J. Agric. Food Chem. 1986, 34, 683−685

Tecklenburg, E.; Zabik, M. E.; Uebersax, M. A.; Dietz, J. C.; Lusas, E. W. *J. Food Sci.* **1984**, *49*, 569.
Thompson, D. B.; Erdman, J. W. *Cereal Chem.* **1982**, *59*(6), 525.
Wade, H. E.; Morgan, D. M. *Biochem. J* **1955**, *60*, 264.
Wheeler, E. L.; Ferrel, R. E. *Cereal Chem.* **1971**, *48*, 312.
Winkler, R. L.; Hays, W. L. *Statistics: Probability, Inference and Decision*; Holt, Rinehart and Winston: New York, 1975; p 450.

Received for review December 10, 1984. Revised manuscript received March 25, 1986. Accepted April 28, 1986.

Water Stress and Seasonal Effects on Rubber Quality in Irrigated Guayule

William W. Schloman, Jr.,* Donald J. Garrot, Jr., and Dennis T. Ray

Guayule (line N396) was harvested monthly from January through July, 1984, from irrigated plots grown in Tucson, AZ. Irrigations were scheduled according to the crop water stress index (CWSI) to maintain three irrigation regimes. Plots were irrigated when their respective CWSI values reached or exceeded 0.3 (wet), 0.6 (medium), and 0.9 (dry). Individual plants from four replicated plots of each regime were analyzed for rubber molecular weight by gel permeation chromatography. The molecular weight ($\bar{M}_w$) values for all three irrigation regimes increased from January through March during the plants' winter quiescent period. Molecular weight decreased between March and April for all regimes when active growth resumed. By May, $\bar{M}_w$ had increased above March values. Between irrigation regimes, significant differences in $\bar{M}_w$ were observed only in January and June, with the dry plots yielding the highest molecular weight in both instances.

The processing behavior of natural rubber (*cis*-polyisoprene) reflects the physical properties of the raw polymer. Properties such as bulk viscosity can be correlated with the structure of the rubber molecule itself, including its molecular weight distribution (Bristow, 1982; Ong and Lim, 1983; Subramaniam, 1972). Natural rubber produced from wild stands of guayule (*Parthenium argentatum* Gray) has a processing profile that compares favorably with that of natural rubber from *Hevea brasiliensis* Muell. Arg. (Winkler et al., 1977; Ponce and Ramirez, 1981). Although the chemical structure of rubber from guayule and *Hevea* are quite similar (Ramos-de Valle and Aramburo, 1981; Swanson et al., 1979), Hager et al. (1979) reported that juvenile guayule, estimated to be 6 months old, has a significantly lower weight-average molecular weight ($\bar{M}_w$) and broader molecular weight distribution (MWD: $\bar{M}_w$ divided by the number-average molecular weight, $\bar{M}_n$). Rubber from these juvenile plants had a $\bar{M}_w$ of (6.4–7.8) × 10^5 and MWD of 6.2–10.4. Mature plants yielded rubber with a $\bar{M}_w$ of (9.8–14.6) × 10^5 and MWD of 1.7–2.4. Meeks et al. (1950) reported that the yield of acetone-soluble, low molecular weight guayule rubber was seasonally dependent. These observations raise the question of whether the quality of guayule rubber, and ultimately its commercial acceptability, is dependent on shrub age and harvest date. One method of determining rubber yield (Smith, 1985) is based on the assumption that the molecular weight of guayule rubber is constant.

Rubber accumulation in guayule is cyclical. Spence and McCallum (1935) reported that there is relatively little accumulation during periods of vegetative growth, with accumulation being greatest in the fall and winter. Environmental factors that affect the production and accumulation of rubber in guayule include temperature, light intensity, nutrient availability, and water stress (Hammond and Polhamus, 1965). When plants were subjected to temperatures above 7 °C, the rate of rubber accumulation dropped significantly (Bonner, 1943). Rubber precursor incorporation is higher in cold-treated plant tissue (Goss et al., 1984). Light intensity has an effect on both plant growth and rubber production: a reduction in incident sunlight produced a proportional reduction in dry weight and rubber accumulation (Bonner and Galston, 1947). These effects are related to rubber precursor availability. Spence and McCallum (1935) reported that shrub defoliated in the fall, a period of rapid rubber accumulation, halts almost all rubber production in the remaining woody tissue. Carbohydrate reserves in the wood were slowly metabolized, but not used for rubber production. Normally, rubber precursors are synthesized in the leaves and transported to the bark for rubber formation.

Water stress management has been studied as a means of controlling rubber production. Benedict et al. (1947) grew plants under alternating low- and high-stress periods. Plants were forced to divert isoprenoid metabolism toward rubber production during short, nonwinter stress periods. Maximum growth rates were resumed by reducing moisture stress. In this way, rubber accumulation was optimized. If adequate nutrition is maintained, water is the only independent variable by which guayule rubber production can be controlled. The impact of the resulting stress-induced shifts in rubber synthesis on rubber quality has not been addressed to date.

We report here the characterization of rubber in a single guayule line from irrigated plots sampled over a 7-month period beginning 17 months from seed germination. Three irrigation regimes were maintained by the crop water stress index (CWSI) (Idso et al., 1981; Jackson et al., 1981). The sample interval includes the period of cold-weather stress associated with rubber formation (Bonner, 1943) and the period of renewed vegetative growth.

EXPERIMENTAL SECTION

Shrub Origin. Seeds of guayule line N396 growing within increase plots at Mesa, AZ, were harvested in October 1981 from single plants exhibiting common phen-

---

Central Research Laboratories, The Firestone Tire & Rubber Company, Akron, Ohio 44317 (W.W.S.), and Department of Plant Sciences, University of Arizona, Tucson, Arizona 85721 (D.J.G., D.T.R.).

0021-8561/86/1434-0683$01.50/0

Table I. Whole-Shrub Rubber Level and Molecular Weight, January-June 1984[a]

		rubber			
month	dry wt, g/plant	wt %	g/plant	$\bar{M}_w \times 10^{-3}$	MWD
Jan	300 ± 68ab	2.8 ± 0.5a	8.6 ± 2.6a	933 ± 206a	4.4 ± 1.2abc
Feb	265 ± 71a	3.5 ± 0.6b	9.0 ± 2.4ab	1167 ± 188bc	3.8 ± 0.6a
March	292 ± 75ab	3.6 ± 0.6b	10.3 ± 2.7bc	1204 ± 91cd	4.2 ± 0.9ab
April	*b*	3.8 ± 0.7c	*b*	1021 ± 229ab	4.5 ± 0.8bcd
May	325 ± 82b	3.4 ± 0.6b	10.9 ± 3.2c	1410 ± 125e	4.5 ± 0.7abc
June	404 ± 119c	2.1 ± 0.4d	8.3 ± 2.9a	1350 ± 244de	4.9 ± 1.0cd
July	490 ± 37d	2.7 ± 0.1a	13.5 ± 1.2d	1168 ± 302bc	5.2 ± 1.0d

[a] Data are averages of 12 values: one sample from each of four replicated plots of irrigation regime. Values are compared between months. Comparisons significant at the 0.05 level have the same letter suffix. [b] Not determined.

Table II. Whole-Shrub Rubber Characterization: Effects of Water Management by Month, January-June 1984.

month	irrig regime	$\bar{M}_w \times 10^{-3}$ [a]	MWD
Jan	wet	817 ± 85b	3.8 ± 0.7a
	medium	828 ± 154b	5.3 ± 1.6a
	dry	1152 ± 167a	3.9 ± 0.8a
Feb	wet	1071 ± 94a	4.1 ± 0.7a
	medium	1151 ± 270a	3.4 ± 0.4a
	dry	1278 ± 136a	3.7 ± 0.4a
March	wet	1148 ± 41a	3.8 ± 0.1a
	medium	1196 ± 126a	4.7 ± 1.5a
	dry	1268 ± 57a	4.0 ± 0.5a
	control[b]	1168	4.1
April	wet	1097 ± 283a	4.1 ± 0.9a
	medium	1008 ± 211a	4.7 ± 0.5a
	dry	958 ± 287a	4.7 ± 1.0a
May	wet	1462 ± 78a	4.5 ± 0.7a
	medium	1413 ± 46a	4.2 ± 0.6a
	dry	1356 ± 204a	4.7 ± 0.8a
June	wet	1163 ± 163b	5.1 ± 0.6a
	medium	1328 ± 151ab	5.0 ± 0.6a
	dry	1558 ± 252a	4.6 ± 1.6a
	control[b]	1349	4.0
July	wet	966 ± 331a	5.3 ± 1.4a
	medium	1273 ± 200a	5.2 ± 0.2a
	dry	1236 ± 354a	5.1 ± 1.3a

[a] For each month, values were compared between treatment levels. Comparisons significant at the 0.05 level have the same letter suffix. [b] Unirrigated check sample.

totypic characteristics. After germination (August 1982), seedlings were maintained under greenhouse conditions until hand transplanted in October 1982 to a field site at the Campus Agricultural Research Center of the University of Arizona in Tucson. Planting density was 36 650 shrubs/ha. Shrub growth was maintained by using a drip irrigation system. Plots were irrigated when their respective CWSI values reached or exceeded 0.3 (wet), 0.6 (medium), and 0.9 (dry). CWSI measurements were taken between 11:00 and 13:00 mountain standard time at approximately 3-day intervals on seven plants spaced so as to cover the length of each plot. Measurements were taken only when the sky was clear. The number of irrigations required over the regular June through October growing season was 8 (wet), 6 (medium), and 5 (dry). By June 1984 the total water applied was 124 (wet), 112 (medium), and 76 cm (dry). Individual plants from four replicated plots of each regime were harvested monthly from January through July 1984. Only plants bordered by others on all four sides were harvested. Plants for check samples were harvested in March and June from a control basin to which no irrigation water was applied after establishment (May 1983). Flooding associated with the early onset of seasonal rains precluded further maintenance and sampling in accordance with this protocol.

Shrub Processing and Extraction. Whole plants were processed for rubber yield determination by sequential extraction with acetone and hexane as described in Garrot et al. (1981). Table I summarizes rubber yield data. For rubber qualitative determinations, additional 10-g samples were exhaustively extracted in a Soxhlet apparatus with acetone to remove resin, followed by dry tetrahydrofuran (THF) to remove rubber. The THF solutions, stabilized with an alkyl-*p*-phenylenediamine antioxidant, were used directly for gel permeation chromatography (GPC) analyses. For leaf rubber characterization, six additional shrubs were harvested and defoliated. A composite leaf sample was extracted as described above.

Rubber Characterization. Reagent THF was passed through a 0.45-μm filter before use. Solutions were adjusted to concentrations of 0.02 g/dL and then passed through an asbestos filter with a porosity <1 μm. The chromatograph was a Waters Associates Model 200 fitted with a Model M-45 pump and a Model R-401 differential refractometer. Four 7.8 mm i.d. × 122 cm Styragel columns were used: 1×10^6 Å, 1×10^5 Å, 1×10^4 Å, 1×10^3 Å. Solvent flow rate was 1 mL/min. A universal calibration curve (Grubisic et al., 1967) was prepared against standard polystyrene samples from Waters Associates. Mark–Houwink constants for rubber were those determined for anionically prepared linear *cis*-polyisoprene: $K = 1.91 \times 10^{-4}$ dL/g, $a = 0.74$.

Results for monthly sample composites are summarized in Table I. Data comparing irrigation treatments by month are summarized in Table II. A single-factor main-effects analysis of variance model (SAS procedure ANOVA) was used to run least significant difference tests on all main-effects means.

RESULTS AND DISCUSSION

Guayule has been reported incapable of using stored rubber as a food reserve (Traub, 1946; Benedict, 1949). In our study, rubber accumulation continued through April, followed by a loss in yield in June (Table I). Rather than being a result of rubber metabolism, this yield decrease is an artifact of the partial degradation of rubber in desiccated plant tissue. By the June harvest, the plants had developed a complete canopy; lower branches were heavily shaded. We observed that the shaded portions of the plants had lost their leaves and suffered substantial dieback. Curtis (1947) attributes rubber loss in this dead tissue to oxidative or photooxidative degradation to low molecular weight, acetone-soluble byproducts. With high temperatures, June proved to be the month of greatest water stress. An early onset of seasonal rains in July produced substantial new growth and a concomitant increase in rubber yield.

Guayule rubber quality reflects a long-term upward trend in molecular weight resulting from synthesis and accumulation of higher molecular weight rubber. Superimposed on this are short-term variations in molecular weight resulting from intervals of rapid accumulation of low molecular weight rubber. Hager et al. (1979) consider the first stable rubber particles formed on the surface of the rubber polymerase enzyme to have low molecular weight ($<10^5$). These particles further polymerize on the same or another enzyme site until size factors hinder polymer chain elongation. Leaf rubber can substantially affect whole-shrub rubber quality. While whole-shrub rubber had a $\bar{M}_w$ of $(9.3–14.1) \times 10^5$ and MWD of 3.8–5.2 (Table I), leaf rubber from freshly harvested lush shrub had a $\bar{M}_w$ of $(4.5–5.6) \times 10^5$ and MWD of 5.0–6.4. The broad molecular weight distribution of leaf rubber is symptomatic of a substantial low molecular weight component. Conversion to high molecular weight rubber does not occur to any appreciable extent in leaves.

The impact of this low molecular weight rubber was minimal between January and March, an interval of reduced plant growth and increased leaf loss due to cold stress. The MWD of whole-shrub rubber was at a minimum and the molecular weight at a relative maximum. Renewed shrub growth in March–April was accompanied by low molecular weight rubber synthesis. Rubber levels (weight percent of shrub) were highest at this time. The molecular weight reached a relative minimum, while the MWD began its long-term increase. The broadening MWD reflects in part the contribution of leaf rubber as growth proceeds. The subsequent conversion to high molecular weight rubber gave a maximum in $\bar{M}_w$ in May–June. Finally, molecular weight decreased as a result of accelerated growth in July.

The influence of water management practices on rubber quality was apparent only in January and June (Table II). In both instances, the dry-treatment regime resulted in significantly higher molecular weight rubber than did the wet-treatment regime. January conditions of low temperature combined with water stress supported formation of high molecular weight rubber. However, the highest molecular weight rubber observed during the study was formed in June as water stress promoted conversion of the lower molecular weight rubber formed earlier. As a desert shrub, guayule is well adapted to survive severe water stress. We conclude that the range in CWSI used in this study could be broadened to produce maximum treatment effects.

We have detailed the extent to which rubber molecular weight and molecular weight distribution vary over a 7-month period in a single shrub line. These data clearly demonstrate that no single sample can provide a complete measure of rubber quality. The shrub used in this study was relatively young. Lloyd (1911) estimated that most guayule found in the wild is at least 12 years old. Cultivated guayule has typically been harvested at 4–5 years of age. However, shorter harvest cycles of 2–3 years have also been investigated (Hammond and Polhamus, 1965). Still younger shrub tissue has been processed as part of pollarding experiments. The aerial portions of guayule have been harvested and then subsequently reharvested after as little as 1 year's regrowth (Hildreth, 1946; Hunter et al., 1959). Had further controlled sampling of our shrub population been possible, it would have been desirable to monitor rubber quality through the end of the growing season and into the following cold-stress period.

For the seasonal interval under study, the periods of highest rubber quality were the end of the cold-stress period and late spring. The cold-stress period gave rubber with a somewhat narrower molecular weight distribution. Water stress served to optimize molecular weight during this period. We recognize that, as guayule matures and accumulates higher molecular weight rubber, the impact of the lower molecular weight rubber component on overall rubber quality will be attenuated. The magnitude of seasonal variations in rubber quality would be reduced. Commercial shrub processors will prefer a constant supply of feedstock as they operate throughout the year. Processing economics and the demand for natural rubber do not favor guayule's management as a seasonal crop (Weihe and Nivert, 1983). Commercially viable shrub processing will have to accommodate seasonal variation in rubber quality, whatever the magnitude.

ACKNOWLEDGMENT

R. W. Biro and K. E. Lawson were responsible for sample preparation and extraction. M. J. Hackathorn performed chromatographic analyses. We thank D. McIntyre, R. Seeger, and R. Kozulla for helpful discussions regarding rubber characterization methodology. D.J.G. and D.T.R. acknowledge funding provided by the U.S. Department of Agriculture under the Special Grants Program of the Native Latex Act. W.W.S. acknowledges funding provided by the Gila River Indian Community under Department of the Navy (NAVAIR) Contract No. N00019-82-C-0486. This work was presented at the 4th International Conference on Guayule Research and Development, Tucson, AZ, Oct 16–19, 1985.

LITERATURE CITED

Benedict, H. M. *Bot. Gaz. (Chicago)* **1949**, *111*, 36.

Benedict, H. M.; McRary, W. L.; Slattery, M. C. *Bot. Gaz. (Chicago)* **1947**, *108*, 535.

Bonner, J. *Bot. Gaz. (Chicago)* **1943**, *105*, 233.

Bonner, J.; Galston, A. W. *Bot. Rev.* **1947**, *13*, 543.

Bristow, G. M. *NR Technol.* **1982**, *13*, 56.

Curtis, O. F., Jr. *Plant Physiol.* **1947**, *22*, 333.

Garrot, D. J., Jr.; Johnson, D. L.; Rubis, D. D.; Dill, G. M. *Anal. Chem.* **1981**, *53*, 543.

Goss, R. A.; Benedict, C. R.; Keithly, J. H.; Nessler, C. L.; Stipanovic, R. D. *Plant Physiol.* **1984**, *74*, 534.

Grubisic, Z.; Rempp, P.; Benoit, H. *J. Polym. Sci., Part B: Polym. Lett.* **1967**, *5*, 753.

Hager, T.; MacArthur, A.; McIntyre, D.; Seeger, R. *Rubber Chem. Technol.* **1979**, *52*, 693.

Hammond, B. L.; Polhamus, L. G. *Research on Guayule (Parthenium argentatum): 1942–1959*; U.S. Department of Agriculture: Washington, DC, 1965; pp 11–114.

Hildreth, A. C. *India Rubber World* **1946**, *114*, 55.

Hunter, A. S.; Burtch, L. M.; McDowell, C. Ch. *Prod. Res. Rep.* **1959**, *No. 24*, 14–15.

Idso, S. B.; Jackson, R. J.; Pinter, P. J., Jr.; Reginato, R. J.; Hatfield, J. L. *Agric. Meteorol.* **1981**, *24*, 45.

Jackson, R. J.; Idso, S. B.; Reginato, R. J.; Pinter, P. J., Jr. *Water Resour. Res.* **1981**, *17*, 1133.

Lloyd, F. E. *Guayule, a Rubber-Plant of the Chihuahuan Desert*; Carnegie Institute: Washington, DC 1911; pp 56, 57, 85–89.

Meeks, J. W.; Banigan, T. F., Jr.; Planck, R. M. *India Rubber World* **1950**, *122*, 301.

Ong, E. L.; Lim, C. L. *NR Technol.* **1983**, *14*, 25.

Ponce, M. A.; Ramirez, R. R. *Rubber Chem. Technol.* **1981**, *54*, 211.

Ramos-de Valle, L. F.; Aramburo, F. *J. Rheol. (NY)* **1981**, *25*, 379.

Smith, M. K. *J. Agric. Food Chem.* **1985**, *33*, 928.

Spence, D.; McCallum, W. J. *Trans. Inst. Rubber Ind.* **1935**, *11*, 119.

Subramaniam, J. *Rubber Chem. Technol.* **1972**, *45*, 346.

Swanson, C. L.; Buchanan, R. A.; Otey, F. H. *J. Appl. Polym. Sci.* **1979**, *23*, 743.

Traub, H. P. *Plant Physiol.* **1946**, *21*, 425.

Weihe, D. L.; Nivert, J. J. In *Proceedings of the Third International Guayule Conference*, Pasadena, CA, 1980; Gregg, E. C., Tipton, J. L., Huang, H. T., Eds.; Guayule Rubber Society: Riverside, CA, 1983; pp 115–125.

Winkler, D. S.; Schostarez, H. J.; Stephens, H. L. *Rubber Chem. Technol.* **1977**, *50*, 981.

Received for review September 12, 1985. Revised manuscript received March 4, 1986. Accepted April 8, 1986.