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# 2012 Industrial Crops: Developing Sustainable Solutions

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## Program



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## ABSTRACTS

# Keynote Speaker

### HOW AGRICULTURAL RESEARCH HELPS BUILD THE BIOECONOMY AND A SUSTAINABLE FUTURE

Catherine Woteki

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The United States Department of Agriculture (USDA) is a global leader in science innovation that promotes food security and economic growth, helps mitigate the effects of climate change, and helps create cost-effective and diverse renewable energy options as the foundation of the American bioeconomy.

Reducing reliance on fossil and petroleum-based sources of carbon and promoting a society that meets its needs with renewable biomass from sustainable agriculture practices are two key Departmental goals. USDA policy works to: (1) encourage bioeconomy R&D investments; (2) facilitate the commercialization of bioinventions from lab to market; (3)

develop and reform regulations to reduce barriers and costs of production while protecting human and environmental health; (4) meet the national workforce needs to support the bioeconomy; and (5) promote and support public-private partnerships.

USDA's research and education programs support all components of the bioeconomy policy. USDA has established regional centers, educational programs and projects with private partners that focus on the development of biofuels and biobased products to support and build the bioeconomy. The Agricultural Research Service, the National Institute of Food and Agriculture, and the Forest Service support regional programs that focus on improving biomass varieties and production systems and enable higher value uses of co-products while protecting or enhancing vital ecosystem services. The National Agricultural Statistics Service (NASS) currently coordinates with USDA's other agencies to collect statistics to inform decisions in both the public and private sectors. Similarly, the Economic Research Service and the Office of the Chief Economist conduct analyses using NASS data and data from external sources to inform decisions made by other USDA agencies and understand the status and economic feasibility of the bioeconomy.

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# Plenary Session

## THE DEVELOPMENT OF LIQUID FUELS FROM LIGNOCELLULOSE

Chris Somerville

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The efficient production of lignocellulosic fuels by biochemical routes will require innovation in three main areas: sustainable production of feedstocks that do not compete with food production, depolymerization of feedstocks, and conversion of feedstocks to liquid fuels. In this respect there is renewed interest in identifying plants that have optimal biomass accumulation and understanding the production issues associated with large-scale cultivation and sustainable harvesting of such species. Additionally, the importance of enhancing soil carbon and nutrient retention while minimizing inputs will require an integrated approach to the development of cellulosic energy crops. The challenges on the processing side include the development of improved chemical or biological catalysts for polysaccharide and lignin depolymerization and conversion to fuels, the development of microbial strains that can convert a wide range of sugars to next generation fuels under harsh conditions, and numerous innovations in chemical engineering. There appear to be many different routes to improved processes for cellulosic fuels production. However, because of the interdependencies of elements of the overall path from biomass to liquid fuels, research on the design of optimized processes is at a preliminary stage of technical maturity.

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## IMPLEMENTING CERTIFIABLE SUSTAINABILITY STANDARDS FOR PHARMACOPOEIAL-QUALITY MEDICINAL PLANT CROPS

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Botanical ingredients are produced and traded in a range of defined grades and qualities. Botanicals often have

multiple end-uses and purposes, each necessitating a different quality standard in order to deliver the intended effect of the final product. There are significant qualitative and quantitative differences between the specification requirements for cosmetic, food or spice grades vs. therapeutic or pharmacopoeial quality standards. Manufacturers of finished herbal medicinal products require a steady supply of pharmacopoeial-quality botanical raw materials because this defined quality is the foundation of predictable safety and efficacy.

Pharmacopoeial standards aim to establish the minimum quality necessary for reproducible therapeutic effects and as such they provide companies with a basis for purchasing and quality specifications in order to evaluate the composition, identity, purity, quality, and strength of a botanical. As a successful company's annual demand requirements increase, the challenges faced by medicinal plant producers to sustainably scale-up to match the defined qualities and quantities are considerable. This is especially the case for botanicals that require several years' maturity, e.g. many medicinal roots or rhizomes require minimum 5 to 6 years maturity before harvest and inner barks of tree branches or stems can require 10 to 15 years of growth before first harvest.

The objective of this presentation is to summarize the nearly 40-year experience of a California herbal product company collaborating with its network of producers and suppliers in the implementation of independently certifiable sustainability standards for the production of specified qualities of certain medicinal plants.

For methodology, the company has invested in the implementation of standards including the United States Department of Agriculture (USDA) National Organic Program (NOP) for cultivated crops as well as the NOP Wild-crop Harvesting Practice Standard, the International Standard for Sustainable Wild Collection of Medicinal and Aromatic Plants (ISSC-MAP), the Fair Wild Foundation (FWF) Fair Wild Standard, the Fairtrade International (FLO) Fairtrade Standard for Herbs and Herbal Teas, and the Non-GMO Project Standard. Experience to date provides evidence that implementation of site-appropriate standards, with independent auditing and reporting, contributes to assuring conformance to specified qualitative and quantitative pharmacopoeial standards. This practice also facilitates compliance with production and process control system requirements of Current Good Manufacturing Practice (cGMP) and tracking and traceability requirements of food safety regulations.

The collaborative implementation of certifiable sustainability standards for supply of medicinal plant ingredients has enabled the company to develop and maintain long-term fair trade relationships with producers and suppliers. Fair trade helps to improve quality of life for local, rural and indigenous communities who farm and/or wild-collect plants and also empowers them to sustainably manage the biodiverse ecosystems that are the native habitat of many botanical raw materials used in traditional herbal medicinal products. The price premiums paid to producers for compliance with quality and sustainability standards corresponds to nature conservation and to a sustainable supply of the specific quality materials. In conclusion, it just makes good business sense.

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## RECOVERING VALUE FROM THE POMACE PILE

Torey Arvik, Ph.D.

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Sustainable agriculture is now perceived as a minimum requirement by many consumers. Agricultural research is focusing on finding alternative uses to traditional by-products of crop production. This research can help bring nutrients back to the table that would normally be thrown away in large agricultural operations. Grape seeds and skins represent approximately 20% of the wet weight entering the winery and are typically discarded as 'pomace'. The vast majority of grape pomace in the United States is composted and returned to the land in one form or another. This is a good use, but certainly not the best, considering that as much as half of the nutrient value of grapes remains in what leaves the winery. Recent studies show that micronutrients that support human health are being sent back to the vineyard instead of people who could really use them. This presentation describes the technical and functional

attributes of grape skin and seed flours and how their use can close the loop on winery sustainability as well support human health.

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### NIFA PROGRAMS TO SUPPORT INDUSTRIAL CROPS AND PRODUCTS

Carmela A. Bailey

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The mission of the National Institute of Food and Agriculture (NIFA) is to advance knowledge for agriculture, the environment, human health and well-being, and communities by supporting research, education and extension programs in the land grant university system and other partner organizations (www.nifa.usda.gov). Within this mission, bioenergy and biobased industrial products have been identified as priority topics to reduce dependence on fossil-based resources and expand agricultural markets. Research and development is supported in the context of a sustainable supply chain that encompasses crop development through product development and evaluation.

From an agricultural perspective, sustainability is defined as satisfying America's needs for food, fiber, feed, (and fuel), while at the same time maintaining or enhancing environmental quality, rural economic viability, and quality of life. Sustainability is an overarching theme for all bioenergy programs in NIFA and some programs require the incorporation of environmental, economic and social considerations during project planning and implementation.

NIFA has a portfolio of funding opportunities that support industrial crop development and production, and development of bioenergy and biobased products. Competitive grant programs support the R&D continuum from basic and early applied research through pre-commercialization. This presentation is an overview of these programs with examples of funded projects and how they have incorporated the elements of sustainability.

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## Oilseeds I

### BIOENGINEERING CAMELINA OILSEEDS FOR ENHANCED BIOFUELS AND OLEOCHEMICALS

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Energy rich plant oils are increasingly used for manufacturing biofuels and for providing renewable raw materials of numerous industrial products. To harvest this important natural resource for industrial uses, non-food oilseed crops with great potential for genetic engineering are needed. Camelina (Camelina sativa) has a long history of cultivation and the oil is not a food source in the United States. In addition, its advantageous agronomic features and the ease of

genetic transformation make camelina an ideal platform to produce enhanced oils for biofuels and biomaterials. In this presentation, I will give an overview of our recent projects in genetic engineering of camelina to increase oil content in seeds, and then focus on our approaches to improve the oil fatty acid composition for biofuels and oleochemicals.

Camelina seeds contain about 30-40% of oil. The main drawback of camelina oil for industrial uses is its high content of polyunsaturated fatty acids (PUFA), which are subject to oxidation and render oil instability. It is possible to decrease the PUFA content and to increase the monounsaturated oleic acid by traditional mutation breeding. The high oleic camelina oil can also be effectively achieved by down-regulation of key fatty acid desaturases and elongases through genetic transformation.

Many seed oils contain special fatty acid structures that provide unique physical or chemical properties that are useful for manufacturing numerous industrial products. However most of these plants are not suitable for modern agriculture or are restricted to grow in certain regions. Camelina may serve as a platform to produce these industrial fatty acids in transgenic seeds. For example, the hydroxylated fatty acids found in castorbean and Physaria oils can be generated in camelina by transforming the key fatty acid hydroxylases; jojoba type wax esters can be produced in camelina seeds by introducing the jojoba biosynthetic pathway. Efforts are underway to increase the amount of these novel oils in transgenic seeds using biochemical and genomics tools. To this end, knowledge gained from the model plant Arabidopsis, the close relative to camelina, will be greatly beneficial.

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## DOES IRRIGATION INCREASE CAMELINA SEED YIELD?

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The nationwide search for renewable, non-imported sources of energy has led to increased use of agricultural sources of oil to make biodiesel for fuel. We found no evidence of oilseed-to-biodiesel research or commercialization efforts in the arid south central Oregon plateau, including the Klamath Basin, prior to 2005. Much of this region has irrigation water available, although amounts vary by year and location. Recently, national interest in camelina (Camelina sativa) has increased due to its reported need for lower inputs (including water use), than other oilseeds such as canola and soybean, as well as its ready conversion to high value fuels such as aviation jet fuel. Despite the lack of local research and experience, a local farmer partnership built a 3.8 M liter per year biodiesel production facility in 2006, hoping to contract local oilseed production, while using geothermal heat for some of their energy needs. Other biodiesel companies are also active in Oregon (mostly utilizing canola oil), but several small fields of camelina have been grown in southern Oregon and northern California since 2008, often without irrigation.

The objective of this study was to evaluate spring-seeded camelina's response to irrigation along with response to other agronomic factors to evaluate its suitability as a 'low-input" crop under the cool, high elevation conditions in the Klamath Basin. We grew camelina using standard research-scale field equipment in 2007, 2008, 2009, 2011, and 2012, testing the effect of various agronomic factors on camelina growth and seed yield, including seeding date, nitrogen fertilizer response, harvest method, and soil type, in addition to response to irrigation. At least two irrigation rates were evaluated each year.

As expected, seed yields varied over the years due to differences in seeding date, weather, and agronomic management, but there was a general trend for increased irrigation to produce higher seed yields within the range tested. Depending on particular agronomic treatments, mean seed yields ranged from 348 kg ha-1 to 3146 kg ha-1, with yield falling between 550 and 2250 kg ha-1 in most cases. When seed yield for all years and management methods was regressed against received moisture (irrigation plus precipitation), there was a weak positive correlation between moisture and yield (R2 = 0.21). However, if data for only 2007-2009 is considered, this correlation was stronger (R2 = 0.68). In an effort to standardize year-to-year variation in weather, seeding dates, and harvest dates, we

also calculated a measure of seasonal moisture relative to water demand to regress against seed yield. This standardized measure was calculated by dividing the sum of irrigation plus precipitation by the seasonal calculated Jensen-Penman evapotranspiration (Et) between seeding and harvest dates. Using this measure [(Irr + Precip) / Et] we found similar results. Regression against seed yield for 2007-2009 resulted in positive correlation (R2 = 0.68). When the data for 2011 and 2012 were added, the correlation was still positive, but the linear regression was not as strong (R2 = 0.15).

The poorer correlation coefficient when including data from 2011 and 2012 was due to improved yield even at relatively low levels of irrigation (better yield with less irrigation than in previous years). In essence, as our expertise with this crop has improved, we have been able to increase yields at relatively lower irrigation rates (while still using similar agronomic inputs otherwise) than we did in earlier years. Thus while camelina does seem to respond positively to increased moisture availability throughout the range we have tested, it also has the ability to produce reasonable seed yields even while receiving far less irrigation than is typically given to other crops in this region.

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## INFLUENCE OF GENOTYPE AND SOWING DATE ON CAMELINA SEED YIELD AND OIL CONTENT IN THE NORTHERN CORN BELT

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Camelina (Camelina sativa L.) has recently gained considerable attention in the U.S. as a potential oilseed feedstock for advanced biofuels and bioproducts. Although progress has been made towards characterizing camelina's production potential for the western U.S. and Canada, very little research has been done to evaluate its potential further east in the fertile northern Corn Belt region.

The objectives of the following study were to determine the best camelina cultivar and time of sowing in the northern Corn Belt while evaluating the effects of sowing date and genotype on seed yield, oil content, and plant population density.

The study was conducted over three growing seasons between 2008 and 2010 in west central Minnesota, USA on a Barnes loam soil. The experimental design was a split plot randomized complete block with four replications. A total of ten camelina genotypes were evaluated and sowing dates ranged from April 16 to June 15 over the 3-year study.

Seed yield and oil content and plant population density were greatly affected by sowing date, which all three tended to decline with delayed spring sowing. Seed yield was only significantly affected by genotype in 2009, whereas oil content was consistently affected by genotype all 3 years. Average seed yields were as high as 2300 kg ha-1 to as low as 669 kg ha-1 and were generally greatest for sowings between late April to mid-May. Across sowing dates and cultivars, oil content ranged from about 36 to 43% (wt wt-1) and declined with delayed sowing. Although yield differences among about the top five to six genotypes were generally not great, the cultivars Calena and CO46 consistently produced high seed yields and oil content. Plant lodging, which was also measured during the study, was generally low across cultivars and sowing dates throughout the study.

Results indicate that the best time to sow spring camelina in west central Minnesota is from about April 20 to mid-May. Seeding past mid-May resulted in significant losses in yield and oil content across all cultivars. Further research is needed to optimize other inputs for camelina production in the northern Corn Belt.

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## GENETIC AND PHENOTYPIC DIVERSITY IN CAMELINA GERMPLASM

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Camelina is a new crop targeted for agronomic systems across the Midwest. Camelina is a promising bioenergy crop fitting the requirements for the biodiesel industry, especially for production of JP-5 fuel used in the aircraft industry. Moreover, its fatty acid profile satisfies the standards for nutraceutical and animal feed uses.

Thirty-five accessions of camelina from the USDA-ARS-NCRPIS germplasm collection were selected based on geographic representation. The germplasm was evaluated in three environments during the years 2010 and 2011 in a randomized complete block design. Oil content from harvested seed was determined by nuclear magnetic resonance (NMR) and fatty acid profiles by gas chromatography (GC). Genetic diversity was assessed by simple-sequence repeats markers (SSR). 74 SSR were tested to determine the diversity among camelina accessions. A principal component analysis (PCA) and an UPGMA dendogram were developed from the genetic distance matrix and used to evaluate the genetic relatedness amongst camelina accessions.

Camelina accessions showed significant variation in oil content (30% - 40%). The main fatty acid profile also show large variations, linoleic acid ranged from 16.2 to 27.6% and linolenic acid content ranged from 28.7 to 43.5%. Linoleic and linolenic content were negatively correlated. A phenotypic dendogram (Ward method) shows relationships within and among seven groups of accessions based on their seed yield, oil concentration and oil quality.

The variation in fatty acid composition and oil content allows selection of promising accessions to amplify the genetic base in camelina enhancement programs.

The results will be used as a tool to aid genetic collection management, and to provide insights on whether collection quality would be improved by expanded (new accessions) and whether selection of divergent accessions for breeding programs is appropriate.

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## INDUSTRIAL OILSEED CROPS IN TEXAS AND THEIR ADAPTATION ACROSS CLIMATIC REGIONS OF THE STATE

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Oilseeds are significantly missing from the crop mix in Texas, with the exception of cotton, which, although an oilseed, more than half of the seed is used as whole fat dairy rations. With 22.5 million acres (9.1 million ha) of crops planted in state, oilseeds other than cotton represent approximately1.2% of acres planted. While the amount of land planted to oilseeds remains low, the price of vegetable oil has remained strong, running on a parallel with petroleum prices. As the US strives to meet federally mandated renewable fuel standards (RFS2), oilseeds are a quick and

efficient way to efficiently produce large quantities of oils which can be used for biofuels or biopolymers. We estimate that for every 1% of the crop base of Texas converted to dedicated oilseed crops, over 22 million gallons (83 million liters) of vegetable oil could be produced annually.

The objective of this study was to indentify species and germplasm of industrial oilseed adapted to the broad climatic variations in agricultural production systems across the state of Texas as well as identifying and refine best management practices (BMPs) on candidate crops which would be economically competitive with present day crops. Our goal was to identify species, cultivars and production systems that would yield a minimum 100 gallons/ac (3784 l/ha) as a dedicated bioenergy crop.

The study compared seed yield and oil content of available germplasm among cool season annuals (winter and spring canola, brown mustard (Brassica juncea), cool season safflower, oilseed radish, camelina, crambe, flax and others) and warm season annuals (castor, sesame, sunflower and warm season safflower, peanut) at Amarillo, Lubbock, Vernon, Commerce, Beaumont, College Station, Yoakum, Uvalde and Weslaco, Texas. In most locations, plots were combine harvested, samples were cleaned and weighed and oil content analyzed by nuclear magnetic resonance (NMR).

Statewide, three winners were found, consistently producing high oil yields across wide environments: sunflower, castor and winter safflower. The cool season Brassica species had a good fit over many ecosystems, but yield was limited by stand establishment problems and vernalization issues of the winter types in warm climates. Flax had an excellent fit in Central and South Texas but was limited by cold winter temperatures. Peanut produced very high yields of oil, but was limited to irrigated production on sandy soils.

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## DORMANT AND SPRING SEEDED CANOLA COMPARISONS

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Dormant seeding of spring canola (Brassica napus L.) in the fall offers potentially higher seed yields and better workload distribution, but also carries risks associated with seed germination prior to winter and resultant poor stands the following spring with low yield performance.

The research objective was to compare crop performance from fall and spring canola seeding dates in North Dakota where approximately 85% of the U.S. canola is produced.

The experiment was a RCBD with two fall and two spring seeding dates and four replicates conducted at Prosper, ND. A glyphosate resistant spring canola hybrid was fall sown on Oct. 28 and Nov. 11, 2011, and spring sown on April 9 and May 14, 2012. All seeding dates were sown into tilled hard red spring wheat (Triticum aestivium L.) stubble. Standard agronomic practices were applied for other crop management regarding stand establishment, fertility, pests, and harvest.

Seed yield was similar for the two, fall and late spring seeding dates, and averaged 1578 kg/ha. Yield was 40% greater from the early spring seeding date at 2221 kg/ha. Greater yield for this date, in comparison to the dormant fall dates, was related to almost twice as great stand establishment for the spring sowing. Reasons for reduced dormant stands are unclear, since fall emergence was not observed, although seed germination could have been initiated prior to winter. Stand losses from cool temperatures could also have occurred in the spring, since emergence was first observed on March 24 from the dormant seedings. Lower yield from the late compared to early spring seeding date was expected and related to greater crop heat stress with later seeding, since flowering was pushed into the warmer temperatures of July for this date.

The study will be repeated in 2012/2013 before drawing conclusions and making recommendations.

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## EVALUATION OF REGIONAL FIELD PENNYCRESS (Thlaspi arvense L.) POPULATIONS

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The common winter annual weed Thlapsi arvense L. (field pennycress) has been recently identified as a potential new source for fuel and industrial products. Field pennycress offers a very short life cycle allowing for opportunities for double cropping in the Midwest region. Identifying traits under genetic control or influenced by the environment become critical in understanding the genetic diversity available for a plant breeding program.

The objective of this study was to evaluate wild populations of field pennycress (Thlaspi arvense L.) collected from the central United States for potential inclusion in a plant breeding program. In the spring 2011, 28 wild populations of field pennycress seed were collected along with GPS coordinates and altitude data. Original populations were evaluated for seed traits and seed oil properties, including total seed oil and fatty acid methyl ester content. Total oil content was determined by nondestructive pulsed NMR on whole pennycress seed. Fatty methyl esters were quantified utilizing gas chromatography. Seed oil properties from each population were compared between seed from the original site of collection and seed grown in Macomb Illinois in 2012.

Total oil content for the 28 original populations ranged from 24.5% to 34.0%. When grown in Macomb IL, total oil content ranged from 29.4% to 33.0%. Greater latitude and longitude distance and altitude from Macomb had only a slight positive effect on total oil content. More Northern locations had increased Erucic acid levels. However in Macomb in 2012, there was no significant difference in Erucic acid levels across all populations. The hot dry conditions of 2012 lead to significantly increased levels in linolenic acid (C18:3) and significantly decreased levels of seed yield, 1000 seed weight, and oleic acid (C18:1). Linoleic acid (C18:2) levels increased in 2012 but were not significant.

Understanding the environmental influences on important agronomic and seed traits in pennycress will help in determining ideal locations for commercial seed production and assist in variety development and selection.

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## ISOLATION AND ENRICHMENT OF THE ERUCIC ACID FROM THLASPI ARVENSE (PENNYCRESS) OIL

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Pennycress is being developed as an off-season rotation crop between annual corn and soybean production. This rotation scheme may offer distinct advantages to farmers by providing additional farm income from an otherwise fallow season with little impact on the subsequent soybean production. The seed contains up to 36% oil with the major fatty acid as erucic (36.6%). The fatty acid composition in thlaspi has been shown to have physical properties suitable for biofuels like biodiesel and hydro-treated renewable jet fuel (HRJ). Like many developing new crops, the chemical characterization and methods for enrichment of the individual components of the triglyceride have yet to be reported. Of particular interest, is the isolation and enrichment of erucic acid, which has a number of potential industrial applications.

The triglyceride composition of pennycress oil was determined by HPLC using a C8 column which separated the individual triacylglycerides into 9 individual triglyceride groups of carbon chain lengths that ranged from 48 to 64. Each individual peak was isolated on a HPLC by 25 repeated 100 µL injections of a 20 mg/mL solution of pennycress oil dissolved in acetone, followed by elution using a gradient of acetonitrile/acetone from 50:50 to 0:100 over 20 minutes then collecting at 0.2 min/fraction. Pure individual peaks were obtained and concentrated from the fraction collector and subsequently analyzed by GC as their methyl esters and the whole triglycerides analyzed by NMR. In addition, known standards were injected on the HPLC to confirm retention time assignments for eluted peaks.

Enrichment of erucic acid was accomplished by three independent methods on fatty acid, methyl esters and potassium soaps from pennycress oil. Molecular distillation of the methyl esters at 90$_o$C under 50 mTorr of pressure enriched the erucic acid content from 36.6% to 64.6% in a single pass with the erucic fraction consisting of 76% of the mass balance. A second pass distillation of the enriched erucic fraction increased the 22:1 content to 70.7% with the second pass mass balance of 60% and an overall mass balance of the erucic fraction of 42.2%. Molecular distillation of the fatty acids provided a less effective enrichment, only providing a 52.5% erucic acid fraction in a 68.9% overall mass balance in a two pass distillation. Fractionation by crystallization of the potassium soap during hydrolysis of the triglyceride provided the largest enrichment of the erucic acid. When the potassium erucate was allowed to crystallize from the mixture of pennycress soaps in a 90:10 methanol water solution (5:1 volume/mass solvent to soap) gave 66% erucic acid fraction that was 71% of theoretical erucic acid content. Higher purity erucic acid (87%) could be obtained when ethanol water was used as a crystallization solvent but theoretical erucic acid mass recovery was low (23%).

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## HIGH-THROUGHPUT GENOTYPING OF LESQUERELLA (PHYSARIA AND PAYSONIA) GERMPLASM COLLECTIONS USING DART MARKERS

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The Physaria (syn. Lesquerella) and Paysonia germplasm collection of the U.S. National Plant Germplasm System has not been fully characterized for genetic diversity. There are currently more than 200 accessions being managed ex situ to support new crop research and development activities.

To better understand the extent of diversity in the collection of Physaria and Paysonia, DArT (Diversity Array Technology) marker platforms were developed and utilized to characterize the P. fendleri collection together with other representative species. A total of 86 accessions from 12 species were used to develop two types of marker platforms. The first resulted in 2,833 polymorphic markers, and the second, 27,748 high quality markers, both have a scoring reproducibility greater than 99%.

Cluster analysis successfully classified the representative species by geographical source, and breeding status. Previous taxonomic group classification was also found concordant with results of molecular marker analysis. The analysis of genetic structure revealed significant genetic differentiation among the assigned population groups in P. fendleri.

The additional information provided by molecular characterization of the Physaria and Paysonia collection will make possible managing their germplasm resources more efficient. The DArT platforms will also be useful in future molecular breeding work in P. fendleri and other species.

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### EXPLORE THE OIL BIOSYNTESIS PATHWAYS IN LESQUERELLA FENDLERI

Grace Chen

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Lesquerella fendleri (A. Gray) S. Wats. (Brassicaceae), being developed as a new industrial oilseed crop in the southwestern region of the United States, is valued for its unusual hydroxy fatty acid (HFA) in seed. 55-60% of total L. fendleri seed fatty acids is HFA and its main component is lesquerolic acid (14-hydroxy-eicos-cis-11-enoic acid: 20:1OH). HFA is an important industrial raw material, which is, conventionally, made from castor (Ricinus communis). The production of castor oil, however, is hampered by the presence of the toxin ricin and hyper-allergic 2S albumins. L. fendleri, on the other hand, does not have such biological toxic components, thus its oil represents a safe source of HFA.

Lesquerella oil contains 55-60% HFAs, comparing 90% in castor oil. It is known that most of the HFAs in L. fendleri are located only at sn-1 and sn-3 positions of TAG. So it is possible to enhance HFA accumulation in L. fendleri seeds by manipulating a lysophosphatidic acid acyltransferase (LPAT) that favors incorporation of hydroxy acyl groups at the sn-2 position. To explore the potential, a seed specific napin promoter was fused to the LPAT2 gene from castor, and the resulting vector was used to transform L fendleri. Seeds of fifteen independent transgenic lines were analyzed for changes in fatty acid (FA) composition, HFA content and oil content.

The results from preliminary study showed that the common seed FA composition remained unchanged between wild type and transgenic lines. However, all transgenic lines showed increase in ricinoleic (18:1OH) and densipolic acid (18:2OH) content. The percentage of 18:1OH and 18:2OH in combine is 1.2% in wild type, but varied from 1.5% to 4.8% among transgenic lines. The third minor HFA, auricolic acid (20:2OH), also increased slightly, from 2.7% in wild type to 3.5-3.7% in transgenic lines. Surprisingly, the major HFA 20:1OH content had 4-6% decrease in transgenic lines. The total HFA content and oil content in wild type parent was measured at 56% and 18%, while in transgenic lines the level ranged from 52-60% and 13-21% respectively.

Although we did not observe a significant increase in total HFA content in transgenic lines carrying napin-LPAT2, the results advanced our understanding of the mechanism underlying HFA synthesis and will direct the exploration of other pathways involved in oil accumulation in L. fendleri.

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## Medicinal and Nutraceutical Plants

### MEASURING CHEMICAL COMPOSITION IN YERBA MANSA POPULATIONS, A MEDICINAL PLANT IN THE US SOUTHWEST

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Many people living in the southwestern region of the United States actively wildcraft medicinal plants. These plants are used for a wide range of conditions. Yerba mansa (Anemopsis californica (Nutt.) Hook. & Arn.) is one of the most widely used medicinal plants with a native range from California through western Texas. Compositional differences in extracts of bioactive compounds may be due to genetic differences in plant populations and/or environmental

influences on the accumulation of these compounds.

A series of studies were conducted to establish an extraction method for analysis of the essential oils from multiple individuals representing multiple populations of yerba mansa. This method was then used to determine if there were chemotypes distinguishing yerba mansa populations; and if environmental conditions altered essential oil composition. Finally the ethnobotanical reports for specific bioactivities (anti-microbial and anti-cancer) were tested. Rhizome samples were collected from plants representing 17 distinct populations throughout New Mexico. Plants were collected in seven counties, located at altitudes between 1198 and 1793 m, representing USDA zones 4–8. Rhizomes were dried and then ground. Supercritical fluid extraction conditions were established that produced an extraction profile similar to a traditional steam distilled oil: 0.5 g root in a thimble pressurized with CO2 to a density of 0.72 g/mL (5150 psi, 100 °C, ISCO SFX3560). The composition of the sample was then determined by GC/MS. In vitro cell growth assays were used to determine IC50 values against specific human cancer cell lines; turbidity measures were used to determine anti-microbial bioactivity.

The leaf essential oil generated by steam distillation contained over 38 compounds detected as peaks by GC/MS. The most abundant compounds in leaf essential oil were: elemicin, methyleugenol, and piperitone. The rhizome essential oil was also very complex; the most abundant compounds were methyleugenol, thymol and elemicin. Greenhouse reared clones of a wild population of A. californica had an identical leaf volatile composition with the parent plants. Steam-distilled oil had antimicrobial properties against 3 (Staphylococcus aureus, Streptococcus pneumoniae, and Geotrichim candidum) of 11 microbial species tested. Some of this bioactivity could be accounted for by the α-pinene in the oil.

Three distinct chemotypes were detected using a hierarchical clustering analysis on the concentration of 10 different analytes (α-pinene, 1,8-cineole, myrtenol, methyleugenol, isoeugenol, elemicin, piperitone, limonene, and cymene) in three individuals from each of 17 populations. One chemotype was characterized by high elemicin concentrations, a second chemotype by high methyleugenol concentrations and the third by high piperitone and thymol concentrations. The essential oil from yerba mansa rhizome inhibited the growth of AN3CA (uterine cancer) and HeLa (cervical cancer) cells in vitro but had no inhibitory activity against lung, breast, prostate or colon cancer cells. The IC50 values for the root oil were 0.056% and 0.052% (v/v) for the AN3CA and HeLa cells, respectively.

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## DEVELOPMENT AND AGRONOMIC SCALE-UP OF SPECIALTY CROPS FOR PRODUCTION OF TARGET MOLECULES

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The overall goal of Kemin's specialty crop improvement (SCI) program is to develop plant cultivars that can accumulate optimized levels of target molecules combined with desirable agronomic traits. The SCI program at Kemin is a vertically integrated research, development and production initiative focused on the breeding and agronomic scale-up of new cultivars of rosemary (Rosmarinus officinalis L.) and spearmint (Mentha spicata L.) for optimal accumulation of antioxidants. Kemin is currently one of the largest commercial growers of proprietary rosemary in the world with over 1000 acres planted in Texas and New Mexico. Kemin's spearmint extract is derived from proprietary spearmint varieties that are grown in Indiana; and harvested and dried using a patent-pending technology. SCI is actively engaged in developing proprietary rosemary and mint varieties with the highest levels of antioxidant molecules combined with higher biomass and winter hardiness making it a highly valuable resource for Kemin's commercial business units.

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## GENETIC DIVERSITY OF RIPARIAN POPULATIONS OF GLYCYRRHIZA LEPIDOTA ALONG THE SALMON AND SNAKE RIVERS.

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Glycyrrhiza lepidota Pursh (Fabaceae; American wild licorice), is a nitrogen-fixing, perennial, facultative riparian species present along many dryland rivers in western North America, including the U.S., southern Canada and northern Mexico. The Native Americans who lived along the Missouri and Columbia Rivers used the root, which is fifty times sweeter than sugar, for food, medicine, and ceremonial purposes. Lewis and Clark purchased a large amount of licorice from the Indians during "The Expedition". Like Glycyrrhiza glabra, common licorice native to Europe, Central Asia and China, Glyccyrhiza lepidota has been used to treat coughing, hoarseness , and sore throat. Licorice has been selected by a panel from the University of Würzburg and two conservation groups, the World Wildlife Federation and TRAFFIC (an organization promoting the sustainable use of wild-collected species), as the medicinal plant of the year for 2012.Knowledge of the extent, distribution, and structure of the North American Glycyrrhiza species genetic diversity can provide a tool for breeders to exploit and improve its medicinal qualities.

The objective of this study was to assess the genetic diversity and structure within and among populations along two different river environments in the Northwest.

We sampled 10 populations along a 30 mile stretch of the Snake River in a mostly wide open and relatively linear environment. We also sampled 5 populations along 50 miles of the Salmon River in a narrow, canyon-like environment accessible mostly by raft. Unlike the wide and linear environment of the Snake River, the Salmon River environment was narrow and winding with steep walls, which may have limited movement of pollinators among populations. Amplified fragment length polymorphism (AFLP) and Targeted Region Amplified Polymorphism (TRAP) markers were used to measure genetic diversity. The TRAP markers were designed to target regions in the genome corresponding to metabolites specific to Glycyrrhiza.

STRUCTURE analysis of the Snake River populations showed a large, panmictic population, while Salmon River populations were more distinct from one another, as well as from the Snake River populations.

River environments can have an effect on genetic diversity, likely from isolation of populations from pollinators. Interestingly, the largest populations along the Snake River were the most similar within and differentiated somewhat from the smaller populations; possibly as a result of restricted dispersal of pollinators from an abundant source.

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## FIELD PRODUCTION ECONOMICS AND BEST MANAGEMENT PRACTICES OF OENOTHERA ELATA (HOOKER'S EVENING PRIMROSE)

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Increasingly the usage of native plants as neutraceuticals has become a recognized practice. As additional applications for these materials have been identified, the production practices and identified markets have adjusted to meet the demand. The traditional production of gamma linolenic acid (GLA) has been from the U.S. native plant Oenothera biennis (Evening primrose). Evening primrose oil has been used for skin conditions, arthritis and auto-immune

disorders. In recent years, field production has moved offshore to take advantage of cheap labor and increased yields in cool moist climates. In an effort to identify a high value niche crop, Oenothera elata (Hooker's evening primrose) has been investigated as a potential commercial source of GLA which may be produced in regions with greater environmental demands.

The objective of this study was to identify the best management practices for this new commercial crop including spacing, irrigation, fertility, herbicide usage, and harvesting methods.

From 2009-2011, O. elata seedlings were produced using standard greenhouse practices. Seedlings were transplanted into the field in early spring using a commercial transplanter. Irrigation and fertility treatments were implemented across a row spacing study resulting in a split-split plot design. In addition, pre and post emergence herbicide experiments were conducted in the greenhouse using field soil. Finally, harvesting techniques were evaluated using three different defoliants, as well as, two different methods of harvesting as a randomized block design. All production costs were identified and built into a production budget.

It was determined Hooker's evening primrose produces the greatest plant growth index and floral density at 0.9 m spacings without impacting yield. Irrigation at 66% evapotranspiration (ET) was an effective irrigation rate for oil production. Multiple chemistries may be used as pre-emergence treatments while fluazifop and fenoxaprop are identified as the most suited post-emergence herbicide treatments.

Hooker's evening primrose has an excellent potential for production of GLA to be used in cosmetics as well as a neutraceutical. Best management practices have been determined for a semiarid environment allowing for production of GLA within the USA. Production costs were estimated to be $1906.89 per acre in 2011. Growers will need to receive $0.48 per pound in order to break even at this expense level.

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## EVALUATION OF FENUGREEK (TRIGONELLA FOENUM-GRAECUM L.) AS NEW CROP IN WESTERN NEBRASKA

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Fenugreek (Trigonella foenum-graecum L.) is an annual legume and known to possess a numbers of medicinal properties (anti-diabetic, anti-cholesterol and anti-cancer). This medicinal property is mostly because of several health-enhancing bioactive compounds (diosgenin, galactomannan, and 4-hydroxy isoleucine) present in matured seed. Fenugreek is grown under conditions very similar to that found in western Nebraska. So, can western Nebraska take the lead and become the source of fenugreek products for nutraceutical market in North America? The objectives were (1) to evaluate fenugreek germplasm for regional adaptation and seed yield potential, and (2) to study genetic diversity at genome level based on molecular markers. Germplasm available at USDA-National Plant Germplasm System and publicly available varieties were grown in the field. Common agronomic characteristics (plant stand, flowering, seed maturity, plant height, seed yield) were measured. These lines were also genotyped for molecular markers (ISSR, and SSR). Here, we will report extent of variation in the morpho-agronomic traits and DNA marker-based genetic relationship among the germplasm set tested. We found significant genetic variability for biomass production and seed yield among the genotypes. This set of germplasm will serve as base for the development of cultivars suitable for production in the western Nebraska and neighboring region.

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## *VARIATION OF L-DOPA CONCENTRATION IN THE LEAF AND FLOWER TISSUES OF SEVEN FABA BEAN ACCESSIONS WITH DIFFERENT FLOWER COLORS*

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Faba bean (Vicia faba L.) has been selected to adapt to a wide range of environments worldwide and is grown for different end-uses such as food, feed, forage and green manure. Particularly noteworthy in faba bean is the medicinally important component L-3,4-dihydroxy phenylalanine (L-DOPA), the major ingredient in medicines used to treat Parkinson's disease (PD) patients. L-DOPA can cross the blood-brain barrier into the brain where it is converted to dopamine, a monoamine neurotransmitter. It has been reported that L-DOPA is present in several tissues of faba bean. Although synthetic L-DOPA has been playing a major role in PD treatment, it is documented that the anti-PD effects of faba bean is superior to those of synthetic L-DOPA and considerably more lasting. Therefore, faba bean may be used as a crop for molecular pharming of natural L-DOPA. The objective of this preliminary study was to examine the variation of L-DOPA concentration in the leaf and flower tissues of seven faba bean accessions with various flower colors. Leaf and flower samples were taken from field grown plants with different flower colors, namely, pink with purple lines and black dot, white with a black dot, pure white, brown, and crimson. Samples were freeze-dried and L-DOPA was quantified by an ACQUITY UPLC system with a HSS T3 column. MS analysis was performed on an inline Synapt G2 HDMS time-of-flight mass spectrometer.

Very little variation of L-DOPA concentration was observed in both flower and leaf tissues within an accession. However, the difference between the seven accessions was significant. The average L-DOPA concentration in flowers ranged from 27.8 to 63.5 mg/g-DW (dry weight) and that in leaf tissues ranged from 18.2 to 48.7 mg/g DW. There was no significant correlation between L-DOPA concentrations in flowers and leaves. The accession with crimson colored flowers had the highest L-DOPA concentration (63.5 mg/g) in flowers but low levels (20.5 mg/g) in leaves. The accession with brown-colored flowers had high L-DOPA concentrations in both flowers (55.7 mg/g) and leaves (48.7 mg/g). Our study revealed a high level of variation of L-DOPA concentration in the leaf and flower tissues among the seven faba bean accessions studied. Further investigation is needed to elucidate the genetic basis of this useful trait.

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## ANTIFUNGAL PROPIETIES IN VITRO OF PLANT EXTRACTS AGAINST PHYTOPHTHORA CINNAMOMI

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Several crops of commercial importance in Mexico are affected by Phytophthora cinnamomi fungus that causes the root rot disease. At the present time, the control of this disease is principally carried out by the use of chemical

products; due to the environmental concerns, more research is required to obtain natural bioactive products for the control of P. cinnamomi.

The aim of this research was to evaluate the in vitro effect of Agave lechuguilla, Opuntia ficus-indica, Lippia graveolens, Carya illinoensis and Yucca filifera extracts on the mycelial growth inhibition of P. cinnamomi.

Plants were collected in the southeast of Saltillo, Mexico, the polyphenols were extracted from dry leaves with water, ethanol, cocoa butter and lanolin emulsion in a ratio of 1:6 (plant: solvent), during 7 h at 60°C. The concentration of hydrolysable and condensate tannins was determined by spectrophotometric methods. The antifungal in vitro activity was determined by mycelial growth of P. cinnamomi in Petri dishes containing potato dextrose agar (PDA). A complete random design with four repetitions was evaluated. Moreover, a PROBIT analysis was carried out to determine the concentration of inhibition for each extract at 50% and 90%.

The obtained results showed that the inhibition on mycelial growth varied from 0% in the control to 100% in treatments with different extracts. It was observed, that the increase in total polyphenols concentration caused the significantly reduction of P. cinnamomi mycelial growth. The plant extracts under study showed a high effect on the inhibition of mycelial growth, particularly, the aqueous and ethanolic extracts.

The analysis of IC50 reveals that the lowest concentrations to inhibiting in 50% the mycelial growth of P. cinnamomi are: L. graveolens 23.07 in lanolin; A. lechuguilla 28.87 in ethanol. Inhibitory concentrations higher than 50% were Opuntia in water (13039 ppm), Y. filifera in ethanol (5378 ppm), C. illinoensis in cocoa butter (2887 ppm), and L. graveolens in ethanol (2032 ppm). The extracts that showed no inhibition were: Y. filifera both lanolin as cocoa butter; O. ficus-indica in lanolin, cocoa butter and ethanol; A. lechuguilla in lanolin and cocoa butter; L. graveolens in cocoa butter; and C. illinoensis in water, lanolin and ethanol.

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# Oilseeds II

### *GREEN PROCESS FOR BIOMASS-BASED ENERGY: PRODCUTION OF CASTOR-DERIVED SYNTHETIC LUBRICANTS*

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The present consumption of fossil fuels has led to significant levels of environmental pollution and is rapidly diminishing petrochemical and energy reserves. Biomass would be a promising carbon-based alternative energy source and is a sustainable chemical feedstock. However, more-efficient processes for the selective conversion of biomass into useful organic intermediates and energy need to be developed. In this context, biodegradable lubricant and biodiesel are two examples for promising energy products based on castor oil.

Castor, being regarded as one of non-food, non-wood biomass with easy growing, less land and labor consumption, is a key industrial crop and the only commercial source of hydroxy fatty acid with numerous industrial applications, including bio-based plastics, lubricants, bio-fuel additives and coatings. Crude castor oil is used in many nonfood applications such as polyurethane polymers, pharmaceuticals and cosmetics, soaps, inks and paints, and others. However, one of the problems associated with castor seeds is that they contain the toxin ricin and hyper-allergenic 2S albumins which are detrimental to growers and processors as well as the environment. Furthermore, current processes for castor utilization generally suffer from high energy consumption and heavy pollutant to environment. In this context, it is highly desirable to develop eco-friendly and sustainable technology for castor crop improvement and

utilization.

Environmental friendly lubricating greases may be produced by solely replacing the mineral base oil with vegetable oil. Castor oil has high viscosity and is a unique raw material to manufacture specific lubricant used at low temperatures. Biodiesel composed of monoalkyl esters of fatty acids is a renewable alternative to fossil diesel. Waste castor fatty acids, by products generated in the manufacturing of sebacic acid, are feasible raw materials for producing economical biodiesel. Developing efficient and cost-effective green processes for catalytic conversion of castor oil to value-added products such as biodiesel and high performance lubricants are extremely desirable.

We have set up a pilot plant for manufacturing environmental friendly castor-based lubricant with annual capacity of 5000 tones. This kind of lubricant has many advantages including (1) biodegradability and eco-compatibility, (2) facile formation of thin film on the metal surface, (3) outstanding performance, such as anti-oxidation, anti-wear properties, low-temp, fluidity, detergency and dispersion, and long life-time, (4) high performance in petrol and diesel engines, (5) sustainable production based on renewable, non-food, non-wood biomass raw material, and (6) tunable lubricating property by structure modification.

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## BIODEGRADATION OF CASTOR-BASED LUBRICANT WASTES

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With the development of mechanical engineering, the demand for lubricant oil is growing largely. Nearly 40 million tons of lubricant oil is consumed all over the world every year. On the other hand, the lubricant dispersion after using or leaking causes not only more lubricant needed, but also serious pollution to the soil and water, leading to deterioration of ecological environment. Therefore, the degradable and environment-friendly bio-lubricants are in urgent need.

Compared with the petroleum-based lubricants, plant-derived lubricants have excellent characteristics such as good lubricity, higher viscosity index, non-toxic and easy to biodegradation. Plant-derived lubricants after use can be decomposed into CO2 and H2O by microorganisms with reasonable time, leaving no or little impact on the environment. In this context, we have set up a pilot plant for manufacturing environmental friendly castor-based lubricant (CBL) with annual capacity of 5000 tones.

This study aims at seeking for efficacious indigenous microorganisms to evaluate the degradability of the CBL, and developing effective methods for biodegradation of waste lubricants. Bacterial screening was conducted using CBL wastes as a sole carbon source. Bacterial strains Klebsiella sp. L-3 and Alternaria sp. M-4 isolated from the sewage and sludge in a castor oil refinery were used in the study of the degradation of CBL wastes.

Results showed 80% and 90% of degradation rate by employing L-3 and M-4 strains for 7 days, respectively. An improved method of oil detection was established based on CEC-L-33-A-93 test method. The metabolites were analyzed by FT-IR and GC-MS. It was found that the main carbon backbone of CBL wastes molecule can be cleaved into smaller molecules, and the peak area of metabolite decreased significantly. The biodegradability of CBL demonstrates the environmental friendly nature of CBL.

This is the first report of Alternaria sp. capable of degrading lubricants such as CBL wastes. Moreover, the strains had better growth and stronger degradation in high concentrations of lubricant. The detailed degradation mechanism is

under investigation in our laboratory.

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## THE CHALLENGES FOR PROMOTING WORLWIDE PRODUCTION OF CASTOR

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We bring to discussion the main findings of an extensive literature review on castor elaborated by 22 scientists from 16 institution and 8 countries (A review on the challenges for increased production of castor, Agronomy Journal, 2012).

This oilseed has an increasing demand in the specialty oil chemical industry, but the consumption is being limited by the supply rather than by the worldwide demand. Only three countries (India, China, and Brazil) concentrate 90% of the castor production. Those countries are experiencing a fast economical development, and the labor costs for hand harvesting are becoming prohibitive. Therefore, adapting castor for mechanized harvest is the top priority at a global level.

Some important challenges for castor breeding are: (i) adapting the crop for new growing regions and cropping systems, (ii) promoting international scientific cooperation in the use of genetic resources, and (iii) understanding the inheritance of traits with agronomical importance, such as tolerance to diseases and abiotic stresses. Studies for development of transgenic castor had little progress because the regeneration of castor is limiting. Furthermore, it is questionable if deregulation of a transgenic castor would be economically feasible.

Increments in castor productivity depend largely on crop management. A post-emergent herbicide selective for castor was discovered. The slow seed germination in cold soils deserves attention particularly on Northern growing regions. Gray mold (Botryotinia ricini) is regarded as the main disease for castor, but little progress was made in the last decades to understand its biology, improve its management, and develop tolerant varieties.

The use of castor oil for biodiesel is still controversial because it finds a higher value in the chemical industry. Regarding the oil properties, castor can be used for biodiesel production with some minor limitations. New uses for castor oil are continually being developed.

Ricin has a negative public perception in the United States. Evidences are accumulating about the reduced risk for the use of castor meal for ruminant feed. The development of ricin-free varieties and of methods for castor meal detoxification is limited by the lack of adequate methods for measuring ricin toxicity. Other noxious compounds found in castor plant (allergens and ricinine) deserve more attention.

Organic fertilizer is another potential use of castor meal, particularly due to its noticeable anti-nematode effect. Castor has also an unexploited potential for remediation of soil contaminated with heavy metals. Apparently, the use of castor oil would be considerably higher if this feedstock were available in large scale, with a reliable supply, and at a reasonable price.

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## SEED ABORTION AS A MECHANISM OF SOURCE/SINK ADJUSTMEN IN CASTOR PLANTS

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Castor (Ricinus communis L.) is an industrial oilseed crop. Presently, the main demands for technological development in castor are the adaptation of the crop to mechanical harvest, yield increase, and reduction of ricin content in the seed. Previous studies found that around 10% of the seeds in a regular raceme are aborted, and they seem to contribute for a reduced productivity. An aborted seed has a seed coat and regular size, but they do not accumulate reserves. This study aimed to investigate the effect of source and sink manipulations in the rate of castor seed abortion.

Two varieties of castor ('AL Guarany' and 'BRS Energia') were raised in 12 L pots in a greenhouse (Texas Tech University, Lubbock, TX), in a completely randomized design with six replications. The treatments consisted of: (i) a source reduction imposed by weekly defoliations that limited the plant to 50% of the leaf area observed in the day the first raceme was launched; (ii) a sink reduction imposed by clipping all the racemes except the first one; and (iii) a control treatment with no manipulation.

When the sink was reduced, assimilates were shifted to vegetative growth, and the leaf area increased to 6406$cm_2$ compared with 5341 $cm_2$ in the control plants. The defoliation increased the frequency of aborted seeds to 14.8%, but the sink reduction did not influence the frequency of aborted seeds (6.7%) which was similar to the control treatment (4.5%). The sink reduction did not influence the number of seeds in the first raceme. The mean seed weight of the first raceme was equal in all the treatments. The control plants had a higher seed production because they produced additional racemes. The sink-limited plants were not able to adjust the seed number and seed weight to compensate for the clipped racemes. The source and sink manipulation did not influence the seed filling duration which was 71.4 days on average.

It seems that seed abortion is a mechanism for adjusting the sink size to the source. This mechanism plays an important role because the plant can postpone the adjustment of the sink by ca. 20 days which is the initial phase of seed development. It has a low energetic cost because the seed is still empty when abortion occurs. The loss of assimilates is negligible (2.5% of the total seed weight). It is unlikely that castor seed yield would be reduced due to seed abortion. We found little plasticity in the number of seeds per raceme and in the seed weight, corroborating the hypothesis found in other studies that the number of racemes is the most plastic yield component in castor plants.

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## POLLEN DISPERSAL AND GENE FLOW IN CASTOR (RICINUS COMMUNIS L.)

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Castor has been the focus of substantial research. The fatty acid in its seed, ricinoleic acid, is highly reactive and acts as a precursor for chemical reactions to generate substances in everyday life. Because of its chemical reactivity, it has the potential to replace petroleum in many situations. Studies conducted in north Mississippi/southern Tennessee indicate seed yields of 2018-2354 kg/ha (1800-2100 lbs/acre) can be harvested from castor at these locations. However, the most commonly planted cultivar is Hale, developed near Plainview TX, and as such, would be expected to have maximum yields at or near similar latitudes and climates. New cultivars are needed to expand castor's

productive range in the US. To achieve this goal, the pollination habit and breeding nature of castor must be investigated. Castor is reported to be wind pollinated, although insects frequent male flowers. Literature, especially from western locations of the US, suggests castor pollen travels great distances which confounds isolation of breeding lines. Our objectives were to test distance of pollen travel in Mississippi and Oklahoma and evaluate the crossing rate at various distances from the pollen source. Three fields were planted; one at Lane, OK and two at Starkville, MS. The field design was a block of 289 plants, equidistantly planted on 1m (3.28') centers. The nine plants in the center of the block were a homozygous dwarf red leafed genotype (Homma) developed specifically for this experiment. The other 280 plants were the cultivar Hale (green leaf). Plants were allowed to pollinate under ambient conditions. Seed was harvested by plant and identified with their original field position. The following spring, seed of the three test locations were sown plant-to row in separate blocks at Starkville, MS and screened for bronze (heterozygous) progeny. Of the 59,241 progeny evaluated, 861 were bronze phenotype. Individual rates of crossing indicated a 1.13% crossing rate at the MS north field; 1.5% outcrossing at the MS south field; and 2% outcrossing at the OK site. Non-linear regression was used to determine a curve predicting distance pollen traveled from the source.

Distance varied by location, with the greatest distance of pollen travel 7.32 m (24') (á=0.002) at MS south field location. Based on this information, breeder's blocks need to be 8 m (26') from one another, with or without intervening crops, to prevent contamination of germplasm.

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## INDUSTRIAL OILSEED CROPS AND ECOSYSTEM SERVICES: POLLINATORS

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Many industrial and specialty oilseed crops have showy flowers that are highly attractive to insect pollinators. Moreover, across the spectrum of species and cultivars that can be grown in frigid regions, such as Minnesota, floral phenology can be manipulated to vary from early spring (April) to late autumn (November). This time period corresponds to that for transient honey bees, which typically are shipped to California in November and return to Minnesota in April. Currently, honey bee colonies are sustained in April-May and September-October by artificial diets comprised largely of high-fructose corn syrup (for energy) and soy meal (for protein) because floral resources are unavailable at those times. About one-third of transient colonies are lost annually and another large proportion under-perform in terms of pollination efficiency while in California. Causal mechanisms are many, but apiarists agree that poor nutrition during critical phases of a colony's life cycle (spring and autumn) has wide-ranging and over-riding effects.

Our goal was to examine a broad array of oilseed crops, each with known economic value, for their flexibility in floral phenology and pollinator attractiveness.

Minnesota-hardy crops included winter camelina, pennycress, winter canola, spring camelina, spring canola, calendula, flax, borage, echium, sunflower, and cuphea. They were sown in 3 by 7.5 m plots in a RCB design near Morris, MN. Planting dates and harvesting methods were used to manipulate floral phenology of some species. Weekly observational surveys of insect visitors and floral abundance were conducted over two growing seasons.

Although some oilseed species clearly were more attractive to some pollinators (e.g., echium and honey bee) than others, in general the more flowers present the greater the abundance of pollinating insects regardless of season. In essence, if seasonal conditions (e.g., air temperatures) are conducive to anthesis, irrespective of crop species, insect pollinators (including honey bees) will be present, often en masse, to tap that highly valued resource. Future research will examine pollinator health as a consequence of wider seasonal availability of floral resources.

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**DEVELOPMENT OF HRJ FUEL FROM BRASSICA IN ROTATION WITH WHEAT FOR THE WESTERN U.S.**

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The aviation industry has expressed a strong interest in the development of renewable jet fuel from oilseed crops within the U.S. to supplement its fuel needs and provide a smaller carbon footprint for its industry. The USDA NIFA identified objectives within its recent BRDI grant program to address the challenges for the advancement of feedstocks to provide raw materials for Hydrotreated Renewable Jet (HRJ) fuel. In response, the USDA-ARS successfully assembled a project team to evaluate the potential to optimize each sector of the feedstock supply change for Brassica rotations within the Western U.S. Wheat Belt.

This project is organized around six objectives: Objective 1: Genetically improve feedstocks to enhance oil yield and quality stability across varying western U.S. production conditions and compatibility with hydro-treated renewable jet (HRJ) fuel conversion processes; Objective 2: Provide regionalized strategies to integrate sustainable oil seed production into existing land uses in ways that increase farm profitability and rural economic opportunities, while providing biofuel refiners dependable supplies of high quality feedstocks; Objective 3: Develop cost-effective processes to remove feedstock oil impurities and identify co-product market opportunities to decrease HRJ fuel production costs and increase system profitability through value-added income streams; Objective 4: Lower HRJ production costs by optimizing (a) conversion technology for genetically improved oilseed feedstocks and pre-treatment requirements, and (b) operational settings to genetically plant oils enhanced for conversion and processing efficiency; Objective 5: Develop analyses to provide strategic guidance addressing the uncertainties of expanded oilseed-based HRJ fuel production on select economic, social, and environmental indicators of sustainability; and Objective 6: Align participant and stakeholder interests along the supply chain to promote effective development of partnerships for creating new rural economic development opportunities centered on HRJ fuel production. This presentation will provide an overview of research that has begun on this four year project.

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# Natural Rubber and Resins

**ERICAMERIA NAUSEOSA (RUBBER RABBITBRUSH):
A COMPLEMENTARY RUBBER FEEDSTOCK TO AUGMENT THE GUAYULE RUBBER PRODUCTION STREAM**

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Ericameria nauseosa (rubber rabbitbrush) is a highly prolific desert shrub that produces high quality natural rubber. Over the past several years we have investigated rabbitbrush's potential as a commercial rubber feedstock. Like guayule, rabbitbrush produces natural rubber within its bark tissues and can be harvest for latex and solid rubber. As such, it may be possible to use rabbitbrush as a drop-in replacement in guayule rubber extraction facilities. Several properties of rabbitbrush make this prospect particularly attractive. First, rabbitbrush has a large geographic range spanning the Mexican and Canadian borders of the Western United States, greatly expanding the acreage for rubber feedstock production. Second, because rabbitbrush rubber yields are highest in the summer instead of the winter like guayule, rabbitbrush could supply idle guayule rubber extraction plants during the off season. Third, in addition to rubber, rabbitbrush produces high levels of terpenoid resins and lignocellulosic biomass that could be used as bioenergy co-products. Finally, rabbitbrush thrives in saline and alkaline soils with very low water requirements under both high and low temperature extremes. As such rabbitbrush can be used to bring land previously viewed as unarable into commercial production. While rabbitbrush is currently a wild species, because of its high genetic diversity and diploid genome, rapid genetic improvement should be possible using modern marker assisted breeding approaches. In total, rabbitbrush provides a potential feedstock that could augment production at guayule rubber extraction facilities and increase North American rubber acreage without competing with traditional food crops for resources.

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## OVEREXPRESSION OF HMGR IN GUAYULE: LABORATORY AND FIELD STUDIES

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In 2011, 10 million metric tons of the biobased product, natural rubber (NR) was harvested from the Hevea rubber tree for use in commerce, valued over $10B USD. Natural rubber biosynthesized via the isoprenoid pathway by alternative plant sources, such as guayule (Parthenium argentatum) may be more economically sustainable with improved yields, through breeding or targeted metabolic engineering of the isoprenoid pathway. The enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) is considered a key regulatory enzyme of isoprenoid carbon flux in mammals, in microbial systems, and possibly in plants. The objective of our study was to modify isoprenoid production in guayule through overexpression of the HMGR gene.

A transformation plasmid pND4-HMGR(tAN) was constructed with a modified binary vector and the HMGR gene from A. nidulans containing only the 465 amino acid catalytic domain driven by a constitutive promoter. Five independent transgenic lines were obtained via leaf disc Agrobacterium-mediated transformation. In the laboratory, the NR content of two-month old in vitro plantlets showed a 65% increase in rubber over the control for one line (HMGR6), and lower resin production for another (HMGR2). Field evaluations were conducted over a 4 year period in Maricopa, AZ. The genetically modified HMGR6 line was differentiated from control lines in size, biomass, and plant morphology descriptors, but not in rubber or resin content. Following pollarding harvest, plant survival and regrowth in the field were evaluated. Remarkably, the survival rate of all HMGR-modified plants was better than controls, with

the highest survival rate for line HMGR6.

In conclusion, we report the first genetic modification of guayule to overexpress the isoprenoid pathway enzyme HMGR. Survival during regrowth was significantly improved for HMGR overexpressing plants, suggesting enhanced carbon flux to important secondary isoprenoid metabolites, such as growth phytohormones.

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## WILL TOMATO SPOTTED WILT VIRUS LIMIT WIDESPREAD TARAXACUM KOK-SAGHYZ CULTIVATION IN NORTH AMERICA?

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Sowing of wild-collected seed of putative Taraxacum kok-saghyz (TKS) plants in climate controlled greenhouses and open fields managed by OARDC (Wooster, OH) began in 2006. Since then, multiple commercial species susceptible to thrip-transmitted Tomato Spotted Wilt Virus (TSWV) and TKS have been grown in close proximity. In late 2008, a TSWV outbreak occurred in two commercial, greenhouse-grown species. In early 2009, ELISA-based testing of leaf samples of all species in the research complex (except weeds) yielded positive results. However, interference with the ELISA by TKS rubber left the results equivocal. Also, the ELISA also can detect INSV (Impatiens Necrotic Spot Virus). Regardless, until now, TKS was regarded as a symptomless harbor of TSWV and, therefore, a possible threat to TSWV-susceptible crops.

Subsequent ELISA-based testing of TKS root and leaf samples from clonal plants often yielded negative results for root samples but, occasionally, positive results for leaf samples with negative roots. This suggested that TSWV may not persist in TKS roots and that asexual propagation through root cuttings could eradicate TSWV infection from a TKS population. Beginning in 2008-09, asexual propagation via root cuttings and the purposeful co-production of TKS and commercial, TSWV-susceptible species (lettuce, petunia, tomato) in the same spaces has been standard. TKS and susceptible 'indicator' species have also been grown in adjacent research fields in separate experiments. Moreover, seed traced to a second wild-collection of TKS seed (Hellier et al., 2011) have been sown in the same spaces. It has been learned that the first collection also contained seed of T. brevicorniculatum (TB), which co-occurs with TKS in natural populations. More recently, three plants from 24 lines of TKS and five lines TB and two plants from five lines of T. officinale were tested for TSWV using ELISA. Extracts were made from a pool of positive plants and used to inoculate TSWV-susceptible tobacco plants.

For three years, indicator plants have been symptomless. However, plantings were sprayed regularly; this result may reflect excellent thrip control. Positive ELISA results were obtained from one plant of each TKS line and from a single TB plant. None of the T. offinale plants tested positive. Diseased phenotypes of susceptible tobacco plants were obtained following inoculation with leaf extracts of the five ELISA- positive TKS. Therefore, TKS can be a phenotypically-asymptomatic carrier of TSWV. RT-PCR confirmed the presence of TSWV and showed that this particular TSWV is >98% identical with other strains (amino acid and nucleotide sequences) originating in the southern USA. Thus, our TKS likely was infected after arriving in the USA, and the USDA collection did not co-introduce a European or Asian strain of TSWV. However, since TKS can harbor TSWV, we are developing appropriate seed treatment and testing methods.

In conclusion, T. kok-saghyz does not seem an original source of TSWV and appears eligible for widespread commercial planting.

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## CONVERT INULIN TO RUBBER IN RUSSIAN DANDELION

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Taraxacum kok-saghyz (TKS) is a promising alternative natural rubber crop. In addition to high quality rubber, the TKS root also contains inulin, a β(2-1) fructan, which accumulates during the growing season and becomes a major storage carbohydrate for plant over the winter. Since previous studies indicate that TKS roots contain less rubber in the fall than in the ensuing spring, it is possible that substrates released from inulin dissimilation during the winter are used for rubber synthesis. This raises interest in converting inulin directly to increase rubber yield and allowing an autumn harvest.

In this study, we will employ genetic engineering to either boost inulin degradation or block inulin synthesis in order to release the photosynthetic products from storage. To ensure that most of the liberated substrates are directed to rubber synthesis or other secondary metabolites of commercial interest, like terpenes, it is equally important to up-regulate the key enzymes involved in the biosynthesis of these products. As a foundation to this research we have established a hairy root transformation system for TKS to allow more efficient verification of transformed genes in TKS roots than traditional Agrobacterium tumefaciens methods which require regeneration of full plants after transformation.

TKS seedlings were germinated from collected seeds of KAZ08-017. TKS tissue culture was initiated from leaves of germinated seedlings. The hairy root system was established using Agrobacterium rhizogenes mediated transformation of the pCambia1300 plant transformation vector with green fluorescence protein (GFP) as a reporter gene. Meanwhile, the pEarleyGate vector harboring the Arabidopsis thaliana 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGR) gene or 1-deoxy-D-xylulose-5-phosphate synthase (DXS) gene, both driven by a constitutive promoter were transformed using tissue cultured TKS leaf discs to serve as a preliminary transformation test.

TKS hairy root system was successfully established. The presence of the GFP gene in transformants was confirmed by visualizing the fluorescence from induced roots under UV light. Aside from our original intention, this method also opens up possibility to introduce transgene into TKS root while boosting the total yield of the transformed root. Since latex is known to accumulate mainly in the TKS taproot, our immediate future work will focus on regenerating a full transgenic plant from the transformed TKS root.

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## GUAYULE RESIN EFFECTS ON TERMITE FEEDING ACTIVITY

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Guayule, a desert adapted plant, yields hypoallergenic rubber that is used primarily by the medical profession for rubber gloves, catheters, etc. Recently there also has been renewed interest in using guayule rubber for tire production

due to the expected rubber shortage. Terpene resin is a . The resin is potentially a valuable a co-product from guayule that can be extracted prior to rubber extraction. The resin has termiticidal properties. As such, this resin could serve as a natural and renewable product for termite pest management. Several previous studies have shown that the resin can provide resistance to several species of termites. However, quantification of the termite damage and resin concentrations needed to provide termite resistance when the resin is impregnated into other materials is lacking. The objective of this study was to quantify the effect that guayule resin has on the feeding behavior of the subterranean termite, Heterotermes aureus. A series of choice and no-choice feeding studies were conducted to quantify the effect that guayule resin has on the feeding behavior of the subterranean termite. In the choice study, composite wood blocks were impregnated with guayule resin using an acetone carrier so that their weights increased by either 5% or 35%. Then, termites were given a choice to feed on wood blocks impregnated with 0% (untreated control), 0% (acetone treated control), 5%, and 35% guayule resin, respectively. The consumption of each guayule treatment was determined by calculating the weight loss of the blocks over a 28 day period. Data showed that the untreated and acetone-treated blocks were readily fed on by the termites, but the guayule-treated blocks were not. The absence of significant differences in consumption of the 5% and 35% blocks suggests that low concentrations of guayule resin can deter termite feeding activity. No-choice feeding studies were also conducted to determine if termites would feed on guayule-treated foodstuff in the absence of other dietary options. Termites were placed in various feeding arenas that only contained a single piece of filter paper treated with 0%, 2.5% or 5% guayule resin in acetone. The amount of filter paper consumed for each treatment was determined by measuring the surface area missing from the filter papers. Data showed that the termites readily fed on the untreated and acetone treated filter paper, but were reticent to feed on the filter paper containing 2.5% and 5% resin. The findings show that guayule resin might be used to prevent property damage by infusion of guayule resin into lumber or as a paint additive. The use of guayule resin to control termites could lead toward more environmentally benign and cost effective termite control.

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## FARM TO MARKET: DRIVING INNOVATIVE APPLICATIONS OF GUAYULE THROUGH INTEGRATION OF THE VALUE CHAIN

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

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## ANALYSIS OF COLD-ACCLIMATED GUAYULE TRANSCRIPTOME

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The perennial shrub commonly know as guayule (Partheniun argentatum), native of the Chihuahuan desert, is a rubber producing plant currently being develop as a domestic source of high quality natural rubber essential for the manufacturing of several consumer products for which the use of synthetic rubber is not an option. Rubber synthesis in guayule occurs in the bark parenchyma tissue, mainly during the winter when night temperatures are moderately cold (6-15 °C). The molecular mechanisms triggered by such cold induction of rubber synthesis are unknown, however, are important to understand for the purpose of improving the plant to maximize rubber production yields.

To begin to understand the molecular basis of cold-induced rubber production in guayule, we performed a computational analysis of the transcriptome of a 2 year-old cold-acclimated guayule shrub. A cDNA library from bark parenchyma tissue poly(A) mRNA was made using a modified oligo(dT) primer. Double-stranded cDNA fragments >600 bps were introduced into plasmid DNA maintained in ~1.3 x $10^6$ clones of Escherichia coli. 11,748 quality sequences were generated by randomly sequencing clones, of which 9,021 were unique transcripts. Identities of the cDNA library sequences were assigned based on BlastX searches of the non-redundant NCBI database.

The most abundant sequences in our collection were related to stress responses (biotic and abiotic). Several of other the abundant transcripts have also been reported to be highly abundant in Hevea brasiliensis (the rubber tree). In agreement with previous protein analysis of guayule, cold-acclimated rubber-producing tissue was enriched with allene oxide synthase (AOS) transcript (AOS protein is present in high abundance and intimately associated with guayule rubber particles). Interestingly, transcripts encoding other proteins believed to be associated with rubber particles (e.g. cis-prenyl transferase and small rubber particle protein), or the isoprenoid pathway enzymes that make the precursors for rubber biosynthesis (e.g. the mevalonate pathway enzymes and farnesyl pyrophosphate synthase) were not highly abundant. Altogether, transcripts of enzymes relevant to rubber biosynthesis constituted about 1.1% of the unique transcripts population.

Our analysis of cold-acclimated guayule transcriptome, in combination with a seasonal gene expression profile of selected rubber synthesis-related genes, suggest that the cold temperatures associated with abundant rubber synthesis in field plants is not controlling rubber synthesis at the gene expression level. This conclusion opens the possibility of a post-transcriptional point of regulation, and/or the critical cold-inducible gene(s) encoding members of the rubber synthesis machinery remain to be identified.

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## BIOBASED ALTERNATIVES TO GUAR GUM AS TACKIFIERS FOR HYDROMULCH

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NOT PRESENTED

Guar gum, obtained from guar [Cyamopsis tetragonoloba (L.) Taub.] seeds, is currently the principal gum used as a tackifier (binder) for hydraulically-applied mulches (hydromulches) used in erosion control. The oil industry's increased use of guar gum in hydraulic fracturing together with lower global production has driven up the price to levels never previously reached, resulting in the wholesale price of guar gum increasing to as much as $18.00/kg, when

historically prices ranged from $1.80 to $2.20/kg.

The objective of this research was to determine if other biobased tackifying agents which are research group has been studying are acceptable as alternatives to guar gum. These include defatted camelina and lesquerella seedmeals, corn starch, corn starch-sodium palmitate inclusion complexes prepared from either normal or high amylose starch, pectin, lignin, plantago gum or xanthan gum.

Camelina and lesquerella seedmeals were obtained after oil extraction with hexane. Corn starch-sodium palmitate inclusion complexes were produced by jet cooking. Corn starch, pectin, lignin, plantago gum and xanthan gum were used without modification from the suppliers.

All of the tackifiers were mixed as dry powders (2.0 g each) with hydromulch (Hydrostraw®, 48.0 g) formulated without a tackifier, to which deionized water (400 ml) was added to form a paste, which was applied evenly to 38.8 cm x 25.9 cm baking pans. The pans were placed in a drying oven at 40 $_{o}$C for 24 h, and then subjected to simulated rainfall for 1 h at a rate of 6 cm/h in a research track sprayer. The pans were removed from the sprayer and placed in a drying oven for an additional 24 h. Rainfastness index was calculated as: [Weight of hydromulch + tackifier after rainfall (g)/50.0 g] x 100%.

Our results indicate that lesquerella and camelina seedmeals, the high amylose corn-starch-sodium palmitate inclusion complex and xanthan gum had higher rainfastness indices than guar. Pectin, plantago gum, methyl cellulose, the normal corn starch-sodium palmitate complex and lignin were equal to guar in rainfastness indices. Only waxy starch had a lower rainfastness index.

These results indicate that other tackifiers would be acceptable as replacements for guar gum with cost and availability determining which materials would be used.

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## GROWTH AND BIOMASS YIELD OF GRINDELIA CAMPORUM and G. CHILOENSIS GROWN IN THE KLAMATH BASIN OF OREGON, USA

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Worldwide demand for resins (used to make high quality copier paper and specialty chemicals) continues to increase. The supply of the traditional raw material (pine rosin) fluctuates dramatically with wood and pulp prices. The USA and Argentina both import nearly 100% of these resins. Grindelia camporum, a native of California, and Grindelia chiloensis, a native of Argentina, both produce significant quantities of grindelic acid and related resins on their leaves, flowers, and stems. Earlier studies in Oregon and Argentina showed that G. camporum and G. chiloensis could grow well and produce large amounts of biomass and crude resin, but optimum conditions were not well understood.

This study was done to compare the effects of irrigation rate, plant spacing, and nitrogen fertility on crop growth, biomass yield, resin production, and winter survival for several cultivars of these two species in the Klamath Basin, a drier and colder location than the site of earlier Oregon studies. Seedlings of eight G. camporum and five G. chiloensis cultivars were transplanted to the field in the spring, 2010 in several field experiments near Klamath Falls, Oregon, USA. In one study, plots of all cultivars were grown under "High", "Medium", and "Low" irrigation treatments, with irrigation rates set to equal 60%, 40% and 20% of calculated Penman-Jensen evapotranspiration rate (Et) during the growing season. In another study, a single G. camporum and two G. chiloensis cultivars were planted at four plant densities, all grown under the "Medium" irrigation rate.

Whole plants were harvested in the fall. Biomass yield differences due to irrigation rate were significant for G. camporum: Yields ranged from 0.5 to 6.3 Mg/ha, with some cultivars clearly larger than others at all irrigation rates. Irrigation treatment differences were not significant for G. chiloensis, but differences between cultivars were significant: Yields ranged from 0.7 to 1.6 Mg/ha. The effect of plant density on biomass per plant was not significant for either species, but biomass per hectare was significantly different, directly following the density pattern. Biomass yields ranged from 0.8 to 11.4 Mg/ha for G. camporum, and from 0.8 to 3.1 Mg/ha for G. chiloensis.

Differential cutting heights were imposed during the fall 2010 harvest. Cutting stems just above ground level significantly reduced winter survival for both species compared to cutting 20cm above ground level. Survival of G. camporum was generally better than G. chiloensis, but survival of both species was worse in the Klamath Basin than had been observed previously in the warmer Rogue Valley. For the plants that survived, biomass yields in 2011 were dramatically higher than in 2010, ranging from 2.8 to 135.9 Mg/ha for G. camporum and from 7.5 to 52.4 Mg/ha for G. chiloensis. Irrigation effects on yield were not significant overall for either species, although some cultivars appeared to benefit from some irrigation. Yield differences due to plant density were significant for both species, and followed the same pattern observed in 2010, where closer plant spacing resulted in higher per-hectare biomass yield, even though canopy closure was complete or nearly so for most densities. Mid-season application of 56 kg N/ha did not significantly increase biomass yield. Resin analysis has not yet been completed for these studies, but based on the biomass results it appears that both G. camporum and G. chiloensis are good low-input crop candidates for the semiarid, temperate zone to fill industry's need for commercial resin production. Biomass yields have been dramatically higher in the second (and presumably subsequent) years for this perennial but winter survival may limit their production to warmer winter areas within these regions.

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## PRODUCTION OF GUAR FOR HIGH GUM AND SEED YIELDS

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Guar (Cyamopsis teragonoloba (L.) Taub.) is a highly drought-tolerant, annual legume crop that is thought to have originated in India that has historically been grown in the semi-arid regions of the Eastern Pakistan and Western India. In the United States, commercial guar production has been limited to the semi-arid regions of the High and Rolling Plains of Northern Texas and Southwestern Oklahoma. Guar is the primary source of galactomannan gum that is used for thickening, sizing, and strengthening materials in the paper, textile, mining, pharmaceutical, and food industry. The United States is also a major importer of guar gum for use in hydraulic fracking in the petroleum industry. Halliburton Corp. supported a breeding program at Texas Tech University on guar from 1998 to 2007 that generated two guar varieties, Matador (PVP 200400235) and Monument (PVP 200400301). Matador has a highly branched growth habit and produces seed pods on short racemes. Monument has a non-branching growth habit and produces seed pods on long racemes with many unfilled pods. Our central hypothesis is that these two guar cultivars will respond very differently to plant populations, row spacing, and irrigation regimes. We intend to exploit these differences in growth habit in designing an agronomic production system that targets seed yields in excess of 3,000 kg/ha. Higher seed and gum yields will improve the economics of guar gum production in the U.S. in comparison to guar gum historically produced in Pakistan and India. Domestic production of guar gum for use in hydraulic fracking could help enhance the Energy Independence of our country.

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## SECURING THE FUTURE OF NATURAL RUBBER – AN AMERICAN TIRE AND BIO-ENERGY PLATFORM FROM GUAYULE

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There is a high level of interest in alternative sources of natural rubber for the tire industry due to both the price and supply volatility of Hevea brasiliensis. The guayule plant (Parthenium argentatum) has served as a major source of domestic natural rubber in the early 20th century and is being evaluated by several groups as an alternative today.

The objective of this presentation is to outline technical and agronomic areas which must be addressed if guayule is to be a viable candidate to replace Hevea.

A $6.9 million DOE/USDA grant was recently awarded to a consortium lead by Cooper Tire & Rubber under the Biomass Research and Development Initiative (BRDI) to look at all aspects of guayule rubber production and the development of a concept tire using only guayule or guayule derived rubber. The study includes genome sequencing work, advanced breeding, agronomic development for guayule, isolation/stabilization of the guayule rubber, modification of the guayule rubber for tire applications, tire compound development, tire building and tire evaluation. Bagasse will be evaluated in both a cogeneration facility and a biomass-to-fuel facility. Finally, the program will include a sustainability/life cycle analysis of the production of guayule and its use in tires which will involve the economic, environmental and social effects of production on society.

The program will be outlined and critical questions for the commercialization of guayule rubber will be considered.

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## PERFORMANCE OF LATEX PRODUCTS CONTAINING MACRO, MICRO AND NANO FILLERS MADE FROM AGRICULTURAL, INDUSTRIAL AND FOOD PROCESSING WASTES

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Many natural rubber latex consumer products contain fillers with the intention of diminishing overhead costs, or creating new desirable physical properties that aid in processability or utility. Yet these commonly used fillers are not sustainable industries, and dedicate energy and natural resources such as petroleum for their production. Thus, sustainable alternatives for natural rubber latex composites need to be developed.

The objective of this study was to investigate how natural rubber latex composites differ in tensile properties due to lattice origin, type of sustainable filler, as well as filler loading and particle size.

Lattices used in this study include: Hevea latex (NRL) with high protein content (Centex, Centrotrade), Hevea Vytex

NRL with no soluble protein (Centrotrade), and Guayule latex (GNRL). The fillers in this study include calcium carbonate derived from eggshells, carbon fly ash, and guayule bark bagasse. The macro fillers were dry milled and sieved to a particle size of 300 microns to 38 microns. The micro fillers were dry milled and sieved to a particle size of 38 microns and smaller. The nano fillers were wet milled in water using a pebble mill (Glen Mills, PM 100), and particle size was confirmed using SEM and TEM at the Molecular and Cellular Imaging Center, OARDC.

Latex composites were manufactured using coagulant dipping with an automated dipper (Dip Tech Systems). The film was then vulcanized, and four dumbbell specimens of each compound were cut. Evaluation of the tensile mechanical properties followed ASTM D 412 and were measured using an tensiometer (Instron).

In general, all latex composites had stronger tensile properties with smaller particle sizes at lower loadings. The tensile strength of latex composites were often inferior compared to latex films without fillers, but a reinforcing effect was found in low loadings of nano sized fillers. In general, the elongation at break was increased in latex composites compared to latex films without fillers. Many of the composites of all three lattices exceeded the tensile requirements described in ASTM D 3577, the surgical glove standard.

Latex composites with sustainable fillers can create polymer films with qualities desirable in many applications, such as increased elongation at break. The use of such fillers can create novel materials and decrease cost of manufacture.

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# Fiber and Cellulosics

## STARCH-BASED BIOPRODUCTS

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Starch is one of the least expensive and most abundant biopolymers available. Commercial supplies are typically available from corn, wheat, potato, and cassava. Starch is a hydrophilic biopolymer that is readily degradable in most environments. Because of its relatively low cost and abundant supply, starch continues to be considered as a replacement for non-renewable polymers in many products. The objective of this presentation is to provide an overview of current progress of the Bioproduct Chemistry and Engineering research unit in developing starch-based food packaging, controlled release devices, and fire retardant coatings. Starch-based food packaging was developed using a baking process similar to that used in the food industry. A composite material of starch, fiber and calcium carbonate was developed that had the strength to be demolded without breaking. The parts were trimmed and coated with a polyester film to provide moisture resistance. These products have the functional properties needed for commercial food packaging and readily degrade in a composting environment. Starch-based foam microparticles were made by atomizing a 5% aqueous gelatinized starch solution. The droplets were air-classified and collected in ethanol and air dried. The foam particles were infiltrated with essential plants oils as an active ingredient. The particles were used as a controlled released delivery system for controlling parasitic mites in honeybee colonies. Starch-based coatings were used as a means of providing fire protection in emergency situations due to wildfires. The starch-based coatings provided up to forty minutes of control against heat in excess of 500°C.

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## CARYOPSIS EXTRACTION FROM BIG BLUESTEM SPIKELETS (ANDROPOGON GERARDII) WITH SEED CONDITIONING EQUIPMENT: OPTIMAL WATER ACTIVITY TO ACHIEVE HIGH SEED

## QUALITY

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Big bluestem is a perennial, warm-season grass that may be used as a bioenergy crop for cellulosic bioethanol production. Big bluestem produces spikelets with appendages and awns, and is considered a chaffy grass species that does not readily flow through commonly available field sowing equipment. In addition, spikelets may not be filled resulting in decreased Pure Live Seed (PLS). Intact caryopses can be extracted from spikelets with seed conditioning equipment; however, mechanical damage may occur.

The objective of this study was to determine the optimum water activity to minimize mechanical damage during conditioning and thus achieve high seed quality. Equations were developed to increase seed water activity to a desired level

X-ray and scanning electron microscopy was performed to illustrate Big bluestem filled seed and mechanical damage induced during seed conditioning. Water activity of big bluestem spikelets were adjusted to 0.55, 0.65, 0.75 or 0.85 aw levels, and brushed with a laboratory brushing machine (Westrup LA-H) for 1, 2 or 3 minutes. A seed aging study was conducted to compare longevity of extracted caryopses and spikelets.

The greatest germination ($7_{th}$ day count and percentage normal seedlings) was obtained with caryopses extracted from spikelets adjusted to 0.75 aw and brushed for one minute. Brushing spikelets with lower water activity or for longer durations resulted in greater mechanical damage to the caryopses. Recovery of caryopses from spikelets was less than 50 percent in all treatments. Intact spikelets and caryopses, obtained from 0.75 aw and one minute brushing and were aged at 50% RH at $30_oC$ up to four months. Caryopses and spikelets showed similar ageing trends thus brushing did not reduce longevity.

A commercial brushing machine that is routinely used in seed conditioning was effective in extracting caryopses from intact spikelets. Increasing water activity level before brushing protected big bluestem caryopses from detrimental effect of brushing machine and caryopses quality was significantly improved. Moreover, the resulting caryopses were free flowing so they could be sown in conventional planting equipment.

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## OPTIMIZING BIOMASS BLENDS FOR MANUFACTURING MOLDED PACKAGING MATERIALS USING MYCELIUM

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Polystyrene is one of the most widely used plastics and is commonly produced in three forms: 1) Extruded polystyrene – disposable utensils, CD/DVD cases, yogurt containers, smoke alarm housing, etc.; 2) Expanded polystyrene foam – molded packaging materials and packaging "peanuts"; 3) Extruded polystyrene foam – insulation boards. Extruded polystyrene foam is commonly sold under the name of StyrofoamTM. Polystyrene packaging and insulation is a multibillion dollar a year industry. Since polystyrene is non-biodegradable, a biodegradable material that is eco-friendly was sought as a substitute for packaging and insulation board consumers. The material produced from a

process, developed by Ecovative Design, LLC, involved growing fungal species on biomass substrates to produce an eco-friendly packaging product (EcoCradleTM ) and insulation panels (GreensulateTM ). Previous research had shown desirable physical and mechanical properties from molded packaging materials produced with cotton gin byproducts (CGB) as a substrate. However, when CGB was blended with other biomass substrates, physical and mechanical properties were improved. The objective of this research was to evaluate and determine the optimal biomass blend(s) producing the best overall physical and mechanical properties for composites produced using Ecovative's technology.

Eight biomass substrates, kenaf fibers, kenaf pith, hemp pith, switchgrass, sorghum stover, rice straw, flax shive, and CGB were evaluated for optimal performance using twenty-one response variables. Evaluations were conducted on samples produced using a fungus selected from the Ganoderma tsugae group. All biomass materials were processed at USDA-ARS, Cotton Production and Processing Research Unit in accordance with predetermined particle size requirements. The substrates were sent to Ecovative's facility in Green Island, NY where samples were made from predetermined blends of each material. Properties evaluated included: density, strength (compressive and flexural), dimensional stability, modulus of elasticity, energy absorption, thermal conductivity, and accelerated aging.

Results from a D-Optimal mixture design revealed different optimal blends involving two to four biomasses depending on end-user specifications and properties deemed most desirable. The CGB were most effective when dynamic energy absorption and compressive strength were of interest. Sorghum stover was a good ingredient for energy absorption when strength was not as desirable. Kenaf pith was a primary ingredient when lower density was valued. Cost of the biomass was not a response variable in determining the optimal blends.

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## EVALUATION OF VARIOUS HEAT TREATMENTS TO IMPROVE PHYSICAL AND MECHANICAL PROPERTIES OF COMPOSITES MADE FROM COTTON BURS, COTTON STALKS, KENAF, FLAX, AND SOUTHERN PINE BLENDS.

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Previous studies evaluating physical and mechanical properties of composites produced from blends of cotton carpel (burs), cotton stalks, kenaf, and southern yellow pine indicated water absorption and thickness swell properties higher than composites made from 100% southern yellow pine. In the previous studies, Urea-Melamine-Formaldehyde (UMF) was the resin. As a continuation of the previous studies, this study evaluated composites produced from blends of cotton burs (B), cotton stalks (S), kenaf (K), flax, (F), and southern yellow pine (P) using two resins, UMF and Phenol-Fomaldehyde (PF). The objective of this study was to assess the impact of select heat treatments (pre and post) on the physical and mechanical properties of composites produced from various blends of B, S, K, F, and P, using two resins, compared to composites (heat treated and untreated) produced from 100% P and 100% commercial hardwood fibers (H).

Experiments were conducted on 10 composite board blends. All fibers were processed through a hammermill and a shaker table so particles were within 0.42 to 4mm. All fibers were dried to less than 5% moisture content and blended with one of two binding agents comprised of either 10% UMF or 8% PF adhesive with 1.5% wax emulsion. Heat treatments consisted of heating fibers either pre- or post board fabrication using an oven at 185C for 30 min. Board construction was accomplished using a 91-Mg capacity oil-heated hydraulic press. Composite boards, 0.635 cm by 40.6cm by 43.2cm, were produced using the following blend of fibers: 100% B, 50B/50K, 50B/50S, 50B/50F, 50B/50P, 100P, 100H, 100F, 100S, and 100K. Three specimens from each board were subjected to water absorption, thickness swelling, internal bond, and static bending stresses (modulus of rupture

[MOR] and modulus of elasticity [MOE]). The testing was performed in accordance with methods described in Part B of the American Society for Testing and Materials (ASTM) D 1037-06a.

Results indicate heat-treating the fibers post-fabrication improved water absorption in all boards compared to untreated specimens. Heat-treating had mixed effects on MOE, MOR, and internal bond with some fiber composites having improved values while others had lower values. Composites with flax fibers exhibited water resistance equivalent to the 100% P and H composites. Composites made with PF resin were more dimensionally stable than those made with UMF.

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## COVER CROPS IMPACT ON ENERGY CROPS PRODUCTIVITY IN NORTH DAKOTA

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Forage crops have gained interest as a potential source of lignocellulosic feedstock to produce ethanol. However, more focus should be on developing sustainable cropping systems to improve biomass productivity. This study was done to identify the agronomic potential of six different cover crops on four different annual biomass crops, as sources of feedstock. The experiment was conducted at two locations in a randomized complete block design with a split-plot arrangement where the cover crop was in the main plot and the biomass crop in the sub-plot with three replicates. Cover crop species were planted on 8 to 9 August in 2010 and 2011 following oat (Avena sativa L.), and just before the expected hard frost (October 14 according to previous data), nitrogen uptake, biomass yield and quality was evaluated. In the following spring, four biomass crops were planted after the cover crops in each successive year, and their biomass yield and forage quality parameters were analyzed. Results across environments indicated the cover crop forage radish (Raphanus sativus var. niger cv. Daikon) produced the highest dry matter yield (3.8 Mg/ha) followed by forage pea (Pisum sativum L.) (3.4 Mg/ha). Forage pea N uptake was 127 kg N/ha and significantly higher than all other cover crops. Forage pea biological dinitrogen fixation was about 59 kg of N/ha in only 40 days growth in the fall. This was calculated by subtracting the total N uptake by the peas from the average N uptake of the non-legume cover crops. The annual dedicated bioenergy crops corn (Zea mays L.), sweet sorghum (Sorghum bicolor L), and forage sorghum biomass yields were 2 to 3 Mg/ha higher than their check yields when following a legume cover crop. Forage sorghum and sweet sorghum biomass yields were also greater than their check yields when grown after forage radish. This increased biomass might be explained by the improved water infiltration associated with tap-rooted cover crops and the redistribution of leached nutrients back into the rooting zone by previous deep rooted scavenger brassica cover crops. Sweet and forage sorghum produced greater biomass yield (19-29 Mg/ha) than corn, forage oat, and forage barley (Hordeum sativum L.). Clearly, the additional N fixed by the legume cover crops enhanced the biomass yield in these bioenergy crops reducing costly nitrogen fertilizer needs.

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## WINTER CAMELINA AND FORAGE SORGHUM RELAY- AND DOUBLE-CROPPING FOR OIL AND BIOMASS FEEDSTOCK PRODUCTION IN THE NORTH CENTRAL REGION

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Short life cycle, high seed oil content winter-annual species like camelina (Camelina sativa L.), grows exceptionally well in Minnesota, North Dakota, and Montana. Recent findings indicate that camelina, especially when grown as a winter annual, matures early enough to allow double- or relay-cropping with forage crops such as sorghum (Sorghum bicolor L.). Additionally, double-cropping reduces the pressure on food production resulting from the introduction of energy crops while providing certain environmental benefits such as minimizing soil erosion, lengthening the growing season, and taking up excess soil N and moisture. Forage sorghum is an energy-efficient annual forage crop candidate that can produce high yield under droughty conditions and has low production inputs. The objective of this project was to determine the biomass-oil yield of double- and relay-cropping sequences in the North Central Region. Winter camelina, cv. Joelle, was planted at Prosper and Carrington, ND, and Morris, MN, on 29 August, 1 September, and 14 September, 2011, respectively. The experiment was a RCBD with a split-plot arrangement, where the main plot was winter camelina or fallow, and the sub-plot , forage sorghum, soybean (Glycine max L.), and corn (Zea mays L.). Relay seeding was performed in late April/early May with corn and soybean and by 15 May with forage sorghum. Double-cropping with sorghum or soybean followed camelina harvest at the end of June. Germination, emergence, and winter survival of camelina was successful at all sites. Camelina seed yield fluctuated between 1295 and 1885 kg $ha^{-1}$ at Prosper, 713 and 1069 kg $ha^{-1}$ at Carrington and 217 and 374 kg $ha^{-1}$ at Morris. There was no significant difference in camelina seed yield when a relay crop was in place, whether the relay crop was corn, soybean, or forage sorghum. This indicates that seeding a relay crop did not cause damage to the camelina plants nor enough competition to reduce seed yield. The relay crops were seeded earlier than their double cropped counterparts resulting in higher biomass and grain yield. Biomass and grain yield were expected to be greater with relay cropping since crop development occurred earlier in the season when temperatures were cooler and precipitation was greater as compared to growth conditions from later seeding for double cropping. The lowest biomass and grain yield was for the double crops planted after camelina harvest. Winter camelina and forage sorghum in relay- or double- cropping is an alternative system with potential for bioenergy feedstock production in North Dakota.

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## EFFECT OF COUPLING AGENTS ON THE WEATHERING CHARACTERISTICS OF BIO-FIBER COMPOSITES

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Bio-fiber polymer composites (BFPC) are composite materials made from a thermoplastic or thermoset resin (substrate) with cellulosic fibers as fillers or reinforcement. BFPC have shown a significant growth in the last decade as a building product, automotive parts and landscaping products. BFPC combine the superior mechanical properties of cellulosic fibers and the long-term performance of plastic. Some of the advantages of BFPC include low cost, recyclability, minimal health hazards, low density, and high strength to weight ratio.

A study was conducted to evaluate the effect of coupling and compatabilizing agents on the long term performance of cotton burr/stem fraction (CBS) of cotton gin waste as cellulosic fiber reinforcement in the polymer composite matrix. The composites were manufactured commercially using 5 different treatments. The base recipe constituted of 50% filler, 40% thermoplastic, 6% lubricant and two levels of coupling and compatibilizing agents.

The samples were subjected to accelerated UV weathering for 2200 hrs. following the guidelines established in ASTM D 6662 and ASTM G 154. The weathered samples were further tested to determine the changes in physical and

mechanical properties of CBS fiber reinforced polymer composites treated with additives. Physical properties tested include water absorption, color shift, surface defects, and polymer degradation. Mechanical properties tested were flexural modulus, flexural strength, impact, hardness and coefficient of thermal expansion. Overall this study reports the effect of specialty additives on the long term performance of CBS based bio-fiber composites.

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# POSTER PRESENTATIONS (Oilseeds)

## OIL DIVERSITY IN WILD Manihot SPECIES – A NEW INDUSTRIAL CROP?

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Wild cassava species (Manihot spp.; Euphorbiaceae) may have a potential to become a new industrial crop due to their seed oil content. Manihot esculenta Crantz is the only commercial species of the Manihot genus, cultivated for its starchy tubers used to produce flower. Cassava seeds are known to have high oil content with no commercial value. Reports on the oil and the fatty acid profile in wild cassava seeds are limited. Wild cassava plants usually produce a higher number of seeds than M. esculenta and their tuber roots are not used for consumption.

The objective of our study was to determine the oil content and the fatty acid profile in seeds of selected wild Manihot species.

Seed samples of 11 wild Manihot species and the cultivated M. esculenta from the Embrapa germplasm collection in Brazil were analyzed for oil (106 accessions) and fatty acid (FA) profile (75 accessions). The oil content was determined by a Nuclear Magnetic Resonance (NMR) technique and the FA profile was analyzed by gas chromatography (GC), in three replications. Data were evaluated by ANOVA.

The oil content varied from 17 (M. caerulescens) to 30% (M. flabellifolia). All species contained five predominant fatty acids. The concentration of the fatty acids was: palmitic (C16:0) 9.0 – 21.7%, stearic (C18:0) 1.9 – 11.2%, oleic (C18:1) 12.4 – 31.2%, linoleic (C18:2) 45.9 – 72.3%, linolenic (C18:3) 0.8 – 3.2%. The concentration of oil and the fatty acids varied significantly within species and between accessions.

Seeds of wild cassava species may be a source of oil for biofuel production and other industrial uses; however, the exact applications are subject to testing. Our study showed the rich content of oil and its wide variation in the 11 wild Manihot spp. studied. The germplasm is also a good source for future crop improvement.

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## CASTOR BEAN INBRED ADVANCED LINES EVALUATION IN SÃO PAULO STATE – BRAZIL

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Castor bean (Ricinus communis L.) is an oilseed crop of great industrial importance for its peculiar characteristics and oil quality, in Brazil is cultivated mainly in semi-arid regions. The demand for information about castor bean crop has increased due to interest in alternative fuels based on vegetable oils. The success of agricultural production depends heavily on the use of cultivars with superior performance, and genotypes adapted to different environments is crucial to ensure the success of any crop.

In this context, the genetic improvement of castor bean is essential and the objective of the study was to evaluate castor bean inbred advanced lines of the "Instituto Agronômico – IAC" crop breeding program, in the North Central region of the state of São Paulo, Brazil.

The experiment was conducted in the "Pólo Regional de Desenvolvimento Tecnológico dos Agronegócios do Centro Norte", located in the city of Pindorama, SP, in 2011/2012 harvest. It was used a randomized complete block design with 18 treatments (lines) and three replications. The experimental plot consisted of a line of 7.0 m, spaced 2.00 m between rows and 0.90 m between plants, floor area of 12.6 $m^2$. The sowing occurred on 15 january/2012. The characters evaluated were plant stand, plant height, stem diameter, number of internodes and grain yield.

The average yield was of 1.007,67 kg $ha^{-1}$, which is considered low, in spite of overcoming the national average. This may have occurred because the experiment was conducted in late summer period. The analysis of variance showed a significant difference between treatments, by Scott-Knott it was observed that the line 02 obtained the highest grain yield 1.730,00 kg $ha^{-1}$, exceeding the controls and also presented a semi dwarf type. The line 10 had the highest average height (2,21 m) and line 08 the lower average height (0.91 m). It is worth mentioning that the plant height is extremely important for the crop mechanization, therefore, the shorter plant height more suitable for mechanized harvesting

It is concluded that it was possible to evaluate promising castor bean inbred advanced lines of short height of the IAC Castor Bean Breeding Program.

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## COMPILATION OF STUDIES ON JATROPHA NUTRITION IN BRAZIL: LEAF DIAGNOSIS, SEASONAL VARIATION, AND NUTRIENTS REDISTRIBUTION

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Jatropha (Jatropha curcas L.) is an oilseed tree that was largely promoted as a promising option for cultivation in marginal land for production of oil for biodiesel. However, there is limited technical information to support the cultivation of jatropha in large scale.

This report in a compilation of a series of studies on the nutrition and fertilization of jatropha carried out by our research team belonging to four research institutions in Brazil.

Large variations in the nutrient content among parts of the plant were found, particularly among developmental phases along the growing season (vegetative growth, blooming, and seed filling). The nutrients P, K, Cu, and Zn are largely redistributed from old leaves to actively growing tissues such as new leaves and seeds. We detected little redistribution of N, Ca, Mg, Fe, and Mn. The low redistribution of nitrogen was unexpected and needs more attention. The loss of

nutrients through leaf abscission was quantified in order to support a fertilization program for this crop.

We studied the requirements of sampling for leaf tissue analysis. We found that for an adequate leaf analysis the samples should be composed of at least four fully expanded leaves, gathered from two or three vegetative stems in the middle portion of the canopy, from at least 20 plants, at 30 days after the growth was resumed after the physiological break. The adequate range of nutrients content in the leaf tissue was: 33.9 to 39.7 g $kg^{-1}$ of nitrogen, 3.0 to 3.1 g $kg^{-1}$ of phosphorus, 18.1 to 21.8 g $kg^{-1}$ of potassium, 12.5 to 16.7 g $kg^{-1}$ of calcium, 15.0 to 17.1 g $kg^{-1}$ of magnesium, and 1.6 to 1.9 g $kg^{-1}$ of sulfur.

All these studies were published as peer reviewed articles. However, as many of them were published in Portuguese, we will be glad to discuss them further if you are interested.

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## COMPILATION OF STUDIES ON JATROPHA IN BRAZIL: SUBSTRATES AND CONTAINERS FOR SEEDLING PRODUCTION, FERTILIZATION, AND ROOT SYSTEM FORMATION

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Jatropha (Jatropha curcas L.) is an oilseed tree that was largely promoted as a promising option for cultivation in marginal land for production of oil for biodiesel. However, there is limited technical information to support the cultivation of jatropha in large scale.

This report in a compilation of a series of studies on propagation of jatropha carried out by our team since 2006 at Embrapa Algodão and Universidade Federal de Campina Grande (Campina Grande, Brazil).

We observed that vegetative propagation of jatropha is relatively easy since stem cuttings are able to root in a short time without aid of hormones or any conditioning. Wider cuttings clipped from the middle part of the stem were found to have higher rooting capacity. The optimum substrate for jatropha seedling growth is made of a blend of low-density high-porosity materials with a source of organic matter and nutrients.

Propagation by seedling in plastic bags or root plugs and using either stem cuttings or seeds is considered easy and fast. However, any kind of container caused malformed root system that negatively impacted the root system efficiency in adult plants. Jatropha plants grow faster in the nursery than at field conditions, particularly if the substrate has adequate properties.

Fertilization with phosphorus was particularly important for an adequate growth of the seedlings. The influence of the nutrient content in the substrate was evaluated in the nutrient content in the leaf tissue. The period for growing seedlings in nurseries using plastic bags or root plugs was around 60 days.

All these studies were published as peer reviewed articles. However, as many of them were published in Portuguese, we will be glad to discuss them further if you are interested.

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## NEW CROP OILS - PROPERTIES AS POTENTIAL LUBRICANTS

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New crops oils such as lesquerella, field pennycress, meadowfoam, and cuphea were investigated and compared to common commodity vegetable oils for their fatty acid profiles, low temperature, and lubricating properties. The fatty acid profile investigation showed that: lesquerella is high in hydroxy fatty acid (FA), greater than 52%; cuphea had high levels of saturated FA, greater than 82%. Low temperature and viscosity properties were also measured and compared to common commodity vegetable oils as well as commercial petroleum-based oils.

The higher levels of saturated FA in cuphea led to high pour points (PP) of 3$_{o}$C. Pennycress and lesquerella had some of the best PP among the new crop oils, -21 and -24$_{o}$C respectively, while castor had a PP of -30$_{o}$C. All the plant based oils, except castor and lesquerella, had excellent viscosity index in the range of 167 – 231. New crop oils with anti-oxidant additive (1-3%) were oxidatively stable as current commercial petroleum products based on the rotating pressurized vessel oxidation test (RPVOT) and gave times > 200 min. Wear scar diameters (wsd) of the vegetable based oils from 4-ball anti-wear test showed best results for cuphea and pennycress with wsd of 0.530 and 0.594 mm, respectively.

In general, the new crop oils were found to have specific unique advantages over traditional commodity vegetable oils. Spider plots were used to compare new crop oils on a defined goodness scale to commercial oils.

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## EXTRACTION OF OIL FROM EUPHORBIA LAGASCAE SEEDS BY SCREW PRESSING

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Euphorbia lagascae (Spreng.) is a drought tolerant plant native to Spain. Euphorbia seeds contain 45-50% oil with 60-65% of its fatty acids as vernolic (12S,13R-epoxy-cis-9-octadecenoic) acid. Vernolic acid potentially has wide applications in paints and coatings, plasticizers, adhesives, polymers, and lubricants. Current sources of epoxy fatty acids include epoxidized soybean oil and processed petrochemicals. The seed also contains hydrolytic and hydrating enzymes which become active when seeds are crushed, resulting in changes in free fatty acid (FFA) content, iodine value, and epoxy content in the oil. High temperature treatment or seed conditioning is necessary to inactivate these enzymes. Although conventional seed processing routes, such as hydraulic pressing, mechanical expelling, and solvent extraction, have been explored to extract oil from euphorbia, information on these studies are very limited. The goal of this study was to determine the optimum cooking temperature and moisture content (MC) of euphorbia seeds for oil extraction by screw pressing.

The seeds were obtained from field trials conducted at Klamath Basin Research & Extension Center, Klamath Falls, Oregon. The seeds (2.5 kg ), with 5% (as stored) and 10% moisture contents (MC), were heated to 37.8, 60.0, 82.2, and 104.4 $_{o}$C and held at these temperatures for 20 min using a fluidized bed dryer (Endecotts FBD2000). The dryer was preheated to 148 $_{o}$C before use. The heated seeds were screw-pressed immediately using a tubular radial expeller (Scott Tech ERT60II). The oil in the seed and the residual oil in the press cakes were determined and the oil yields were calculated. The oils were also analyzed for solids and FFA content.

The bulk euphorbia seeds contained 41.1% (dry basis, db) oil. Clean seeds (after removing seeds incased in shell) had

an oil content of 46.1% (db). The highest oil yield (66%) was obtained from seeds with 10% MC and heated to 82.2 or 104.4 $^{o}$C. The amount of solids in the oil ranged from 13.6 to 55.5%. The amounts of solids in the oil were lower when the MCs of the heated seeds were between 3 and 5%. Oil from unheated seeds had the highest FFA (10%). The amount of FFA decreased with increasing heating temperature but leveled off around 6.1 to 6.6%. Also, heated seeds with MC below 4% tend to have lower amounts of FFA. Therefore, the optimum conditions for screw pressing euphorbia seeds include tempering the seeds to about 10% MC, cooking the seeds at between 82 to 104 $^{o}$C, and keeping the final MC between 3-5%. We anticipate that double pressing the seeds will improve the oil yield to >90% as the residual oil in the press cake goes down to about 5% as one would expect in a commercial-scale pressing operation.

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## STUDY ON THE DEGRADATION OF CB1A ALLERGEN AND RICININE FROM THE CASTOR BEAN MEAL

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Shortage of feed protein resources has become a bottleneck in animal husbandry and feed industry development all over the world. Oilseed meal could be regarded as one of the main sources of protein feed. Ricinus communis is one of the most important oil-bearing crops. Castor bean meal (CBM) contains rich and various nutrients including protein up to 33%-35%. However, CBM contains harmful components including toxin ricin, potent CB1A allergen, less toxic alkaloid (ricinine) and hemagglutinins. These components can severely limit the utilization of the CBM.

The CBM used in this study was heat-treated during oil extraction, and a study indicates that ricin and hemagglutinins are largely denatured, but not ricinine and CB1A allergen. This work is to develop a simple, economical, effective and safe method to reduce ricinine and CB1A allergen in CBM. In this context, indigenous fungi were screened for efficient degradation of ricinine and CB1A allergen. CB1A fraction was obtained by extracting through macroporous adsorption resin and polydextran gel from CBM. The molecular structure and purity of the CB1A allergens were determined by employing UV scanning, DSC, HPLC, IR and NMR techniques. The fermentation process with different fungus strains was monitored by PCR-SSCP (Polymerase Chain Reaction – Single Strand Conformation Polymorphism).

The results showed that enzymatic hydrolysis by fungi was effective on degradation of ricinine; and β-galactitol enzyme combined with protease converted CB1A allergen into β- link polysaccharide and galactitol. Ricinine and CB1A allergens in CBM were markedly reduced. In an animal feeding experiment, a group of mice grew healthy after feeding with 15% and 20% of the treated CBM, showing no damages to liver, kidney or spleen. Therefore, the fungi-treated CBM at 15%-20% dosage was recommended in protein feed industry. This study provides evidence that CBM can be used as an alternative protein feed in livestock farming.

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## IMPACT OF HEAT STRESS ON FIELD PENNYCRESS SEED YIELD AND POLLEN VIABILITY

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Field pennycress (Thlapsi arvense L.) is a new crop being investigated for its potential as a possible off season source for biodiesel. Related to the Brassica species of mustard and canola, winter annual field pennycress is also susceptible to heat stress during the reproductive stage of development. The objective of this study was to determine at what temperature seed development was inhibited in pennycress. The hypothesis of our experiment is that pollen viability becomes diminished as temperatures rise above 30$^{o}$C as seen in the 2012 growing season. A growth chamber experiment was conducted on spring pennycress 'Spring 32' over an 8 week period. Thirty plants in individual pots were grown to anthesis at 24$^{o}$C day /18$^{o}$C night. Five plants were transferred to 30, 32, 33, 34, and 35$^{o}$C for 7 days and returned to normal conditions (24$^{o}$C). Plant height, pod number, seed number, seed mass , dry biomass, seeds per pod, and harvest index were calculated for each plant. Pollen was collected from each plant after treatment, fixed in Carnoy's fixative, and examined for viability under a dissecting microscope. Pollen was also placed on growing media to evaluate percentage of pollen germination.
Plant height was not significantly impacted by increased temperatures when compared to plants which remained under 24$^{o}$C conditions. However, temperatures above 30$^{o}$C had a significant impact on pod and seed numbers. Pollen viability decreased by 84% at 30$^{o}$C and 100% at 32$^{o}$C. Pollen germination could not be determined due to the small size and poor visibility.

Pennycress's ability to handle heat stress will greatly impact which regions of the country are best suited for commercialization and aid breeders in evaluating lines for improved heat tolerance.

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## FIELD PENNYCRESS (Thlaspi arvense L.) RESPONSE TO NITROGEN RATES

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Field pennycress (Thlaspi arvense L.) is a new potential oilseed crop that is currently being evaluated as a domestic source of biodiesel fuel. Knowledge of pennycress nitrogen requirements will help producers apply optimal nitrogen and sulfur rates while minimizing production costs.

This study was initiated to evaluate the effect of nitrogen and sulfur fertilization rates on pennycress plant height, pod number, seed and biomass yield, and oil content and quality. Two growth chamber experiments were conducted on spring and winter annual pennycress lines, 'Spring 32' and 'W12', with six nitrogen rates (0, 25, 50, 75, 100, and 125 lbs. nitrogen per acre) and two sulfur rates at 10 and 25 lbs. per acre. Each study was replicated twice over 1 year.

Nitrogen fertilizer rate was a significant factor influencing plant height, pod and seed number per plant, seed and biomass yield for both the spring and winter lines of pennycress. A rate of 100 lbs. of nitrogen resulted in the greatest number of pods and seeds per plant, 83 and 650 respectively. Nitrogen fertilization had no effect on the number of seeds per pod, 1000 seed weight, harvest index, total oil content, and fatty acid constituents. Seed yields increased significantly for 100 and 125 lbs. of nitrogen per acre in combination with 25 lbs. of sulfur. Winter and spring pennycress lines were not significantly different from each other in response to increasing nitrogen rates. Overall nitrogen use efficiency decreased with increasing nitrogen rates.

Understanding the ideal nitrogen and sulfur levels for pennycress to obtain optimal yields will further improve a producer's ability to successfully integrate this new crop into the already well-established corn and soybean rotation in the Midwest.

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## HAND EMASCULATION AND INDUCED MALE STERILITY TO IMPROVE FIELD PENNYCRESS (THLASPI ARVENSE L.) BREEDING

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Field pennycress (Thlaspi arvense L.) is a self-pollinated winter annual weed species that is currently being studied as a new source of industrial products and biodiesel. Conducting controlled crosses between small flowered pennycress varieties are difficult and time consuming.

The objective of this study was to develop a reliable method of inducing pollen sterility to assist in conducting controlled crosses in this self-pollinated crop.

A total of 146 controlled crosses between winter and spring lines were conducted during the morning (8-10 am) and afternoon (3-5 pm) hours resulting in 32.1% and 44.6% fertilization, respectively. The spring to winter line crosses resulted in 46% fertilization. For winter to spring crosses, only 30% were successful. A secondary experiment was initiated to investigate the role of sulphonylurea herbicide, tribenuron-methyl, as a potential aid to induce male sterility in pennycress. Pennycress seedlings were grown in a controlled environment. When all plants reached reproductive stage, applications of 0.1, 0.2, 0.3 and 0.4 μg/ml tribenuron-methyl per plant were applied to the leaves and repeated 10 days later. An application consisted of a single 1 ml mist spray to each of 8 replicated plants in a treatment group. All applications of the herbicide resulted in severe stunting of the plants and a delay in flowering. Control plants flowered within 2 days, while 0.1 μg/ml application flowered in 10 days. Plants with applications of 0.2, 0.3 and 0.4 μg/ml resulted in severe yellowing of young tissue and did not set seed after 20 days of treatment.

The development of a reliable induced male sterility protocol will greatly improve the efficiency of pennycress breeding and will help lead to the development of new varieties of pennycress.

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## CAMELINA SATIVA IN THE HIGH PLAINS

Garret Jewett

One of the major hurdles to the adoption of on-farm produced biofuel is the lack of appropriate crops for large areas of the United States. Camelina sativa can be grown to produce diesel substitutes or edible food oil. It has been the focus of study in the high plains region because of its drought tolerance and high yields under harsh climatic conditions. The economic value of growing and crushing camelina is determined by the potential yield, seed oil content and the composition of the oil. We have carried out a multi-location variety yield trial of 23 varieties of camelina across 11 different locations in Colorado, Arizona, Wyoming, Montana and Washington in 2011 and 2012. The results of this variety trial will give a better idea of how genotype, environment and genotype by environment interaction (GEI) affects the yield, oil profile and oil content of the varieties. For 5 of the locations we have gathered data related to

yield components such as seeds per pod, thousand seed weight, and pods per plant. When correlated to overall yield and compared across environments we can get an idea of the stability of these yield component traits and how they vary among varieties. This information will give breeders an idea of what specific traits should be focused on in their quest to increase overall yield.

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# POSTER PRESENTATIONS (Natural Rubber and Resins)

## BUTANOL PRODUCTION FROM INULIN RICH BIOMASS

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In the last decade with increasing crude oil prices, exhausting oil resources and growing environmental concerns have brought much attention to butanol as an alternate fuel for gasoline. However, substrate cost still makes up at least 50% of the total production cost of butanol. In order to make butanol production cost effective and competitive with gasoline, inexpensive and locally-available substrates have to be utilized. One such waste is derived from the alternate rubber crop Taraxacum kok-saghyz (TKS). TKS produces a large amount of inulin in addition to rubber. Commercial-scale TKS production could be a good source of substrate for significant butanol production.

The primary objective of this study was to determine the best clostridial strain to ferment unhydrolysed and hydrolyzed TKS inulin and optimize the fermentation conditions to maximize butanol yield.

The amount and molecular weight of inulin extracted from TKS roots during latex extraction (a cold water extraction) and Eskew extraction (hot water extraction at 70° C) was compared. Several strains of Clostridia.sp were screened for their ability to ferment raw inulin and fructose, a major component of inulin hydrolysis. Endo-inulinase (Novozyme) was adopted for enzymatic hydrolysis and the amount of enzyme required to achieve complete hydrolysis was evaluated.

Batch fermentations were performed anaerobically at 35°C, with no agitation or pH adjustment. The amount of sugars in the fermentation medium was limited to ~50 g/L and the initial pH was adjusted to 6.3-6.5. Samples were taken at intervals to measure pH, cell density, residual sugars, ABE, acetic acid and butyric acid.

The addition of calcium carbonate in the fermentation medium had immense impact on inulin and fructose consumption by Clostridia.sp. Clostridium saccharobutylicum exhibited inherent inulinase activity and fermented raw inulin well compared to other species. Clostridium beijerinckii outperformed other strains when fructose was the primary sugar compound in the medium.

Fermentation of inulin derived from TKS can be scaled up into an industrial fermentation process that could improve economics and helps commercialization of TKS as a viable natural rubber producing crop in USA. Also, in-situ fermentation of inulin during rubber extraction could provide solvents needed by other industries.

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## RABBITBRUSH: A RENEWABLE SOURCE OF RUBBER AND BIOFUEL

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Chrysothamnus nauseosus (Rabbitbrush) is a potential crop for rubber and biofuel production because it has the remarkable ability to produce more than 6 % rubber, and 36 % of oleoresin which can be used for biofuel production. Rabbitbrush can be grown on marginal, alkaline lands under drought conditions, making it an ideal crop for arid environments, currently considered unusable for traditional agricultural applications. The goal of this project is to investigate the potential of Rabbitbrush as a new domestic commercial source of natural rubber, resin and biomass. In this study, rubber, resin, and biomass energy content of Chrysothamnus nauseosus subsp. consimilis from Austin, NV, were analyzed. Gel permeation chromatography (GPC), plasticity retention index (PRI) and nuclear magnetic resonance (NMR) analyses confirmed that the polymer molecular weight and chemical uniformity of the rubber were comparable to current commercial natural rubber producers, such as Hevea brasiliensis. Calorimetric analyses showed that energy content of the plant and bagasse, were similar to other biomass energy sources. The chemical component analyses using gas chromatography and the energy content of the resin are underway. Overall, Chrysothamnus nauseosus subsp. consimilis has the potential to be a domestic renewable rubber and biofuel crop.

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## GUAYULE RESIN DETECTION AND INFLUENCE ON GUAYULE RUBBER

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Guayule (Parthenium argentatum) is a natural rubber (cis-1,4-polyisoprene) producing crop, native to North America. Guayule also produces organic resins, complex mixtures of terpenes, triglycerides, guayulins, triterpenoids and other components. During natural rubber extraction, guayule resins can be co-extracted with the polymer, sometimes leading to a significant fraction of low molecular weight extractables in the rubber. Resin present in guayule rubber can affect viscosity, thermal stability and mechanical properties of rubber compounds. The objectives of this study were: 1) quantify the extractable content of guayule rubber and other polymers, 2) evaluate the use of Gel Permeation Chromatography (GPC) to characterize the resin in rubber, 3) measure the effect of the resin addition on physical properties of cast polymer films.

The four polymers investigated were: 1) solvent extracted Guayule rubber (Sacaton simultaneous extraction process) 2) dried Guayule latex (aqueous process), 3) synthetic Polyisoprene (Natsyn™), 4) dried Hevea latex (RRIM 600 Campinas, Brazil). Acetone soluble extracts were quantified as function of temperature by Accelerated Solvent Extraction (ASE). GPC was used to characterize the polymers before and after extraction, and the extracts. Guayule resin (Sacaton simultaneous extraction process) was added at +5% and +10% to a solution of guayule rubber in THF, and films cast for physical property testing. The evaluations included bulk viscosity, thermal stability (by Plasticity Retention Index (PRI)), and green strength of cast polymer films.

Resin addition and subtraction was readily quantified by the GPC UV detector at 254 nm and by Refractive Index; over a range of molecular weight from ~75 to ~500 g/mol. This demonstrated the potential of the GPC to measure rubber molecular weight and resin content simultaneously. The correlation between resin quantified by GPC and by ASE extractables was determined for all four polymers. Addition of Sacaton resin to guayule rubber led to decreased bulk viscosity, and softening of green tensile properties due to plasticization, in agreement with other published results. However, thermal stability (PRI) was improved, possibility due to antioxidant present in the Sacaton guayule resin.

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## PHENOTYPE ANALYSIS OF RUSSIAN DANDELION ROOT TISSUES FROM THE NATIONAL PLANT GERMPLASM SYSTEM COLLECTION

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Russian dandelion (Taraxacum kok-saghyz) (TKS) produces high quality natural rubber (NR), cis-1,4 polyisoprene, by biosynthesis, and has been used historically as a source of NR during times of short supply or high prices for Hevea NR. The rubber is primarily located in root tissues along with appreciable levels of inulin, a storage carbohydrate used in foods and as a source of carbon for biobased products. Along with other temperate NR producing crops, such as guayule, TKS can be used for domestic production of natural rubber and inulin as a source of biobased raw materials for replacement of petroleum-based polymers and fuels.

In order to facilitate development of TKS in the USA, a National Plant Germplasm System (NPGS)-sponsored collection expedition took place in 2008. Twenty accessions were collected and subsequently planted at the Western Regional Plant Introduction Station in Pullman, WA for evaluation. The objective of this study was to quantify the plant phenotypes of the new NPGS collection in terms of plant biomass, root morphology, and amount of rubber and inulin stored in the root tissue.

Russian dandelion roots were harvested in October 2010 from plants approximately 1.5 years old grown from original seed and root pieces and shipped to the Western Regional Research Center in Albany, CA for testing. Roots were air dried and stored at -20°C until processing. Roots were categorized as tap, lateral, or a combination. A break test was conducted to give a visual score of the quantity of rubber in the root. Fructan content was measured using Megazyme Fructan Assay Kit, AOAC Method 999.03 and AACC Method 32.32. Rubber content was determined using Accelerated Solvent Extraction (ASE), with cyclohexane solvent at 100°C.

The NR content of the roots varied from 5-19% as determined by ASE. Comparison of the break test measurements with ASE results indicated the break test was capable of distinguishing high and low rubber producers. The inulin content varied from 13-46%. No correlation was found between NR or inulin content and root morphology nor whether plants were grown from seeds vs. roots pieces. Moreover, the amount of rubber stored in the root was independent of the inulin content. With additional data collected from 2011 and 2012 root harvests, which will help to better characterize the accessions phenotypes, the next steps include correlation of plant phenotype to genotypes, and development of molecular markers for breeding lines.

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# POSTER PRESENTATIONS (Medicinals and Nutraceutical Plants)

## CALYX DIVERSITY OF FLAVONOLS AND FATTY ACIDS IN ROSELLE (HIBISCUS SABDARIFFA L.) FOR USE AS A POTENTIAL NUTRACEUTICAL CROP

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Flavonols and fatty acids in plants have potential to be used as antioxidants, lowering of cholesterol, and for cancer prevention. Dried roselle calyces are used worldwide as a health tea. However, there is limited research for flavonol and fatty acid diversity among roselle accessions in the USDA, ARS collection.

The objectives of this study were to evaluate roselle calyces for quercetin, kaempferol, myricetin, and fatty acid diversity in a greenhouse.
Thirteen roselle accessions were planted in 27.5 cm x 27.5 cm plastic pots containing potting soil grown in a greenhouse from August 1, 2009 – April 1, 2010.
Freeze dried calyces from 6 accessions were ground to a fine powder. 0.5 g – 1.2 g of calyx tissue per accession was placed into 5 ml test tubes. Each tube containing calyx tissue plus solvent was vortexed and incubated in a water bath for 5 hrs at 80º C. An aliquot from each sample was then transferred to a 2 ml test tube and centrifuged at 12000 rpm for 5 min. Afterwards, 1 ml of the supernatant was removed from each tube and filtered into an injection vial. Flavonols were separated and identified by HPLC.

Freshly ground roselle calyces from 13 accessions were dried in an oven at 55º C for 2 hrs. The tissue was then transferred to test tubes containing 6 mL of hexane plus isopropanol (3:1). The test tubes with calyx tissue were incubated overnight at 65º C. Oil from the ground calyces was extracted in 3 ml heptane and converted to fatty acid methyl esters (FAMEs) with 500 µl of a 0.5 M sodium methoxide in methanol solution and were vortexed for 30 sec followed by incubation at 65º C for another 2 hrs. An aliquot from the heptane layer was transferred to a vial for injection into the GC on an Agilent 7890A with a split/splitless (S/Sl) inlet and flame ionization detector (FID). Peak separations were performed on a DB-225 capillary column and were identified by retention time comparison to a FAME standard mix RM-3.

Variation for all traits were found among the roselle calyces. PI 265319 produced the most quercetin (1.1 mg/g) and myricetin (0.97 mg/g). However, PI 286316 produced the most kaempferol (1.9 mg/g). Several roselle accessions differed significantly in fatty acid content. PI 286319 produced the most palmitoleic (0.05 %) and gadoleic acid (0.2 %), however PI 468409 produced the most oleic acid (5.09 %). PI 286319 also produced the most linoleic acid (6.2 %) while PI 268097 produced the most γ-linolenic acid (0.09 %). PI 286316 produced the most eicosapentaenoic acid (0.04 %), however PI 265319 produced significantly more docsahexaenoic acid (5.3 %) than all other roselle accessions.

Sufficient variability exists in these Hibiscus accessions for quercetin, kaempferol, myricetin, and fatty acid breeding projects and use as a nutraceutical crop.

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## MEXICAN CANDELILLA WAX COVERS EFFECT ON AVOCADO FRUITS SHELF LIFE

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At the present time, exists an elevate demand of high quality products containing only natural ingredients due to their good sensorial and nutritional properties. Edibles coatings are transparent films that cover foods against humidity and oxygen. The conservation mechanism consist in create a barrier that works as a modified atmosphere around the product and also acts as a physical obstruction against microorganisms.

The objective of the present work was to study the effect of natural wax as nutraceutical covers with antioxidant compounds applied in avocado for extended shelf life time.

Edibles coatings of Euphorbia antisyphilitica Zucc. (candelilla), were used as natural wax mixed with natural antioxidants compounds. The tested antioxidant compounds were ellagic and gallic acids, obtained by fungal fermentation. The blanks tested were: a) covers without antioxidants, and b) fruit without cover. Quality physicochemical parameters of avocado shelf life were evaluated.

The obtained results showed that candelilla wax covers with ellagic acid improved the shelf life quality in avocado fruit.

Edibles coatings of candelilla with ellagic acid represent an interesting alternative in order to maintain fruits quality and its nutritional values in post-harvest storage conditions.

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## SENSITIVITY IN VITRO OF RHIZOCTONIA SOLANI TO POLYPHENOLS DERIVED FROM LARREA TRIDENTATA, FLOURENSIA CERNUA, AGAVE LECHUGUILLA, YUCCA FILIFERA, AND OPUNTIA FICUS-INDICA

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In Mexico, the potato crop is affected by Rhizoctonia solani fungus, causing the black scab disease. The control of this disease is made by mean of chemical products, but due to the environmental concerns, more research is required to obtain natural bioactive products for the control of R. solani.

The aim of this research was to evaluate the in vitro polyphenols effect from Larrea tridentata, Flourensia cernua, Agave lechuguilla, Yucca filifera, and Opuntia ficus-indica on the mycelia growth inhibition of R. solani. The plant were collected in Chihuahua Desert; the polyphenols were extracted from dry leaves with ethanol in a ratio of 1:6 (plant: solvent), during 7 h al 60°C. The concentrations of hydrolyzable and condensed tannins were determined by spectrophotometry. The antifungal activity was determined in vitro by inhibition of mycelial growth of R. solani on Petri dishes containing potato dextrose agar (PDA). A completely randomized design with four replications was used. Moreover a PROBIT analysis was performed to determine the concentration of each extract inhibition at 50% and 90%.

The effect of different ethanolic extracts on R. solani was highly significant (p = 0.05) at 72 h. The extracts of the studied species showed a high effect on the inhibition of mycelium fungal growth. Most of the extracts inhibited the mycelium growth of R. solani at 100% except the extract of Y. filifera (46%) with the highest dose tested (3000 ppm).

According to PROBIT analysis, the $IC_{50}$ of the tested extracts was very variable. The lowest IC50 was obtained with F. cernua to 16.3 ppm and the higher with Y. filifera 54.46 x $10_2$ ppm. The total polyphenol IC50 (ppm) obtained with ethanolic extracts were lower than those obtained with water, cocoa butter, and lanolin.

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