Physiologia Plantarum

An International Journal for Plant Biology

Physiologia Plantarum 140: 368–379. 2010

Photosynthesis and assimilate partitioning between carbohydrates and isoprenoid products in vegetatively active and dormant guayule: physiological and environmental constraints on rubber accumulation in a semiarid shrub

Michael E. Salvucci[a,*], Csengele Barta[a], John A. Byers[a] and Alberto Canarini[b]

[a]US Department of Agriculture, Agricultural Research Service, Arid-Land Agricultural Research Center, Maricopa, AZ 85138, USA
[b]Department of Evolutionary and Experimental Biology, University of Bologna, Bologna, Italy

Correspondence
*Corresponding author,
e-mail: mike.salvucci@ars.usda.gov

Received 29 May 2010;
revised 19 July 2010

doi:10.1111/j.1399-3054.2010.01409.x

The stems and roots of the semiarid shrub guayule, *Parthenium argentatum*, contain a significant amount of natural rubber. Rubber accumulates in guayule when plants are vegetatively and reproductively dormant, complicating the relationship between growth/reproduction and product synthesis. To evaluate the factors regulating the partitioning of carbon to rubber, carbon assimilation and partitioning were measured in guayule plants that were grown under simulated summer- and winter-like conditions and under winter-like conditions with CO_2 enrichment. These conditions were used to induce vegetatively active and dormant states and to increase the source strength of vegetatively dormant plants, respectively. Rates of CO_2 assimilation, measured under growth temperatures and CO_2, were similar for plants grown under summer- and winter-like conditions, but were higher with elevated CO_2. After 5 months, plants grown under summer-like conditions had the greatest aboveground biomass, but the lowest levels of non-structural carbohydrates and rubber. In contrast, the amount of resin in the stems was similar under all growth conditions. Emission of biogenic volatile compounds was more than three-fold higher in plants grown under summer- compared with winter-like conditions. Taken together, the results show that guayule plants maintain a high rate of photosynthesis and accumulate non-structural carbohydrates and rubber in the vegetatively dormant state, but emit volatile compounds at a lower rate when compared with more vegetatively active plants. Enrichment with CO_2 in the vegetatively dormant state increased carbohydrate content but not the amount of rubber, suggesting that partitioning of assimilate to rubber is limited by sink strength in guayule.

Introduction

Parthenium argentatum A. Gray, commonly known as guayule, is an arid-land shrub native to north-central Mexico and the adjacent Big Bend areas of Texas in North America (Hammond and Polhamus 1965, Jasso de Rodríguez et al. 2006). The bark of this plant contains latex that has been used as a source of natural rubber since prehistoric times (Hammond and Polhamus 1965). Interest in cultivating guayule has waxed and

---

Abbreviations – Ci, internal CO_2 concentration; ETR, electron transport rate; GC–MS, gas chromatography–mass spectrometry; HPAEC-PAD, high-performance anion-exchange chromatography-pulsed amperometric detection; PAR, photosynthetically active radiation.

waned repeatedly throughout the last century and a half, generally coinciding with changes in the availability of natural rubber from the world's primary source, *Hevea brasiliensis*, the Brazilian rubber tree (Finlay 2004). The onset of type I latex allergies, caused in part by the hasty production of latex gloves in response to the AIDS epidemic of the 1980s, has spurred much of the recent commercial interest in guayule (Cornish et al. 2001) as a potential source of natural, but hypoallergenic rubber (Cornish et al. 2008). In addition, concerns that future production of natural rubber will not meet projected demands, coupled with the twin fears of a *H. brasiliensis* leaf blight and the increasing costs of the petroleum products needed for the production of synthetic rubber, have intensified interest in alternative sources of rubber (Mann 2009, van Beilen and Poirer 2007).

Rubber is an end-product of carbon metabolism in guayule, produced in the plant during periods of vegetative and reproductive dormancy. This fact was recognized in the 1940s by Bonner and co-workers who observed that rubber accumulated under stress or cold nights, i.e. conditions that caused a cessation of vegetative growth (Bonner 1943, Bonner and Galston 1947). Citing the results of defoliation experiments, Bonner and Galston (1947) concluded that continued assimilation of carbon by the leaves during dormancy was necessary for rubber accumulation. A similar conclusion was reached by Downes and Tonnet (1985) who attributed reduced rubber yield at one of their field sites to excessive leaf death and defoliation brought about by frost damage at this location. The notion of an inverse relationship between vegetative growth and rubber synthesis has been explored in detail by van Staden and colleagues (Appleton and van Staden 1989, 1991, Kelly and van Staden 1993) who showed that the capacity for rubber synthesis increases with cold temperatures and short day lengths and decreases just before bud break and the resumption of vegetative/reproductive growth. Defoliation (Bonner and Galston 1947) and radiotracer experiments (Gilliland et al. 1985, Kelly and van Staden 1993) established that sucrose synthesized in the leaves and translocated to the stem provides most of the carbon for rubber synthesis rather than carbon stored in the stem, which is mainly in the form of fructans.

Increasing the rubber content of guayule would enhance its potential as a commercial source of natural rubber. However, increasing rubber synthesis in guayule is complicated by the fact that rubber is a product of 'dormant physiology' unlike most other plant products, which are derived from or produced during active vegetative or reproductive growth. To better understand the factors limiting rubber production, guayule plants were grown under simulated summer- and winter-like conditions of temperature and photoperiod to induce vegetatively active and dormant states. Growth, photosynthesis, the partitioning of carbon between carbohydrates and isoprenoid pathway products, including biogenic leaf volatiles, were examined in the two physiological states to elucidate the constraints on rubber accumulation. Enrichment with CO_2 was used as a separate treatment to determine whether increasing source strength in the physiologically dormant state affects rubber accumulation.

Materials and methods

Materials

The volatile standards used for gas chromatography–mass spectrometry (GC–MS) quantification were purchased from Sigma-Aldrich-Fluka (St. Louis, MO), Bedoukian Research Inc. (Danbury, CT), International Flavors and Fragrances Inc. (New York, NY), Good Scents Co. (Oak Creek, WI) and Chromadex Inc. (Irvine, CA). Chemical standards of the identified volatile compounds and the internal standard, (+)-carvone were all $>98\%$ purity.

Plant material and growth conditions

Guayule (*P. argentatum*) plants, PI 599675, cultivar AZ-2 (Ray et al. 1999) were grown from seed. Seeds were germinated in vermiculite cell packs, and the seedlings were transplanted into a 10-cm pot containing a soilless mixture of peat, perlite and vermiculite. Seedlings were grown under controlled environment conditions (30°C, 12-h day/20°C, 12-h night), 200 μmol photon m^{-2} s^{-1} photosynthetically active radiation (PAR). Once seedlings had developed three to four true leaves, they were transplanted to 3.8-l pots and maintained in the growth chamber for 1 week before transfer to a greenhouse under natural light (i.e. <1800 μmol photon m^{-2} s^{-1} PAR) and a temperature regime of 28/22°C. For the first 2 weeks, transplants were watered only when the surface of the soil was dry, after which they were watered every 4 days and fertilized once a month with a complete nutrient solution containing 2 g l^{-1} of 20-20-20 Peters® professional water-soluble fertilizer (Scotts-Sierra Horticultural Products Co., Marysville, OH) and 0.5 ml l^{-1} of a micronutrient solution composed of 2 m*M* $MgCl_2$, 10 m*M* H_3BO_3, 0.4 m*M* $ZnSO_4$, 0.2 m*M* $CuSO_4$, 0.4 m*M* Na_2MoO_4 and 0.1 m*M* $NiCl_2$. As the plants increased in size, irrigation was adjusted as needed. After 10 months, plants required watering once a day and were transplanted into 19-l pots. Plants were watered sparingly for the first week after transplanting to allow recovery. Twelve plants of similar height and morphology were moved into one of two identical plant growth chambers (Conviron E15, Winnipeg, Canada) for the start of the experiment (described below). After 1 week in the growth chamber, plants were watered twice a week alternating between nutrient solution applied at half strength and water.

All plants were initially grown under the same conditions of temperature and photoperiod (Fig. 1A). After 20 days, the plants were randomized and divided among three growth chambers, one used to produce summer-like conditions and two to produce winter-like conditions. Three plants, one from each chamber, were harvested at the beginning of the experimental treatment to determine the starting levels of resin and rubber (see below). The day and night temperatures (Fig. 1A), as well as the photoperiod were adjusted incrementally over the next 2 weeks for the remaining plants to a final summer- or winter-like condition of 13-h photoperiod, 30/22°C day/night or 9.5-h photoperiod, 18/8°C day/night, respectively (Appendix S1). Relative humidity in all chambers was maintained at 70%. On the 60th day after transfer to the growth chamber, the atmosphere in one of the winter-like chambers was enriched with CO_2 at a concentration of 1000 $\mu l\ l^{-1}$ during the photoperiod. After 166 days in the growth chamber, aboveground material was harvested from each plant and processed as described below.

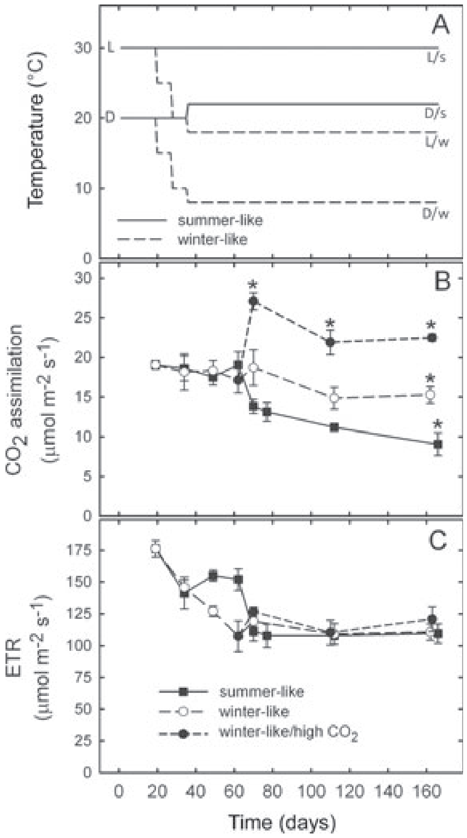

Fig. 1. CO_2 assimilation and the apparent rate of electron transport in guayule plants during a time course of temperature changes used to simulate summer- and winter-like conditions. Ten-month-old guayule plants were transferred from a greenhouse to growth chambers at time zero. Panel A: Temperatures during the dark (D) and light (L) period were adjusted over time to obtain a summer- (solid line) or winter-like (dotted line) conditions. L/s, L/w, D/s and D/w refer to temperatures in the light/summer, light/winter, dark/summer and dark/winter, respectively. CO_2 assimilation (Panel B) and the apparent rate of electron transport (Panel C) were measured at 1000 $\mu mol\ m^{-2}\ s^{-1}$ PAR for plants grown and measured under simulated summer- (solid squares) and winter-like (open circles) conditions and for plants grown and measured under winter-like conditions in an atmosphere enriched with 1000 $\mu l\ l^{-1}$ CO_2 (solid circles). Differences in CO_2 assimilation rates among treatments that are statistically different at the $P < 0.05$ level are indicated by the asterisks.

Plant processing

Individual plants were divided into stems or leaves (including flowers and floral stems) and the aboveground biomass was determined. Leaves were dried for 48 h at 60°C in a forced draft oven. Stem material was frozen at −80°C and freeze-dried for 48 h in a lyophilizer. Dried material was weighed and then ground to a powder using a Wiley Mill (Coffelt et al. 2005) before storage in a desiccator. Chemical analysis was performed within 4 weeks of harvest.

Chemical analysis

The resin and rubber content of the freeze-dried stem material was determined by UV spectroscopy and light scattering after automated solvent extraction with acetonitrile and cyclohexane, respectively (Salvucci et al. 2009). The carbohydrate content of the stems and leaves was determined by pulsed amperometric detection (PAD) after separation by high-performance anion-exchange chromatography (HPAEC) on a Dionex (Sunnyvale, CA) ICS-3000 ion-chromatograph equipped with two 4 × 250 mm Dionex PA-1 columns linked in series (Hendrix and Wei 1994). Dried stem and leaf material was extracted at 80°C first with 80% ethanol and then with water. The ethanol-soluble fraction containing the monosaccharides and disaccharides plus ethanol-soluble inulin was dried under vacuum after passage through activated charcoal (Norita A, Sigma-Aldrich) as described by Hendrix (1993). The dried material was dissolved in water, and carbohydrates were separated and determined by HPAEC-PAD using a curvilinear gradient of 0 to 0.5 *M* sodium acetate in 100 m*M* NaOH (Hendrix and Wei 1994). Inulin was determined from the amounts of fructose and glucose liberated after hydrolysis with inulinase. Ethanol- and water-soluble extracts, dissolved in water, were incubated with 1 μl of inulinase (Sigma-Aldrich Chemical Co., Cat. No. I2017) for 2 h at 30°C in 0.1 *M* sodium acetate, pH 5.5 in a total volume of 0.1 ml. Analysis of the chromatograms showed that these conditions were sufficient to completely convert all the oligosaccharides to monosaccharides. The glucose and fructose liberated in the reaction with inulinase were determined by HPAEC-PAD. Starch was determined in leaves by extraction of the ethanol- and water-insoluble material with 0.2 *M* NaOH. Following neutralization, the solution was digested sequentially with amylase and amylopectin (Hendrix 1993), and then analyzed for glucose using HPAEC-PAD.

Gas-exchange measurements and leaf volatile collection

Gas-exchange and chlorophyll fluorescence measurements were conducted at various times during the growth period using a LiCor (Lincoln, NE) Li-6400 Portable Photosynthesis System equipped with a Li-6400-40 Leaf Chamber Fluorometer. Fully expanded leaves near the top of the canopy were dark-adapted for 20 min by enclosure of 2 cm^2 of leaf area in the darkened leaf chamber prior to measurement of gas exchange and selected fluorescence parameters. Measurements were conducted at 1800 $\mu mol\ m^{-2}\ s^{-2}$ PAR with 10% blue light on four leaves from each of the three plants, using a relative humidity in the leaf chamber of about 40% and a temperature and CO_2 concentrations that matched those used for growth (Fig. 1).

Leaf volatiles were collected during the last week of the experiment from plants grown under summer- and winter-like conditions and under winter-like conditions in a CO_2-enriched atmosphere. Small clusters of two or three healthy, mature leaves of approximately 20 cm^2 total leaf area were selected from positions high enough in the canopy to provide an irradiance of 500 $\mu mol\ m^{-2}\ s^{-1}$ PAR. Leaves were enclosed in a spherical, transparent chamber (LiCor Conifer Chamber, Li-6400-22) attached to a Li-6400 portable photosynthesis system. The leaf cluster was maintained at constant temperature for 1 h prior to volatile collection. Measurement temperatures of 20, 30 and 40°C were obtained by adjusting the temperature of the leaf chamber and growth cabinet to the desired leaf temperature. The leaves of plants grown under summer- and winter-like conditions were supplied with 380 $\mu l\ l^{-1}$ CO_2 during the measurement, while those grown under winter-like conditions with CO_2 enrichment were supplied with 1000 $\mu l\ l^{-1}$ CO_2.

After recording steady state photosynthesis, volatiles were collected by diverting the air stream from the chamber outlet through a 0.62 × 10.2 cm volatile collection trap packed with 30-mg Super-Q (Analytical Research Systems Inc., Gainesville, FL). Volatiles in the air stream were collected for 60 min at 50% relative humidity using a flow rate of 0.65 $l\ min^{-1}$. To maintain a constant flow rate through the trap, an external vacuum pump was attached to the outlet of the volatile collection trap and used in combination with the internal pump of the photosynthesis system. Inlet air was filtered through activated charcoal prior to entry into the chamber. Between samplings, leaves were removed from the chamber and the chamber was flushed with clean air to remove residual volatile contaminants. Prior to each sampling cycle, a no-leaf sample was collected from the chamber.

Immediately after collection, volatiles collected on the Super-Q matrix were eluted with 200 μl dichloromethane containing 1 $ng\ \mu l^{-1}$ (+)-carvone as an internal standard. Samples were stored at −80°C in airtight vials (Agilent Technologies, Santa Clara, CA) until analysis. Samples were analyzed by GC−MS within 12 h of collection.

Identification of leaf volatiles by gas chromatography−mass spectrometry

Volatile compounds were separated on a Varian 3800 gas-chromatograph (Varian Inc., Palo Alto, CA) equipped with a 60 m × 0.25 mm Cyclodex-B fused-silica capillary column (J and W Scientific of Agilent Technologies, Santa Clara, CA) using a temperature program optimized for volatiles. The helium carrier gas was programmed for a constant flow of 1.2 $ml\ min^{-1}$. Samples of 1 μl were injected at 250°C using a Varian CP-8400 auto-sampler. The injection mode was split-less for 0.75 min, then 60:1 split for 5 min, thereafter 20:1. The oven/column temperature was initially held at 40°C for 2 min, and then increased to 60°C at a rate of 10°C min^{-1}. After 10 min at 60°C, the temperature was increased at 3°C min^{-1} to 150°C and then to 230°C at 20°C min^{-1} before being held at 230°C for 10 min. For chemical identification, the mass of the compounds separated by GC was determined with a Varian Saturn 2000 Mass Spectrometer using the NIST05 (National Institute of Standards, USA) and Wiley7 (John Wiley & Sons Inc., Hoboken, NJ) spectral databases and by comparison with the spectra of commercial standards. Quantification was based on the MS response factors of the commercial standards (or similar compounds/isomers), and the internal standard (1 $ng\ \mu l^{-1}$ (+)-carvone), adjusted for leaf area, collection time and the volume of solvent used for extraction.

Statistical analysis

All analyses were conducted at least in triplicate for each plant, and the mean results were used to determine the means ± SE for all the plants from each treatment. Statistical differences between the three treatments were evaluated by ANOVA using the Holm–Sidak pairwise method for multiple comparisons.

Results

Growth and photosynthesis under simulated summer- and winter-like conditions

Guayule plants were grown for about 5 months under controlled environment conditions (Fig. 1A and Appendix S1) that simulated early summer- or winter-like conditions in south-central Arizona, USA (33°04′37″N, 111°58′26″W), a potential production area for guayule. Visual inspection revealed that plants increased in size during the course of the experiment. Growth was more vigorous under summer-like conditions, although differences in aboveground biomass among treatments were not statistically significant at the $P < 0.05$ level (Table 1). After growth under summer- and winter-like conditions, the dry:fresh weight ratio and the percentage of dry weight that was partitioned between stems and leaves were similar between treatments. Somewhat surprisingly, enrichment with CO_2 under winter-like conditions did not increase the total amount of aboveground biomass.

The assimilation of CO_2 by photosynthesis was determined at various times throughout the time course of the experiment at saturating irradiance and with the conditions of temperature and CO_2 concentration used for growth (Fig. 1B). Rates of CO_2 assimilation were similar under summer- and winter-like conditions up through day 60. After day 60, the CO_2 assimilation rates of plants grown under summer-like conditions decreased more rapidly than in plants grown under winter-like conditions. The decrease coincided with a decrease in stomatal conductance (Fig. 2A).

The rate of CO_2 assimilation in plants grown and measured under winter-like conditions with elevated CO_2 was about 1.6-fold higher than in plants grown and measured at air levels of CO_2. Transient changes in the apparent rate of electron transport occurred during the time course under both summer- and winter-like conditions prior to day 60, but afterwards the rates were similar between treatments and constant for the duration of the experiment, both in air and with elevated CO_2 (Fig. 1C).

Stomatal conductance was higher under winter- compared with summer-like conditions, but lowest for plants grown and measured with elevated CO_2 (Fig. 2A). Stomatal conductance remained relatively constant over the duration of the experiment under winter-like conditions, but decreased under summer-like conditions, particularly after day 60. The internal CO_2 concentration of the leaves was similar and relatively constant under summer- and winter-like conditions, but higher in plants that received CO_2 enrichment during growth and measurement (Fig. 2B).

Carbohydrate accumulation under summer- and winter-like conditions

The content of non-structural carbohydrates in the stems and leaves of guayule was determined after growth under summer- and winter-like conditions and under winter-like conditions with CO_2 enrichment. In stems, the total amount of simple sugars (i.e. glucose, fructose and sucrose) was higher under winter- than under summer-like conditions and highest when plants were

Table 1. Effect of growth condition on the aboveground biomass of guayule plants. [a,b]Differences among treatments that were statistically different at the $P < 0.05$ level are indicated by different letters.

	Growth condition		
Parameter	Summer-like	Winter-like	Winter-like/high CO_2
Fresh weight (FW)			
Total (g)	780 ± 36^{a}	$717 \pm 17^{a,b}$	613 ± 42^{b}
Leaves (g)	367 ± 23^{a}	403 ± 22^{a}	344 ± 23^{a}
Stems (g)	360 ± 12^{a}	282 ± 5^{b}	246 ± 18^{b}
Stems (% total FW)	46.2 ± 0.9^{a}	39.4 ± 0.6^{b}	40.2 ± 0.4^{b}
Dry weight			
Total (g)	381 ± 24^{a}	332 ± 6^{a}	292 ± 23^{a}
Leaves (g)	197 ± 15^{a}	$172 \pm 5^{a,b}$	144 ± 10^{b}
Stems (g)	184 ± 9^{a}	159 ± 1^{a}	148 ± 13^{a}
Stems (% total dry weight)	48.4 ± 0.9^{a}	48.1 ± 0.7^{a}	50.5 ± 0.9^{a}
Dry weight (% of FW)	48.8 ± 0.9^{a}	46.3 ± 0.2^{a}	47.6 ± 0.7^{a}

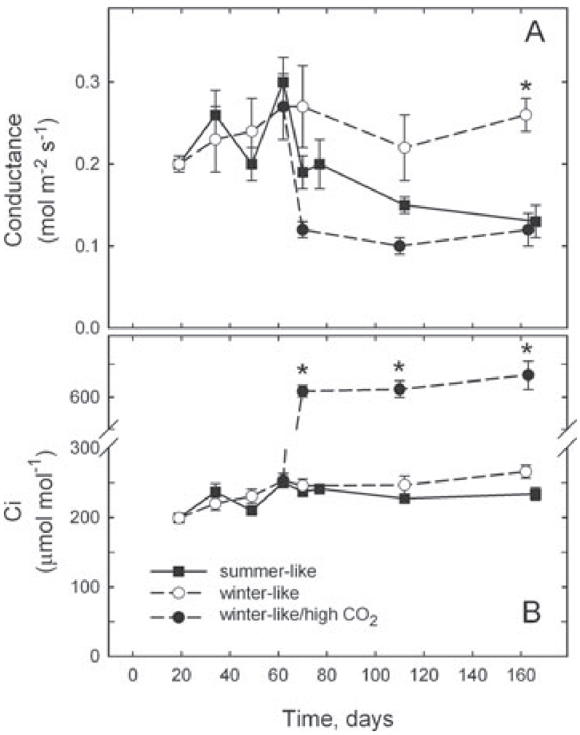

Fig. 2. Stomatal conductance and internal CO_2 concentration (Ci) in guayule plants under simulated summer- and winter-like conditions. Guayule plants were grown in growth chambers under the conditions described in Fig. 1A and Appendix S1 and measured over the time course. Stomatal conductance (Panel A) and Ci (Panel B) were measured at 1000 µmol m^{-2} s^{-1} PAR for plants grown and measured under simulated summer- (solid squares) and winter-like (open circles) conditions and for plants grown and measured under winter-like conditions in an atmosphere enriched with 1000 µl l^{-1} CO_2 (solid circles). Differences in stomatal conductance and Ci among treatments that are statistically different at the $P < 0.05$ level are indicated by the asterisks.

grown under winter-like conditions with CO_2 enrichment (Table 2). Even greater differences between treatments were observed for the content of inulin. For example, total inulin was more than two-fold higher under winter- compared with summer-like conditions, and more than four-fold higher when plants were grown under winter-like conditions with CO_2 enrichment. The degree of polymerization of water-soluble inulin determined from the relative amounts of glucose and fructose liberated by digestion with inulinase was similar under all growth conditions, about 8–10 fructosyl units per glucose (data not shown). In contrast, the degree of polymerization of the ethanol-soluble inulin was 22, 6 and 9 fructosyl units per glucose under summer-, winter- and winter-like conditions with CO_2 enrichment, respectively.

In leaves, soluble carbohydrates were highest under summer-like conditions, but starch levels were similar under summer- and winter-like conditions (Table 3). In contrast, starch, which is present only at very low levels in the stem (Kelly and van Staden 1994), was about three-fold higher in the leaves of plants grown under winter-like conditions with CO_2 enrichment compared with plants grown in air. As observed with the stems, inulin levels in leaves were more than three-fold higher under winter- compared with summer-like conditions and more than four-fold higher under winter-like conditions with CO_2 enrichment. The degree of polymerization of leaf inulin was similar under all conditions, i.e. about 9 fructosyl units per glucose (data not shown).

Accumulation of rubber and resin under summer- and winter-like conditions

Compared with the level at the start of the experiment, the resin and rubber content of the stems increased over the course of the experiment in plants grown under summer- and winter-like conditions, as well as in plants grown under winter-like conditions with elevated CO_2 (Table 4). The increase in resin content was similar under all three growth conditions. In contrast, rubber content differed among treatments. Rubber content was highest under winter-like conditions, almost two-fold higher than under summer-like conditions. Plants grown under winter-like conditions in an atmosphere enriched with CO_2 had levels of rubber that were higher than under summer-like conditions, but lower than under winter-like conditions without CO_2 enrichment.

Table 2. Effect of growth condition on the non-structural carbohydrates in the stems of guayule plants. ^{a,b}Differences among treatments that are statistically different at the $P < 0.05$ level are indicated by different letters.

	Carbohydrate level (nmol mg^{-1} dry weight)						
	Mono- and disaccharides				Inulin		
Growth condition	Glucose	Fructose	Sucrose	Total	Ethanol soluble	Water soluble	Total
Summer-like	8.3 ± 1.3^{a}	4.6 ± 1.2^{a}	24.1 ± 2.7^{a}	37.0 ± 2.9^{a}	37.4 ± 5.0^{a}	54.4 ± 7.6^{a}	91.9 ± 12.4^{a}
Winter-like	$15.4 \pm 3.3^{a,b}$	20.1 ± 5.0^{a}	19.4 ± 0.7^{a}	57.9 ± 7.9^{a}	$72.8 \pm 19.8^{a,b}$	189.1 ± 5.7^{b}	261.9 ± 23.1^{b}
Winter-like/high CO_2	21.4 ± 2.2^{b}	51.2 ± 7.8^{b}	19.5 ± 0.3^{a}	92.0 ± 9.8^{b}	154.6 ± 30.5^{b}	225.8 ± 22.5^{b}	380.5 ± 48.5^{c}

Table 3. Effect of growth condition on the non-structural carbohydrates in the leaves of guayule plants. [a,b]Differences among treatments that are statistically different at the $P < 0.05$ level are indicated by different letters.

	Carbohydrate level (nmol mg^{-1} dry weight)					
Growth condition	Glucose	Fructose	Sucrose	Total soluble	Starch	Iinulin
Summer-like	49.6 ± 1.1^a	33.4 ± 2.3^a	17.9 ± 3.9^a	100.8 ± 7.3^a	33.7 ± 10.3^a	72.6 ± 10.2^a
Winter-like	18.1 ± 1.6^b	19.2 ± 2.8^a	25.3 ± 4.2^a	62.6 ± 1.2^b	26.2 ± 7.6^a	252.1 ± 7.6^b
Winter-like/high CO_2	28.1 ± 3.4^c	30.7 ± 6.8^a	31.0 ± 5.3^a	$89.8 \pm 11.8^{a,b}$	97.0 ± 34.4^a	301.1 ± 31.4^b

Table 4. Effect of growth condition on the resin and rubber content of guayule plants. [a,b,c,d]Differences among treatments that are statistically different at the $P < 0.05$ level are indicated by different letters.

Growth condition	Resin (% dry weight)	Rubber (% dry weight)
Start of experiment	2.7 ± 0.2^a	3.7 ± 0.2^a
Summer-like	$3.5 \pm 0.1^{a,b}$	5.3 ± 0.3^b
Winter-like	3.7 ± 0.2^b	9.3 ± 0.5^c
Winter-like/high CO_2	$3.5 \pm 0.2^{a,b}$	6.9 ± 0.2^d

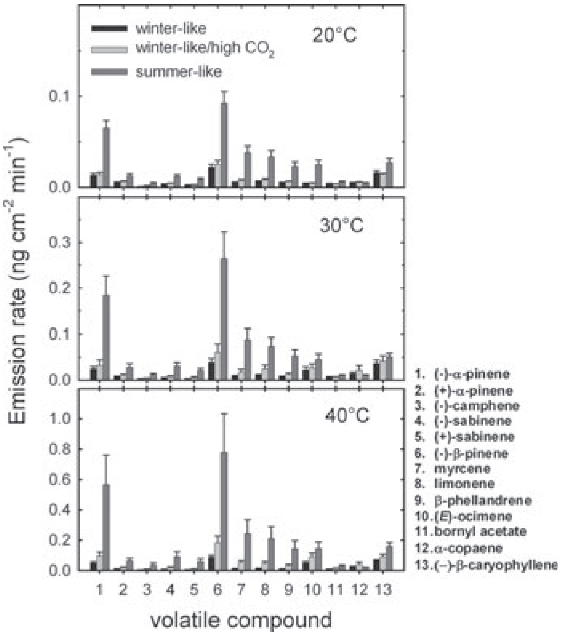

Fig. 3. Emission of volatile compounds by guayule plants under simulated summer- and winter-like conditions. Guayule plants were grown in growth chambers under the conditions described in Fig. 1A and Appendix S1 in Supporting Information. Volatile compounds were collected at 20 (top panel), 30 (middle panel) and 40°C (bottom panel) for 1 h at 500 μmol m^{-2} s^{-1} PAR from plants grown and measured under simulated summer- (black bars) and winter-like (light gray bars) conditions and for plants grown and measured under winter-like conditions in an atmosphere enriched with 1000 μl l^{-1} CO_2 (light gray bars). Volatile compounds were separated and identified by GC–MS and their identities are indicated.

Emission of biogenic volatile compounds under summer- and winter-like conditions

Volatile compounds emitted from guayule leaves were collected in the light at 20, 30 and 40°C from plants grown under summer- and winter-like conditions and under winter-like conditions with CO_2 enrichment. Analysis of the volatiles by GC–MS identified 13 compounds (Fig. 3). Of these compounds, β- and α-pinene were emitted at the highest rates from all plants under the three collection temperatures. Emission rates increased markedly with increasing leaf temperature, although the composition of the emitted volatile blend was not altered appreciably. Total emissions increased by about 2- to 2.5-fold with an increase in measurement temperature from 20 to 30°C and by about 4- to 7-fold with the increase from 30 to 40°C (Table 5). Volatile emission at each temperature was much higher under summer- compared with winter-like conditions. For example, emission rates of plants grown under winter-like conditions were only 18 to 26% of those under summer-like conditions. Interestingly, volatile emission rates were considerably higher under winter-like conditions in a CO_2-enriched atmosphere than under winter-like conditions in air, but still much lower than under summer-like conditions.

At each temperature, the rates of CO_2 assimilation measured just prior to collection of volatiles were similar in plants grown and measured in air under summer- and winter-like conditions (Table 6). Rates of CO_2 assimilation were highest in plants grown and measured under winter-like conditions with elevated CO_2, even though stomatal conductance was lower. Under all treatment conditions, the rate of CO_2 assimilation was inhibited at 40 compared with 20 and 30°C, even though volatile emission rates were highest at the highest temperature.

Discussion

Induction of vegetatively dormant and active states in guayule

The environmental conditions used here produced the desired physiological states in guayule; a vegetatively dormant state characterized by accumulation of rubber in the stems, as well as carbohydrate in the leaves and

Table 5. Total volatile production at three temperatures by guayule plants grown under different environmental conditions. The numbers in parenthesis indicate the relative rate at each temperature compared with the rate for plants grown under summer-like conditions. ^{a,b}Differences among treatments that are statistically different at the $P < 0.05$ level are indicated by different letters.

	Total volatile emission (ng cm^{-2} min^{-1})		
Growth condition	20°C	30°C	40°C
Summer-like	0.353 ± 0.052^a (1)	1.013 ± 0.235^a (1)	1.765 ± 0.245^a (1)
Winter-like	0.093 ± 0.012^b (0.26)	0.185 ± 0.033^b (0.18)	0.365 ± 0.018^b (0.21)
Winter-like/high CO$_2$	0.115 ± 0.011^b (0.32)	$0.316 \pm 0.080^{a,b}$ (0.32)	0.868 ± 0.021^b (0.49)

Table 6. Effect of growth condition on the rates of CO$_2$ assimilation, stomatal conductance, the internal CO$_2$ concentration (C_i) and transpiration in guayule plants. Gas exchange was conducted at the indicated leaf temperature and an irradiance of 500 μmol m^{-2} s^{-1} PAR using a CO$_2$ concentration of either 380 or 1000 μl l^{-1}. ^{a}Measured at 380 μl l^{-1} CO$_2$. ^{b}Measured at 1000 μl l^{-1} CO$_2$.

Growth condition	Temperature (°C)	Assimilation (μmol m^{-2}s^{-1})	Conductance (mol m^{-2} s^{-1})	C_i (μmol m^{-2} s^{-1})	Transpiration (mmol m^{-2}s^{-1})
Summer-like^a	20	12.8 ± 1.2	0.24 ± 0.02	275.4 ± 3.1	2.63 ± 0.23
	30	12.2 ± 0.7	0.26 ± 0.02	280.7 ± 5.5	4.65 ± 0.33
	40	7.1 ± 0.5	0.11 ± 0.01	239.0 ± 12.3	4.33 ± 0.35
Winter-like^a	20	12.9 ± 0.6	0.31 ± 0.05	292.2 ± 9.1	2.95 ± 0.40
	30	13.1 ± 1.2	0.28 ± 0.08	263.4 ± 28.3	4.74 ± 0.93
	40	6.3 ± 1.3	0.08 ± 0.02	210.5 ± 19.4	3.26 ± 0.58
Winter-like/high CO$_2^b$	20	15.7 ± 1.1	0.09 ± 0.01	697.2 ± 22.9	1.08 ± 0.11
	30	16.4 ± 0.9	0.10 ± 0.02	668.8 ± 44.9	2.11 ± 0.44
	40	11.8 ± 2.1	0.06 ± 0.02	579.2 ± 85.1	2.32 ± 0.72

stems and a more vegetatively active state characterized by lower levels of rubber in the stem and much lower levels of carbohydrates in the leaves and stems. Accumulation of inulin was perhaps the best indicator of vegetative dormancy because fructans accumulate in guayule during winter months and are remobilized upon bud break and the onset of vegetative growth (Kelly and van Staden 1991, 1994).

Accumulation of rubber was greater under winter- compared with summer-like conditions, consistent with induction of a vegetatively dormant state. However, compared with the start of the experiment, rubber levels increased even under summer-like conditions as plants grew and matured. The amount of rubber that accumulated under winter-like conditions, i.e. 9.3% of the stem dry weight or about 4.5% of the total plant dry weight, is higher than the amounts generally reported in the literature for 15-month-old plants (Appleton and van Staden 1989, Coffelt et al. 2005, Dissanayake et al. 2007, Veatch-Blohm et al. 2007). The amount of resin that accumulated in the stems was remarkably similar under the three growth conditions, indicating that resin accumulation in the stem was unaffected by the physiological state of the plant. A lack of response of resin levels to environmental conditions has been reported previously (Bonner 1943, Dissanayake et al. 2007, Veatch-Blohm et al. 2007) and indicates that resin accumulation is regulated differently from the accumulation of rubber and the production of volatile terpenoid compounds (see below).

Although carbohydrate and rubber levels indicated a more active state under summer-like conditions, the aboveground biomass of plants grown under this condition was only slightly higher than under winter-like conditions. That differences in aboveground biomass between plants grown under summer- and winter-like conditions were not larger can be attributed to several factors including higher respiratory activity in the dark under summer-like conditions coupled with rates of CO$_2$ assimilation that were generally lower than for plants grown under winter-like conditions. In addition, it is possible that pot size might have restricted growth under summer-like conditions. In fact, the decline in CO$_2$ assimilation rate and stomatal conductance that occurred over the time course is a possible indication of a progressive constraint on the roots that might have limited growth under summer-like conditions. These results, as well as similar results from others (Bonner and Galston 1947), suggest that biomass accumulation is not always a good indicator of the physiological state of guayule.

Carbon assimilation in the vegetatively dormant and active states

The rate of CO$_2$ assimilation by guayule has been measured in a limited number of studies either seasonally or in response to conditions like night temperature or water-deficit that affect rubber accumulation (Allen et al. 1987, Sundar and Reddy 2000, Veatch-Blohm et al. 2007). To gauge the potential for CO_2 assimilation in the vegetatively active and dormant states, we measured photosynthetic activity in guayule plants under different growth conditions as they acclimated (Fig. 1B) and after several months of acclimation (Fig. 1B, Table 6). The data showed that the rates of CO_2 assimilation and the apparent rates of electron transport activity in guayule plants in the dormant state were similar to or greater than the rates in more vegetatively active plants. Thus, guayule plants that were actively accumulating rubber in their stems and storing carbohydrate in their stems and leaves still had a capacity for photosynthesis that equaled or exceeded the capacity exhibited by vegetatively active plants. That rates of CO_2 assimilation were high in vegetatively dormant plants was consistent with the hypothesis that rubber production serves as a repository for fixed carbon when excess assimilation capacity occurs during times of restricted growth.

Stomatal conductance in guayule was lower under summer- compared with winter-like conditions, probably because of the higher leaf temperature. Compared with C_3 crops like cotton that can be cultivated in the desert (Lu et al. 1994), stomatal conductance in guayule was relatively low regardless of physiological state. These data are consistent with previous assumptions about guayule stomatal conductance based on its superior water-use efficiency compared with wheat (Downes and Tonnet 1985) as well as expectations for plants like guayule that are native to desert or semiarid habitats (Smith et al. 1997).

Effects of CO_2 enrichment on guayule plants in the dormant state

Elevated CO_2 increased the rate of CO_2 assimilation in guayule plants grown under winter-like conditions at saturating irradiance (Fig. 1B) and, to a lesser extent, under the lower irradiances used for growth (Table 6). As has been observed in other studies (Hendrix et al. 1994, Rogers et al. 2004), plants grown with CO_2 enrichment had higher levels of non-structural carbohydrates in the stems (i.e. fructose and inulin) and leaves (i.e. starch and inulin) than plants grown in air. Curiously, even though net photosynthetic rates were higher with elevated CO_2, the aboveground biomass produced by plants that received CO_2 enrichment during growth under winter-like conditions was lower than in plants grown under the same conditions but in air. These data indicate that the greater source strength produced by elevated CO_2 did not increase the net carbon gain. Midday measurements of CO_2 assimilation even during the last days of the experiments (Table 6) showed no evidence of feedback inhibition of photosynthesis by elevated CO_2, but it is possible that inhibition could have occurred late in the photoperiod when measurements were not taken. Alternatively, higher starch and soluble sugar levels in the leaves might have stimulated respiration during the long dark period, counteracting the increase in CO_2 assimilation that occurred under short days with elevated CO_2.

Cold temperatures, particularly at night, reduce respiratory activity and the rate of carbon export/translocation from the leaves (Bunce 2004). Consistent with a limitation on carbon export from the leaves was the high inulin and starch concentration in the leaves, particularly in plants grown with CO_2 enrichment. Interestingly, rubber content like biomass was lower with CO_2 enrichment. A similar result was observed in a separate, preliminary experiment conducted with guayule, but for only 3 months (data not shown). Thus, although CO_2 assimilation and the levels of non-structural carbohydrates in the stem were significantly higher under elevated CO_2, the additional carbon was not used for increased rubber synthesis. We do not have an explanation for this result, but it suggests that rubber accumulation under optimal conditions is sink–rather than source–limited. This limitation could involve anatomical, biochemical or a combination of constrains.

Emission of biogenic volatile compounds by guayule plants in the vegetatively active and dormant states

In addition to the terpenoid compounds that comprise the resin, guayule, like many arid-land shrubs, synthesizes a suite of volatile compounds derived from the isoprenoid pathway (Nakayama 1984). Early studies with guayule reported that the concentration of volatile compounds in leaves was greater in spring and summer than in winter (Bonner and Galston 1947). In the present study, volatile emission rates at each of three different measurement temperatures were considerably higher in plants grown under summer- compared with winter-like conditions, even though the rates of CO_2 assimilation were similar. Thus, volatile emission increased in the more vegetatively active plants and decreased when plants became dormant. The role of these volatiles in guayule is unknown, but could involve one or more of the functions proposed for biogenic volatiles (reviewed in Laothawornkitkul et al. 2009), including the well-known role of monoterpenes and sesquiterpenes as insect repellants (Byers et al. 2004, Langenheim 1994, Wheeler et al. 2003). As generalist insect herbivores in arid regions of North America are more abundant in the summer (Naranjo et al. 2008), higher rates of monoterpenes and sesquiterpenes emission might decrease the vulnerability of guayule to herbivory.

Analysis of the physiological factors affecting the synthesis of rubber in guayule

The complex relationship between biomass and rubber accumulation in guayule was recognized by Bonner and Galston (1947) who state that 'rubber accumulation occurs particularly under conditions favorable for assimilation but unfavorable for excessive growth'. Rubber is generally synthesized during the colder winter months when the plant enters a dormant phase, characterized by a cessation of vegetative and reproductive growth. Dormancy occurs in response to cold night time temperatures and short photoperiods, characteristic of winter in guayule's native habitat (Hammond and Polhamus 1965, Jasso de Rodríguez et al. 2006). Cold temperatures reduce mitochondrial respiration and slow carbon export and translocation (Bunce 2004), both required for vegetative growth. In contrast, day-time temperatures and solar radiation can be relatively high in this environment during the winter months, conducive for high rates of photosynthesis. In fact, even though the plant is vegetatively dormant, CO_2 assimilation during winter months is probably essential for dissipating excess light energy, particularly during cold mornings (Hutchison et al. 2000).

Vegetative growth by guayule during the spring and summer months produces the leaves and stems that are then active in the winter in assimilating CO_2, translocating reduced carbon in the form of sucrose to the stems and roots and converting the sucrose to polymers such as inulin and rubber (Appleton and van Staden 1989, Kelly and van Staden 1991, 1993). The gas exchange results presented here showed that under winter-like conditions, guayule leaves were very active in CO_2 assimilation even though the plants were vegetatively dormant. The synthesis of rubber and inulin in the stems and roots provides a storage outlet for assimilated carbon during the winter when the demand for fixed carbon for vegetative growth is minimal. Upon resumption of vegetative and reproductive growth, the carbon stored as inulin is remobilized, while rubber remains in the bark parenchyma and is not degraded (Bonner and Galston 1947). Interestingly, fructose is not a substrate for rubber synthesis; consequently most of the carbon liberated from inulin during remobilization for vegetative growth is unavailable for rubber synthesis. That rubber is not remobilized along with inulin suggests that rubber does not function as a carbon reserve for vegetative growth, but fulfills another role, perhaps serving as a feeding deterrent against herbivores (Agrawal and Konno 2009).

In guayule, the enzymatic machinery required for rubber synthesis is cold-induced (Cornish and Backhaus 2003, Goss et al. 1984, Ji et al. 1993, Madhavan et al. 1989, Sundar and Reddy 2000). Thus, as plants become vegetatively dormant and the stems become major sinks for assimilated carbon, rubber synthesis provides an alternate pathway for carbon. The mechanism for induction of the rubber pathway enzymes by cold and possibly day length is unknown, but could involve metabolic changes brought about by the increased availability of substrate from reduced respiratory activity and the cessation of meristematic growth. Upon resumption of vegetative and reproductive growth, the enzymatic capacity for rubber synthesis is no longer required because meristematic tissue provides an expanding sink for newly assimilated carbon (Appleton and van Staden 1989).

Constraints on increasing rubber content in guayule

Because of its potential as an alternate source of natural rubber, there is interest in increasing the rubber content of guayule through conventional breeding (Ray et al. 1999) or transgenic approaches (Cornish et al. 2005, Veatch et al. 2005). Unfortunately, increasing sink strength by over-expressing enzymes of the rubber synthesis pathway or by eliminating the requirement for cold induction is unlikely to succeed as a strategy unless (1) the period of vegetative dormancy can be lengthened to allow stems to function longer as sinks and (2) sufficient bark material is available to store the additional rubber. Increasing the length of the dormant period or diverting some of the remobilized carbon from inulin to rubber through conversion of fructose to glucose might lead to increased rubber, but would reduce the amount of carbon available for re-growth. Thus, increasing the rubber content of guayule is a conundrum because obvious strategies for producing more rubber adversely affect the vegetative growth that is needed to produce and store the next season's rubber.

Acknowledgements – The authors would like to acknowledge the expert technical assistance of Gwen Coyle, Stephanie Johnson and LeAnne Elhoff (USDA-ARS, Maricopa, Arizona, USA).

References

Agrawal AA, Konno K (2009) Latex: a model for understanding mechanisms, ecology, and evolution of plant defense against herbivory. Annu Rev Ecol Evol Syst 40: 311–331

Allen SG, Nakayama FS, Dierig DA, Rasnick BA (1987) Plant water relations, photosynthesis, and rubber content of young guayule plants during water stress. Agron J 79: 1030–1035

Appleton MR, van Staden J (1989) The relationship between season, growth and isoprenoid biosynthesis in *Parthenium argentatum*. J Plant Physiol 134: 524–532

Appleton MR, van Staden J (1991) Influence of temperature and daylength on growth and isoprenoid biosynthesis in guayule under controlled environmental conditions. Biores Technol 35: 147–152

Bonner J (1943) Effects of temperature on rubber accumulation in guayule plant. Bot Gaz 105: 233–243

Bonner J, Galston AW (1947) The physiology and biochemistry of rubber formation in plants. Bot Rev 13: 543–596

Bunce JA (2004) A comparison of the effects of carbon dioxide concentration and temperature on respiration, translocation and nitrate reduction in darkened soybean leaves. Ann Bot 93: 665–669

Byers JA, Zhang QH, Birgersson G (2004) Avoidance of nonhost plants by a bark beetle, *Pityogenes bidentatus*, in a forest of odors. Naturwissenschaften 91: 215–219

Coffelt TA, Ray DT, Nakayama FS, Dierig DA (2005) Genotypic and environmental effects on guayule (*Parthenium argentatum*) latex and growth. Ind Crops Prod 22: 95–99

Cornish K, Backhaus RA (2003) Inductions of rubber transferase activity in guayule (*Parthenium argentatum* Gray) by low temperatures. Ind Crops Prod 17: 83–92

Cornish K, Williams J, Hall JL, McCoy RG III (2008) Production and properties of Yulex® – the natural solution to latex allergy. Rubber Chem Tech 81: 709–722

Cornish K, McMahan CM, Pearson CH, Ray DT, Shintani DK (2005) Biotechnological development of domestic rubber producing crops. Rubber World 233: 40–44

Cornish K, Brichta JL, Yu P, Wood DF, McGlothlin MW, Martin JA (2001) Guayule latex provides a solution for the critical demands of the non-allergenic medical products market. Agro-Food-Industry *hi-tech* 12: 27–31

Downes RW, Tonnet ML (1985) Effect of environmental conditions on growth and rubber production of guayule (*Parthenium argentatum*). Aust J Agric Res 36: 7285–7294

Dissanayake P, George DL, Gupta ML (2007) Improved guayule lines outperform old lines in south-east Queensland. Ind Crops Prod 25: 178–189

Finlay MR (2004) Old efforts at new uses: a brief history of chemurgy and the American search for biobased materials. J Indust Ecol 7: 33–46

Gilliland MG, van Staden J, Mitchell JJ (1985) CO_2 fixation and rubber deposition in the bark and leaves of guayule during winter. J Plant Physiol 121: 369–379

Goss RA, Benedict CR, Keithly JH, Nessler CL, Stipanovic RD (1984) *cis*-Polyisoprene synthesis in guayule plants (*Parthenium argentatum* Gray) exposed to low, nonfreezing temperatures. Plant Physiol 74: 534–537

Hammond BL, Polhamus LG (1965) Research on guayule (*Parthenium argentatum*): 1942-1959. USDA-ARS Technical Bulletin 1327: 157 pp

Hendrix DL (1993) Rapid extraction and analysis of nonstructural carbohydrates in plant tissues. Crop Sci 33: 1306–1311

Hendrix DL, Wei Y (1994) Bemisiose: an unusual trisaccharide in Bemisia honeydew. Carbohydr Res 253: 329–334

Hendrix DL, Mauney JR, Kimball BA, Lewin K, Nagy J, Hendry GR (1994) Influence of elevated CO_2 and mild water stress on nonstructural carbohydrates in field-grown cotton tissues. Agric For Meteor 70: 153–162

Hutchison RS, Groom Q, Ort DR (2000) Differential effects of chilling-induced photooxidation on the redox regulation of photosynthetic enzymes. Biochemistry 39: 6679–6688

Ji W, Benedict CR, Foster MA (1993) Seasonal variation in rubber biosynthesis, 3-hydroxy-3-methylglutaryl-Coenzyme A reductase, and rubber transferase activities in *Parthenium argentatum* in the Chiuahuan desert. Plant Physiol 103: 535–542

Jasso de Rodríguez D, Angulo-Sánchez JL, Rodríguez-García R (2006) An overview of guayule research and development in Mexico. Ind. Crops Prod 24: 269–273

Kelly KM, van Staden J (1991) A preliminary study of the carbohydrate metabolism in *Parthenium argentatum*. Bioresource Technol 35: 127–132

Kelly KM, van Staden J (1993) A comparison of transport and metabolism of [^{14}C]sucrose and [^{14}C]fructose in summer and winter in guayule (*Parthenium argentatum* Gray). J. Plant Physiol 141: 436–443

Kelly KM, van Staden J (1994) Carbohydrate production in guayule. S Afri J Bot 60: 193–198

Langenheim JH (1994) Higher plant terpenoids: a phytocentric overview of their ecological roles. J Chem Ecol 20: 1223–1279

Laothawornkitkul J, Taylor JE, Paul ND, Hewitt CN (2009) Biogenic volatile organic compounds in the earth system. New Phytol 183: 27–51

Lu Z, Radin JW, Turcotte EL, Percy R, Zeiger E (1994) High yields in advanced lines of Pima cotton are associated with higher stomatal conductance, reduced leaf area and lower leaf temperature. Physiol Plant 92: 266–272

Madhavan S, Greenblatt GA, Foster MA, Benedict CR (1989) Stimulation of isopentenyl pyrophosphate incorporation into polyisoprene in extracts from guayule plants (*Parthenium argentatum* Gray) by low temperature and 2-(3,4-dichlorophenoxy)triethylamine. Plant Physiol 89: 506–511

Mann CC (2009) Addicted to rubber. Science 325: 564–566

Nakayama FS (1984) Hydrocarbon emission and carbon balance of guayule. J Arid Environ 7: 353–357

Naranjo SE, Dierig D, Ellsworth PC (2008) Survey and evaluation of Lygus bugs on lesquerella and guayule, two new desert crops in the western USA. J Insect Sci 8: 49

Ray DT, Dierig DA, Thompson AE, Coffelt TA (1999) Registration of six guayule germplasms with high yielding ability. Crop Sci 39: 300

Rogers A, Allen DJ, Davey PA, Morgan PB, Ainsworth EA, Bernacchi CJ, Cornic G, Dermody O, Dohleman FG, Heaton EA, Mahoney J, Zhu X-G, Delucia EH, Ort DR, Long SP (2004) Leaf photosynthesis and carbohydrate dynamics of soybeans grown throughout their life-cycle under free-air carbon dioxide enrichment. Plant Cell Environ 27: 449–458

Salvucci ME, Coffelt TA, Cornish K (2009) Improved methods for the extraction and quantification of resin and rubber from guayule. Ind Crops Prod 30: 9–16

Smith SD, Monson RK, Anderson JE (1997) Physiological Ecology of North American Desert Plants. Springer-Verlag, Berlin, 286 pp

Sundar D, Reddy AR (2000) Low night temperature-induced changes in photosynthesis and rubber accumulation in guayule (*Parthenium argentatum* Gray). Photosynthetica 38: 421–427

van Beilen JB, Poirer Y (2007) Establishment of new crops for the production of natural rubber. Trends Biotech 25: 522–529

Veatch ME, Ray DT, Mau CJD, Cornish K (2005) Growth, rubber and resin evaluation two-year-old transgenic guayule. Ind Crops Prod 22: 65–74

Veatch-Blohm ME, Ray DT, Gehrels A (2007) Night temperature, rubber productions, carbon exchange in guayule. Ind Crops Prod 25: 34–43

Wheeler GS, Massey LM, Southwell IA (2003) Dietary influences on terpenoids sequestered by the biological control agent *Oxyops vitiosa*: effect of plant volatiles from different *Melaleuca quinquenervia* chemotypes and laboratory host species. J Chem Ecol 29: 189–208

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Diurnal time course of temperature and photoperiod used to simulate summer- and winter-like conditions for guayule plants. Guayule plants were transferred from a greenhouse to growth chambers under the conditions described in Fig. 1A. Temperature and photoperiod were adjusted over the first 40 days to obtain a final summer- (solid line) or winter-like (dotted line) condition of temperature (A) and photoperiod (B).

Edited by R. Scheibe