

Presentation abstracts

1. EU-PEARLS: EU-based Production and Exploitation of Alternative Rubber and Latex Sources. Hans Mooibroek
2. The Many Futures of Alternative Rubber Plants. Mark Finlay
3. *Taraxacum kok-saghyz* as a sustainable source of natural rubber and inulin: development of biological feedstocks and root processing and rubber quality validation. M.D. Kleinhenz, F.C. Michel, R. Roseberg, S. St. Martin, R. Seiple, C.J. Ohlemacher, C.M. McMahan⁶, D.K. Shintani, S.C. Myers, K. Cornish and F.W. Ravlin
4. Natural rubber in tyres: present status and future trends. Nico Gevers
5. Is the production of natural rubber from rubber tree really threatened? Franck Rivano, C. Mattos, D. Garcia
6. Natural rubber and latex allergies. Katrina Cornish
7. The biotechnology of isoprenoids - all things considered? Tobacco BY-2 cells as a model system. Thomas J. Bach
8. Summary of U.S. Efforts to Identify Rubber Biosynthetic Genes. David Shintani, Huma Nural-Taban, Jillian Collins, Upul Hathwaik, Katrina Cornish, Colleen McMahan, Maureen Whalen, Wenshuang Xie, Debbie Scott, Gerald Lazo, Mark Distefano, Amanda Degraw, Olivier Henry
9. Dissection and molecular characterisation of the rubber synthetic pathway and latex rheology in *Taraxacum koksaghyz*. Christian Schulze Gronover
10. Genetic and genomic analyses of SALB resistance in rubber tree (*Hevea* spp.). Marc Seguin, Granet F, Cubry P, Doaré F, Espéout S, Garcia D, Mattos C R R, Pujade-Renaud V and Le Guen V
11. Quantitative proteomics and flow cytometric analysis of *Taraxacum koksaghyz* accessions. Ingrid van der Meer, J.P.F.G. Helsper, A.H.P. America, J.H.G. Cordewener, J.H.W. Bergervoet, J. van Arkel, D. Willemsz and A.J. Koops
12. Evaluating and improving guayule germplasm. Dennis T. Ray
13. Guayule USDA-ARS Agronomic and Breeding Research – An Update. Terry A. Coffelt
14. Mexico: The largest genetic reservoir of guayule. Diana Jasso de Rodríguez and Raúl Rodríguez García
15. Taxonomy and diversity of natural populations and *ex-situ* collections of the Russian dandelion (*Taraxacum koksaghyz* Rod.), a potential rubber crop. Peter J. van Dijk, Peter de Heer, Jan Kirschner, Jan Štěpánek, Issa Omarovich Baitulin, Tomáš Černý, Karin Molenveld and Christian Schulze Gronover
16. Agronomic Performance and Latex characteristics of *Taraxacum* accessions. Enrique Ritter, M. Hernandez, N. Remondegui, M. Arias
17. Progress of guayule trials in Europe (Spain and France). Early evaluation. Didier Snoeck, R. Van Loo, T. Chapuset, P. Visser, R. Metral, L. Corentin, S. Palu
18. Economic and Policy Conditions for the Development of alternative rubber plants. Michel Petit
19. Guayule rubber production at Sacaton, Arizona 1987-1990. William W. Schloman
20. *Taraxacum kok-saghyz*: Production of high quality seeds and development of extraction technologies for natural rubber, latex and inulin. Anvar U. Buranov, Yuriy Kim

21. Variability in the wild species of *T. koksaghyz* as measured via several analyses techniques. Karin Molenveld, Marinella van Leeuwen and Wouter Teunissen
22. Fast pyrolysis of guayule shrub and bagasse. Colleen M. McMahan, Akwasi A. Boateng, Charles A. Mullen, Katrina Cornish
23. Energy-saving tyres based on natural rubber. Siti S. Sarkawi, W. K. Dierkes and J .W. M. Noordermeer

Poster abstracts

1. Pathways towards the creation of an agamospermous hybrid dandelion rich in rubber. Tomáš Černý, Jan Kirschner, Jan Štěpánek
2. A yeast model for isoprenoid biosynthesis Matthé Wagemaker, Jan Springer, Hans Mooibroek.
3. Modulation of isoprenoid production in *Nicotiana tabacum* and *Taraxacum koksaghyz*. Nicole van Deenen, J. Post, Christian Schulze Gronover and Dirk Prüfer
4. Genetic studies of natural rubber biosynthesis in *Hevea* and dandelion. Stephen B. Ryu and Yong Jik Lee
5. Biochemical and anatomical analysis of alternative rubber crops. Hunseung Kang, Yong Jik Lee, and Stephen B. Ryu
6. Proteins involved in rubber biosynthesis in *Taraxacum koksaghyz*. Andrea Hillebrand, Thomas Schmidt, Christian Schulze Gronover and Dirk Prüfer
7. Approaches to increase biomass production in *Taraxacum koksaghyz*. Oliver Munt, Christian Schulze Gronover and Dirk Prüfer
8. Valorisation of latex of the pulp of argan fruit (*Argania spinosa*) Daniel Pioch, Serge Palu, André Collet, Frederic Bonfils, Christine Char-Raluy
9. Comment satisfaire la demande de caoutchouc naturel, à laquelle le latex tiré de l'hévéa ne suffit plus ? En améliorant deux plantes : le guayule et le pissenlit russe. Du caoutchouc naturel en Europe. Serge Palu, Daniel Pioch,
10. Linkage mapping in *Taraxacum* using AFLP and COS and SSR markers. Enrique Ritter, Maria Hernandez, N. Remondegui, Marina Arias, Koen Huvernaars, Peter J. van Dijk

PRESENTATION ABSTRACTS

General session Natural Rubber

1. EU-PEARLS: EU-based Production and Exploitation of Alternative Rubber and Latex Sources

Hans Mooibroek¹ and Jan B. van Beilen²

¹ Wageningen UR, AFSG, Biobased products, Bioconversion, PO Box 17, 6700 AA Wageningen, The Netherlands. E-mail: hans.mooibroek@wur.nl

²Department of Molecular Plant Sciences, Le Biophore, Quartier Sorge, CH-1015 Lausanne, Switzerland.

Abstract: With the world economy barely recovering from a major crisis, natural rubber prices are reaching record highs due to increased worldwide demand for natural rubber and latex, adverse weather attributed to climate change affecting SE Asia, and increasing prices for synthetic rubber due to the high oil price. Furthermore, a fungal disease threatening *Hevea brasiliensis* plantations in South-East Asia, can break the current quarantine any time. Therefore, alternative sources of latex and natural rubber are urgently needed as they are unique and valuable raw materials essential to industry, medicine, personal care, and transportation. In many of these applications it cannot be replaced by synthetic – petroleum – based materials, which means that secure access to natural rubber is a strategic issue.

The EU-PEARLS Consortium comprising partners from The Netherlands, Germany, Switzerland, Czech Republic, Kazakhstan, Spain and France focuses on the two most promising plants for temperate regions: the North-American shrub *Parthenium argentatum* (guayule) and *Taraxacum koksaghyz* (Russian dandelion).

The FP7 EU-PEARLS project includes the collection and creation of new germplasm, biochemistry and genetics of rubber biosynthesis, breeding, agronomy, harvesting of latex and rubber, processing and the production of prototypes (surgical gloves, tires). Analysis of the rubber biosynthetic pathway in these crops using the helper organisms *Arabidopsis thaliana* and Baker's yeast *Saccharomyces cerevisiae*, and mapping of genes involved in rubber biosynthesis will accelerate conventional breeding for commercially-viable rubber yields. Plants are being tested for growth and rubber production under different climatic conditions in Europe. Furthermore, the economic, environmental and societal effects, water and land requirements will be investigated.

2. The Many Futures of Alternative Rubber Plants

Mark R. Finlay, Professor of History, Armstrong Atlantic State University

Abstract: In this talk, Dr. Finlay will take a long and multinational view of the history of alternative rubber plants. For more than a century, the industrial world has remained dependent upon natural rubber derived from one principal source, the tropical tree *Hevea brasiliensis*. As a result, policymakers and industrialists alike have repeatedly pressed experts to identify a workable substitute for Hevea.

Throughout the twentieth century, a wide range of circumstances impelled searches for alternative rubber plants: political revolution in rubber producing countries; war and naval blockades; trade wars and economic embargoes; autarkic political regimes; Cold War rivalries; and opportunities presented by various advances in biotechnologies. Although these occurrences proved too short-lived to sustain the political and economic viability of alternative crops, economic, geopolitical, and ecological threats imperiled the industrial

world's access to Hevea often enough that surrogates like guayule and the Russian Dandelion (or *Taraxacum kok-saghyz*, or TKS), have experienced many imagined futures. The question remains whether the current future may be a bright one.

This presentation will go beyond the theme of Finlay's 2009 book, *Growing American Rubber*, to consider several aspects of the search for alternative rubber plants that took place beyond the United States. By touching upon examples from the history of British, German, French, Canadian, Australian, Russian, Italian, and eastern European rubber crop research, the talk will aim to be of interest to an international audience.

Based on historical evidence, Finlay will also try to assess the political, strategic, and economic circumstances that may be necessary to achieve a twenty-first century resolution to a current issue with a rather long past.

3. *Taraxacum kok-saghyz* as a sustainable source of natural rubber and inulin: development of biological feedstocks and root processing and rubber quality validation

M.D. Kleinhenz^{*1,3}, *F.C. Michel*^{2,3}, *R. Roseberg*⁴, *S. St. Martin*^{1,3}, *R. Seiple*⁵, *C.J. Ohlemacher*⁵, *C.M. McMahan*⁶, *D.K. Shintani*⁷, *S.C. Myers*⁸, *K. Cornish*^{1,2,3} and *F.W. Ravlin*³

* presenting author; Matthew D. Kleinhenz; kleinhenz.1@osu.edu; ph. 330.263.3810

¹ Department of Horticulture and Crop Science ² Department of Food, Agricultural and Biological Engineering

³ The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison Avenue, Wooster, Ohio 44691-4096 ⁴ Oregon State University, Klamath Basin Research and Extension Center, 6941 Washburn Way, Klamath Falls, Oregon 97603-9365, ⁵ University of Akron Goodyear Polymer Center, 170 University Avenue, Akron, Ohio 44325-3909, ⁶ United States Department of Agriculture, Agricultural Research Service, Western Regional Research Center, 800 Buchanan Street, Albany, California 94710

⁷ Department of Biochemistry, University of Nevada-Reno, Reno/MS 200, 1664 North Virginia Street, Reno, Nevada 89557, ⁸ The Ohio State University, College of Food, Agriculture, and Environmental Sciences, Ohio BioProducts Innovation Center, Room 152 Howlett Hall, 2001 Fyffe Court, Columbus, OH 43210

Keywords: bagasse, biofuel, bioproduct, butadiene, cis-1,4-polyisoprene, Eskew, Russian dandelion

Abstract: The Ohio BioProducts Innovation Center (OBIC) and its private- and public-sector collaborators employ a market-pull approach to accelerate the commercialization of renewable specialty chemicals, polymers/plastics and advanced materials. *Taraxacum kok-saghyz* (TKS) is a promising but as of yet non-commercial source of inulin, natural rubber and other products. Since 2006, participants in the Program of Excellence in Natural Rubber Alternatives (penra.org) have worked to position TKS as a viable alternative source of these products by addressing major links in the emerging crop's value chain. Classic and molecular approaches are employed to develop TKS biological feedstocks and agronomic production practices. Bench-pilot scale rubber and inulin extraction tests are used to optimize root processing methods and fuel projections of TKS usage within a "total consumption" model. Outcomes from industry-accepted protocols provide evidence of the potential modern commercial appeal of TKS root components. Also, operating within a Life Cycle Assessment framework assists in setting goals and objectives and assessing progress. Russian dandelion produces cis-1,4-polyisoprene of the same stereochemistry and similar molecular weight to that of *Hevea*. Roots of unselected, wild-collected TKS genotypes contain, on average, 1.4 and 56.4 percent rubber and inulin, respectively. The PENRA-based TKS biological inventory now includes two major phenotypes, nearly four-hundred half- and full-

sib families and many thousands of selected F₁-F₂ individuals (and others awaiting evaluation), selfs, and clones. Root rubber levels and stand establishment metrics exceed commercialization thresholds in some families and many individuals. Seed availability has increased 80-fold since project initiation, in part through the integration of field, greenhouse and high tunnel breeding and germplasm enhancement activities. Fresh roots are lifted from direct-sown or transplanted, flat ground or raised bed field plots using a two-stage, semi-automated process followed by whole plant, root and rubber measures. A rubber extraction method for stored dried roots has been developed using multi-stage aqueous counter current extraction of inulin, fructose and glucose, followed by pebble milling and rubber flotation separation. This method yields a syrup with a hexose concentration greater than 100 g/l that upon hydrolysis is suitable for commercial ethanol fermentation. Also, the extracted natural rubber has molecular weight, cure behavior and vulcanizate mechanical properties similar to the *Hevea*-based NR, Standard Malaysian Rubber SMR20.

4. Natural rubber in tyres: present status and future trends

Nico Gevers, Apollo Vredestein, Po Box 27 7500 AA Enschede the Netherlands

tel: +31 53 4888343 e-mail: nico.gevers@apollovredestein.com

Abstract: Since the invention of the pneumatic tyre in the 19th century natural rubber has been used in tyres as one of the major polymers. Also in 2010 natural rubber is still an important raw material in the production of tyres. In this presentation the focus will be on the use of Natural rubber in modern (Passenger car radial) tyres: Where, why, how much natural is used .

Also the future of natural rubber in tyres will be presented: future tyre requirements like the increasing emphasis on rolling resistance and wet grip as well the availability of natural rubber and oil related polymers will have an impact on Natural rubber market and the tyre industry. Several scenario's on the use of Natural rubber will be shown.

5. Is the production of natural rubber from rubber tree really threatened?

F. Rivano¹, C. Mattos², D. Garcia³

1. CIRAD-PERSYST; 2. CIRAD-BIOS; 3. Michelin-Brazil

Keywords : *Microcyclus ulei*, SALB, resistance, *Hevea brasiliensis*, escape zones

Abstract: Although rubber tree is native to the Amazon, Brazilian production of natural rubber represents only 1% of the world production. This situation is due to South American Leaf Blight (SALB), caused by the fungus *Microcyclus ulei*, which precludes the cultivation of Asian clones in areas where the disease pressure is severe. Besides the search for escape zones, the most effective track for a sustainable control that has been adopted for over half a century, is the genetic resistance. In 1992, a partnership project between CIRAD and Michelin was launched to develop rubber tree varieties combining a good level of resistance and yields at least equal to traditional clones. Sources of resistance were found on original material in a rubber collection in Brazil and used within a breeding program in order to genetically improve the species for Latin America. The first step consisted in selecting 13 clones resistant to most of the known races of the fungus. These clones were then sent to several Latin America countries, as well as to Asia and Africa, to set up an experimental network. The second step was to use these clones as genitors to create new resistant cultivars by artificial pollination and to select the best resistant cultivars when putting them under conditions of high disease pressure. This material, once tested both in the field and in controlled conditions, will in turn be shipped to Latin America countries and worldwide. By assessing its adaptation under different climatic conditions, this material shall be recommended for the development of new plantations, where the *Microcyclus* pressure could be a limiting factor. Indeed the risk of an accidental introduction of the disease into the major

rubber growing areas is important, hence the only way to avoid any catastrophic situation is to supply farmers with resistant clones, with good agronomic performances.

6. Natural rubber and latex allergies

Katrina Cornish

Katrina Cornish, Ph.D., FAAAS, Professor and Ohio Research Scholar, Bioemergent Materials, Department of Horticulture and Crop Science, Department of Food, Agricultural and Biological Engineering, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison Avenue, Wooster, OH 44691-4096

Abstract: Facts and perceptions underlying latex and rubber allergies will be discussed, in a background of scientific data and regulatory concerns.

Session isoprenoid synthesis

7. The biotechnology of isoprenoids - all things considered? Tobacco BY-2 cells as a model system.

Thomas J. Bach

Institut de Biologie Moléculaire des Plantes, CNRS UPR 2357, Université de Strasbourg, 28 rue Goethe, F-67083 Strasbourg

Abstract: For quite a long time we are using tobacco Bright Yellow 2 (BY-2) cells as a model system for the study of isoprenoid biosynthesis and function. This cell line bears a number of advantages, for instance that cells can be highly synchronized in the cell cycle of about 14h. Furthermore, BY-2 cells are known for their very easily taking up biosynthetic precursors and inhibitors for tracing down pathways, to which it adds their easy transformation and use in studies relying on fluorescence and confocal microscopy techniques.

Of course, in view of the importance of number of isoprenoid derivatives in medicine and in technical applications, it is always desirable to increase the plants' productivity, for instance through genetic manipulation. During evolution, biochemical reactions and regulatory mechanisms have undergone "optimization" to fit environmental challenges and to guarantee the survival of a species. Until recently, the selection of positive traits through breeding was more or less by chance, while today the tools of molecular biology might help to arrive at a wanted trait more rapidly. However, without having a clear idea of rate-limiting steps within a pathway and their regulation at different levels, over-expression of "a gene of interest" might lead to a quite unexpected result. Of course, when we consider major sinks for cytoplasmic isoprenoid biosynthesis like sterols, then an enzyme like 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) is responsible of coarse control, but we cannot neglect fine-tuning at later steps, for instance at the level of squalene synthase, cycloartenol synthase and sterol methyltransferases I and II. For instance, the "sink" capacity must exceed that of the production of early intermediates in order to avoid an accumulation of potentially regulatory or even toxic compounds. As an example, I will present some data on farnesol, which can be formed from the central biosynthetic intermediate farnesyl diphosphate by activity of \pm unspecific phosphatases, of by the degradation of farnesylated proteins. BY-2 cells, like mammalian cells react upon treatment with farnesol beyond a threshold value with programmed cell death, but, surprisingly, through induction of HMGR activity instead of its inhibition. A major interest is also focused on the cross-talk between the cytoplasmic mevalonic acid (MVA) and the plastidial methylerythritol phosphate (MEP) pathways. To this

end, we have developed a visualization system, based on expression of a green fluorescent protein (GFP) modified to become isoprenylated and if so, being targeted to the plasma membrane, but if not being mislocated into the nucleus. Application of pathway-specific inhibitors revealed that modification by geranylgeranyl transferase type I, a process that occurs in the cytosol, was clearly dependent on a functional MEP pathway in BY-2 cells. In the meantime, we have developed this system further for the identification of inhibitors that interfere with the MEP pathway and/or the process of protein isoprenylation, using half-automated confocal microscopy techniques. By adjusting the experimental conditions, i.e., by controlling the degree of inducible transgene expression, such a system can also be used to evaluate the pool size of isoprenoid precursors.

8. Summary of U.S. Efforts to Identify Rubber Biosynthetic Genes

*David Shintani*¹, *Huma Nural-Taban*¹, *Jillian Collins*¹, *Upul Hathwaik*¹, *Katrina Cornish*², *Colleen McMahan*³, *Maureen Whalen*³, *Wenshuang Xie*³, *Debbie Scott*³, *Gerald Lazo*³, *Mark Distefano*⁴, *Amanda Degraw*⁴, *Olivier Henry*⁴

¹Department of Biochemistry and Molecular Biology, University of Nevada, Reno, NV, 89557 USA; ²Yulex Corporation, Maricopa, AZ 85238, USA (current address: Horticulture and Crop Sciences, The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, Ohio, 44691, USA); ³Crop Improvement and Utilization Research, Agricultural Research Service, United States Department of Agriculture, Albany, CA 94710, USA; ⁴Department of Chemistry, University of Minnesota, St. Paul, MN, USA

Abstract: Over the past several years, the National Science Foundation Plant Genome Research Program, the USDA Agricultural Research Service and Yulex supported efforts to investigate rubber biosynthesis in plants. The overall program employed a combination of genomic, proteomic and reverse genetic approaches to functionally identify rubber biosynthetic genes. Through this effort we generated over 10,000 expression sequence tags (ESTs) each from rubber producing tissues of *Parthenium argentatum* (guayule), *Taraxacum kok-saghyz* (Russian dandelion) and *Ficus elastica* (rubber fig). A comparative genomic analysis was conducted between these three species and *Hevea brasiliensis* to identify commonly expressed rubber related genes. Additionally, mass spectrometric protein sequencing allowed for the identification of over 300 proteins from rubber particles purified from a number of different rubber producing plant species. Targeted proteomic analysis was also performed using benzophenone analogs of farnesyl pyrophosphate to photoaffinity tag proteins associated with the rubber transferase on isolated Hevea and guayule rubber particles. Through these combined studies, we identified a small number of candidate rubber biosynthetic genes including a cis-prenyltransferase, small rubber particle proteins, rubber elongation factor and allene oxide synthase. Selected genes were subsequently functionally analyzed for their role in rubber biosynthesis using a reverse genetic approach in transgenic *Taraxacum kok-saghyz*. The results of these cumulative studies will be summarized in this presentation.

9. Dissection and molecular characterisation of the rubber synthetic pathway and latex rheology in *Taraxacum koksaghyz*

Christian Schulze Gronover

Fraunhofer Institute for Molecular Biology and Applied Ecology, Forckenbeckstr. 6, 52074 Aachen, Germany

Abstract: The biosynthesis of natural rubber poly(cis-1,4-isoprene) takes place in the latex of laticifers or specialized parenchyma cells in the bark, where it is stored in rubber particles as an end product. Rubber particles are the active site of natural rubber biosynthesis and thus should contain all necessary enzymes for the chain elongation process to form poly(cis-1,4-isoprene) of high molar mass. Molecular and biochemical characterisation of latex and

rubber particles resulted in the identification of several enzymes involved in natural rubber biosynthesis or influencing latex rheology.

10. Genetic and genomic analyses of SALB resistance in rubber tree (*Hevea* spp.),

Seguin M¹, Granet F⁴, Cubry P^{1,4}, Doaré F², Espéout S^{1,4}, Garcia D¹, Mattos C R R⁵, Pujade-Renaud V^{1,3} and Le Guen V¹

1. Cirad, UMR-dap, Montpellier, France. 2. Cirad, UR31, Kourou, French Guiana. 3. Cirad, UMR-piaf, Clermont-Ferrand, France. 4. Michelin, Clermont-Ferrand, France. 5. Michelin, Ituberà, Bahia, Brazil

Corresponding author: *Dr Marc Seguin, Cirad, UMR-dap, Av. Agropolis, TA A96/03, 34398 Montpellier, France. Phone: 33 (0) 467 61 71 27; e-mail: marc.seguin@cirad.fr*

Keywords: rubber tree, *Microcyclus ulei*, disease resistance, genetic resources, genome mapping, QTL

Abstract: South American Leaf Blight (SALB) is of strategic concern for world supply of natural rubber. Indeed, current production of natural rubber is based almost only on the cultivation of elite cultivars of the rubber tree (*Hevea brasiliensis*), which are fully susceptible to this fungal disease. The introduction of the causal agent (*Microcyclus ulei*, Ascomycota) from Latin America to South-East Asia or West Africa, would probably lead to a rapid collapse of *Hevea* plantations. We estimate that the genetic approach is the only way firstly to succeed in the development of rubber tree cultivation in Latin America, and this would help facing the increasing demand in latex and rubber, and secondly to prevent the devastating effects of an accidental introduction of *M. ulei* in Asia or Africa. For nearly 20 years, we conduct a large breeding program in Brazil for the creation of cultivars combining both SALB tolerance and high latex yield, by introgression of resistance factors identified in the *Hevea* genetic resources. In tight connection with the genetic improvement program, we develop researches on genetic analysis of natural resistances through classical genetic diversity analyses and genetic mapping. For that purpose, we produced *Hevea* molecular resources for genome analysis: collections of genetic markers (SSRs, EST-SSRs, AFLPs), EST/cDNA libraries of candidate genes and genetic maps. We obtained significant results on 1) the establishment of genetic determinism of several sources of resistance, 2) the evaluation of the genetic variability of resistance factors in *Hevea* genetic resources and 3) the identification of molecular markers linked to major resistance genes or QRLs (Quantitative Resistance Loci) that can be used for marker aided selection (MAS).

11. Quantitative proteomics and flow cytometric analysis of *Taraxacum koksaghyz* accessions

I. van der Meer, J.P.F.G. Helsper, A.H.P. America, J.H.G. Cordewener, J.H.W. Bergervoet, J. van Arkel, D. Willemsz and A.J. Koops

Plant Research International, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

Abstract: Within the EU-PEARLS consortium we have investigated differences between rubber particles from various accessions of *Taraxacum koksaghyz* and other *T.* species with regard to protein composition and size distribution. Various accessions of *T. koksaghyz* showed more than one particle size within the population of rubber particles. To allow a more accurate comparison between accessions, rubber particles (latex) were isolated at various stages during plant development. Using SDS-PAGE a large variation in protein composition was observed between accessions of *Taraxacum koksaghyz*. This in contrast to small

differences between accessions of *T. hybernum*. A more quantitative comparison of protein profiles was obtained by LC-MS analysis of tryptic digests of the various protein isolations. A solvent partitioning protocol was developed to allow efficient extraction and concentration of the proteins from the latex before digestion. LC-MS/MS analysis revealed a large redundancy in the form of homologous peptides between the various proteins identified in rubber particles of each *T. koksaghyz* accession. Investigations on the origin of this redundancy with regard to protein composition of the latex are in progress. To allow such detailed investigations a genetically homogenous population of the same accession is required. Due to self incompatibility of *T. koksaghyz* sexual plant propagation is not feasible. *In vitro* culture and propagation, followed by culture under greenhouse conditions appeared the most feasible approach for this purpose.

Session : Agronomy and genetics

12. Evaluating and improving guayule germplasm

Dennis T. Ray

School of Plant Sciences, Forbes Building, Rm. 303, The University of Arizona, Tucson, AZ 85721, USA

Abstract: Breeding a new industrial crop, such as guayule, is not appreciably different from enhancement and breeding of conventional crops. In both, plant breeders take the extant germplasm and search for genetic variability in desired traits. The major differences are that in new crops plant breeders are often working with an unfamiliar species that is not yet fully domesticated and the available germplasm is often limited.

The main objective of the guayule breeding program is to facilitate successful commercialization by developing higher yielding cultivars. Improvement has been accomplished, with newer lines yielding up to 250% more rubber than lines developed in the 1940s and 1950s. This is surprising since the genetic base from which improvement has been made appears to be very narrow, and because guayule reproduces predominately by apomixis (asexual reproduction by seed).

Improvement through plant breeding is dependent upon having genetic diversity within the available germplasm, and being able to identify different genotypes. Guayule germplasm exhibits extreme variability both within and between lines for morphological traits such as height, width and biomass, and chemical constituents such as rubber, resin and latex content.

The measured variation is due partly to the facultative nature of apomixis in guayule (asexual reproduction and sexuality coexisting), which periodically releases genetic variation among progeny. It has also been shown that a great amount of this measured variation is due to environment, and selections, to take advantage of only the genetic differences, must be made within the first two-years of growth. However, heritability estimates using midparent and single parent regressions at 2 years of growth, were significant, except for rubber and guayulin B concentrations. For these two traits selection will probably have to be made within the first year of growth.

There have been relatively few individuals involved in guayule breeding. Thus, with limited resources and time, most of the improvement has been made by single-plant selections from within populations. Although this method has the potential for only modest long-term gains, it requires a relatively short time period to realize improvements. To facilitate the selection process, indirect measures have been developed so that many more plants can be evaluated by relatively few individuals. For instance, most selections are made for plant height, width and biomass because they have been found to be highly correlated with rubber yield. By making selections in this manner we may be inadvertently changing the plant in ways that

are not necessarily desirable, such as the increased resin to rubber ratio and lower rubber concentrations found in some newer lines.

Contact: Dennis T. Ray, Department of Plant Sciences, Forbes Building, Rm. 303, The University of Arizona, Tucson, AZ 85721, USA. Tel: 520-621-7612. E-mail: dtray@email.arizona.edu

13. Guayule USDA-ARS Agronomic and Breeding Research – An Update

Terry A. Coffelt

Research Geneticist and Acting Research Leader, Plant Physiology and Genetics USDA-ARS-USALARC 21881 North Cardon Lane Maricopa, AZ 85138 USA Voice: +1 520-316-6359 Email: Terry.Coffelt@ars.usda.gov

Abstract: The latest guayule research effort by USDA-ARS at the U.S. Arid-Land Agricultural Research Center began in the late 1990's. The first objective was to develop a plot harvesting method that could be used to analyze samples for latex, rubber, and resin. A new method was developed that could be used to harvest agronomic and breeding plots for rubber, resin, and latex analyses. This method has been successfully used in all of our field tests for over ten years. This research has included agronomic, breeding, and post harvest studies. Results from a few of these tests will be discussed. Agronomic research has involved plant population, planting and harvesting dates, cutting height, and herbicide tests. Work on planting date and population effects on latex, rubber and resin yields showed little effect of transplanting date but higher plant population generally resulted in higher rubber, resin, and biomass yields. Other research has shown that guayule can be harvested year round and possibly selection of different lines can be used to maximize yields at various harvest dates. Harvesting at 100% after 4 years growth gave higher yields than harvesting at 50%. Optimum harvest schemes will depend upon location, line and production goals. The current recommendation is to harvest after two years of growth and then harvest the regrowth every two years after that. Herbicide studies have shown that herbicides recommended for other field crops such as cotton are safe and practical to use for establishing guayule. Breeding research has shown that more progress can still be made using traditional breeding efforts than using transgenic approaches. Development of a rapid method for determining the ploidy levels in individual plants has shown that plants can vary from diploid to pentaploid in traditional breeding populations and in germplasm in the National Germplasm System. We have developed a set of 29 descriptors for evaluating germplasm and providing a basis for registration and protection of germplasm. Fourteen of these descriptors are designated as a minimum set for preliminary evaluations. We conducted a two year study to evaluate different post harvest storage conditions on latex, rubber, and resin yields. Harvested shrub was allowed to stay in various wet and dry storage conditions for up to four weeks. Harvests were conducted in the spring, summer, and fall. Shrub in treatments without moisture was lower in latex than the moist or freshly harvested shrub, while shrub from treatments with moisture was higher in latex than the fresh shrub. Increases in latex yields from shrub in the moist treatments were due to increases in extracted latex rather than higher biomass or total rubber. Latex yield increases were about 100% higher than fresh shrub under moist storage, especially when plants were allowed to stay in storage for 2-3 weeks following harvest. Rubber and resin contents and yields were unaffected by the moist storage. While many advances have been made, much work stills needs to be done to maximize guayule production and yield.

14. Mexico: The largest genetic reservoir of guayule

Diana Jasso de Rodríguez and Raúl Rodríguez García

Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro No. 1923, Colonia Buenavista, 25315, Saltillo, Coahuila, México

Abstract: Guayule (*Parthenium argentatum* Gray, Asteraceae) is native to semiarid lands in the Northern States of Mexico where it grows wild. The commercialization of guayule began in 1903, and peaking in the 1940s when industrial extraction facilities were operating in the states of Coahuila, Zacatecas, and Durango. Three commercial periods are defined during 1903–1951. Fourteen processing plants were operating from 1903 to 1922. From 1922 to 1935, the Continental Mexican Rubber Company modified the rubber production process and five industrial units were in operation. The third period spanned from 1936 to 1951, and despite the high production during the World War II with seven industrial units in operation, this marked the end of commercial activity. During the third period, collection and planting (over 3000 ha) were performed in Cartagena, Durango. In 1970, a pilot unit was established and a new extraction process developed. However, the commercial stage was not reached. Recently, in 1998, a project for latex production using the solvent extraction technology was carried out; but again no commercial unit was developed. Plant breeding and genetic studies were carried out from 1973 until the present to identify high yielding genotypes, improve rubber and resin content, and molecular weight characteristics. We have also studied genotype by environment and ontological effects on rubber production, as well as, agriculture management and product development. This report outlines the work.

Our studies on native plant accessions in Mexico resulted in defining amount of variability in the states of Coahuila, Zacatecas, and Durango for rubber content with values up to 21% (Jasso de Rodríguez *et al.*, 2004). These unimproved accessions may be used to supply the initial requirements of an industrial processing plant of guayule aiming for solid rubber and latex production. These accessions may also be used for unique traits in breeding programs. An opportunity to conduct biosynthetic studies is envisaged especially in the high rubber content shrubs. The continuous operation of a rubber industrial exploitation plant requires the establishment of guayule as a crop that guarantees the proper supply of shrubs. Molecular weight characterization of the rubber showed seasonal effects on rubber quality. This allows the selection for the best harvest time when rubber does not have low molecular weight and quality is better. The guayule plants in the Mapimi region are a potential source of seeds and vegetative tissue for successful breeding programs. This is the only site with diploid shrubs and the rubber content is particularly high. Agriculture management (irrigation) had no effect on the rubber content in plants irrigated for 5 years (Rodríguez-García *et al.*, 2002), but increased drastically the rubber and resin yielding from 18 to 105 g plant⁻¹.

Keywords: Guayule; Native collections; Rubber; Resin; New crop Production; Irrigation; Biomass.

References

Jasso de Rodríguez, D., Rodríguez-García, R., Angulo-Sánchez, J.L., 2004. Biomass rubber and resin potential of Mexican guayule cultivated under natural environmental conditions and evaluated at different ages. Annual Meeting Association for the Advancement of Industrial Crops, 19–22 September 2004, Minneapolis, MN p.13.

Rodríguez-García, R., Jasso de Rodríguez, D., Angulo-Sánchez, J.L., 2002. Guayule production: rubber and biomass response to irrigation. *In*: Janick, J., Whipkey, A. (Eds.), Trends in New Crops and New Uses. Am. Soc. Hort. Sci, Alexandria, VA, pp. 240–244.

Contact: D. Jasso de Rodríguez, Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro 1923, Colonia Buenavista, 25315, Saltillo, Coahuila, México. Tel: (844) 4110212. E-mail:dianajassocantu@yahoo.com.mx

15. Taxonomy and diversity of natural populations and *ex-situ* collections of the Russian dandelion (*Taraxacum koksaghyz* Rod.), a potential rubber crop.

Peter J. van Dijk¹, Peter de Heer¹, Jan Kirschner², Jan Štěpánek², Issa Omarovich Baitulin³, Tomáš Černý², Karin Molenveld⁴ and Christian Schulze Gronover⁵

¹ Keygene N.V. Agro Business Park 90, 6708 PW Wageningen, The Netherlands

² Institute of Botany, Academy of Sciences, CZ-25243 Průhonice 1, Czech Republic

³ ECER Establishment Center "Ecological Reconstruction", Timiriyev street, 36 'D', 050040 Almaty, Republic of Kazakhstan

⁴ A&F Wageningen UR Food & Biobased Research, Wageningen, The Netherlands

⁵ Westphalian Wilhelms-University of Münster, Institute of Biochemistry and Biotechnology of Plants, 48143, Münster, Germany

Abstract: The Russian dandelion (*Taraxacum koksaghyz* Rod.) was identified in the 20th century as a potential rubber crop, producing considerable amounts of high quality rubber in its roots. However, cultivation turned out to be not economical and ended about 50 years ago. Today there is a renewed interest in the Russian dandelion, partly driven by the potential vulnerability of the *Hevea* rubber production system. Recently two large *T. koksaghyz* research programs have been launched: PENRA (Program of Excellence in Natural Rubber Alternatives) in the USA and EU-PEARLS (Production and Exploitation of Alternative Rubber and Latex Sources) in Europe. Half a century of neglect has resulted in the loss of germplasm and taxonomic knowledge of the Russian dandelion and related species. In this paper we describe the occurrence of the Russian dandelion and related species in *ex-situ* collections and in wild populations in Kazakhstan. We will also report on the genetic diversity in wild populations and on the rubber content and rubber molecular weight of these accessions.

Van Dijk, P., Štěpánek, J., Baitulin, I.O., Černý, T., and Kischner, J. 2010. *Taraxacum koksaghyz* Rodin definitely is not an example of overcollecting in the past. A reply to S. Volis et al. (2009). Journal of Applied Botany and Food Quality 83, 1, in press

16. Agronomic Performance and Latex characteristics of *Taraxacum* accessions

E. Ritter, M. Hernandez, N. Remondegui, M. Arias

NEIKER – Granja Modelo, Apartado 46, E-01080 Vitoria-Gasteiz, Spain. eritter@neiker.net

Abstract: An Accession-Planting Date Trial was performed using a total of 19 *Taraxacum kok-saghyz* (TKS) and nine *T. brevicorniculatum* (TBC) populations which were planted in May and June 2009, respectively. In addition, seven *Taraxacum* populations were planted in September 2009 for over wintering. Plant development in terms of morphological and physiological characteristics, biomass production and latex and rubber contents were evaluated in this trial. Large phenotypic variation between as well as within populations exists and exceptional genotypes were detected for all traits under study, suggesting that breeding can lead to fast gains in trait expression.

Generally, early planting favours morphological differentiation with ramifications and rosette formation. Also biomass and latex production is increased. Biomass production and latex contents increases with plant development. Plants planted at a later stage may compensate reduced latex contents with increased rubber concentration. TKS accession produce less leaf and also root biomass than TBC but the latex and rubber contents in manifold increased in TKS. Over wintering of *Taraxacum* spec. has no advantage. Biomass is reduced and nearly no latex is formed in the 2nd year.

In addition nutrient requirements were analysed for two *Taraxacum* accessions in an NPK fertilizer trial using a randomised block design with 3 repetitions. Two levels of each element N, P and K were assayed. Leaf (LDW) and root production (RDW) were evaluated in the trials. Increasing N and P fertilization showed a significant effect for both variables, while K fertilization level has no effect under our conditions. Significant N*K and P*K interactions were observed for both variables under study while a significant N*P interaction was found only for variable RDW. The significant highest root production was obtained with high N but lower P and K fertilization. The lowest root production is obtained with the lowest fertilizer levels. Under field conditions without growth limitations sufficient N and to a certain degree

also P supply is required to produce maximum root and leaf biomass. K requirements are apparently lower, at least under our field conditions, although the degree of natural supply of this soluble nutrient in our soil can be considered as low. A high potassium dosage can have even a negative effect under high N and P fertilization levels.

17. Progress of guayule trials in Europe (Spain and France). Early evaluation

D. Snoeck (1), R. Van Loo (2), T. Chapuset (1), P. Visser (3), R. Metral (4), M. Calleja (4), L. Corentin (1), S. Palu (1).

1. CIRAD, Montpellier, France. 2. Wageningen UR, The Netherlands. 3. Nunhems, Spain. 4. Supagro Inra, Montpellier, France

Abstract:

The main objectives of the project were to define the potential for cultivation of guayule (*Parthenium Argentatum*) in Southern Europe and to assess the economical justification as an alternative source for natural rubber produced in Europe.

The best available subsets of guayule lines from Arizona germplasm were collected from USDA, National germplasm and US Universities (Arizona, Texas). The seeds from the germplasm collection both in France and Spain were used to produce enough guayule plants for local field trials and to settle a guayule collection of selected accessions to produce seeds for European projects. The genetic diversity of 40 guayule imported varieties was tested in two locations: Murcia in Spain, and Montpellier in France. The results showed clear genetic differences. First results showed that Mexican varieties are best adapted to South of Spain. Germination rates of seeds produced by the project varied from 5% to 60% depending on origin, age, line and seeds cleaning.

A fertilization and irrigation trial was set-up in Murcia and Montpellier in May 2009. Three levels of irrigation and 3 levels of fertilization were compared using AZ2 seeds as genetic material. In Montpellier, different levels of irrigation did not alter the growth, but less watering reduced the mortality rate. While in Murcia, watering resulted in significantly higher yields.

The low rubber content (3.2% in March 2010) and a high mortality of the plants (> 60%) observed in Montpellier after the 2009-2010 winter showed that France is not yet adapted for commercial cultivation of guayule.

Rubber content of guayule plants in Murcia (Spain) harvested was 7.4% in March 2010 (for 17-months-old plants). Less than 1% mortality was observed, and the average yields of the irrigation trial was above 10 tons of dry matter (i.e.: 700 kg/ha of rubber) after two years, showing that the area is fully adapted for commercial cultivation of guayule, provided good watering.

Session processing

19. Guayule rubber production at Sacaton, Arizona 1987-1990

W. W. Schloman, Jr.

Consulting Chemist, Stow, OH 44224-1577, USA

E-mail: wwschlo@uakron.edu. Tel: 1-330-673-8899.

Keywords: Guayule; *Parthenium argentatum*; rubber; processing

Abstract: In 1986, The Firestone Tire & Rubber Co. (later: Bridgestone/Firestone, Inc.) was contracted by the U.S. Department of Agriculture (USDA) to produce a total of 54 t (53 long tons) of rubber (GR) from guayule (*Parthenium argentatum* Gray). The process involved simultaneous extraction of rubber and non-rubber components (“resin”) using a two-component monophasic solvent. Extracted rubber would be isolated from the resulting rubber-resin miscella by coagulation with the more polar component. Firestone designed and built a prototype processing facility at Sacaton, Arizona. By December, 1990, a total of 8.8 t of GR had been produced, 5.5 t (61%) of which met either TSR20 specifications for NR or modified FEMA specifications for GR. This material was later used to fabricate aircraft (TSR20) and light truck tires (FEMA).¹

What factors relating to guayule itself influenced the design process?^{2,3} What is known now that might have proven beneficial to design and operation?⁴⁻⁶ What was learned from plant operations that might facilitate development of commercially viable guayule processing?^{7,8}

References:

- ¹Schloman, W. W., Jr. **2005** *Ind. Crops Prod.*, 22(1), 41-47.
- ²Cole, W. M.; Schloman, W. W., Jr.; Beinor, R. T.; Compton, J. B.; Dembek, J. A., Jr. **1989** *Guayule Rubber Project: Processing and By-Product Application Studies, Progress and Status Report 10*, pp 2-11.
- ³Cole, W. M.; Schloman, W. W., Jr.; Sagar, V. R.; Beattie, J. L.; Beinor, R. T. **1986** *Guayule Rubber Project: Processing and By-Product Application Studies, Progress and Status Report*, (November 23) pp 53-58.
- ⁴Veatch, M. E.; Ray, D. T.; Mau, C. J. D.; Cornish, K. **2005** *Ind. Crops Prod.*, 22(1), 65-74.
- ⁵Schloman, W.W., Jr.; McIntyre, D.; Hilton, A. S.; Beinor, R. T. **1996** *J. Appl. Polym. Sci.*, 60(7), 1015-1023.
- ⁶Schloman, W. W., Jr.; McIntyre, D.; Siler, D. J.; Stumpf, D. K.; Hoffmann, J. J. **1997** *Ind. Crops Prod.*, 7(1), 27-36.
- ⁷Cole, W. M.; Hilton, A. S.; Schloman, W. W., Jr.; Compton, J. B.; Dembek, J. A., Jr. **1991** *Guayule Rubber Project Final Report*, pp 87-163.
- ⁸Schloman, W. W., Jr.; Hilton, A. S. **1992** *Guayule Rubber Stabilization In: Proceedings of the First International Conference on New Industrial Crops and Products, October 8-12, 1990, Riverside, Calif.*, H. H. Naqvi, A. Estilai, I. P. Ting (eds), OALS:Tucson, Ariz, pp 123-126.

20. *Taraxacum kok-saghyz*: Production of high quality seeds and development of extraction technologies for natural rubber, latex and inulin.

Anvar U. Buranov¹, Yuriy Kim²

¹Kok Technologies, Inc., Surrey, BC, Canada. E-mail: buranov@koktech.com Tel: 778-554-9459;

²Institute of Oil and Fiber crops, Yuqori Chirchiq district, Tashkent region, Uzbekistan

Keywords: *Taraxacum kok-saghyz*, production of seeds, extraction of natural rubber, latex, inulin.

Abstract: *Taraxacum kok-saghyz* (TKS) is recognized as one of the most promising rubber-bearing plants for the commercialization due to high content of rubber (24%) and inulin (40%).¹⁻³ Its unique properties such as fast growth and adaptability in various colder climates make it an ideal candidate for large-scale growing activities in North America and Europe.

TKS in the wild fields grows in close neighborhood with many rogue species and therefore it is important to differentiate them for the production of high quality seeds. Our experience indicates that harvesting the seeds directly from wild fields by visual observation leads to harvesting of rogue species as well. Therefore, we have harvested the roots by checking for

the rubber sheath and latex viscosity. The roots were transplanted onto two tests plots in different climatic regions. The seeds produced were of high quality and contained only high rubber-yield varieties.⁴ These seeds were delivered to Oregon State University and Ohio State University in 2004. TKS plants from these seeds yielded 10% rubber upon first year of cultivation and 15% rubber after selection activities.⁵

Development of green and efficient extraction technologies for natural rubber, latex and inulin from TKS represents a big commercial interest. TKS contains rubber in the form of both latex and solid natural rubber. The ratio of latex and solid natural rubber in the cultivated TKS varies depending on the season and climate. Previously developed latex extraction technologies was able to remove only 50 % total rubber in the form of latex.⁴ Our newly-developed know-how latex extraction technology is able to remove about 60% of all available rubber as latex via affordable and efficient green process based on green extraction agents. Previously developed technologies for natural rubber were based on the use of caustic chemicals and extensive pebble milling in water. Our patented dry rubber recovery process has significantly reduced the extraction costs and eliminated the use of caustic chemicals and water-milling.⁶ The newly-designed rubber extractors (patent-pending) can recover the solid natural rubber from dry rubber-bearing plants in one-step with mechanical forces. No chemicals or water are used. Inulin is further extracted from the resulting dry ground biomass.

Kok Technologies Inc (Kok) is currently offering patented technology for rubber extraction, extraction equipment and extraction agents. Kok also offers know-how extraction technology for latex and extraction agents. Kok is currently collaborating with Canadian Universities and open to collaboration with interested parties worldwide on the study of aerial parts of TKS and other bioproducts.

References:

Whaley GW; Bowen JS (1947). Russian Dandelion (kok-saghyz). An Emergency source of Natural Rubber. USDA, Washington.

Buranov AU, Elmuradov BJ, Shakhidoyatov KM, Anderson FC., Lawrence JP, (2005). Rubber-bearing plants of Central Asia. Proc. 2005 Annu. Meeting of AAIC. 17–21 Sept. 2005. pp.639-647

van Beilen JB; Poirier Y (2007). Crit. Rev. Biotechnol. 27(4): 217-231

Buranov AU; Elmuradov BJ (2010). *J. Agric. Food Chem.*, 58 (2), pp 734–743

Kleinhenz MD et al. Abstract #40. 176th Technical Meeting. Rubber Division of ACS. Oct 13-15, 2009.

Buranov AU. US Patent No 7,540,438. June 2, 2009.

21. Variability in the wild species of *T. koksaghyz* as measured via several analyses techniques

Ir Karin Molenveld, Marinella van Leeuwen Ing. and Wouter Teunissen Ing.

A&F Wageningen UR Food & Biobased Research. Bornse Weiland 9, 6708WG, WAGENINGEN, The Netherlands

Abstract: Within the EU-PEARLS project the potential of *Taraxacum koksaghyz* as an alternative source for natural rubber is researched. Since the currently available *T. koksaghyz* is a wild species and within the 4 year project there is limited time to develop crosses and selections, WP6 is challenged to find methods on how to deal with variability (in root size and rubber content) in *T. koksaghyz* and how to measure rubber content.

This presentation focuses on the comparison of various methods to measure rubber content and combines data with plant weight (dry and wet) and root weight.

When comparing the various rubber content measurements (latex concentration, breaking test and hexane extractable rubber) it was concluded that is difficult to compare the various tests since they all measure very different parameters in different parts of the plant. Also, the various tests all have their strengths and weaknesses and combining methods can be very helpful in plant selection.

22. Fast pyrolysis of guayule shrub and bagasse

Colleen M. McMahan¹, Akwasi A. Boateng², Charles A. Mullen², Katrina Cornish³

¹USDA-ARS, Western Regional Research Laboratory , 800 Buchanan St., Albany, CA, 94710 USA, colleen.mcmahan@ars.usda.gov 510.558.5816

²USDA-ARS, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038 USA

³The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, OH USA

Keywords: guayule, pyrolysis, bio-oil, coproducts

Abstract: Economic sustainability of guayule, an alternative crop for production of natural rubber, can be significantly enhanced by utilization of biomass residues. Guayule bagasse, a free-flowing solid residue from latex extraction, represents an attractive bioenergy feedstock due to its high energy content, small particle size, and high density. Importantly, guayule is harvested year-round providing a continuous source of feedstock. Guayule bagasse resembles other hardwood feedstocks and can be used in processes designed for such. One option for feedstock conversion is fast pyrolysis. The bioenergy production potential for guayule as a thermochemical conversion feedstock was determined based on Py-GC/MS pyrograms, and the contributions of natural resin and rubber components quantified. The stem-derived latex-extracted bagasse had the highest energy-containing thermal products.

Guayule bagasse and whole shrub were converted into bio-oil, charcoal, and non-condensable gases by fast pyrolysis at ~ 500°C in a bench-scale fluidized bed reactor. Over a sand medium, bio-oil was produced in the 60% yield range without catalyst. Bio-oils from guayule had energy content around 30 MJ/kg, 75% of the value of heavy fuel oil. Bioproducts from fast pyrolysis included limonene, a cyclic terpene not usually found in pyrolysis products. Guayule bio-oil, with its low water content heat of combustion, may have advantages for diesel fuel conversion compared to other feedstocks.

23. Energy-saving tyres based on natural rubber

S. S. Sarkawi, W. K. Dierkes and J. W. M. Noordermeer

University of Twente, Elastomer Technology and Engineering, P.O. Box 217, 7500 AE Enschede, the Netherlands, E-mail: j.w.m.noordermeer@utwente.nl; tel.: 0031-53-4892529

Keywords: Natural Rubber, Silica, Reinforcement, Tyres, Rolling Resistance

Abstract: Natural Rubber (NR) is a strategic, green material with unique properties and many of its applications cannot be rivalled by synthetic alternatives. NR represents 48% of the total rubber market and is growing, the main application being in truck tyres and also partly in passenger tyres.

Based on legislation issued in 2008 within the European Union, in the year 2012 a labelling system will be introduced for passenger tires, defining scaling and minimum requirements for rolling resistance, wet traction and noise generation. Rolling resistance is a measure for fuel consumption of a tyre, a low value resulting in significant fuel savings of the automobile and reduction of CO₂-emissions to the environment.

Rubber polymers, including NR, need to be reinforced with fillers in order to provide the necessary strength for most applications. In recent years high-dispersion silica has become a major alternative to more traditional carbon-black. Its use in tyre tread compounds greatly reduces their rolling resistance, while maintaining the other performance characteristics of the tyres. Silica-rubber technology encompasses four important elements: the rubber polymer, the special type of silica, a coupling agent and the appropriate mixing technology. Since silica is highly polar and hydrophilic, it is not compatible with less polar rubbers such as NR. A bi-functional silane coupling agent is needed to enhance their interaction on nano-scale by the creation of chemical links between the primary silica particles and the rubber molecules.

Nonetheless, at present the silica-technology in passenger tyre treads employs solution-polymerised rubber polymers and is not feasible with emulsion-based polymers such as NR. In the presentation the merits of silica-reinforcement are discussed, including the limitations for NR and possibilities for improvement, as presently investigated in our group.

POSTER ABSTRACTS

1. Pathways towards the creation of an agamospermous hybrid dandelion rich in rubber

Tomáš Černý, Jan Kirschner, Jan Štěpánek

Institute of Botany of the ASCR, v.v.i.

Keywords: *Taraxacum koksaghyz*, Kazakhstan, agamospermy, hybridization, polyploidy, conventional breeding

Abstract: Russian dandelion (*Taraxacum koksaghyz* Rodin, Tks) is a diploid sexual species with high concentrations of rubber in its roots (10–15% of root dry weight). The breeding program, which aims at improving crop vigor and yield, root size and rubber yield, started with collection of wild germplasm material of various dandelion species (roots and seeds) in southeastern Kazakhstan in 2008. After measuring the ploidy levels of all plants (FCM analysis) we performed the conventional hybridization by controlled hand-made pollination of Tks (as mother plants) with other related dandelions with different ploidy levels and breeding systems. The following pollen donors were used: artificial tetraploid of Tks, *T. brevicorniculatum* (triploid apomict), *T. sect. Ceratoidea* (triploid and tetraploid apomicts), *T. sect. Macrocornuta*, *T. sect. Ruderalia* (both diploid sexuals), *T. serotinum*, *T. haussknechtii*, *T. bicornis*, *T. bessarabicum* (all diploid sexuals) and *T. stenolepium* (tetraploid sexual). Thus, over 2500 crosses were made in the season 2009 to obtain F1 achenes.

The most successful crosses were those between Tks and diploid and tetraploid sexuals (diploid and triploid F1 progeny, respectively), whereas those between Tks and apomicts gave only several true F1 hybrids. The reason is in small portion of normally developed pollen grains in apomicts (about 1%) and also in effective genetic barrier between sympatric species. Progeny originating from the crosses is cultivated under ca. 500 cultivation numbers. In parallel, controlled within-population crosses of Tks were made and over 10000 achenes were so obtained for further experiments.

In the season 2010 the program has continued with controlled backcrosses (BC) between all F1 plants and Tks (as mother plants again). In total, approx. 3700 heads of *T. koksaghyz* were pollinated (~ 120 000 individual flowers), of them ca 650 heads (18%) contain some visibly ripe achenes. Such relatively low success is given by high pollen sterility of F1 hybrids (99-100% of aborted pollen grains is a frequent phenomenon). During the growing almost no seeds from F1 plants have been obtained, due to sterility of F1 hybrids. As an exception four F1 plants (*T. brevicorniculatum* × Tks) proved to be apomictic. Three of them according to FCM test are triploid and one probably is pentaploid. A selection of BC seeds was sown to obtain new potentially perspective progeny.

In the course of controlled crosses a certain portion of plants proved to be of non hybrid origin (diploid progeny morphologically identical with Tks) as a result of the breakdown of self-incompatibility (Mentor Effect).

Kirschner J. & Štěpánek, J. 2008. The most common dandelions in Middle Asia: The problem of *Taraxacum* sect. *Macrocornuta*, *T. sect. Ceratoidea* sect. *Nova* and the identity of *T. halophilum*. *Phyton* 48, 61–78.

Van Dijk, P., Kirschner, J., Štěpánek, J., Baitulin I.O. & Černý, T. (in press). *Taraxacum koksaghyz* Rodin definitely is not an example of overcollecting in the past. A reply to S. Volis et al. (2009). *J. Appl. Bot. Food Qual.* 83.

2. A yeast model for isoprenoid biosynthesis

Wagemaker M.J.M., Springer J., Mooibroek H.

Wageningen UR - Food & Biobased Research, Wageningen, Netherlands

Abstract: Cultivation of rubber producing crops other than the rubber tree (*Hevea brasiliensis*) fit to grow in parts of the world other than Southeast Asia, is seen as a potential alternative resource of natural rubber. Russian dandelion (*Taraxacum koksaghyz*) and guayule (*Parthenium argentatum*) grow in moderate and dry climates, respectively, and produce high quality rubber. These plant species have been selected as most promising crops for the FP7 subsidized EU-PEARLS research project. Part of the research is aimed at creating more fundamental knowledge concerning the physiology and regulation of (poly) isoprene-biosynthesis necessary for a progressive development in breeding and processing of the rubber producing plants.

Natural rubber, *cis*-1,4-polyisoprene, is formed by condensation of isoprenyl diphosphate molecules (IPP-molecules) to an allylic initiator (farnesyl diphosphate) by the action of a *cis*-prenyltransferase (CPT). The rubber is formed in particles surrounded by a monolayer membrane, onto which the CPT and other, SRPPs, REF and yet undefined, proteins are attached. Recent studies demonstrated that besides CPT, other undefined proteins, co-factors and IPP/allylic initiator ratios play an important role in synthesis of high grade rubber.

Baker's yeast *Saccharomyces cerevisiae* is selected as model-organism and will be used to study and engineer poly-isoprene biosynthesis and the physiological impact of isoprene accumulation. The species serves as a convenient eucaryotic model for functional genomics and proteomics technologies allowing the analysis of heterologous plant genes. Valuable tools include the yeast deletion strain collection, synthetic genetic arrays, microarrays, proteome arrays, and the two-hybrid-system. The yeast endogenous mevalonate (MVA) pathway can be used as a source of (poly)isoprenoid precursors and redirection cellular recourses can be optimized while the downstream branch-point enzymes can be added in a modular fashion to generate *cis*-1,4-polyisoprene. When expression of a *cpt*-gene is established successfully, complex interactions with other proteins and physical factors can be studied in detail.

Our current efforts to be highlighted include the engineering and heterologous expression of a number of *Taraxacum CPT* genes in yeast and some of the effects observed.

3. Modulation of isoprenoid production in *Nicotiana tabacum* and *Taraxacum koksaghyz*.

Nicole van Deenen^a, J. Post^a, C. Schulze Gronover^b and Dirk Prüfer

^aDepartment for Plant Biochemistry and Biotechnology, Hindenburgplatz 55, Westphalian Wilhelm's University Muenster, 48143 Münster, Germany

^bFraunhofer Institute for Molecular Biology and Applied Ecology (IME), branch Münster, Hindenburgplatz 55, 48143 Münster, Germany

nicki.v.d@uni-muenster.de, Tel.: 0049-251-8324716

Abstract: The biosynthetic basis for the formation of natural rubber is the isoprenoid pathway that provides isopentenyl diphosphate (IPP) as the precursor for several different isoprenoids in all higher plant als well as for the formation of the polyisoprenes in rubber producing plants. Two separate, compartmentalized isoprenoids pathways are present in the cell: the cytosolic mevalonic acid (MVA) and the plastid localized methylerythritol phosphate (MEP) pathway, whereas the MVA pathway derived IPP is described to be the main precursor for the formation of the rubber chains.

In the present work, we describe the identification and functional characterisation of MVA pathway genes in the rubber producing plant *T. koksaghyz* and their specific expression

patterns in different tissues indicating the importance of TkHMGR1 in providing IPP in the latex for rubber synthesis.

In a second part one example is shown how metabolic engineering of the MVA pathway can be used to modulate the isoprenoid content in a plant system. Two derivatives of a truncated HMG-CoA reductase (HMGR) - described to be the key enzyme of the MVA pathway - were heterologously expressed alone in *N. tabacum* and in combination with a farnesyl diphosphate synthase (FPS) - another described high turnover enzyme processing IPP to subsequent products - and the metabolic changes in the plant were presented.

4. Genetic studies of natural rubber biosynthesis in *Hevea* and dandelion

Stephen B. Ryu and Yong Jik Lee

Environmental Biotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon 305-806, Korea

Abstract: The *Hevea brasiliensis* tree is currently the only commercial rubber producing crop. Because of the poor genetic variations, there is always a potential danger of crop failure with diseases. *Hevea* also has another fundamental problem of life-threatening allergy caused by the proteins in the latex. It is, therefore, highly desirable to develop alternative rubber crops that produce a high quality rubber without allergy. Most candidates for the alternative rubber crops have some drawbacks such as relatively shorter length of rubber polymer and/or lower rubber production compared to the *Hevea*. In order to make them commercially viable rubber crops, it is required to either increase the size of rubber polymer or improve rubber production. We propose to genetically manipulate the pathway of rubber biosynthesis by introducing functional genes and/or by blocking the expression of certain genes. We are investigating the gene(s) that determine the rubber polymer size or rubber production by transforming plants such as Russian dandelion and sunflower with potential genes. Ultimately we aim to apply the basic technology to an alternative target crop and produce high quality natural rubber without allergy.

5. Biochemical and anatomical analysis of alternative rubber crops

Hunseung Kang¹, Yong Jik Lee², and Stephen B. Ryu²

¹Division of Applied Plant Science, Chonnam National University, Gwangju 500-757, Korea

²Environmental Biotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon 305-806, Korea

Abstract: Fig tree (*Ficus carica*) has been studied for natural rubber biosynthesis activity, and anatomically compared with *Hevea* and *Ficus benghalensis*. The molecular size of the natural rubber from fig tree is about 190 kD. Major proteins in latex of fig tree were 25 and 48 kD in size and several proteins were below 20 and above 100 kD. The effect of EDTA and Mg²⁺ ion on in vitro rubber biosynthesis in fig tree and rubber tree suggested that divalent metal ion present in the latex serum is an important factor in determining the different rubber biosynthetic activities in fig tree and rubber tree. The micromorphology of rubber particles from fig tree was examined by electron microscopy in comparison with *Hevea* and *F. benghalensis*. Rubber particles in the three different plant species investigated share some degree of similarity in architecture.

6. Linkage mapping in *Taraxacum* using AFLP and COS and SSR markers

E. Ritter, M. Hernandez, N. Remondegui, M. Arias, K. Huvernaars, P.J. van Dijk

NEIKER – Granja Modelo, Apartado 46, E-01080 Vitoria-Gasteiz, Spain, eritter@neiker.net

Abstract: In 2009 KEYGENE established a mapping population derived from a controlled cross between a high and a low rubber producing *Taraxacum koksaghyz* parent. In total 119 progeny plants were grown. AFLP analyses were performed with these materials using 20 primer combinations. A total of 763 polymorphic markers were scored.

On the other hand NEIKER evaluated over 100 COS and SSR primers from different origins in the mapping population. A linkage map using the mapping Software MAPRF6 was established. After producing initially individual parental linkage maps, an integrated map was generated based on a total of 62 common anchor fragments. This integrated map contains 448 displayed markers, has a length of 858 cM, and an average linkage group length of 107 cM. Individual linkage groups vary between 80 and 136 cM in length and contain between 42 and 70 markers each. On average a linkage group is composed of 23.1 P1-specific markers, 23.5 P2-specific markers, and 7.8 common markers, summing up to 56 markers per linkage group. In addition, a total of 208 so-called RF0 markers (i.e: markers which are linked with a recombination frequency (RF) of zero to other mapped markers) were mapped. Furthermore, 47 so-called associated markers were determined. These markers do not fit precisely in the existing framework maps (probably due to scoring errors), but they show reduced RF values (<10cM) with other mapped markers and therefore they are “associated” to them. Thus, the actual marker number in the final integrated map of this cross including RF0 and associated fragments is 703 with an average of 88 markers per linkage group. A total of 62 (mainly distorted) Fragments could not be integrated into the linkage map. This linkage map and the corresponding mapping data were also used to integrate the segregating COS and SSR markers. Although many of these markers were monomorphic or even no amplification products were obtained with the corresponding primers, several COS and SSR markers could be integrated in the established linkage groups.

7. Proteins involved in rubber biosynthesis in *Taraxacum koksaghyz*

Andrea Hillebrand^a, T. Schmidt^a, C. Schulze Gronover^b and Dirk Prüfer^{a,b}

^aDepartment for Plant Biochemistry and Biotechnology, Hindenburgplatz 55, Westphalian Wilhelm's University Muenster, 48143 Münster, Germany

^bFraunhofer Institute for Molecular Biology and Applied Ecology (IME), branch Münster, Hindenburgplatz 55, 48143 Münster, Germany

a_hill05@uni-muenster.de; Tel.: 0049-251-8324716

Abstract: Natural rubber (*cis*-1,4 polyisoprene) is one of the most important raw materials in the world and nowadays the para rubber tree *Hevea brasiliensis* is the sole crop whose latex is used for commercial rubber production. As the global demand for natural rubber increases it probably can no longer be satisfied by *Hevea* rubber. Since the special characteristics of natural rubber, like enormous elasticity and capacitance, can not be mimicked by artificially produced polymers, there is a need for alternative rubber crops.

Amongst others *T. koksaghyz* has been considered as a potential alternative rubber source due to its ability to produce large amounts of high quality rubber. The elucidation of rubber biosynthesis and proteins involved in this process in *T. koksaghyz* is crucial to improve rubber harvest and production. In *H. brasiliensis* a *cis*-prenyltransferase is supposed to be responsible for polymerization of IPP subunits to form long chains of rubber, while small rubber particle protein and rubber elongation factor influence rubber biosynthesis in a supportive manner.

In *T. koksaghyz* we identified orthologous cDNAs encoding three *cis*-prenyltransferases (*TkCPT1-3*) and five small rubber particle proteins (*TkSRPP1-5*). The expression profile, localization studies as well as functional analyses implicate these proteins in rubber biosynthesis.

8. Approaches to increase biomass production in *Taraxacum koksaghyz*

Oliver Munt^a, C. Schulze Gronover^b and Dirk Prüfer^{a,b}

^aDepartment for Plant Biochemistry and Biotechnology, Hindenburgplatz 55, Westphalian Wilhelm's University Muenster, 48143 Münster, Germany

^bFraunhofer Institute for Molecular Biology and Applied Ecology (IME), branch Münster, Hindenburgplatz 55, 48143 Münster, Germany

o.munt@uni-muenster.de; Tel.: 0049-251-8324716

Abstract: The dandelion *Taraxacum koksaghyz* produces high quality natural rubber and could be therefore used as a potential alternative rubber producer which can be cultivated in moderate climate zones all over the world. Since the plant itself is rather small it is necessary to increase plant biomass for optimum rubber yield. The increase of root biomass is of special interest since the root is the major latex storage organ.

The growth conditions for highest possible biomass production, the nutritional needs and especially nutritional compositions that will lead to higher plant biomass production have been determined. Further approaches for higher biomass output include plant cultivation in the field and manipulation on molecular level.

9. Valorisation of latex of the pulp of argan fruit (*Argania spinosa*)

D. Pioch, S.Palu (1), A. Collet (2), F. Bonfils, C. Char (3)

UMR GPEB (CIRAD/UM2/UM1) (1), ICG/UM2 (2). UMR IATE (Cirad,UM2,INRA/SupAgro) (3)

Abstract: Argan, tree (*Argania spinosa*) belongs to the Sapotacea family. It is a perfect tree for harsh environment, surviving heat, drought and poor soil. It is an endemic species of south west Morocco. Argan takes the place of the olive as a source of forage for millions of goats, as cooking-oil, as a source of energy and home-furniture, but more and more for cosmetic applications. The argan oil is extracted from the nut. The argan tree is part of 2.000 species of latex plants that produce natural rubber or polyisoprene with a cis 1.4 (*Hevea*, *Parthenium argentatum* or *Taraxacum kok-saghyz*) or a trans 1-4 stereo-isomer (gutta percha). Battino (1929) was the first to isolate latex from the argan fruit pulp and showed it was a trans-isomer. Sandret (1957) showed that the latex of argan fruit contains rubber at a concentration of 0.11 to 0.48% (fresh pulp weight). Fellat-Zarrouck et al, (1987) have shown that the polyisoprene of the argan pulp is either of the cis and trans isomer, but that cis form was the main rubber polymer. Within the project RARGA PROD 2, CIRAD and Agropolis have studied the valorization of the argan pulp, and mainly the rubber fraction

10. Comment satisfaire la demande de caoutchouc naturel, à laquelle le latex tiré de l'hévéa ne suffit plus ? En améliorant deux plantes: le guayule et le pissenlit russe Du caoutchouc naturel en Europe

Serge Palu, Daniel Pioch

UMR Génie des procédés Eau Bioproduits, CIRAD, Jean Jacques Perrier, revue Pour La Science

Production of natural rubber in Europe ? How to comply with demand in natural rubber, that *Hevea* cannot meet due to increasing needs? By improving two latex plants: Guayule and Russian Dandelion. *Hevea brasiliensis* plantations mainly in Asia, will not cover the growing world demand for natural rubber. In Europe, France, Germany, the Netherlands, and Spain, palm to produce it not by adapting *Hevea* to temperate climate, but by growing two plants producing of rubber with identical properties to standard *Hevea* rubber, the first a bush from Mexico, GUAYULE (*Parthenium argentatum*), and the second a herb from Kazakhstan, Russian dandelion (*Taraxacum kok-saghyz*).