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## 2007 AAIC Annual Meeting: Bringing Industrial Crops into the Future



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### ABSTRACTS

#### Keynote Speaker

#### THE ROLE OF INDUSTRIAL CROPS IN A BIOBASED ECONOMY

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In the last 18 months, there has been a sea-change in the attitude of national policymakers regarding climate change, with many Congressional hearings being held and numerous bills being introduced. It has become a mainstream issue – now the question is focused on what policy measures are needed to address this enormous problem. For the past several years, energy security has been in the forefront of national policy discussions, with nearly 300 bills introduced in Congress on various energy issues since January 2007. Last year, more than \$300 billion was spent on the import of oil to the United States – and that number is expected to grow. Sixty-eight percent of the oil consumed in the United States is in the transportation sector, a few percent is used in the generation of electricity and for heating oil, and a significant percent is used for the ubiquitous petrochemical feedstocks and products that dominate our lives. Therefore, concerns about oil and, increasingly, climate change – and, of course, rural economic development interests play an important role -- are driving policy changes that have created a major emphasis on the role of biofuels, particularly ethanol and cellulosic ethanol. While the 2002 farm bill included some provisions for biobased products and the development of biorefineries, there is a gap in the understanding and appreciation of the role of biobased products can play to reduce our reliance on oil, reduce greenhouse gas emissions,

and protect human health. How are we going to address that gap? Who are allies in this effort? Where do biobased products fit in a policy climate that seems to be fixated on biofuels?

Given the twin imperatives of addressing oil and climate change, this should be a time of enormous opportunity and excitement for those involved in the development of biobased products. It will require dedicated efforts across the country to help determine which feedstocks, i.e., industrial crops, are most appropriate in various regions, given the differing climate, soil, and input requirements. Harvesting, storage and transportation requirements will be a key piece of the picture as well in helping growers determine what makes the most sense for them overall. This process must be accelerated. Industrial crops will play a critical role in helping the country move to a sustainable future. Integrated biorefineries can produce both fuels and a wealth of biobased products that can replace our dependence upon petrochemicals and oil and provide multiple, cascading benefits by addressing multiple problems. The time is now.

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## Plenary Session

### STATUS OF INDUSTRIAL CROPS IN THE UNITED STATES

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As a result of increased consumption of fuel and petroleum derived products, interest in fuels and products derived from agriculture materials has significantly increased leading to a new age for agriculture. To meet this demand for fuels, focus has been placed on carbohydrates and fats derived from traditional and new crops.

This presentation will be directed toward the utilization of seed oils as fuels and industrial chemicals. The fast- and short-term answers to biodiesel have always been soybean. Unfortunately, soybean oil has several shortcomings in its effort to supply the U.S. market. First and foremost is the fact that, if all current soybean oil was converted to biodiesel, we could only supply 12% of the U.S. diesel demand. Furthermore, we would not begin to address all of the specialty chemical needs such as plastics, lubricants, coatings, and the thousands of other products derived from petroleum.

Established oilseed crops such as canola, rapeseed, sunflower, and flax have high oil yields and can meet some of the burden for fuel production. However, these oils are used extensively in food and the resulting balance between food vs. fuel must be considered.

New crops that can be grown in rotation with the more traditional crops, off-season production, and utilization of land not currently under cultivation will also play a role in meeting these industrial needs. Pennycress, camelina, and *Brassica juncea* have potential as over winter crops in rotation with soybean production throughout the Midwest. The high oil content and the fatty acid profiles of these mustard crops make them suitable for utilization as both fuels and base stocks for functionalized industrial chemicals. Coriander, meadowfoam, cuphea, and lesquerella provide novel chemical moieties that make them desirable for a wide range of industrial chemicals.

Lastly, exploration of new plant species, particularly plants native to regions where there is little current agriculture activity will be necessary to identify potentially novel materials and growing regions if we are to fully meet the needs of a future with limited petroleum resources.

### TRENDS AND FUNDING OPPORTUNITIES FOR INDUSTRIAL CROPS

Carmela A. Bailey

USDA Cooperative State Research, Education, and Extension Service, Washington, DC, USA

The USDA has a portfolio of funding opportunities that support research and development in the emerging bioeconomy. This presentation describes the various programs that make a significant contribution to the development and adoption of alternative technologies to increase energy independence and to offer new opportunities for industrial crops.

The Cooperative State Research, Education, and Extension Service (CSREES) competitive grant programs support fundamental and early-applied research through the National Research Initiative, pre-commercialization research through the Small Business Innovation Research Program, and academic training is supported through Science and Education Resources Development programs (<http://csrees.usda.gov>). CSREES collaborates with the Department of Energy through joint solicitations for the Plant Feedstock Genomics program and for the Biomass Research and Development Initiative (<http://www.brdisolutions.com/default.aspx>). The Biodiesel Fuel Education Program, co-administered with USDA's Office of Energy Policy and New Uses since 2003, has played a significant role in educating the public about the benefits of biodiesel use, and for identifying fuel quality issues that arise with increasing production and use ([www.biodiesel.org](http://www.biodiesel.org)).

The USDA's Rural Development offices offer grant and loan programs to demonstrate value-added processing and renewable energy technologies ([www.rurdev.usda.gov](http://www.rurdev.usda.gov)).

The current focus for USDA research programs is on biofuels, but biobased industrial products can also contribute to reduced reliance on petroleum and improve returns to the farmer and the local community. The USDA's Biopreferred Purchasing Program creates a tremendous market pull for new products through the purchasing power of the Federal government. USDA has recently designated the first of many biobased items that will be given first preference for purchase by Federal agencies ([www.biobased.oce.usda.gov](http://www.biobased.oce.usda.gov)). This program will allow agencies to meet their environmental goals, and will open new opportunities for industrial crops to meet the expanding market for biobased products that are cost competitive and can meet performance requirements.

As the USDA moves forward in the development of new technologies to provide liquid fuels, power, and products from renewable agricultural and forestry resources, programs must also include priorities that address sustainability. The investment in R&D should address the economic, environmental, and social implications of technology, management practices, and policies.

## **THE PROMISE OF DEDICATED ENERGY CROPS**

Anna Rath

Director of Business Development, Ceres, Inc., Thousand Oaks, CA, USA

Conventional wisdom offers multiple reasons as to why biofuels will never displace a significant fraction of the world's transportation fuel demand – inability to attain critical mass, lack of available land, insufficient incentives for farmers, and lack of cost competitiveness.

We believe that high-yielding dedicated energy crops offer the potential to overcome all of these issues. Higher yield densities lead to more gallons of biofuel per acre than can be derived from traditional crops. Crops designed to grow on marginal land enable use of vast areas not currently used in agriculture. High yields and reduced input requirements create farmer economics that are competitive with the most attractive traditional crops. Higher yield densities also offer significant economies in harvest and transportation costs, thus improving the overall economics of the resulting biofuels. Ceres is rapidly developing these high-yielding dedicated energy crops.

## **GREEN PRODUCTS AND SUSTAINABILITY IN THE SURFACTANT INDUSTRY**

Franz J. Luxem

R&D Manager, Stepan Chemical Co., Northfield, IL, USA

It is easily recognized that the realization of limited petroleum resources and the run-up of oil prices, along with political instability in regions, have finally reached a point where the public and governments are paying close

attention to alternatives, which are based on renewable (sustainable) resources.

Green products are not a new invention and neither is the concept of sustainability. New, however, seems to be that the current trend is in fact “sustainable” economically, as many major corporations have committed considerable resources to developing processes that use biomass rather than petroleum as a feedstock. At the same time, new products are created and marketed as “green” or from renewable resources, making their way onto the shelves of large chains, rather than specialty shops. The presentation today will take a look at what “green” and “sustainability” mean in the context of the chemical industry, and the surfactant industry in particular.

## **BIOBASED LUBRICANTS AND GREASES: FROM RESEARCH TO COMMERCIALIZATION**

Lou Honary

Professor and Director, National Ag-Based Lubricants (NABL) Center,  
University of Northern Iowa, IA, USA

This presentation reports on the developmental activities of the University of Northern Iowa’s National Ag-Based Lubricants (NABL) Center and the commercialization of lubricants for Industrial and for Automotive use. Since 1991, the Center’s work has been focused on the creation of soybean oil-based lubricants, primarily for *industrial* markets. The Center’s efforts have resulted in the successful commercialization of over 30 industrial lubricants and grease.

Since 2006, the Center’s scope has been expanded to also include research and development into the use of vegetable oils for *automotive* oil and fuel applications. Vegetable oils, due to their polar nature, adhere better to metal surfaces and offer better protection against friction and wear. They have about 100°C higher flash and fire points than their equivalent petroleum counterparts, resulting in safer performance. At the same time, vegetable oil-based lubricants have shortcomings, including an inherent lack of oxidation stability and a higher pour point.

This report reviews approaches to addressing these shortcomings, as well as field test reports and commercialization summaries of biobased hydraulic fluids, machining fluids, gear oils, tractor/transmission fluids, and GC-LB rated greases. In addition, the promotion and use of biobased products is a part of the United States strategy for reducing dependence on imported petroleum. This report provides a brief overview of the USDA’s efforts to label biobased products, as well as federal purchasing requirements for the use of these products.

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### **General Crops**

#### **EVALUATION OF SWEET SORGHUM (*SORGHUM BICOLOR* (L.) MOENCH) GERMPLASM AS A POSSIBLE FEEDSTOCK FOR ETHANOL PRODUCTION IN ARIZONA**

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Sweet sorghum (*Sorghum bicolor* (L.) Moench) has been grown traditionally as a syrup and sugar crop in the southeastern United States, but increasing demand for alternative fuels has led to renewed interest in cultivating sweet sorghum as a source of fermentable carbohydrates. The purpose of this study was to evaluate the potential of sweet sorghum as a feedstock for ethanol production in Arizona.

Thirty-one lines of sweet sorghum were planted on 25 April 2006. All lines were evaluated for height, lodging and insect damage at the end of June. Five lines were evaluated weekly through June for height, leaf number, and tiller number. Each line was harvested 30 days after it reached 50% bloom and juice was extracted from a 15-stalk sub-sample. Potential ethanol yields for each variety were calculated based on sugar content (°Brix), juice extraction, and

plot weight using a conversion rate of 1.58 kg sugar per l (13.2 lb per gallon) of ethanol. Ten lines were harvested at different times during the growing season to monitor sugar development. Juice samples were analyzed by High-Performance Liquid Chromatography (HPLC) for sucrose, glucose, and fructose contents.

Plant height, number of leaves, and number of tillers differed significantly among the five lines measured weekly. Plant height and the extent of lodging and insect damage for all lines differed significantly by rep and by type of sorghum (sugar, syrup, or forage). Predicted ethanol yields of the lines tested varied significantly and ranged from  $635.8 \pm 144.0$  l ha<sup>-1</sup> to  $3588.5 \pm 447.9$  l ha<sup>-1</sup>. Sugar content and plot weight tended to increase with later maturity, whereas juice extraction decreased in later-maturing lines. Glucose and fructose concentrations were highly correlated, and both were negatively correlated with sucrose concentration.

The results of this study suggest that sweet sorghum may be suitable for ethanol production in Arizona. Future work should include screening of additional germplasm and breeding sweet sorghum lines specifically adapted to Arizona's climate and agricultural infrastructure.

## HYBRID AND PROXIMATE COMPOSITION EFFECTS ON ETHANOL YIELD FROM PEARL MILLET

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Investors have committed to the construction of new ethanol plants in the southeast in spite of the grain-deficit status of this region. Pearl millet is a likely viable supplemental feedstock. The DDGS has a greater nutritional value, resulting in a lower net cost of ethanol production from pearl millet. Because the fermentation process concentrates mycotoxins in grain, DDGS from pearl millet will have lower mycotoxin levels due to its resistance to fungi that produce aflatoxins and fumonisins. No information exists on the differences in fermentability among pearl millet hybrids, or genotype x environment interactions in feedstock quality. The objective of this preliminary study was to assess experimental pearl millet hybrids for genotype and environment effects on yield, proximate composition, ethanol yield, and fermentation efficiency.

Yield trials were established at Moultrie, Tifton, Watkinsville, and Newton GA in 2006. Fertilizer was applied at 88 kg N/ha. Grain was combine-harvested, and yields were corrected to 15.5% moisture. Grain was evaluated for protein, fat, and starch content on a dry basis, and for fermentation efficiency.

Experimental hybrid (606 x 2304) was among the top yielding hybrids at all locations. Across all locations, hybrid (606 x 2304) had 17% greater yield than TG102. The hybrid (606 x 2304) had 40% higher 100-grain weight, 7% lower protein content, similar fat, 50% greater starch, and 14% greater fermentation efficiency than TG102. For all hybrids, ethanol yield was correlated with starch content ( $R=0.91$ ,  $P<0.0001$ ) and fermentation efficiency ( $R=0.37$ ,  $P=0.03$ ), but was negatively correlated with protein content ( $R= -0.88$ ,  $P<0.0001$ ).

It will be possible to select hybrids that produce higher levels of ethanol for the developing bioenergy industry in the southeast. The negative correlation of ethanol yield with protein content may affect the economics of fermentation by reducing the DDGS quantity, and may also affect grain value for the alternative use markets.

## OPTIMIZING PRODUCTION OF BIODIESEL FEEDSTOCKS ON THE TEXAS HIGH PLAINS

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Increasingly limited supplies of irrigation water available from the Ogallala Aquifer and the semi-arid climate limits the sustainable production of biodiesel feedstocks on the 7.3 million hectares of cropland of the Southern Great Plains of Texas.

The two strategies being pursued to reduce water needed to produce biodiesel feedstocks are to develop fall planted oilseed crops that grow during the cooler months and to select drought tolerant genotypes of spring planted oilseed crops.

In the 2006-2007 growing season, three trials were established at Lubbock, TX. The first trial evaluated the winter survival and seed yield potential of three spring cultivars and 59 accessions from the USDA Germplasm Collection of safflower (*Carthamus tinctorius* L.). The 10 accessions with over 50% survival produced seed yields, which ranged from 1413 to 3,448 kg/ha. In the second trial, 228 accessions from the USDA castor (*Ricinus communis* L.) germplasm collection, four Brazilian varieties, and two U.S. varieties were screened for drought tolerance. The third trial determined the relative water production functions for two cultivars of eight spring-planted oilseed crops grown under subsurface drip irrigation at 0, 20, 40, 60, and 80% of potential evapotranspiration (PET). The species evaluated in this trial included castor, safflower, sunflower (*Helianthus annuus* L.), soybean (*Glycine max* L.), cotton (*Gossypium hirsutum* L.), Oriental mustard (*Brassica juncea* (L.) Czern. & Cross), yellow mustard (*Sinapsis alba* L.), and camelina (*Camelina sativa* L.). The information gained from these trials will help develop sustainable biodiesel feedstock production across this extensive crop production region.

## CROPS FOR BIOMASS, PROTEIN, AND SMALL MOLECULE PRODUCTION

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The decline in tobacco production in southern Ontario has led many growers searching for alternative crops. One possible diversification opportunity is the cultivation of crops that generate multiple revenue streams. For example, crops such as tobacco and amaranth not only produce large amounts of biomass, which in the future could be used as a feedstock for ethanol or bio-gas production, but they also produce very high quality protein that could be used in the cosmetic and food industries. Furthermore, refinements in processing offer the potential for the isolation of other economically valuable chemicals from biomass crops.

The objective of this preliminary study was to examine the feasibility of growing tobacco and amaranth at high density for the production of native protein, recombinant protein, small molecules, and biomass. Data collected will be used to develop new agronomic practices and economic models for this novel type of agriculture.

In the summer of 2007, multiple, replicated field experiments were carried out to examine the influence of different factors on the production of biomass, protein, and small molecules by different varieties of tobacco. The treatments included plant density and nitrogen fertilization rates, seedling production in trays with different cell numbers, application of fungicide to newly transplanted seedlings, and seeding/transplanting methods. Plants were harvested at the stretch bud stage, leaving about 15.2 cm of stem for regrowth in the field. Leaf sub-samples were

taken for chemical and protein analyses. Fresh and dry weights were recorded and used to estimate biomass yields. A second harvest was performed on the plant material that re-grew after the initial harvest.

We will report on the small molecule, protein, and biomass yields for the 2007 field experiments and discuss the challenges faced when moving from low- to high-density crop plantings. We will also provide a preliminary comparison of the cost of using transplants vs. direct seeding for stand establishment.

## FORMULATION AND APPLICATIONS OF GLYCEROL POLYMERS

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Increased production of biodiesel in the U.S. and Europe during the past several years has created an oversupply of glycerol and lowered domestic profitability. In addition, increased petroleum prices have increased the cost of synthetic polymers made from petrochemicals. Glycerol can be polymerized with phthalic acid to form polyesters that are commonly referred to as glyptal resins. Glycerol can also react with other di- or tri-carboxylic acids such as azeleic acid or citric acid to form polymers with variable properties.

The objective of this study was to examine the physical and chemical properties of polyesters formed by reacting di- and tri-carboxylic acids with glycerol, and to evaluate the applications of these polymers.

Glycerol was reacted at temperatures ranging from 75 to 290°C with the following organic acids: malonic (propanedioic); citric (2-hydroxy-1,2,3-propanetricioic); succinic (butanedioic); maleic ( $\alpha$ -butenedioic); adipic (hexanedioic); phthalic (1,2-benzenedioic); suberic (octanedioic); azelaic (nonanedioic); and sebacic (decanedioic).

Generally, temperatures below 100°C did not result in polymerization. Higher reaction temperatures generally created porous polymers, whereas lower (100-125°C) temperatures created dense polymers. Malonic acid tended to sublime at temperatures of 125°C or greater, and malonic, citric, succinic, and maleic acid-glycerol polymers decomposed when placed in water, whereas phthalic acid polymers tended to soften when placed in water. Adipic, suberic, azeleic, and sebacic acid-glycerol polymers were water-fast and flexible, although the longer-chain acids required extra reaction time. Adipic acid-glycerol polymers combined with a variety of organic compounds with differing chemical characteristics were cast in 20-cm-diameter pie pans to produce biopolymer mats. Preliminary data indicate that improved plant growth and pest protection can be achieved by the use of these mats around horticultural plants such as tomatoes.

The use of glycerol to produce glycerol-organic acid polymers for horticultural applications demonstrates an attractive novel use of this commodity.

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### Medicinals and Nutraceuticals

## SAPONARIA VACCARIA – A POTENTIAL INDUSTRIAL/MEDICINAL CROP FOR THE CANADIAN PRAIRIES

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*Saponaria vaccaria* (syn. *Vaccaria pyramidata*, *V. hispanica*, com. cow cockle), a weed species introduced into

North America from Eurasia, is a member of the Caryophyllaceae family that was originally investigated in the 1970s as a potential new starch crop for the northern Great Plains. Since that time, significant phytochemical work on the seed, which is used in traditional Chinese medicine, was carried out and led to renewed interest in the species as a potential new crop for production, not only for specialty small-grained starches, but also of various phytochemicals with industrial and medicinal applications.

The objective of this work was to evaluate the agronomics of various landraces for selection and production of true breeding, high yielding plants with 'appropriate' chemical profiles.

Seeds from a variety of sources were obtained and grown at several sites in Saskatchewan over a five-year period. Various agronomic characteristics were evaluated, double haploidy protocols were developed, as well as chemical analyses for the various classes of compounds.

In general, *Saponaria* was amenable to large-scale cultivation. The species responded well to nitrogen application, tolerated early season frosts and summer droughts, was not impacted by insects, and was competitive with many indigenous weeds. Some disease problems were noted, however, particularly with *Alternaria*. Evaluation of haploidy protocols was successful and led to the production of several double haploid lines. Chemical analysis methods for phenolics, saponins, and cyclopeptides were developed and used to examine the variability of these components.

In conclusion, the plant *Saponaria* possesses attractive agronomic characteristics making it amenable to large-scale cultivation in the Canadian Prairies utilizing available equipment. It produces a number of products, which in theory, should be marketable if efficient large-scale processing methods can be developed. Currently, a local company, Saponin Inc., is working to produce and market products from this plant.

## QUALITY ASSESSMENT OF HIBISCUS (*HIBISCUS SABDARIFFA*) CALYCES FROM SENEGAL

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*Hibiscus sabdariffa* L. (*Malvaceae*) is an annual shrub, widely grown in Central and West Africa. Hibiscus is utilized world wide in the production of drinks (e.g., herbal or ice teas), jellies, sauces, chutneys, wines, preserves, and natural food colorants (anthocyanins). The production of hibiscus calyces in developing countries is becoming important for income generation activities for the benefit of rural communities.

The objective of this study was to assess the quality of hibiscus calyces grown in Senegal during the 2004 and 2005 production years.

Hibiscus calyces were grown in different regions in Senegal during the production season of 2004 (29 samples) and 2005 (24 samples). Quality control analyses (color, foreign matter, moisture, ash, and total anthocyanin) were conducted on the hibiscus calyces.

The 2004 samples had a high variation in their color with 40% of the calyces being medium red or pink. The color of the 2005 production was more homogeneous with the majority of the samples being dark red. The foreign matter content of the 2004 calyces varied from almost 0% to 0.8%. The 2005 production had much lower levels of foreign parts in the dried hibiscus calyces (less than 0.2%). The moisture content was also higher in the 2004 samples with some of these higher than 12%, whereas the 2005 samples had lower levels (less than 8%). The acid insoluble ashes showed that 44% of the 2004 samples exhibited values higher than 1% indicating that these samples were contaminated with sand and soil.

All the 2005 samples had low levels (less than 1%) of acid insoluble ashes. A maximum of 1% is usually acceptable for international standards. A method to measure total anthocyanins was adapted for the hibiscus calyces. The 2005 samples were more homogeneous and had higher anthocyanin content than the 2004 samples.

By assessing the quality of the hibiscus calyces in the 2004 production in Senegal, it was possible to increase significantly the quality of hibiscus calyces in the 2005 production. Due to the quality assessment and improvement,

Senegal is now commercializing high quality calyces for the U.S. market.

## **GERANIUM AS AN ESSENTIAL OIL CROP FOR EMERGING FARMERS IN SOUTH AFRICA**

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South Africa needs information on crops suitable for the small farmer that are low risk and drought tolerant. At present, South Africa imports €5.5 million worth of essential oils per year. They are used in the consumer market (culinary, perfumery, cosmetics, pharmaceutical) and industry. International demand for natural products is growing at a rate of 15% per year. South Africa's share of the world exports in essential oils is estimated at 1.03%. A worldwide demand exists for the rose geranium essential oil of the *Pelargonium* species that contains geraniol, linalool, and citronellol as substitutes for the expensive attar of rose in the perfume trade. *Pelargonium* species are indigenous to South Africa and were known for their medicinal value by the earliest indigenous people. Geranium oil production was not sustainable in the past in African countries. Most rural areas of South Africa are densely populated and poverty stricken. These communities rely on subsistence farming as a livelihood. Limited information on geranium cultivation methods such as plant density, yields, harvesting methods and co-operative farming and markets has led us to undertake this study. There have been signs developing for growing essential oils in South Africa by commercial and emerging farmers since 2000. However, the lack of affordable access, and suitable and applicable information prevented many farmers from entering the essential oil business.

The objective of this study was to determine the farming potential in terms of maximum yield for emerging farmers. This study focuses on planting density, harvesting techniques, and yield. The ultimate objective was to transfer the knowledge acquired to the emerging farmers in South Africa.

Initial trials were established at the Lowveld College of Agriculture and with various commercial farmers located in different climatic zones. Plant densities were varied over the range of 15000, 25000, and 35000 plants per ha. The results were compared with the production obtained at the various sites from commercial farmers, community projects, and agricultural trials. Investigations also compared harvesting methods, and different sizes and distillation units.

Larger plant densities than the prescribed South African norm of 15000 plants per ha, and harvesting methods were investigated to promote this crop as a profitable option for the emerging farmers.

Geranium can be grown as a profitable crop by the emerging farmers in South Africa provided suitable agronomic and technological information are provided.

## ***ALOE VERA* CO<sub>2</sub> ASSIMILATION INDEX OVER TWO LOCALITIES AND DIFFERENT SOIL WATER DEFICITS**

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The objective of this work was to determinate the influence of climatic condition changes and soil water deficit on CO<sub>2</sub> assimilation index in order to establish optimal *Aloe vera* growth conditions.

The experiments were conducted at two locations: (1) Marín Experimental Station, at the Facultad de Agronomía, Universidad Autónoma de Nuevo León, latitude 25° 23' N, longitude 100° 02' W, altitude 400 masl, semiarid warm climate and an average temperature of 22°C during the year; (2) Buenavista Experimental Station at the Universidad Autónoma Agraria Antonio Narro in Saltillo Coahuila, México, latitude 25° 21' N, longitude 100° 00' W, altitude 1743 masl, semiarid mild climate, and an annual average temperature of 17°C.

For estimating the influence of soil water deficit over sabila assimilation index, two different treatments were

designed. A control treatment where irrigation was applied two times per week to maintain the soil moisture at field capacity (low deficit), and a drought treatment, where after each initial irrigation, the following irrigation was applied only when the soil water content level was lower than the permanent wilting point (high deficit). Two drought periods were evaluated at each location.

Measurements of CO<sub>2</sub> assimilation index (AI), transpiration (T), stomatal conductance (SC), leaf temperature (LT), active photosynthetic radiation (PAR), and internal CO<sub>2</sub> (Ci) were carried out with a LI-6400 portable system. The equipment was modified to include a camera for the sabila leaves, in which part of the leaf was placed into a 40 cm long chamber. Physiological changes were measured over a 24-h period with intervals of 1.5 to 2 h at the beginning and end point of the drought cycle using four plants and one leaf per plant per treatment.

## ANTIFUNGAL EFFECTS *IN VITRO* OF SEMIARID PLANT EXTRACTS AGAINST POST-HARVEST FUNGI

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The crop losses from post-harvest diseases were estimated from 10 to 30% of total production, and in some perishable crops greater than 30%. Mexico has a great diversity of plants that have been found to have inhibitors of some phytopathogenic fungus, resulting in additional sources of plants with antifungal activity.

The objective of the present work was to determine *in vitro* the antifungal effects of *Fluorensia cernua*, *Fluorensia retinophylla*, *Fluorensia microphylla*, *Agave lechuguilla*, and *Larrea tridentata* against *Colletotrichum gloesporoides*, *Alternaria alternata*, and *Rhizopus* sp.

Plant leaves were placed into flasks with solvents (ethanol and mixture of methanol-chloroform (1:1)) and constantly agitated for 22 h. The solutions were filtered and the solvent was removed with a vacuum system. Extracts were tested in concentrations of 500, 1000, 2000, 4000, and 5000 µl l<sup>-1</sup>. Mycelial growth inhibition was evaluated.

Results from mean comparison of the three *Fluorensia* species showed that the 4000 and 5000 µl l<sup>-1</sup> doses had high inhibition with values of 84.8 and 86.7% for *C. gloesporoides*, respectively, and against *A. alternata* were 90.6 and 95.9% and for *Rhizopus* sp. were 91.0 and 95.9%, respectively. The plant with the least fungal growth was *F. retinophylla* in ethanol extract. For *L. tridentata* and *A. lechuguilla*, the 4000 µl l<sup>-1</sup> dose had higher inhibition over *C. gloesporoides* (100%), *A. alternata* (66.4%), and *Rhizopus* sp. (78.9%). The species with the highest inhibition was *L. tridentata* at 80.5 %.

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## Fiber and Cellulosics

### HESPERALOE FIBRE FOR PAPERMAKING

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The unusual fibre properties of hesperaloe (Agavaceae), an arid-zone plant, were discovered in the late 1980s by scientists at the University of Arizona. During the past 15 years, considerable advances have been made in the

domestication of this plant as a sustainable source of agricultural fibre for the pulp and paper industry.

The unique fibre morphology was subsequently identified in the late 1990s to be well suited for overcoming the technical barrier in the manufacture of a new class of ultra lightweight printing paper.

The physical strengths of hesperaloe pulp have been demonstrated to be 3 to 4 time greater than those of premium softwood kraft pulp. It has been estimated that one hectare of hesperaloe crop could offset the harvesting of about 60 hectares of boreal-forest trees for papermaking. Pre-commercial demonstration of the performance of the hesperaloe printing paper is being undertaken presently with the cooperation of Greenpeace Germany.

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## Oilseeds

### **PHYSIOLOGICAL CHANGES DURING SEED DEVELOPMENT OF CUPHEA PSR23**

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Cuphea (*Cuphea viscosissima* Jacq. x *C. lanceolata* W.T. Aiton, PSR23) is a new oilseed crop being developed in the northcentral USA. Cuphea oil is composed of high amounts of medium-chain fatty acids that are suitable for detergent/cleaner applications and have potential for cosmetics.

The objectives of this study were to determine the effects of seed development on seed moisture, weight, oil content, fatty acid composition, germination, and vigor.

Two thousand cuphea flowers were tagged at anthesis in the field each year at Prosper, ND in 2004, 2005, and 2006. Each flower (that developed into a seed capsule) was tagged with the date of anthesis. Two hundred developed capsules from the labeled flowers were harvested at 3- to 4-d intervals from 5- to 35-d post anthesis corresponding with 37 to 295 growing degree-days (GDD). The GDD were calculated using a base temperature of 10°C.

Seed development required approximately 253 GDD or 30 d post anthesis to reach physiological maturity. Maximum seed germination was reached at 33 d post anthesis. Seed oil content increased and oil composition changed as seed matured. Seed oil was high in linoleic and palmitic acids from 0 to 10 d post anthesis and declined thereafter. Capric acid began to accumulate at 10 d post anthesis and reached above 70% at physiological maturity.

### **OIL, SEED, AND FATTY ACID DEVELOPMENT IN FALL- AND SPRING-PLANTED LESQUERELLA**

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An understanding about the development of seeds and their storage oils is needed for plant improvement. This research followed seed weight, and the accumulation of oil content and the predominate fatty acids found in the seed oil from anthesis until maturity.

This study was made on both fall-planted (11 October 2006) and spring-planted (15 March 2007) lesquerella (*Lesquerella fendleri*) by tagging flowers and analyzing the seeds produced. The sampling dates of developed fruits (siliques) for both plantings were 7, 12, 15, 19, 22, 26, 29, 33, 36, and 41 days after flowering (DAF). On 27 April 2007, 200 flowers were tagged in the fall-planted field and sampling occurred from 3 May (7 DAF) until 6 June 6 (41

DAF). On 1 June 2007, 300 flowers were tagged in the spring-planted field and sampling occurred from 8 June (7 DAF) until 11 July (41 DAF).

The sigmoidal curve for the seed dry weight over 41 DAF was steeper in the spring- than the fall-planted dry weight. Lesquerolic acid accumulated faster and began at an earlier date in the spring planting than the fall planting. Other fatty acids followed a similar trend. The oil quality and quantity were unaffected by these temperatures. Spring planting of lesquerella would greatly reduce the length of time a grower would have land in production than the fall planting, and thereby reduce production costs.

## **YIELD COMPONENTS OF SPRING- AND FALL-PLANTED LESQUERELLA**

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We determined yield components of lesquerella (*Lesquerella fendleri*) cultivated on three variable soils and sown in either the fall of 2006 or the spring of 2007. Plants from 0.125 m<sup>2</sup> of land area were harvested weekly and the number of buds, flowers, and siliques were determined.

Plants sown in the fall on a heavy clay soil senesced rapidly and prematurely upon commencement of irrigation treatments initiated near the beginning of reproductive development. Plants sown in either the fall or the spring on well-drained sandy loam soils required irrigation every 7 to 10 days and produced comparable seed yields. Yield development was characterized by a steady decline in flowers per m<sup>2</sup> and a steady increase in siliques per m<sup>2</sup>. Buds per m<sup>2</sup> declined gradually after peaking during the early stages of reproductive growth. All phases of reproductive development were much faster for the spring- than the fall-planted crop.

We conclude that (1) knowledge of soil type is critical to lesquerella cultivation due to the extreme sensitivity of the crop to water logging and (2) spring planting may be a viable alternative to the traditional fall planting of the crop.

## **LESQUERELLA TOLERANCE TO PREEMERGENCE HERBICIDES**

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Management of weedy vegetation in fall-planted *Lesquerella fendleri* (Gray) S. Wats. is critical for successful crop production due to lesquerella's slow growth during establishment and short stature. Lesquerella is a broadcast planted crop that makes mechanical cultivation impossible and hand weeding difficult and expensive. Thus, chemical control of weeds with herbicides will be necessary for successful lesquerella crop production.

The objective of this initial study was to determine the tolerance of lesquerella to several rates of various preemergence herbicides when the herbicides were applied at various times after planting under sprinkler irrigation, and when they were applied after planting and before the first flood irrigation.

Two experiments were conducted in which lesquerella was planted in level basins in October 2006 and was flood irrigated except that in Experiment I the field was sprinkler irrigated three times, once after three different herbicide application times. In both experiments, two treatments, benefin (Balan) and pendimethalin (Prowl H<sub>2</sub>O) were applied preplant incorporated (PPI) and all other treatments were preemergence applications where irrigation was used to incorporate or move the herbicide into the soil. In experiment I, a temporary sprinkler irrigation system was installed after the crop was planted. Two rates of the herbicides pendimethalin (Prowl H<sub>2</sub>O), oxyfluorfen (GoalTender), flumioxazin (Chateau), metolochlor (Dual Magnum), bensulide (Prefar), and pronamide (Kerb) were applied using a CO<sub>2</sub>-pressurized, backpack sprayer in both experiments. In Experiment I, after the first set of herbicide treatments was sprayed, the field was sprinkler irrigated to incorporate the herbicide and initiate crop germination. A second and third

set of treatments was applied at about 1 week intervals after planting when the soil surfaced dried out and the field were sprinkler irrigated again after the applications. Experiment II included the same herbicides as experiment I, but they were applied preemergence only once after planting and the field was flood irrigated to incorporate the herbicides and initiate crop germination. Data collected included population densities, visual injury ratings, and seed yield.

Population densities in Experiment I (sprinklers) were 561 plants/m<sup>2</sup> in the untreated control, and in the Balan PPI and Prowl H<sub>2</sub>O PPI treatments were 185 and 291 plants/m<sup>2</sup>, respectively. The Prowl H<sub>2</sub>O, GoalTender, and Chateau treatments almost completely eliminated lesquerella emergence when the herbicides were applied before the first or second sprinkler irrigation, but crop emergence was normal when the herbicides were applied before the third sprinkler irrigation. The other herbicides had more complicated injury patterns, whereas Prefar was relatively safe. The flood-irrigated Experiment II results were similar to Experiment I.

Herbicide tolerance in lesquerella is limited and additional research is needed to develop weed management programs that can be utilized by growers.

## **CANOLA AND CAMELINA RESEARCH AND COMMERCIALIZATION IN THE KLAMATH BASIN OF OREGON**

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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, before 2005. Much of this region has irrigation water available, and oilseed crops that are suited to a cooler climate such as canola and industrial rapeseed (*Brassica napus*), mustards (*Sinapsis alba* and *Brassica juncea*), and camelina (*Camelina sativa*) seemed to have a possible fit with existing crops such as potatoes, grass and alfalfa hay, and small grains. 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.

The objective of this preliminary study was to evaluate the seed yield potential of several public and private varieties of spring-planted canola, mustard, and camelina over a range of Klamath basin growing conditions.

In spring 2006, we planted six canola varieties, one industrial rapeseed variety, and two mustard varieties at two locations. One was an upland mineral soil; the other was a high organic matter lake bottom soil. The trial was fertilized and irrigated uniformly at each location. In fall 2006, we planted six canola varieties at the mineral soil site to evaluate winter-hardiness. In spring 2007, we planted a variety trial on the mineral soil including 15 canola, two rapeseed, two mustard, and one camelina varieties. This trial was laid out in a way that allowed all varieties to be tested under two levels of irrigation to evaluate the response to reduced moisture availability. Both areas were fertilized uniformly. In spring 2007, we also planted a simpler trial of replicated canola and camelina strips on the high organic matter soil. The plots were swathed or pushed as the seedpods began to approach maturity, and were threshed with a Hege plot combine. Seed was cleaned and weighed, but oil analysis was not done.

In the spring 2006 trial, despite greater than expected weed pressure, canola seed yields ranged from 1570 to 2550 kg/ha, with a mean of 2090 kg/ha. The two mustard varieties had yields of 2250 and 2080 kg/ha. The fall-planted 2006 trial did not survive a period of very cold temperatures with no snow cover in December. The 2007 spring-planted trials grew well and plants were larger and appeared to have more seedpods in the areas receiving greater irrigation. The camelina matured earlier than almost all canola varieties. It appears that canola and camelina both respond to supplemental irrigation in this climate, although camelina may be better suited to conditions of low moisture availability.

Further details of the 2007 trial, along with an update on the local commercial biodiesel production facility, will be discussed. Ongoing research, including the effects of multiple fall planting dates on winter survival and seed yield of canola and camelina, will also be discussed.

## **THE DEVELOPING CAMELINA INDUSTRY IN THE WESTERN UNITED STATES**

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Bio-based fuels such as “bio-diesel” are limited due to raw material costs. Over 80% of biodiesel manufacturing costs today are from purchase of increasingly expensive soybean and canola oil feedstocks. Low input costs of crops such as camelina (*Camelina sativa* L.) offer a very low cost alternative material for use in biodiesel manufacture. However, having a low cost material is only a part of the issue. Production volume must also be established in order for camelina to be used as a supplement or replacement for the more common feedstock oils. Camelina is also being developed as a nutraceutical oil as a replacement for the less stable flax oil industry.

The study involved developing a camelina production system suitable for growers to produce 20,000 hectares of camelina in 2007. To complicate this, camelina would have to compete for land in a year when wheat prices are at their highest in 30 years.

Producer meetings were scheduled from February through March 2007 in rural Montana. Production contracts were provided and production systems explained. Contracts were offered with a signing bonus making the crop price to the grower at \$0.095-0.10/lb (\$0.24-0.25/kg).

Commercial production of camelina increased by 100 fold using a system of advertising, grower meeting, and government research. Growers were able to deliver to elevators or railcars. Concerns were basically weed control, costs of production, and speed of harvest. In general, growers were satisfied with camelina as a rotational crop.

Camelina does provide a reasonable alternative crop for the wheat production areas of the western United States. Production is expected to increase to possibly 0.5 million hectares in 2008 and 1.5 to 3 million hectares in 2009.

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## Natural Rubber and Resins

### THE ROLE OF THE SMALL RUBBER PARTICLE PROTEIN IN DETERMINING RUBBER YIELDS AND POLYMER LENGTH IN RUSSIAN DANDELION

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The Small Rubber Particle Protein (SRPP) is an acidic low molecular weight protein that is abundant in the latex of the rubber producing plant species *Taraxacum kok-saghyz* (Russian dandelion). SRPPs are tightly associated with the surface of cytosolic vesicles known as rubber particles (RP), which represent the site of *de novo* rubber biosynthesis and sequestration. While the role of SRPPs in rubber biosynthesis is unclear, it has been reported that the addition of recombinant SRPP to *in vitro* rubber synthase assays significantly increases the rate of isopentenyl pyrophosphate incorporation into rubber. These results suggest that SRPP is either an ancillary protein that accentuates rubber synthase activity or an actual component of the rubber synthase complex.

Due to its putative importance to rubber biosynthesis, we have set out to characterize the SRPP gene family in Russian dandelion. We have identified three unique cDNA clones that correspond to the major RP localized SRPPs. The temporal pattern of mRNA and protein accumulation of each SRPP isozyme corresponds directly with the pattern of rubber accumulation in Russian dandelion. To obtain *in vivo* evidence for SRPPs role in rubber biosynthesis, we have generated transgenic Russian dandelion lines with increased and decreased SRPP levels. We are in the process of analyzing these transgenic lines for changes in SRPP content, rubber transferase activity, rubber yield, and polymer length.

### SEASONAL VARIATION IN GUAYULE PRODUCTION FIELDS

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Commercial production of guayule for latex production presents quite different logistical challenges than small-scale field trials. It is necessary to produce high-yielding shrub on a consistent basis over a range of field types and growers, and also to protect the shrub from dehydration post-harvest before processing. These issues are complicated by the changing morphology of the plant throughout the year and potentially by intrinsic differences among guayule lines.

In this study, several commercial guayule fields in different parts of Arizona were monitored over time following the first winter after stand establishment. In addition to latex yield and water content (and its impact on latex yield), we measured the percentages of (1) the bark (rubber-producing tissue) from different stem diameters, (2) leaf percentage, (3) plant size (height and width), and (4) plant age, in different lines, to determine the plant characteristics that had the most impact on processing variables and latex yield.

The amount of bark was generally inversely correlated with stem diameter, and the proportion of leaves was highest in the spring and summer months. Plant growth continued throughout the year except for the winter dormancy period. The most critical factor affecting latex yield, in this study, was the water content of the shrub at the time of harvest, with excellent results up to 11.8% latex (dry weight basis) being observed in shrub from well maintained fields. The data indicate that a water content of  $\geq 40\%$  at harvest is necessary for good processing results. However, we also have demonstrated that shrubs with as low as a 30% water content can be re-irrigated and then harvested once their water content has risen to 40% without adverse effect. Shrubs that have dropped to below 30% water content suffer irreversible latex loss (mainly thorough in situ coagulation) and appear to require another winter rubber production season in the field to generate acceptable latex yields.

Field monitoring is continuing indefinitely, as well as extensive post-harvest studies to determine the best pragmatic methods for protecting latex yields during transportation to the processing plant under different weather conditions throughout the year. These studies assist us to improve agronomic practices, determine quality parameters for growers, and provide the essential underpinning for the design of transportation and staging equipment before processing.

## **COLD TOLERANCE OF GUAYULE ON THE SOUTHERN HIGH PLAINS**

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Native guayule populations are scattered throughout 300,000 km<sup>2</sup> of rangeland in the Chihuahuan Desert and surrounding regions. The only native indigenous U.S. stands occur in the Trans Pecos region of southwestern Texas, and represent the most northern extension of the plant's habitat. Maximum air temperatures of over 38°C are frequent, and minimum temperatures of -23°C have been recorded.

The objective of our study was to determine whether guayule production could be successful further north on the Southern High Plains near Plainview, TX.

Seed used in the experiment included four released lines, AZ-1, AZ-2, AZ-3, and AZ-4; a released USDA cultivar (11591); and three unreleased breeding lines, N9-3, N6-5, and N13-1. Seeds were planted 14 March 2006, in the greenhouse at the Texas A&M University Agricultural Research Station near Pecos. Guayule seedlings were transplanted on 18 May 2006, at the Texas A&M University Agricultural Research Station at Plainview. The experimental design was a randomized complete block with four replications. Plots were single rows (each containing 18 plants of each line) spaced 1 m apart and 6 m long. Plant height was measured 7 November 2006 and 6 June 2007. Guayule cold damage was estimated June 6, 2007, using the following index: (1) no damage, (2) slight – injury of terminals to 6 cm, (3) moderate – 2/3 of plant volume injured, (4) severe – all aerial portions killed but resprouting, and

(5) complete – beyond recovery with no regrowth.

Plant height measured on 7 November 2006, varied from 31 cm for 11591 to 56 cm for AZ-1. Only one line had increased height measured on 6 June 2007. The 11591 had increased from 31 to 38 cm. Line N6-5 remained the same, whereas all other lines decreased in height. The cold damage index ranged from 1.2 in line 11591 to 3.8 in line AZ-1. The maximum and minimum air temperatures recorded during the study were 38°C and -14°C, respectively.

Line 11591 had the least cold damage and holds promise for establishment and rubber production on the Southern High Plains. Guayule grown here will not produce as much biomass as those cultivated in the Southwest, but certain production criteria may make the Plains an additional production site: annual rainfall averages 46 cm, irrigation water salinity is less than 1 E.C. and is pumped from only 90 m, and center pivot irrigation is available for establishing guayule by direct-seeding.

## RUBBER AND RESIN QUANTIFICATION IN YOUNG GUAYULE PLANTS

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Natural rubber, biosynthesized *cis*-1, 4-polyisoprene, is the largest single volume elastomer in commerce, at about 9 million tons sold globally per year. It is a strategic raw material used in thousands of applications that is critically in medical devices, personal protective equipment, and aircraft tires. Almost all natural rubber used in the United States is imported from *Hevea brasiliensis* rubber tree plantations of Asia and Africa. Natural rubber latex prices are increasing. A 3-million-ton shortage has been predicted over the next 10 to 15 years, driven primarily by Asian utilization.

Guayule (*Parthenium argentatum*), a woody desert shrub indigenous to the southwestern USA, produces high molecular weight *cis*-1, 4-polyisoprene, and has entered the commercial arena as an alternative latex material for the manufacture of medical devices safe for people suffering from Type I IgE-mediated *Hevea* latex allergies. Cultivation of guayule for latex production provides the favourable economics to support commercialization; however, increasing rubber biosynthesis in guayule could have a major impact on sustainability of this new industrial crop.

Overexpression of key metabolic pathway genes in *Agrobacterium*-mediated transformed guayule has been successful, but to date no studies have confirmed an increase in rubber production. A major hurdle to progress in the application of biotechnology is the time required to assess phenotypes. Confirmation of gene expression does not guarantee an increase in any single metabolite in the complex isoprenoid pathway. Changes in rubber production for field-grown plants, the true test of efficacy, require 1 to 2 years to assess.

The objective of our work was to determine whether reproducible measurements of rubber and resin production could be made in plants less than one year old grown in a laboratory or greenhouse environment.

Young plant tissues were extracted with acetone and cyclohexane to remove the resin and rubber residues, respectively. Extracts were quantified by gravimetry for both fractions. Dried cyclohexane fractions were re-solubilized in THF and tested by HPLC against rubber mass standards and polystyrene molecular weight standards. Results indicate sufficiently high levels of resins in three-month-old plants to be quantified by gravimetry; rubber could also be detected in these plants by SEC-MALLS. Differences between control and genetically modified plants suggest the method may be useful in early detection of the efficacy of metabolic engineering of rubber in guayule.

## POTENTIAL RECYCLING OF ANTIOXIDANT SOLUTION FOR GUAYULE LATEX EXTRACTION

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Commercialization of guayule for producing latex for medical products and other uses is underway. As commercialization reaches full scale, the liquid waste stream from latex extraction will potentially become an increasing problem. The objectives of this study were to evaluate the properties of the liquid waste stream after latex extraction and determine whether any or all of the waste liquid could be recycled for latex extraction before disposal.

Waste liquid was collected from three waste stream sources in a latex extraction facility. Currently, wastes from all three sources are discharged from the facility. Each of the three sources was analyzed for Na, K, Ca, Mg, SAR, and pH. Based on these analyses, six treatments were developed for recycling two of the waste streams. Treatment 1 was a control using 100% original/new antioxidant solution for each extraction. Treatment 2 was a mixture of 70% original and 30% waste stream #3. Treatment 3 was a mixture of 50% original, 30% waste stream #3, and 20% waste stream #2. Treatment 4 was a mixture of 20% original, 30% waste stream #3, and 50% waste stream #2. Treatment 5 was 100% waste stream #2. Treatment 6 was 100% waste stream #3. All mixtures are based on the volume percent of the final mixture. After the mixtures for the treatments were made, each was analyzed for Na, K, Ca, Mg, SAR, C, N, and pH. The treatments were used as the antioxidant solution in the laboratory latex extraction process of freshly chipped guayule shrub. Each treatment was replicated four times in a completely randomized design. Following latex extraction, the homogenate from each sample was analyzed for Na, K, Ca, Mg, SAR, EC, C, and N. The total latex extracted was also determined.

Results showed that using any of the treatments to recycle waste liquid resulted in a lower SAR of the waste being discharged (26.95 to 10.5). The N content of the waste liquid averaged about 0.2% and could be recaptured as fertilizer. The K values were also high (500-600 ppm), indicating that guayule probably has a high K requirement and may require extra K fertilizer especially where soil tests are low in K. The solids in the waste stream and the obnoxious odor characteristic of the waste liquid could be overcome by lowering the pH of the waste liquid from over 10 to less than 7. If this were done with phosphoric acid, then the waste solids would be high in N, P, and K and could be an acceptable fertilizer for plant use. The best treatment for latex extraction was Treatment 4 with an increase of 2.4x the amount of latex extracted than the control (Treatment 1). This increase is probably due to recovery of latex from the waste liquid rather than increased extraction from the shrub as indicated by similar changes in SAR values between the treatments.

The waste liquid obtained during latex extraction can be recycled for latex extraction, thus reducing the amount of waste liquid discharged by about 50%. The waste that is discharged should be of value for use as a fertilizer.

## **LACK OF GUAYULIN-INDUCIBLE IRRITATION AND SENSITIZATION**

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Guayulins are a major terpenoid component in guayule, Guayulin A (cinnamic acid ester) making up 1 to 10% of the resin fraction. The compound is suggested as being a contact sensitizer (possibly able to cause Type IV allergies) because of reactions it appeared to induce in guinea pig in an early study. Guayule latex is under development as the base material for various medical devices because it is safe for humans suffering from Type I Hevea latex allergy. Thus, it was necessary to determine the levels of guayulin in purified latex, determine the levels needed to induce irritation and/or sensitization, and to reduce the levels in latex to well below an active concentration should it prove necessary.

Guayule resin and guayulin A were supplied to MB Research Laboratories, for evaluation using the localized lymph node assay (LLNA) in mice. Resin (2.5, 5, and 10%), guayulin A (0.1, 0.25, and 0.5%), and resin mixed with additional guayulin A (5% resin + 0.5% guayulin A) was evaluated. The resin mixtures containing 1.54% guayulin A and 0.27% guayulin B (anisic acid ester) and additional guayulin A were used to assess possible adjuvant effects of the resin components on the irritation/sensitization ability of guayulin A. In addition, the levels of guayulin A and B in purified latex were assayed by HPLC and the effect of pH on guayulin degradation through hydrolysis (and

concomitant appearance of degradation products) was determined. In a separate study, guayule latex examination glove films were tested in an FDA-required nine-week Repeated Patch Dermal Guinea Pig Sensitization Test (Buehler method modified for medical devices, and ISO 10993-10 2002, “Biological Evaluation of Medical Devices, Part 10, Tests for Irritation and Delayed-Type Hypersensitivity”), performed by AppTec Laboratory Services, St Paul, MN, USA.

Even at the highest concentrations used, no irritation or sensitization was observed in the mouse LLNA, although all mice in the positive control group strongly reacted. The pH level of commercially available guayule latex, nominally pH 12.0, is sufficient to reduce the concentration of guayulin A by 73% from an average of 0.22% to 0.06% (g/g wet latex). In addition, the guayule latex examination glove films did not cause sensitization or irritation in the Repeated Patch Dermal Guinea Pig Sensitization Test guinea pig trial, and were classified as acceptable in regard to dermal sensitization.

In conclusion, it seems extremely unlikely that guayule latex products will cause sensitization due to the presence of guayulins co-purified with the latex.

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## **POSTER PRESENTATIONS (General Crops)**

### **LONG-TERM PRESERVATION OF NEW INDUSTRIAL CROP GERMPLASM AT THE NATIONAL CENTER FOR GENETIC RESOURCES PRESERVATION**

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The Cropdatabase of the New Crops Crop Germplasm Committee within the Germplasm Resource Information System (GRIN) lists 118 species in 42 genera as having potential uses as industrial crops ([http://www.ars-grin.gov/npgs/cgc\\_reports/newcrops2.htm](http://www.ars-grin.gov/npgs/cgc_reports/newcrops2.htm)). It is challenging to choose an appropriate long-term storage conditions for the new industrial crops due to the variety and diverse chemical composition of seeds and the fact that optimal seed storage conditions have not been described for these crops. In most cases, because of their ‘new crop’ status, limited research resources have been devoted to preserving these crops. The National Center for Genetic Resources Preservation (NCGRP) is a storage site for base collections of all accessions in the USDA, ARS, National Plant Germplasm System (NPGS). The base collections serve as a back up for the 475,000 accessions preserved in the NPGS. One goal of the NCGRP is to back up 100% of the NPGS accessions. At present, only about 78% of the accessions are stored at the NCGRP.

The purpose of this study was to review the back up storage status of the new industrial crops germplasm, to identify species that are not backed up at the NCGRP, and to raise awareness of the germplasm collections lacking information on long-term seed storage techniques. Data used in this study were obtained from the GRIN database.

Of the new industrial crops, 92% of the accessions are in long-term storage at the NCGRP. Ten of the 42 genera (23%) have all their accessions backed up at the Center, yet 6% of the genera (*Agave*, *Dimorphoteca*, *Hesperaloe*, *Panax*, *Physaria*, *Salicornia*, and *Salvia*) have no backup seed samples at the NCGRP or any other site. Of the remaining genera, a relatively small fraction (10%), which represents over 60% of the species listed as new industrial crops (71 species) have  $\leq 55\%$  of their accessions in the long-term storage. The majority of the collections in long-term storage at the NCGRP are stored at  $-18^{\circ}\text{C}$  (67.9%); the remaining collections (seed or shoot tips) are stored in liquid nitrogen (24.2%) with the exception of a few *Cuphea* sp. accessions being stored in  $4^{\circ}\text{C}$ . Of particular interest is that seeds of some high oil containing accessions in the genera of *Lesquerella*, *Limnanthes*, or *Simmondsia* are stored in liquid nitrogen or  $-18^{\circ}\text{C}$ . This is believed to be the optimal storage conditions at the time the germplasm was backed up. Recent research has suggested that these storage conditions for high oil seed may not be optimal. Research is also needed in the areas of defining germination testing procedures for these crops for monitoring seed viability in storage.

To secure these valuable genetic resources for future improvement and cultivar development, studies on conditions

promoting seed longevity in storage as well as establishing procedures and frequency of viability monitoring are needed.

## **PIGMENT SEPARATION AND PHYSICOCHEMICAL CHARACTERISTICS OF DIFFERENT COLORED WHOLE WHEAT GRAIN SEEDS**

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Wheat seed color related pigments and bioactive compounds were separated from black, blue, and red wheat seeds to evaluate their potential as commercially available functional foodstuff.

To determine an efficient condition for separation of the pigments, two extraction methods were used. These consisted of a direct methanol extraction without any hydrolysis treatment; a methanol extraction after hydrolysis by 1N HCl solution at 100°C for 90 min; and three thin-layer chromatograph separation methods with different mobile phases consisting of (a) ethyl acetate-formic acid-water (65:15:20, v/v/v), (b) 1-butanol-ethanol-water (40:10:20, v/v/v), and (c) chloroform-methanol (100:10, v/v).

The extraction effectiveness was better with the direct methanol extraction method without any hydrolysis than that after hydrolysis with 1N HCl. The best separation efficiency was obtained from the mobile phase of ethyl acetate-formic acid-water (65:15:20, v/v/v). Research of the various colored seeds for antioxidant activities and physicochemical characteristics including ash content, crude fat, and dietary fiber will be discussed.

## **EVALUATION OF TOTAL AMINO ACIDS AND FREE AMINO ACIDS IN KOREAN SOYBEAN VARIETIES**

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Soybean [*Glycine max* (L.) Merr.] is an inexpensive, high quality protein source and has long been a staple of the traditional diet in Korea. Experiment was carried out to get the basic information of total amino acids (TAA) and free amino acids (FAA) in Korean soybean varieties.

The content and composition of TAA and FAA were analyzed by amino acid autoanalyzer with ion exchange column (4.6 mm ID x 60 mm, 2622SC-PF & 2622SC-PH, Hitachi, Japan). The TAA contents of soybean seeds were considerably variable, whereas the amino acid composition of TAA showed a slight variance among the tested 30 soybean varieties. The results indicated that the monoamino monocarboxylic acid (MMA) and monoamino dicarboxylic acid (MDA) constitute approximately 52% of the total amino acids. All soybean varieties showed low sulfur containing amino acids. However, the FAA content and composition of soybean seeds were much different than those of TAA. The FAA, cysteine, aspartic acid, and glutamic acid were high and constituted approximately 43.7% of free amino acids. There was a significant quadratic relationship between free amino acids (FAA) and protein compositional amino acids (TAA) ( $y = 0.0036x^2 - 28.741x + 89832$ ,  $R^2 = 0.477$ ,  $P < 0.05$ ), whereas there was no significance between soybean seed size and TAA, and FAA.

## **IMPACT OF PLANTING DATE AND VARIETY ON SWEET SORGHUM ETHANOL PRODUCTION POTENTIAL IN URUGUAY**

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The oil price increase together with the need for improving the environment are two main reasons for using ethanol as an alternative fuel. Sweet sorghum [*Sorghum bicolor* (L.) Moench], because of its fast growth rate, early maturity (90-150 d), elevated total energy value, and wide agronomic adaptability, has frequently been suggested as a good source of ethanol production. Uruguay has a good biomass potential for C4 species without irrigation, but there is no information about sweet sorghum potential.

The objective of this preliminary study was to identify the best variety and planting date combination for sweet sorghum based on stem yield, juice quality, and theoretical ethanol production per hectare for Uruguayan conditions.

We conducted a two-year field study on loamy clay soil (Argiudol typic) in Paysandú, Uruguay (32° S, 56° W; annual precipitation = 1100 mm). Two sweet sorghum varieties (Topper 76-6 and M81-E), and three planting date (October [PD1], December [PD2], and January [PD3]) were evaluated in a strip-plot design with three replications. We measured fresh stem weight, stem high, stem diameter, extraction of juice (%), °Brix content, and theoretical ethanol production.

The PD2 had the highest fresh stem weight (68.73 Mg ha<sup>-1</sup>, 20% and 65% more than PD1 and PD3, respectively; P≤0.05). This better behavior per area unit in PD2 was associated with the individual stem weight due to the taller stem height (2.69 m) and diameter (15.3 cm). No significance differences (P≤0.10) between varieties were detected for these yield parameters. However, the M81-E had better °Brix content (total solid in stem juice) than Topper 76-6, but no difference in stem extraction was detected. For sweet sorghum, the best planting date × variety combinations could result in 4350 l ha<sup>-1</sup> of theoretical ethanol production.

Integrating sweet sorghum in this region using an appropriate planting date can benefit farmers, allowing extra income for them, improving environmental quality, and decreasing non-renewable fuel import into Uruguay.

## PESTICIDE CLEARANCES FOR SPECIALITY CROPS

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Major U.S. crops, such as wheat, corn, cotton, and soybeans provide ample economic incentives for pesticide development in the U.S. However, far fewer pesticides are registered for small-acreage and speciality/industrial crops due to high regulatory costs and market risks. Although numerous weed, insect, and disease pests attack specialty and industrial crops, these crops provide 40% or more of all crop revenues in the U.S.

The IR-4 program provides mechanisms for pesticide clearances for minor crops through partnerships with US EPA, land grant universities, USDA/ARS, pesticide registrants, and grower organizations. Pesticide Clearance Requests are first prepared by grower groups, land grant personnel, or others, and are submitted to the IR-4 headquarters office. Annual priorities are established, GLP protocols are developed, and field residue samples are obtained. Pesticide clearance processes are usually less difficult when there is no food or animal feed uses involved in the specialty crop or its byproducts.

Once residue data are generated, IR-4 prepares petitions to expand labels of new and existing pesticides. For greater efficiency, US EPA and IR-4 have organized 800 crops into 20 Crop Groups, based on botanical similarities and edible plant parts, to enhance the minor crop registration process. Representative crops are designated within each Group and act as surrogates for extending a label to other crops in the Group. Crop Groupings provide for more efficient registrations that would benefit growers and consumers. Since 1963, more than 7,300 tolerances have resulted from the IR-4 work, representing 42% of all tolerances granted by EPA.

## **ANALYSIS OF RICE AMYLOPECTIN STRUCTURE BETWEEN KOREAN AND JAPANESE VARIETIES ACCORDING TO PHYSICAL CHARACTERISTICS**

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Rice is one of the most important crops in the world. Structural analysis of amylopectin is one of the most important approaches to evaluate it for consumers or industrial uses.

This study was carried out to investigate the chain-length distribution of amylopectins and pasting properties of Korean (Nampyungbyeo, Dasanbyeo, Goami 2) and Japanese cultivars (Koshihikari, Kirara 397). These are strikingly different from the Korean varieties Nampyeonbyeo (soft type) and Dasanbyeo (hard type) and the Japanese varieties Koshihikari (soft type) and Kirara 397 (hard type). Cooked rice of Goami2 was significantly different in rice surface, hardness, and stickiness between the Korean varieties and Japanese varieties measured by tensipresser (Myboy System, Taketomo Electric LTD).

Amylopectin distributions and its content of four varieties except Goami2 showed similar trends and distributed in the order DP13~24 (50.4~51.1%), DP6~12 (50.4~51.1%), DP25~36 (12.0~12.4%), and DP>37 (8.5~8.9%), respectively. The surface hardness and chain-length distribution of amylopectin in each rice variety had a negative correlation to DP 6~12, and a positive correlation to DP>13. However, the surface and stickiness had a positive correlation to DP 6~12, and a negative correlation to >DP13, indicating that amylopectin distribution influenced significantly the texture of cooked rice.

## **PHYSICAL CHARACTERISTIC CHANGE AND RETROGRADTION IN SURFACE AND OVERALL GRAIN OF COOKED RICE AFTER STORAGE**

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Rice is a staple food and its safe storage is very important. Changes in the physical and chemical properties of rice grains occur during storage. The physicochemical processes include properties such as hydration, swelling, solubility, viscosity, and pasting.

This study investigated the surface and overall properties of five rice cultivars (Ilpumbyeo, Odaebyeo, Nampyeongbyeo, Taebongbyeo, and Chuchungbyeo), that were stored at room and low temperatures (15°C) for 1 year using a tensipresser (My Boy System, Taketomo Electric LTD). Also, taste testing was made with a Taste analyzer (SATAKE, STA-1A, Japan) and gelatinization properties (RVA-3D, Newport Scientific, Sydney, Australia). Properties of cooked and stored rice at 4°C for 24 h were measured.

At room temperature, high maximum viscosity and breakdown were found, whereas low stickiness of rice surface was observed. Furthermore, the surface properties of rice stored at room temperature had higher hardness and lower stickiness than those at low temperature (15° C). Hardness and stickiness also had the same tendency. Also, water absorption had a high rate of change in the initial stage during storage condition and low temperature. Taste and appearance was lowered at room temperature storage.

## **ULTRA-HIGH CARBON DIOXIDE APPLICATIONS ACCELERATES PLANT ESTABLISHMENT AND GROWTH**

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In a typical crop-breeding scheme, collected seed crosses are germinated and grown in pots in a greenhouse and/or nursery setting for 3 to 12 months before transplanting into the field. Perennial crop breeding, being long-term, are particularly subjected to long periods for seedling soil establishment and nursery growth. Decreasing the time required for seedling establishment in soil and improving the subsequent early growth would benefit breeders and propagators. Applications of high CO<sub>2</sub> levels ( $\geq 700 \mu\text{mol}\cdot\text{mol}^{-1}$  CO<sub>2</sub>) have been employed to accelerate rooting and overall plant growth in various species.

The objective of this study was to determine whether various concentrations of CO<sub>2</sub> (e.g., 350 to 30,000  $\mu\text{mol}\cdot\text{mol}^{-1}$  CO<sub>2</sub>) could improve plant establishment and growth. This study tested both established (sweetgum (*Liquidambar styraciflua* L.) and loblolly pine (*Pinus taeda* L.)), potential (Osage-orange (*Maclura pomifera*) perennial tree crops, and a potential herbaceous crop (Cuphea (*C. lanceolata* x *C. viscosissima*)) in ultra-high CO<sub>2</sub> environments.

Two-week-old Cuphea, loblolly pine, and Osage-Orange seedlings were exposed to 350, 1,500, 3,000, 10,000, and 30,000  $\mu\text{mol}\cdot\text{mol}^{-1}$  CO<sub>2</sub> for 30 days within transparent test chambers under greenhouse conditions. The CO<sub>2</sub> concentrations were attained through mixing of compressed air and CO<sub>2</sub> via a flowmeter and administered during the day light photoperiod. Single 1- and 2-cm sterile Sweetgum shoots derived from proliferating axillary shoot clumps were exposed to a non-sterile environment employing the several CO<sub>2</sub> levels for 30 days. Average daily temperature was 25°C and illumination was provided by natural sunlight. At the end of the test period, seedlings and shoots were examined for survival, fresh weight, leaf number, and root number.

All seedlings benefited from ultra-high CO<sub>2</sub> treatments compared with ambient air (350  $\mu\text{mol}\cdot\text{mol}^{-1}$  CO<sub>2</sub>) controls. However, the rate of growth response increases varied among the different species tested. For example, the fresh weight of Osage-Orange seedlings increased 130 % when given 10,000  $\mu\text{mol}\cdot\text{mol}^{-1}$  CO<sub>2</sub> over the untreated control. Short term treatments with ultra-high CO<sub>2</sub> has merit to improve plant growth responses and sped overall growth process in both trees and herbaceous crops.

## **BREEDING FOR VALUE-ADDED RICE WITH IRON AND ZINC ENRICHED GRAIN**

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Dietary iron deficiency is the most common nutritional disorder in the world. An accelerated rate of widespread micronutrient malnutrition is observed among children, and women of childbearing age whose staple food is rice (ACC/SCN 1992). Among micronutrients, Fe, Zn, Ca, iodine, and vitamin A are highly deficient for many people consuming a staple food crop such as rice. Micronutrient enrichment in rice grains through selection and breeding is the most sustainable way in improving the health of people whose staple food is rice.

This study was aimed to identify a high micronutrient germplasm that could increase the grain concentrations of iron and zinc and to estimate genetic and genotype-by-environment (G × E) variance components for rice genotypes with iron and zinc density.

The 246 rice germplasm were analyzed for iron and zinc concentrations by Inductively Coupled Argon Plasma (ICP). Elite lines selected from the ICP result were used to estimate genetic and genotype-by-environment (G × E) variance components with the Additive Main effects and Multiplicative Interaction (AMMI) model.

Iron concentration ranged from 10 to 22mg/kg with a mean of 14.5mg/kg. For zinc, the range was from 14 to

43mg/kg with a mean of 28.9mg/kg in brown rice. The range in milled rice was 2 to 12mg/kg with a mean of 4.3mg/kg for iron, and 10 to 33mg/kg with a mean of 22.8mg/kg for zinc. The AMMI model used to determine the interaction between genotype and environment showed that genotypes,  $G \times E$  interaction and environment component accounted for 55% (theoretically the most important factor), 20% and 5%, respectively. The AMMI (partitioned into 4 IPCA effects) explained that grain Fe and Zn density in IPCA1 and IPCA2 sufficiently accounts for more than 80% of  $G \times E$  variation.

Iron is deposited in high concentration in the outer layer of brown rice. In contrast, the zinc is deposited in equally in the grain in all varieties. The most promising rice varieties across various environments were obtained for better nutrition.

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## **POSTER PRESENTATIONS (Medicinal and Nutraceuticals)**

### **EVALUATION OF SEED DEVELOPMENT AND FATTY ACID ACCUMULATION IN BORAGE**

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Borage (*Borage officinalis* L.) seed oil is rich in gamma-linolenic acid (GLA) utilized mostly in the pharmaceutical, cosmetic, and nutritional industries. Borage exhibits indeterminate growth with flowering and seed development commencing from the lower to upper aerial regions of the plant. This results in physiologically mature seed at the base of the stem and new inflorescence development from the apical meristems. Mature borage seed has an oil content ranging from 290 to 380 g kg<sup>-1</sup> with a GLA content between 17% and 25%. Commercial processors prefer a borage seed GLA content of 22% or greater. Recognizing when maximum GLA occurs during seed development could identify management strategies, pertaining to seeding and harvest timing, to assure that preferred GLA levels are attained.

The objective of this study was to determine seed weight, oil content, and fatty acid composition changes during borage seed development with particular focus on the changes of GLA content.

The study was conducted over the 2006 growing season at a North Dakota State University field research site near Prosper, ND. To evaluate seed development, 500 flowers were tagged at anthesis in each of four replicates for two different flowering dates spaced two weeks apart. Harvest dates for seed collections started 5 d after initial anthesis and continued to physiological maturity. Harvest dates were spaced at 3-d intervals. Seed collections at each harvest date consisted of approximately 50 seeds from each replication. Seed oil content analysis was performed by Soxhlet oil extraction and oil composition analysis by gas chromatography.

Analysis of the first harvest date showed seed GLA content increased from 10 d post anthesis to physiological maturity. Seed moisture decreased from 850 g kg<sup>-1</sup> at 5 d post anthesis to 350 g kg<sup>-1</sup> at physiological maturity. Maximum seed dry weight showed a linear increase from 5 d post anthesis until physiological maturity. Seed physiological maturity was attained at 20 days post anthesis or approximately 241 growing degree-days. Maximum seed GLA and dry weight occurred at physiological maturity for individual seeds.

### **SEMIDESERT PLANT EXTRACTS EFFECT AS TOLERANCE INDUCTORS IN TOMATO (*LYCOPERSICON ESCULENTUM* Mill.) AGAINST *FUSARIUM OXYSPORUM* AND ITS INFLUENCE ON PLANT GROWTH**

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An alternative to chemical agents used against tomato diseases is the application of natural products. Because they can be assimilated by the plant and soil microorganisms, they do not affect the environment, even though they can promote resistance to diseases. Phytoalexins affect plant growth and fruit quality.

The objectives of the present work were: (a) to obtain ethanolic extracts from *Chenopodium quinoa* Willd. (quinoa) and other species of the Chihuahuan Semidesert; *Yucca carnerosana* Trel. (yuca). *Agave lechuguilla* Torr. (lechuguilla), *Larrea tridentata* Sesse & Moc. ex DC. (gobernadora); and *Flourensia cernua* DC. (tarbush); (b) to evaluate their extracts on *Fusarium oxysporum*, and (c) to determine the incidence of *F. oxysporum* on tomato growth.

Five plant extracts were evaluated for mycelial growth of *F. oxysporum* in Petri dishes with a culture medium (agar, dextrose, potato PDA). Mycelial inhibition was estimated based on 0% mycelial inhibition as mycelial growth of inoculated control. The mycelial inhibition values were compared by random experimental design with four replications.

Gobernadora extracts (1000 and 2000  $\mu\text{l l}^{-1}$ ) inhibited *F. oxysporum* 100% at 48 and 96 h, and had the best results. The tarbush extract at 96 h inhibited 78.0% with 1000  $\mu\text{l l}^{-1}$ , and with 500  $\mu\text{l l}^{-1}$  inhibited growth at 73.3%; yuca extract (4000  $\mu\text{l l}^{-1}$ ) inhibited 18.3% and 9.6% with 200  $\mu\text{l l}^{-1}$ . Lechuguilla and quinoa extracts did not inhibit mycelial growth of *F. oxysporum*.

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## ANTIFUNGAL COMPOUNDS FROM MEXICAN *FLOURENSIA CERNUA*

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Mexican plants from semiarid lands have been widely investigated as sources of important bioactive agents. The *Flourensia* genus, tribe Heliantheae consists of 32 species of resinous shrubs. *Flourensia cernua* D.C. (Asteraceae) is a bitter tasting shrub that grows on the semiarid lands in northern Mexico. Because of its chemical composition, *F. cernua* leaves have been used as folklore medicine for treating various health disorders.

The objectives of the present work were to: (a) extract the *F. cernua* chemical compounds with different solvents; (b) isolate into fractions the different compounds, and (c) identify the chemical structures of the components with major biological activities against fungus.

Ethanolic extract was obtained by sequential extraction with constant agitation at room temperature. Column chromatography with solvent gradient was used for fractionation. The fractions obtained were tested against *Fusarium oxysporum* at different concentrations (500, 1000, and 2000  $\mu\text{l l}^{-1}$ ). The samples with fungal controls were further characterized by infrared spectroscopy (IR) and gas chromatography coupled with mass spectroscopy (GC-MS).

The results showed that the ethanolic extract had the major fungicide control over *F. oxysporum*. The IR spectra consist of conjugated carbonyl functionalities including alkane at 2924-2853  $\text{cm}^{-1}$  and carboxylic acid at 1719  $\text{cm}^{-1}$ .

## SELF-INCOMPATIBILITY AND EMBRYO DEVELOPMENT IN *ASTRAGALI RADIX*

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*Astragalus membranaceus* (*Astragali Radix*) is an important medicinal plant propagated by seed with the root used for medicine. The flower shape is similar to soybean. It does not set seed by self-pollination. Therefore, to improve the breeding of *A. membranaceus*, it is important to find why it does not self-fertilize. This study was conducted at the National Institute of Crop Science's medicinal plant experimental field and laboratory to determine the characteristic of fertilization process and embryo development of *A. membranaceus* to provide basic data needed in breeding.

Natural pollination, artificial pollination, and selfing were done in this study. The packet used parchment paper and net. To observe fertilization process and embryo development by histology after fertilization of *A. membranaceus* flower, self-pollination involved covering the bud with parchment paper when the flower bud began to form, and cross-pollination did depollination on the afternoon 3 days before flowering, and artificial pollination the next morning between 9 to 10 am. In the fertilization investigation, self-pollinated flowers were picked when the third small flower was completed, whereas artificial pollinated flowers were picked at 0.5, 1, 3, 6, 24 hours, and 2, 3, 5, 10, 15 days after pollination. The picked flowers were examined and stored in hypotonic solution after placing them into fixing solution, and used to produce samples. Samples were prepared with paraffin, dyed by aceto-carmine (1% iron aceto-carmine), and observed with the optical microscope.

*Astragalus membranaceus* showed poor seed bearing when self-pollination was induced. When artificial pollination was induced, it showed less than 5% bearing in late August, but more than 13% bearing from the beginning of 4 September. The flower size was about 17.0 mm×4.0 mm and pistils and stamens had the same length of 15.0 mm at flowering stage. When self-pollination or cross-pollination was induced, pollen tubes extended to an ovule. While pollen tube extended to the ovule, reproductive cell was split and formed two male generative nuclei and a vegetative nucleus. In the case of self-pollination, fertilized embryo was not observed, but was formed in the case of cross-pollination. *A. membranaceus* is noted to have zygote self-incompatibility. In the case of cross-pollination, fertilization was observed in 6 to 8 h after pollination, where apical cell derivatives continuously split after fertilization. A spherical pre-embryo was then formed three days after fertilization. The seed attained full shape with a seed coat showing its distinctive contour 15 days after fertilization.

Thus, *A. membranaceus* in Leguminosae is found to have zygote self-incompatibility although its flower shape is shown to match the self-compatibility plant.

## ANTHOCYANIN CONTENT IN LEAVES AND FLOWERS OF SEVERAL HIBISCUS SPECIES

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*Hibiscus* species contain bioactive phytochemicals and nutraceuticals to be utilized in the pharmaceutical and nutraceutical markets. Twenty-eight *Hibiscus* species consisting of more than 300 accessions are conserved at the USDA, ARS, Plant Genetic Resources Conservation Unit, Griffin, GA. Anthocyanins as well as related flavonoid compounds (quercetin and kaempferol) are present in flowers, leaves, and calyxes. Anthocyanins are not only responsible for leaf, stem, flower, and seed color, but can inhibit LDL oxidation as well as hypertension in humans, whereas the antioxidants, quercetin, and kaempferol are known to be anticancer compounds.

The objective of this study was to determine the amount of anthocyanin from leaves, flowers, and calyxes of *H. brackenridgei*, *H. calyphyllus*, *H. cannabinus*, *H. laevis*, *H. ludwigii*, *H. radiatus*, and *H. sabdariffa*.

An Opti Sciences CCM-200 chlorophyll content meter was converted to an experimental hand held anthocyanin meter. The manufacturer replaced the 655 nm light emitting diode (LED) of the CCM with a 520 nm LED in order to measure absorbance near the wavelength at which free anthocyanin aglycones, cyaniding, and pelargonidin monoglucosides absorb. Anthocyanin indexes were recorded from each of three leaves using this modified anthocyanin

meter.

Preliminary analysis revealed that leaf anthocyanin indexes ranged from 2.9 to 30.5 for *H. cannabinus*, *H. calophyllus*, *H. laevis*, *H. ludwigii*, and *H. sabdariffa*. However, flower anthocyanin indexes from *H. cannabinus* ranged from 1 to 167.7. The extremely low anthocyanin indexes (1) were recorded from the white petal portion of the corolla lobes, whereas the very high anthocyanin indexes (167.7) were recorded from the inner purple to red corolla lobes. The isoflavones, quercetin, and kaempferol have similar biochemical pathways as the anthocyanins, cyaniding, and pelargonidin. *Hibiscus sabdariffa* leaves can produce 280.8 ng/μl of quercetin and 183.4 ng/μl of kaempferol. This indicates that leaf anthocyanins produced in *H. sabdariffa* include both quercetin and kaempferol.

A useful, quantitative method employed in the quantification of anthocyanin indexes from different *Hibiscus* species will be demonstrated. The *Hibiscus* species identified in this study can serve as potential new sources of high anthocyanins to be introduced into breeding lines or cultivars and/or used as a pharmaceutical or nutraceutical crop in the southeastern U.S.

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## **POSTER PRESENTATIONS (Oilseeds)**

### **BLOWN LESQUERELLA AND CASTOR ESTOLIDE OILS**

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*Lesquerella fendleri* is a member of the Brassicaceae (mustard) family. Lesquerella is a winter annual seed oil crop native to the desert southwestern United States and is currently undergoing intensive research efforts for its successful introduction into agriculture. Lesquerella produces a small seed that has 25 to 30% oil that contains 55 to 64% hydroxy fatty acid. The hydroxy fatty acids of lesquerella are lesquerolic (55 to 60%, 14-hydroxy-*cis*-11-eicosenoic acid) and auricolic (2–4%, 14- hydroxy-*cis*-11-*cis*-17-eicosenoic acid). The distribution of TG is 10% nonhydroxy acyl, 15% monohydroxyacyl, and 73% dihydroxyacyl, which indicates that lesquerella oil is essentially a difunctional triglyceride in terms of hydroxy functionalities.

The objective of this study was to develop industrial products with lesquerella and castor oils as a starting material. In the past, estolides have been used to develop new products from new industrial crops. Estolides from lesquerella have been synthesized with two different methods either 200°C from the fatty acid or 130°C using a tin catalyst from the fatty acid or the triglyceride. These estolides were then blown with a steady airflow and elevated temperatures to increase the viscosity of the estolide oils.

Triglyceride estolides were synthesized from the hydroxy moieties of lesquerella and castor oils with oleic and saturated fatty acids. These materials were characterized and physical properties recorded (pour points <-27°C). The oil estolides were then subjected to a steady airflow at 100°C for different duration (12-168 h). The viscosities at 40°C for the blown estolide oils increased from 126.6 to 928.5 cSt in the lesquerella series and from 125.6 to 473.5 cSt in the castor series while maintaining acceptable low temperature properties.

Many new and useful products and applications have been developed for lesquerella and castor oil estolides.

### **EFFECT OF EXTRUSION COOKING OF LESQUERELLA SEEDS ON THE QUALITY OF THE EXTRACTED OIL**

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*Lesquerella fendleri* is an oilseed crop belonging to the *Brassicaceae* family that is native to the desert southwestern United States. The seed has 28% oil, which contains about 64% hydroxy fatty acid (HFA). HFA is used in a variety of industrial applications such as lubricants, corrosion inhibitors, engineering plastics, plasticizers, emulsifiers, and coatings. Production of *lesquerella* is currently being scaled up for commercialization.

The seeds also contain glucosinolates that, upon hydrolysis by thioglucosidase (TGSase), produce undesirable sulfur-containing compounds in the meal and may end up in the oil during extraction. Inactivation of TGSase is an important step in preparing seeds for oil extraction. This study investigated the use of extrusion cooking in inactivating TGSase and its effect on the quality of the crude oil.

Whole *lesquerella* seeds with 6% (as is) and 11% moisture were extruded at 270 and 470 rpm screw speeds. The temperature of the extrudate was recorded and its moisture content was determined. The extent of seed cooking was evaluated by measuring the protein solubility and TGSase activity in the extrudate. Uncooked and extrusion-cooked seeds were screw pressed and the crude oils obtained were analyzed for solids, free fatty acid, sulfur, and phospholipids contents and color.

Running the extruder at 270 and 490 rpm provided residence times of 110 and 80 s, respectively. The exit temperature of the extrudate ranged from 124<sup>B</sup>C to 289<sup>B</sup>C, with the latter obtained from 6% MC seed extruded at 490 rpm. Seeds with 6% initial MC dried down to 2.3%, while seeds with 11% initial MC came out at 7% MC. The protein solubility decreased by 55% and 70% for seeds with 6% and 12% initial MC, respectively, when extruded at 270 rpm. All extruded seeds tested negative for TGSase activity. Extruded seeds with 6% initial MC resulted in much higher solids (6.4-9.4%) in the oil compared than the 11% MC seeds (1-1.7%). Free fatty acid content increased with increasing moisture, but was not affected by cooking. However, the sulfur content significantly increased from 9-33 ppm to 102-112 ppm after cooking. Cooking also increased the phosphorus content (a measure of total phospholipids) from 3 ppm to 16 ppm. This higher phosphorus content is still less than the values for good water-degummed oils (60-200 ppm), indicating that degumming may be unnecessary. Crude oil from extruded seeds was darker (13-14 Gardner) than the oil from uncooked seeds.

## ROTATIONAL EFFECTS OF CUPHEA WITH CORN, SOYBEAN, AND WHEAT IN THE NORTHERN CORN BELT

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*Cuphea* (*Cuphea viscosissima* Jacq. x *C. lanceolata* W.T. Aiton; line PSR23) is a recently domesticated, new oilseed crop that can serve as a replacement for saturated small and medium chain fatty acids (i.e., C8:0 to C14:0) presently imported by the United States. Although diversifying crop rotations can have economic and environmental benefits, some species can negatively impact the following year's crop through allelopathic effects, introduction of new pests or pathogens, or excessive depletion of subsoil moisture and nutrients. While management practices for growing *cuphea* have been developed, little is known about the effects of *cuphea* in rotation with conventional crops.

In the northern Corn Belt where *cuphea* has been commercially produced, the predominant crops are corn (*Zea mays* L.), soybean [*Glycine max* (L.) Merr.], and wheat (*Triticum aestivum* L.). A study was conducted in west central Minnesota from 2004 to 2006 to determine where *cuphea* might best fit into rotation with these crops. The experimental design was a strip plot randomized complete block replicated four times. The crops were initially planted in 9 m x 37 m strips in either a north-south or east-west orientation and then planted in the opposite direction the following year to give all combinations of the current and previous crop in a two-year rotational scheme. The main effect analyzed was the impact of the previous crop on the current crop.

*Cuphea* seed yield and final plant population density were not affected by the previous crop (i.e., corn, soybean, wheat, or *cuphea*) in 2005 and 2006. In 2006, *cuphea* oil content was greatest when the previous crop was wheat (32 % wt wt<sup>-1</sup>) and lowest when following corn (29.5 %). Corn and wheat grain yields were unaffected when following *cuphea* in rotation, and yields tended to be similar to or greater than those in the establishment year (2004). In 2005,

the yield of soybean that followed cuphea in rotation was about 427 kg ha<sup>-1</sup> lower than when the previous crop was corn or wheat. However, in 2006 there were no significant differences in soybean yields regardless of the previous crop. When soybean followed cuphea in rotation, the final stands were 24 and 9 % less in 2005 and 2006, respectively, than when corn was the previous crop.

For west central Minnesota, evidence indicates that it may be best to rotate cuphea after soybean or wheat, and before corn or wheat. Results indicate that cuphea might negatively affect soybean that is planted the subsequent year, although more research will be needed to verify this.

## **INTRASPECIFIC VARIATION IN FATTY ACID COMPOSITION OF *CUPHEA* (LYTHRACEAE) SEED OILS: GENETIC VS ENVIRONMENTAL EFFECTS**

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*Cuphea* P. Browne is one of the few flowering plant genera that have the potential to serve as a temperate oil replacement for MCFAs, especially to develop high-yielding capric-lauric acid plants. Several genetic and agronomic studies have been performed aiming at evaluating species for fatty acid content, determining the feasibility of domesticating *Cuphea*, and understanding the barriers for domestication. Although such efforts resulted in few germplasm lines with favorable agronomic traits including partial non-shattering and non-dormancy (e.g., *Cuphea* PSR23), many questions about fatty acid production and commercialization of *Cuphea* still remain unanswered. One of such questions is how much of the variation in fatty acid composition among populations is contributed by either genetic or environment. Previous studies in *Cuphea* considered seed oil composition as a genetically controlled and conserved trait. However, recent studies in *Arabidopsis* have shown that seed lipid composition may be regarded as an adaptive trait and could result in the existence of natural intraspecific variation within any given species.

The objective of this study was to examine the extent of natural variation that occurs within species. This will allow researchers to predict the type of oil that may be produced in a particular area. Furthermore, it will give us insight into whether natural variants containing fatty acids of particular interest exist among the different populations of *Cuphea* plants. Secondly, an attempt was made to determine whether variation in MCFAs could be correlated with environmental factors, especially temperature, latitude, altitude, and precipitation.

Twenty-eight populations representing three widely distributed *Cuphea* species were used for this study. Fatty acid composition in seeds was determined by a standard GC instrument using the Methyl Ester method. Locality and climatic data for all the samples were also compiled and, using these locality data, climatic data were gathered using geographic information system (GIS) environmental data layers. For purposes of the present study, we used four GIS data layers: elevation (m), annual mean temperature (°C), mean diurnal temperature (°C), and annual precipitation (mm). Data were analyzed using redundancy analysis (RA).

The main fatty acids were determined as caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), myristoleic acid (C14:1), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and linoleic acids (C18:2). In each species, a single fatty acid was dominant in all populations, whereas the proportion of the different fatty acids varied from one population to another. Among the environmental factors analyzed, elevation, latitude, and their interaction were by far the most influential determinants of fatty acid composition in the seed oils, particularly with respect to the ratios of lauric:capric and lauric:myristic acids.

## **WILD PERENNIAL *HELIANTHUS PUMILUS* AS A POTENTIAL SOURCE FOR IMPROVED OIL CONTENT AND QUALITY IN CULTIVATED SUNFLOWER**

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The genus *Helianthus* consists of 51 species and 19 subspecies with 14 annual and 37 perennial species. The narrow genetic base of cultivated sunflower has been broadened by the infusion of genes from the wild species, which have provided a continued source of desirable agronomic traits. There has been an increased interest in using wild species in breeding programs, but there have been concerns about the introgression of low oil content and quality from the wild species. *Helianthus pumilus* (Dwarf sunflower) is a perennial species with potential genes for oil improvement based on its xerophytic habitat. Unfortunately, due to the demand for achenes of this species and the difficulties of regenerating achenes from the original populations, few achenes have been available for research for almost 20 years.

The objective of the study was to undertake an exploration to Colorado and Wyoming, USA to collect achenes from the entire distributional range of the species and assess the potential of the populations for improving oil content and quality in cultivated sunflower.

The sunflower exploration took place from August 7 to August 19, 2005 and covered 5150 kilometers in Colorado and Wyoming. Heads were collected from 10 to 100 plants within each population and were bulked into a single sample. For each population, a composite sample of 10 randomly sampled achenes was analyzed for fatty acids composition using organic base-catalyzed transesterification of fatty acid methyl esters and capillary gas chromatography. Oil content was determined on a 2-ml achene sample using nuclear magnetic resonance (NMR). The achene samples were deposited at the USDA-ARS, NPGS, North Central Regional Plant Introduction Station, Ames, IA, where they are maintained and distributed. Voucher specimens are maintained at the USDA-ARS wild sunflower species herbarium at Fargo, ND.

It had been 20 to 30 years since six known locations of this species were last visited. Achenes of 47 additional populations were collected and placed in the wild sunflower germplasm collection. The exploration was successful in collecting representative populations from the entire distributional range.

The *H. pumilus* populations had oil content of 254 g/kg, which is considerably lower than cultivated sunflower of 470 g/kg. The highest oil content of an *H. pumilus* population was 294 g/kg, 30% higher than previously reported. The linoleic acid concentration approached 750 g/kg, much higher than the 540 g/kg expected from a semi-arid environment. The combined saturated palmitic and stearic fatty acids in *H. pumilus* was 102 g/kg, about equal to cultivated sunflower oil of 110 g/kg.

The higher concentrations of linoleic acid in *H. pumilus* could be a potential source of genes for increasing linoleic acid concentration in traditional sunflower oil. The low oil content of this species can be increased by backcrossing to a high oil cultivar. Further research will be needed to determine the inheritance of the fatty acids and oil content traits.

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