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Seasonal and Clonal Variations in the Latex and Raw Rubber of Hevea brasiliensis

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The variation in the quality of raw natural rubber causes serious problems associated with processing and grading with the quality of processed crop varying with changes in season, clonal origin and especially with the maturation time of the cuplump material. This study was undertaken to evaluate the contribution of these sources of variation and where possible, identify latex physiological parameters as early markers of properties of the processed crumb. Relationships between certain physiological parameters of latex analysed by micro-latex diagnosis technique and some relevant technological properties of the raw rubber were estimated for some selected clones. These were found to be greatly influenced by eco-climatic conditions. Highly significant clonal variations were also observed in the physiological parameters of latex while these variations were not so consistent for most of the technological properties. Finally, significant correlations were observed between some technological properties and physiological parameters notably between the inorganic phosphorus and plasticity retention index and between the total solid content and the Mooney viscosity.

Natural rubber (NR) must be technically specified and graded for its wide industrial applications such as in the manufacture of heavy duty vehicle tyres, items of medical and paramedical use, adhesives, footwear, *etc*. The quality of a rubber product is amongst others a function of the quality of the raw rubber, which explains the persistent demand by manufacturers for more consistent raw materials.

The quality of processed raw NR and its variability depend in part on the quantities and qualities of the non-rubber elements it contains. The physiological parameters, described by the 'Latex Diagnosis' (DL) technique to evaluate the status of the laticiferous system of the *Hevea brasiliensis*[1], could be implicated in this variability. They are specific for all clones and strongly linked to environmental factors such as exploitation and climatic conditions[2]. Hence, the tapping system, the stimulation with *Ethrel* and the height of cut can modify their relative values[3–5]. Important seasonal variations are described for certain parameters of latex-like sugar content, pH and total solid content[1]. In particular, a strong relation is suggested for the clone GT 1 between its dry rubber content and climatic factors like sunshine and cumulative

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rainfall[6] An exhaustive review of these seasonal climatic effects on the expression of the different physiological parameters is presented by Jacob *et al* [1] Technological properties, meanwhile, will vary with duration of maturation[7], season and clonal origin[8–10] This study was undertaken to evaluate, in the environmental conditions of the South West Province of Cameroon, the causes of variation in specification parameters of raw NR The main objectives were to determine the seasonal and clonal variability of processed NR and seek possible correlations with the physiological parameters of corresponding latex

MATERIALS AND METHODS

Planting Materials

The study involved three clones representing the main metabolic groups found in *Hevea brasiliensis* PB 235 — a high metabolism clone, GT 1 — a clone of intermediate metabolism and PB 217 — a slow metabolism clone All plantings were of similar ages (GT 1 1983, PB 235 / PB 217 1985), same panel opening dates (PB 217 / GT 1 1990 and PB 235 1991) and panel history (*Panel B*, 90 cm above the union) Trees were tapped every four days on half-spiral Stimulation was with 2 5% Ethrel®, eleven rounds per year on the panel for PB 217 and GT 1 clones [d/4, ½S↘, ET 2 5% Pa 11/y(m)] and four rounds per year for PB 235 clone [d/4, ½S↘, ET 2 5% Pa 4/y]

Determination of Physiological Parameters of Latex

Plots for each clone consisted of tasks of 100 trees in a homogeneous stand Latex sampling and analysis were in accordance with the micro-latex diagnosis (MLD) technique[11] After latex collection, 1 mL of blended latex was reserved for total solid content (TSC) measurement and another 1 mL was immediately coagulated in 9 parts of an acidic solution (TCA 2 5% + EDTA 0 1%) and preserved at 4°C for sucrose (SUC), phosphorus (Pi) and thiols (RSH) analysis This exercise was conducted monthly on a day before the first tapping of each month The SUC, Pi and RSH contents were analysed using a spectrophotometer Spectro-22 RS (Labomed Inc)

Determination of Technological Properties of Raw NR

After tapping of all the selected trees, the latex was left to coagulate naturally and the cuplumps allowed to mature for 3 days in cups and then stored for 15 days in the factory before processing They were processed under industrial conditions Test pieces were cut from dried crumbs for analysis Laboratory samples were homogenised in conformity with *ISO 1795*[12] The technological properties determined were the Mooney viscosity [ML (1+4)], the initial Wallace plasticity number (P_0), the plasticity retention index (PRI) and the ash content, all in conformity with their respective norms prescribed by the *ISO 2000*[13]

RESULTS

Climatic Characterisation of the Study Sites

The evolution in climatic factors for the period from July 1998 to June 1999 for the two estates where the study was undertaken (Sonne and Missellele) are presented in *Figure 1* (*a*) cumulative rainfall, (*b*) mean monthly temperature, (*c*) sunshine and (*d*) mean monthly relative humidity

The mean monthly temperature and the relative humidity were relatively constant

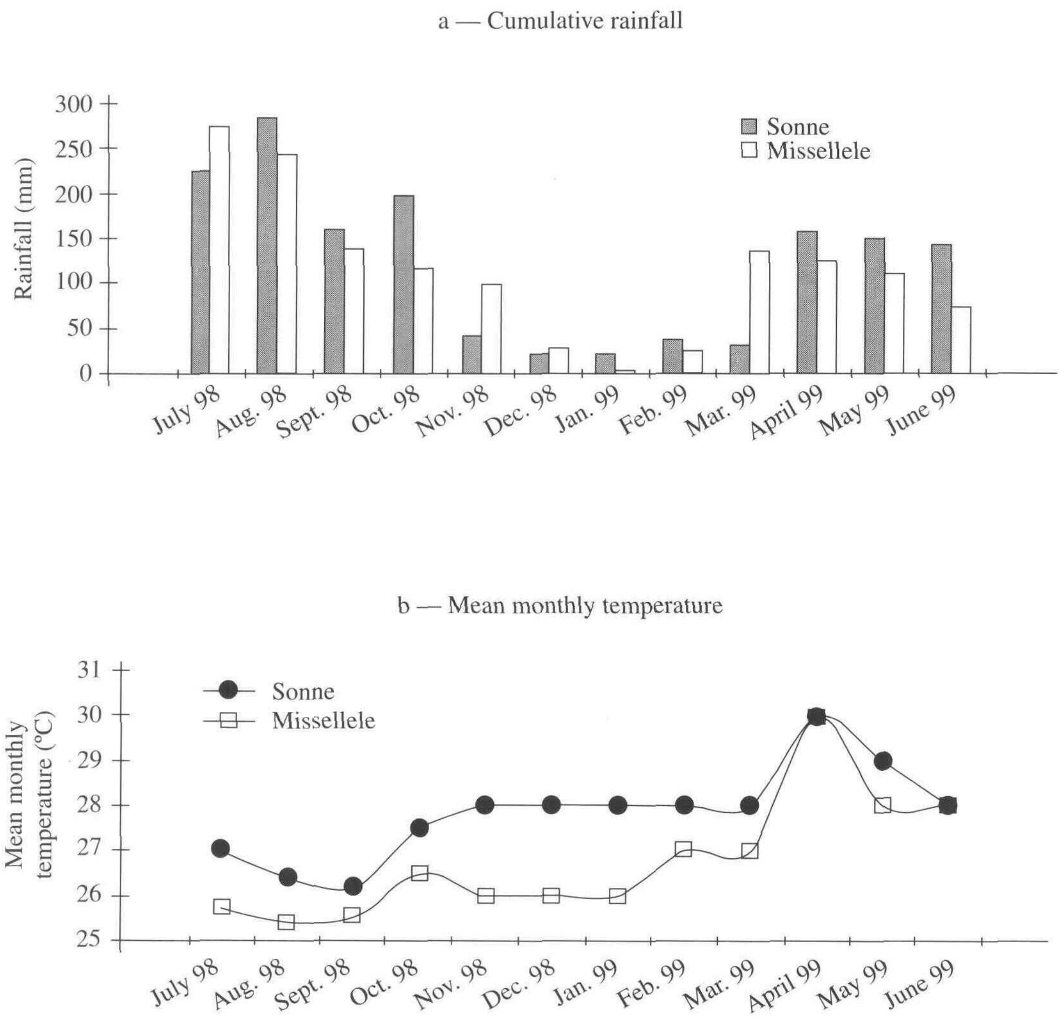
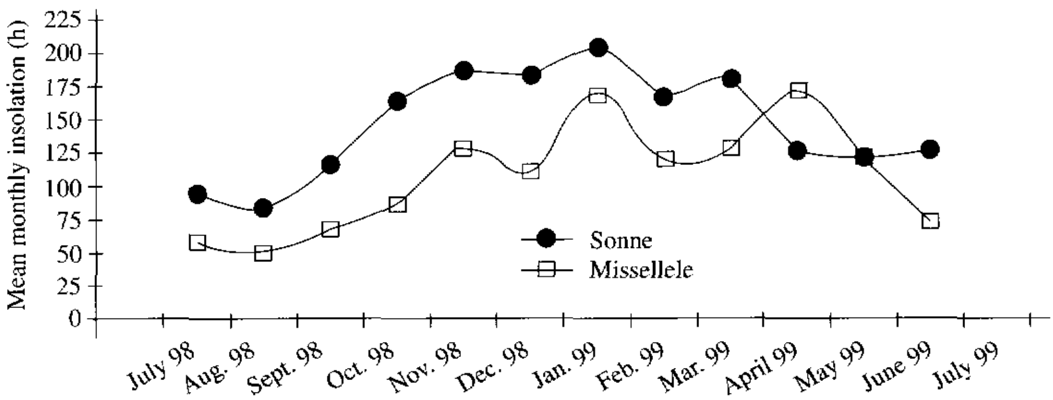


Figure 1. Evolution of climatic factors in Sonne and Missellele.

c — Mean monthly insolation



d — Mean monthly relative humidity

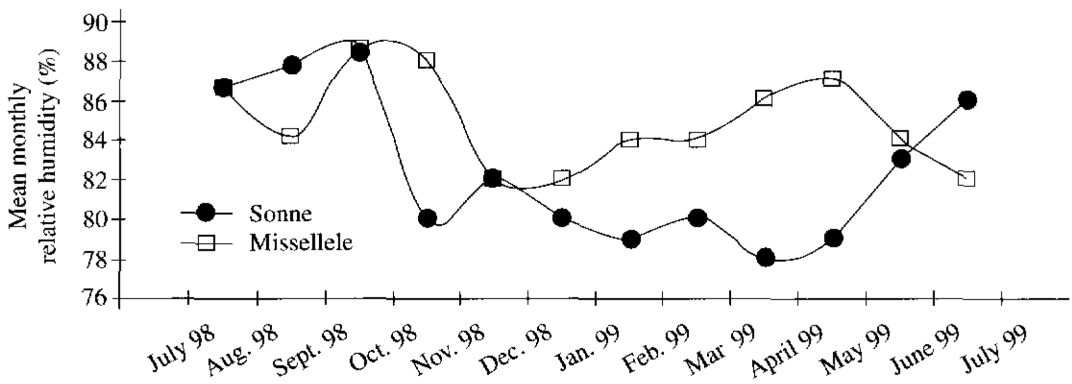


Figure 1 (cont.). Evolution of climatic factors in Sonne and Missellele.

throughout the year (25°C – 30°C and 78% – 88%, respectively) and as such the seasons could principally be distinguished by the pattern of rainfall distribution and the number of insolation hours: a rainy and cloudy season from July to October and a dry and sunny season from December to February/March. The rains were heaviest in July/August (200 mm – 300 mm) and lowest in December/January (5 mm – 15 mm). The months of April/June and November corresponded to intermediate seasons with rainfall of 100 mm – 150 mm. The monthly insolation generally increased from August to January for both sites.

Two main seasons could be distinguished, separated by an intermediate season: (*i*) *mid-June to October*: a major rainy season of overcast and misty weather, continuous rains and drizzle; (*ii*) *mid-November to mid-March*: a major dry season of dry and foggy weather, harmattan and diffused radiance; (*iii*) *mid-October to mid-November/mid-March to mid-June*: Intermediate seasons of changeable weather, storms and rain showers alternating with bright intervals.

Some climatic differences were observed between the two sites. Maximum rainfall during the major rainy season (284 mm) was observed in Sonne during the month of August while in Missellele it was in July (274 m). The maximum rainfall during the intermediate season was also observed one month later in Sonne (158 mm in April) than in Missellele (136 mm in March). The lowest mean monthly temperature (~ 25°C) was recorded respectively, in August and September for Missellele and Sonne. Sonne was generally hotter, more insolated (except in April) and had a lower relative humidity which decreased during the small and big rainy seasons (from ~ 89% in September to ~ 78% in March). The differences observed between the 2 sites with respect to the amplitude and phase of climatic factors could be in part explained by their respective geographical locations. Sonne is closer to the sea and benefits more from the seaside climatic influence.

Variations in the Physiological Parameters

The evolution of the physiological parameters is presented in *Figure 2 a, b, c* and *d* for TSC, SUC, Pi and RSH, respectively for the 3 clones. The existence of significant seasonal effects on these parameters was statistically verified (*Table 1*).

The evolution of the TSC for the 3 clones was similar, varying from ~ 40% (average level for the 3 clones during the major rainy season) to ~55% (very high clonal levels) during the major dry season. The clonal TSC values were very low (less than 40% in June for clone PB 235) during the rainy season. The significant negative correlation of the parameter with rainfall (B) for clones GT 1 and PB 217 (*Table 1*) reveals the importance of water availability in latex dilution. During the rainy season, when water availability is not limiting, circulation in plant tissues is stimulated, bringing about a drop in viscosity and improving flow. It is interesting to note the instantaneous response of TSC to climatic factors (*cf.* influence of I_0, B_0 and H_0) even if the climatic factors present a certain residual effect (*cf.* influence of B_1 and B_3 for clone GT 1).

Sugar content (SUC), varied similarly for the 3 clones (*Figure 2b*). However, the clone PB 235 (high metabolism) presented low monthly levels of sugar reserves (<5 mM from May to December — except in July), whereas PB 217 (a low metabolism clone) showed high sugar content (>10 mM for the period November/April). Clone GT 1 confirms its classification under the medium metabolic group with intermediate levels of sugar. For GT 1 and PB 235 cones, the decrease in SUC

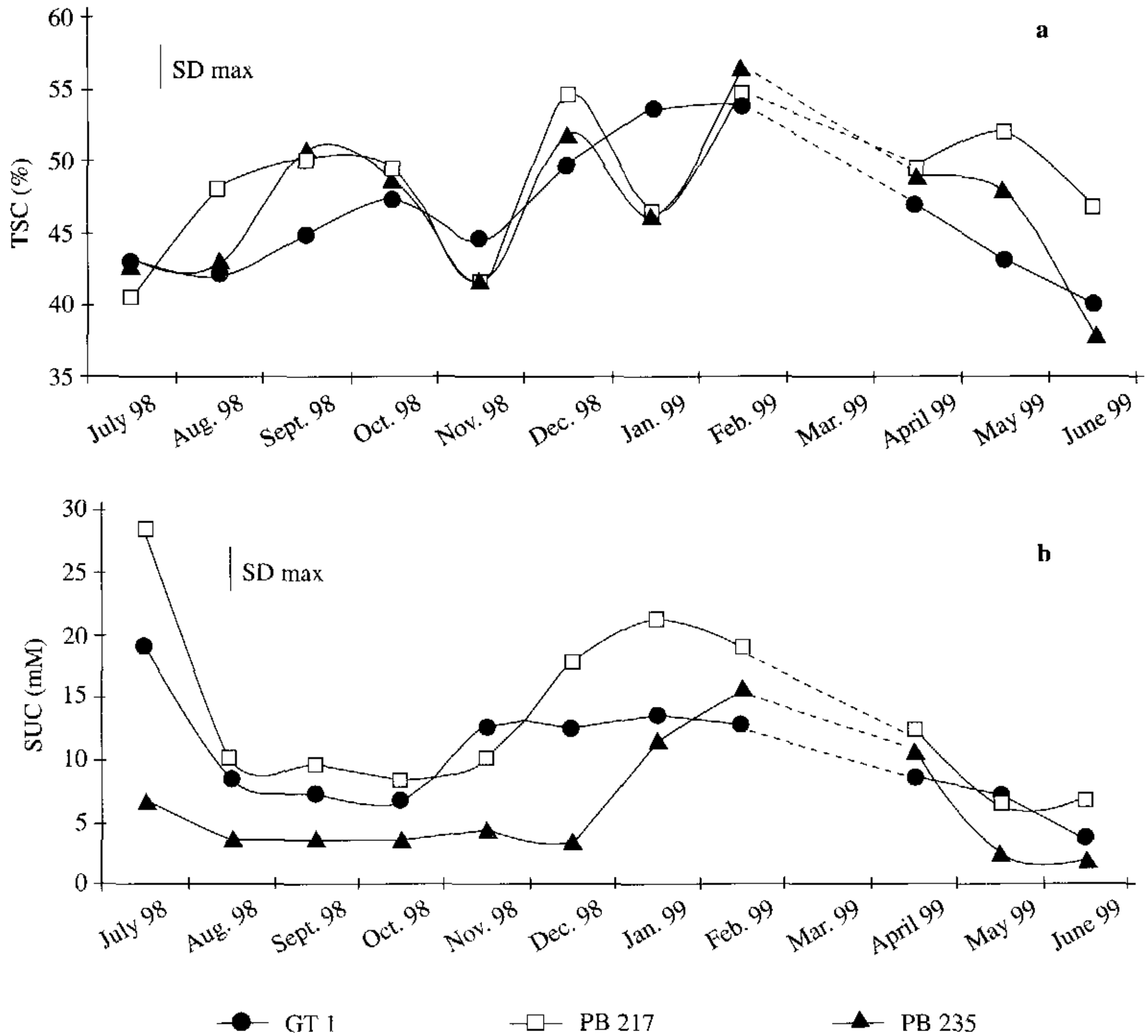


Figure 2. Evolution with time of the various physiological parameters for the three clones.

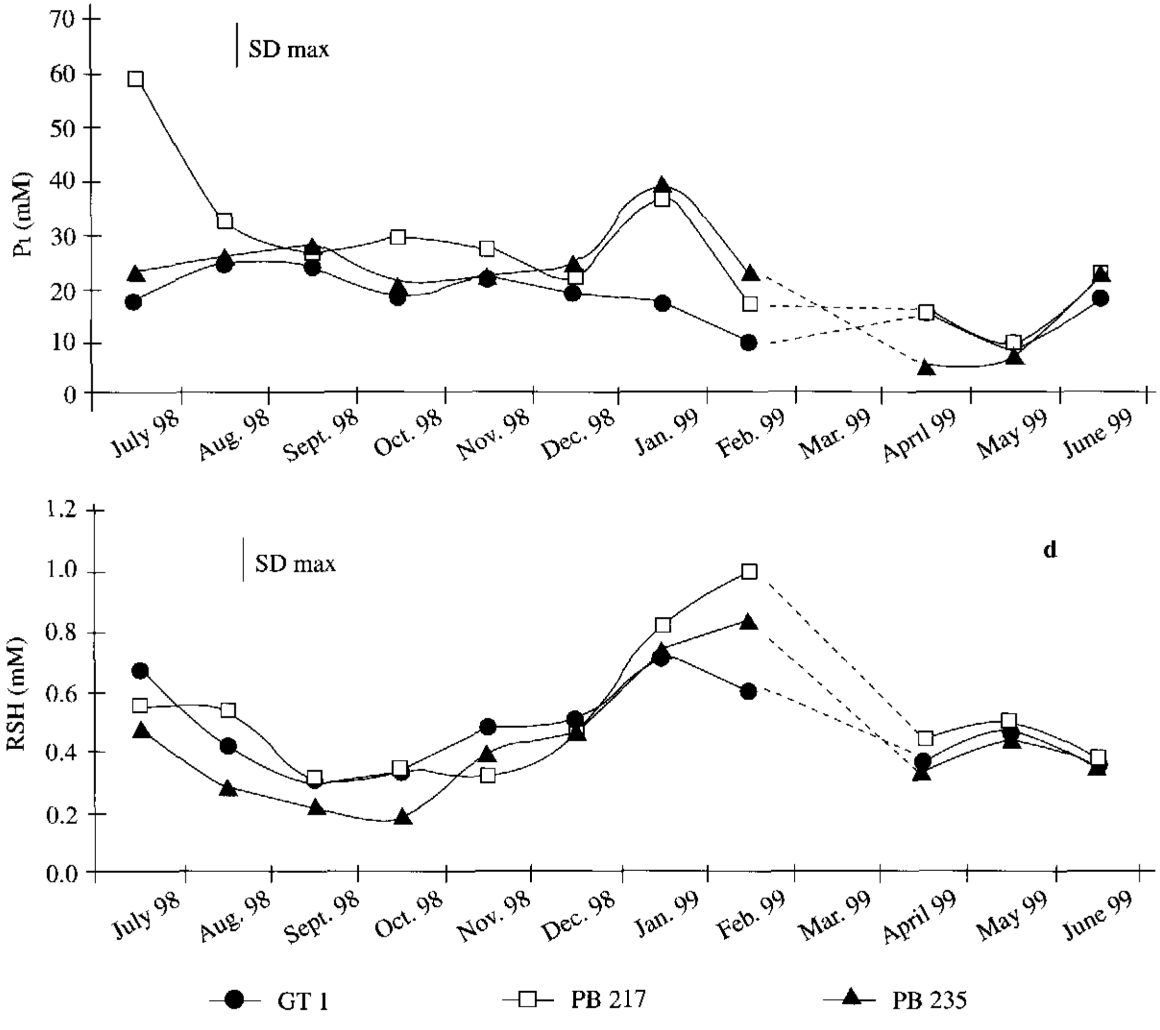


Figure 2 (cont.). Evolution with time of the various physiological parameters for the three clones.

TABLE 1. SIGNIFICANT LINEAR CORRELATIONS BETWEEN THE PHYSIOLOGICAL PARAMETERS AND THE CLIMATIC FACTORS (BASED ON THE MAGNITUDE OF THE LINEAR CORRELATION COEFFICIENTS)

Parameters	MISSELLELE				SONNE	
	GT 1		PB 217		PB 235	
	(+)	(–)	(+)	(–)	(+)	(–)
TSC	I_0, I_1	B_0, B_2, B_3, H_3		B_0		
SUC		B_1, B_3, H_1, H_3		B_1, B_3, H_1, H_3	I_1, I_3	B_0, B_1, B_3
Pi	B_1, B_3	I_1, I_3				
RSH		B_1, B_3, H_1, H_3		B_3, H_3		

(+) = Positive significant correlation; (–) = Negative significant correlation;
I = Hours of sunshine; B = Rainfall; H = Relative humidity.
Significance of numeric suffix : 0 – climatic factor of the month preceding the sampling;
1 – *Average climatic factor of the two months preceding the sampling*; 3 – *average climatic factor of* the three months preceding the sampling.

from July to November is associated with a high level of energetic metabolism [*cf.* (Pi) >20 mM during this period]. Clone PB 235 on its part, presented a high positive correlation between SUC and cumulative insolation (I_1 and I_3) indicating the prominent role of sunshine on its production. The negative effect of rainfall B_1 and B_3 on SUC also shows the strong impact of present climatic factors on eventual sugar synthesis.

The level of energetic metabolism revealed by inorganic phosphorus content (Pi) globally *decreased with increasing SUC, confirming the* relationship between these two physiological parameters. The highest Pi was observed in January for clones PB 235 and PB 217 (~40 mM) though the level decreased dramatically the following month, just before the wintering period. For clone GT 1, as presented in *Table 1*, Pi is inversely related to the cumulative sunshine I_1 and I_3, respectively and positively by rainfall B_1 and B_3, respectively of the 2–3 months preceding the analysis. This increase in Pi with cumulative rainfall indicates in particular that water availability regulated by high rainfall, low mean temperature and insolation facilitates the exploitation of the contents of the laticiferous vessels.

As for the other parameters, the trends in the evolution of RSH content were similar for the three clones (*Figure 2d*) though the clone PB 217 globally presented the best level of cell membrane protection with the highest RSH. The RSH generally tripled for all the clones during the major dry season, from 0.32 mM in November to 0.99 mM in February for clone PB 217 which synchronised with the accumulation of sugar reserves. As for SUC, a high negative correlation between rainfall and RSH was observed (*Table 1*), particularly in Missellele for clones GT 1 and PB 217. The maximum effect of this climatic factor on thiols turn-over was generally expressed 3 months later (*cf.* influence of B3 and H3 on RSH).

Variations in Technological Properties of Processed Crumb

Seasonal variations. The evolution in technological properties of processed crumb from the 3 clones is presented in *Figure 3* (*a*: initial Wallace plasticity; *b*: Mooney viscosity; *c*: plasticity retention index; *d*: ash content).

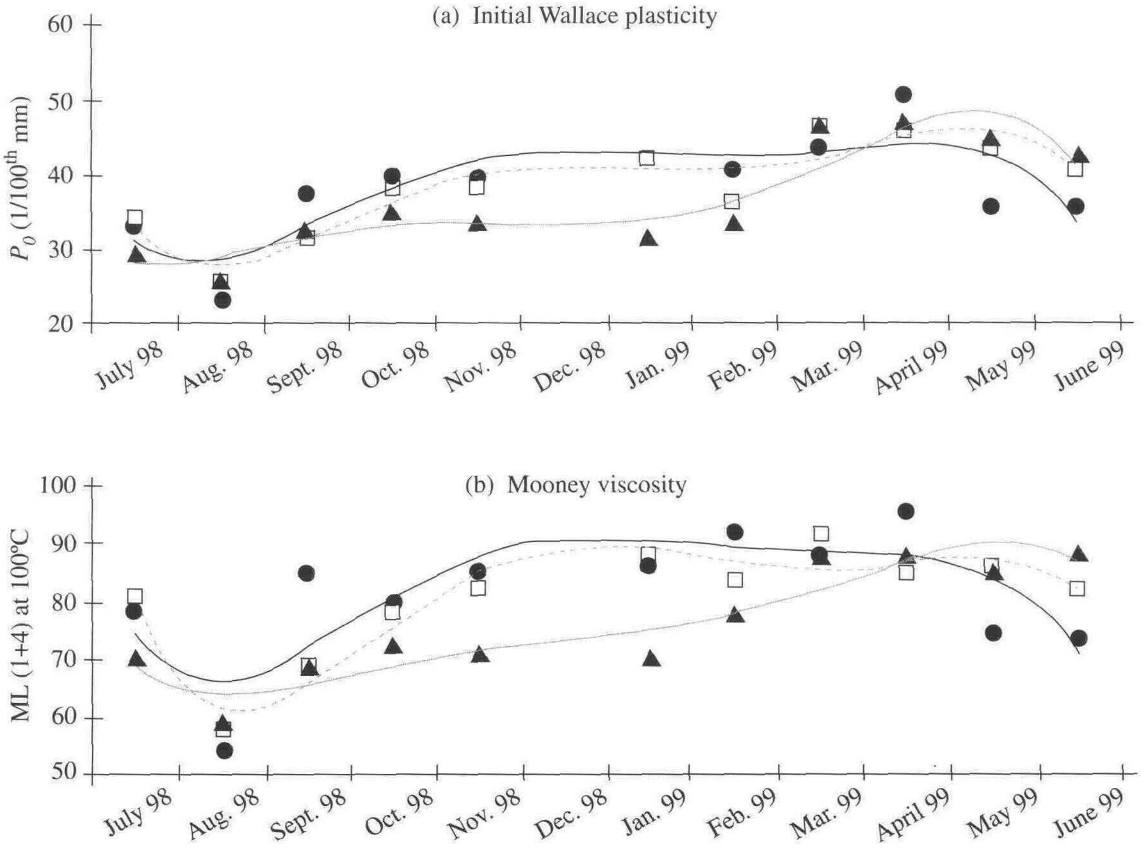
The general distribution of the three clones in terms of the initial Wallace plasticity number (P_0) and the Mooney viscosity was generally identical. For clones GT 1 and PB 217, these rheological properties were lowest during the month of August characterised by heavy rainfall and low sunshine. During the transition period (from September to November), these values increased by ~40% and then levelled off till the month of March. Then, P_0 and Mooney viscosity increased to a maximum in the month of May (which corresponds to the transition climatic period) and then dropped significantly thereafter. Even though PB 235 presented a similar trend all year round, the increase of these properties after the major rainy season was not as marked as for the other clones. However, the increase was significantly higher during the transition period that preceded the heavy rainy season, such that the values at this period were the same as for the other clones.

A high variability of the plasticity retention index (from ~30% to 80%) was observed throughout the period of investigation. The general trends of the PRI for clones GT 1 and PB 217 were similar to those of P_0 and Mooney viscosity, except that the plateau observed during the major dry season was not stable. For clone PB 235 which globally presented the lowest PRI values, there was a gradual drop from the month of May (75%) to December (35%) followed by a progressive increase till April. This was not the case for the other clones for which the PRI doubled from August to May (~40% to ~80%).

The clones GT 1 and PB 235, in terms of their ash contents, presented similar trends with a gradual increase from the major rainy season (~0.30% in July) to the major dry season (~0.55% in February) and then a progressive decrease to May–June. This trend was the same for clone PB 217 though with a lower amplitude (~0.30% in October to ~0.50% in March) and a phase lag of about four months compared to the two other clones.

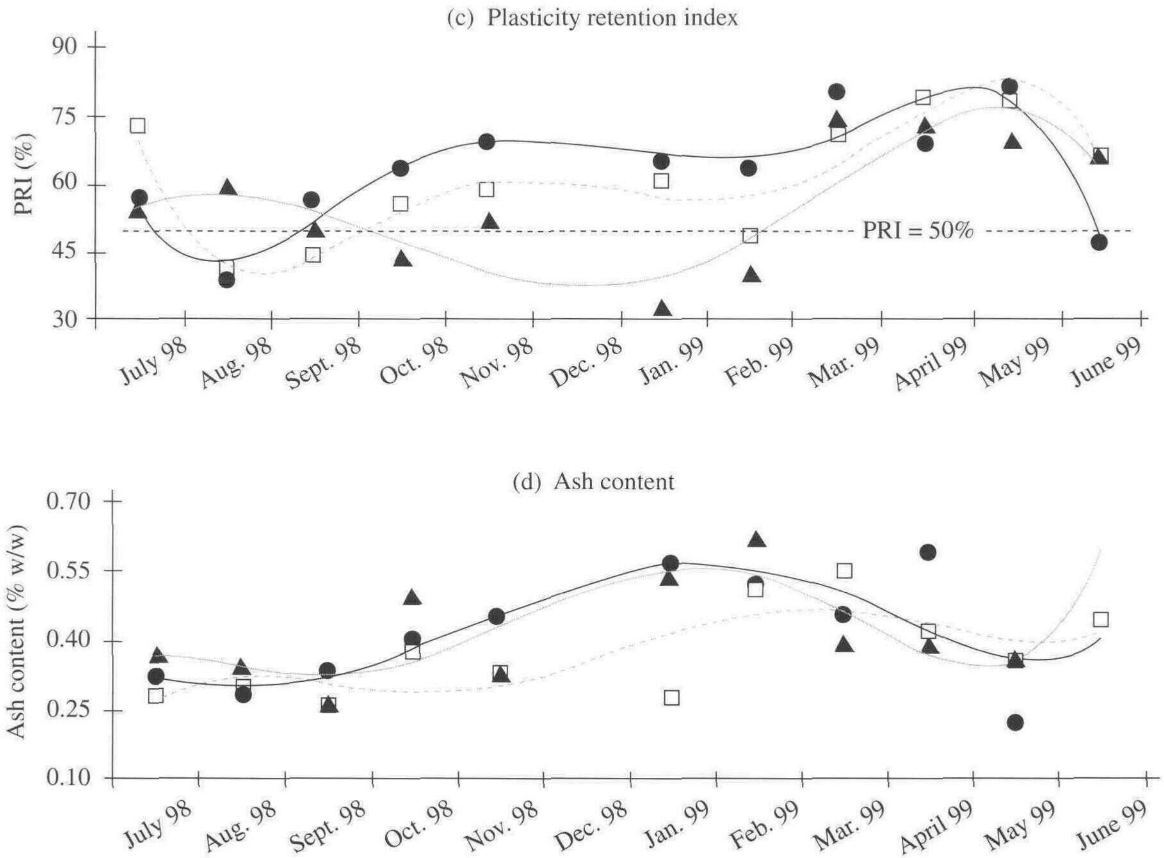
Clonal variations. The existence of significant effects of clonal origin on these technological properties was tested for each month of observation (using a general linear model procedure, SAS System) revealing in particular a differential effect of clonal behaviour on the technological properties with seasons. Thus, during the transition climatic period (October to November), the P_0 and the Mooney viscosity for clone PB 235 were significantly lower than for the other clones. Furthermore, clone GT 1 presented in February a significantly low PRI indicating the sensitivity of this property to clonal origin during the month preceding defoliation of trees. The ash content levels of clones GT 1 and PB 217 were significantly higher during the month of January (major dry season), whereas those of the clone PB 235 were higher during the month of March (climatic transition season).

Correlation between physiological and technological properties. Correlation analyses between the physiological and technological variables were conducted for each clone, the results of which are presented in *Table 2*. The TSC was positively correlated to Mooney viscosity, ash content and to a lesser extent, P_0 for clone GT 1. The SUC and RSH were tightly positively correlated to ash content for



● = GT 1; □ = PB 217; ▲ = PB 235
5th order polynomial best fit curves :
—— GT 1; - - - - - PB 217; —— PB 235)

Figure 3. Evolution in technological properties of crude processed rubber.



● = GT 1; □ = PB 217; ▲ = PB 235
5th order polynomial best fit curves :
—— GT 1; - - - - PB 217; ······ PB 235)

Figure 3 (cont.). Evolution in technological properties of crude processed rubber.

TABLE 2. PEARSON CORRELATION COEFFICIENTS BETWEEN TECHNOLOGICAL AND PHYSIOLOGICAL PARAMETERS

Parameters	P_0			PRI			ML (1+4)			Ash		
	GT 1	PB 235	PB 217	GT 1	PB 235	PB 217	GT 1	PB 235	PB 217	GT 1	PB 235	PB 217
TSC	−0.001	0.041	0.048	0.134	−0.391	−0.341	**0.651**	0.088	0.307	0.648	0.350	−0.271
SUC	0.557	−0.356	0.104	0.405	−0.105	0.382	0.107	0.116	0.126	0.120	0.636	0.399
Pi	−0.389	**−0.767**	−0.397	**−0.655**	**−0.786**	−0.072	−0.386	−0.547	−0.291	0.074	0.301	−0.547
RSH	−0.032	−0.191	−0.033	0.196	−0.368	−0.122	0.072	0.115	0.263	0.181	0.608	0.233

Correlations in bold were significant at $P<0.05$ (Pearson correlation coefficients, $|R| > 0.65$)

clone PB 235 (Pearson correlation coefficients of 0.636 and 0.608, respectively). On the other hand, Pi was negatively correlated to P_0 and Mooney viscosity (clone PB 235), PRI (clones GT 1 and PB 217) and ash content (PB 217).

DISCUSSION AND CONCLUSION

After describing the significant variations in climatic factors and the correlations existing between them, the physiological parameters were observed to be influenced most significantly by two groups of climatic factors (temperature and sunshine negatively correlated to relative humidity and rainfall).

The TSC that reveals the level of biosynthetic activity strongly depends on the seasonal variations, particularly rainfall. Water availability is not limiting following heavy rains, decreases its TSC and viscosity, and so facilitates latex flow[2,14]. This study illustrates that rainfall during the month of investigation affects the dilution of latex more than the cumulative rainfall of the months preceding analysis (*cf.* the high negative relationship observed between B_0 and TSC). Sucrose, the essential molecule in carbohydrate assimilates produced during photosynthesis, was influenced by seasonal variations, increasing with the number of sunshine hours. The positive correlation observed between SUC and I_1/I_3 suggests that the sugar content depends essentially on the cumulative rate of photosynthesis during the months preceding analysis. The decrease in sucrose content associated with an increased isoprenic synthesis logically synchronises with an increase in TSC, confirming previous observations[15]. Inorganic phosphorus content reveals the level of energetic metabolism in *cis*-polyisoprene synthesis and reflects the regeneration intensity in the latex-producing tissue. In this experiment, the SUC has confirmed its dependence on the level of energetic cell metabolism. Moreover, the increase in Pi wth cumulative rainfall indicates the importance of water availability in activating the energetic metabolism process. Finally, thiols which protect cell membrane against oxidative degradation[16] were strongly influenced principally by climatic factors (*cf.* the relationship between RSH and B_1, B_3, I_1, I_3). Concluding therefore, the variation in the physiological parameters of latex showed that water availability controlled by climatic factors is a limiting factor for latex synthesis and determines metabolism activation.

Significant correlations were observed between technological properties of processed crumb and the physiological parameters of latex. In particular, a positive relationship was found between the bulk viscosity and the TSC latex for clone GT 1. Latex with high TSC also has a high dry rubber content (DRC) so the positive correlation between TSC and Mooney viscosity could be subsequent to an increased probability for the formation of extended crosslinks with the increase in DRC. The clonal variation observed in the initial plasticity of processed crumb could be due to differences in the molecular size and structural arrangements of the rubber hydrocarbon as suggested by Bloomfield[17]. A hypothesis to account for the absence of such a relationship for PB 235 clone could be its high metabolism that may lead to the formation of shorter rubber hydrocarbon chains.

Thiols protect latex organelle membrane and are potential activators of key enzymes in rubber synthesis[2]. As protective antioxidants of cell membrane, they occupy the active sites for crosslinking, thereby inhibiting the crosslinking reaction that causes bulk viscosity increase[18]. The RSH would be expected to correlate positively with the level of degradation of the rubber as indicated by the level of PRI. However, the absence of this correlation could have been due to breakdown of the carbon-sulphur bonds in the thiols during processing and drying.

A highly significant negative correlation was observed between Pi and PRI for clone PB 235 and to a lesser extent, for clone GT 1. This could indicate that a high rate of metabolism in clones which are inherently activated leads to the production of macromolecular chains with incomplete protection after synthesis, leading to a rather high sensitivity to thermo-oxidative degradation. This relationship was not observed in the low metabolism clone (PB 217) where the slow formation of macromolecular chains could allow for their complete protection against ageing.

This study has enlightened some complex phenomena governing variability in the technological properties of processed raw rubber. The variations were greatly associated with eco-climatic conditions which also influence the physiological parameters of latex. The most significant correlations between the technological and physiological parameters could now be mathematically formalised so that rheological properties could be subsequently deduced from early field physiological observations. The physiological parameters could then serve as early markers of the technological properties of processed crumb.

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