during the study period ranges from an average of 8.78 kg to 6.11 kg for the 25 cup bases of the File-blocs indicating a decrease of about 30.41%.

A Pearson correlation between leaf density and latex production was determine and presented in **Figure 5**. Statistical analyses for PB 235 gave a Pearson correlation between leaf density and cup-bottom latex mass per block file equal to 0.90 ($r = 0.90$) for a value of $p < 0.0001$ (**Figure 5**). From this analysis taking in to account r^2 (0.81), it appears that 81% of production is influenced by leaf density.

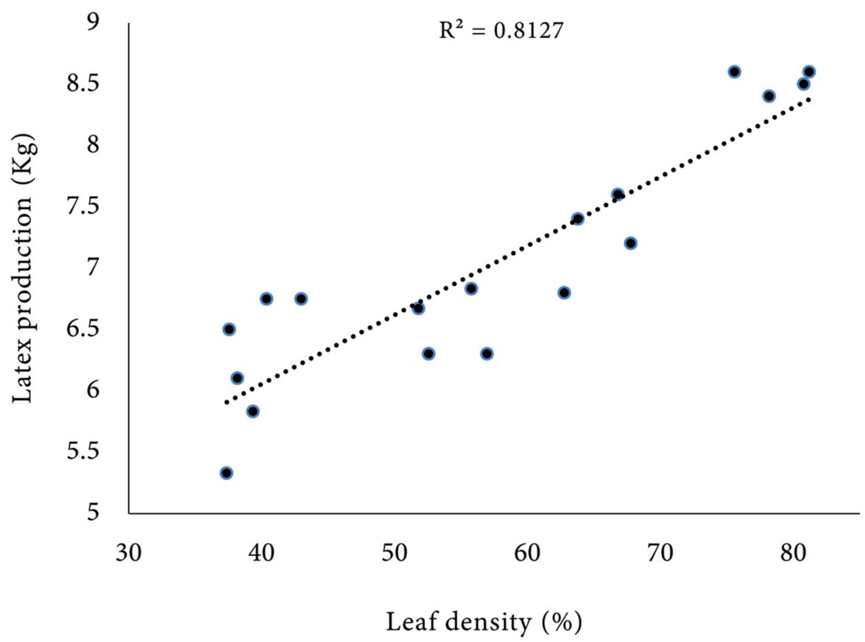

Figure 5. Correlation between leaf density and wet coagulated cup-bottom latex mass of the clone PB235.

3.4. Evolution of Leaf Density and Production of Clone PB260

Table 5 shows the evolution of leaf density, wet coagulated cup-bottom latex mass and leaf emission periods (Stage A) of clone PB260.

Table 5. Variation in leaf density (LD) and wet coagulated cup-bottom latex mass (CB) per block file (BF) of the PB 260 clone.

Periods	LD (%)	CB/BF (Kg)	Rating scale	Stage A (%)
P1	83.33 ± 4.83^{a}	8.75 ± 0.30^{b}	4	0
P2	79.27 ± 6.45^{a}	8.92 ± 0.33^{ab}	4	0
P3	62.87 ± 2.53^{b}	8.66 ± 0.15^{b}	3	0
P4	50.67 ± 7.81^{b}	8.50 ± 0.40^{bc}	2	0
P5	35.95 ± 7.16^{c}	8.15 ± 0.17^{c}	2	9.33
P6	30.73 ± 6.30^{c}	7.86 ± 0.12^{c}	2	40

The averages followed by the same letter on the columns are not significantly different according to the Tukey-Kramer test at 5%.

The leaf density of the PB260 clone during the experimental period is characterized by three main rating scales. The first scale of 4 for a leaf density between 79.27 and 83.33% (period 1), the second scale of 3 observed in period 3 for a leaf density of 62.87%, and finally the second scale characterized by a leaf density between 30.73 and 50.67% (periods 4, 5 and 6). The clone PB 260 has a more homogeneous foliage, although having defoliated this clone had issued many young leaves at the end of September. The latter presented a marked refoliation period and all the block-files of this clone issued new leaves at the beginning of October. The trees in rather poor condition on the foliar level were able to reconstitute a correct canopy (+9.33%) more in line with the usual appearance of this clone. The PB60 clone recorded a decrease in leaf density of 52.60% during the study period from July (period 1) to period 6, corresponding to the end of September or the beginning of October. Production during the study period ranges from an average of 8.75 kg to 7.86 kg for the 25 cup bases of the File-blocs indicating a drop of about 10.17%.

Statistical analyses for PB 260 gave a Pearson correlation between leaf density and cup-bottom latex mass per block file equal to 0.88 ($r = 0.88$) for a value of $p < 0.0001$ (Figure 6). From this analysis, with r^2 (0.7744) it appears that 77.44% of production is influenced by leaf density.

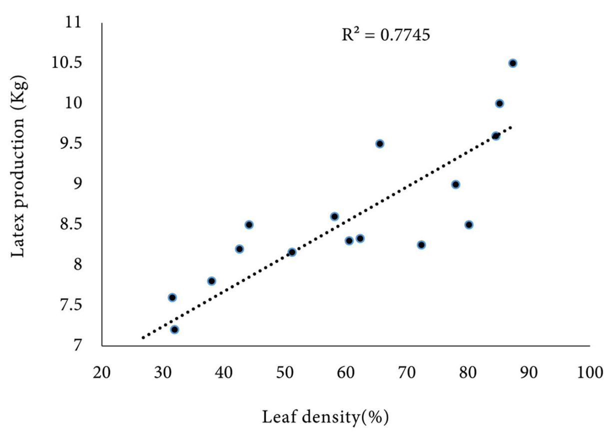

Figure 6. Correlation between leaf density and wet coagulated cup-bottom latex mass of the clone PB260.

3.5. Evolution of Leaf Density and Production of Clone RRIM600

The evolution of leaf density, wet coagulated cup-bottom latex mass and leaf emission periods (Stage A) of clone RRIM 600 are presented in Table 6.

Table 6. Variation in leaf density (LD) and wet coagulated cup-bottom latex mass (CB) per block file (BF) of the RRIM 600 clone.

Periods	LDF (%)	CB/BF (Kg)	Rating scale	Stage A (%)
P1	55.87 ± 2.58[a]	9.05 ± 0.22[a]	3	0
P2	42.27 ± 3.06[b]	8.93 ± 0.12[ab]	2	0
P3	31.05 ± 2.24[c]	8.80 ± 0.15[ab]	2	0
P4	23.27 ± 1.57[d]	8.52 ± 0.32[b]	1	0
P5	16.33 ± 1.72[e]	8.27 ± 0.28[bc]	1	24
P6	24.20 ± 0.60[d]	7.98 ± 0.32[c]	1	58.66

The averages followed by the same letter on the columns are not significantly different according to the Tukey-Kramer test at 5%.

The leaf density of this clone during the experimental period is characterized by three main rating scales. The first scale of 3 for a leaf density of 55.87% (period 1), the second scale of 2 observed in periods 2, 3 for a leaf density between 42.27 and 31.05%, and finally the scale 1 characterized by a leaf density between 23.27 and 24.20% (periods 4, 5 and 6). Between periods 1 and 5, we observed a decrease in leaf density of 70.77% on average over the three (3) File-blocks monitored. On the other hand, between periods 5 and 6, we observe an increase in leaf density of 32.52%, corresponding to a period of marked leaf emission (58.66%), located at the end of September following a very clear defoliation that occurred at the end of August. The behavior of this clone was similar to that of GT 1: clear defoliation in August followed by refoliation in mid-September. On this clone, defoliation gave rise to very marked and very spreading foliar emissions. Production during the study period ranges from an average of 9.05 kg to 7.98 kg for the 25 cup bases of the File-blocs, with a drop of about 11.82%.

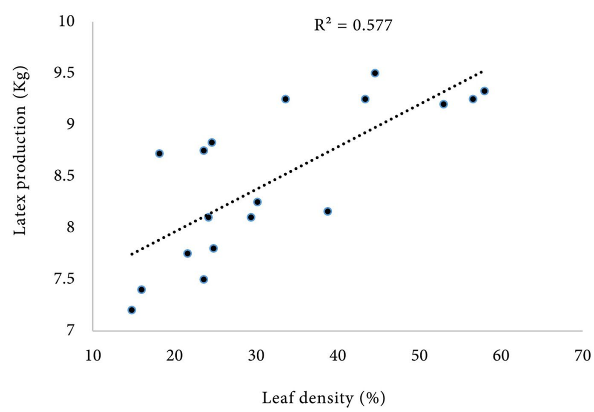

Figure 7. Correlation between leaf density and wet coagulated cup-bottom latex mass of the clone RRIM600.

Statistical analyses for RRIM600 yielded a Pearson correlation between leaf density and cup bottom per block file equal to 0.76 ($r = 0.76$) for a value of $p < 0,0001$ (**Figure 7**). From this analysis and taking into account r^2 (0.5776), it appears that 57.76% of the latex production is influenced by leaf density.

4. Discussion

Over the last five targeted years (2009-2014) at Siat Kango, rainfall during the defoliation and refoliation months (June, July, August and September) varied annually, with 2013 seeing no rain from July to September, while other years experienced significant September rainfall that peaked at 306.3 mm in 2014. This indicates that rainfall varied from one month to another and does not depend on years. With climate changes, it has been well establishing that rainfall varied from years to years [10] [11].

The study tracks a severe defoliation and subsequent partial refoliation cycle over a three-month period for all the four clones studied. The canopy collapsed from a baseline density of 60.80% (GT1), 80% (PB235), 79.27% (PB260), 55.87% down to critical levels between 16% and 38% representing Scale 1 (GT1 and RRIM600) and Scale 2 (PB235 and PB260). This represents a massive 73.26% maximum drop in leaf volume across the monitored blocks and clones. Wintering pattern depends on the types of hevea clone, age of the plant and climate parameter [12]-[15]. It is well established that latex yield varies among clones due to genetic variability, anatomical variations, and climate conditions [16]-[18].

High individual leaf density is supposed to increase yield and more photosynthate (sugar) reserves available to convert into latex, while defoliation (low leaf density) decreases yield leading to severe reduction in energy production during leaf-dropping periods.

A distinct leaf emission pulse greater than 40% was observed on clones GT1, PB260 and RRIM600, while PB235 leaf emission was around 17% triggered more than 14% increase in leaf density shifting from period 5 to 6. This highlights the natural resilience and high vegetative growth capacity of all clones under favorable moisture conditions [19]-[21].

High humidity and rainfall during the late September refoliation provided optimal microclimate conditions for the germination of fungal spores. This ascomycete fungus heavily targeted the highly susceptible young flushes of leaves. The attack was severe enough to completely halt refoliation on multiple trees, cementing a state of secondary defoliation. The vulnerability is compounded by the genetics of clone GT1 and the practice of monoculture cultivation [22] [23]. The extended timeline required for the canopy to recover further escalates disease risk. This correlates with the findings linking elongated refoliation periods to heightened powdery mildew infection rates due to prolonged exposure of vulnerable, immature tissues [24]-[26].

Total latex mass from the 25 cup bottoms dropped by 16.81% (falling from 9.28 kg to 7.72 kg). Latex synthesis in the laticifer vessels relies entirely on sucrose produced via photosynthesis in mature leaves [27] [28]. Severe defoliation starves the tree of metabolic energy, directly limiting rubber regeneration between tapping sessions. The coefficient of determination indicates that 57.76% to 81% of the variance in wet coagulated cup-bottom latex mass is likely associated with leaf density [27] [28]. The remaining 42.24% to 19% can be controlled by tapping frequency, disease pressure, soil nutrients, stimulant concentration or ambient temperature including rainfall.

Among the four evaluated clones, PB260 proved to be the most resilient, restricting leaf loss to 52.60% and minimizing its latex production drop to just 10.17%. Conversely, PB235 was the most vulnerable, suffering a 30.41% yield plunge due to its extreme dependency on leaf density (81%). While RRIM600 managed a strong late September canopy rebound (+32.52%) after a heavy 70.77% defoliation, GT1 experienced the highest initial leaf loss (73.26%) and completely failed to recover due to severe rainy-season *Colletotrichum gloeosporioides* fungal attacks.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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