Qualitative and Quantitative Variations in Rubber Content among Different Guayule Cultivars[†]

O. P. Sidhu, Neelima Ratti, and H. M. Behl*

Department of Tree Biology, National Botanical Research Institute, Lucknow 226 001, India

Quantitative and qualitative variations in rubber from 14 cultivars of guayule were studied. Average molecular weight ($\bar{M}_w$)was determined through gel permeation chromatography. There were wide variations in phenology, resin, rubber content, and rubber quality; however, no correlation could be observed among biomass, resin, and rubber content. Most of the cultivars had a unimodal refractive index response of polyisoprene except in those cultivars where resin content exceeded that of rubber. The high resin percentage affected the quality of rubber and not its quantity. The present study does not specify the essential threshold temperature minimum to trigger *cis*-polyisoprene synthesis. However, since all of the cultivars had low $\bar{M}_w$ values, the temperature minimum may be critical for controlling the balance between *cis*-polyisoprenoid and *trans*-resin biosynthesis. This paper provides additional information on the rubber-resin relationship and the quality of rubber produced by different cultivars of guayule. It also points to the need for additional research on correlation of genetic makeup with temperature/environmental factors in rubber yield and yield quality.

Parthenium argentatum Gray of Asteraceae is an alternative source of natural rubber. Commercialization of guayule depends ultimately on economically viable yields of rubber. Rubber and resin are both present in the shrub in appreciable but variable quantities. Guayule was introduced into India in the early 1980s. Several elite germ plasms were introduced; however, results after nearly 10 years of research indicate that the crop is not economically feasible because of erratic yields. Resin, though present in substantial quantities, is left unutilized and often discarded in the processing of rubber.

Isoprenoid synthesis in guayule is a highly regulated and complex pathway affected by an array of genetic, developmental, and environmental factors. Specific influences on rubber yield under agronomic conditions include varietal differences, plant age, water and nutrient availability, cold and disease stress, seasonal rhythms and dormancy, etc. (Hammond and Polhamus, 1965). Spence and McCallum (1935) reported that rubber accumulation is cyclical and is greatest during the fall and winter. Experiments conducted by Appleton and Staden (1991) confirmed that enzymatic potential for rubber biosynthesis is stimulated by minimum temperatures (7-10 °C), in combination with duration of the plants' exposure to these temperatures. They also identified increasing daily temperature ranges above a threshold value for day temperatures of between 10 and 15 °C as favoring rubber synthesis. Rubber-producing potential according to them was reduced to a residual level when plants were exposed to a thermal regime of 15/33 °C. On the other hand, freezing temperatures adversely affect the enzymatic potential for rubber synthesis (Downes and Tonnet, 1985). The role of the environment in regulating isoprenoid synthesis in guayule is of interest but not well understood.

Variations in rubber were attributed to genetic variations and facultative apomixis in guayule (Naqvi, 1985). He studied variability in rubber content among U.S. Department of Agriculture (USDA) guayule lines and reported a range in rubber content of 3-7% in these lines. However, in another study Naqvi (1986) found certain other cultivars to be homogeneous for rubber and resin. Estilai (1991) investigated various guayule entries for rubber, resin, and biomass content. Of the eight entries that were harvested at the ages of 21, 33, and 45 months, seven had significantly higher rubber content at the age of 21 months as compared to the same at 33 and 45 months. The resin content was higher in most of the entries at the age of 45 months compared with 21- and 33-month-old plants. Kuruvadi (1991) estimated the rubber and resin content in 38 guayule collections from a diverse breeding population in Mexico. The analysis of variance showed significant differences for percentage of rubber, whereas no significant difference was found for resin content. However, none of these workers has studied qualitative variations in rubber of available germ plasms.

Recent studies by Appleton and Staden (1989) and Norton et al. (1991) show that environmental factors such as light (photoperiod), temperature, sucrose levels, nitrogen source, and osmotic effects can influence the quality and quantity of rubber and resin. Goss et al. (1984) recorded increased incorporation of [^{14}C]acetate and [^{14}C]mevalonate into rubber fraction from plants exposed to low, nonfreezing temperatures. The effect of environment on resin production or the effect of resin percentage on quality or quantity of rubber has not received much attention. However, the quality of synthesized rubber is important as it determines the physical properties of rubber such as tensile strength and elasticity, which are so crucial for guayule (Martin et al., 1972).

In this study, the quantitative variability of resin and rubber of 14 guayule cultivars and qualitative variations in rubber of these cultivars have been reported. A gel permeation chromatography (GPC) method was used to determine molecular weight average ($\bar{M}_w$) and polydispersity (M_{wd}) of rubber. An attempt has also been made to discuss the relationship of the effect of resin content and local environmental conditions on rubber quality.

MATERIALS AND METHODS

Three- to four-year-old guayule cultivars (ARIZ-101, GH-9, UCR-7, UCR-1, N575, USDA-10, USDA-5, USDA-17, ALI-10, USS2X, G-89, G-97, C-244, and C-254) under trial at the research station of National Botanical Research Institute, Lucknow, India,

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* Author to whom correspondence should be addressed.

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Table I. Yields of Rubber, Resin, $\bar{M}_w$, $\bar{M}_n$, and Polydispersity (M_{wd}) among Guayule Cultivars

cultivar	rubber, %	resin, %	$\bar{M}_w$	$\bar{M}_n$	M_{wd}
USDA-5	8.21	8.07	2.5×10^5	7.2×10^4	3.55
C-244	8.04	8.92	2.9×10^5	9.2×10^4	3.19
C-254	7.86	6.15	1.0×10^5	3.4×10^4	3.12
N575	7.56	6.40	2.9×10^4	1.0×10^4	2.84
ALI-10	7.54	7.06	1.0×10^5	3.3×10^4	3.00
UCR-1	7.32	5.50	7.2×10^4	3.1×10^4	2.34
USDA-17	7.14	8.94	1.3×10^5	1.9×10^4	7.02
ARIZ-101	7.13	6.80	1.0×10^5	3.7×10^4	2.88
UCR-7	7.00	5.40	1.2×10^5	3.5×10^4	3.46
USDA-10	6.40	10.68	1.4×10^5	1.8×10^4	7.49
G-97	6.34	9.97	1.0×10^5	1.5×10^4	6.94
G-89	5.78	6.27	5.6×10^4	1.7×10^4	3.25
GH-9	5.50	7.30	1.2×10^5	2.2×10^4	5.58
USS2X	4.98	6.22	1.6×10^5	5.4×10^4	3.00

were investigated. These cultivars have been made of collections from University of California, Riverside, trials (USDA-5, USDA-10, USDA-17, UCR-1, UCR-7, and N-575), University of California, Davis, trials (C-244, C-254, ALI-10, and USS2X), and University of Arizona trials (ARIZ-101). Sources of G-series cultivars (G-89, G-97, and GH-9) are not known. Material for examination was harvested during January. Leaves, stem, and branches were separated. Larger branches were cut in 1–2-cm segments and dried in an oven at 45 °C. Samples were then ground to fine powder in a Wiley mill with a 2-mm mesh screen and again dried in an oven at 45 °C until a constant dry weight was achieved. There were four replications for each experiment.

Five-gram samples were extracted with acetone by using a Kinematica Polytron homogenizer and then filtered; the bulk of the solvent was removed using a Buchi rotary evaporator. The shrub resin content was calculated on the basis of the weight of the acetone extract residue. The air-dried, deresinated shrub was then extracted with hexane for 5 min, and the extract was worked up in a manner similar to that for the acetone extract to determine the rubber content of the shrub. The concentrated hexane extract thus obtained was precipitated with excess MeOH and centrifuged at 5000 rpm for 15 min. The coagulated rubber was then redissolved in HPLC grade tetrahydrofuran, filtered through a 0.5-mm Teflon filter, and used for gel permeation chromatographic analysis.

Rubber Analysis. The GPC measurements of hexane extractables were performed on a Waters HPLC system that consisted of a Waters 501 solvent delivery system, a Rheodyne 7125 sample injector, and a Shimadzu RID-6A refractive index detector. Ultrastyragel columns (10^6, 10^5, 10^4, and 10^3 Å) were connected in series and maintained at 25 °C. Flow rate of tetrahydrofuran (mobile phase) was 1 mL min^{-1}. To calculate the actual average molecular weight ($\bar{M}_w$), average molecular number ($\bar{M}_n$), and polydispersity of polyisoprenes (M_{wd}), the magnitude of the detected response was first determined manually by measuring the height of the chromatogram peak above the base line recorded response following the procedures of West (1986) and Margerison et al. (1973). Average molecular weights were calculated from the chromatogram at a resolution of 1-mm interval corresponding to 0.1 min of time. Analysis of GPC data was aided by a computer program in BASIC which produced calculations of yields of rubber.

RESULTS AND DISCUSSION

Rubber and Resin Content. Fourteen guayule cultivars were screened for their rubber and resin contents, $\bar{M}_w$, $\bar{M}_n$, and polydispersity. The same are presented in Table I. Rubber and resin values (average of 10 samples) are given as percentage of shrub dry weight and arranged in descending order of rubber concentration. The rubber percentage ranged from 4.98 to 8.21%, while resin percentage ranged from 5.4 to 10.68%. Rubber and resin in certain cultivars (USDA-5, C-244, and ALI-10) were present in nearly equal quantities, whereas the values in other cultivars varied. Rubber content in most of the cultivars was between 6 and 8% except GH-9 (5.5%) and USS2X (4.98%). There were considerable phenotypic variations even in the cultivars with similar quantities of rubber/resin, suggesting that phenology and rubber or resin content were not correlated. The regression output with rubber as independent variable and resin as dependent variable is given in Table II. R^2 value was 0.00028 with a regression constant of 7.2087. The analysis of variance for percentage rubber and resin between guayule cultivars showed significant differences.

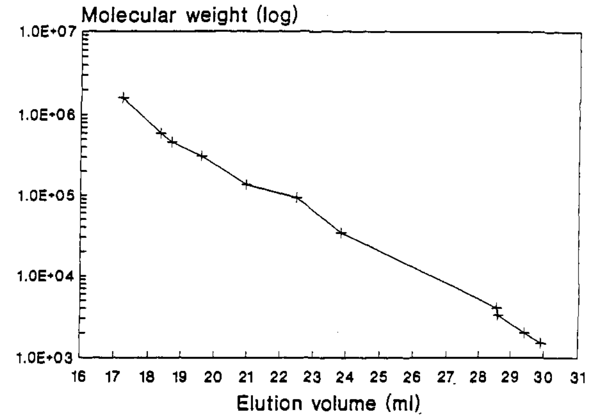

Figure 1. Calibration curve of polyisoprene standards.

Table II. Regression Output of Rubber and Resin Content in Guayule Cultivars with Rubber as Independent Variable

constant	7.208690	degree of freedom	12
standard error of Y est	1.727497	X coefficient(s)	0.028495
R^2	0.000284	standard error of coefficients	0.487811
no. of observations	14		

Quality of Rubber. $\bar{M}_w$, $\bar{M}_n$, and M_{wd} were calculated by gel permeation chromatography, which had the advantage that it resolved components of different molecular weight in a complex mixture of hydrocarbons. A calibration curve was drawn by using a wide range of polyisoprene standards with M_w from 1500 to 1.6×10^6. A plot of the logarithm of molecular weight vs elution volume for a set of standards indicated a linear relationship (Figure 1). The refractive index detector responses were found to depend linearly on the concentration of polyisoprene regardless of molecular weight. This is in conformity with earlier studies (Norton et al., 1991).

The average molecular weight ($\bar{M}_w$) ranged from 2.9×10^4 in N575 to 2.9×10^5 in C-244 (Table I). The average molecular number ($\bar{M}_n$) was in the range 1.0×10^4 to 9.2×10^4. There was no correlation in rubber quantity and quality, suggesting there are other variables that affect rubber quality. Cultivars with relatively high rubber output, such as N575, had low M_w and vice versa as in the case of USS2X, a diploid cultivar.

Campos-Lopez and Angulo-Sanchez (1976) measured the $\bar{M}_w$ and $\bar{M}_n$ of guayule rubber by osmometry and light scattering methods and reported values of 2.5×10^6 and 1.2×10^6, respectively. Benedict et al. (1950) and Hauser and LeBeau (1946) determined the same by a viscometry method and reported that guayule has a $\bar{M}_w$ of $1–2 \times 10^5$. Black et al. (1985) reported $\bar{M}_w$ values of 5×10^5 and 6×10^5 when guayule was examined by GPC. West (1986) reported that $\bar{M}_w$ of guayule was 1×10^6. Most of these workers did not mention the cultivars, selections, or entries of guayule investigated and have reported a single $\bar{M}_w$ value. They have not examined the intravarietal range. Hager et al. (1979) reported that 6-month-old guayule has a lower weight-average molecular weight, whereas mature plants yielded rubber with a $\bar{M}_w$ of $9.8–14.6 \times 10^5$ and M_{wd} of 1.7–2.4.

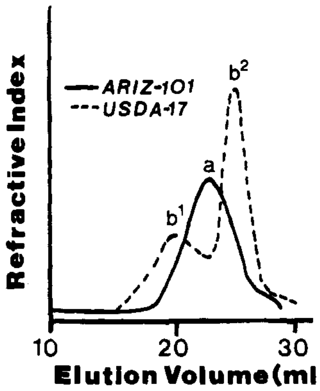

Figure 2. GPC chromatograms of guayule rubber. (—) Unimodal curve of ARIZ-101 with a peak maximum (a) at 266 000; (- - -) bimodal curve of USDA-17 with peak maxima at 440 000 (b^1) and (b^2) 18 750.

The M_{wd} for most of the cultivars in the present study was between 2.3 and 3.6, which is well within the acceptable limits. The GPC chromatograms of most of the samples were unimodal, showing little polydispersity. In Figure 2, a unimodal chromatogram of ARIZ-101 is shown with a major peak at 266 000. However, in those cultivars where resin percentage exceeded that of rubber, the M_{wd} was very high. It was 7.49 in USDA-10, where resin was 10.68% as compared to 6.4% rubber. In such cases, the GPC chromatogram was bimodal. This trend was observed in USDA-17 with high end peaks of 440 000 and 18 750 (Figure 2). Other cultivars in this category were G-97 and GH-9. USS2X, which is a diploid cultivar, was the only exception; its resin content was relatively higher than its rubber content, its M_{wd} was 3.00, and the chromatogram was unimodal. This cultivar is unique in the sense that it is slow growing, has a relatively low biomass, and synthesizes low quantities but good quality rubber. It is a useful genetic material for breeding studies.

It was interesting to observe that in cultivars where resin content was higher than that of rubber, it was not only the quantity but the quality of rubber which was affected. The data suggest that resin-producing potential has an influence on rubber-producing potential, although resin is produced continually whereas rubber synthesis is seasonal and accumulates in cold winter months. It appears that increased resin-producing potential or synthesis of *trans*-terpene resins interferes with the biosynthesis pathway leading to the synthesis of *cis*-polyisoprene. The same can be quantified from the M_{wd} patterns, and accordingly the quality of rubber can be predicted by analyzing the quantity of rubber and resin. The results also suggest that those cultivars which are rich in resin have poor utility as rubber producers and should be developed mainly for resin potential.

No direct correlation could be established between biomass and quality or quantity of rubber. Similar results have been reported by Hammond and Polhamus (1965) and Thompson et al. (1988).

Environmental Influence. Guayule in India has been found to have rubber with relatively low $\bar{M}_w$ as compared to the same in papers from other parts of the world. Even the M_{wd} in certain cultivars is relatively high. The effect of local environment cannot be ruled out. Appleton and Staden (1989) have demonstrated that minimum temperature, in combination with its duration and the extent to which it falls below the threshold value (below 6 °C), induces the enzymatic potential for *cis*-polyisoprene biosynthesis in guayule. The same was shown to have no effect on the enzymatic potential for *trans*-terpene resin.

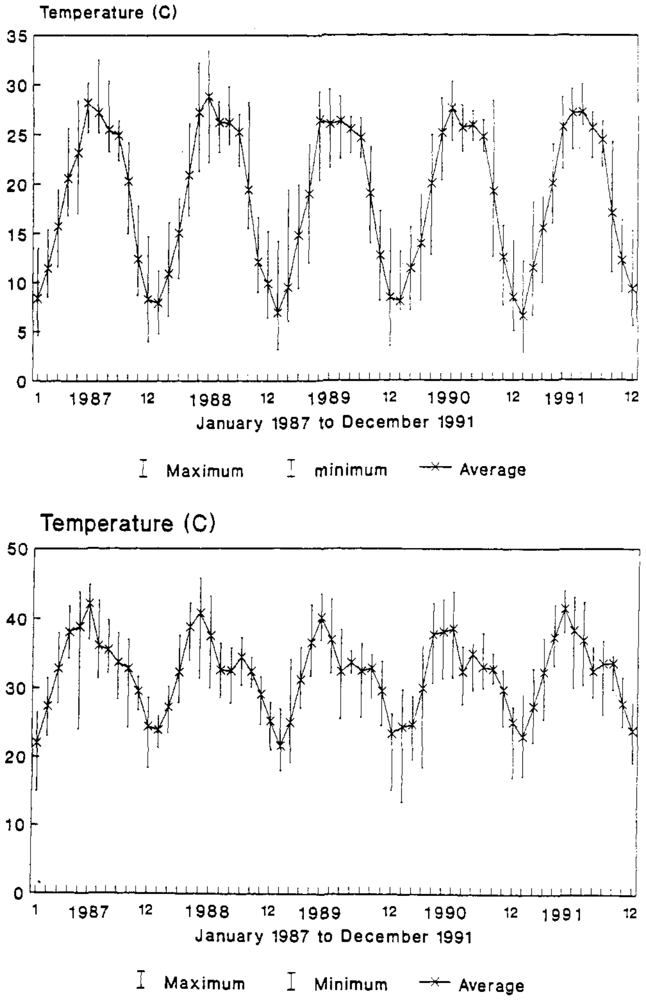

Figure 3. (A, Top) Monthly average of minimum temperature, with maximum and minimum deviations during 1987 through 1991. (B, Bottom) Monthly average of maximum temperature, with maximum and minimum deviations during 1987 through 1991.

Mean daily temperatures were monitored throughout the growth period of guayule during the present study. The mean minimum temperature in Lucknow (the experimental site) and most of the areas proposed for guayule cultivation in India was 18.62 °C (Figure 3A), and it rarely goes below the threshold limit of the minimum temperature of 6 °C proposed by Appleton and Staden (1989). On the other hand, the high temperature crosses 40 °C during the summer months (Figure 3B). Plants experienced a very short time at low temperatures (5–10 °C) during the year. Exposure of plants to high night temperatures could be the reason for relatively low rubber content. How critical the threshold limit of low temperature is remains to be established but, if true, explains the reason for low $\bar{M}_w$ of guayule in India. Appleton and Staden (1989) through the use of nondestructive radiochemical assay indicated that the initial steps leading to the *trans*-terpene resins are not limited by temperature variables and thus facilitate the establishment of a correlation between minimum temperatures and a penultimate stage in the biosynthesis pathway leading to the formation of *cis*-polyisoprene.

Benedict et al. (1986) stated that low temperatures do not stimulate the synthesis of *trans*-terpene resin. However, Appleton and Staden (1989) found that resin-accumulating potential increased during early winter, implicating a cold effect, but monitoring over longer periods produced no correlation between annual variations in environmental conditions and resin-accumulating potential. Abundant sunshine for most of the year and correspondingly high temperature appear to favor resin synthesis; however, different cultivars vary in their genetic makeup to make use of the abundant light.

The present study does not specify the essential threshold temperature minimum to trigger *cis*-polyisoprene synthesis, as many cultivars were found to have appreciable quantities of rubber on percentage dry weight basis. However, since all of the cultivars had low M_w values, the temperature minimum may be critical for controlling the balance between *cis*-polyisoprenoid and *trans*-resin biosynthesis and increase in molecular weight of the polyisoprene molecules. The potential for resin or rubber accumulation did not appear to be influenced by temperature or environmental conditions alone, as various cultivars examined showed different trends. Genetic makeup, leaf area index, and subsequently availability of assimilates available for rubber formation may affect the final products. Cytological studies revealed that these cultivars had different ploidy levels and showed other cytological variations (unpublished data). The role of genetic makeup needs further research.

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