

Critical Reviews in Biotechnology, 27:217-231, 2007

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ISSN: 0738-8551 print / 1549-7801 online

DOI: 10.1080/07388550701775927

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Guayule and Russian Dandelion as Alternative Sources of Natural Rubber

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ABSTRACT Natural rubber, obtained almost exclusively from the Para rubber tree (*Hevea brasiliensis*), is a unique biopolymer of strategic importance that, in many of its most significant applications, cannot be replaced by synthetic rubber alternatives. Several pressing motives lead to the search for alternative sources of natural rubber. These include increased evidence of allergenic reactions to *Hevea* rubber, the danger that the fungal pathogen *Microcyclus ulei*, causative agent of South American Leaf Blight (SALB), might spread to Southeast Asia, which would severely disrupt rubber production, potential shortages of supply due to increasing demand and changes in land use, and a general trend towards the replacement of petroleum-derived chemicals with renewables. Two plant species have received considerable attention as potential alternative sources of natural rubber: the Mexican shrub Guayule (*Parthenium argentatum* Gray) and the Russian dandelion (*Taraxacum koksaghyz*). This review will summarize the current production methods and applications of natural rubber (dry rubber and latex), the threats to the production of natural rubber from the rubber tree, and describe the current knowledge of the production of natural rubber from guayule and Russian dandelion.

KEYWORDS guayule, *Parthenium argentatum*, Russian dandelion, *Taraxacum koksaghyz*, rubber tree, *Hevea brasiliensis*, latex

INTRODUCTION

It is likely that during this century renewable materials will gradually replace industrial polymers based on petrochemicals for reasons of sustainability and the limited availability of petroleum. Presently, most biopolymers used in non-food applications are not competitive with petrochemical equivalents on a price/performance basis, and significant efforts on raw materials, processing technology, and applications, are required to change this (van Beilen and Poirier, 2007). The one outstanding exception is natural rubber produced from the rubber tree *Hevea brasiliensis*. This material, a polymer consisting of isoprene units (C_5H_8)_n linked together in a *cis*-configuration, is unique in that for many applications it has no affordable synthetic equivalent. Aeroplanes cannot safely land with tires made from synthetic rubber; only truck tires containing natural rubber are resilient enough to withstand heavy shear and loads; car tires containing natural rubber are safer than synthetic rubber tires because of the

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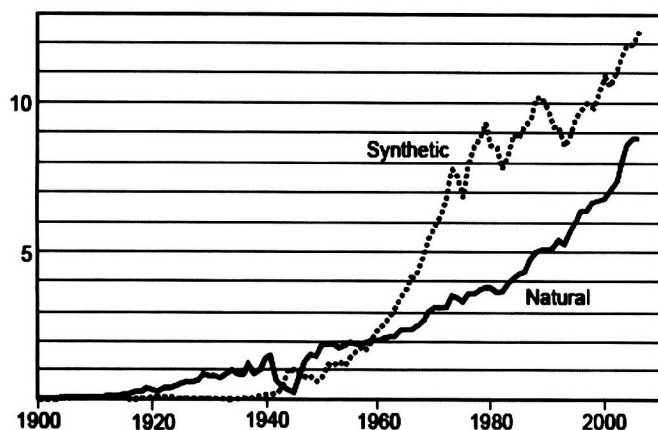


FIGURE 1 Global production in million tons per year of synthetic and natural rubber during the 20th century. Based on (Cornish, 2001) and data from the International Rubber Study Group (<http://www.rubberstudy.com/statistics-quarstat.aspx>).

unique heat dispersion abilities of natural rubber. Rubber is used in over 40,000 consumer products, including more than 400 medical devices, due to its unique properties, which include resilience, elasticity, abrasion and impact resistance, efficient heat dispersion, and malleability at cold temperatures (Cornish, 2001). Significantly, six decades of industrial research have not produced synthetic rubbers with similar price-quality ratios, and in recent decades the market share of natural rubber has even increased from close to 30% in the 1970s to nearly 40% at present (Figure 1). Moreover, synthetic rubbers are derived from petroleum, a non-renewable resource. A decline in global oil production may begin within the next 10 to 20 years (Hirsch *et al.*, 2006), leading to sharply increasing prices of synthetic rubbers.

Rapid economic development in China and India is now fuelling growth of natural rubber demand, and prices have become increasingly volatile. At the same time a plant disease named South American Leaf Blight (SALB) could potentially wipe out the production in Southeast Asia (80% of world production). Evidently, tire companies, which consume 70% of world production, are concerned since a single feedstock of natural rubber supplies their total global business. However, national governments are major stakeholders as well, as natural rubber is a strategic resource. Without natural rubber, many sectors of the economy depending on heavy-duty tires would experience great difficulties.

Approximately ten percent of natural rubber is used as latex to produce gloves, condoms, catheters, and other medical products. Especially in the USA, but also in Europe and Japan, increasing numbers of people are

allergic to proteins in *Hevea* rubber. Thus, alternative crops for natural rubber production would not only help to secure supply, but could also provide a source of hypoallergenic rubber.

For all of the above reasons, there is interest in countries depending on imported natural rubber to develop large-scale alternative (domestic) natural rubber production (Mooibroek and Cornish, 2000). This has been recognized at various times in the past, leading to research and development programs during which many plants were investigated. Eight botanical families, 300 genera, and 1,800 species have been identified that produce natural rubber in their latex, but only a few of these are known to produce large amounts of high molecular weight rubber (Bushman *et al.*, 2006).

Guayule, a shrub growing in semi-arid regions in Mexico and the Southern US, is the only non-tropical plant that has been used as a commercial source of natural rubber (early 20th century). Several other potential sources of natural rubber were investigated as well. One of these, the Russian dandelion, was promising enough to justify large research and development programmes in the USSR (1931–1950), in the US in the framework of the Emergency Rubber Program during WWII, and in other countries. Many other species have not been studied in sufficient detail to establish their utility or were found to contain rubber of low molecular weight (Table 1).

NATURAL RUBBER PRODUCTION

The annual worldwide natural rubber production is estimated to be close to 8,800,000 tons (<http://www.rubberstudy.com/statistics-quarstat.aspx>), almost all of it from one biological source: the Brazilian rubber tree (*H. brasiliensis*), and 80% coming from three Southeast Asian countries, namely Malaysia, Indonesia, and Thailand. The latex is tapped from trees, starting 5–7 years after planting. At the end of their productive lives (25 to 30 years) (Jones, 1997), the trees are felled for timber production, which, on average yields 13 tons ha⁻¹ y⁻¹ of rubberwood. The rubber yield varies from 500 kg ha⁻¹ y⁻¹ in smallholder plots to more than 1500 kg ha⁻¹ y⁻¹ in large plantations (Balsiger *et al.*, 2000). In experimental plots with new *Hevea* lines yields of up to 3000 kg ha⁻¹ y⁻¹ have been obtained.

Natural rubber from *H. brasiliensis* mainly consists of *cis*-1,4-polyisoprene, with many minor additional

TABLE 1 Guayule and Russian dandelion as alternative sources of natural rubber

Rubber source	Content (%)	Mw (kD)	Prod. T/Y (year)	Yield (kg ha ⁻¹ y ⁻¹)	References
Rubber tree <i>H. brasiliensis</i>	30–50 of latex 2% of tree dw	1,310	8,800,000 (2005)	500–3000	(Blanc <i>et al.</i> , 2006; Han <i>et al.</i> , 2000; Mooibroek and Cornish, 2000; Priya <i>et al.</i> , 2006)
Guayule <i>P. argentatum</i> Gray	3–12	1,280	10,000 (1910)	300–1000	(Kim <i>et al.</i> , 2004; Mooibroek and Cornish, 2000; Polhamus, 1962; Swanson <i>et al.</i> , 1979)
Russian dandelion <i>T. koksaghyz</i>	trace–30	2,180	3,000 (1943)	150–500	(Hallahan and Keiper-Hrynko, 2004; Mooibroek and Cornish, 2000; Whaley and Bowen, 1947)

Dw., dry weight; Mw; molecular weight of the rubber in kiloDaltons.

components (such as proteins, minerals, and lipids) that are—in part—responsible for the superior properties of this material compared to synthetic materials. The rubber polymer chains are produced from isoprenoid precursors that are synthesised as part of the general mevalonic acid (MVA) pathway located in the cytoplasm (Bouvier *et al.*, 2005). A rubber transferase (EC 2.5.1.20) located in the cytoplasm of plant laticifer cells progressively adds isopentenyl moieties onto a single allylic diphosphate primer molecule to form the rubber biopolymer. The rubber molecules accumulate in particles that are surrounded by a species-specific fatty acid monolayer and rubber particle-associated proteins. The biochemistry and mechanism of rubber biosynthesis have been reviewed recently (Cornish, 2001; Mooibroek and Cornish, 2000; Puskas *et al.*, 2006; van Beilen and Poirier, 2007).

Hevea Rubber Market and Applications

Natural rubber is a highly valuable and strategically important biomaterial; unlike most other biopolymers, it cannot be replaced by synthetic materials in many of its applications. For example, heavy-duty tires for trucks, busses, and airplanes, as well as many latex products for the medical profession, cannot be made exclusively from synthetic rubber, or only at a significantly higher cost.

About 10% of the latex harvested from *Hevea* trees is manufactured into latex products. Latex films form an excellent barrier to pathogens, including viruses, giving condoms and gloves high protection against infection. This is largely due to the excellent film-forming nature of natural rubber latex. In this respect, natural rubber

latex is vastly superior to vinyl films and to most other competitive materials. Natural rubber films are also very strong and are closely fitting (tactile performance is not impaired). Gloves made from this material both protect the wearer and the object (scientific samples, objects, drugs, foodstuffs, electronic components, etc.) or the person being handled. Much of the rubber-processing industry is located in Malaysia and employs four times more workers than the rubber plantations: 80% of all catheters, 70% of latex threads and 60% of latex gloves are made in Malaysia.

The harvested latex is concentrated by centrifuging, removing some of the water and much of the proteins, and is preserved with ammonia. Products, such as gloves and condoms, are produced by dipping a porcelain or glass former into the latex. Usually, the latex is prevulcanized by mixing with sulfur and accelerators. In the next steps, the coated former is dipped into a coagulant (typically calcium nitrate) to gel the latex, and then heated in a continuous oven, which dries and vulcanizes the latex films.

The remaining 90% of latex is coagulated and converted into dry rubber. Usually, smallholder rubber has been coagulated prior to sale, already in the tapping cup. The coagulated rubber is milled and washed to remove contaminants introduced by collection and transportation. Most natural rubber is dried using fossil fuel, which, as a rule of thumb, consumes about a tenth of the fossil fuel required to produce synthetic rubbers. Rubber sheets can also be dried by solar heating.

Dry natural rubber is mainly applied in high performance tires, such as aircraft and truck tires (more than 70% in the USA). These tires are much safer than those made from synthetic rubber due to the ability of natural rubber to dissipate heat.

The rapid economic development in Asia, especially in China (the world's largest rubber consumer imported 1.5 million tons in 2005) and India, is resulting in strongly rising prices. According to the International Rubber Study Group (<http://www.rubberstudy.com>), the production deficit for 2006 was around 250,000 tons. The group forecasts a 50% increase in rubber consumption by 2020. Accordingly, after a low point in 2000, the price of natural rubber has increased 5-fold to €2.30 per kg in June 2006. It is now extremely volatile, losing ground to €1.50 per kg in December 2006, and subsequently gaining again to €1.90 per kg in June 2007. The increasing oil price is likely to act in two directions: while it makes natural rubber a better competitor for synthetic rubber, it also tends to lower the demand for natural rubber by producers of heavy-duty tyres for the car industry.

Rubberwood

An important side product of *Hevea* rubber production is rubberwood (Killmann, 2001). This tropical wood was originally used merely as a fuel for drying and smoking rubber and to provide a source of charcoal for local cooking. However, rubberwood has favorable qualities and light color, making it a good timber for furniture making and other applications. For example, it can be easily steam-bent or stained to resemble other timber. In 1998, Malaysia exported rubberwood furniture with a value of 683.3 million \$US, and rubberwood is now one of the most successful export timbers of Southeast Asia. In some cases, *Hevea* is grown primarily for timber harvesting, with the latex as a co-product.

Biological Threats to Natural Rubber Production

A critical issue is the threat to natural rubber production from plant diseases, as *H. brasiliensis* is genetically very homogeneous: the millions of hectares of rubber plantations are all derived from a small sample of seeds collected in Brazil by Dr. Henry Wickam in 1876 (Davis, 1997). Therefore, plant diseases are an important theme, which can be illustrated by the example of Brazilian rubber production: in 1934, the endemic leaf blight pathogen *Microcyclus ulei* or South American Leaf Blight (SALB) wiped out the local large-scale

production of rubber, and it has not been possible to restart large-scale production since. The present production on marginal lands in Brazil, where SALB is less of a problem, is only 96,000 T/Y, about 1% of world production. Accidental spread of SALB in Southeast Asia could reduce production by millions of tons in a matter of years, leading to serious shortages, and consequently drastically higher prices.

Attempts in the 1980s and 1990s in Brazil to develop SALB resistant *Hevea* clones have not met with success. Although some progress has been made (Le Guen *et al.*, 2003), all promising lines finally succumbed to the fungus in the field (Le Guen *et al.*, 2007; Lespinasse *et al.*, 2000). Apart from efforts in Asia on common plant diseases, yield, and agronomics, *H. brasiliensis* is studied in France and Brazil to generate leaf-blight resistant varieties, increased yield, and altered properties. Recently, efficient transformation of calli and regeneration of plants was shown to be possible (Blanc *et al.*, 2006). Several genes involved in rubber synthesis have also been cloned (for a brief overview see van Beilen and Poirier, 2007). However, the narrow genetic base, prolonged breeding cycles and juvenile period, and highly heterozygous nature of *H. brasiliensis* make breeding complex, time-consuming and labor-intensive. In view of the critical importance of rubber, these efforts appear to be rather limited.

Economic Threats to Natural Rubber Production from *Hevea*

An additional and relatively recent threat to natural rubber production from *Hevea* is competition for land by palm oil plantations: in only three decades the palm oil acreage in Malaysia increased from less than 100,000 hectares to almost 2,000,000 hectares, which is slightly more than the rubber tree acreage. Together, the two crops account for more than 70% of the total agricultural land use of that country. Thus, new palm tree plantations replace either rubber trees or natural forests. Similar developments are also occurring in Thailand and Indonesia.

Increasing labor costs are an issue especially in Malaysia, as latex harvesting is very labor intensive, and the Malaysian economy is shifting to an industrial basis. As with all agricultural commodities the effects of climate change, pollution, economic development, and population growth are unpredictable factors, which may



FIGURE 2 Left panel: Guayule (from USDA-NRCS Plants Database, <http://plants.usda.gov/java/profile?symbol=PAAR5>). Right panel: Russian dandelion (provided by Dirk Prüfer).

induce major changes in available acreage, yield, and demand for natural rubber.

Latex Allergies

Hevea latex products are responsible for moderate to severe allergic reactions due largely to proteins naturally associated with the rubber particles. The incidence of such reactions has increased dramatically in the last 15 years and it is now accepted that 1–6% of the general population suffer from latex allergies. Some studies have shown that up to 17% of healthcare workers are at risk of reactions (Bousquet *et al.*, 2006). In response to this issue, the American Society for Testing and Materials has developed a new standard (ASTM 1076-06) for Category 4 natural rubber latex. Previous standards measured physical performance rather than protein content and applied only to *Hevea*-based raw materials. The new standard offers an opportunity for medical product manufacturers looking to develop safe protein-free high performance latex from alternative plant sources.

H. brasiliensis latex allergens have also been reported to occur in the particulates derived from rub-off and wear of tires. The presence of allergens in particulates possibly worsens asthma symptoms in sensitized patients (Namork *et al.*, 2006). Other research, however, indicates that dry rubber products (cut thread, hot water bottles and divers' flippers were tested) contain extremely low amounts of residual extractable protein

contents, and show very low or negligible allergenicity (Yip *et al.*, 1995).

GUAYULE AS AN ALTERNATIVE SOURCE OF NATURAL RUBBER

Just as *H. brasiliensis* and related trees served as a source of rubber balls for the Indians of the Americas at least as far back as the Mayan civilization, guayule (*Parthenium argentatum* Gray) also served as a source of rubber for the local population in present Mexico and the Southern USA (Figure 2, left panel). It was obtained by chewing the plant material, separating the fibrous material, thus accumulating a mass of rubber. Commercial exploitation of guayule started in the early 20th century when rubber production in Amazonia became a significant enterprise, demand for rubber increased rapidly, and import prices in the USA were high. In 1910, 10,000 tons per year of rubber were produced from natural stands of the guayule shrub (Ray *et al.*, 2005). As rubber production from *H. brasiliensis* became more efficient, and natural stands of guayule were exhausted, this production strategy was gradually abandoned.

Among the new or alternative crops considered for rubber production, guayule is special in that it has a long history of commercialisation, and emergency projects with intensive research efforts (Table 2). Unfortunately, the extended periods of neglect have caused the loss of experience and genetic material, and have limited progress.

TABLE 2 Brief chronology of events in the use of guayule and Russian dandelion as alternative sources of natural rubber

1888	First involvement of a US company with guayule rubber production
1902	Solvent extraction method developed (discontinued in 1905)
1903	Water flotation method (Saltillo process) developed
1904	First shipment of 50 pounds of guayule rubber to the Manhattan Rubber Company
1905	First large plant constructed in Mexico (7–8 tons per day)
1907	First facility built in Texas (Marathon), operated until 1916
1910	50% of rubber imported in the US is from guayule (10% of world production)
1912	All production halted due to the Mexican Revolution, first collection of guayule germplasm by McCallum in Texas and Mexico (smuggled seeds)
1916	Start of a breeding and selection program in Continental, Arizona (principle cultivar resulting from this program was "593")
1929	Production in the US ceased due to the Great Depression
1931	Discovery of Russian dandelion as a source of natural rubber in the framework of a screening program to make the USSR self-sufficient in strategic materials
1942	Initiation of the Emergency Rubber Project involving over 1,000 scientists and technicians, and 9,000 laborers. New guayule germplasm collected in Mexico and Texas. Russian dandelion germplasm obtained from the USSR
1944	Russian dandelion project terminated in June 1944
1946	All guayule stands were liquidated (due to cheap <i>Hevea</i> rubber, and the expectation that synthetic rubber would fully replace natural rubber). A small USDA breeding program continued, including the evaluation of 25 selections
1950	Emergency program to stockpile seeds and seedlings (stopped in 1952)
1951	End of Russian dandelion cultivation in the USSR
1959	Termination of the USDA guayule breeding program in Salinas, 24 germplasm lines plus line "593" were stored at the USDA National Seed Storage Laboratory
1973	First oil crisis
1976	Saltillo plant opened in Mexico (closed early 1980 ties), source of all test rubber used in making tires until 1990
1977	National Academy of Sciences report on guayule
1978	Native Latex Commercialisation and Economic Development Act (US Congress)
1979	Second oil crisis
1984	Critical Agricultural Materials Act (US Congress)
1988	Bridgestone/Firestone prototype plant (closed 1991)
1990s	Type I allergy to <i>Hevea</i> rubber recognized as a major problem, and renewed interest in alternative sources of natural rubber
1997	Yulex Corporation founded to provide hypoallergenic latex from guayule
2004	Patent by DuPont on cis-prenyl transferase homologs cloned from Russian dandelion
2006	Production facility for guayule latex (Yulex Corporation)
2007	Oil price reached \$98 per barrel in November 2007

Guayule Breeding

The intermittent breeding efforts during the 20th century have made guayule a partially domesticated crop. The general status of breeding programs was reviewed in 1991 (Estilai and Ray, 1991) and in 2005 (Table 3) (Ray, Coffelt, and Dierig, 2005). Currently, research on breeding is only carried out in the USA. Guayule breeding benefits from (and is complicated by) extreme variability within and between lines for every trait that has been evaluated (rubber quality and quantity, dry weight, resin content, yield, etc.). The facultative nature of apomixis in polyploid guayule continually releases new variability, which may be exploited by plant breeders (Ray, Coffelt, and Dierig, 2005). How-

ever, from a breeder's point of view, guayule (but also *H. brasiliensis*) is a difficult species to work with because it is a perennial, requiring relatively large amounts of land for breeding programs. Guayule is physiologically immature for 2 years, and reproduction is essentially asexual (by apomixis). Thus breeding depends mainly on the selection of high-yielding plants.

The primary target of the breeding program has been the development of higher yielding cultivars. This target has clearly met with success, as new lines available from the United States Department of Agriculture (USDA) yield up to 250% more rubber than the old lines from the 1940s and 1950s. Nevertheless, breeding for increased rubber content has been frustrating as progenies of individual selections failed to repeat the high

TABLE 3 Guayule breeding targets and results

Target	Results	Comment
Rubber yield	300–1,000 kg ha ⁻¹ y ⁻¹	Product of dry matter per hectare and mean rubber content on an annual basis
Rubber content	Up to 8.9%, av. 6% Salinas up to 12.4%	(Whitworth and Whitehead, 1991) (Cole <i>et al.</i> , 1991)
Biomass	Up to 26,000 kg/ha/y, av. 13,000 kg/ha/y	Relevant because of bagasse fuel value
Rubber quality	Not well defined for guayule, high Mw in bark, low Mw in wood	High variability between lines and between growth conditions, some lines like Gila are failures
Multiple harvest	Probably amenable to selection	Survival rates of plants cut at ground level varies from 11–100% between lines, best results were obtained during dormancy
Post harvest rubber degradation	Genetic basis not investigated	Storage always reduces Mw, grinding of plant material causes rapid deterioration due to the absence of a natural antioxidant
Disease and insect resistance	Triploids and tetraploids are more resistant to <i>Verticillium</i>	Probably great reservoir for disease resistance genes in guayule. Damage from insects is not considered serious.
Cold tolerance	Native stands withstand –18°C	Interspecific hybridization may be used; however, hybrids typically have bad rubber quality
Drought tolerance	Inadequate funding for evaluation	Rubber yield increases with increased irrigation
Salt tolerance	Inadequate funding for evaluation	Guayule is only slightly salt tolerant; the genetic basis not known
Seed quality	Inadequate funding for evaluation	Seed size, quality and germination are probably amenable to breeding
Co-products	Inadequate funding for evaluation	Higher ratio of rubber to resin is desirable

Summarized from (Estilai and Ray, 1991; Ray, Coffelt, and Dierig, 2005).

rubber content of the selected parents. One of the other breeding targets listed in Table 3 concerns seed characteristics. Guayule seeds are small, dormancy must be broken by special treatments, and germination is not reliable. Future breeding activities might be directed toward more novel techniques like recurrent selection in sexually reproducing diploids followed by chromosome doubling, crossing high yielding apomictic plants or crossing apomictic plants with sexually reproducing plants. Research in Mexico is primarily focused on the properties and potential of wild stands of guayule (Jasso de Rodriguez *et al.*, 2006). This material constitutes an important source of properties for further breeding. The progress made with *H. brasiliensis* shows the potential: in four decades the rubber yield increased ten-fold from 300 kg ha⁻¹ y⁻¹ to 3000 kg ha⁻¹ y⁻¹.

Guayule Molecular Genetics and Genetic Engineering

Wild guayule stands contain a natural polyploid series of diploids ($2n = 2x = 36$), triploids ($2n = 3x = 54$) and tetraploids ($2n = 4x = 72$); and under cultiva-

tion, individual plants have been identified with chromosome numbers up to octaploid ($2n = 8x = 144$). Diploids reproduce predominantly sexually, and polyploids reproduce by facultative apomixis.

Several genes encoding enzymes and proteins associated with rubber synthesis have been cloned. This includes the major guayule rubber particle protein (RPP) gene (Backhaus and Pan, 1997), and a 24 kDa protein tightly associated with the so-called small rubber particle protein (SSRP) (Kim *et al.*, 2004). As it is very similar to the SSRP of *H. brasiliensis* it was designated guayule homologue of SSRP (GHS). *In vitro* functional analysis by heterologous expression in *E. coli* revealed that it plays a positive role in isopentenyl incorporation *in vitro* (GHS incorporates IPP monomers in washed rubber particles). A series of other proteins associated with rubber particles have been isolated or investigated (cited in Kim *et al.*, 2004), but their functions have not been established. The major protein associated with guayule rubber particles was shown to be a 53 kD cytochrome P450 enzyme of the CYP74 family highly homologous to allene oxide synthase (AOS). It comprises about 50% of the total protein in guayule

rubber particles, and has been implicated as a rubber transferase (Pan *et al.*, 1995). Although it is catalytically active as an AOS (converting 13(*S*)-hydroperoxy-(9*Z*,11*E*)-octadecadienoic acid to α - and γ -ketol fatty acids), and it is the major protein in washed rubber particles catalyzing IPP polymerization (Benedict *et al.*, 1990), its role in rubber synthesis has not been proven. Moreover, it does not show any sequence similarity with *cis*-prenyltransferases. For either guayule or rubber tree, reconstitution of a complete long-chain rubber biosynthesis machinery from its individual components has not been demonstrated, most likely because not all components are known, and the functions of the individual components cannot be adequately tested in isolation. The question of the nature of the protein(s) required for high molecular weight rubber synthesis is one of the most significant outstanding questions relating to the biochemistry of rubber biosynthesis.

Tissue culture techniques for asexual propagation of guayule have been developed in the 1980s (see, for example, Radin *et al.*, 1982, and Lovelace *et al.*, 1982). The use of tissue culture may be important to maintain the genetic stocks of selected guayule germplasms and cultivars, because the identity of selected lines cannot be maintained in successive generations due to lack of control over sexual reproduction and apomixis. A new method for guayule tissue culture, using low light and ammonium was recently published (Dong *et al.*, 2006). In this work a resistance gene to the herbicide ammonium-glufosinate was introduced into guayule. Various allylic diphosphate synthetase genes were also introduced in tissue-culture generated transgenic guayule plants (Veatch *et al.*, 2005). Some differences in plant height and width and rubber and resin content were observed, but these were not consistent.

Guayule Agronomy and Harvesting

Guayule is the dominant perennial shrub found on the semi-arid limestone bajadas (mild slopes at the feet of mountains) and hillsides of the Big Bend region of Texas and the Chihuahuan desert of north central Mexico, areas with a temperature range between -18 and 49.5°C . High temperature does not appear to affect growth, but temperatures below 4°C induce semi-dormancy and extended freezing temperatures can cause plant death. One of the few problems encountered in Guayule cultivation is root disease, especially

in standing water (Mihail *et al.*, 1991). Thus, preferred soils are mainly well-drained calcareous or sandy-loam soils with relatively low concentrations of nutrients. Guayule has been grown in desert-like environments, humid warm conditions, hot dry semi-arid conditions, and regions with moderate temperature and rainfall.

Germplasm from the USA breeding program has been tested in Argentina (Coates *et al.*, 2001), Australia (Dissanayake *et al.*, 2004; Ferraris, 1993; George *et al.*, 2005) and Israel (Mills *et al.*, 1990). Guayule lines were also grown in Greece, Morocco, and South Africa, but in these cases reports or publications are not publicly available. In Mexico cultivation experiments have been carried out with native germplasm (Jasso de Rodriguez *et al.*, 2002; Jasso de Rodriguez *et al.*, 2005). The agronomy studies have been reviewed recently (Foster and Coffelt, 2005).

Good seed quality is a prerequisite for good plant establishment in the greenhouse or in the field. Guayule seeds are very small (about 1000–1500 seeds per gram). Primary dormancy in guayule seeds is caused by an inner seed coat dormancy and embryo dormancy, but can be broken by seed treatment, with the advantage of uniform and fast germination (Foster and Coffelt, 2005). Currently, seedlings are grown in nursery trays in the greenhouse and fields are established using typical commercial transplanting systems. Transplanted seedlings grow slowly in the field and require frequent irrigation with 100–250 mm of water with a low salinity. A pre-emergence treatment is also required as guayule is a poor competitor. The costs of this method are substantial at around US\$ 900–1600 per ha (Foster and Coffelt, 2005). Direct seeding might be possible for less than US\$ 400 per ha, but this method appears to be much less reliable due to the small size of the seed, poor germination and competition with weeds (Foster *et al.*, 1999; Thompson and Ray, 1989).

Guayule prefers areas with annual precipitation between 280 and 640 mm, but for maximum rubber yields, moderate to heavy applications of irrigation are necessary. Dry matter production and resin and rubber yields have been shown to increase proportionally with increased water availability (Nakayama, 1991). Although sufficient water is required to allow crop growth, guayule is quite resistant to drought conditions: it will interrupt rubber accumulation, but survive extended periods of limited water availability.

Several procedures for harvesting are being considered. The entire plant including the root system may be

harvested, the plant may be clipped above the crown and have only the branches and stem removed, or it may be undercut 15 to 20 cm below the soil surface (digging), leaving the top of the taproot intact. Results so far do not indicate that clipping will increase the total yield over the cultivation period (Mills, Werner, and Benzioni, 1990).

Harvested guayule can be pressed into bales with a density of up to 280 kg per m³ (Coates, 1993). Reducing the volume of the shrub by chopping has been suggested to reduce transport costs to the processing factory. This may be possible for short transport distances and immediate processing. However, longer transport distances and increased storage time causes rubber degradation (Coates, 1991). This is due to the fact that guayule does not contain the natural antioxidants that protect *H. brasiliensis* rubber from being degraded. Exposure to oxygen due to cuts and tears reduces the molecular weight of the rubber (Malani and Clark, 1986). This may be prevented by adding an antioxidant to chopped plant material or otherwise processed material (Schloman *et al.*, 1989). High temperatures during storage of the crop (in whichever form) must be avoided, because triglycerides in the guayule material trigger oxidative deterioration of the rubber, even in the presence of antioxidant (Schloman *et al.*, 1996).

Rubber yield and dry mass yield vary strongly between various lines. Moreover, yields from the same line often strongly vary in various regions, soils, or under different conditions. It has been reported that the season and the age at which the guayule plants are harvested affects the rubber and resin yield. In some cultivars the percentage of rubber in the shrub varies with the season while in other cultivars the rubber content is more stable. For a set of eight cultivars tested the biomass yield per year increased (Estilai, 1991). However, rubber yield per year did not show a similar pattern. Circumstantial evidence indicates that old stands may contain very high percentages of rubber. In the various published studies, biomass yields reached 15 tons, and rubber yields approach 1000 kg ha⁻¹ y⁻¹. Yulex has reported biomass and rubber yields of 20 and 2 tons ha⁻¹ y⁻¹, respectively (Katrina Cornish, personal communication).

Processing of Guayule Rubber

Because guayule stores its rubber within the cells of its bark and woody tissue, the latex cannot be harvested

by tapping. To gain access to the rubber, the plant cells must be ruptured. This can be accomplished by subjecting coarsely ground material to compressive and shear forces in differential roll mills or extruders. Several approaches have been tried to recover rubber from the thoroughly ground shrub: flotation, sequential extraction, simultaneous extraction (Wagner and Schloman, 1991), and latex harvesting by centrifugation (Cornish *et al.*, 2006; Jones, 1948). The first procedure to be used on a large scale (the Saltillo process) was based on flotation of the rubber in water after parboiling, hammer milling, and wet milling (Jones, 1948; Tint and Murray, 1949), and involved the use of large amounts of a dilute caustic solution to bring about the coagulation of the rubber particles. One of the facilities, operated by Continental-Mexican, had a capacity of 180 tons of shrub, yielding 25 tons of rubber per day. Due to the high-water requirements, the nature of the waste-streams, and the limited quality of the rubber, this method became obsolete.

The isolation of uncoagulated rubber particles (latex) from the guayule shrub was originally developed quite early (Jones, 1948), and was modified and refined by Cornish and co-workers (Cornish, 1996; Cornish *et al.*, 2006). In this method, the plant material is chopped up, homogenized thoroughly in a basic buffer using pebble or hammermills, and the mixture is filtered to remove plant fibers and other solid material. Subsequent pressing, washing and centrifugation steps separate the latex droplets contained in the plant cells from cell debris and resin. The filtrate is then centrifuged yielding a creamy top layer containing the rubber particles, which can be washed and stored.

It is critical that the method is applied on freshly harvested plant material to obtain low-protein uncoagulated latex that can be used to make gloves, condoms, and materials for medical applications. Thus, the shrub must remain in a hydrated condition throughout harvest, shipping, and storage until homogenized in the aqueous extraction medium. Coagulation within the plant already begins after 10% of the defoliated branch water is lost, and almost complete coagulation ensues after 50% water loss (Cornish *et al.*, 2005).

As isolated, the guayule latex is probably unsuitable for dry rubber applications. An early publication on latex production (Jones, 1948) indicates that contamination by resin cannot be excluded in latex production. Moreover, a fraction of the rubber is of low molecular weight, which reduces the strength of the rubber (Cole

et al., 1991). However, these contaminants can be removed by solvent extraction.

Solvent extraction methods were developed already in 1902. While this was too expensive in the early 20th century, the method was re-investigated again in the 1980s by the USDA and Bridgestone/Firestone (Cole *et al.*, 1991). Extraction directly addressed one of the problems of guayule: it contains as much resin as rubber, and the rubber contains a low MW fraction, removal of which yields a better quality rubber than the Saltillo process. After extraction of all components using a carefully chosen solvent or mixture of solvents (Kay and Gutierrez, 1987; Wagner and Parma, 1988) a polar solvent is added, which precipitates the high MW rubber, followed by the low MW rubber (Schloman, 1992; Wagner *et al.*, 1991). The method was tested at two experimental processing facilities operated by Texas A&M University and Bridgestone/Firestone (Cole *et al.*, 1991). Although the plant was successful in extracting and producing rubber meeting the specifications, engineering difficulties in handling the shrub plagued both facilities (Cole *et al.*, 1991; Wagner and Schloman, 1991). Supercritical CO₂ (scCO₂) extraction technology is also claimed for use in the separation of resins and rubber from guayule shrub and other rubber and/or resin containing plant materials (Cornish *et al.*, 2006).

Although guayule processing yields high quality rubber, the cost of cultivation, harvesting and processing is significant: presently used agronomy methods would lead to costs of €1.60 to 2.05 per kg rubber without considering the processing costs (van Beilen *et al.*, 2006). With crop improvements and better cultivation practices these costs could be substantially reduced. In view of the current price of €1.70 per kg of *Hevea* rubber, a competitive advantage can only be achieved if crop yields are improved, and economic processing methods are developed. Guayule latex is hypoallergenic, justifying the added cost. However, dry rubber from guayule would have to compete directly with *H. brasiliensis* rubber. Thus, it is essential to find uses for the co-products. Depending on the harvesting and processing procedures several useful by-products or waste streams are generated: resin, low molecular weight rubber, and bagasse (McIntyre *et al.*, 2001).

Guayule resin is a very complex mixture of sesquiterpene ethers, triterpenoids, and fatty acid triglycerides (Nakayama, 2005; Schloman *et al.*, 1983). Resin may be used as a rubber additive (Schloman, 1988) or as a wood preservative (Nakayama *et al.*, 2001). Unfortunately, the

resin composition varies with shrub line, cultivation site, harvest date, and processing history (Schloman and Wagner, 1991). Low molecular weight rubber could serve as a feedstock for depolymerised rubber (liquid natural rubber), which has wide applications in adhesive and moulded product manufacture (Nor and Ebdon, 1998; Schloman and Wagner, 1991). Bagasse remaining after rubber and resin extraction was already used to fuel the early processing plants in Mexico. Potentially it has many other applications: free flowing soil amendment (Schloman, 1991), or a starting material for Fischer-Tropic biomass-to-liquid conversions (Kuester, 1991). Attempts to convert guayule cellulose to ethanol were not very successful, as the bagasse appears to be very resistant to hydrolysis (Chang and Tsao, 1980).

Advantages of Guayule Rubber

While guayule rubber has the same molecular weight and general properties as *H. brasiliensis* rubber, it does not contain the proteins that can cause severe allergic reactions (Cornish, 1996). This development has revived interest in guayule rubber, and the US-based company Yulex is now building a small-scale production facility of low-protein guayule latex, providing a new source of hypoallergenic natural rubber gloves and medical items conforming to the new Category 4 ASTM rubber standard. Initial target applications for guayule rubber include gloves and other products for which the strength and resiliency of natural rubber is desired without the potential allergic reactions.

Disadvantages of Guayule Rubber

Dry guayule rubber isolated directly from the shrub may contain as much as 20–40% resin by weight. If the rubber is isolated by solvent extraction and deresinated, the bulk viscosities of the rubber are lower than those of *H. brasiliensis* rubber. Only selective coagulation to isolate the high molecular weight fraction of the guayule rubber produces material with properties similar to *Hevea* rubber (Schloman, 2005). *H. brasiliensis* rubber contains proteins and reactive functional groups that cause storage hardening with an increase in bulk viscosity when stored over long periods of time. This results from cross-linking reactions that produce branched polymer chains. In contrast, guayule rubber undergoes irreversible, heat-induced chain cleavage, thus, is less stable to oxygen and heat than *Hevea* latex. Unsaturated fatty

acid triglycerides present in the resin facilitate oxidation of the polymer chain. Combinations of an amine antioxidant and a zinc dialkyldithiocarbamate have been used to counter these effects, improving guayule rubber stability (Schloman, 2005).

RUSSIAN DANDELION

The ideal rubber-producing crop plant would be annual, fast-growing, and produce large amounts of biomass. Annual crops can be readily planted and plowed-out in response to market needs and farmer production considerations, and are also more readily included in on-farm crop rotations and farming systems. Russian dandelion (*Taraxacum koksaghyz* Rodin) might fit these requirements better than guayule (Figure 2, right panel).

T. koksaghyz was discovered in Kazakhstan in the course of a strategic program in 1931–32 to develop a domestic source of natural rubber in the USSR. It was selected as the best candidate rubber crop out of almost 1,100 indigenous plant species tested (Ulmann, 1951). The roots of wild Russian dandelion contain 4–5% high quality rubber, produced in laticifers accompanying the vascular bundles throughout the plant (Kekwick, 2001).

Russian dandelion was cultivated extensively in the USSR in the 1930s. In 1941, the 67,000 ha of Russian dandelion covered 30% of the total USSR rubber consumption (Ulmann, 1951). Due to natural rubber shortages during World War II, several other countries independently started growing Russian dandelion in emergency rubber programs: the USA (Whaley and Bowen, 1947), the UK (Anonymous, 1945), Germany (Heim, 2003), Sweden, Spain, and other countries (Polhamus, 1962). The best USA cultivation fields yielded about 110 kg rubber per hectare, while the best Russian yields were 200 kg rubber per hectare.

Unfortunately, the agronomic properties of Russian dandelion were reported to be rather unfavorable. Cultivation was very labor intensive and expensive: because the seedlings were rather small they were out-competed by weeds, making intensive tilling necessary. After World War II cheap *H. brasiliensis* rubber became available again and in all countries the Russian dandelion programs were terminated. Cultivation of Russian dandelion continued in the USSR until the early 1950s, after which it was abolished, probably for economic reasons.

Breeding of Russian Dandelion

Only limited breeding for rubber yield and vigor has been carried out, in part because of the short duration of the emergency rubber programs. Breeding of *T. koksaghyz* is complicated by the fact that the species has a self-incompatibility system, preventing self-fertilization. The germplasm used in the USA emergency rubber program was basically unimproved, wild material, with the best plants producing 5–6% rubber in root dry weight, but most plants only producing 2–3% rubber. According to Kupzow (1980) the best USSR accessions produced 15% rubber (Kupzow, 1980). Tysdal and Rands (1953) showed that in open pollinated polycrosses, both the rubber percentage and the root size could be increased, resulting in doubled prospective rubber yields (Tysdal and Rands, 1953). Based on these results Polhamus (1962) predicted that the rubber percentage in Russian dandelion could potentially be increased by breeding “to 15 to 25% of dry-weight” (Polhamus, 1962).

Converting Russian dandelion to a commercially attractive crop would require an increase of vigor and agronomic properties, for example by hybridization of the Russian dandelion with the common dandelion, *Taraxacum officinale*. According to Whaley and Bowen (1947) “greatly increased vigour is a basic requirement in the improvement of *T. koksaghyz*” (Whaley and Bowen, 1947). *T. officinale* is cultivated as a product for health food shops, and in France it is grown commercially as a salad herb. Field trials in New Zealand produced 6–9 tons of dried root per ha after six month of growth (Parmenter, 2002). If improved rubber content of 20% of *T. koksaghyz* could be combined by hybridization with the large roots of *T. officinale*, theoretically a rubber yield of 1200–1800 kg rubber per hectare could be produced.

Several features make Russian dandelion a very attractive model species to study rubber biosynthesis. Russian dandelion has a short life-cycle (6–8 months) relative to other plants, such as *H. brasiliensis*, where several years of growth are needed before clear phenotypes can be determined. Similarly, guayule can only be assessed after at least two years. Furthermore, Russian dandelion is both amenable to tissue culture and can be relatively easily transformed, with the analysis of meaningful rubber phenotypes possible within six months after transformation. Development of Russian dandelion as a model rubber-producing plant would require building up the molecular tools; BAC libraries, inbred lines,

FLOW DIAGRAM FOR KOK-SAGHYZ FACTORY TEN LONG TONS OF RUBBER PER DAY

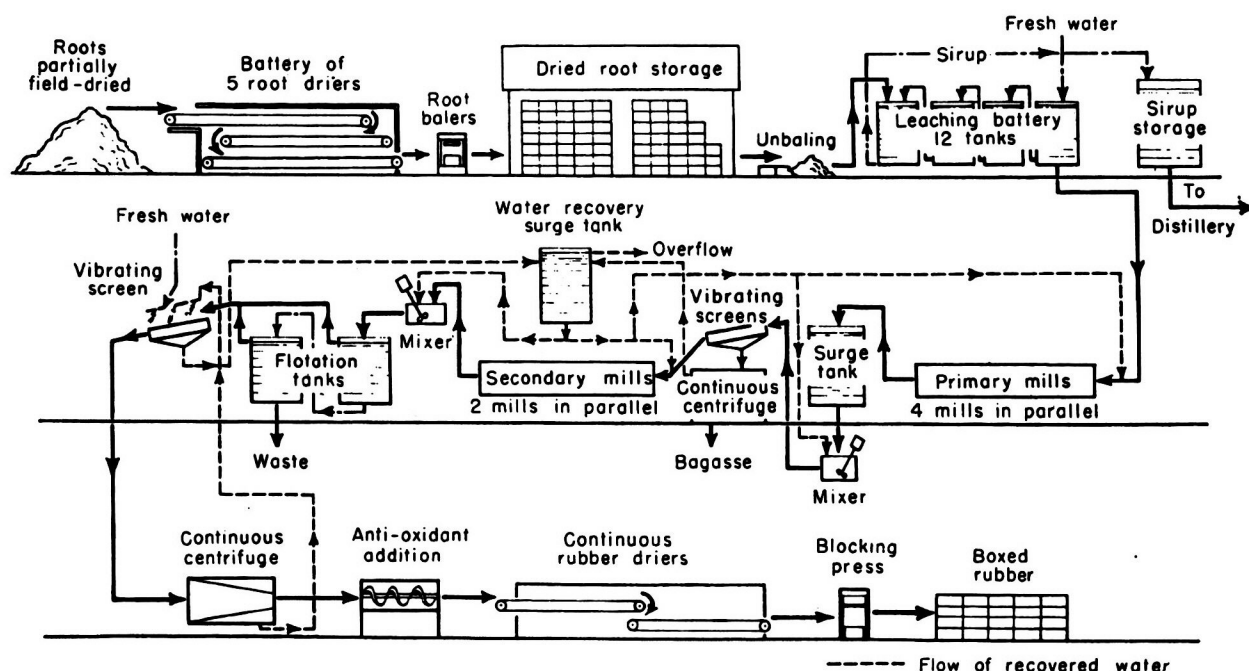


FIGURE 3 Diagram showing processing steps in pilot-mill rubber extraction from Russian dandelion roots (from Whaley and Bowen, 1947).

tilling populations, EST libraries, improved transformation protocols, RNAi silencing, promoter and enhancer sequences, microarrays, etc. These tools are presently available only to a limited extent. Regarding the biology of Russian dandelion, the functioning and development of laticifers (special cell networks containing latex and rubber particles) should be investigated. DuPont carried out some work on genes involved in rubber biosynthesis, showing that at least some of the *Taraxacum* genes involved in rubber biosynthesis are quite similar to the corresponding *Hevea* genes (Hallahan and Keiper-Hrynko, 2004).

Processing of Russian Dandelion

In the US Emergency Rubber project, processing technologies for Russian dandelion were developed (Polhamus, 1962; Whaley and Bowen, 1947) (Figure 3). After harvesting, the roots need to be washed to remove dirt and soil. If roots cannot be processed immediately, it is advantageous to dry the roots for storage. This is also relevant to transport and processing in year-round operations. However, fresh roots can be stored for several months under appropriate conditions. Processing is started by leaching the roots in hot water. This removes the soluble carbohydrates (mainly inulin) that can be

used for ethanol production. The leaching process softens the tissue and coagulates the latex in fine filament. Subsequent pebble milling steps loosen the plant tissue and rolls together the rubber filaments, which can be separated from the root solids by passing over a vibrating screen and flotation. This process recovered well over 90% of the total rubber in the roots.

The rubber can also be extracted as latex. In contrast to guayule, Russian dandelion latex is contained in laticifers. Although these cannot be tapped, the latex can be released by cutting up the tissue; it is not required to rupture all individual cells. The latex can be captured as a dilute solution in water and stabilised with suitable anticoagulants (Polhamus, 1962).

By-Products

As is the case with guayule, the economic viability of rubber production from Russian dandelion will require the exploitation of the crop in the context of a biorefinery, in which all components of the plant are used. Valuable side products from Russian dandelion processing may include inulin, which is the major storage sugar of dandelions (25–40% of dry root weight), and could be used directly in non-food applications or fermented to bioethanol (Polhamus, 1962; Whaley and Bowen,

1947). The remaining plant matter could be used for biogas production.

Advantages and Disadvantages of Russian Dandelion Rubber

Laboratory tests on the physical and chemical properties of *T. koksaghyz* rubber showed that it was of excellent quality. Tires made from Russian dandelion rubber were as resilient as those made from *H. brasiliensis*, and better than guayule-based tires (Heim, 2003; Whaley and Bowen, 1947). A recent US Patent reinforced these findings by reporting a molecular weight as high as 2.2×10^6 Da (Hallahan and Keiper-Hrynko, 2004).

One potential disadvantage of Russian dandelion is that rubber particles contain possibly more associated proteins than *H. brasiliensis* rubber particles, raising the possibility that people already sensitized to natural rubber from *H. brasiliensis* may also be sensitized to rubber from Russian dandelion (Cornish *et al.*, 2005). Therefore, Russian dandelion should be considered also for conventional, non-medical applications, such as tires and other dry rubber applications.

CONCLUSIONS

The development of crops for the renewable production of energy and materials has been given new impetus by the convergence of numerous factors, including uncertainty about the planet's reserves of oil, gas and coal, and global warming due to the rise in atmospheric CO₂ and other greenhouse gasses. Rubber is not only one of the most important polymers used in our society, it is also a renewable biopolymer the source of which is threatened by biological agents (SALB), economical developments and climate change. The development of alternative sources of natural rubber appears essential in the mid- to long-term to both secure our access to this essential polymer and decrease our dependence on fossil carbon for the production of synthetic equivalents. Crops synthesizing rubber could also be used for the sustainable production of other products, such as bioethanol from the lignocellulosic residues, or other valuable compounds, such as inulin from Russian dandelion in the framework of biorefineries. Although alternative sources for natural rubber were investigated in the past, recent advancements in numerous areas of plant sciences, including at the molecular level with the tools of genomics, metabolomics, and proteomics, as well as at the agronomic level, with

marker-assisted breeding, should ensure rapid progress in our understanding of rubber biosynthesis and the transfer of this knowledge to the development of new rubber-producing crops.

ACKNOWLEDGMENTS

This work was supported by the Sixth Framework Programme of the European Commission (EPOBIO, SSP/022681). The authors gratefully acknowledge valuable comments from Hans Mooibroek and Dirk Prüfer.

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