### CIRAD

### French Agricultural Research Centre for International Development

### BIOWOOEB

### Biomass, Wood, Energy, Bioproducts research unit

# DEVELOPMENT OF INNOVATIVE ALTERNATIVE CROPS FOR THE PRODUCTION OF NATURAL RUBBER



#### **INTRODUCTION**

The last conference organized by CIRAD on the same topic was in October 2010 in Montpellier as part of the European EU-PEARLS project. The 2019 international conference is open to researchers, farmers, government institutions, industrialists and students with an interest in natural rubber (NR) alternatives, guayule (*Parthenium argentatum*) and Russian or Kazakh dandelion (*Taraxacum kok sagyz*), and their potential commercial development worldwide. Europe imports 1.2 million tons of hevea NR each year and is dependant on Asia for 90% of its NR import. NR is listed in Europe as a critical material, which justifies an interest in developing over the next 5 to 10 years, new agricultural commodities such as guayule for mediterranean or semi-arid climates and Kz dandelion for continental climates.

The Agriculture and chemicals industries are undergoing a bio-revolution. They both face constraints linked to the depletion of fossil resources, the need to reduce energy consumption and CO2 emissions, global warming and increased pressure on water accessibility. They must continue to meet the growing needs of society while at the same time addressing the great global challenges of the future. They must develop more environmentally friendly and sustainable alternative process, to meet the future needs of humanity (food, energy, medicines and hygiene ...) while respecting our environment (COP21). They must move towards using more renewable resources, towards bio-based polymers within a biorefinery concept.

Chemistry, before the advent of petrochemicals, was a chemistry of renewable resources, and polymers before synthetics were natural. Thus, latex which first appearance in south America is one of the oldest natural polymer materials known to civilization. Latex was brought to Europe in the 15th century by the conquistadors. At the start of the 20th century, when there were blockades due to conflicts, the industry of synthetic elastomers developped. Today,NR is still widely used and represents 39% of the worldwide rubber demand. The NR demand which was 12 million tons in 2018, is expected to reach 17 millions tons in 2025, with a growth rate of 5.3% for tyres and 3.5% for the gloves. Natural poly-isoprene is indispensable for tires for our airplanes, our tractors and our trucks.

The production of hevea rubber from Southeast Asia (95% of world production) could be threatened by a fungus (*Microcyclus Ulei*) which could spread from South America to Africa and Asia. Hevea plantations are at risk from the oil palm industry, which is more lucrative. Hevea latex has allergenic properties which affect many people. Chemistry and biology must find new ways to meet the global demand, and develop opportunities to re-locate production of NR latex to semi-arid developing countries.

To support and encourage these efforts, CIRAD and its partners (Languedoc-Roussillon Occitanie Region, Agropolis Fondation,...) wish to play their part by organizing this conference. I am convinced that there is a strong convergence between the objectives of researchers, farmers and industrialists. The concept of "sustainable development" that brings us together during the conference will provide a positive direction for the evolution of our society. Faced with the major challenges affecting the future of our planet (climate change, threats to biodiversity and water resources), we must find practical and pragmatic solutions, adapted to the territory and to the different patterns of consumption. Thus the challenge we face with regard to bio-based products and renewable resources.is to bring supply to meet demand in the context of sustainable development. The development of environmental technologies based on the two alternative rubber plants will create the jobs of tomorrow.

I hope you will make use of this conference, to meet the actors of the two industrial crops, to learn more about guayule and Kz Dandelion for future diversification of the agriculture of tomorrow, but also I encourage you all to take advantage of these three days to develop some concrete solutions and take some time to visit our beautiful region of Occitanie.

The aim of the conference is to bring together academics, farmers, end-users, government and private agencies, and university students to discuss the interest in developing two major alternative sources of natural rubber (NR): guayule (*Parthenium argentatum*) a shrub native to Mexico, adapted to semi-arid and Mediterranean climates, and Russian dandelion (*Taraxacum kok sagyz*), native to Kazakhstan, adapted to continental climates. Interest in the two plants has increased around the world. Chemistry, before the advent of petrochemicals, was a chemistry of renewable resources. Polymers before synthetics were natural. NR is still widely used. The demand for NR was 12 mt. in 2018 and is expected to reach 19 mt. in 2025, with a growth rate of 5.3% for tires and 3.5% for gloves.

To support and encourage these efforts, CIRAD and its partners wish to play their part by organizing this conference. There is a strong convergence between the objectives of researchers, farmers and industrialists. The concept of "sustainable development" that will bring us together during the conference will provide a positive direction for the evolution of our society. This conference will provide information on recent developments of research projects worldwide. Development experts, agri-chain professionals and innovators are all encouraged to share their experience and interest for the two plants. Academics working on other potential latex plants with interesting new co-products are also welcome.

Discussions and presentations are expected to cover the following topics:

- 1. Agriculture and genomics,
- 2. Processing and extraction,
- 3. Bio-refinery and co-products,
- 4. Economics and sustainability.

Information is available on the conference website at https://diacpnr.sciencesconf.org/.

### **Sponsors**















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- Ingrid M. VAN DER MEER, Wageningen University & Research, The Netherlands

# **KEYNOTE SPEAKERS**

# **PLENERY SESSIONS**

#### Mrs Fazilet Cinaralp (Belgium)

Keynote Speaker

*General Secretary of the European Tyre & Rubber Manufacturers Association* <u>http://www.etrma.org/</u>

#### Abstract:

The Rubber industry is well positioned to help the automotive value chain as well policy makers to meet sustainable mobility challenges.

New materials, greater transparency in the supply chain, resource efficient manufacturing and use, changing vehicle designs and needs, electrification, autonomous driving, circular economy, etc... lead to looking at 360° spectrum of opportunities. Alternatives to the critical hevea rubber is part of it! The Industry is advancing at fast pace with its own challenges and many more innovations and opportunities.

Subject: oral Topics: sustainability of natural rubber Keywords: Guayule, automotive value chain, materials, innovations, alternative sources

### Dr Katrina Cornish (USA)

Keynote Speaker

#### Valorization and Scalability of Alternative Rubber through Properties and Production Process

The Ohio State University, Wooster, Ohio, USA

Guayule (Parthenium argentatum) and rubber dandelion (Taraxacum kok-saghyz) face similar commercialization challenges with respect to scalability. It is impossible to produce rubber from either crop on a small scale at a cost or volume that would permit entry into the commodity natural rubber market currently served by rubber from Hevea brasiliensis. Guayule rubber has physical properties distinct from *H. brasiliensis* rubber which can be valorized and access high value niche markets. Current progress will be described. However, dandelion rubber from field-grown plants is similar to H. brasiliensis rubber which makes valorization for niche markets more challenging. Also, most of the rubber coagulates in the roots while the plants are still alive. Indoor hydroponic production allows production of dirt-free rubber, and may affect the relative proportion of latex and solid rubber in living roots, which will affect process selection. Also, we are investigating the impact of multiple root harvests from the same plants on rubber quality, including the amount of gel. This approach may identify value added niche markets for this rubber.

Subject: oral Topics: guayule and R. dandelion Keywords: Guayule, Partheniun argentatum, Rubber dandelion, Taraxacum kok-saghyz

#### David Shaw (UK)

#### Why the tire industry needs alternatives to Hevea-NR

#### David Shaw<sup>1</sup>

1: Tire Industry Research - <u>Website</u> 34 Marchmont Road Wallington Surrey SM6 9NU - United Kingdom

Natural rubber (NR) is a critical material for the world's tire industry. Current analysis of supply and demand suggest a structural overcapacity until the mid- to late 2020s, and then a structural shortage. This shortage will become critical by the early 2030s, unless there is significant planting of *hevea Brasiliensis* trees in SE Asia. That looks unlikely.

Furthermore, the impacts of climate change present risks for the current planted area of h. *Brasiliensis* in terms of extreme weather, droughts and floods, and also in terms of sustainable growth conditions. The tire industry uses rubber from natural sources, such as *h Brasiliensis* and synthetic sources, derived from fossil resources.

Recent technical demands in the passenger car tire sector – driven largely by legislation around the world – have pushed the synthetic rubber industry and its allies in the carbon black and silica sectors, to develop new materials and compounds that go a long way to meeting the technical requirements of the tire industry.

Meanwhile, the established hevea-NR community has stood by and made no significant technical improvements, nor has it listened to the requirements of the tire industry.

The world's leading tire makers are desperate to build a portfolio of alternatives to Hevea-NR that can be employed to manage the risks associated with a structural shortage of NR a decade, or so.

This paper examines the background to this potentially-disastrous situation; reviews the different technologies that the tire makers are bringing to bear; analyses their strategic plans and offers some insights into the development of these alternatives in the coming decades.

The portfolio of alternative solutions examined in this paper include:

- Cutting weight and resource per tire
- Improving SR-based compounds
- Developing better synthetic polyisoprene
- Different mixing techniques for better dispersion
- Improving the wear-performance of tires.
- Developing material sources derived from end-of-life tires
- Developing alternative NR sources: TKS and guayule
- Improving the production technologies and reporting at NR factories
- Spreading the risk away from S E Asia
- Engaging with NGOs and others who are active in NR growing regions
- Improving the wear-performance of tires.

Clearly, the development of TKS and guayule play a significant role in this strategic development, but the paper attempts to put these developments into a wider perspective of tire makers' efforts to manage risks of shortages of NR over the mid- to long term.

#### Subject: oral

Topics: Economy, environment

Keywords : Tire ; strategy ; risks ; risk management ; legislation ; vehicles ; Guayule ; Dandelion ; isoprene ; supply ; demand

#### Sophia ALAMI (France)

Guayule (Parthenium argentatum).: Towards a model of sustainable bio-sourced value chains that contribute to the agro-ecological transition

Sophia ALAMI<sup>1</sup> and Hassan BOUKCIM<sup>2</sup>

(1) AGAP, Montpellier Univ, CIRAD, INRA, INRA, SupAgro –F 34398 Montpelier-France (2) President and CEO VALORHIZ

Agriculture is often pointed out as a contributor to environmental disruption. However, it offers the means to mitigate climate change and build innovative, resilient and productive agroecosystems. These multifunctional ecosystems would enable diversified and sustainable exploitation of natural resources, while preserving and restoring ecosystem services. The condition would be the design of an innovative alternative model of sustainable and restorative multifunctional agriculture. The French economic social and environmental council (the consultative assembly « CESE ») calls for the integration of sustainability goals to those of added value to achieve a sustainable bioeconomy. A bioeconomy which implements agronomic practices that preserve and maintain soil fertility while respecting biodiversity1 A paradigmatic innovation is therefore necessary for the emergence of a new model of a so-called sustainable and responsible bioeconomy.

1 Vers une bioéconomie durable Jean-David Abel et Marc Blanc, Avis du CESE(Conseil économique, social et environnemental) Mars 2017

https://www.lecese.fr/sites/default/files/pdf/Avis/2017/2017\_08\_bioeconomie\_durable.pdf

2 FAO 2018. 10 elements of agroecology that can guide us toward sustainable food systems

*3 HLPE. 2019.* Agroecological and other innovative approaches for sustainable agriculture and food systems that enhance food security and nutrition. A report by the High Level Panel of Experts on Food Security and Nutrition of the Committee on World Food Security, Rome 2019. Full report forthcoming at www.fao.org/cfs/cfs-hlpe.

This communication is the result of a reflexive approach to a cluster of projects aiming at the construction of a guayule production and processing sector in the South of France. This exercise offers an opportunity to draw from research projects finished or under way, a generic methodology to develop innovative agricultural value chains that are part of a performing, shared (multi- stakeholders) and sustainable bioeconomy. Sobriety and effectiveness will aim at the recyclability and the optimization of the use of resources at the level of the territory with, ideally, a multi-valuation of the products and by-products, according to the principles of circular economy and biorefinery. We hypothesize that the principles of agroecology as defined by FAO2 and HLPE3 are building blocks for a sustainable transition bioeconomy. We mobilize them to identify sustainability components using a systemic approach that brings together production, supply and processing of bioresources, product valuation and ecosystem maintenance activities within the territories. The creation of a network of stakeholders for the co-construction of the sector on the territory is highlighted as well as the circular economy features. Through this analysis of creation of an innovative sector of production and valuation of Guayule, we demonstrate (evidence base) the possibility of building a sector according to agroecological principles. The approach is based on systemic integration of the different modules of the value chain to satisfy, in fine and jointly, the very often opposed objectives of productivity, profitability, sustainability, health, equity.

Subject: oral

Topics: guayule

Keywords: Guayule, value chains, bioeconomy, sustainability, innovation, resilience, climate change, agroecology, systemic approach

#### **D.Pioch (France)**

#### History of research activities on guayule (Parthenium argentatum) at Cirad since the 1980s.

#### S.Palu, D.Pioch, T.Chapuset<sup>1</sup>

1-The French agricultural research and international cooperation organization working for the sustainable development of tropical and Mediterranean (CIRAD)

Interest for guayule (*Parthenium argentatum*) in France started in the 1950s at the French Rubber institute (IFC), in parallel with research on *Hevea Brasiliensis* in Indochina. With the first oil crisis in the 1980s, the Rubber research Institute in Africa (IRCA) continues the researches on Hevea in Africa for IFC, and also started a programme on guayule in Morocco and in West Africa (JB Serrier). Interest for guayule continued but at a smaller scale with the creation of CIRAD in 1984 and was then abandoned in the 1990s. A new interest started in 2008, when the CIRAD and 17 European partners worked on a KBBE/FP7 European project (EU-based Production and Exploitation of Alternative Rubber and Latex Sources (FP7, P212827) until 2012. The EU-PEARLS project started a growing interest in Europe for alternatives sources of natural rubber based on guayule and Kazakh dandelion (*Taraxacum kok saghtz*). With the collaboration of tyres companies (Continental, Vredestein/Apollo, Bridgestone, CooperTires and Yulex), guayule and dandelion were developed at an industrial scale. In case of a shortage of NR expected in the coming years, both innovative alternative crops could have a large scale development, as NR has been labelized by Europe as a critical raw material.

Subject: oral Topics: guayule Keywords: history, development experiment in southern countries

#### **Dr.M.Dorget** (France)

#### **Towards a European Guayule Rubber Supply Chain!** M.Dorget

CENTRE DE TRANSFERT DE TECHNOLOGIE DU MANS Pôle Matériaux 20 rue Thalès de Milet 72000 LE MANS

Nearly 93% of the commercial natural rubber (NR) is produced in Asia from the rubber tree. Whole world is therefore dependent on a single plant mainly grown in one area. Europe has clearly identified this and targets at least 20% of self-supplied rubber. European Tire Rubber Manufacturers Association (ETRMA), and companies such as VERSALIS and MAPA support this approach. Among the envisaged solutions, the use of other lactiferous plants is proposed: dandelion and guayule, adapted to continental and Mediterranean European climates, respectively.

Subject: oral Topics: Economy, environment Keywords: profitable and sustainable guayule latex

#### Dr Ingrid van der Meer (The Netherlands) Keynote speaker

#### Development of Russian dandelion into a crop for natural rubber production

#### Dr. Ingrid M. van der Meer

Wageningen University & Research, The Netherlands

The EU-funded project DRIVE4EU, Dandelion Rubber and Inulin Valorization and Exploitation for Europe, coordinated by Wageningen University & Research, was aimed at the development of the production chain of natural rubber and inulin from *Taraxacum koksaghyz* (TKS or Rubber dandelion). In total, 13 partners from academia and industry joined forces in this demonstration project (<u>http://drive4eu.eu/</u>). The objective of the project was to set up a European chain for the production and processing of natural rubber and inulin, enabling the EU to become less dependent on the import of natural rubber and at the same time to respond to the threat of a global rubber shortage. The combination of rubber and inulin makes Rubber dandelion very interesting as a production platform for two biobased materials, because inulin can be used as raw material for furan-based polymers.

The main activities and results of DRIVE4EU will be discussed:

- Breeding of plant genotypes with high root biomass, high rubber and inulin yield,
- Optimized cultivation and harvest methods for Rubber dandelion, and setup of large scale demo field trials,
- Ecological analysis of the gene flow between *Taraxacum koksaghyz* and other, wild, dandelion species,
- Scaled-up and optimized extraction and refinery protocol for natural rubber and inulin from Rubber dandelion,
- Testing and application of natural rubber from Rubber dandelion in end product uses, and
- Demonstration of the economic viability of the DRIVE4EU production chain for natural rubber and inulin.

#### Dr. D.A. DIERIG (USA) Keynote speaker

### DEVELOPMENT OF GUAYULE AS A NATURAL RUBBER SOURCE IN THE U.S. BY BRIDGESTONE

1 : D.A. DIERIGI, V.M.V. CRUZI, G. WANGI, AND, W.S. NIAURA2 Bridgestone Americas, Inc., Agricultural Operations, Guayule Research Farm, Eloy, AZ 85131 - United States

There are many challenges to overcome for guayule (*Parthenium argentatum*, Gray) to become a commercially viable crop. Some of the improvements needed include higher rubber content and yield, faster/earlier phenotypic evaluation for breeding, agronomic management, and market development for coproducts. Since 2012, Bridgestone has become the industrial leader and the first tire company to make significant investments toward research and development as a supplemental source of natural rubber. For the first time in guayule history, a major company is leading development rather than another government-led effort. A 3-year joint research and development effort was initiated between Bridgestone Corporation and Versalis SpA in 2018. Government funding has strengthened this effort through a Coordinated Agriculture Project (CAP) grant from U.S. Department of Agriculture, Sustainable Bio-economy for Arid Regions (SBAR). This presentation will address how Bridgestone is participating; and how Bridgestone is proceeding with a research initiative to address the challenges that must be overcome to reach mass production.

Subject: oral Topics: Agronomy Keywords: guayule breeding , agronomy

#### **Helge Fluess (Germany)**

## Development of population genetic tools supporting the breeding progress of Russian dandelion

<u>Helge Fluess</u><sup>1</sup>, Roland Geyer<sup>2</sup>, Marie Eggert<sup>3</sup>, Katja Thiele<sup>3</sup>, Brigitte Ruge-Wehling<sup>1</sup> 1: Julius Kühn-Institut; Institute for Breeding Research on Agricultural Crops 2: lifespin GmbH 3: Julius Kühn-Institut; Institute for Biosafety in Plant Biotechnology

Russian dandelion (Taraxacum koksaghyz, Tks), with its ability to produce and store high quality rubber in its roots, appears to become one of the most promising alternative resources for natural rubber and has raised the interest of many researchers and rubber processing industries.

In order to establish TKS as a sustainable resource crop for natural rubber and offer at least a complementary alternative to rubber from Hevea tree plantations, many projects are dealing with questions in genetics, genomics, proteomics, agronomy, processing and technical issues. As part of the TAKOWIND II project, funded by the Federal Ministry of Food and Agriculture, we focus on breeding research by developing molecular markers and population genetic tools in order to support the breeding progress of Tks.

On that account, we provide genetic background information on several accessions of Tks and other Taraxacum species such as T. brevicorniculatum and T. officinale through AFLP and SSR-marker analysis. One of our major goals though, was the development of a high resolution genetic map based on a mapping population segregating for rubber content. In order to develop sufficient SNP-markers for genetic mapping, a Genotyping-by-Sequencing (GBS) approach was applied. Additionally, AFLP and SSR markers were also included in this map. In order to identify genomic regions that are related to high or low rubber content, field trials with the cloned mapping population were performed over three years and at three different locations. Rubber contents of the complete mapping population have been quantified for all years and locations by a specific NMR-protocol (lifespin). Based on this phenotypic data and the established genotypic data, we apply a QTL-analysis for rubber content and incorporate identified QTL in the genetic map of Tks. Furthermore, RNAseq and MACE (Massive Analysis of cDNA Ends) analysis have been applied with the parents of the mapping population and respective bulks of the offspring with high vs. low rubber contents. The identified differential expressed transcripts in combination with known gene sequences of the rubber synthetic pathway are used as basis for the evaluation of trait-associated molecular markers. In total, we developed a set of different marker resources genome-wide (GBS/SNP, AFLP, SSR) as well as transcript-based, which can be applied in ongoing breeding programs and expand the genetic background knowledge of Tks in general.

Subject: oral

Topics: Agronomy

Keywords: Russian dandelion ; breeding ; molecular markers ; QTL ; analysis ; genetic mapping

#### **Dr.Frédéric Bakry (France)**

## Adaptation of the guayule to Mediterranean climate: characterization and enhancement of genetic diversity

<u>Frédéric Bakry</u>, Chloé Cotard, Bénédicte Favreau, Najate Maghnaoui, Thierry Quaak, Hassan Boukcim, Julie Leclercq<sup>1</sup> 1: UMR AGAP-BURST, CIRAD

The objective of Agroguayule project (co-financing ADEME, Call for projects GRAINE) is to select lines that combine good adaptation to the Mediterranean climate (dry and cold), high vegetative vigour and a high concentration in natural rubber. Guayule varieties are mainly apomictically propagated. The descendants are homogeneous. However, since apomixis is facultative, there is some diversity in the descendants.

In our studies, we started with a very low initial genetic diversity (5 cultivated varieties, uncultivated diploids and close relatives of P incanum). First of all, we checked the ploidy of the accessions available as well as their pollen fertility. In complement, we looked for and characterized off-types in the progeny of these 5 cultivated varieties.

On nearly 100 selected off-types, we observed a high variation of ploidy levels (3X to 7X) compared to the initial cultivars. Our preliminary observations on agro-morphological features of these off-types (growth, foliar development, plant habit...) have shown that there is no relationship between the level of ploidy and plant vigour. These results raise questions about the formation of polyploid gametes (2N) in the guayule. This question will be addressed by comparing parental and polyploid descendant genotypes.

Additional studies on parents and the most outstanding offsprings are ongoing in a plant-byplant using analytical chemistry, fingerprinting metabolomics and SPIR spectroscopy. First, overall classification of plants will be performed. Then, we will focus on rubber biosynthesis according to seasonality, distribution and concentration in plants (bark, leaf, flower, wood) according to agro-climatic conditions.

The outputs of this work will contribute to better design and implement a seed field. It also aims to select varieties that are more productive and better adapted to the cold and water deficit conditions characteristic of the Mediterranean climate.

Subject: oral Topics: Agronomy Keywords: guayule; abiotic stress; ploidy; phenotyping

#### Dr. Anvar Buranov (Canada)

## Development of sustainable and hypoallergenic latex from Taraxacum kok-saghyz (TKS) Anvar Buranov $^{\rm 1}$

1: Nova-Biorubber Green Technologies Inc - Website

27266-15350 Fraser Hwy, Surrey, British Columbia. www.novabiorubber.net and www.novabiorubber.com - Canada

Sustainable and hypoallergenic latex can be produced from annual rubber plant Taraxacum kok-saghyz (TKS) worldwide. It takes only 4 months to grow TKS. TKS contains up to 24% latex/biorubber and 40% inulin. Domestic production of latex from TKS can save significant transportation costs and provide thousands of job opportunities in agriculture, processing and manufacturing of latex products.

NovaBioRubber has produced and tested latex and biorubber from TKS in Canada. NovaBioRubber products such as TKS plants, green extraction proces, proprietary rubber extractor, biorubber, latex and inulin were tested by the third parties extensively. Third party evaluation reports will be presented. Preclinical tests indicate latex from TKS is hypoallergenic. Hypoallergenic latex can be used for the production of medical gloves, catheters, condoms, latex mattress, pillows, toys etc. Solid biorubber and inulin are also produced for manufacturing rubber and food products.

The goal of any processing technology must be to extract biorubber and latex as pure as possible at lower cost without altering quality. Some processes cause significant physical and chemical changes in the quality of biorubber. Production of high quality biorubber at lower cost is critical in order to produce competitive biorubber for various applications. Also, environmental impact of these technologies should be carefully evaluated before choosing a process for commercial activities. NovaBioRubber has already has patented and demonstrated a "green" extraction process and proprietary processing equipment for biorubber extraction from rubber-bearing plants.

Currently, NovaBioRubber is conducting the field trials to grow TKS on various farms in British Columbia, Canada. Biorubber content in TKS plants reached 17% after growing just 4 months. It is important to choose the right climate and right farm for good yields.

National Research Council Canada tested green biorubber extraction process, prototype equipment, and TKS plants grown in Canada for 8 months. Latex from TKS have also been tested extensively and confirmed to be safe due to the lowest amounts of antigenic proteins similar to lettuces.

The third party evaluation reports are available from National Research Council Canada and Akron Rubber Development Labs. The results will be discussed.

Subject: oral

Topics: Agronomy, process, products, economy, environment

Keywords : Taxacum kok ; saghyz ; biorubber ; latex ; inulin ; green process ; rubber extractor ; crop production; third party evaluations

#### Kamila Magzieva (Kazakhstan)

#### Industrial agenda of Kazakhstan. Industrial cultivation of dandelion Taraxacum Kok-Saghyz and production of natural rubber in Kazakhstan – KZ-RUBBER", Dr. Kamila Magzieva, Kok-Saghyz TM LLP, Kazakhstan

Dandelion Taraxacum Kok-Saghyz Rodin (TKS), the perennial rubber plant, Asteraceae family, is Kazakhstan endemic, introduced in the "Red Book" of Kazakhstan under №251. The area of natural habitat – the eastern Tien Shan, southeast of Almaty region. In the period from 1931 to 1960 was the source of production the natural rubber (NR) in the USSR. More than 500 thousand hectares were sown in 15 regions of the USSR, 300 plants produced NR for the automotive and light industry. In 1960, the production of TKS NR stopped because of the unwillingness of the Soviet Union to invest in mechanization of TKS treatment. Up-to-date worldwide studies have shown that TKS is a real alternative to Hevea Braziliensis and that dandelion treatment is much cheaper and more effective than rubber trees.

All research activities of EU-PEARLS project, as well as demonstration actions of its successor Drive4EU, have been realized thanks to the non-domesticated TKS species, which provided to the project by the Kazakh Government in accordance to its Decree No. 1046 from 09.07.2009, exclusively for the research purposes. The Drive4EU project was success in breeding higher yields and improved cultivation and harvesting as well as scaling up extraction. In accordance of the formal request of the Kazakh Government, coordinator of the Drive4EU sent information on all results of the project to the Kazakh Foreign Ministry by the end of 2018. KZ-RUBBER, which is a successor to two EU-funded projects ("EU-PEARLS" and "Drive4EU"), and "KZ PEARLS" project funded by the Kazakh Government, represents the commercial phase of rubber production from the TKS Dandelion. Kazakhstan is the perfect location for the future home of the New Rubber Economy because the TKS dandelion is the Kazakh endemic well adopted to its climate conditions. As such, Kazakhstan is poised to play a strategic and vital role in the commercial development of TKS-derived rubber. The project will also ensure that Kazakhstan country is a leader in Sustainability and Green Industry Innovation in Central Asia. Furthermore, its strategic Eurasian location will enable the KZ-Rubber project to benefit from logistical/infrastructure networks connecting to Europe and Asia.

The Government of the Republic of Kazakhstan supports KZ-RUBBER project by the "Action plan (Road Map) on realization of the project «Industrial cultivation of Taraxacum Kok-Saghyz and production of natural rubber and other Taraxacum Kok-Saghyz dandelion derivative products in Kazakhstan» for 2019-2024" accepted on 8th April 2019. The project is also included in the Map of the industrialization of the country. The protocol decision of the Regional Coordination Council of the Almaty region No. 11 of December 26, 2018 recognized the project as an investment one and for the needs of the project more than 500 hectares of land are currently available in village Kapal of Aksu district in the Almaty region, followed by an increase in acreage and production areas. The Kazakh Foreign Ministry included the project in the list for investment projects negotiated with OPIC (USA) for the amount of 188 US\$.

The Almaty Regional Government is going to drive the growth and development of the sustainable natural rubber industry in Kazakhstan via supporting activities of Kok-Saghyz TM and its partners: KeyGene (The Netherlands), The Bassiouni Group (USA), and Kamizhan Ltd (Kazakhstan). Kok-Saghyz TM LLP (KSTM), based in Almaty, Kazakhstan, is leading the implementation of KZ-RUBBER project. Given its extensive experience in the EU-PEARLS, KZ-PEARLS, and Drive4EU projects, KSTM possesses unique knowledge and expertise required to establish TKS rubber production in Kazakhstan. KSTM is seeking partnerships with governments, international institutions, corporations and strategic investors as well as with the EU research community inviting them to join the project.

Subject: oral

Topics : Russian dandelion, economy, environment Keywords : dandelion, economy, new project

#### **E.Tardan**

#### Development of guayule (Parthenium argentatum) at Cirad, France after the EU-PEARLS project.

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Research activities on guayule started again in France in 2008 with the European project, EU-PEARLS. Guayule research activities have continued in France with implementation of several experimental fields after 2012, in the Languedoc-Roussillon region (LR) near Montpellier and near the town of Perpignan. The aim of the French research on guayule was; (i) to produce sufficient quantities of biomass to develop a latex extraction process; (ii) to study the plant growth and yields based on rubber and resins contents control by ASE and NIRS methods; (iii) to produce enough seeds to extend guayule fields as an innovative crops on abandoned wine lands; (iv) to interest farmers with guayule plantations. An experiment plot, of 0.25 ha with 2000 plants of five former USDA varieties used for the EU-PEARLS project, was planted from May 2014 and 2015 in Lansargues near Montpellier. The mortality, growth of plants and rubber and resin content were monitored for several years. Differences between five selected lines were compared depending on the type of soils, local climate, planting process, irrigation. Cirad and its partners have enough biomass accessible to study the development of a latex extraction process based on a CIRAD/CTTM/SATT international patent in 2018. The paper summarizes our research and development activities on guayule in France.

Subject: oral Topics: Agronomy Keywords: guayule ; field experiment ; process ; rubber

#### Dr.Federica Zanetti Keynote speaker

## Guayule rubber a strategic raw material for Europe with a long-term history within the AAIC (Association for the Advancement of Industrial Crops)

Federica Zanetti, Andrea Monti DISTAL - Dept. of Agricultural and Food Sciences, Alma Mater Studiorum, University of Bologna (Bologna), Italy

The world rubber production is for about 40% covered by natural rubber. Among plants sourcing natural rubber Hevea brasiliensis is by far the most important and only commercial species, in terms of supplied quantity. With the increasing number of applications of natural rubber and its volatile price the search for alternative crops able to grow also in other environmental conditions than tropical forest, like Hevea, has been the objective of studies in Europe and USA since the beginning of the 20th century. Among others guayule (Parthenium argentatum) was identified early after the first world war as a crop able to grow in some areas of the Southern Mediterranean region (i.e. southern Spain, and southern Italy), as well as the northern part of the Mediterranean Africa. Being an evolutionary product of the Chihuahua desert (Central America), the guayule plant has the characteristics that adapt it to those arid environments. The principal point of difference between guayule and other latex-producing plants seems to be that the latter have no capacity (or necessity) for storing rubber. On the other hand, guayule under conditions found in its natural habitat and simulated under cultivation practices has a very definite need and use for stored rubber in the form of an impervious sheath to minimize the loss of water by evaporation. For this reason, rubber concentration in the parenchyma cells guayule, mainly in the bark, can be as high as 40-50% making the whole plant sourcing up to 15-20% rubber, otherwise Hevea despite the outstanding biomass produced it can store about 1-2% of rubber on it. One advantage of guayule rubber is that its latex contains fewer proteins, both in terms of diversity and total amount, than the latex from Hevea, and this makes allergenic reactions to guayule rubber by consumers sensitized to Hevea rubber. Furthermore, in addition to rubber a certain amount of resin is formed in the guayule plant. Different from the rubber, the resin is formed in a definite system of resin-secreting glands, thus the two compounds never mix in the guayule plant. In guayule mechanical harvest is widely adopted thus the future uptake of this crop in Europe would be much quite easy from this point of view. In the AAIC (Association for the Advancement of Industrial Crops), which is an international science society bringing together scientists from all over the world a dedicated division on natural rubber and resin sources exists. Actually the AAIC predecessor organization, the Guayule Rubber Society, dated back in late 80's, was incorporated in the AAIC with the scope to foster and promote the production of natural rubber from the guayule shrub internationally.

Subject: oral, Keynote speaker Topics: Agronomy Keywords: guayule, dandelion, association AAIC

#### Dr. Sylvie Sabatier

### Modelling the Guayule plant growth and development with a Functional Structural Plant Model.

Sylvie Sabatier<sup>1,2</sup>, Marc Jaeger<sup>1,2</sup>, Nicolas Hemery<sup>3</sup>, Christ Mougani<sup>1,2</sup>, Ali Abed Alsater<sup>1,2</sup>, Philippe de Reffye<sup>1,2</sup>, Serge Palu<sup>3</sup>, and Loic Brancheriau<sup>3</sup> 1 AMAP, Univ Montpellier, CIRAD, CNRS, INRA, IRD, Montpellier, France, marc.jaeger@cirad.fr 2 CIRAD, UMR AMAP, F-34398 Montpellier, France 3 UR BioWooEB, CIRAD, Univ Montpellier, 34398 Montpellier, France

The Guayule (Parthenium argentatum, Asteraceae), is a small ramified tree native to the northern Mexico and southwestern United States. The guayule shows a growing interest in research and agriculture (Ray, 1993) due to its hypoallergenic latex properties (Taurines et al., 2019), and seems adapted to South France climate (Sfeir and al., 2014). However, the production itineraries in relation to latex production are still not assessed, and so far little studies were done on the plant structure and functioning. This study aims to propose a first FSPM of the species using the GreenLab model, calibrated from data issued from two varieties in different environmental conditions.

The studying methodology is first based on a qualitative architectural analysis (Barthelemy and al., 2007). Second, on the various axis typologies, the development and branching stochastic rules can then be retrieved from field internode distributions collections. Finally, the organ source and sink relations parameters can be fitted from dedicated dry weight measurements (Kang et al., 2018).

Experimental plots were hold south of France, close to Montpellier on two varieties CL1 and CLA1, with six environmental conditions related to density (9091 and 62500 plants per hectare) and hydric pressure (no stress, low stress and high stress). 50 plants per environmental conditions were measured. The sampling was optimized to the plant structure and to quantify the polyisoprene and resins contents. The guayule shows a sympodial development is composed of modules with terminal inflorescence. Its architecture corresponds to the Leeuwenberg's model (Hallé et al., 1978). The axes are constituted of successive modules. Over a year, the plant produces eight to nine successive modules. Studying the plant structure, we found out that the number of relay axis per module follows a binomial distribution. The modules are ordered from the plant base to the top. And these modules are composed of internodes whose number also follows a binomial law, which parameters are quite stable from one order to another. In the further modelling process, we thus did consider that the plant elementary unit was the module, called as a meta-phytomer.

Under this assumption, we summarized the total dry weight of leaves and internodes per module to build the axis organic series (Buis and Barthou, 1984). Field measurements issued from these two series constituted then a target to be adjusted by the structural functional GreenLab model (Kang et al, 2018) in order to calibrate the organ source parameters. An initial analysis calculated the strength sink of leaves and internodes in a context of free growth and analysed the differences between the two varieties. We are currently applying the methodology to assess the impact on the parameters of development and growth, the effects of planting density and irrigation.

This first modelling study hold on two varieties on the Guayule tree shows that the plant structure can be efficiently modelled using a simple module approach. The development parameters, defining the module number of phytomers and branching rules are nearly stable and close for both varieties under the various environmental conditions. First functioning parameters were also retrieved from the measurements. These parameters make it possible to obtain the first stochastic 3D simulations of the Guayule's growth and architecture for both varieties.

Subject: oral Topics : Keywords : guayule

#### Dr. O.Taugourdeau

#### Guayule cultivation as a sustainable leverage to rehabilitate urban brownfields

Hassan Boukcim<sup>1</sup>, <u>Olivier Taugourdeau<sup>1</sup></u>, Estelle Hedri<sup>1</sup>, Jerome Nespoulous<sup>1</sup>, Julie Berder<sup>1</sup>, Serge Palu<sup>2</sup>, Julie Leclerc<sup>2</sup>. 1: Valorhiz, 1900, Boulevard de la Lironde, PSIII, Parc Scientifique Agropolis, F34980 Montferrier sur Lez 2: CIRAD

Brownfields are a common species of modern urban landscape. Urban brownfields can have various origin (industrial, residential or commercial ...) but they often present similar issues: degraded soils (compaction, lack of fertility, pollutants ...), limited biodiversity and associated ecosystem services furniture, unwanted species (including humans) and vague future. Producing high value biomasses on these brownfields is a promising leverage to "use" these areas. Cultivating biomasses on urban brownfield will improve soil quality, increase ecosystem services furniture and prevent unwanted species installation. For this purpose, Guayule is a promising crop as it can be economically profitable on small areas (>1ha, Sfeir et al. 2014) and is able to grow on low fertility soils.

AgroGuayule project (ADEME-GRAINE cofounding) aims at breaking numerous technicaleconomical barriers to allow Guayule cultivation on brownfields. Pilot plantations were made across various brownfields to access the feasibility of guayule growing in various conditions. Several scenarios of soil and plant fertilizers are currently tested to estimate their cost-benefice balance.

During the talk, we will present the AgroGuayule projects aims and the first results from the pilot plantations and how they can be scale up at a national scale with an ecological engineering and agroecological perspective.

Subject: oral Topics: Agronomy Keywords: guayule ; field experiment ; process ; rubber

#### Dr. Teerasak Punvichai

# Effect of Supercritical CO2 and type of co-solvent for extraction of lipids and terpenics from Guayule biomass (Parthenium argentatum)

Teerasak Punvichai<sup>2</sup>, Daniel Pioch<sup>1</sup>, Serge Palu<sup>1</sup>, and Eric Tardan<sup>1</sup> 1 CIRAD, Centre de cooperation internationale en recherche agronomique pour le developpement, CIRAD/UPR114, France 2. University Prince of Songkla, Thailande

Guayule (Parthenium argentatum), a perennial crop under semi-arid climate, producing polyisoprene (Guayule rubber, GR) and resin, is the most probable alternative source of natural rubber. GR is similar to Hevea's. To date e\_orts aimed at marketing GR, while the bagasse (GB) (90%-dw, dry weight) left after water-based extraction remains underused. Thus Cirad is investigating a "green" process for extracting valuable compounds from GB, including lipids. After having obtained preliminary results, showing that a co-solvent is necessary in addition to SC-CO2, we now investigate the inuence of operating parameters.

Under SC-CO2, resin extract is higher with ethanol as co-solvent (12.1%-dw) compared to acetone (7.8%-dw), at temperature 35\_C and 300 bar, co-solvent ow rate 3mL/min, CO2 ow rate 34.4 g/min. Acetone-based extraction under pressurized conditions above boiling point (ASE-acetone) is used as reference method. The selectivity for aromatic carboxylic acids (cinnamic, p-anisic) initially linked to sesquiterpenes, is better with SC-CO2-ethanol compared to SC-CO2-acetone. The minor components are lipids rich in C18:2, C16:0, C18:3, in between cotton and soybean oils, although poorer in C18:1. The unsaponifiable contains sesquiterpenes, whose extraction is mainly dependent on pressure but not temperature with SC-CO2-ethanol. From these trials SC-CO2-ethanol shows a higher efficiency compared to SC-CO2-acetone, for extracting aromatic carboxylic acids as potential industrial feedstock.

Subject: oral Topics: guayule, resins Keywords: Guayule (Parthenium argentatum), Super-critical fluid, sc-CO2

#### **Dr Jean-Luc VERDEIL**

## "Imaging as a knowledge tool for guayule biomass: new insights into compartmentalization of metabolites of interest"

Guayule (*Parthenium argentatum*, Asteracea) is a rubber plant recently domesticated by humans who must benefit from innovative approaches to speed-up knowledge acquisition in order to fasten the mastery of its culture but also to optimize the biomass valorization processes. Imaging is an evolving discipline that today allows us to acquire knowledge about anatomy, histolochemistry or the localization of secondary metabolites. It allows to acquire a 3D vision of the biomass but also to understand cellular dynamics (*in vivo* imaging). In our talk, we will show how conventional histology, X-ray tomography and spectral analysis techniques in multiphitonic microscopy make it possible to have a good knowledge of the structure of guayule stems. We will also show how imaging approaches can allow to visualize and to understand the effect of the different steps of isoprene extraction and thus open new routes for the optimization of post-harvesting processings .

Subject: oral Topics: guayule, resins Keywords: Guayule (Parthenium argentatum), imaging, histolochemistry, tomography and spectral analysis.

#### **CHEN DONG/C.McMAHAN**

(by visio conference)

## Transcriptome analysis of guayule reveals rubber biosynthesis pathways' response to drought stress

Authors: <u>Chen Dong<sup>1</sup></u>, Grisel Ponciano<sup>1</sup>, Yi Wang<sup>1</sup>, Naxin Huo<sup>1</sup>, Yong Q. Gu<sup>1</sup>, Colleen McMahan<sup>1</sup>

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Guayule (Parthenium argentatum A. Gray) is a promising alternative species to Hevea brasiliensis for a commercial source of natural rubber (NR, cis-1,4,-polyisoprene), a strategic raw material necessary for national defense, modern transportation, and medicine. Previous studies have shown that guayule yields more rubber under various environment stress, including drought stress, under which guayule is naturally native habituated. At the molecular level drought stress results in differential expression of various metabolic pathways. A comparative transcriptome analysis of stembark tissues harvested in two-year-old guayule field plants following high and low irrigation treatments was performed to elucidate drought stress responses in rubber biosynthesis pathways as well as identification of drought stress related genes in guayule. A comprehensive transcriptome database was built using genome-guided and de novo RNA-seq assembly, following which, annotation and comparison of gene express was carried out. A total of 1475 differentially expressed genes (DEGs) were identified, with 796 up regulated and 679 down regulated under the water limited condition. Candidate secondary metabolite genes related to rubber synthesis, rubber particle associated genes, and genes related to drought stress response were selected and experimentally validated for their expression using q-PCR analysis. Rubber and resin yield were quantified, and plants subjected to drought conditions were found to produce more rubber. In conclusion, we report the identification of a large set of guayule cDNA unigenes from stembark tissues, providing insights into the genetic and molecular basis of rubber production and response to drought stress in guayule.

Subject: oral (visio conference) Topics: guayule, Keywords: transcriptome analysis,gene,drought

#### S. PALU

# Implementation of guayule experimental plots in Languedoc-Roussillon/Occitanie Region (Southern France) as an alternative crop for agricultural diversification for local farmers

Authors: S. Palu, E. Tardan, D. Pioch, N. Boutahar, A. Napoli<sup>1</sup> Centre international en recherche agronomique pour le developpement (Biowooeb, Cirad, France)

With the support of the European Agricultural Fund for Rural Development (FEADER) and the Occitanie-Region, a research program (2017-2020) has been implemented by Cirad (Biowooeb Research Uunit) with several farmers partners (SCEA Mas St Jean, BIO-ORB-PPAM, Claira city Town Hall, and GAEC Hautes-Coumes) and Chamber of Agriculture-66, to develop guayule cultivation in the area. The objectives of the project are: i) to transfer the results of five years of research on guayule by Cirad; ii) to set several experimental plots on abandoned former vineyards; iii) to select the pedo-climatic conditions under which guayule can be grown in the Mediterranean climate (Departments 34-Herault and 66-Oriental Pyrenees), especially with limited water resources; iv) to advertise and attract other farmers for initiating the development of this new alternative crop for the production of natural rubber and co-products in Europe; v) to produce and supply guayule biomass for natural rubber latex production under the frame of other research projects.

A description is given on the implementation of five experimental sites of 0.25 ha each, showing a range of soil types, climatic conditions and cropping techniques, from nursery production, field planting, survival vs planting season, down to the yield per hectare of biomass, rubber and resin. Five guayule lines selected during the former European project EU-PEARLS were investigated. Plant growth, and evolution of the rubber and resin content, were monitored during the 3 years of the project; suitable planting and harvest seasons were also investigated.

The obtained encouraging results will be used to assess the economic viability of guayule as an innovative biorefinery alternative for local farmers, keeping in view climate change and limited access to water for agriculture.

Subject: oral Topics: guayule Keywords: guayule experimentation, Occitanie, farmers

### **POSTER SESSION**

| Authors                                 | Title of abstract   | Thematic          |
|---|---|-------------------|
| Yield performance of wild               | Marie Eggert 1, Kai Eggert 2,   | R.Dandelion       |
| Taraxacum koksaghyz under               | Brigitte Ruge-Wehling 3, Helge  |                   |
| different N regimes in the              | Flüß 3, Katja Thiele 1  |                   |
| field                                   | 1: Julius Kühn-Institut, Biosafety in Plant Biotechnology,  |                   |
|   | Quedlinburg, Germany<br>2: Molecular Plant Nutrition group, Department of   |                   |
|   | Physiology and Cell Biology, Leibniz Institute of Plant   |                   |
|   | Genetics and Crop Plant Research, Gatersleben, Germany<br>3: Julius Kühn-Institut, Institute for Breeding Research on               |                   |
|   | Agricultural Crops, Groβ Lüsewitz, Germany  |                   |
| Identification of powdery               | Brigitte Ruge-Wehling 1, Stephanie  | R.Dandelion       |
| mildew resistant T. officinale          | Nehrlich 1, Helge Fluess, Katja   |                   |
| allowed new hybrid                      | Thiele  |                   |
| production                              | 1 : Julius Kühn-Institut; Institute for Breeding Research on  |                   |
| -                                       | Agricultural Crops<br>2 : Julius Kühn-Institut; Institute for Biosafety in Plant  |                   |
|   | Biotechnology   |                   |
| Competitiveness of Russian              | Ingo Uhlemann 1, Marie Eggert,  | R.Dandelion       |
| Dandelion (Taraxacum                    | Helge Flüß, Brigitte Ruge-Wehling,  |                   |
| koksaghyz) and its hybrid               | Katja Thiele 1  |                   |
| lines in native plant                   | 1 : Julius Kühn-Institute, Biosafety in Plant Biotechnology,<br>Quedlinburg, Germany  |                   |
| communities                             | 2 : Julius Kühn-Institute, Institute for Breeding Research on   |                   |
|   | Agricultural Crops, Groß Lüsewitz, Germany  |                   |
| Bioactive compounds from                | Giovanni Antonio Re <sup>a*</sup> , Maria Giovanna  | R.Dandelion       |
| leaves of Taraxacum kok-                | Molinu <sup>b</sup> , Giovanna Piluzza <sup>a</sup> Maria   |                   |
| saghyz                                  | Maddalena Sassu <sup>a</sup> , Anton Pietro   |                   |
|   | Stangoni <sup>a</sup> , Giuseppe Campesi <sup>a</sup> ,   |                   |
|   | Leonardo Sulas <sup>a</sup>   |                   |
|   | <sup>a</sup> National Research Council, Institute for the Animal<br>Production System in Mediterranean Environment, Traversa        |                   |
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|   | Sassari, Italy  |                   |
| The AIR project (Aerial Inulin          | <u>Marina Arias-Royo</u> <sup>1</sup> , Rolf Mank <sup>1</sup> ,  | R.Dandelion       |
| and Rubber)                             | Anker S_rensen <sup>1</sup> , and Peter J. Van Dijk <sup>1</sup>  |                   |
|   | 1 Keygene { Agro Business Park 90 6708 PW, Wageningen,<br>Netherlands   |                   |
| Bromatological composition              | Leonardo Sulas <sup>1</sup> <sup>*</sup> , Giuseppe Campesi,  |                   |
| in leaves of guayule grown in           | Giovanni Antonio Re <sup>1</sup> , Maddalena  | Guayule           |
| a Mediterranean environment             | Sassu <sup>1</sup> Tony Stangoni and Giovanna   | -                 |
|   | Piluzza <sup>1</sup>  |                   |
|   | <sup>1</sup> National Research Council (CNR), Institute for the Animal  |                   |
|   | Production System in Mediterranean Environment, Sassari,<br>Italy   |                   |
| Study of guayule bagasse                | <u>Napoli A.,</u> Boutahar N., Tardan E.,   | Guayule           |
|   | Valette J., Palu S., Picoh D.   | ···· <b>·</b> ··· |
| pyrolysis: Effects of pyrolysis         | UR BioWooEB - Persyst Department - CIRAD – France   |                   |
| parameters on its heat and mass balance |   |                   |
| mass barance                            |   |                   |

Yield performance of wild Taraxacum koksaghyz under different N regimes in the field <u>Marie Eggert</u> 1, Kai Eggert 2, Brigitte Ruge-Wehling 3, Helge Flüß 3, Katja Thiele 1
1: Julius Kühn-Institut, Biosafety in Plant Biotechnology, Quedlinburg, Germany
2: Molecular Plant Nutrition group, Department of Physiology and Cell Biology, Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany
3: Julius Kühn-Institut, Institute for Breeding Research on Agricultural Crops, Groß Lüsewitz, Germany

As in other countries of the temperate climate zone, in Germany, the Russian dandelion (*Taraxacum koksaghyz*, Tks) was recently re-discovered as a promising alternative to the tropical *Hevea* tree, producing natural rubber on a larger industrial scale.

Belonging to the widely spread Taraxacum species, Tks is assumed to be of low demand regarding nutrient supply. However, commercialization of dandelion rubber strongly relies on the increase of root yield. Greenhouse experiments suggested a strong positive response between root dry weight and increasing nitrogen (N) supply. Hence, we wanted to test whether recent yields of wild-type Tks stands can be improved by N fertilization. We conducted a field trial over two years on two different locations in Germany applying different types and doses of N fertilizers. Tks was established as a summer crop by direct seeding in April and harvest in the middle of October. N was applied in three different growth stages (field emergence, bud appearance, end of flowering). After harvest, root yield, rubber concentration and element concentration in roots of Tks was measured. There were no significant effects resulting from N application on Tks yield in either year or location, implying that N supply was sufficient for Tks under these environmental conditions. The effect on rubber concentration was not consistent. Element analyses via N analyzer and ICP-OES revealed the nutrient demand of Tks under the recent yield performance and revealed no demand for fertilization at the locations tested. In 2019, a third trial year, also with Tks x Taraxacum officinale hybrids in addition to Tks, was initiated to verify the results that N fertilization is not indicated on locations with similar soil conditions and with the current plant material.

Subject: poster Topics : Agronomy Keywords : Russian Dandelion ; Taraxacum koksaghyz ; fertilization ; yield ; element concentration Identification of powdery mildew resistant T. officinale allowed new hybrid production

Brigitte Ruge-Wehling 1, Stephanie Nehrlich 1, Helge Fluess, Katja Thiele

1 : Julius Kühn-Institut; Institute for Breeding Research on Agricultural Crops

2 : Julius Kühn-Institut; Institute for Biosafety in Plant Biotechnology

So far, Russian Dandelion (*Taraxacum koksaghyz, Tks*) has proven to be the most promising alternative resource crop for natural rubber next to the para rubber tree (*Hevea brasiliensis*). In accordance with focus on a sustainable bioeconomy, *Tks* attracts the attention of many researchers and rubber related industries.

One of the agronomic aspects in breeding of dandelion is the growing of interspecific hybrids that combine favorable agronomic traits from Taraxacum officinale, To, and high rubber content of Tks. To is characterized through high biomass but unfortunately To leaves response highly sensitive to powdery mildew. A sustainable management of the disease requires the selection of resistant To genotypes. A simple and reliable test technique has been recently established in our institute. The pathogen was sampled in our local field and has been identified as Podosphaera erigerontis-canadensis via BLAST analysis of fungal ITS regions. Due to the fact that Podosphaera erigerontis-canadensis is obligate biothrophic, the fungus has to be propagated on adult plants under greenhouse conditions. Determination of susceptible and resistant/tolerant plants was performed using six week old greenhouse plants. Infection was initiated by spraying the plants with fungus suspension of 1x105 conidia. 10 and 20 days after inoculation, the plants were scored on a scale of IR1 (resistant) to IR5 (susceptible). T. officinale (RT, triploid) which is known to be highly susceptible, reacts with 100% infestation in our experiments. Different accessions of Tks showed an intermediate infection ratio (IR3). Surprisingly we were able to select a diploid To accession that was symptomless after two repetitions. The resistant genotypes are currently used for intraspecific crosses with highly susceptible plants to produce segregating populations that are informative for genetic analyses. For marker development, a MACE analysis of resistant and susceptible plants will be conducted. The experiment aims to the selection of differential expressed transcripts which exclusively occur in the bulk of resistant plants. Those sequences will be checked for their linkage to powdery mildew resistance in Taraxacum.

Subject: poster Topics : Agronomy Keywords : Taraxacum officinale ; resistance ; powdery mildew ; genetic analysis ; breeding

## Competitiveness of Russian Dandelion (Taraxacum koksaghyz) and its hybrid lines in native plant communities

Ingo Uhlemann 1, Marie Eggert, Helge Flüß, Brigitte Ruge-Wehling, <u>Katja Thiele</u> 1 1: Julius Kühn-Institute, Biosafety in Plant Biotechnology, Quedlinburg, Germany 2: Julius Kühn-Institute, Institute for Breeding Research on Agricultural Crops, Groß Lüsewitz, Germany

Russian Dandelion (*Taraxacum koksaghyz*, Tks), the most promising source of natural rubber in temperate climate, originated from the Tian Shan Mountains in Kazakhstan and is therefore an alien species in central Europe. During the last years many efforts were made to establish Tks as a crop plant on increasing acreage. To prevent the possibility of dissemination followed by stable appearance of Tks in the environment, we tested the competitiveness of Tks in two plant communities adjacent to our field sites. Succession trials were established in a grassland cared by mowing several times per year and on a plot on arable land surrounded by conventional crops. In these trials, both established in spring, at the end of the season all Tks plants had disappeared on the plots and no seedlings were detected on the plots and nearby.

One strategy to increase fitness and biomass of Tks is producing hybrids of Tks with other more vigorous *Taraxacum* species. Hence, we generated several hybrid lines with triploid/apomictic individuals of *Taraxacum officinale* (To), as well as progenies with a diploid/sexual To accession. Description of taxonomic traits as well as flowering behavior, reproductive biology and ploidy level is ongoing for a number of hybrid lines. Hybrids show significantly increased biomass which is potentially correlated with higher competitiveness and therefore succession trials will be repeated with Tks-To-Hybrids starting this season.

Subject: poster Topics : Economy, environment Keywords : guayule, Russian Dandelion ; Taraxacum koksaghyz ; competitiveness ; hybridization

#### Bromatological composition in leaves of guayule grown in a Mediterranean environment

Leonardo Sulas<sup>1\*</sup>, Giuseppe Campesi, Giovanni Antonio Re<sup>1</sup>, Maddalena Sassu<sup>1</sup> Tony Stangoni and Giovanna Piluzza<sup>1</sup> <sup>1</sup>National Research Council (CNR), Institute for the Animal Production System in Mediterranean Environment, Sassari, Italy \*Corresponding author: <u>leonardo.sulas@cnr.it</u>

Guayule is a perennial shrub endemic to northern Mexico, which has received attention as an alternative source of natural rubber. As the guayule leaves represent a byproduct, their exploitation might contribute to improve the sustainability of whole rubber chain. In semiarid regions where guayule grows wild its leaves are grazed by sheep, goats and rabbits, even if information regarding bromatological traits are still missing. Native shrubs are essential dietary sources for ruminants in Southern Europe to alleviate feed shortages. It is important to investigate if guayule leaves might be an opportunity for feeding livestock in Mediterranean area. Within a research aimed at evaluating the guayule performances, our objective was to investigate the bromatological composition of guayule leaves in view of their exploitation as a fodder resource.

Guayule was grown on experimental plots in Southern Sardinia, Italy. In late summer, leaf samples were hand plucked, to simulate grazing, from undisturbed three-year-old plants. Leaves were weighted, oven dried at 65 °C, then ground to 1 mm screen. Total N was determined using Kjeldahl method and crude protein (CP) was calculated by multiplying the N content by 6.25. Neutral and acid detergent fibres (NDF and ADF) and acid detergent lignin (ADL) were determined by using the procedure of Van Soest et al. (1991) and ether extract using (EE) Soxhlet extraction. Total digestible nutrients (TDN), digestible dry matter (DDM), dry matter intake (DMI), relative feed value (RFV) and net energy for lactation (NE<sub>1</sub>) of leaves were estimated according to the equations adapted from Lithourgidis et al. (2006).

Green leaves represented up to 15% dry of shoot biomass. Leaf DM content was in the range 100 - 130 g kg<sup>-1</sup> dry matter (DM). CP concentration of leaves ranged from 137 to 175 g kg<sup>-1</sup> DM. NDF did not exceed 374 g kg<sup>-1</sup> DM and ash content ranged from 180 to 230 g kg<sup>-1</sup> DM.

On average, the values of TDN, DDM, DMI, RFV and NE<sub>1</sub> in leaves were 774 and 775 g kg<sup>-1</sup> DM, 33.4 g kg<sup>-1</sup> of body weight, 193% and 1.84 Mcal kg<sup>-1</sup>, respectively.

As regard Mediterranean conditions of Sardinia, CP of standing hay (i.e., ungrazed dry residuals in natural and improved pastures) decreased to 4% in summer, whereas the CP values in guayule leaves reached 17.5%. It is worth of note that leaf RFV exceeded 151 value, which is indicative of a prime forage. The potential of guayule to maintain green leaves at the end of summer coupled with its peculiar chemical composition can be exploited for compensating seasonal fodder shortages associated to drought season and/or the delayed establishment of annual forage crops. Our results give clues that guayule has the potential to supply high quality green forage for complementing conventional forage resources of Mediterranean farming systems. Results also encourage further investigations dealing with the chemical characterization of protein fractions, palatability and bioactive compounds in guayule leaves.

Subject: poster Poster Keywords: *Parthenium argentatum*, byproducts, fodder.

#### Bioactive compounds from leaves of Taraxacum kok-saghyz

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Taraxacum kok-saghyz L.E.Rodin (rubber or Russian dandelion, thereafter referred to as TKS) is a dandelion species native to Kazakhstan, Uzbekistan and north-west China, considered as a promising alternative source of natural rubber (NR) from its roots. It has been stated that, within the process for producing of an economic supply of NR from TKS roots, one of the major challenges is the exploitation of large quantity of byproducts that result from separating and purifying its NR. These include the non-rubber parts of roots and leaves, which must also be separated and converted into products and/or raw materials of value to different commercial sectors. A plenty of literature deals with the antioxidants from leaves in *Taraxacum* genus species except than in TKS. Thus, the specific aim of this study was to investigate the possible exploitation of TKS leaves, which represent a rubber byproduct, as a source of phenolic compounds with antioxidant properties for potential applications in forage, nutraceutical and pharmacological fields. Two accessions (TKS016, TKS018) grown in Sardinia (Italy) under Mediterranean conditions were investigated at vegetative and flowering stages, respectively. Leaf samples were kept on ice and stored at -80 °C, until lyophilisation. The procedures for sample preparation were according to (Re et al., 2019). Antioxidant capacity [ABTS ((2,2'azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt)) and by DPPH (1,1diphenyl-2-picrylhydrazyl) assays], total phenolics and total flavonoids were quantified following procedures previously reported by Sulas et al., 2016. Analysis of phenolic compounds was performed on an HPLC instrument and the chromatographic separation was carried out according to Piluzza et al., 2019.

The leaves of TKS018 had the highest antioxidant capacity values (19.6 mmol trolox equivalent antioxidant capacity 100 g<sup>-1</sup>), total phenolic (106.4 g gallic acid equivalent kg<sup>-1</sup>), and total flavonoid contents (22.9 g catechin equivalent kg<sup>-1</sup>). At both phenological stages, TKS016 showed significantly lower values than TKS018 in DPPH, and total phenolic. Among twentyone phenolic compounds screened, six individual molecules were identified, namely chlorogenic, cryptochlorogenic, caffeic, sinapic, chicoric and 3,4-dimethoxycinnamic acids. In particular, chicoric acid (8.53 - 10.68 mg  $g^{-1}$  DW) and chlorogenic acid (4.18 - 7.04 mg  $g^{-1}$  DW) were the most abundant identified in leaves of TKS. Chicoric acid has been already identified as the major compound occurring in T. officinalis aerial part as our results. A study on T. formosanum Kitam, a chinese medicinal herb, quantified chicoric acid as the predominant phenolic acid in its plant extracts. It has been reported that chicoric acid possesses various functional properties including antioxidant, antivirus and immunoregulation. Based on our results, T. kok-saghyz represents an important source of chicoric acid with potential application as antioxidant to be used as herbal medicine and nutrition for production of healthy food/feed. Additionally, leaves exploitation as an antioxidant source might contribute to an economic supply of TKS rubber chain.

Keywords: Russian dandelion• biomaterials • antioxidant• cinnamic acid derivatives • industrial crop

#### The AIR project (Aerial Inulin and Rubber)

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#### Abstract

In the last years, lots of efforts have been made to study and improve the growth conditions of the rubber dandelions. In the AIR project, we are researching and optimizing the growth of rubber dandelions under controlled conditions such as hydroponic cultivation, variable light spectra by LEDs, salt and mechanical stress. Our efforts are put in increasing the root biomass production and rubber yield as well as in the improvement of the production system. Through digital phenotyping and gene expression analyses we will monitor how different light recipes and intensities affect rubber and inulin production and the plant phenotype.

Keywords: innovation, rubber, inulin, Taraxacum koksaghyz, soilless, hydroponics, LED, stress, digital phenotyping

## Study of guayule bagasse pyrolysis: Effects of pyrolysis parameters on its heat and mass balance

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The valorization of solid co-products from the exploitation of biomass is proving to be an increasingly important step in the current context of circular economy and eco-efficiency of the use of sustainable natural resources. In this context, the guayule-latex sector generates a significant amount of bagasse (of the order of 80% in dry mass) which needs to be valorized.

A promising technical option currently under study is the transformation of this bagasse into solid fuel (charcoal), liquid fuel (bio-oil) and gas combustible by pyrolysis processes. One of the important issue concerns the mass and energy balances of the pyrolysis transformation; the aim being to optimize the industrial thermochemical process as best as possible by ideally ensuring an energy autonomy of the process.

Thus, the present work aims to establish heat and mass balances of the pyrolysis of bagasse guayule. The pyrolysis was performed under different operating conditions (temperature, heating rate, residence time) to evaluate its effects on the yields and quality of energy vectors mentioned above. The experiments were conducted on a CIRAD laboratory pilot covering the classic pyrolysis conditions encountered at the industrial level in terms of temperature (up to 800 ° C), heating rate (5 ° C / min) and residence time (up to 2 hours). Charcoals, bio-oils and gases were collected and analyzed afterwards. The mass yields of charcoal and bio-oils were determined by weighing. The yield of the pyrolysis gases part was obtained by calculation after determining the composition of the main gas compounds (CO, CO<sub>2</sub>, CH<sub>4</sub>, H<sub>2</sub>) by gas chromatograph.

The heat balances were then determined through calorimetry measurements (higher and ower Heating values) of guayule bagasse, charcoals and bio-oils. The calorific values of pyrolysis gases have been classically calculated from their chemical compositions.

These results are needed in future optimization and scale-up studies of a pyrolysis unit. They will help to predict qualitatively and quantitatively marketable fuel products, mainly charcoal and bio-fuel. These studies will also contribute to the estimation of the possible energy valorization of the pyrolysis gases for the own needs of the pyrolysis process in heat and power.

Topics: guayule Keywords: Guayule, co-products, bagasse, biochar