# **BioRubber for Europe** in Global Perspective

**EU-PEARLS Final Congress** 

**Programme and abstract book** 

24 - 25 September 2012 Wageningen The Netherlands

BioRubber for Europe in Global Perspective EU-PEARLS Final Congress 24 - 25 September 2011 Wageningen The Netherlands

#### Contents

Sponsors 5
Organizing committee 5
Welcome7
Programme9
Programme11
Monday, September 24 <sup>th</sup> 201211
Tuesday, September 25 <sup>th</sup> 201213
Welcome lecture15
New materials from nature, base of the biobased economy
Keynote lectures19
Natural Rubber: the critical success factors and the challenges to be overcome in delivering a sustainable global rubber industry going forward21
Progress in alternative natural rubber research and development in North America22
Future of sustainable development of China rubber industry - Progress of biorubber in China23
The story of <i>Taraxacum koksaghyz</i> : from basic research towards the applications24
Chances and threats for natural rubber for use in low rolling resistance tyres25
Alternative sources of natural rubber: new developments in the USA26
Regular oral presentations27
NEIKER Contribution to the launching of <i>Taraxacum koksaghyz</i> as an alternative source of natural rubber
Rubber Elongation Factor (REF), a major allergen component in <i>Hevea brasiliensis</i> latex has amyloid properties
Commercialization of a new green technology for processing <i>Taraxacum koksaghyz</i> (TKS)31
Latex and rubber composite materials exploiting wastes as fillers32
Economic success of guayule
Use of dandelion and guayule natural rubber in tyre formulations34
A comparison of guayule and standard natural rubber and its potential in rubber recycling35
Taraxacum koksaghyz biological feedstock development: process components, approach and team progress
Agronomic performance of guayule ( <i>Parthenium argentatum</i> ) as alternative source of rubber and latex in Europe: genetic variation and effects of irrigation and fertilization
The assessment and introduction of productive guayule and <i>Taraxacum koksaghyz</i> genotypes, and technology for large-scale production of natural rubber in Kazakhstan38

	Farm to market: driving innovative applications of guayule through integration of the value chain	
	Modulating acetyl-CoA pool in the cytosol for isoprenoid biosynthesis40	
	Poly(cis-1,4-isoprene) – synthesis and storage of the major polymer in natural rubber41	
	Assessment of guayule profitability in Southern Europe	
	Identification of key elements in the proteome of Russian dandelion ( <i>Taraxacum koksaghyz</i> ) involved in rubber synthesis	
	The genomic organization of rubber biosynthesis genes in the Russian dandelion ( <i>Taraxacum koksaghyz</i> ) and the common dandelion ( <i>T. officinale</i> )44	
	Guayule resin improves the controlled release of linalool from alginate microcapsules45	
(	Closing lecture47	
	EU-PEARLS: EU-based production and Exploitation of Alternative Rubber and Latex Sources - The perspective	
F	Posters 51	
	Taraxacum koksaghyz linkage map construction53	
	Optimization of a method to extract polyisoprene in guayule without macromolecular structure degradation	
	Approaches to weed management in Russian dandelion55	
	Quality and allergenicity of alternative natural rubber lattices	
	Analysis of protein extracts from films produced with <i>Hevea</i> and guayule latex57	
	Guayle / Hevea latex glove comparison58	
	Pilot plant for the extraction of rubber and inulin from <i>Taraxacum koksaghyz</i> (TKS)59	
	Laticifer tissue-specific activation of the <i>Hevea SRPP</i> promoter in <i>Taraxacum</i> brevicorniculatum and its regulation by light, tapping and cold stress60	

#### **Sponsors**

The organising committee gratefully acknowledges the support of:







#### Organizing committee

Hans Mooibroek, Wageningen UR Food & Biobased Research, The Netherlands

Ingrid van der Meer, Plant Research International, The Netherlands

Robert van Loo, Plant Research International, The Netherlands

Luisa Trindade, Wageningen UR Plant Breeding, The Netherlands

Peter van Dijk, Keygene, The Netherlands

Nico Gevers, Apollo-Vredestein, Enschede, The Netherlands

Jan van Beilen, Université de Lausanne, Switzerland

Sabine Meijerink, Wageningen UR Communication Services, The Netherlands

Diana Beljaars, Wageningen UR Communication Services, The Netherlands



The EU-PEARLS project (EU-based Production and Exploitation of Alternative Rubber and Latex Sources) has been financed in part by the European Commission under the 7th Framework KBBE-2007-3-1-06 Program, Activity 2.3 Life Sciences, Biotechnology and Biochemistry for Sustainable Non-food Products and Processes; 2.3.1 Biological Polymers from Plants. Grant number P212827, April 2008 – September 2012.

#### Welcome

EU-PEARLS Final Congress: BioRubber for Europe in Global Perspective

Wageningen, September 2012

Dear colleague, friend,



**BioRubber for Europe in Global Perspective**, organized by the EU-PEARLS consortium marks the end of the FP7 EU-PEARLS research project. Contributions from EU-PEARLS partners as well as from China, the United States, Kazakhstan, Russia and many others reflect the global importance of the work we have carried out and will continue in the future. In addition to the scientific importance of understanding the biosynthesis and applications of natural rubber, one of the major natural biopolymers, governments and funding organizations have become aware of the strategic importance of natural rubber.

With this Congress, which follows the well-received Mid-term Workshop: **The Future of Natural Rubber**, organized by CIRAD in Montpellier, France in October 2010, we hope to answer the question as to what role Europe can play in securing access to natural rubber. Is there, in addition to *Hevea brasiliensis*, room for Russian dandelion and guayule as new crops for the production of natural rubber? How do we arrive at economically viable natural rubber industry, also in Europe? What role for by-products such as bagasse, inulin and resins? Clearly, there is much to do.

With these questions in mind, this congress marks the end of EU-PEARLS, but also the beginning of the next phase, with an invitation to all participants to bring this project forward, to strengthen our ties, and to build new initiatives.

We all learned a lot. I learned a lot as the coordinator of a large EU project, and made many new contacts and friends. After this Congress, I hope to see you again in that next initiative!

Hans

Dr. Hans Mooibroek

Wageningen UR Food & Biobased Research, Wageningen, The Netherlands

T: +31 317 480214; E: hans.mooibroek@wur.nl

## Programme

#### Programme

#### Monday, September 24th 2012

Location:

Lectures, keynotes, presentations and poster sessions – Ir Haak room Lunch – Terras room

#### 08.00 Registration

#### 09.00 Welcome and opening lecture

Raoul Bino, Wageningen UR, The Netherlands.

New materials from nature, base of the biobased economy

### 09.35 – 12.25 Session 1: The economical prospects of natural rubber in a changing world

Chair: Dennis Ray

#### **KEYNOTE**

Fazilet Cinaralp, Secretary General of the ETRMA, the European tyre and rubber manufacturers' association, Belgium – 30 min

Natural Rubber: the critical success factors and the challenges to be overcome in delivering a sustainable global rubber industry going forward

#### **KEYNOTE**

Dennis Ray, University of Arizona, USA – 30 min Alternative sources of natural rubber: new developments in the US

15 minute break

#### **KEYNOTE**

Zhang Liqun, Beijing University of Chemistry and Technology, China – 30 min

Future of sustainable development of China rubber industry - Progress of biorubber in China

Murat Karabayev, CIMMYT-Kazakhstan - 20 min

The assessment and introduction of productive guayule and *koksagyz* genotypes, and technology for large-scale production of natural rubber in Kazakhstan

Didier Snoeck, CIRAD, France – 20 min Feasibility of guayule commodity chain in the Mediterranean

#### 12.25 - 13.30 **Lunch / Poster session 1**

## 13.30 – 16.55 **Session 2: Plant breeding and genetics, isoprenoid biosynthesis, analytical methods**

Chair: Yves Poirier

#### **KEYNOTE**

Jan Kirschner, IBOT, Czech Republic – 30 min

The story of *Taraxacum koksaghyz*: from basic research towards the applications

Peter van Dijk, KeyGene, The Netherlands - 20 min

The genomic organization of rubber biosynthesis genes in the Russian dandelion, *Taraxacum koksaghyz* and the common dandelion, *T. officinale* 

Ingrid van der Meer, Wageningen UR Plant Research International, The Netherlands – 20 min

Identifying key elements in TKS rubber biosynthesis using proteomics

20 minute break

*Yves Poirier, Université de Lausanne, Switzerland – 20 min*Modulating acetyl-CoA pool in the cytosol for isoprenoid biosynthesis

Christian Schulze Gronover, Fraunhofer Institute for Molecular Biology and Applied Ecology, Germany – 20 min

Poly[cis-1,4-isoprene] synthesis and storage of the major polymer in natural rubber

Karine Berthelot, CNRS UMR, France - 20 min

Rubber Elongation Factor (REF), a major allergen component in *Hevea brasiliensis* latex has amyloid properties

Oral poster presentations, presented poster:

Marina Arias, NEIKER, Spain – 5 min Taraxacum koksaghyz linkage map construction

15 minute break

#### 16.55 – 18.00 Session 3: Agronomy, processing and biorefining

Chair: Robert van Loo

#### **KEYNOTE**

Katrina Cornish, Ohio State University, USA - 30 min

Progress in Alternate Rubber Research and Development in North America

Mike Fraley, PanAridus, USA – 20 min Economic Success of Guayule

#### 18.00 **Poster session 2**

19.00 Departure to congress diner

19.30 Congress diner at Hotel de Wereld

#### Tuesday, September 25<sup>th</sup> 2012

Location:

Lectures, keynotes, presentations and poster sessions – Ir Haak room Lunch – Terras room

#### 09.00 – 11.35 **Session 3: Agronomy, processing and biorefining (continued)**

Chair: Katrina Cornish

Robert van Loo, Wageningen UR Plant Breeding, The Netherlands – 20 min Agronomic performance of guayule *Parthenium argentatum* as alternative source of rubber and latex in Europe: genetic variation and effects of irrigation and fertilization

Daniel Pioch, CIRAD, France - 20 min

The challenge of applying the biorefinery concept to the water-based extraction of polyisoprene from guayule biomass

Matthew Kleinhenz, Ohio State University, USA – 20 min
Taraxacum koksaghyz biological feedstock development: process components, approach and team progress

30 minute break

Marina Arias, NEIKER, Spain - 20 min

Contribution to the launching of *Taraxacum koksaghyz* as an alternative source of natural rubber

Anvar Buranov, Nova-BioRubber Green Technologies Inc., Canada – 20 min Commercialization of a new green technology for processing *Taraxacum* koksaghyz (TKS)

Oral poster presentations, presented posters:

Stephen Beungtae Ryu, KRIBB, Korea – 5 min

Laticifer tissue-specific activation of the *Hevea* SRPP promoter in *Taraxacum* brevicorniculatum and its regulation by light, tapping and cold stress

Frederick Michel, Ohio State University, USA - 5 min

Pilot Plant for the Extraction of Rubber and Inulin from *Taraxacum koksaghyz* (TKS)

John Cardina, Ohio State University, USA – 5 min Approaches to Weed Management in Russian dandelion

G. Fakou Allah, Ibn Zohr University, Agadir Institut, Morocco – 5 min Variability in Guayule seeds germination

Frederic Bonfils CIRAD, France - 5 min

Optimization of a method to extract polyisoprene in Guayule without macromolecular structure degradation

#### 11.35 - 12.25 **Poster session 3**

#### 12.25 - 13.30 **Lunch**

## 13.30 – 16.30 Session 4: Applications of latex/rubber and co-products from (alternative) sources of natural rubber

Chair: Jan van Beilen

#### **KEYNOTE**

Wilma Dierkes, University of Twente, Enschede, The Netherlands – 30 min Chances and threats for natural rubber for use in low rolling resistance tyres

*Nico Gevers, Apollo Vredestein, The Netherlands – 20 min*Use of Dandelion and Guayule Natural rubber in tyre formulations

Robert Kind, MIMMM, GPRI, United Kingdom - 20 min

A comparison of Guayule and Standard Natural Rubber and its potential in rubber recycling

20 minute break

Katrina Cornish, Ohio State University, USA – 20 min Latex and rubber composite materials exploiting wastes as fillers

Maria Pascual-Villalobos, IMIDA, Spain - 20 min

Guayule resin improves the controlled release of lialool from alginate microcapsules

Rodolfo Manzone, Yulex, USA - 20 min

Farm to market: driving innovative applications of Guayule through integration of the value chain

#### 16.30 – 17.20 Break and Poster session 4

Oral poster presentations, presented posters:

Michel Dorget, CIRAD / CTTM / PIERCAN, France – 5 min Guayule / Hevea latex glove comparison

Katrina Cornish, Ohio State University, USA – 5 min Quality and allergenicity of alternative natural rubber lattices

Chantal Mourton-Gilles, ANSM, Vendargues, France - 5 min

Analysis of protein extracts from films produced with Hevea and guayule latex

#### 17.20 Closing lecture

Jan van Beilen and Hans Mooibroek, Université de Lausanne, Switzerland and Wageningen UR, The Netherlands – 30 min

EU-PEARLS: EU-based production and Exploitation of Alternative Rubber and Latex Sources - The perspective

#### 17.45 Closure and farewell by Hans Mooibroek

## Welcome lecture

#### New materials from nature, base of the biobased economy

Raoul J. Bino

Wageningen University & Research centre, Agrotechnology & Food Sciences Group (AFSG), POB 17, 6700 AA Wageningen, The Netherlands

Wageningen UR Food & Biobased Research performs research and development to convert biomass efficiently, cost effective and sustainable into high-value chemicals, materials and energy. We work on the establishment of a green, biobased economy by creating breakthroughs in the production of innovative high performance materials, chemicals and processes. The joint expertise enables cost-effective solutions for every step in the production of these biobased products. Our focus is to develop innovative solutions for new products from biobased materials. The development of bio-rubber from natural products combines the expertise of Wageningen UR: genetics, process optimization and control and market analysis.

## Keynote lectures

## Natural Rubber: the critical success factors and the challenges to be overcome in delivering a sustainable global rubber industry going forward

#### Fazilet Cinaralp

Secretary General of the ETRMA, the European tyre and rubber manufacturers' association, Belgium

#### Facts are:

- GDP growth is the main driver of elastomer demand given the strong linkages between GDP and demand in tyre / automotive markets.
- Global GDP grew by 5.3% in 2010 and 2.9% in 2011, with growth slowing down during the second half of the year. Growth has remained weak in 2012 as the Eurozone crisis continues. The emerging markets remain the drivers of this growth, although growth is slowing as export demand slows.
- With highly remunerative prices and more favorable weather conditions than those seen in 2009 and 2010, global natural rubber production grew by about 7% in 2011, reaching a record level of 10.7 million tonnes.
- The high prices around 2005 have encouraged a new round of planting and interest in rubber cultivation → natural rubber production to rise to 16.4 million tonnes in 2015 (Source LMC).
- Natural rubber's <u>specific chemical characteristics</u> have made it the source of choice in many specialized applications<sup>1</sup>. Almost all <u>natural rubber is extracted from one biological source</u>: the Brazilian rubber tree (<u>Hevea brasiliensis</u>). Malaysia, Indonesia, and Thailand together account for about three quarters of the world supply. The yield of rubber varies from 500 kg per ha per year in smallholder plots to almost 2 tons per ha per year in large plantations <sup>2</sup>.
- NR demand is dominated by the tyre industry: At worldwide level, the tyre industry is by far the <u>largest end-use market</u> for natural rubber, consuming ~ 70 % of the NR produced.
- As a renewable resource, the natural rubber sector is well placed to play an important role
  in lowering the environmental impact of vehicle-based transport and contributing to
  sustainable agricultural development.

#### Key elements to consider:

- Natural Rubber as a Sustainable Source of Rubber Supply. As a by-product of rubber trees, natural rubber has the potential to generate a number of positive environmental benefits that are not associated with synthetic rubber.
- Substitution between natural and synthetic rubber.
- Substitution between rubber from Hevea brasiliensis and other sources.

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.

<sup>&</sup>lt;sup>2</sup> Balsiger et al., 2000. In experimental plots with new Hevea lines, yields of up to 3 tons per ha per year have been obtained.

## Progress in alternative natural rubber research and development in North America

Katrina Cornish, Mark A. Bennett, John Cardina, Alba Clivati McIntyre, Robert Grohs, Barbara Hellier, Rafiq Islam, Pablo S. Jourdan, Matthew Kleinhenz, Colleen M. McMahan, Frederick C. Michel Jr, Crittenden J. Ohlemacher, W. Forrest Ravlin, Richard J. Roseberg, David K. Shintani, James Todd, David J. Wolyn, Wenshuang Xie

Many developments have been taking place in North America alternative natural rubber research, development and production as concerns about global natural rubber supplies increase. Partnerships have formalized and industrial investment has greatly increased. Pilot processing facilities have been built and are operating at The Ohio State University. These plants use water-based extraction processes and can produce tons of rubber and latex from rubber-producing species. Research efforts around the rubber (or Russian) dandelion, TKS (Taraxacum koksaghyz, Ohio crop name "Buckeye Gold") have (i) coordinated around the USDA germplasm collection (ii) advanced seed production and quality, (iii) selected improved germplasm, (iv) improved stand establishment and crop management, (v) developed new methods of weed control, (vi) correlated root and plant morphology with root and rubber yield, and (vii) initiated biotechnological approaches to improve rubber yield and agronomic characteristics. In addition, we are investigating the risk and control of gene flow between TKS crops and common dandelion weeds. Guayule (Parthenium argentatum) research is focused on biotechnological crop improvement, adaptation to new growing areas, and new latex and rubber product development. Crop profitability of both quayule and TKS is being enhanced by research in utilization of bagasse and waste water in multiple applications. Commercial guayule production is increasing with the independent efforts of Yulex Corporation, Bridgestone Tire and Rubber Company, Cooper Tire, Apollo Tire and PanAridus LLC.

## Future of sustainable development of China rubber industry - Progress of biorubber in China

Zhang Jichuan, Sun Shuquan, Wang Runguo, Kang Hailan, Wang Zhao, Shi Jinwei, Zhang Ligun\*

Key Laboratory of Beijing City on Preparation and Processing of Novel Polymer Materials; Center of Advanced Elastomer Materials, College of Material Science and Engineering, Beijing University of Chemical Technology, Beijing 100029, P.R. China

Abstract: China needs more and more rubber resources to support its rapid development of economy which has become the world's second largest economy at present. Rubber materials could be obtained from two sources. One is natural rubber (NR) which comes from *Hevea brasiliensis*, but it is shortage in China; the other is synthetic rubber (SR) which is unsustainable because of its background of fossil resources. Therefore, using solar energy to develop biorubber is the future of sustainable development of China rubber industry which includes the bio-based engineering elastomer (BEE) that was synthesized from bio-based monomers fermented from annually renewable agricultural and biomass feedstock and the second natural rubber (SNR) that was extracted from other rubber bearing plants than *Hevea brasiliensis*. At the same time, developing recycled rubber from scrap rubber is an important component of China's rubber industry for sustainable development.

## The story of *Taraxacum koksaghyz*: from basic research towards the applications

<u>Jan Kirschner</u><sup>1</sup>, Jan Štěpánek<sup>1</sup> & Tomáš Černý<sup>1</sup>

The underestimated science of taxonomy is shown to be of great importance in the cases when the success of an experimental research depends on the ability of the researcher to find the experimental model in the field and identify and name it correctly. Taraxacum koksaghyz Rodin (TKS), a species detected in the early 1930s and popular as a potential or real alternative source of natural rubber during the WWII, has several features and attributes that complicate its exploitation as a regular technical crop. These properties are discussed. The distribution, ecology and gross morphology of TKS may pose one type of problems. Another source of difficulties is associated with the reproduction system of TKS; the reproduction is discussed and compared with the distribution of sexuality and asexuality in the genus Taraxacum. Also the prevailing evolutionary force in the genus, i.e. the extensive historical hybridization, represents a complication because of the existence of closely related taxa (allopatric sexuals and sympatric agamosperms). All these factors and the generally difficult taxonomy of the genus caused a gradual disappearance of TKS from the germplasm collections. In order to get a vigorous and robust rubber producer based on TKS an extensive hybridization scheme was developed and results of thousands of artificial crosses are briefly summarized. Among the most promising results there are numerous polyploid agamospermous hybrids between TKS and its close relatives. An outline of future research of TKS and its hybrids is given.

<sup>&</sup>lt;sup>1</sup> Institute of Botany, Academy of Sciences, CZ-25243 Průhonice 1, Czech Republic

## Chances and threats for natural rubber for use in low rolling resistance tyres

J.W.M. Noordermeer<sup>1</sup>, W.K. Dierkes<sup>1</sup>, S.S. Sarkawi<sup>1,2</sup>, W. Kaewsakul<sup>1,3</sup> and K. Sahakaro<sup>1,3</sup>

<sup>1</sup>Department of Elastomer Technology and Engineering, University of Twente, 7500 AE Enschede, the Netherlands

The need to save natural resources puts an ever increasing pressure on the tyre world to develop energy-saving tyres. The high-dispersion silica technology for low rolling resistance passenger car tyres, has become a generally accepted practice in Europe. The elastomers employed for this technology are primarily synthetic rubbers: solution – styrene butadiene rubber (sSBR) and butadiene rubber (BR). In contrast to this, truck tyres are mainly made from natural rubber (NR); being the major application of the currently used 11 million tons of NR. Unfortunately, the combination of NR with silica and a coupling agent remains a challenge, but it offers a tremendous potential for a reduction of energy consumption for transport at the same time

The key element of the high-dispersion silica technology is the nano-scale reaction of the silanol groups on the surface of silica with a coupling agent. The latter eventually creates a chemical link between the silica particles and the rubber molecules during vulcanization. NR is intrinsically less thermally stable than the above mentioned synthetic polymers, while rather high temperatures and long reaction time are needed for the commonly used coupling agents to react with the silanol groups on the silica surface. Optimization of the mixing and processing conditions is essential for the silanization reaction in silica reinforced NR.

In addition, NR is a natural product, subject to seasonal variations, and containing non-rubber constituents such as proteins. Our investigation shows that proteins and coupling agent have an antagonistic effect in silica reinforcement of natural rubber. The utilization of modified NR and deproteinized NR is a possibility to enhance the compatibility between silica and NR, as well as to minimize protein adsorption on the silica surfaces, respectively.

The presentation will give an overview of a series of studies made lately in our group, into the problems encountered and the feasibility of implementing silica technology in natural rubber for low rolling resistance tyres.

<sup>&</sup>lt;sup>2</sup> Malaysian Rubber Board, RRIM Research Station Sg. Buloh, 47000 Selangor, Malaysia <sup>3</sup> Centre of Excellence in Natural Rubber Technology (CoE-NR), Department of Rubber Technology and Polymer Science, Faculty of Science and Technology, Prince of Songkla University, Pattani 94000, Thailand

## Alternative sources of natural rubber: new developments in the USA

Dennis T. Ray

School of Plant Sciences, University of Arizona, Tucson, AZ 85721, USA

The improvement of new industrial crops is not appreciably different from the enhancement of germplasm and breeding of conventional food, feed and fiber crops. Although the same basic breeding and genetic methods are utilized, one major difference is that the new crop breeder is often totally unfamiliar with the species, the germplasm, and the final product, and to make matters worse, there is often little information available to help them. This review will trace the commercialization efforts of just one rubber producing crop, guayule (*Parthenium argentatum* Gray), and its current status in the USA.

Interestingly, *Hevea brasiliensis* (A. Juss.) and guayule had parallel early histories. In both, commercialization began by harvesting wild stands, before the establishment of plantations and the initiation of cultural studies. Variability within wild stands lowered yields in both species, and the problem continued through early cultivation efforts because these populations were established with open-pollinated seeds from plants that were very heterogeneous genetically. So why is *Hevea* the dominant rubber crop today? The main difference in the commercialization efforts between the two crops goes back to the establishment of the Rubber Research Institute of Malaya in 1925, which has been responsible for almost 90 years of continuous increases in *Hevea* yields and the production of a uniform and reliable industrial product. Guayule, on the other hand, has suffered from intermittent research efforts, which have been undermined by periods of neglect such that guayule researchers were/are often in the position of "reinventing the wheel," and with really no long term/continuous breeding programs to support an industry.

Breeding new crops is seldom carried out by public plant breeders in the USA because of a funding structure that requires significant progress on any project in a relatively short period of time (usually 2-3 years). The thesis of this talk is that the establishment of plant breeding programs by industry is the critical step in moving a new crop from potential to actual; because by doing so the industry feels strongly enough in a crop's potential to make the necessary long-term investments. In the USA, industry now appears interested in guayule to the point of developing significant plant breeding programs. Thus, although crop champions are often necessary to move a new crop forward, real commercialization will not happen unless the crop/product is pulled by industry, rather than being pushed by a few very enthusiastic and well-meaning individuals.

# Regular oral presentations

## NEIKER Contribution to the launching of *Taraxacum koksaghyz* as an alternative source of natural rubber

M. Arias<sup>1</sup>, M. Hernandez<sup>1</sup>, N. Remondegui<sup>1</sup>, J. Herrero<sup>1</sup>, M. Ricobaraza<sup>1</sup>, E. Ritter<sup>1</sup>.

Natural rubber production has become a target since it has specific properties that cannot be replaced by synthetic rubber and the mail source of commercial rubber, *Hevea brasiliensis*, is nowadays under a severe disease threatening.

The increase of rubber demand and the reduction of rubber tree crop areas are summing up to the need of finding a good quality natural rubber alternative source.

Within the frame of the European Program "EU-based Production and Exploitation of Alternative Rubber and Latex Sources (EU-PEARLS)", NEIKER has developed its work evaluating growth conditions for efficient rubber synthesis in greenhouse and field trials and collaborating to the genetic mapping of rubber synthesis genes.

In this work we try to give a fast and integrated overview of the trials, essays, plant material, methods and techniques we have used, together with the main obtained results.

<sup>&</sup>lt;sup>1</sup> Department of Biotechnology, NEIKER-Tecnalia. The Basque Institute of Agricultural Research and Development, P.O. Box 46, E01080 Vitoria, Spain

## Rubber Elongation Factor (REF), a major allergen component in *Hevea brasiliensis* latex has amyloid properties

<u>Karine Berthelot<sup>1\*</sup></u>, Sophie Lecomte<sup>2</sup>, Yannick Estevez<sup>1</sup>, Bénédicte Coulary-Salin<sup>3</sup>, Ahmed Bentaleb<sup>4</sup>, Christophe Cullin<sup>3</sup>, Alain Deffieux<sup>1</sup>, Frédéric Peruch<sup>1\*</sup>

<sup>1</sup> Laboratoire de Chimie des Polymères Organiques, CNRS UMR 5629, F-33600 Pessac, France
 <sup>2</sup> Chimie et Biologie des Membranes et Nano-Objets, CNRS UMR 5248, F-33600 Pessac, France
 <sup>3</sup> Institut de Biochimie et Génétique Cellulaires, University Bordeaux 2, CNRS UMR 5095,
 F-33000 Bordeaux, France

Hevea brasiliensis is a tropical plant belonging to the Euphorbiaceae family, which is cultivated worldwide, but mainly in south-east Asia, to produce natural rubber (NR), a poly(cis-1,4-polyisoprene) biopolymer of high economic importance. NR is produced from the latex after tapping. Latex is a complex white cytoplasmic system produced by plant laticifers, which contains mainly rubber and non-rubber particles, organites, proteins and cytoplasmic C-serum. The hevea latex contains two major components and allergens Hevb3 and Hevb1, which are also named Small Rubber Particle Protein (SRPP) and the Rubber Elongation Factor (REF). REF and SRPP have been respectively localized on the Large Rubber Particles and the Small Rubber Particles. Genes encoding for REF and SRPP proteins have been cloned and various isoforms identified, but their real functional role has never been uncovered.

By phylogenetic analysis, we evidenced that REF and SRPP are mainly plant proteins from the same family, and related to stress-proteins.

In order to elucidate their possible functions, we purified both proteins and discovered that REF displays aggregation properties. Performing biochemical (PK resistance, dye-binding) and structural analyses (TEM, CD, ATR-FTIR, WAXS) we characterized REF as an amyloid able to form fibrils, whereas SRPP has mainly the characteristics of an alpha-helical protein.

In addition, we investigated the interaction of both proteins with membrane models. We performed lipid dot blots and ellipsometry measurements. Both proteins are highly hydrophobic but clearly differ in their interaction with lipid monolayers used to mimic the monomembrane surrounding the rubber particles.

With this work, we have the evidence that REF is probably the first amyloid protein identified in plants and we propose the first lead in elucidating the role of REF and SRPP in hevea latex, and their potential involvement in rubber biosynthesis.

<sup>&</sup>lt;sup>4</sup> Centre de Recherche Paul Pascal, CNRS UPR 8641, F-33600 Pessac, France E-mail: kberthelot@enscbp.fr or peruch@enscbp.fr

#### Commercialization of a new green technology for processing *Taraxacum koksaghyz* (TKS)

Anvar Buranov and Jason Rite

Nova-BioRubber Green Technologies Inc., 115-887 Great Northern Way, Vancouver, BC, V5T 4T5, Canada

Tel: 1-778-554-9459; E-mail: buranov@novabiorubber.com Website: www.novabiorubber.com

Nova-BioRubber Green Technologies Inc (Nova) develops innovative green technologies and processing equipment for the production of natural rubber, latex and inulin from *Taraxacum koksaghyz* (TKS). Previous processing technologies (developed in 1940s) require the extensive use of toxic chemicals, water, energy and labor to produce lower quality of natural rubber. The use of chemicals and higher temperature contributed to the deterioration of rubber quality and formation of toxic wastes. Therefore, there is an urgent need to develop a new technology that is environmentally friendly and cost-effective.

We have recently developed and patented a dry processing technology.<sup>2-4</sup> Our technology employs unique mechanical forces to recover 98% natural rubber in TKS instantaneously and continuously at room temperature. Our technology is green. Our technology does not produce toxic wastewater and no pollution observed. The advantage of our technology arises from the use of mechanical forces in dry media that provides cost savings in terms of energy (50%), labour (50%), chemicals (100%) and water consumption (90%). No greenhouse gas emission is observed.

Our green technology has been in development for the last eight years, going through various stages of R&D from conceptual to full prototype manufacturing and testing under financial support from Canadian government. Our team conducted the bulk of early-stage conceptual development of extraction technology and the production of sample products. Natural rubber and latex samples were rigorously tested by our industrial partners.

Our technology was further validated through prototype rubber extractor fabrications in partnership with several machine manufacturing companies and rigorous bench top and field-testing. For the last year, the focus has been on refining the processing equipment that is reliable, cost-effective, and delivers the performance customers seek. Nova has already manufactured prototype pilot-scale and larger-scale rubber extractors (design is patent pending) to be built close to the farms worldwide.

#### References:

- Whaley, G.W.; Bowen, J.S. Russian Dandelion (kok-saghyz). An Emergency source of Natural Rubber. *Miscellaneous Publication No.618, USDA*, US government printing office, Washington, 1947. 210 pp.
- 2. Anvar U. Buranov. Process for natural rubber recovery from rubber bearing plants with a gristmill. United States Patent No 7,540,438. **Issued on June 02, 2009**.
- 3. Anvar U. Buranov and Burkhon Elmuradov. Extraction and Characterization of Latex and Natural Rubber from Rubber-bearing Plants. *J. Agric. Food Chem.*, **2010**, *58* (2), 734–743.
- 4. Anvar U. Buranov, Burkhon J. Elmuradov, Khusnutdin M. Shakhidoyatov, Frederick C. Anderson, John P. Lawrence. RUBBER-BEARING PLANTS OF CENTRAL ASIA. Proceedings of 2005 Annual meeting of AAIC: International Conference on Industrial Crops and Rural Development. September 17-21, 2005. Murcia, Spain. Pages 639-647.

## Latex and rubber composite materials exploiting wastes as fillers

Katrina Cornish, J. Lauren Slutzky and Richard S. Kamenik

The development of sustainable alternatives to petroleum, has led to increasing utilization of biobased materials in industry and an increased desired to minimize environmental footprint. Natural rubber can be used in place of synthetic rubber in many applications should sufficient competitively-priced natural rubber become available. Many different plant species produce natural rubber but only a few of them have a suitable combination of yield, polymer molecular weight, rubber composition, and agronomic characteristics that renders them attractive as potential commercially-viable crops plants. These include the industrial giant Hevea brasiliensis, the latex of which can be modified in various ways post-tapping, and the temperature-zone species Parthenium argentatum and Taraxacum koksaghyz, now under commercial development. Fillers are used in many rubber products, some of which have reinforcing effects, such as carbon black in rubber tires, while others are simply used as cheap diluents of the more expensive rubber polymers. We have tested macro, micro and nano fillers we made from plant and mineral wastes from the agricultural and food processing industries in three different lattices and three different solid rubbers. Different materials and sizes reacted differently with the latices and rubber from the different sources. These differences are probably largely due to the non-rubber components of the rubber and rubber latex, such as the rubber particle monolayer biomembrane, the latex serum and the media used to stabilize the latices and rubber during shipping and storage.

<sup>&</sup>lt;sup>1</sup> Department of Food, Agricultural and Biological Engineering, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison Avenue, Wooster, OH 44691-4096, USA

#### **Economic success of guayule**

Michael R. Fraley

PanAridus, USA

For several years PanAridus has been quietly working on identifying the major issues necessary for commercial success of guayule. The most critical aspect to economic success is the ability to produce guayule rubber that is profitable in a commodity based market. This not only requires a keen vision and understand of agribusiness, it requires genetic superiority.

PanAridus has not only developed the world's largest germplasm bank in guayule, we are now commercializing superior propriety genetics that will finally afford "Economic Success of Guayule".

## Use of dandelion and guayule natural rubber in tyre formulations

Nico Gevers<sup>1</sup> and Frans Kappen<sup>2</sup>

When tyres are developed for the European market, the focus in the coming years will be more and more on tyre performance properties like wet grip performance, rolling resistance and noise. Especially wet grip and rolling resistance can be influenced by the properties of the rubber compound used in the tread of a tyre. Also tread compounds with new sources of natural rubber have to fulfill these (future) requirements. The effect of these new sources of Natural rubber, Dandelion and Guayule NR, on the relevant compound properties will be shown. Also the manufacturing process of Dandelion NR and the effect of processing on properties will be presented.

<sup>&</sup>lt;sup>1:</sup> Apollo Vredestein B.V., Ir. E.L.C. Schiffstraat 370, 7547 RD Enschede, The Netherlands <sup>2:</sup> Wageningen UR Food & Biobased Research, P.O. Box 17, NL-6708 WG Wageningen, The Netherlands

## A comparison of guayule and standard natural rubber and its potential in rubber recycling

Robert C Kind, MIMMM, GPRI

Polymer Recyclers Ltd, Gascoigne Wood Interchange, New Lennerton Lane, Sherburn in Elmet, Leeds, LS25 6LH UK. E-mail: bob.kind@polymer-recyclers.com

Applications of latex/rubber and co-products from (alternative) sources of natural rubber.

Natural rubber is technically specified using methods originally developed by the Malaysian Rubber Board and at the time represented a significant improvement in the marketing of natural rubber. Standard Malaysian Rubbers meet specified criteria covering quality and presentation. This specification has been adopted worldwide and forms the basis of many international standards which are used by all major natural rubber producers.

This paper shows how rubber from the shrub *Parthenium argentatum* 'guayule' fits the specification. The author comments on its physical form and properties and how this might translate into traditional rubber applications.

Polymer Recyclers have a patented process for treating powdered rubber from recycled tyres. Natural rubber can be used as a binder to make a high quality master batch. Guayule has been substituted for natural rubber in this process and comparative results are shown.

## Taraxacum koksaghyz biological feedstock development: process components, approach and team progress

M.D. Kleinhenz\*<sup>1,3</sup>, M.A. Bennett<sup>1,4</sup>, J. Cardina<sup>1,3</sup>, F.C. Michel<sup>2,3</sup>, R. Roseberg<sup>5</sup>, R. Grohs<sup>6</sup>, J. Todd<sup>7</sup>, C.J. Ohlemacher<sup>8</sup>, F.W. Ravlin<sup>3</sup>, and K. Cornish<sup>1,2,3</sup>

Ontario, Canada, N3Y 4N5

<sup>7</sup> Ontario Ministry of Agricultural and Rural Affairs, Simcoe Research Centre, P.O. Box 587, 1283 Blueline Road, Simcoe, Ontario, Canada n3Y 4N5

There are strong incentives to develop *Taraxacum koksaghyz* (TKS) as a new source of inulin, natural rubber and other products and the potential for doing so is high. Still, the rate at which TKS is domesticated and commercialized is a direct function of the ability of multi-disciplinary teams to optimize each component in the process. A North America-based biological feedstock development team tracks its progress in terms of rubber production potential, a metric calculated from ten values related to germplasm, agronomy, engineering and rubber qualification and testing. Breeding and genetics and propagation activities identify and multiply promising geno- and phenotypes, using criteria related to downstream use. Collaborative stand establishment and protection efforts contribute to the separation of genetic and environmental effects on crop performance and to the development of commercially-viable TKS production systems. Engineering activities raise the efficiency of rubber extraction. Rubber qualification and testing indicate whether upstream processes result in commercially-viable rubber. The success with which these component processes have been integrated can be expressed in terms of rubber production potential - generally described as the ratio of viable rubber extracted to seed sown. At project initiation, in 2006, rubber production potential (RPP) did not exceed 4 g viable rubber per kilogram seed sown. RPP values up to fifteen times greater are now common. In 2006, seedlots contained a mixture of species and were unselected and unimproved. In 2012, nearly fifty-fold increases in the rate of TKS seed production (g filled seed harvested per g seed sown) have been achieved through roguing, selection and methods improvement.

<sup>&</sup>lt;sup>8</sup> Applied Polymer Research Center, The University of Akron, Akron, Ohio 44325-3909

# Agronomic performance of guayule (*Parthenium* argentatum) as alternative source of rubber and latex in Europe: genetic variation and effects of irrigation and fertilization

E.N. van Loo (Robert) 1, Snoeck, D.2, Pioch, D.2, Chapuset, T.2, Visser, P.3 and Palu S.2

Guayule (*Parthenium argentatum*) has been extensively tested in North America as a potential new crop that can provide latex and rubber, but it's suitability was never tested in Europe. We will present results of almost four years of agronomy testing of guayule that was carried out at two locations in Europe (in France and Spain).

In a first experiment, a set of about 30 accessions of guayule was tested at optimal irrigation and fertilization in order to identify accessions that can grow well in Europe.

First year yield amounted to up to 20 tons of total dry matter and 15 tons of stem. Rubber contents varied between 3 % and 10 % of stem dry matter. Guayule also produces resins as a valuable second product (containing drying oils and terpenes). The resin content varied less (between 7 and 12 %).

In a second experiment, we investigated the effect of irrigation level (no water shortage (=100 % irrigation), 66 % irrigation and 33 % irrigation) and fertilizer application (apply according to expected uptake (100 N), and 50 % of 100 N and no fertilizer) using the accession AZ2. At 33 % irrigation, yields were halved. Using the data, the water use efficiency and optimal water use of guayule can be determined.

Distinct differences in adaptation of guayule were found between Spain (Cartagena) and France (Montpellier) that are related to climate and soil conditions. In France, due to a relatively heavy soil some waterlogging occurred during winter and spring. In combination with extraordinary cold winters with snow in Montpellier, mortality in the trial in France was high. Selections of guayule accessions and genotypes were made that could survive these conditions.

In Spain, with a drier climate and a easily draining soil and mild winters, no such mortality was observed and high yields could be obtained. Growth curves over three seasons for the different fertigation treatments have been obtained and will be presented. Annual yields of over 25 tons/ha total dry matter and stem yields over 20 tons/ha are possible under the Spanish conditions. An analysis is presented also of the dry matter distribution to root, stem and leaves.

In a third trial, on the basis of intermittent harvests, growth of six cultivars/accessions of guayule has been studied, including an analysis of dry matter distribution over root, stem and leaves.

On basis of first analyses of rubber and resin content, it is concluded that both an annual rubber yield and an annual resin yield of 1500-2500 kg/ha can be obtained in Europe under the right conditions.

<sup>&</sup>lt;sup>1</sup> Plant Breeding Wageningen UR, Plant Research International, P.O. Box 16 6700 AA, Wageningen, The Netherlands, robert.vanloo@wur.nl; <sup>2</sup> CIRAD, Montpellier, France; <sup>3</sup> El Molinar, Cartagena, Spain

#### The assessment and introduction of productive guayule and Taraxacum koksaghyz genotypes, and technology for largescale production of natural rubber in Kazakhstan

K. Magzieva<sup>1</sup>, S. Magziyeva<sup>1</sup>, M. Karabayev<sup>2</sup>, K. Zhambakin<sup>3</sup>

<sup>1</sup>: FP7 NCP-Kz; <sup>2</sup>: CIMMYT-Kazakhstan; <sup>3</sup>: Institute of Plant Biology and Biotechnology, Kazakhstan

Natural rubber is a unique biopolymer that is essential to industry, medicine, personal care, and transportation, especially because it cannot be replaced by synthetic – petroleum – based materials in many of these applications. Its major source now is the rubber tree *Hevea brasiliensis*. However, throughout the world the needs in natural rubber and rubber tools have risen sharply, which means an urgent search for alternative sources. The early testing and introduction of alternative sources of rubber, able to grow in most parts of the country, establishment of seed production system, development of technologies of their rapid cost-effective micro propagation, processing of plant raw materials to produce high value-added products is the most effective solution. Activities in Kazakhstan based on use of rubber plants *Taraxacum koksaghyz* and *Parthenium argentatum* (guayule) and resource-saving agricultural technologies of their cultivation, in the case of a positive effect can create a sustainable basis in the country for the production of the rubber.

### Farm to market: driving innovative applications of guayule through integration of the value chain

Rodolfo Manzone and Paul Zorner

Yulex Corporation, 4050 E Cotton Center Blvd., Suite 68, Phoenix, AZ 85040, USA

Yulex has expanded its agronomic development and breeding program in order to both increase latex yield and consistency of that yield. We are sequencing the guayule genome and developing a portfolio of molecular markers to accelerate our breeding efforts. Yulex is also initiating programs with the USDA and leading academic institutions to both develop and deploy these breeding tools, as well as to develop commercial seed amplification and seed quality protocols to support greater commercial acreage and to transition establishment of these plantings from greenhouse grown transplants to direct seeding. Using as starting platform its established industrial aqueous extraction process for emulsions, Yulex has developed processes for solid rubber extraction which have generated a complete portfolio of materials (liquid and solids) suitable for applications ranging from medical products and devices to consumer products, in markets such as action sports, cosmetics, clothing and apparel, juvenile items etc. Yulex materials can be converted with a wide range of technologies ranging from dipping, casting, injection and compression molding, extrusion etc.

### Modulating acetyl-CoA pool in the cytosol for isoprenoid biosynthesis

Shufan Xing and Yves Poirier

Department of Plant Molecular Biology, University of Lausanne, CH-1015 Lausanne, Switzerland

Arabidopsis thaliana was used as a model system to study the regulation of acetyl-CoA availability in the cytosol. ATP citrate lyase (ACL) is the main enzyme involved in the synthesis of cytosolic acetyl-CoA from citrate. Plant overexpressing the genes encoding the ACL-A and -B subunits showed 2.5- to 3-fold increase in ACL activity compared to control. Arabidopsis plants producing the polymer polyhydroxybutyrate (PHB) from cytosolic acetyl-coA show strong negative effect on growth arising from the depletion of acetyl-CoA. Overexpression of ACL in plant producing PHB resulted in improvement in plant growth concurrently with an increase in PHB synthesis, indicating an increased availability of acetyl-CoA for metabolic pathways in the cytosol. While plants overexpressing ACL showed marginal changes in sterol content, there was a significant increase in wax deposition, indicating an increase in long-chain fatty acid synthesis. Surprisingly, synthesis of PHB led by itself to an increase in ACL activity. Plants producing PHB were more sensitive to the HMG-CoA reductase inhibitor mevinolin, while plants overexpressing ACL were more sensitive to mevinolin. Together, these results indicate that cytosolic acetyl-CoA may stimulate ACL activity but inhibit HMG-CoA reductase activity, indicating a role for acetyl-CoA in modulating the activity of both ACL and HMG-CoA reductase. The effect of ACL overexpression on the synthesis of natural rubber in dandelion will be the focus of future investigation.

### Poly(cis-1,4-isoprene) – synthesis and storage of the major polymer in natural rubber

Christian Schulze Gronover

Fraunhofer Institute for Molecular Biology and Applied Ecology, Hindenburgplatz 55, 48143 Münster, Germany; Email: christian.schulze.gronover@ime.fraunhofer.de

The main component in natural rubber is poly(cis-1,4-isoprene), which is synthesized from isopentenyldiphosphate (IPP) being one of the central molecules in plants' primary and secondary metabolism. In natural rubber producing plants IPPs are progressively condensed in a chain elongation process to form polymeric molecules with molecular masses of about  $10^6$  g mol<sup>-1</sup>. This chain elongation process takes place at rubber particles in the cytosol of specialized plant cells. The presentation will give an overview about recent findings in IPP biosynthesis, poly(cis-1,4-isoprene) chain elongation and rubber particle integrity in dandelion.

#### Assessment of guayule profitability in Southern Europe

Nisrine SFEIR <sup>1</sup>, José G.GARCIA <sup>2</sup>, Eibertus N. van LOO <sup>3</sup>, Serge PALU <sup>4</sup>, Thierry CHAPUSET <sup>4</sup>, <u>Didier SNOECK</u> <sup>4</sup>

In a context of limited natural rubber resource, improvement of the performance of alternative sources of natural rubber, are investigated. Native to the Mexican desert, guayule (*Parthenium Argentatum*) is a perennial shrub that has proven capable of adapting to the Mediterranean climate. To understand its ecological potential, CIRAD and PRI, in partnership with the European project EU-PEARLS, have conducted on-farm guayule trials in southern Europe (Fraqnce and Spain, respectively). Satisfactory and promising results have prompted studies to assess its technical and economic potential and its profitability if introduced in the Mediterranean region. This study was conducted within this framework.

Data were collected from trials conducted in Spain and the costs of inputs and products were obtained from farmers in southern Spain and southern France. A software program was used to draw different scenarios of incomes from guayule biomass (rubber, resin, and bagasse) and costs at the farmer level. The results are for a farm of 1 ha growing guayule during 10 years. We could demonstrate that an annual harvest of guayule provides the farmer with an average income between  $\mathfrak{E}$  -124 / year and  $\mathfrak{E}$  2,563 / year depending on the scenario.

In the circumstances of climate change where existing crops lose their value in the Mediterranean region, guayule can be considered an alternative crop for dry areas, especially in the context of sustainable development.

<sup>&</sup>lt;sup>1</sup>Institut Agronomique Méditerranéen de Montpellier, 3191 route de Mende, 34093 Montpellier (France)

<sup>&</sup>lt;sup>2</sup> Instituto Murciano de Investigación y Desarrollo Ágrario y Alimentario. C/ Mayor, s/n. 30150 La Alberca (Spain)

Wageningen UR, Plant Research International, 6700 AA Wageningen (The Netherlands)
 CIRAD, Avenue Agropolis, 34398 Montpellier (France)

## Identification of key elements in the proteome of Russian dandelion (*Taraxacum koksaghyz*) involved in rubber synthesis

<u>Ingrid van der Meer</u>, Jan Cordewener, Jeroen van Arkel, Thamara Hesselink, Andries Koops and Twan America

Plant Research International, Wageningen UR, PO Box 16, 6700 AA Wageningen, The Netherlands

Taraxacum koksaghyz (Russian dandelion) produces high molecular weight rubber in its roots and can be considered as a potential rubber crop for the temperate regions. In contrast, the common dandelion (Taraxacum officinale) produces no useful rubber. The biosynthesis of natural rubber takes place in the latex of laticifers, where it is stored in rubber particles as an end product. Rubber particles show a globular structure containing a homogeneous hydrophobic rubber core surrounded by an intact monolayer membrane. This membrane includes a mixture of lipids, proteins and other molecules. A number of rubber biosynthesis genes have been identified in EST libraries of Taraxacum species and cDNAs have been cloned and sequenced (Schmidt et al., 2010). However, it is not yet fully understood which genes (enzymes) are the key elements in high rubber yield and quality, and make the difference between a good rubber producing subspecies or accession and a bad performing one. This could be (one of the) rubber synthesis enzymes, but also enzymes involved in linked metabolic pathways (e.g. mevalonate pathway) or proteins involved in the synthesis and assembly of the rubber particles.

We report on the use of proteomics analysis for the identification of key elements involved in rubber synthesis in Russian dandelion. To characterise differences in latex protein composition between accessions, latex was harvested from different TKS accessions and from different progeny lines of a crossing between two TKS accessions. Based on rubber quality variance found in breeding population, a set of lines was depicted for quantitative comparative proteomics analysis. Proteins were extracted from the rubber fraction (3 plants per accession) using two-phase separation (CMW extraction).

For comparative LC-MS analysis of the protein samples the same amount of (total) protein was digested with trypsin. The resulting complex peptide mixtures were analysed by two-dimensional UPLC-MS in two different acquisition modes (MS<sup>E</sup> and DDA). For quantitative analysis the LC-MS<sup>E</sup> data were aligned using Progenesis software. Detected peaks were reported as feature tables. Principal Component Analysis of peak intensity data displayed clear quantitative differences between different accessions of *T. koksaghyz*. More detailed comparison at the peptide level to identify the proteins causing the major differences between the accessions has been performed. Quantitative expression profiles have been produced for proteins involved in rubber synthesis. These data have been correlated to rubber quality and rubber yield.

Schmidt, T. et al. (2010) "Molecular Cloning and Characterization of Rubber Biosynthetic Genes from *Taraxacum koksaghyz*." Plant Molecular Biology Reporter 28(2): 277-284.

## The genomic organization of rubber biosynthesis genes in the Russian dandelion (*Taraxacum koksaghyz*) and the common dandelion (*T. officinale*).

Peter J. van Dijk and Sandrine Lecoulant

Keygene N.V., PO Box 216, 6700 AE Wageningen, The Netherlands

The Russian dandelion, *Taraxacum koksaghyz*, is a potential rubber crop for the temperate regions. Its roots contain high molecular weight rubber. In contrast, the common dandelion, *Taraxacum officinale*, produces no useful rubber. A number of rubber biosynthesis genes have been identified in EST libraries of these species and cDNAs have been sequenced in the closely related rubber producing species *Taraxacum brevicorniculatum* (Schmidt et al.,2010). The cisprenyltransferases (CPTs) are involved in isoprene polymerization and the Small Rubber Particle Proteins (SRPP) may function as rubber particle chaperones. Schmidt et al. (2010) reported three different CPTs and five different SRPPs in *T. brevicorniculatum*. In this study we investigated the genomic organization of the CPT and SRPP genes in *T. koksaghyz* and *T. officinale*.

To detect all CPT and SRPP genes in the genomes of these two dandelion species BAC insert libraries were screened with EST-derived hybridization probes. For T. koksaghyz a new BAC library was constructed. AFLP® was used to minimize redundancy in BAC sequencing. For T. officinale an existing BAC library was used, which had been physically mapped by Whole Genome Profiling (WGP $^{\text{TM}}$ ). In total 31 T. koksaghyz and 20 T. officinale BACs were sequenced with a Roche GS FLX next generation sequencer.

Three different CPTs were found in *T. koksaghyz* compared to only a single CPT in *T. officinale*. At the BAC level there is no evidence for clustering of CPT genes. In contrast, the Small Rubber Particle Proteins are highly clustered at the BAC level. In *T. officinale* five different SRPPs are located within 150 kb. In *T. koksaghyz* the order of the SRPPs is the same, but SRPP1 and 2 are duplicated. The genomic structure of the different SRPP genes will be discussed. We conclude that there are differences in copy number of the rubber biosynthesis genes and that this may contribute to the differences in rubber synthesis in the roots of these two *Taraxacum* species.

Schmidt, T. et al. (2010) "Molecular Cloning and Characterization of Rubber Biosynthetic Genes from *Taraxacum koksaghyz*." Plant Molecular Biology Reporter 28: 277-284.

### Guayule resin improves the controlled release of linalool from alginate microcapsules

M.J. Pascual-Villalobos and M.D. López

Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA), Estación Sericícola, c/ Mayor s/n, 30150 La Alberca, Murcia, Spain. Email address: mjesus.pascual@carm.es

Guayule resin is a by-product of the rubber extraction process from *Parthenium argentatum* Gray shrubs. When guayule is cultivated to a large extent, it is possible to achieve a production of 200-2000 kg/ha of resin in addition to the commercial natural rubber production that is sought for use as hypoallergenic latex. In this paper, we evaluated a new application of guayule resin as a plant-based material for use in the microencapsulation of bioactive volatiles for non-food purposes. Microcapsules and beads were prepared by inverse gelation and reticulation, respectively. The formulations for entrapping volatile compounds (linalool or carvone) consisted of blends of alginate and starch with or without the addition of guayule resin at 1 %. The volatile release rate was measured over a 14 day period, and the capsule walls were evaluated with scanning electron microscopy (SEM). Volatile loading was in the range of 4 to 34.2%. Guayule resin improves the loading of linalool. Alginate microcapsules at 25°C readily released the linalool, and a controlled release over time was observed if guayule resin or starch was added due to a reduced membrane porosity.

### Closing lecture

### **EU-PEARLS: EU-based production and Exploitation of Alternative Rubber and Latex Sources - The perspective**

Jan B. van Beilen<sup>1</sup> and Hans Mooibroek<sup>2</sup>

<sup>1</sup>Département de Biologie Moléculaire Végétale, Le Biophore, Quartier Sorge, Université de Lausanne, CH-1015 Lausanne, Switzerland

<sup>2</sup> Wageningen UR Food & Biobased Research, Wageningen, The Netherlands

As the EU-PEARLS project is now concluded it is time to take stock and look forward. The project has shown that it is possible to cultivate alternative crops for the production of natural rubber and latex in Europe. Germplasm of the selected crops Russian dandelion and guayule is now available in Europe as a starting point for further breeding programs, while a great amount of molecular genetic data has been collected, which will greatly accelerate this effort. A large number of Russian dandelion hybrids has been produced. Further improvements especially should aim at higher biomass and rubber yields and the root morphology of Russian dandelion going hand in hand with addressing agronomical aspects including planting and harvest procedures and timing. Also the processing has made great progress even if natural rubber and latex are difficult to extract from Russian dandelion and guayule compared to tapping from the rubber tree. Especially in the case of guayule, the thorough disruption of the plant material and the ensuing separation of dry rubber or latex remain challenging tasks.

During the course of the EU-PEARLS project the significance of by-products for the entire chain economy has become increasingly clear. For Russian dandelion the main by-product is the poly-fructose inulin, which can serve as a sweetener or as a source of building blocks for the chemical industry. Furthermore, the remaining leaf material and extracted root biomass may serve food or feed applications or biofuel and energy production. From guayule considerable additional income can be expected from the resins and the lignin fractions, which also may serve as precursors for a variety of high-value chemicals and products. Here again the remaining extracted biomass may be used for energy or biofuel production.

As for the commercial prospects, alternative sources of natural rubber and latex have received considerable attention over the past years as market prices have reached record levels over the past years. In part these were due to rapidly increasing demand from China and other industrializing countries, in part erratic weather – possibly exacerbated by climate change – has interfered with tapping. Prices remain volatile, as the current economic crisis causes uncertainty concerning future demand. Furthermore, both climate change policies and peak oil could drastically reduce the use and availability of liquid fuels, and consequently the demand for natural rubber, unless transportation can be electrified and emit far less greenhouse gasses.

Looking forward, alternative sources of natural rubber and latex must be developed further as a safeguard for possible production shortfalls, either because of increased demand, or because production in tropical countries is disrupted. For commercial application, both crops have to be optimized by breeding, while processing needs to make great strides. For this, follow-up projects with support from governments and industry are urgently needed until the production and application processes for alternative rubber will have reached sufficient maturity and – in the end – profit. The other activities currently developed in a number of other non-European countries like Kazakhstan, China, Korea, India, Morocco will help to establish a mature market for alternative rubber and latex sources.

### Posters

#### Taraxacum koksaghyz linkage map construction

M. Arias<sup>1</sup>, M. Hernandez<sup>1</sup>, N. Remondegui<sup>1</sup>, K. Huvernaars<sup>2</sup>, P.J. van Dijk<sup>2</sup>, E. Ritter<sup>1</sup>,

<sup>1</sup>Department of Biotechnology, NEIKER-Tecnalia. The Basque Institute of Agricultural Research and Development, P.O. Box 46, E01080 Vitoria, Spain

<sup>2</sup>Keygene N.V. Wageningen

Taraxacum koksaghyz is a proved good source for natural rubber as an alternative to Hevea brasiliensis but a breeding program must be developed in order to launch it as a commercial species. As a base for a marker-assisted breeding program, study of inheritance of quantitative and qualitative traits and for map-based gene cloning, a linkage map is needed. Neiker, in collaboration with Keygene has the objective of establishing a high density map in Taraxacum koksaghyz using AFLP, COS and SSR markers as well as QTLs.

Gene Ontology (GO, http://geneontology.org/)-based annotation was performed using Blast2GO to assign GO molecular function, biological process and cellular component terms. The Quantitative Trait Loci (QTLs) added to the map sum up knowledge of expected polygenic interactions in traits of agronomic interest. In addition, co-located molecular markers with relevant biological meaning could potentially explain these QTL.

Synteny can be expressed as conservation or coherence of gene content and order in cromosoms of different genomes in plants. In order to study synteny with lettuce, mapped SSR sequences have been aligned with lettuce genetic sequences.

In addition useful candidate genes for relevant traits with agronomic interest have been searched via *in silico*. This will let open doors to new primer design that could be tested in the mapping population for inheritance studies.

### Optimization of a method to extract polyisoprene in guayule without macromolecular structure degradation

Sunisa Suchat<sup>1</sup>, Christine Char<sup>2</sup>, Serge Palu<sup>3</sup>, Daniel Pioch<sup>3</sup>, Frederic Bonfils<sup>2</sup>

Prince of Songkla University, P.O.8, Suratthani, Thailand
 CIRAD, UMR IATE, Montpellier, F-34398 France
 CIRAD, UR40, Montpellier, F-34398 France

#### **Abstract**

Guayule (*Parthenium argentatum* Gray) is a potential source of domestic polyisoprene (PI). As other polymers, PI mechanical and rheological properties depend on its macromolecular structure (average molar masses, branching, etc.). The main objective of this study was to know the native macromolecular structure of PI in guayule biomass with the minimum degradation due to postharvest processes (storage, extraction). Therefore, a new method, extraction with tetrahydrofuran (THF) at 30°C during 24 hours, was developed and tested on fresh biomass. The macromolecular structure was studied by size exclusion chromatography coupled to a multi-angle light scattering detector (SEC-MALS) measuring the weight-average molar mass ( $M_w$ ). Compared to a conventional method (accelerated solvent extraction or ASE with hexane at 120°C), this new method gave the same extraction rate and very high  $M_w$  compared to processed biomass (dried and extracted with ASE method).

**Keywords:** Guayule, macromolecular structure, extraction, SEC-MALS, weight-average molar mass

#### Approaches to weed management in Russian dandelion

<u>John Cardina<sup>1</sup></u>, Katrina Cornish<sup>1</sup>, Mark A. Bennett<sup>1</sup>, and Matthew Kleinhenz<sup>1</sup>

<sup>1</sup>Department of Horticulture and Crop Science, Ohio State University, 1680 Madison Ave. Wooster, OH, 44691

Large scale field production of Russian dandelion (Taraxacum koksaghyz) will fail unless weeds are controlled effectively. Managing weeds in this crop is difficult because of slow crop establishment and early growth, low canopy architecture, and the presence of many weed seeds in the seedbank. For proposed production practices, weed control in Russian dandelion will likely rely on herbicides rather than mechanical cultivation alone. Our overall goal is to develop a safe, effective, and environmentally responsible weed control program for this new crop. Preliminary studies have identified a few herbicides that might be useful in preemergence and postemergence applications. Field studies were conducted for further evaluation of crop safety and weed control effectiveness. For preemergence broadleaf weed control, sulfentrazone was safe on the crop at rates up to 35 g a.i per ha. For postemergence broadleaf weed control, the herbicide imazamox was safe at rates up to 39 g a.i. per ha. Postemergence grass herbicides provided excellent control and crop safety. Ways to increase crop safety and allow for use of herbicides with effectiveness on a wider spectrum of broadleaf weeds will include seed protectants and altered application timing. For initial crop establishment, a weed control program will include fine seedbed preparation and stale seedbed techniques followed by careful use of pre and postemergence herbicides.

### Quality and allergenicity of alternative natural rubber lattices

<u>Katrina Cornish<sup>1,2</sup></u>, Wenshuang Xie<sup>1</sup>, Crittenden J. Ohlemacher<sup>3</sup>, David Kostyal<sup>4</sup>, David K. Shintani<sup>5</sup>, Matthew Kleinhenz<sup>1</sup>, Frederick Michel<sup>2</sup>, Robert G. Hamilton<sup>6</sup>, Ph.D., D.ABMLI, Johns Hopkins University School of Medicine, Baltimore, MD

<sup>1</sup>Department of Horticulture and Crop Science,
 Department of Food, Agricultural and Biological Engineering, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH
 <sup>2</sup>Department of Food, Agricultural and Biological Engineering, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH
 <sup>3</sup>Applied Polymer Research Center, The University of Akron, Akron, OH
 <sup>4</sup>Akron Rubber Development Labs, Akron, OH
 <sup>5</sup> Department of Biochemistry and Molecular Biology, University of Nevada, Reno, NV
 <sup>6</sup>Johns Hopkins University School of Medicine, Baltimore, MD

Natural rubber demand is exceeding the global supply. This has led to significant renewed commercial and governmental interest around the world in large scale production of alternate rubber sources, especially Parthenium argentatum and Taraxacum koksaghyz. The interspecific differences in natural rubber composition and macromolecular structure among species indicate that T. kok-saghyz rubber is similar to Hevea brasiliensis NR in many ways. This was confirmed with testing of compounded rubber. Extensive testing using ELISA's and immunoblots has demonstrated that T. kok-saghyz also contains several proteins that crossreact with Hevea brasiliensis antibodies that mediate Type I latex allergy. These experiments employed mouse and rabbit IgG antibodies deliberately raised to latex proteins, as well as human IqE serum pools from 400 severely latex allergic patients. Thus, T. kok-saghyz may be considered a valuable supplement to the NR supply but not a hypoallergenic alternative. In contrast, P. argentatum rubber is distinct from the other two sources, in composition and structure. It can be used to make high performance films and contains no proteins that crossreact with antibodies raised against Hevea allergens. Thus, P. argentatum latex and rubber present an alternative natural rubber which can be used in medical applications where the high physical performance of NR is required for protection against disease transmission, without the risk of allergic reactions.

### Analysis of protein extracts from films produced with *Hevea* and guayule latex

M. Daniau<sup>1</sup>, G. Olivier<sup>1</sup>, E. Tardan<sup>2</sup>, S. Palu<sup>2</sup>, A. Souverain<sup>2</sup>, D. Sauvaire<sup>1</sup>, C. Mourton-Gilles<sup>1</sup>

Natural rubber latex allergenicity is linked to proteins of Hevea latex. Latex from other plants has been developed, particularly latex from guayule (*Parthenium argentatum*). This alternative source of latex could be very useful in medical devices to avoid allergenic reactions of patients who have Type I latex allergy. Furthermore, latex is essential for making catheters and tracheal tubes due to its high elastic properties that are not achieved by PVC tubes. Elastic properties level is almost similar for guayule and Hevea latex. The objective of this study is to analyse the proteins extracted from biomedical products and films made with guayule or Hevea latex. The films were compared in terms of total proteins and allergens content after protein extraction using a modified extraction method of the standard EN 455-3. The protein concentration of a guayule films is about 80 fold lower than Hevea film. Using a specific ELISA fit kits method, the Hevea allergens Hevb1 and Hevb5 were not found in guayule protein extracts. The allergen Hev b6 was present but at very low concentration (around 400 fold) in guayule films. The electrophoretic profiles of guayule and Hevea extracts are different. In addition, by using antibodies directed against Hevea or guayule extracts, different protein profiles were revealed.

In conclusion, taking into account such preliminary results on latex allergenicity potential of both Hevea and guayule latex, it could be considered that guayule latex offers an interesting alternative to Hevea latex for medical devices and other medical moulded manufactured products.

<sup>&</sup>lt;sup>1</sup>: Control of Bioactivity and Radioanalysis unit, National Drug and Health Products Safety Agency (ANSM), 635 Rue de la Garenne, 34740 Vendargues

<sup>&</sup>lt;sup>2</sup>: Centre de Cooperation international en recherche agronomique pour le développement (CIRAD), Rue Agropolis, 34000 Montpellier

#### Guayle / Hevea latex glove comparison

M. Dorget<sup>1</sup>, S. Palu<sup>2</sup> & G. Guerin<sup>3</sup>

<sup>1</sup>CTTM, <sup>2</sup>CIRAD, <sup>3</sup>PIERCAN

HEVEA latex has been currently used in order to produce glove by a coagulation process. Mechanical properties of such glove are very good but more and more allergenic problem occur.

An alternative to HEVEA latex for glove application is the GAYULE latex. Major advantage of this GUAYLE latex is the lower content of allergenic proteins than HEVEA latex. Thus we have to cheek if the GUAYULE latex behavior along the glove process is similar or not to the HEVEA latex.

We will show that the aqueous behavior of GUAYULE latex is very similar to the HEVEA latex. It induces similar formulation stability and a similar flocculation process behavior. We can conclude that the surfaces (shell) of the colloidal particles are similar.

We will also show that the rubber properties of the GUAYULE film are different than the HEVEA film. Casting and coagulating film don't have exactly the same mechanical properties. GUAYULE film modulus and maximal stress is lower than HEVEA film properties (with the same formulation and the same process parameters). Our conclusion is that those particles (core) are made of a different polyisoprene.

But we can also compare latex glove to synthetic glove, especially nitrile glove.

#### Pilot plant for the extraction of rubber and inulin from Taraxacum koksaghyz (TKS)

<u>F.C. Michel Jr.</u><sup>1,3</sup>, D. Pitts<sup>4</sup>, M. Varcho<sup>5</sup>, S.K. Grewal<sup>1,3</sup>, J. Rossington<sup>1,3</sup>, B. Kinnamon<sup>3</sup>, M.D. Kleinhenz<sup>1,3</sup>, K.E. Cornish<sup>1,2,3</sup>, C.J. Ohlemacher<sup>6</sup> and F.W. Ravlin<sup>3</sup>

Department of Food, Agricultural and Biological Engineering
 Department of Horticulture and Crop Science
 The Ohio State University, Ohio Agricultural Research and Development Center, 1680
 Madison Avenue, Wooster, Ohio 44691

Process Engineering Associates, 700 S Illinois Ave # A202, Oak Ridge, TN 37830
 Louis Perry & Associates, Inc, 165 Smokerise Drive, Wadsworth, Ohio 44281

<sup>6</sup> Applied Polymer Research Center, The University of Akron, Akron, OH 44325

#### **Abstract**

Taraxacum koksaghyz (TKS) is currently being developed as an alternative source of natural rubber and other products. As part of this effort, a new pilot plant for the extraction of TKS rubber has been designed, constructed and commissioned in Wooster, Ohio. The purpose of this facility is to generate sufficient quantities of natural rubber and byproducts for evaluation by industry and to enable optimization of scalable unit operations for the design of commercial plants. The pilot plant is designed to use dried roots thereby allowing the storage and year round processing of harvested feed stocks and to extract both rubber and inulin without the use of organic solvents. The system is adapted from the Eskew process developed in 1943 as part of the USDA Emergency Rubber Project. The pilot plant has the capacity to process up to 70 metric tons (mt) of dry TKS roots per year. It separates roots into three fractions; purified rubber, a sugar syrup containing inulin, sucrose and fructose, and bagasse consisting of lignocellulose, proteins and inorganic constituents. Based on the properties of roots harvested in Spring of 2011 in Ohio, and depending on root bulk density and the mode of operation of the extraction system, the plant could produce up to 9 mt of rubber, 192,000 liters of a 5 to 29% (w/w) syrup and 26 mt of bagasse per year. Dry roots are first cut and mashed to pieces 2 to 8 cm long and 1 to 3 mm thick using a cutter/roller mill. The mashed roots are then weighed and conveyed into a multi-stage counter current extraction system where inulin is dissolved using hot water (95 °C). The extract is filtered and pumped to tank storage. The extracted roots are then milled in a 1 m<sup>3</sup> pebble mill that agglomerates the rubber and removes the root skin. The milled slurry is then diluted and sent to a two-stage flotation system that separates rubber from bagasse. The floated rubber is then screened and washed, sent to a screw press to remove excess water, dried and blocked. The pilot plant features programmable logic controllers for each unit operation allowing experimentation, process modification and optimization. A number of key process variables have been identified that will be optimized in 2012.

## Laticifer tissue-specific activation of the *Hevea SRPP*promoter in *Taraxacum brevicorniculatum* and its regulation by light, tapping and cold stress

Sandeep Kumar Tata<sup>1</sup>, Jun Young Choi<sup>2</sup>, Ji-Yul Jung<sup>1</sup>, Ka Yung Lim<sup>3</sup>, Jeong Sheop Shin<sup>2</sup>, <u>Stephen Beungtae Ryu</u><sup>1,3,\*</sup>

<sup>1</sup>Environmental Biotechnology Research Center, Korea Research Institute of Bioscience & Biotechnology (KRIBB), Daejeon 305-806, Korea

<sup>2</sup>School of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Korea

<sup>3</sup>Kumho Life and Environmental Science Laboratory, Gwangju 500-757, Korea

Hevea brasiliensis is an important plant species currently cultivated for the commercial production of natural rubber. As the demand for rubber continues to increase, it is important to identify alternative sources of natural rubber and to increase plant rubber content using molecular approaches. Taraxacum koksaghyz, a Russian dandelion, produces natural rubber that is of high quality. In this study, the SMALL RUBBER PARTICLE PROTEIN (SRPP) promoter from H. brasiliensis was characterized to determine its suitability for the expression of latex-specific genes in Taraxacum brevicorniculatum which is another Russian dandelion species of T. kok-saghyz from the similar geographical areas. Studies using transgenic Taraxacum plants carrying the SRPP promoter:  $\beta$ -glucuronidase (GUS) sequence indicate that the SRPP promoter was regulated by various external conditions including light, tapping, and cold. These findings suggest that the SRPP promoter will be a useful molecular tool for the manipulation of gene expression in the laticiferous tissues of Taraxacum plant species.