Seasonal Effects on Guayule Resin Composition

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Rubber and resin levels in guayule line N396 were monitored on a whole-shrub basis from January through July 1984. This sample interval included the period of cold-weather stress associated with rubber formation and the period of renewed vegetative growth. Monthly levels were also determined for selected resin components. Fatty acid triglycerides were most abundant (>8000 ppm) in January, with lower levels (2300–4400 ppm) in succeeding months. Triterpenoid levels showed relatively little variation (1700–2500 ppm for argentatin A). Sesquiterpene esters were most abundant in March (3600 ppm for guayulin A) and July (2900 ppm) and least abundant in April and May (<50 ppm). These variations have direct implications for shrub processing and byproduct applications.

The economic viability of guayule (*Parthenium argentatum* Gray) as a domestic source of natural rubber would be enhanced if commercial processing yielded high-value byproducts. Guayule byproducts include resin (acetone extractables), leaves, cuticle wax, seed, and bagasse. Nivert et al. (1978) and Weihe and Nivert (1983) have suggested that byproduct credits could reduce gross production costs as much as 26–49%. Making a more conservative estimate, McFadden and Nelson (1981) foresaw byproducts used as fuel for process energy cogeneration. In this case, the reduction in costs would be 15–22%.

Guayule resin, the nonrubber shrub extractables, is a ubiquitous byproduct of shrub processing. Resin is obtained by acetone extraction of milled shrub or coagulated latex (Eagle, 1981) or by coagulation of rubber from a combined rubber–resin miscella (Engler and McIntyre, 1984). Resin contains a wide variety of secondary metabolites (Buchanan et al., 1978; Banigan et al., 1982), including substantial proportions of sesquiterpene esters, triterpene keto alcohols, and fatty acid triglycerides (Schloman et al., 1983). Considered separately, each chemical class of resin components has potential commercial value. However, the practical value of resin-derived byproducts rests on the accessibility as well as the utility of particular components or component classes.

We have reported that resin yields vary with cultivation site, harvest date, and shrub strain (Schloman et al., 1983); Benedict et al. (1947) observed seasonal variations in resin yield. Resin composition can depend on shrub strain, cultivation history, and processing procedures. Prokof'ev (1939) and Bonner and Galston (1947) reported that the processes of terpene and rubber formation in guayule are antagonistic. Terpenes, 3–5% of whole-shrub resin, are produced more rapidly than rubber during periods of vegetative growth. Arreguin et al. (1951) concluded that the biochemical pathways leading to rubber formation are of minor importance relative to other isoprenoid syntheses. Seasonal variations in the yield and composition of mono- and oligosaccharides (Traub and Slattery, 1946) and oligoisoprenoids (Meeks et al., 1950) have been reported for guayule. In other plants, seasonal variations have been reported for various classes of secondary metabolites, including monoterpenoids (Cedarleaf et al., 1983; Banthorpe et al., 1972), phenylpropanoids (Vostrowsky et al., 1981), and fatty acid triglycerides (Tsaregorodtseva, 1975).

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Table I. Whole-Shrub Resin And Rubber Levels

month	dry wt, g/plant	resin		rubber	
		wt %	g/plant	wt %	g/plant
Jan	300	4.7	14.2	2.9	8.6
Feb	265	5.0	13.3	3.5	9.0
March	292	5.0	14.5	3.6	10.3
Apr		5.2		3.8	
May	325	5.2	16.9	3.4	10.9
June	404	4.3	17.5	2.1	8.4
July	490	5.1	24.9	2.7	13.4

We report here the quantitation of rubber, resin, and resin components in a single guayule line sampled over a 7-month period beginning 17 months from seed germination. 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 phentotypic 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. Shrub growth was maintained by a drip irrigation system. Plants were harvested monthly from January through July 1984. Flooding associated with the early onset of seasonal rains precluded further harvesting. In all cases, only plants bordered by others on all four sides were harvested.

Shrub Processing and Extraction. Whole plants were processed and extract yields determined as described in Garrot et al. (1981). Resin for characterization was obtained from 10-g samples of processed shrub by overnight Soxhlet extraction with acetone. Resin and rubber levels for 12-shrub composite samples are summarized in Table I.

Resin Component Quantitation. Resin component HPLC analyses were run on a Supelco 25-cm RP-18 column. Guayulins were quantified by an acetonitrile–water gradient program at 1.5-mL/min flow rate [time, min (% acetonitrile)]: 0 (85), 15 (85), 18 (100), 25 (100), 30 (85), 35 (85). UV detection was at 262 nm. Authentic samples of guayulins A and B (Schloman et al., 1983) were run as external standards. Argentatins A, B, and D ("triterpenoid D"; Schloman et al., 1983) were quantified as the (2,4-dinitrophenyl)hydrazones (DNPH), using epiandrosterone DNPH as the external standard. The following tetrahydrofuran (THF)–water gradient program was used at 1.5 mL/min flow rate [time, min (% THF)]: 0 (50), 10

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Table II. Component Levels In Whole-Shrub Resin

month	ppm, whole-shrub basis[a] guayulin A	guayulin B	argentatin A	argentatins B/D	triglycerides
Jan	1967	275	1967	2911	8537
Feb	242	39	2368	3141	4010
March	3629	526	2525	3156	3261
Apr	27	9	1732	2399	4264
May	29	5	2100	2911	3484
June	841	126	1850	2901	2354
July	2939	385	1879	2987	4384

[a] Relative precision of analyses: ±10% for triglycerides; ±5% for guayulins and argentatins.

(50), 20 (65), 35 (65), 40 (50), 45 (50). UV detection was at 371 nm. The DNPH derivatives of argentatins B and D were not resolved under these conditions. Triglycerides were determined following the procedure of Kruempelman and Danielson (1982). Resin component levels (expressed as ppm of whole shrub) are summarized in Table II.

RESULTS AND DISCUSSION

The variations in resin and rubber levels (Table I) reflect changes in both the total plant biomass and the wood/leaf ratio. The resin level appears to be positively correlated with the amount of leaves on the plant, being highest when leaves are most abundant. Leaves contain higher resin levels than does woody tissue (Meeks et al., 1950), whereas rubber levels are highest in woody tissue bark (Lloyd, 1911; Meeks et al., 1950). Vegetative growth also affects shrub composition: stems no older than 1 year typically show higher resin levels than do older stems (Curtis, 1947).

The resin underwent substantial changes in composition (Table II). Fatty acid triglycerides were most abundant in January, with lower levels in succeeding months. Triterpenoid levels showed relatively little variation. Sesquiterpene esters were most abundant in March and July but present in only minor amounts in April and May. Such variations have direct implications for shrub processing and byproduct applications.

As storage substances in plants, triglycerides are accumulated during the latter part of a growing season and are gradually used during the period of cold weather dormancy or quiescence (Tsaregorodtseva, 1975). Our data document the return of guayule triglycerides to steady-state levels prior to the onset of the spring growth period. Triglyceride-derived unsaturated fatty acids are responsible for the accelerated oxidative degradation of rubber by guayule resin (Keller et al., 1981; Bhowmick et al., 1985). Budiman and McIntyre (1981) have reported that the high molecular weight resin fraction, which includes fatty acid triglycerides, is removed more slowly than lower molecular weight components during deresination of flotation process rubber worms. As a consequence, the residual resin in flotation process rubber is triglyceride enriched. This enrichment would be greatest for rubber processed in the fall and winter. During this period, the rubber would require more rigorous antioxidant protection.

Belmares et al. (1980) suggested that the prooxidant properties of guayule resin could prove valuable in applications calling for rubber peptizers. In addition, resin-based coatings with good water resistance were obtained with formulations containing higher triglyceride levels. Cold-season guayule resin would be a more attractive feedstock for these applications.

Guayulins A and B are sesquiterpene esters of *trans*-cinnamic and *p*-anisic acids, respectively. Both esters readily undergo oxidative degradation under conventional shrub-processing conditions (Schloman et al., 1983). Guayulin A is an elicitor of allergic contact dermatitis in sensitized subjects (Rodriguez et al., 1981). The variations in guayulin levels reported here reflect a strong seasonal influence on the turnover rate of these secondary metabolites. Mears and Larson (1982) consider guayule to have shifted from the sesquiterpene lactone defense system of other *Parthenium* species to an aromatic acid defense system. Cinnamic acid has been implicated as an ephemeral allelopathic agent in guayule (Bonner and Galston, 1944; Bonner, 1946), acting as a soil toxin for young plants. We suggest that the guayulins serve as cinnamate and *p*-anisate storage substances, with metabolic turnover occurring at times when release of the free acids into the environment is required. Such release could be associated with leaf loss—allowing additional degradation of intact guayulins by oxidation or photooxidation—as well as root exudation of free aromatic acids.

Taylor (1946) reported that leaves removed without parboiling depress plant growth and are unsuitable for fertilizer. However, Hammond and Polhamus (1965) indicated that leaves are an excellent soil amendment when composted. Although leaves have relatively lower guayulin levels than does woody tissue (Proksch et al., 1981), the levels (1700–1900 ppm) fall in the range we observe for whole-shrub tissue. Consequently, the minima in whole-shrub guayulin levels reflect corresponding minima in leaf guayulin levels. Outside the relatively brief intervals when guayulin levels are at their lowest, special care will be required to assure that composting has progressed sufficiently to minimize guayulin and free aromatic acid levels and the associated potential for allelopathic effects.

Shrub processors should recognize that the potential allergenicity of guayule will show a strong seasonal dependence as guayulin A levels change.

We have detailed the extent to which a selected group of resin components varies over a 7-month period in a single shrub line. These data clearly demonstrate that no single sample can provide a complete measure of resin composition. We recognize that, had further controlled sampling of this particular shrub population been possible, it would have been desirable to monitor resin composition through the end of the growing season and into the following cold-stress period. We further recognize that the shrub used in this study is relatively young. The resin could differ in composition from that in older shrub harvested during the same period of the year. Cultivated guayule has typically been harvested at 4–5 years of age. Shorter harvest cycles of 2–3 years have also been investigated (Hammond and Polhamus, 1965). It is important to recognize that processing economics and the demand for natural rubber do not favor guayule's management as a seasonal crop (Weihe and Nivert, 1983). Commercial exploitation of resin-derived byproducts will have to accommodate the seasonal variations in composition. Other shrub byproducts with potential economic value may also warrant seasonal analysis. These include volatile terpenoids (essential oils), cuticle wax, and polysaccharide precursors to fermentable sugars. For the most complete assessment of any commercial utility, such profiling must ultimately be correlated with variations in shrub strain and cultivation site.

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Registry No. Guayulin A, 31685-97-9; guayulin B, 31685-98-0; argentatin A, 31324-30-8.

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Effect of the Pollutant Ozone in Ambient Air on Lima Beans

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Eight lima bean genotypes with different phenotype seed characteristics were grown in two ozone atmospheres, i.e., glass houses with nonfiltered (NF) air and charcoal-filtered (CF) air. Tolerances to ozone (O_3) and seed yield were determined for each genotype. Chemical analyses were conducted on the seeds for Kjeldahl nitrogen, 16 amino acids, ammonia, four sugars, and starch. The four genotypes that were most resistant to O_3 had green cotyledons. The cotyledon color may be important for the identification of lima bean genotype resistance to O_3. Seed yield did not correlate with visible leaf tissue damage. Kjeldahl nitrogen and amino acid levels were higher in seeds grown in the low-O_3 atmosphere. Seeds of plants grown in NF air contained higher carbohydrate and starch content than those from plants grown in CF air. Cotyledon color or seed coat color were not related to changes in Kjeldahl nitrogen, amino acids, carbohydrate, or starch associated with the O_3 treatments.

INTRODUCTION

Ozone (O_3) gas is produced by photochemical reactions involving sunlight, the nitrogen oxides, and hydrocarbons from fuel combustion such as auto exhausts. In ambient air, O_3 concentrations are varied with elevated concentrations produced over long distances downwind of industrial complexes or major population centers. Light winds may carry O_3 and the precursor chemicals over extensive rural areas of the U.S. resulting in damage to sensitive plants. Five-year average (1978–1982) O_3 values for the 48 contiguous states are available (Adams et al., 1984). Ozone is considered in the U.S. to cause more plant damage than any other air pollutant (Heggestad and Heck, 1971). Mixtures of O_3 with other gaseous pollutants are also a concern since they may have additive or more than additive effects (Lefohn and Tingey, 1984).

Ozone, as a phytotoxic gas, can produce leaf damage, premature senescence, leaf drop, reduced growth, and lower seed yield in sensitive plant species (Lee et al., 1981). Injury to foliar plant structures occur when sensitive cultivars of some species are exposed for 2–4 h with concentrations of 0.05–0.12 ppm O_3 (Reinert et al., 1982).

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