ALTERNATIVE SOURCES OF NATURAL RUBBER

epobio

Realising the Economic Potential of Sustainable Resources
- Bioproducts from Non-Food Crops

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Outputs from the EPOBIO project

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EPOBIO: Realising the Economic Potential of Sustainable Resources - Bioproducts from Non-food Crops

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EXECUTIVE SUMMARY

EPOBIO is an international project to realise the economic potential of plant-derived raw materials by designing new generations of bio-based products that will reach the market place 10-15 years from now. At a Workshop held in Wageningen in May 2006 a wide range of experts considered the Flagship theme of biopolymers and identified a detailed assessment of the potential for new sources of rubber production as the first target for EPOBIO to consider. This report sets out the conclusion of a detailed literature review and an analysis of environmental impacts and the economic case. It also takes account of inputs from international scientists and industrialists.

Natural rubber is a unique biopolymer of strategic importance that, in many of its most significant applications, cannot be replaced by synthetic alternatives. The raw material is supplied either as latex or in dry rubber form. This study has identified three reasons why new alternative feedstock supplies should be developed:

- Increasing evidence of allergic reaction to the proteins in natural rubber obtained from the rubber tree *Hevea brasiliensis* and an immediate need to develop natural rubber sources that do not cause such allergic responses.
- A disease risk to existing supplies of raw material, from *Hevea brasiliensis* that could potentially decimate current production.
- Predicted shortages of supply of natural rubber.

Hevea rubber in latex applications, which include around 40,000 household items, is responsible for moderate to severe allergic reactions. The incidence of those 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. In the US, the American Society for Testing and Materials has developed a new standard (ASTM 1076-06) for Category 4 natural rubber latex in response to the allergy issue. Previous standards measured physical performance rather than protein content and applied only to *Hevea*-based raw materials. The new standard offers an opportunity for manufacturers looking to develop protein-free high performance latex from other sources. It provides a new level of materials safety for medical product manufacturers and will mean that employers will be able to address these health issues by using alternative products.

The EPOBIO analysis shows that the shrub guayule has greatest potential as an alternative source of rubber that would also meet the protein content requirements of the new ASTM standard. There is a need to develop improved extraction and processing technologies and take forward crop improvement. Two new economic opportunities arise, in the cultivation of the crop and in industrial production of guayule-based rubber products.

The guayule shrub is well suited to the semi-arid areas of Southern Europe and, in the context of the reformed and market-focussed Common Agricultural Policy would offer an alternative production choice in areas such as those currently dominated by cotton production. The EU uses 8% of world production of natural rubber latex, and 14.5 % of world production of natural (dry) rubber. It can be assumed that future guayule lines will produce a yield of 10% natural rubber and since plant biomass is typically 10 tons per hectare per year, natural rubber would be produced from guayule at a rate of 1 tonne per hectare per year. Significantly, current EU demand could be met from 1,205,000 hectares of land. This is equivalent to 9% of arable land in Spain, or 1.5% of arable land in the EU.

Guayule has the additional benefit of being a low input crop with the potential to reduce environmental impact and contribute to sustainable development. Although current varieties could be grown immediately, the species is relatively unimproved and there is potential to improve rubber yield and quality, water use and other agronomic issues. There is therefore a new commercial opportunity for farmers in the short term, as well as the potential to create and sustain employment both in the farming sector and in rural areas in the longer term. This will help maintain and develop the rural infrastructure.

Currently, the existing processing technology and industrial expertise for the delivery of guayule latex and guayule dry rubber is based solely in the US. There is an urgent need for Europe to develop production and processing capability in order to address the potential new market and to avoid the loss of competitive position. We anticipate the development of a new extraction and processing industry using existing guayule varieties will take place in parallel to agronomic improvement of the crop and expansion of its cultivation.

As knowledge of the potential to develop biorefineries grows, there would be opportunity to incorporate the production of guayule latex into integrated, zero waste biorefinery systems. This could provide new income opportunities and further support for rural areas. The current opportunity to develop processing technology in the EU and to improve crops for the EU would also help the economic sustainability of both the agriculture sector and a wider industry.

At the strategic level there is a risk to the existing supply of raw material from *Hevea brasiliensis*. South American Leaf Blight has all but ended *Hevea* rubber production in South America and would have a similar devastating effect if it spread to Asia. Risk mitigation through the early development of guayule production, processing and crop improvement gives Europe an opportunity to establish a platform from which to build quickly in the event of a failure of supply.

The disease risk alone may not give sufficient justification for the development of new, alternative supply and processing capability in Europe. But it should be noted that demand for *Hevea* rubber is expected to exceed supply by 25% by 2020. One reason for this is the replacement of rubber trees with palm trees in order to meet the increasing demand for oil for the manufacture of biofuels, driven by increasing regulatory targets in Europe and the US. Meeting the demand for rubber has strategic importance given its essential use in products such as aeroplane tyres, personal protection products in medical applications, dental equipment and emergency equipment such as intravenous tubing.

This report also identifies a need to examine further sources of supply beyond guayule, for example, from Russian dandelion. This is important in risk mitigation since multiple supply chains are preferable to one alternative source of supply. This brings with it an opportunity to develop crops that could be cultivated beyond the semi-arid regions of Europe and the US. The report recommends that the molecular-based research needed to develop potential alternative sources of supply in the longer term should be put in place.

The expansion of guayule cultivation and processing could readily take place in conjunction with developing countries. The crop is well suited to the climates of many developing countries and cultivation and processing in those geographic regions would also be driven by the shortage of supply issues and growing markets. Collaborative work involving the EU and developing countries should be investigated as a priority.

1 INTRODUCTION

It is likely that during this century polymers based on renewable materials will gradually replace industrial polymers based on petrochemicals. Most biopolymers cannot yet compete with their petrochemical equivalents, and significant efforts on raw materials, processing technology, and applications are still required to change this situation. The one outstanding exception is natural rubber produced from the rubber tree *Hevea brasiliensis.* This biopolymer is unique in that for many applications it has no synthetic equivalent. Aeroplanes cannot safely land with tyres made from synthetic rubber; only truck tyres made from natural rubber are resilient enough to withstand heavy shear and loads; car tyres containing natural rubber are safer than synthetic rubber tyres because of the 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). Many decades of industrial research have not produced synthetic rubbers with similar qualities. While the amount of synthetic rubber produced has reached a plateau, natural rubber production is steadily increasing. In a few years from now, natural rubber production could in fact exceed the synthetic rubber market share, which is presently at 60% (Figure 1). Another issue is that 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.

The rapid economic developments in China and India are now fuelling growth of natural rubber demand, and prices have increased dramatically. At the same time a pathogenic fungus named South American Leaf Blight could wipe out the entire production in Southeast Asia (80% of world production), resulting in a severe supply – demand imbalance. Evidently, tyre 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 constitutes a strategic resource. Without natural rubber, many sections of the economy would experience great difficulties.

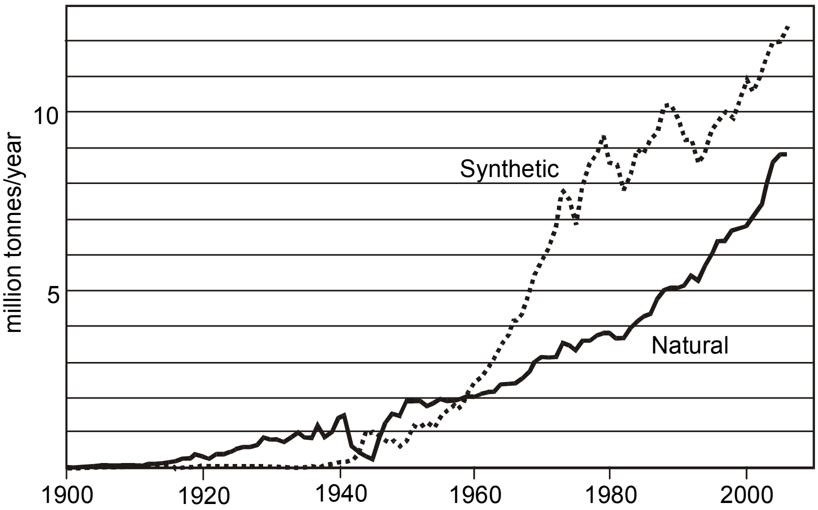

Figure 1 Global production of synthetic and natural rubber during the period 1900-2005. Based on Cornish (2001), International Rubber Study Group and Rubber Statistical Bulletin (2006).

Ten per cent of natural rubber is used as latex to produce gloves, condoms, catheters, and other medical products. Especially in the US but also in Europe and Japan, increasing numbers of people are allergic to proteins in *Hevea* rubber. Thus, alternative crops for natural rubber production not only help to secure supply, but also provide a source of hypoallergenic rubber.

For all of the above reasons it will be necessary to develop large-scale alternative (domestic) natural rubber production. Commercialisation and natural rubber production from domestic crops is in the best short- and long-term interests of developed countries. This has been recognized at various times in the past, leading to research and development programmes 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 alternative source of natural rubber. Several other potential sources of natural rubber were investigated as well, some of them promising enough to justify large research programmes, especially during WWII, while others have not yet been studied in sufficient detail to establish their utility (Table 1). This paper summarizes the production methods and applications of natural rubber (dry rubber and latex), and threats to the production of natural rubber from *Hevea* (Chapter 2); issues in the production of natural rubber from guayule (Chapter 3); and potential other plant sources of natural rubber (Chapter 4). Finally, natural rubber production from the rubber tree and guayule is compared (Chapter 5).

Table 1 Alternative sources of poly-*cis*-isoprenes

Rubber source	**Content (%)**	**Mw (kDa)**	**Production T/Y (year)**	**Yield (kg ha^{-1} y^{-1})**	**R&D related to rubber**	**Refs**
Rubber tree *H. brasiliensis*	n.a.	1,310	9,000,000 (2005)	500 - 3000	Resistance to SALB Rubber polymerase	1
Guayule shrub *P. argentatum* Gray	3 - 12	1,280	10,000 (1910)	300 - 1000	Domestication, Processing, Rubber polymerase	2,6
Russian dandelion *T. kok-saghyz*	0 - 15	2,180 (8)	3,000 (1943)	150 - 500	WWII emergency projects USSR / US Domestication	2,3,8
Rubber Rabbitbrush *C. nauseosus*	< 7	585	-	-	WWII emergency project US	4,6
Goldenrod *S. virgaurea minuta*	5 - 12	160-240	-	110 - 155	Demonstration project in 1931	5,6
Sunflower *Helianthus sp.*	-	279 [a] (6) 69 (8)	Research stage	-	Characterisation, genetic engineering	6,7,8
Lettuce *Lactuca serriola*	1.6 – 2.2 of latex	1,380	Research stage	-	Characterisation, genetic engineering	9

n.a., not applicable; [a] MW of rubber in native plant species; (1) (Blanc *et al.*, 2006); (2) (Mooibroek and Cornish, 2000); (3) (Whaley and Bowen, 1947; Mooibroek and Cornish, 2000); (4) (Weber *et al.*, 1993); (5) (Polhamus, 1962; Swanson *et al.*, 1979); (6) (Polhamus, 1962; Swanson *et al.*, 1979); (7) (Nor and Ebdon, 1998); (8) (Hallahan and Keiper-Hrynko, 2004); (9) (Bushman *et al.*, 2006).

2 NATURAL RUBBER PRODUCTION

2.1 Natural rubber

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 (*Hevea brasiliensis*). Malaysia, Indonesia, and Thailand together produce nearly 80% of the world supply (Table 1). The yield of rubber 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 components that are key to the superior properties of this material compared to all synthetic rubbers.

The rubber molecules are produced from isoprenoid precursors that are thought to be synthesised as part of the general mevalonic acid (MVA) pathway. A rubber transferase (EC 2.5.1.20) located in the cytoplasm of plant laticifer cells progressively adds isopentenyl-diphosphate 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 length of the rubber molecules is one of the main determinants for the functional properties of the resulting natural rubber. For an overview of the biochemistry and mechanism of rubber biosynthesis, please consult (Cornish, 2001) and (Puskas *et al*., 2006).

2.2 *Hevea* rubber market and applications

Natural rubber is a highly valuable biomaterial: in contrast with most other biopolymers it is essential for many applications and cannot be replaced by synthetic materials. For example, heavy-duty tyres for trucks, buses, and airplanes, as well as many latex products for the medical profession, cannot be made with synthetic rubber, or only at great 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: condoms and gloves provide excellent protection from infection. This is largely due to the excellent film-forming nature of natural rubber latex. In this aspect, 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 and may even be enhanced). 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.

The latex harvested from the tree 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 prevulcanised by mixing with sulphur 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 of the natural rubber is dried by 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.

Most of the dry natural rubber is used in tyres, especially those, which call for high performance, notably aircraft and truck tyres (more than 70 % in the USA). The ability of natural rubber to dissipate heat makes these tyres much safer than those made from synthetic rubber.

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. 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 €1.70 per kg in September 2006. An increasing oil price is likely to act in two directions: while it makes natural rubber a better competitor for synthetic rubber, it also reduces demand for natural rubber by the producers of heavy-duty tyres for aeroplanes and the car industry.

2.3 Rubberwood

An important side product of *Hevea* rubber production is rubberwood (Killmann, 2001), which was originally perceived merely as a useful by-product for drying and smoking rubber and to provide a source of charcoal for local cooking. Rubberwood can be easily steam-bent, or stained to resemble any other timber, depending on consumer demand. Its favourable qualities and light colour make it a good timber for furniture making and other applications. The natural colour of rubberwood is one of the principal reasons for its popularity in Japan, where it is increasingly used to replace more traditional timbers. In 1998, Malaysia exported rubberwood furniture with a value of 683.3 million U$, and in general rubberwood is one of the most successful export timbers of Southeast Asia. *Hevea* is sometimes being grown primarily for timber harvesting, with the latex as a co-product.

2.4 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 following example.

In 1934, South American Leaf Blight (SALB) wiped out the production of rubber in Brazil, and it has not been possible to restart large-scale production due to the endemic leaf blight pathogen *Microcyclus ulei.* The present production on marginal lands in Brazil, where SALB is less of a problem, is only 96,000 T/Y, or about 1% of world production. Accidental spread of SALB in South-East 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 (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 example, a recent patent (Hallahan and Keiper-Hrynko, 2003) describes *H. brasiliensis* genes involved in isopentenyl diphosphate biosynthesis. However, the narrow genetic base, prolonged breeding cycles and juvenile period, and highly heterozygous nature of *H. brasiliensis* make breeding complex, time-consuming and labour-intensive. In view of the critical importance of rubber, these efforts appear rather limited.

2.5 Economic threats to natural rubber production from *Hevea*

Other threats to natural rubber production from *Hevea* include competition for land by palm oil plantations: the palm oil acreage in Malaysia increased in only three decades from less than 100,000 hectares to almost 2,000,000 hectares, which is more than the rubber tree acreage. Together the two crops account for more than 70% of the total agricultural land use of that country. Similar developments are taking place in Thailand and Indonesia.

Strongly increasing labour costs are especially an issue in Malaysia, as latex harvesting is very labour-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 induce major changes in available acreage, yield, and demand for natural rubber.

3 GUAYULE AS AN ALTERNATIVE SOURCE OF NATURAL RUBBER

3.1 Introduction

Just as *Hevea* 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*) also served as a source of rubber for the local population in present Mexico and the Southern US. 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 T/Y of rubber was produced from natural stands of the guayule shrub (Ray *et al*., 2005). As rubber production from *Hevea* became more efficient, and natural stands of guayule were exhausted, this production strategy was gradually abandoned.

Among the new or alternative crops considered, 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 as an alternative source 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 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 programme in Continental, Arizona (principal cultivar resulting from this programme was ‘593’)
1929	Production in the US ceased due to the Great Depression
1942	Emergency Rubber Project involving over 1,000 scientists and technicians, and 9,000 labourers. New germplasm collected in Mexico and Texas
1946	All guayule stands were liquidated (due to cheap Hevea 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 programme to stockpile seeds and seedlings (stopped in 1952)
1959	Termination of the USDA guayule breeding programme in Salinas, 24 germplasms plus line ‘593’ were stored at the USDA National Seed Storage Laboratory
1973	First oil crisis
1976	Saltillo plant opened in Mexico (closed early 1980s), source of all test rubber used in making tyres 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 *Hevea* rubber recognized as a major problem
2002	Oil price at 20 $ per barrel
2006	Production facility for guayule latex (Yulex) Oil price reaches 78 $ per barrel before retreating to 60 $

3.2 Advantages of guayule rubber

While guayule rubber has the same molecular weight and general properties as *Hevea* rubber, it does not contain the proteins present in *Hevea* rubber that can cause severe allergic reaction (Cornish, 1996). Natural rubber latex allergy has become an important issue in the United States: approximately 20 million Americans are allergic to the proteins found in *Hevea* rubber (Cornish *et al*., 2001). In Europe, the situation is less dramatic, but latex allergy is also on the rise. This development has revived interest in guayule rubber. Until recently, there was no commercial production of guayule. However, the US-based company Yulex is now building a small-scale production facility of low-protein latex from the alternative natural rubber plant source guayule. If successful, this would be the only source of hypoallergenic natural rubber gloves and medical items. To accommodate this new source of rubber, a new low-protein Category 4 rubber standard has been developed by the ASTM. 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 reaction.

Another aspect of *Hevea* protein allergy is the fact that the latex allergens have been reported to occur in the particulates derived from rub-off and wear of tyres. The presence of allergens in particulates possibly worsens asthma symptoms in sensitised patients (Namork *et al*., 2006). Other research, however, suggests that *Hevea* 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).

3.3 Guayule breeding

The intermittent breeding efforts during the 20th century have made guayule a partially domesticated crop. The general status of breeding programmes was reviewed in 2005 (Ray *et al*., 2005), and in 1991 (Estilai and Ray, 1991). Currently, research on breeding is only carried out in the USA (Latigo, 1996; Foster *et al.*, 1999; Foster *et al*., 2002; Keys *et al*., 2002; Coffelt *et al*., 2005; Jorge and Ray, 2005).

Limited genetic variability is not a problem in guayule breeding, and extreme variability both within and between lines has been found for every trait that has been evaluated (rubber quality and quantity, dry weight, resin content, latex content and yield). The facultative nature of apomixis in polyploid guayule continually releases new variability, which may be exploited by plant breeders (Ray *et al*., 2005). However, from a breeder's point of view, guayule (but also *Hevea*) is a difficult species to work with because it is a perennial, requiring relatively large amounts of land for breeding programmes. Guayule is physiologically immature for 3-7 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 programme has been the development of higher yielding cultivars. This target has clearly met with success, as new lines available from the 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 rubber content of the selected parents. High yield is not the only target. For full domestication, it is necessary to change the seed characteristics (guayule seeds are small, dormancy must be broken by special treatments, and germination is not reliable), growth rate (slow growth in the early stage causes strong competition by weeds), and other properties listed in Table 3. Future breeding activities might be directed toward more novel breeding 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.

Access to guayule germplasm by European groups is currently limited to stands in Greece, Morocco and Madagascar. To gain access to wider germplasm diversity, collaboration with Mexico is essential, also in view of the Convention on Biological Diversity (http://www.biodiv.org/default.shtml). Currently, research in Mexico is primarily focused on the properties and potential of wild stands of guayule (Jasso de Rodriguez *et al*., 2006).

Table 3 Breeding targets and results.

Target	**Results**	**Comment**
Rubber yield	300-600 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 *et al*., 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 *Verticillium*	Probably great reservoir for disease resistance genes in guayule. Damage from insects is not considered serious
Cold tolerance	Native stands withstand -18°C	Interspecific hybridisation may be used, however, hybrids typically have bad rubber quality
Drought tolerance	Inadequate funding	Rubber yield increases with increased irrigation
Salt tolerance	Inadequate funding	guayule is only slightly salt tolerant; the genetic basis not known
Seed quality	Inadequate funding	Seed size, quality and germination are probably amenable to breeding
Co-products	Inadequate funding	Higher ratio of rubber to resin is desirable

Summarized from (Estilai and Ray, 1991; Ray *et al*., 2005)

3.4 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 cultivation, 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.

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 to the herbicide ammonium-glufosinate was introduced into guayule.

In a recent study, various allylic diphosphate synthetase genes were introduced in tissue-culture generated transgenic guayule plants of the USDA lines AZ 101, AZ-2 and N6-5 (Veatch *et al*., 2005). The resulting plants were monitored monthly for plant height and width, and rubber and resin content. Some differences were observed, but these were not consistent: transformed AZ 101 initially grew significantly faster, but final biomass yields were similar, as were the final resin and rubber contents.

3.5 Guayule agronomy

3.5.1 Introduction

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. Preferred soils are mainly well-drained calcareous soils with relatively low concentrations of nutrients. Weather conditions may vary. Guayule has been grown so far in desert-like environments, humid warm conditions, hot dry semi-arid conditions, and regions with moderate temperature and rainfall. Such environments and conditions can be found in many regions of the world; in Europe these regions include large areas in the Mediterranean countries.

Germplasm from the US breeding programme has been tested in Argentina (Coates *et al*., 2001), Australia (Ferraris, 1993; Dissanayake *et al*., 2004; 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. To our knowledge systematic agronomic studies have not been conducted in Europe. 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 were recently reviewed by Foster and Coffelt (Foster and Coffelt, 2005).

One of the few problems encountered in guayule cultivation is root disease, especially in standing water (Mihail *et al*., 1991). Thus, sandy-loam soils are most suitable. Guayule occurs naturally in 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.

Rubber yield and yields in dry mass vary strongly between various lines. Moreover, yields from the same line often strongly vary in various regions, soils, or under different conditions (see section on yield below).

3.5.2 Guayule plant establishment

Cultivation of guayule is started with preparation of the seedbed. In most cases, the field is established with seedlings grown in the greenhouse from seeds. In some instances seeds have been directly sown. Transplanting seedlings is the most reliable method of crop establishment. Plants have a root system, which gives them a growth advantage during establishment. However, costs are estimated around US$ 900-1600 per ha (Foster and Coffelt, 2005). While direct seeding might be possible for less then US$ 400 per ha, this method appears to much less reliable due to the small size of the seed, poor germination and competition with weeds.

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/g). Natural or primary dormancy occurs in guayule seeds and is caused by an inner seed coat dormancy and embryo dormancy. Seed dormancy can be broken by seed treatment, with the added advantage of uniform and fast germination. The currently applied method uses a medium consisting of 25% polyethylene glycol (MW 8000), 0.1 mM gibberelic acid, 0.05% potassium nitrate and 0.1% thiram (tetramethylthiuram disulfide) fungicide adjusted to pH 8.0 with calcium hydroxide. The seeds are treated under aerobic conditions in the light for 3-4 days (Foster and Coffelt, 2005).

A good seedbed is essential for guayule cultivation. Guayule is a poor competitor against annual and perennial broadleaf and grass weeds. Pre-emergence treatment is required for weed control. Weed and pest control on a commercial scale are problematic since no herbicides are labelled for post-emergence treatment of guayule. In experimental trials several herbicides have been shown to prevent weed emergence for periods up to 8 months.

DCPA (4,5 – 11 kg ha^{-1} bensulide (2.2-4.5 kg ha^{-1}), prodiamine (0.3-0.6 kg ha^{-1}) and pendimethalin (0.6-2.2 kg ha^{-1}) have been applied to direct seeded guayule without negative effects. Pendimethalin is safe for use in guayule direct seeding and transplants. The herbicide has a broad spectrum of weed control and is widely used (Table 4). An EU evaluation for plant protection products containing pendimethalin states that these products will fulfil the safety requirements when applied as herbicide on fruit, grapes, vegetables, oil seed, cereals, tobacco and ornamentals. Residues arising from the proposed uses, consequent on application consistent with good plant protection practice, have no harmful effects on human or animal health. Under the proposed and supported conditions of use there are no unacceptable effects on the environment (European Commission, 2003).

Table 4 Herbicidal treatments suggested for guayule cultivation.

Herbicide name	Amount	% weed control	Duration	% yield increase
DCPA	9.0 kg ha^{-1}	60%		127^{b} %
pendimethalin	0.5-1.8 kg ha^{-1}	91%		132^{b} %
pendimethalin	5.6 l ha^{-1}		4 months	
pendimethalin	11.2 l ha^{-1}		6-8 months	
trifluralin	0.6-1.1 kg ha^{-1} ai	69%		89^{b} %
trifluralin	1.1 – 4.4 kg ha^{-1} ai			133^{r} %
oxyfluorfen + oryzalin	2 + 2 kg ha^{-1} ai		6-8 months	
isoxaben	0.6 kg ha^{-1} ai			175^{r} %
pendimetalin	1.1 – 4.4 kg ha^{-1} ai			148^{r} %

Based on (Foster and Coffelt, 2005). ^{b} increase in biomass; ^{r} increase in rubber yield; ai active ingredient.

Presently, stand establishment is accomplished by transplanting. Seeding transplants are produced in nursery trays in greenhouses and fields are established using typical commercial transplanting systems. Transplanting has been extremely successful, but is estimated to be more expensive than establishment by direct seeding (Thompson and Ray, 1989). Transplanted seedlings grow slowly in the field and require frequent irrigation. When transplanted in spring seedlings require 100 – 250 mm of water with a low salinity (below 1.0 dS m^{-1}). Under good conditions the survival rate of the seedlings is 95%.

In direct seeding, seeds can be drilled with a pneumatic planter in rows 1 metre apart, 10 mm deep at 100 seeds per metre. Seed germination starts after 3-5 days and seedlings emerge after 10 days. Seedlings grow slowly and require frequent irrigation during the first 3-4 weeks after drilling. Heavy rain showers can damage the fields. After establishment soils should be allowed to dry to reduce damping-off and other seedling diseases. Due to variation in seed quality and cultivation practices germination rates vary between 10 and 92%. Under good conditions with conditioned seeds high germination rates up to 92% can be obtained (Foster *et al*., 1999).

Current recommendations for direct seeding of guayule are:

1. the use of conditioned seed of high quality
2. accurate planting using fluid drilling or precision drilling techniques
3. careful irrigation (enough for fast establishment, not too much in order to prevent damping-off).

Under these conditions a planting rate of 40 seeds m^{-1} can be used. If establishment via direct seeding becomes the norm, experimental plot work has shown that damping-off of seedlings and weed control will become major areas of concern. Figure 2 shows an established field of guayule.

Figure 2 Guayule grown by Yulex, Ehrenberg, US.

3.5.3 Irrigation, fertilisation and pest control

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 et al., 1991).

Salt tolerance of the established crop is higher than that of alfalfa and is almost as tolerant as upland cotton (salinity levels up to 4.5 dS m^{-1} are acceptable). Higher concentrations of soluble salts in irrigation water will limit water availability to the plant and reduce plant growth.

Irrigation will affect both scrub yields and also rubber accumulation. So far, no direct correlations between water supply, dry matter yield and rubber yield have been established. Although sufficient water is required to allow crop growth, guayule is quite resistant to drought conditions. The plants will interrupt rubber accumulation, but they survive extended periods of limited water availability.

Guayule is a low user of nutrients. Fertilisation requirements are highly dependent on soil fertility. In general, application of nitrogen (18 - 210 kg ha^{-1}) in combination with irrigation increased plant biomass yield. Pests are not a major issue for guayule.

A method to improve production of high molecular weight rubber by adding a growth regulator has been described (Bauman, 1979). This method is also used to improve latex production by *Hevea*, and needs further study.

3.5.4 Harvesting, baling and transport

Several procedures for harvesting are being considered. 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. Typically, the plants are clipped after two years, and harvested by digging on the fourth years. Results so far do not indicate that clipping will increase the total yield over the cultivation period, and it is not clear which procedure is best, particularly when the cost of planting and establishing the crop is taken into account.

Clipping plants just before or after the dormant period (early spring, late autumn) does not affect plant re-growth, while clipping in summer reduces the re-growth percentage. Some studies showed that under irrigated conditions total biomass yield was lower when compared to unclipped plants. At the same time the rubber concentration decreased, while in unclipped plants rubber concentration increased in time (Mills *et al*., 1990).

The shrub might be harvested with a mowing system like swath mowers and special mowers, cutters and saws. Important aspects to consider are the height of the crop and the minimum possible cutting height. Cutting speeds are about 1 ha h^{-1} or higher. Coates (1986, cited in (Coates, 1991) has tested circular saw blades and plough coulters. Coates and Wodrich (Coates and Wodrich, 1989) describe a modified potato harvester to dig up the guayule roots. The optimal field harvest capacity is 0.85 ha h^{-1}.

Guayule can be baled in the field and transported to the factory (Coates, 1993). Large balers with capacities of 0.42 ha h^{-1} or 10.3 tons h^{-1} can process 0.17 ha h^{-1} or 26.3 tons h^{-1} respectively (Coates, 1993), and bales can be pressed up to a density of 280 kg per m^3 (Coates, 1991). For optimal work the large balers require an inverter step. However, if row width can be adjusted to the baler, this step might be omitted. The density of the bales should be sufficient, as typical trucks are loaded to capacity (by weight) if the density of the transported material reaches 262 kg per m^3 (Coates, 1991). Based on a number of assumptions on the size of a rubber factory, and average transportation distances, the price of transport will be less than € 50 per ton of guayule rubber.

Reducing the volume of the shrub by chopping has been suggested to reduce transport costs to the processing factory. This is possible for short transport distances and immediate processing. However, with longer transport distances and increased storage time degradation problems occur (Coates, 1991). This is due to the fact that guayule does not contain the natural antioxidants that protect *Hevea* rubber from being degraded. Exposure to oxygen reduces the molecular weight of the rubber, and every cut and tear results in exposed surface (Malani and Clark, 1986). One proposed solution is to store the shredded, chopped or comminuted material under solvent, thus largely excluding oxygen (Gutierrez *et al.*, 1985). This does not appear to be a practical solution. Alternatively, an antioxidant (typically monohydric hindered phenols such as 2,5-di-t-butyl-p-cresol or 6-t-butyl-2,4-xylenol) is added to chopped plant material or otherwise processed material (Schloman *et al.*, 1989). Because relatively large amounts of antioxidant have to be added (in the order of 1%), the antioxidant should be recovered during processing, for example by distillation of the low-boiling compound. 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).

3.5.5 Yields of guayule cultivation

It has been reported that the season and the age at which the guayule plants are harvested affect 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. For example, part of the guayule shrub used in the Firestone/Bridgestone Sacaton plant ("Salinas") consisted of over 10 year old stands of various lines, which were found to contain at least 12% extractable high quality rubber (Cole *et al*., 1991).

To date, the average biomass yield is around 6 tons ha^{-1} y^{-1} and a rubber yield of 320 kg ha^{-1} y^{-1} is obtained. However, in the various studies biomass yields reach up to 15 tons, and rubber yields approach 1000 kg ha^{-1} y^{-1} (Table 5). Thompson and co-workers estimated a potential yield of 1100 kg ha^{-1} y^{-1} of rubber (Thompson *et al*., 1988a; Thompson *et al*., 1988b), while Estilai (Estilai, 1991) suggested that a yield of 600 to 900 kg ha^{-1} y^{-1} should be feasible. The progress made with *Hevea* shows the potential of breeding: in four decades the rubber yield increased by a factor of ten, from 300 kg ha^{-1} y^{-1} to 3000 kg ha^{-1} y^{-1}.

Table 5a Yield of guayule plant dry matter, rubber yield and resin yield for various guayule varieties in Gatton and Chinchilla, Australia (George *et al*., 2005).

	Gatton year 1			**Gatton year 2**			**Chinchilla year 3**		
	plant dry matter	**rubber yield**	**resin yield**	**plant dry matter**	**rubber yield**	**resin yield**	**plant dry matter**	**rubber yield**	**resin yield**
AZ1	13150	620	727	20300	789	1158	18000	717	1318
AZ2	11380	550	668	19400	771	1115	19000	787	1476
AZ3	10570	467	584	19000	622	906	15100	608	1286
AZ4	n.t.	n.t.	n.t	n.t.	n.t.	n.t	12600	626	970
AZ5	9670	560	537	18700	966	1135	11600	574	922
AZ6	8610	506	455	20100	855	1049	15100	668	1101
11591	8260	391	325	15000	618	497	7200	385	365
N565	6950	371	436	13900	675	727	8300	380	338

Table 5b Yield of guayule plant dry matter, rubber yield and resin yield for various guayule varieties Fort Stockton, Texas (Foster *et al*., 1999; Foster *et al*., 2002) and Cameron Ranch, Texas (Latigo, 1996).

	Fort Stockton year 2 (1995)				Fort Stockton year 3 (1996)				Cameron Ranch year 2			
	plant dry matter	**rubber yield**	**resin yield**	**rubber %**	**plant dry matter**	**rubber yield**	**resin yield**	**rubber %**	**plant dry matter**	**rubber yield**	**resin yield**	**rubber %**
11605	n.t.	n.t.	n.t.		n.t.	n.t.	n.t.		4566	295	315	6.5
AZ-R1	3231	185	395	5.7	6580	302	825	4.6	n.t.	n.t.	n.t.	n.t.
AZ-R2	2280	250	241	11.1	5603	515	668	9.2	n.t.	n.t.	n.t.	n.t.
CAL6	1917	220	208	11.2	4294	392	453	9.3	4938	302	320	6.1
CAL7	2132	203	212	9.6	4833	400	481	8.2	5295	296	356	5.5
N576	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	4671	286	267	6.1
N6-5	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	8859	549	572	6.1
N9-5	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	5178	371	324	7.2
O16-1	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	4488	312	268	6.9
P3-1	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	4927	318	293	6.9
UC101	2220	242	223	11.2	4470	386	486	8.9	8663	576	552	6.7
UC102	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	6880	492	483	7.3
UC104	2289	245	254	10.7	4972	513	518	10.4	n.t.	n.t.	n.t.	n.t.

3.6 Processing of guayule rubber

3.6.1 Introduction

Because guayule stores its rubber within the cells of its bark and woody tissue, the latex cannot be tapped like the rubber tree. 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 (Jones, 1948b; Cornish *et al*., 2006b). 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, 1948a; Tint and Murray, 1949), and involved the use of large amounts of a dilute caustic solution, which could not be easily disposed of or treated. This was required to bring about the coagulation of the rubber particles. Thus, this method has become obsolete. The other methods are described in more detail in the sections below.

All rubber harvesting methods are generally formulated as being applicable to different plant materials, subjected to different mechanical treatments. Thus, any effort to develop guayule, Russian dandelion, or other alternatives would have to take into account the existing and expired patents.

3.6.2 Processing of guayule for latex production

The isolation of uncoagulated rubber particles (latex) from the guayule shrub was originally developed quite early (Jones, 1948b), and taken up again by Cornish and co-workers. In this method, the plant material is chopped up, homogenized thoroughly in a buffer using pebble mills, or other equipment, and the mixture is filtered to remove plant fibres and other solid material. Subsequent pressing, washing and centrifugation steps (Cornish, 1996; Cornish *et al.*, 2006b) separates 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. Subsequently, the latex particles can be washed and stored. It is critical that the method is applied on freshly harvested plant material to obtain low-protein un-coagulated 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 and Schloman, 2005).

It is unclear whether the guayule latex is suitable for dry rubber applications. Presently only small-scale latex production facilities are considered (the most recent publication describes a facility capable of processing 7’000 kg of plant material per day, which contains about 350 kg of rubber (Cornish *et al*., 2006b)). An early publication on latex production (Jones, 1948b) 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). The solvent processing described below allows for the separation of resin and high and low molecular weight rubber, which is seen as an essential feature of the method.

An issue of the latex harvesting process is that rather large (but unspecified) amounts of chemical solutions are used: to stabilize the latex (prevent coagulation) the pH must be slightly basic, and the solution must contain buffering compounds and antioxidants. The purification and recycling of this waste stream is probably a significant cost factor.

3.6.3 Solvent processing

Starting already in 1902, solvent extraction methods were developed. While this appeared to be too expensive in the early 20th century, the method was investigated again in the 1980s by the USDA and Bridgestone/Firestone (Cole *et al*., 1991).

Several extraction methods have been tested on a pilot scale. The first method that was developed directly addressed one of the problems of guayule: it contains as much resin as rubber. By first using a polar extractant, the ground shrub was deresinated. Rubber was then extracted by using a solvent such as hexane in an immersion, percolation or counter current mode (Hamerstrand and Montgomery, 1984; Kay and Gutierrez, 1985). Due to the two steps, the process was prone to solvent loss.

Simultaneous extraction of resin and rubber can be accomplished using a carefully chosen solvent or mixture of solvents (Kay and Gutierrez, 1987; Wagner and Parma, 1988). After extraction, a polar solvent is added which precipitates the rubber. This procedure also allows the separation of low molecular weight rubber from high molecular weight rubber by carefully titrating the mixture (Wagner and Schloman, 1991; Schloman, 1992). It yields a better quality rubber, but also gives rise to an additional co-product (low MW rubber, see below). The method was tested at the experimental processing facilities built by Texas A&M University at College Station, Texas, and by the Bridgestone/Firestone Corporation at Sacaton, Arizona (Cole *et al.*, 1991). The latter facility was designed for simultaneous extraction of rubber and resin using an azeotrope of pentane and acetone (at a later stage pentane was replaced by hexane to facilitate solvent recovery). After separation of the solvent containing rubber and resin (miscella) from the bagasse, the high molecular weight rubber was precipitated by the addition of acetone. The resulting swollen rubber cement was separated from the miscella, and desolventised. The (low molecular weight) rubber and resin remaining in the miscella were also desolventised in pot-type evaporators.

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). Fines, which were difficult to separate from the solvents and accumulated in the final rubber product, were referred to as the most significant unresolved problem in the process used at Sacaton. However, the size of the fines was much smaller than the particles that cause problems in *Hevea* rubber (over 15 µm), and the fines do not contain much copper and manganese. Thus, more ash could be tolerated: as high as 2% ash did not result in a reduction of rubber properties. Terpenes also accumulated due to recycling of the solvents. Recovering the solvents is a major expense, as these have to be evaporated in several steps, and the evaporation of water in the same steps cannot be avoided. The energy input required for solvent and water evaporation is in the order of a quarter to a third of the energy content of the bagasse.

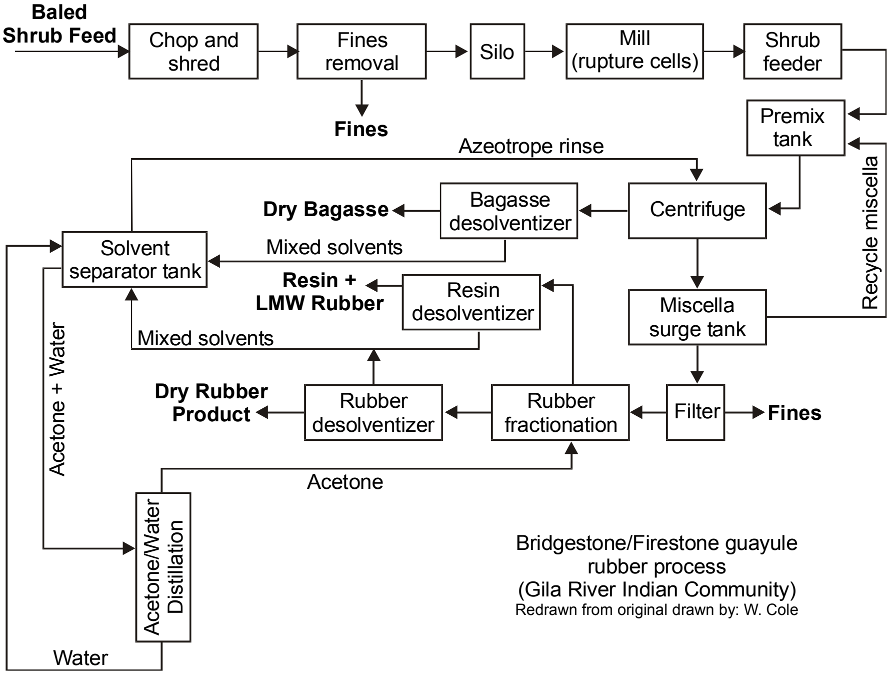

Figure 3 Flow scheme of solvent processing facility (Firestone/Bridgestone).

The solvent losses will in part determine the economic feasibility of the process. However, at this stage these cannot be estimated with any accuracy. After desolventisation the bagasse contains around 0.2% solvent (or 40 g per kg rubber). Losses by evaporation and leaks can be expected in all stages. In similar plant seed oil extraction processes, losses of 0.7 to 7 litres per ton of seeds are typical (Environmental Protection Agency, 2001). Taking this number to rubber extraction would add a loss of 14 - 140 g solvent per kg rubber produced.

3.6.4 Alternative processing methods

Supercritical CO_2 ($scCO_2$) extraction technology is 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.*, 2006a). By itself $scCO_2$ is not a good solvent for rubber, even before the latex has coagulated. To enhance the selective extraction of resins and rubbers from the shrub polar and non-polar co-solvents have to be added to the $scCO_2$. The general advantage of $scCO_2$ extraction is that organic solvent can be avoided. Thus, it is questionable if it makes sense to invest in expensive equipment, and accept the high energy input required for compression and decompression of CO_2 if organic solvents must be added. Pure scCO2 extraction is currently used on an industrial scale to produce more than 100,000 tons of decaffeinated coffee per year, to extract hop acids and oils, and essential oils from a range of plants. The extracted products typically cost € 50 - 300.

The release of rubber from plant tissue can be improved significantly by soaking the plant tissue in guanidine or related compounds, which softens the material, and denatures the protein coats of the rubber particles (Ji, 1994). The particles are then harvested by centrifugation and coagulate in a top layer. The feasibility of this method is unclear, and recovery of the guanidine salt would be necessary for an economical process.

3.6.5 By-products

Although guayule processing yields high quality rubber, the cost of cultivation, harvesting and processing are significant. Guayule latex appears to be hypoallergenic, justifying the added cost. However, dry rubber from guayule must compete directly with *Hevea* rubber. Thus, it is necessary to find uses for the co-products. Depending on the harvesting and processing procedures several by-products or waste streams are generated. Potential uses for these by-products have been investigated (McIntyre *et al.*, 2001).

An indication of the relative amounts is given in (Schloman and Wagner, 1991). Under irrigation conditions, USDA lines produce about 370 kg ha^{-1} y^{-1} of rubber, 360 kg ha^{-1} y^{-1} of resin, and 4,500 kg ha^{-1} y^{-1} of bagasse. Fractionation of the rubber to adjust its physical properties reduces the yield of high Mw rubber to about 310 kg ha^{-1} y^{-1}. On this basis, a commercial-scale processing facility with a nameplate capacity of 25,000 tons y^{-1} of product rubber could be expected to produce 4,000 tons y^{-1} of low molecular weight rubber, 29,000 tons y^{-1} of resin, and 365,000 tons y^{-1} of bagasse (residual biomass).

The resin composition has been determined to be a very complex mixture of sesquiterpene ethers, triterpenoids, and fatty acid triglycerides (Schloman *et al.*, 1983; Nakayama, 2005). 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). The fuel value of the resin is 38’200 kJ kg^{-1}.

Low molecular weight rubber could serve as a feedstock for depolymerised rubber (liquid natural rubber), which has wide applications in adhesive and molded product manufacture (Schloman and Wagner, 1991; Nor and Ebdon, 1998). It can be epoxidised (Schloman, 1992), chlorinated and /or maleinised to obtain added value products (Thames *et al.*, 1994).

Bagasse remaining after rubber and resin extraction was used to fuel the early processing plants in Mexico and unprocessed shrub was used to fuel various processes in the Mexican mining industry. The heating value of the bagasse is 18,300 kJ kg^{-1} (William Schloman, personal communication). Potentially it has many other applications: free flowing soil amendment (Schloman, 1991), fuel (Schloman and Wagner, 1991), BtL (Kuester, 1991), hardboard overlay or fireplace logs

(Wagner *et al*., 1991a; Wagner *et al*., 1991b), or even paper (but due to short cellulose fibres of bad quality) (Marwah *et al*., 1992). Attempts to convert guayule cellulose to ethanol were not very successful, as the bagasse appears very resistant to hydrolysis (Chang and Tsao, 1980). The guayule leaves are a potential source of cuticle wax (Marwah *et al*., 1994). The most likely application of all co-products is the direct use as fuel to provide process heat and electricity for guayule processing. These applications are not unique and are typical of other types of waste lignocellulose (Schloman and Wagner, 1991).

4 ADDITIONAL SOURCES OF NATURAL RUBBER

4.1 Introduction

While many plant species produce rubber, only a few produce large amounts of high molecular weight - good quality rubber (Table 1). Guayule appears to be a good alternative to *Hevea* as it produces relatively high amounts of high quality, low protein rubber. However, it does has a number of disadvantages: it does not tolerate the low winter temperatures in much of Europe and the US; it is currently introduced as a biannual crop because most of the rubber is produced in bark parenchymal cells during winter months; and its processing is complicated and expensive.

The ideal rubber-producing crop plants would be annual, fast-growing, produce large amounts of biomass. Annual crops can be readily planted and ploughed-out in response to market needs and farmer production considerations, and are also more readily included in on-farm crop rotations and farming systems. Some of the alternative plants that are considered might fit these requirements better than guayule. Furthermore, it is advisable to have access to more than one alternative source of natural rubber.

4.2 Russian dandelion

The Russian dandelion (*Taraxacum kok-saghyz*) was identified in Kazakhstan in the course of a strategic program in 1931-32 to develop a native source of natural rubber in the USSR (Ulmann, 1951). The dandelion root was found to be a source of high quality latex, used in making rubber during WWII, with yields of 150 - 500 kg ha^{-1} y^{-1}, and 45 kg of rubber per ton of roots (Whaley and Bowen, 1947; Polhamus, 1962). Tyres made from *Taraxacum* rubber were as resilient as those made from *Hevea*, and better than guayule-based tyres (Whaley and Bowen, 1947). Unfortunately, the agronomic properties are unfavourable (low yield per hectare, labour intensive cultivation, crosses and seed contamination with other dandelions, and weed-potential). Nevertheless, Russian dandelion could be developed as a model species for rubber synthesis. Even in *Hevea*, the rubber synthesis pathway and its regulation have not been completely characterized yet. The rubber transferase gene itself has not been cloned or has not been recognized and proven to encode a rubber transferase. Development of Russian dandelion as a model species would require building up the molecular tools; BAC libraries, inbred lines, tilling populations, EST libraries, 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, functioning and development, in particular, of laticifers (special cell networks containing latex and rubber particles) should be investigated.

An attractive feature of the Russian dandelion is that it could be developed as an annual rubber crop for the temperate regions, and grown in a similar way as chicory. This would require an increase of vigour and agronomic properties, for example by hybridisation of the Russian dandelion with the common dandelion. This is easy and was already recommended in the USDA report (Whaley and Bowen, 1947). Specific targets include breeding for larger roots that are easier to harvest, and genetic engineering of herbicide resistance in rubber producing dandelions (50-70% of the production costs in the WWII emergency project were due to tilling of weeds).

Guayule and Russian dandelion both show polyploidy and apomixis, which affect the breeding programme and possibilities of gene flow to wild relatives. In fact, apomixis-breeding is a desired goal in plant breeding, also to prevent escape of genes into the environment. 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).

4.3 Goldenrod

When the price of rubber soared in the late 1920s, Thomas Edison, Henry Ford and Harvey Firestone combined their efforts, talents and finances in search of a natural source for rubber. Together they established the Edison Botanic Research Company, which screened over 17,000 plants for quality and quantity of rubber they produced. Extensive research proved Goldenrod, a common weed growing to an average height of 3-4 feet, produced 5% yield of latex. Through hybridisation, Edison produced Goldenrod in excess of 12 feet, yielding 12% latex (from http://www.edison-ford-estate.com/lab.asp). In Edison: a biography, the Goldenrod-into-rubber operation in Florida is referred to as “hopeless”, with Henry Ford being too sentimental to shut down the funding. The resulting rubber apparently was of inferior quality, based on tests with 4 tyres made out of the material by Henry Ford. Several Goldenrod species were later shown to contain rubber of an average molecular weight not higher than 200,000 Dalton (Swanson *et al*., 1979), explaining the inferior quality of the tyres. Although Goldenrod is referred to as a crop with good horticultural properties, rubber yields were far too low at 110-155 kg ha^{-1} y^{-1}. Moreover, Canadian Goldenrod is listed as an invasive pest in European countries. Processing of Goldenrod using solvent extraction appeared to be very difficult and expensive (Josephson, 1959).

4.4 Other plant sources of natural rubber

Many tropical vines and trees such as the species *Castilla, Ficus, Funtumia, Manihot,* and *Cryptostegia* also contain rubber (Polhamus, 1962). However, none of these plants grows in Europe or the US. Most were completely ignored after the success of *Hevea*, except for *Ficus* sp., where some work has been done on the biochemistry of rubber biosynthesis.

A desert shrub, Rubber Rabbitbrush (*Chrysothamnus nauseous*), was also investigated in the WWII Emergency Rubber Project, but like guayule, interest waned after 1945. This plant is much more cold tolerant and grows in most arid regions in the US. However, its rubber has a molecular weight of around 500,000 Dalton, which is low compared to the 1,500,000 Dalton of *Hevea* and guayule rubber (Swanson *et al*., 1979; Bhat *et al*., 1990).

Production of rubber in transgenic sunflower or lettuce is being considered in the USA and Canada (Cornish *et al*., 2005). Successful conversion of such candidate crop species into domestic rubber-producing crops yielding commercially viable amounts of high quality rubber will only be possible by using genetic engineering strategies. These strategies require a thorough understanding of biochemical factors affecting rubber yield (biosynthetic rate, substrate availability and rubber particle structure, number and formation) and rubber quality (primarily rubber molecular weight). However, in view of current discussions in the EU, the use of transgenic food-crops for the production of rubber may not be acceptable in the EU.

5 ENVIRONMENTAL AND ECONOMIC COMPARISON OF RUBBER PRODUCTION

5.1 Cultivation and harvesting of guayule

Presently, all natural rubber is produced from the rubber tree *H. brasiliensis*. The latex is tapped from trees, starting 5-7 years after establishment. The trees have a production period of 25 to 30 years and produce up to 1800 kg rubber ha^{-1} y^{-1} (Jones, 1997). At the end of their productive lives, the trees are felled for timber production, which, on average yields 13 tons ha^{-1} y^{-1} of rubberwood. In some cases, guayule has also produced up to 13 tons ha^{-1} y^{-1} of biomass, but typical yields are much lower (George *et al.*, 2005). It is expected that with improved breeding lines and cultivation practices, the average yield can be increased to 15 tons ha^{-1} y^{-1}.

Comparing the environmental impact of guayule and *Hevea* for rubber production for use in tyres has to focus on crop cultivation and the processing phase. No life-cycle analysis studies concerning *Hevea* or guayule rubber production were found in the available scientific literature.

Fertiliser requirements for *Hevea* and guayule are comparable. For *Hevea* an N-requirement and a P/K requirement of 30 kg ha^{-1} y^{-1} is reported (Jones, 1997). For guayule the N-requirement is between 0 and 210 kg ha^{-1} y^{-1}. For standard cultivation (Appendix 1) it has been set to 40 kg ha^{-1} y^{-1}. For future analysis using EPIC (Schmid *et al.*, 2006) we estimate that a biomass yield of 15 tons ha^{-1} y^{-1} and a rubber yield of 1000 kg ha^{-1} y^{-1} can be reached due to progress in the breeding programmes and optimisation of cultivation practices.

The estimation for the cost of guayule cultivation is based on the guayule cultivation manual (Appendix 1). Four situations have been calculated with transplanting seedlings and direct seeding: for each cultivation the current situation with an average yield of 5.5 tons of shrub and 0.32 tons ha^{-1} y^{-1} of rubber, and the foreseen situation based on breeding and cultivation improvements of 15 tons of shrub and 1 ton ha^{-1} y^{-1} of rubber. Prices for seed production and cultivation practices are proxies based on comparable processes in other crops and are indicated in Appendix 1, Table A. Depreciation and interest are indicated as financial costs.

Given the current yield for guayule, cultivation, harvesting and transport to the factory will cost €2.05 per kg of rubber for the transplanted crop and €1.62 for the direct seeded crop (costs for rubber in the shrub, prior to processing). With crop improvements and better cultivation practices these costs can be reduced to respectively to €0.66 and €0.53. 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. The progress made in *Hevea* breeding 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}.

5.2 Natural rubber processing

Hevea rubber needs very limited processing (drying or stabilisation of the latex) as the rubber is harvested directly from the tree. The latex contains natural antioxidants that protect it to some extent from degradation. Guayule on the other hand needs significant mechanical work to rupture the plant cells and release the rubber particles. The ensuing extraction, separation and washing steps are expensive and have a significant environmental impact. To prevent degradation, antioxidants have to be added as soon as the rubber is exposed to oxygen. Due to the large uncertainties in the processing it is not possible to calculate the economic and environmental impact of the processing steps.

Production of one ton of natural rubber from *Hevea* requires 15 to 16 GJ, mainly for transport of the material (Jones, 1997). Solvent extraction of rubber from guayule is a much more energy-intensive activity. If solvent extraction is used, the evaporation of organic solvents alone already consumes 91 GJ per ton of guayule produced (estimation based on (Cole *et al*., 1991)). The bagasse that remains after the process can be used for the generation of energy and yields 369 GJ per ton of rubber produced. This is probably three to four times more than is required to cover the process energy. The remaining bagasse can be sold as fuel. The economic value of the bagasse based on the combustion energy is €40 per ton (Table 6).

Table 6 Energy input for natural rubber production in GJ per ton.

Process	***Hevea*** [a]	**Guayule**
Cultivation, including fertilizers, other chemicals, and harvesting	4	12 [b]
Primary processing	3	> 91 [c]
Transport from producer to consumer	5-8	5-8 [d]
Total	**15-16**	**> 108-111**
Energy production from rest streams including biomass	126 [e]	369 [c]

[a] Jones (1997) UNCTAD/IRSG workshop; [b] estimation based on rapeseed cultivation (Bernesson *et al.*, 2004); [c] estimation based on (Cole *et al.*, 1991), excluding electricity use and cooling and heating; [d] assuming same energy costs for transport; [e] 7.22 tons of rubberwood (may also be used as timber), 17.5 kJ kg^{-1}

5.3 Life-cycle analysis of natural rubber

Subsequent steps in the life-cycle (tyre production, use and end-of-life processing) are the same for *Hevea*- and guayule-derived rubber. A detailed life-cycle analysis, conducted for truck tires, showed that 95% of the environmental impact occurs during the use of the tire (van Beukering and Janssen, 2001). However, this study doesn't provide insight on environmental impact of the production of the natural rubber. A study commissioned by BLIC (European Tyre and Rubber Manufacturers' Association) and conducted by Pré Consultants comes to the same conclusions (http://www.pre.nl/pre/projects.htm#Tyre). This study is not publicly available.

6 EPOBIO RECOMMENDATIONS

Natural rubber is a unique biopolymer that cannot be replaced by synthetic alternatives in many of its most significant applications. Currently, natural rubber is sourced from the rubber tree, *Hevea brasiliensis*. There is a single supply chain from this feedstock supporting global demand for use in the manufacture of dry rubber products such as vehicle and aeroplane tyres and the many high-value applications involving latex. Synthetic rubber cannot replace natural rubber in these uses.

The production base from *Hevea* is under constant threat from a fungal pathogen, which has the potential to destroy the worldwide production capacity if quarantine fails. This security of supply is an issue that must be recognized, and indeed has led to research and development of alternative sources when supply has been disrupted.

An additional risk factor is emerging in the use of *Hevea* rubber. There is now clear evidence that the *Hevea* rubber in latex applications induces allergic responses. This risk factor has recently led the American Society for Testing and Materials to define a new standard (ASTM D1076-06), a Category 4 natural rubber latex that must contain less than 200 micrograms total protein per gram dry weight of latex and no detectable *Hevea* antigenic protein. In addition, this feature of *Hevea* rubber must raise the question of allergenic potential from its use in vehicle tyres and the associated particulates released during wear.

These issues are strong indicators that alternative sources of natural rubber should be investigated. There is an immediate need to address the allergy problem of *Hevea*. There is also a strategic need to mitigate the risk of a single feedstock supporting essential applications on a global scale.

Several additional sources of natural rubber have already been identified. The plant species that has attracted most attention is guayule, an indigenous shrub of Mexico and the southern states of the US. The rubber from guayule has the same properties as that harvested from the rubber tree and significantly, meets the new ASTM standard. To date, research and development on guayule has principally occurred in the US. A single US-based company, Yulex, is commercialising the product.

Given the market opportunities for natural rubber and the risk factors associated with *Hevea*, there would seem to be a considerable economic driver for establishing a European-based supply chain of alternative sources. The EPOBIO study has revealed a strategic need for research and investigation into natural rubbers. This work will be essential to underpin the development of a new industry to address the urgent allergenic issues of *Hevea*. The work will also provide a foundation to develop a robust supply chain in the event that the current single source of natural rubber fails. It is important that Europe builds a competitive position with the potential to respond both to the immediate health risk as well as the strategic supply issue. The first target alternative source of natural rubber with potential for European cultivation and use is guayule. There are additional alternative sources, including those that would be highly relevant for collaborative investigations with developing countries.

6.1 Research and development needs

Targets for delivery of natural rubber from guayule

1. In the short-term, a major bottleneck in realizing the potential of guayule is the processing of the feedstock. The problem is that the rubber is contained within the plant cells. This necessitates cell breakage and extraction protocols to separate the rubber biopolymer from other cellular components. As yet, these steps have not been optimised nor scaled-up for commercial production.

   Therefore, R&D should focus on optimisation of processing guayule using the currently available varieties, and integrating this processing capability into a biorefining process such that co-products can be used either as an energy source, or ultimately as a feedstock for higher value products.

2. In the mid- to long-term, there is considerable potential for guayule crop improvement, although existing varieties can be cultivated in southern Europe. Targets to be addressed include rubber yield and quality, biomass optimisation, water use and other agronomic traits of relevance for European cultivation.

Targets for additional sources of natural rubbers

1. Development of a genetic model to understand the molecular processes controlling rubber biopolymer synthesis and storage is likely to involve characterisation of the Russian dandelion (*Taraxacum kok-saghyz*). Currently, yield and agronomic properties preclude the use of this source on a commercial scale. However, this species has considerable potential for molecular-based research, as well as providing in the longer term the potential for an alternative source of natural rubber to guayule.

   Therefore, R&D should focus on molecular characterisation of Russian dandelion applying genomics and post-genomic technologies.

2. Investigation of alternative sources of natural rubbers should be undertaken in collaboration with developing countries. There is considerable scope for the cultivation of guayule, Russian dandelion and a number of additional rubber species in many geographical regions of the world. In tropical regions, other species could complement the cultivation of *Hevea* and elsewhere in semi-arid regions, guayule for example, could be developed as a new industrial crop providing the processing limitations are overcome.

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APPENDIX 1 GUAYULE CULTIVATION MANUAL

This section describes a guayule cultivation protocol, based on an overview of current literature. Under optimal conditions the results described here can be obtained with current lines and technology.

Establishment

The establishment of a guayule field can be achieved by direct seeding or by transplanting greenhouse-grown seedlings. Transplanting seedlings is the most reliable method of crop establishment while direct seeding is more economical. Seeds need pre-treatment for proper germination.

The currently applied method uses a medium consisting of:

- 25% polyethyleneglycol (MW 8000)
- mM gibberelic acid
- 0.05% potassium nitrate
- 0.1% thiram (tetramethylthiuram disulfide) fungicide adjusted to pH 8.0 with calcium hydroxide

The seeds are treated under aerobic conditions in the light for 3-4 days.

A good seed bed is essential for guayule cultivation. To prevent the growth of weeds a pre-emergence treatment with herbicides like pendimethalin is required. Seedlings are grown in nursery trays and transplanted into the field. Transplanted seedlings grow slowly in the field and require frequent irrigation. Under good conditions the survival rate of the seedlings is 95%.

Direct seeding of seeds can occur with a pneumatic planter in rows 1 metre apart, 10 mm deep at 100 seeds per metre. Seedlings grow slowly and require frequent irrigation during the first 3-4 weeks after drilling. After establishment soils should allow to dry to reduce the damping-off and other seedling diseases. Due to variation in seed quality and cultivation practices germination rates vary between 10 and 92%.

Current recommendations for direct seeding of guayule are:

- the use of conditioned seed of high quality
- accurate planting using fluid drilling or precision drilling techniques
- careful irrigation (enough to allow fast establishment, not too much to prevent damping-off).

Under these conditions a planting rate of 40 seeds/m^2 can be used.

Irrigation is a critical factor influencing guayule establishment and production. At emergence guayule is highly susceptible to salinity. For plant establishment salinity of irrigation water should be below 1.0 dS/m while for established crops salinity levels up to 4.5 dS/m are acceptable. Irrigation will affect both scrub yields and also rubber accumulation. So far, no direct correlations between water supply, dry matter yield and rubber yield have been established. However, sufficient water is required to allow crop growth.

Guayule is a low user of nutrients. Fertilisation requirements are highly dependent on soil fertility.

Table A1 Description and costs of concept guayule cultivation and harvesting

Timepoint	Process [1]	Activities	Materials required	Transplants		Direct sowing	
				€ per ha	€ per ha	€ per ha	€ per ha
				current	foreseen	current	foreseen
Late winter (year 1)	Seed production and conditioning	Seeds are treated under aerobic conditions in the light for 3-4 days. Prices based on cabbage seed: € 7 per 1000 seeds, 30,000 seeds per ha	25% PEG (MW 8000) 0.1 mM gibberelic acid, 0.05% KNO_3 0.1% thiram [2]	210	210	280	280
Late winter	Seedling growth	Sowing seeds, transfer to pots, 10 weeks of growth in greenhouse Price based on cabbage seedlings		510	510	-	-
Early spring	Seed bed preparation	Harrow the field, pre-emergence treatment with herbicide, application of fertiliser	Salary, equipment	30	30	30	30
			Pendimethalin 0.6 kg/ha	58	58	58	58
			40 kg of N	36	36	36	36
Spring	Transplanting	Transplanting of 30,000 seedlings/ha	Salary	24	24	-	-
			Equipment	28	28	-	-
	Drilling and sowing	Precision drilling and sowing machine, 40,000 seeds/ha		-	-	42	42
Spring-summer	Irrigation	Irrigate with drip systems	600 mm	212	212	212	212
Spring (year 2)	Fertilisation	Application of fertiliser	40 kg of N	36	36	36	36
Spring-autumn	Harvest drying and baling	Swath mowing, standard baling equipment		123	123	123	123
Spring-autumn	Transport to factory [3]	Truck, 67 m3, load 17 tons, € 3.10 per km		30	52	30	52

[1] prices for fieldwork were taken from http://www.lfl.bayern.de/ilb/pflanze/; [2] (tetramethylthiuram disulfide) fungicide adjusted to pH 8.0 with calcium hydroxide; [3] transport costs were calculated based on an area with diameter 50 km for the present yields, and 30 km for foreseen yields.

Timepoint	Process [1]	Activities	Materials required	Transplants		Direct sowing	
				€ per ha	€ per ha	€ per ha	€ per ha
				current	foreseen	current	foreseen
Spring-summer	Irrigation	Irrigate with drip systems	600 mm	212	212	212	212
Spring (year 3)	Fertilisation	Application of fertiliser	40 kg of N	36	36	36	36
Spring-summer	Irrigation	Irrigate with drip systems	600 mm	212	212	212	212
Spring-summer (year 4)	Irrigation	Irrigate with drip systems	600 mm	212	212	212	212
Summer-autumn	Harvest, drying baling	Adjusted potato harvester, standard baling equipment		400	400	400	400
Spring-autumn	Transport to factory [3]	Truck, 67 m^3, load 17 tons, € 3.10 per km		30	52	30	52
	Total prod. cost, 4 year period			2,399	2,443	1,949	1,993
	Financing costs			194	194	124	124
	Total costs	Costs inclusive financing		2,593	2,647	2,073	2,117
	Shrub yield over 4 years	5.5 tons ha^{-1} y^{-1} current situation 15 tons ha^{-1} y^{-1} foreseen situation		22 tons	60 tons	22 tons	60 tons
	Cost per ton guayule shrub			110	44	94	35
	Rubber yield over 4 years	0.32 tons ha^{-1} y^{-1} current situation 1.0 tons ha^{-1} y^{-1} foreseen situation		0.32	1	0.32	1
	Cost per ton rubber in shrub			2,050	659	1,620	529

[1] prices for fieldwork were taken from http://www.lfl.bayern.de/ilb/pflanze/; [2] (tetramethylthiuram disulfide) fungicide adjusted to pH 8.0 with calcium hydroxide; [3] transport costs were calculated based on an area with diameter 50 km for the present yields, and 30 km for foreseen yields.

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