Productivity and Cytogenetic Characteristics of Guayule. Screening of Plants from the Mapimi Region

Diana Jasso de Rodríguez[a,*], **José Luis Angulo Sánchez**[b], **Francisca Ramírez Godina**[a], **Regino Morones Reza**[a] and **Raúl Rodríguez García**[a]

[a]Universidad Autonoma Agraria Antonio Narro (UAAAN), 25213 Saltillo and [b]Centro de Investigacion en Quimica Aplicada (CIQA), 25000 Saltillo, Coahuila, México

Guayule plants were screened in the region of Mapimi (Durango, Mexico) to evaluate physicochemical parameters for selection of increased productivity. The objective was to characterize rubber, and resin content, stem diameter, rubber molecular weight and cytology of these plants to identify and select high productivity sites. Samples were collected from nine locations within the Mapimi Region. Inflorescences containing young floral buds of the same plants were selected to determine ploidy level. Cytogenetic results showed the presence of di-, tri- and tetraploids. Samples for rubber and resin analysis were cut from the branches. Genetic variation in molecular weight and molecular weight distributions for rubber was measured. Significant differences were found in resin content and stem diameter between the sampling sites, but differences were not significant for the rubber content between sites. A productivity parameter was defined and used to identify two locations where guayule genotypes exhibited the best characteristics for commercialization. This information may increase the industrial development and understanding of the polymerization bioprocess.

KEY WORDS: **Bimodal, cytogenetic results, diploids, molecular weight distributions, molecular weight, *Parthenium argentatum*, rubber and resin content, stem diameter, tetraploids, triploids, unimodal.**

Guayule (*Parthenium argentatum* Gray) is a source of natural rubber and co-products, such as resin (Re), low-molecular weight rubber (Ru), and woody bagasse. Industrial applications of the co-products could make Guayule utilization more profitable. In terms of quality and physical characteristics, guayule RU is as good as *Hevea* RU (1). It may be used in military aircraft tires and as nonsticking chewing gum base. In addition, blends of guayule RU with polyolefins, such as high-density polyethylene, behave like thermoplastic elastomers. The co-products consist of low-molecular weight (Mw) RU, which may be used as a plasticizer or as processing aids. The low-viscosity co-product polymer can also serve as feedstock for depolymerized RU that is utilized as an adhesive and in the manufacturing of molded products. The guayule RE may be used in wood preservatives, arthropod antifeedants and viscosity modifiers or plasticizers. The RE also acts as a prooxidant and reduces the viscosity of a natural-rubber cement in the presence of air. Phenolic antioxidant retards this peptizing effect. Bagasse is being considered as a cogeneration fuel, a feedstock for gasification and conversion to liquid hydrocarbons and as a source of fermentable sugar or fiber (2).

Greater use of latex products has increased allergic reactions to latex obtained from the *Hevea* tree (3). As many as 10% of health workers exposed to these products have developed latex-related skin problems. Recent studies indicate that guayule latex does not cause allergic responses. Thus, there is a tremendous potential use of guayule latex in the manufacture of medical and toy products. Latex products represent a $4 billion industry.

In Mexico, guayule covers 5,700 km^2 in the states of Coahuila, Durango, San Luis Potosí, Nuevo León and Chihuahua (4). Germplasm collections of high-rubber yielding guayule plants have been made from native Mexican stands for further evaluation and cultivar development (5–10).

Mexico provided germplasm for 21 of the 25 lines developed from "The 1942 Collection, the Emergency Rubber Project Research and the Hammond and Polhamus Collection". These lines are commonly referred to as "USDA varieties" (5,11). Guayule is a genetically diverse species, and different genetic systems are operating, such as polyploidy, apomixis and self-incompatibility (12–14). The level of ploidy has a profound effect on morphological, anatomical, physiological and genetic characteristics in plants. Diploids are normal sexual plants in which both reduction and fertilization occur. Sexual reproduction generates genetic recombination, a prerequisite for continued crop improvement through plant breeding methodologies. In guayule, there are self-incompatible plants with triploids and higher ploidy levels that generally are apomictic in nature. Apomictically produced seed is a clone of the plant from which it was collected and has the same genotype. Cytology studies in guayule germplasm from five different provinces in Mexico showed that the chromosome number varied from $2n = 2X = 36$ to $5n = 5X = 90$ (15). The state of Durango was the only site that had diploid materials (6,10).

Commercial guayule production requires an increase in the plant's RU yield, as well as the development of markets for the co-products, which represent 95% of the harvested plant. The first requirement can be met because natural genetic variation for RU content among native guayule stands ranges from 3.6 to 22.8% (4,5,16).

Researchers are also optimistic that they will be able to regulate environmental factors that influence RU and RE production (water, temperature, nutrients and harvesting), in order to custom-tailor the quality of RU and provide the desired content of high- or low-Mw REs (16).

RE content is the second important co-product of guayule (RU being the first); on the other hand, stem diameter is also important because it is related to the amount of biomass (17). In one study (18), plant height was highly correlated with plant dry weight and RU yield. In another study (19), it was observed that dry weight of a plant was the best predictor of the final RU yield.

Due to the diversity of uses mentioned above, it is important to define a generalized productivity parameter based on RU, RE content and stem diameter. To continue our studies on physicochemical parameters for selection of high-productivity guayule, a screening was conducted in nine sites of Mapimi. The purpose of this work was to evaluate productivity factors, such as RU and RE content, stem diameter, RU Mw and cytogenetic information. These data were used to develop a generalized productivity parameter to identify sites with superior resources.

---

*To whom correspondence should be addressed.

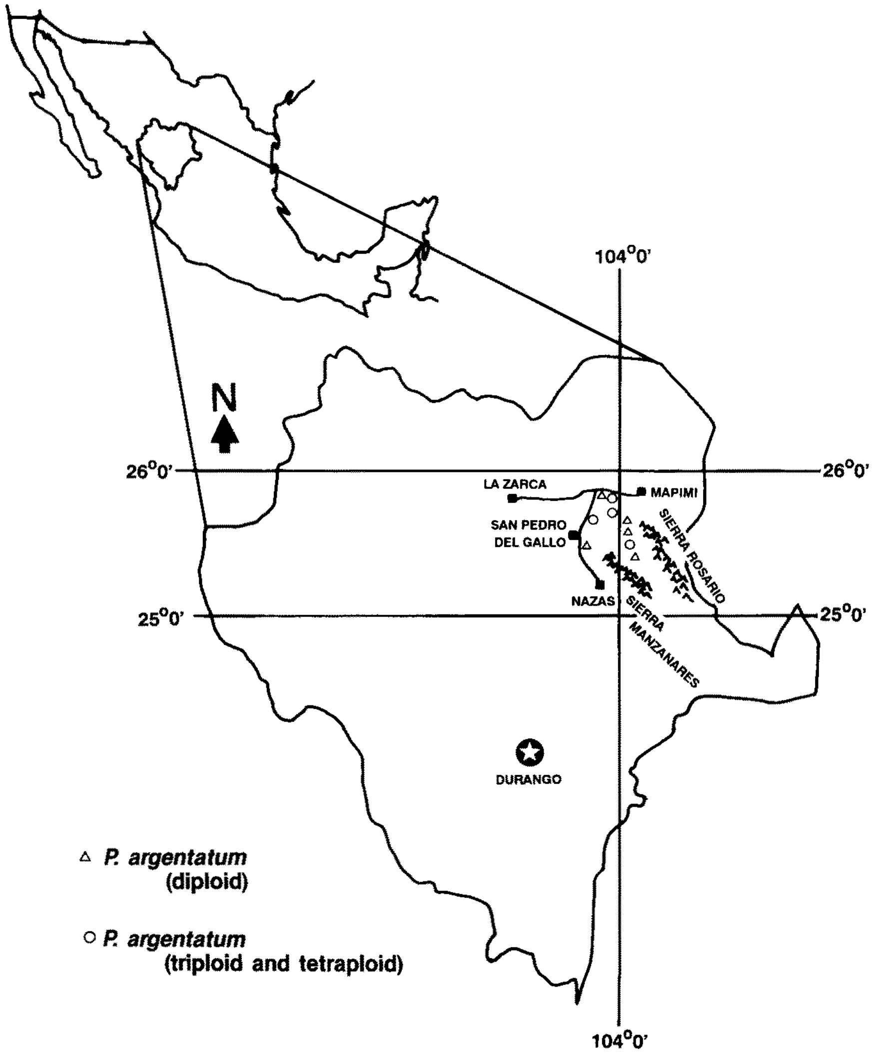

FIG. 1. Geographic distribution of *Parthenium argentatum* in sampling sites of the Mapimi Region (Durango, Mexico).

EXPERIMENTAL PROCEDURES

Samples from a variable number of plants (6–8) were collected from nine sites within the Mapimi Region during July at the beginning of the rainfall season. Samples for % RU and % RE analysis were cut from the branches at the central third of the total plant height, which varied between 40–60 cm. In addition, inflorescences containing young floral buds were selected from each plant. Buds were fixed in 3:1 (vol/vol) propionic acid/ethanol (96%) for 24 h and transferred into 70% (vol/vol) ethanol for preservation until analysis (20). All samples were stored over dry ice and transported to the laboratory, where they were kept at 20°C until analysis.

Stem diameter of samples was measured before grinding them in a Willey mill (Arthur H. Thomas Co., Philadelphia, PA) with a 2-mm mesh screen. Wet and dry weights were determined. Five grams of ground material were used for Soxhlet extraction with acetone and toluene as solvents for the quantitation of REs and RU.

Ground materials (500 mg) were also extracted for 2 h with 10 mL tetrahydrofurane (THF) in a flask at room temperature with agitation. The solution was decanted and 15 mL THF was added to the material. The extraction continued for another three hours; afterwards, the solutions were mixed and the volume was adjusted to 25 mL. This solution was filtered and injected (150 μL) into a size-exclusion chromatograph (Model 150-C, Waters Associates, Palo Alto, CA), with a refractive index detector and three analytical micro-styragel columns (0.3 m length × 0.078 m i.d.) of 10^6, 10^4 and linear nominal porosities. The carrier solvent was THF at 1 mL/min, and the temperature was 30°C.

Mw calculations were based on the universal calibration curve with Mark-Houwink constants (21). No corrections for axial dispersion or skewing were done.

A randomized block design was chosen for analysis of %RU, %RE and stem diameter with nine treatments (sites) and a variable number of replications (plants).

RESULTS AND DISCUSSION

Geographical distributions. Figure 1 shows the state of Durango and the Mapimi Region, where nine sites were sampled. It also shows the localization of diploid and polyploid guayule.

The analysis of variance shows (Table 1) that significant differences were found in RE content and stem diameter between the sampling sites. These data revealed substantial variability in the genetic material and also indicated the possibility of site selection.

The mean values for RE percentage among sites ranged from 8.51 (site 9) to 10.99 (site 1). However, the highest individual percentages obtained were: 9.45 (site 4); 10.04 (site 9); 10.11 (site 6); 10.88 (site 3); 10.99 (site 8); 11.53 (site 2); 11.30 (site 5); 12.23 (site 1) and 12.78 (site 7). The mean for the entire region was 9.6%. Reports (8) on RU and RE analysis in 346 native accessions from five states of Mexico showed a mean of 7.66% RE content for Mapimi. Stem diameter (cm) among sites ranged between 0.58 (site 5) to 1.16 (site 3), with an average mean of 0.864.

In regard to RU content, differences were not significant between sites; however, the average was 11.7% for the region. Naqvi and Hanson (5) reported that native guayule populations west of Mapimi, had 16% RU content. Kuruvadi (9) evaluated 346 indigenous accessions of guayule during three years and reported means of 8.13% and 8.65% RU content for San Pedro el Gallo and Mapimi, (in the state of Durango), respectively. As reported here, the highest individual percentages obtained were: 12.5% (site 1); 13.5% (site 9); 14.5, 15 and 15.4% (sites 3, 5 and 8); 16% (sites 6, 7 and 4); and 17.6% (site 2). Moreover, our finding that guayule plants produced more RU than RE differed with the results obtained by Kuruvadi *et al.* (17). We believe these data to show that the Mapimi Region has excellent genetic materials for the production of natural RU and co-products.

Cytogenetic studies revealed that plants of five of the nine sites sampled (sites 1, 3, 6, 7 and 9) had 18 pairs of chromosomes (2n = 36) (Fig. 2), as reported previously (12–15). These materials had regular meiosis without abnormalities. Triploids (2n = 54) (Fig. 3) were found on two sites only (sites 2 and 6), and tetraploids (2n = 72) (Fig. 4) were found on all sites. This is an important observation, because diploids have sexual reproduction and are useful in the hybridization program. Foster *et al.* (22) reported that tetraploid varieties were utilized for commercial extraction of RU during World War II.

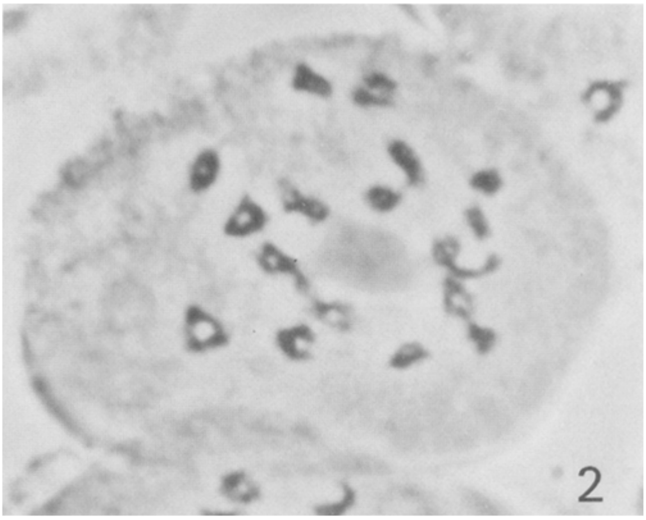

FIG. 2. Photomicrographs of meiotic chromosome × 912.5 in *Parthenium argentatum*, Diploid diakinesis, 18 bivalents (2n = 36).

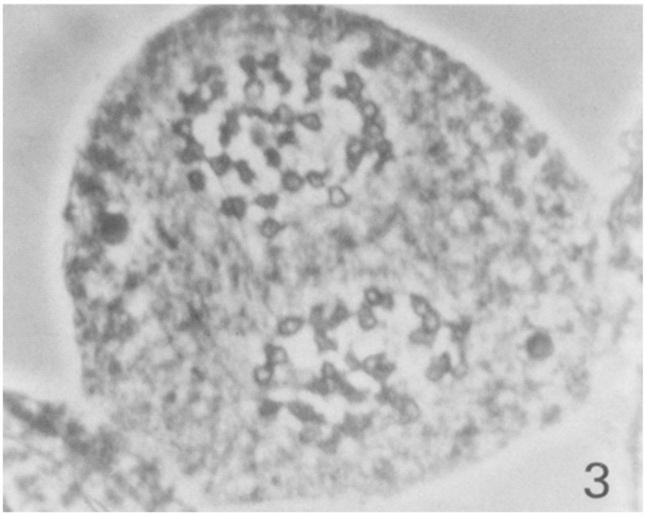

FIG. 3. Photomicrographs of meiotic chromosome × 912.5 in *Parthenium argentatum*. Prophase II, Triploid (2n = 54) showed micronuclei.

TABLE 1

Mean Squares from Analysis of Variance for Rubber, Resin and Stem Diameter at Mapimi Sites

Source	df[a]	Rubber (%)	Resin (%)	Stem diameter (cm)
Sites	8	9.54655 NS[b]	6.80397[c]	0.18114[a,c]
Error	58	5.61430 NS	1.17473	1.85601 E-02
Total	66			
C.V.[d]		20.2	11.3	15.8
Mean[a,e]		11.707	9.602	0.8643

[a]Degrees of freedom.
[b]NS, not significant.
[c]Significant at the 0.01 probability levels.
[d]C.V., coefficient of variation.
[e]Mean of rubber, resin and stem diameter.

In one study (15), the chromosome number was determined for 343 accessions of the guayule germplasm collection. Cytological analysis revealed 6 diploids, 32 triploids, 284 tetraploids, 6 pentaploids and 15 aneuploids. Diploids were not found in the Mapimi Region. Reports (17) on the RE content percentage in diploids show an average of 9.36, and in tetraploids it was 10.69. For diploids, we obtained a mean of 10.75% RE content, and tetraploids had a mean of 9.78%.

Considering the importance of RE content and stem diameter on RU production (2,17–19), we defined a productivity parameter [(% RU + % RE) stem diameter] to identify sites of higher productivity potential. As shown in Figure 5, tetraploids (29.1%) and triploids (25.23%), at site 2 had the highest productivity potential of all

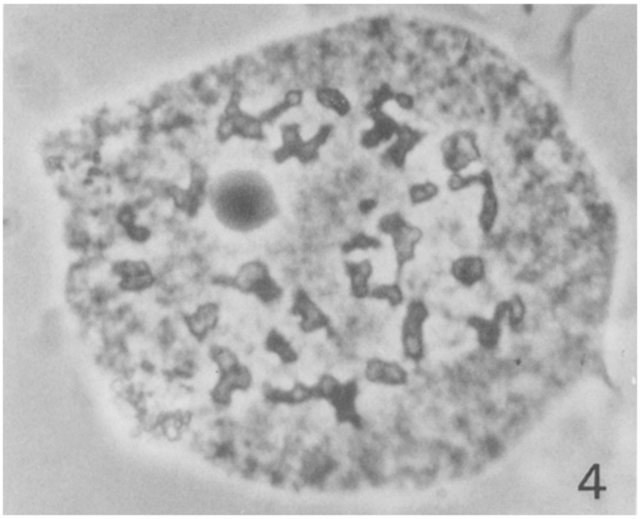

FIG. 4. Photomicrographs of meiotic chromosome × 912.5 in *Parthenium argentatum*. Tetraploid diakinesis (2n = 72).

genotypes at all locations. Kuruvadi *et al.* (17) reported that tetraploid varieties are generally more vigorous, grow rapidly and produce 10.3% more plant height and 8.9% more plant spread; thus, they accounted for more RE, and consequently, more RU per plant compared to diploids. Site 6 has outstanding materials with the three different levels of ploidy. The productivity was 17.1% for tetraploids, 15% for triploids and 13% for diploid accessions. It is possible to select the best materials for breeding programs.

Figure 6 shows mean values of RU Mw from each site. Generally, Mw ranged between 0.96 to 1.46 × 10^6 g/mol. Tetraploids varied from 0.96 × 10^6 g/mol (site 8) to 1.16 × 10^6 g/mol (site 1); triploids had values of 1.19 × 10^6 g/mol (site 2) and 1.46 × 10^6 g/mol (site 6). Diploids produced Mw values ranging from 0.96 × 10^6 g/mol (site 7) to 1.16 × 10^6 g/mol (site 1). Thus, guayule RU from the Mapimi Region showed good quality. In general, high-quality rubber has Mw values ranging between 1.5 × 10^5 g/mol to 2.5 × 10^6 g/mol (4,23).

In regard to RU Mw, some samples showed a bimodal distribution with a relatively high amount of low-Mw polymer chains (Fig. 7, Curve 1). This type of curve has also been found in young plants of *P. argentatum* (24). However, Curve 2 shows a greater proportion of high-Mw polymer chains. Such a distribution is commonly found in *Hevea Brasiliensis* from freshly collected latex (25). These features are apparently correlated with the biosynthetic path, but a general accepted theory is lacking. In any event, the general shape of the Mw distribution curves (unimodal) for RU of the Mapimi Region plants is similar to those of guayule presenting high-Mw polymer chains (Fig. 8, Curve 3), which is the expected result. Currently, we are working on an experiment that will explain the presence of these patterns.

These data suggest that guayule plants in the Mapimi Region are a potential source for successful commercial natural RU production. These findings support continued research on the genetic and biochemical basis for rubber production in guayule to exploit its potential for commercial use.

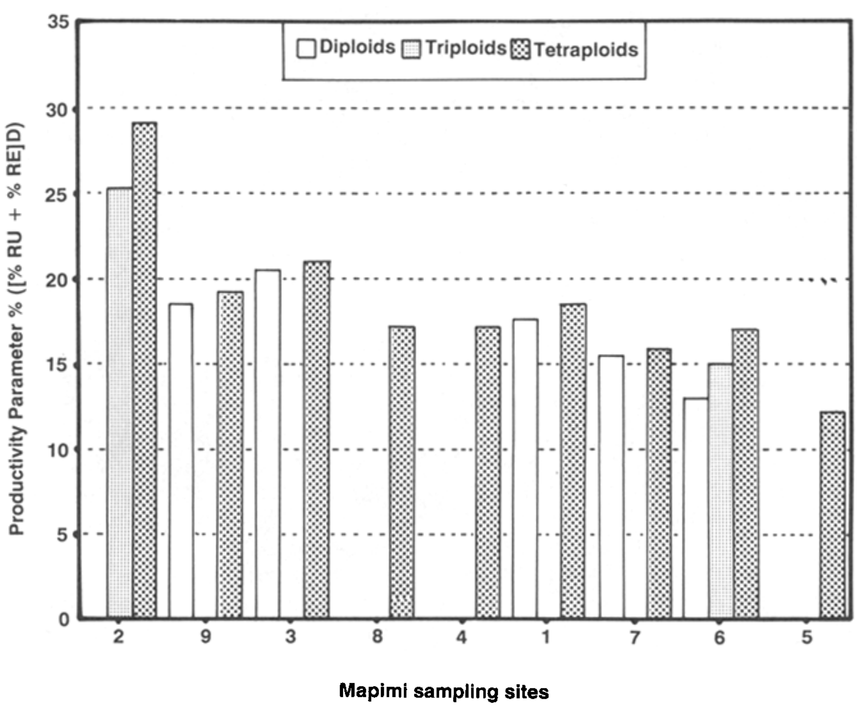

FIG. 5. Productivity parameter ([% RU + % RE]D) to identify sites of higher productivity potential in Mapimi for economical commercialization. % RU, rubber; % RE, resin; D, stem diameter.

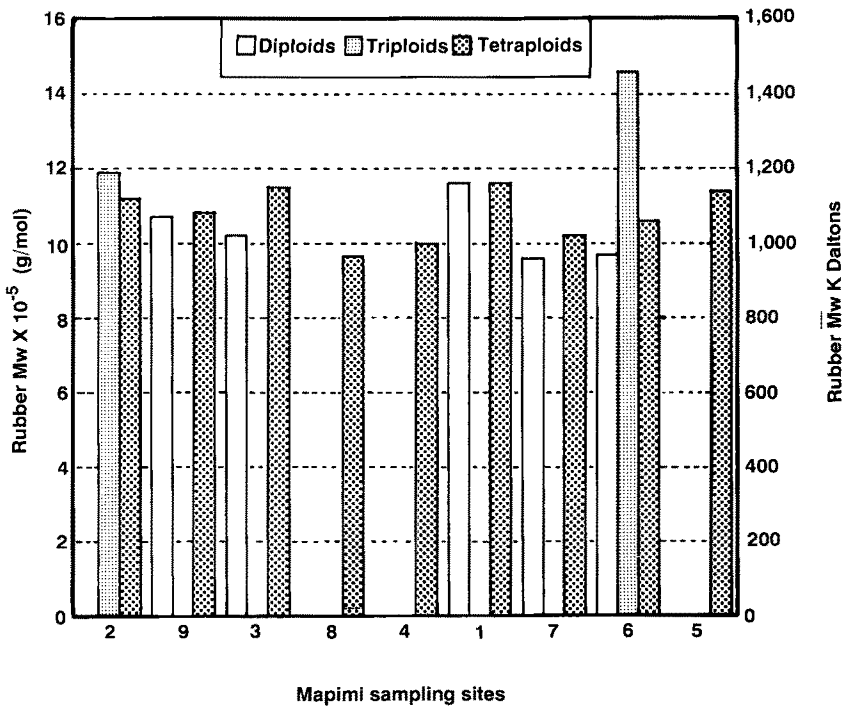

FIG. 6. Weight-average molecular weight (Mw) comparisons of rubber extracted from plants at Mapimi sites.

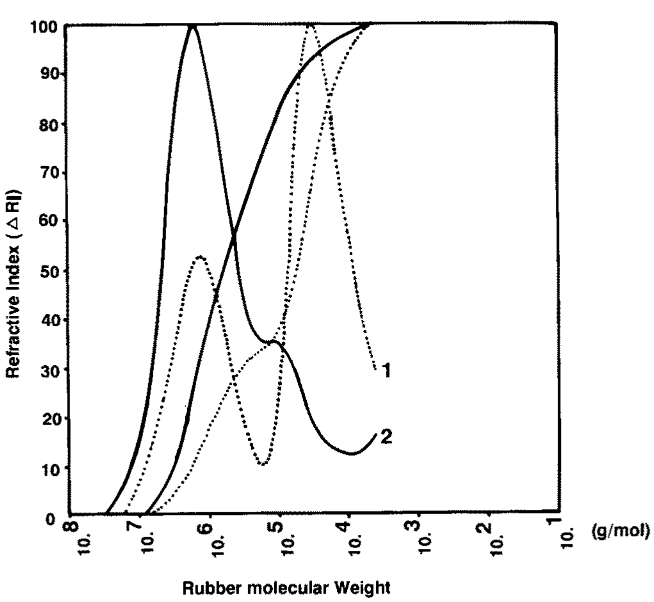

FIG. 7. Molecular weight distributions of guayule rubber from Mapimi plants also showed bimodal curves with rubber chains of low molecular weight.

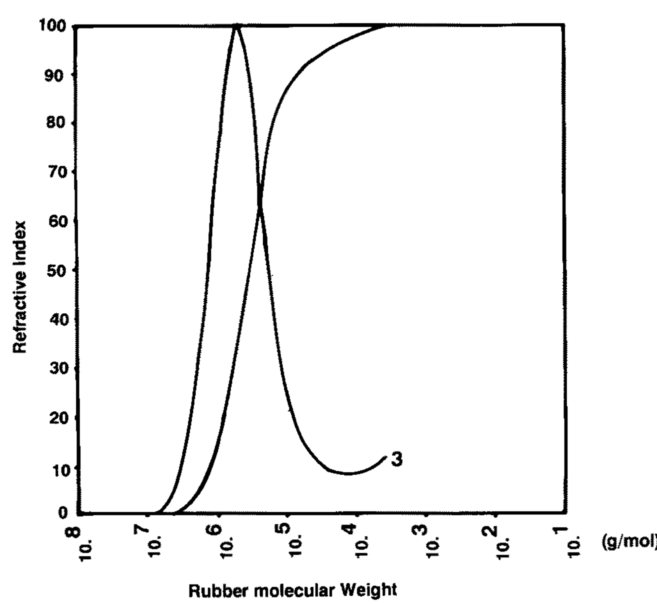

FIG. 8. Molecular weight distributions of natural guayule rubber from Mapimi plants showed unimodal curves.

REFERENCES

1. *National Academy of Sciences*, National Academy of Sciences, Washington, D.C., (1977), p. 80.
2. Schloman, W.W., Jr. and J.P. Wagner, *Guayule Natural Rubber*, edited by J.W. Whiteworth, and E. Whiteheard, GAMC and USDA Cooperative Research Service, Tucson, 1991, pp. 287–310.
3. *The Economist, November: 14* (1992) p. 105; in *AAIC NEWSLETTER 14(2)*: (1993).
4. Angulo Sánchez, J.L., and E. Quero Gutierrez, *J. of Polym. Eng. 5(3)*:183 (1985).
5. Naqvi, H.H., and G.P. Hanson, in *Proceedings of Third International Guayule Conference*, edited by E.C. Greeg, J.L. Tipton and H.T. Huang, Pasadena, 1983, p. 145.
6. Hammond, B.L., and L.G. Polhamus, *Research on Guayule (Parthenium argentatum)*:1942–55, USDA Tech. Bulletin 1327, U.S. Government Printing Office, Washington, D.C., 1965, pp. 101–106.

7. Perry. D.A., H.H. Naqvi and P. Hanson, in *Proceedings of Third International Guayule Conference*, edited by E.C. Gregg, J.L. Tipton, H.T. Huang, Pasadena, 1983, pp. 333–340.
8. Kuruvadi, S., *El Guayulero.* 7:24 (1985).
9. Kuruvadi, S., *Ibid.* *10*:10 (1988).
10. Rollins, R.C., *Contributions from the Gray Herbarium*, Harvard University, 1930, No. 172, pp. 1–72.
11. Estilai, A., and D.T. Ray, *Guayule Natural Rubber*, edited by J.W. Whitworth, and E. Whiteheard, GAMC and USDA Cooperative Research Service, Tucson, 1991, pp. 47–91.
12. Berenger, A.D., *Science 99*:224 (1944).
13. Stebbins, G.L., and M. Kodani, *J. of Hered. 35*:162 (1944).
14. Powers, L., *Genetics 30*:323 (1945).
15. Kuruvadi, S., M.E. Alcala and A. López, *El Guayulero 7*:28 (1986).
16. *New Industrial Uses, New Markets for U.S. Crops: Status of Technology and Commercial Adoption*, edited by Hudson and Harsch, USDA, Maumee, 1992, pp. 38–41.
17. Kuruvadi, S., E.G. Charles and A. López, *El Guayulero 9*:5 (1987).
18. Estilai, A., E. Bahman, H.H. Naqvi, D.A. Dierig, D.T. Ray and A.E. Thompson, *Crop Sci. 32*:July–August (1992).
19. Ray, D.T., D.J. Garrot, Jr. and M.R. Rose, in *Proceedings 4th Annual Guayule Rubber Society Conference*, Riverside, 1983, p. 25.
20. García, V.A., *Manual de Técnicas Citogenéticas*, 2nd edn., Colegio de Postgraduados, Chapingo, México, 1977, p. 118.
21. Angulo-Sánchez, J.L., L.L. Jiménez-Valdes and E. Campos, López, *J. Appl. Polym. Sci 26*:1511 (1981).
22. Foster, K.E., W.G. McGinnies, T.A. Taylor, J.L. Mills, S.J. Alexander, N.G. Wright, D. de Kok, L.J. Gibson, R.R. Wilkinson, F.C. Hopkins, E.W. Lawless and J.L. McCann, *A Sociotechnical Survey of Guayule Rubber Commercialization,* Office of Arid Lands Studies, University of Arizona, Tucson, and Midwest Research Institute, Kansas City, 1979.
23. Estilai, A., *RU Chem. and Technol. 60(2)*:245 (1987).
24. Angulo-Sánchez, J.L., in *Proceedings Third International Guayule Conference,* edited by E.C. Gregg, J.L. Tipton and H.T. Huang, Pasadena, 1980, pp. 443–449.
25. Subramaniam, A., *Rub Chem. Tech. 45*:346 (1972).

[Received November 25, 1992; accepted October 5, 1993]