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2008 New Crops & Bioproduct Development



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ABSTRACTS

Keynote Speaker

BIOFUELS IN BRAZIL: PAST, PRESENT AND FUTURE NEEDS

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The demand for food, fuel, and energy resources continues to increase worldwide. Currently, there are many international efforts aimed at finding renewable, sustainable, and environment friendly solutions for these problems. The spiraling price of petroleum and the adverse effects of using nonrenewable resources are major reasons for increased interest in renewable sources of energy.

Brazil is the largest and most populous country in Latin America and ranks fifth in land area and population in the world. Brazil's location in the tropical and subtropical zones of the world ensures intense solar radiation and year-round water supply for bioenergy production. In addition, the vast untapped land mass allows new land to be used for bioenergy production without reducing the farm area devoted to food production.

Ethanol production in Brazil based on sugarcane has a long, interesting, and turbulent history. Certainly, it is a global model for ethanol production, distribution, and use. Therefore, the Brazilian ethanol industry has attracted interest from scientists, producers, and governments of both developed and developing countries.

The development of flexible-fuel vehicles (FFVs), cars capable of running on gasoline, ethanol, or any combinations of both fuels, has renewed customer interest in biofuels. Brazil's FFVs fleet is the only one in the world that can use 100% of either gasoline or ethanol. In 2007, about 80% of new cars manufactured in Brazil were FFVs.

Like ethanol, biodiesel has received increased interest as a biofuel. Several oleaginous species have been used and investigated: however, soybean is currently the largest source for biodiesel production. Brazil is currently facing challenges to reach goals established in January 2005, when a law introduced into Brazilian Energy Matrix with a mandatory use of at least 2% (B2) biodiesel by 2008 and 5% (B5) by 2013.

Brazil has been working with renewable sources for energy production for over 70 years. However, Brazil's programs must be continually updated and reviewed for future needs. Some of these challenges include the development of new cultivars, the agroecological zoning for biofuels crops, the use of biotechnology to introduce traits of interest, and improve industrial process and products.

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Plenary Session

SELECTION OF NEW INDUSTRIAL OILSEED CROPS

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Global demand for industrial oils is focusing our attention to explore new sources of oils that do not interrupt the food supply chain. The challenges we face are in the selection of new crops that can be regionally produced to minimize transportation costs and disruption in the supply chain. To identify new sources of industrial oil crops, current cropping systems are evaluated based seed output per acre, oil content, current production acres, and type of oil produced. Consideration is given to the overlap into current food production sources. A rich diversity of genetics can be found within the *Brassica* species that is adapted to multiple production environments throughout the world. *Brassica* has potential to be multiuse crop by exploiting the rich genetic diversity for production in high temperature - low water areas and combining the ability to breed new functional oils.

We are exploring the potential of *Brassica napus*, *juncea*, and *carinata* for production in the High Plains as a new sustainable supply of oil. New *Brassica* oil types can be integrated within the current wheat production base. As a rotational crop it has the ability to improve soil structure, reduce disease incidence, and create higher yields for the wheat crop. Considerations in breeding for this area include selection for yield, stress tolerance to heat and water deficit, vigor, disease, maturity, oil content, and harvest ability. Advancements are being made through breeding for long chain fatty acids to create triglyceride structures that improve lubrication properties.

Brassica species provides a new base of oil production in the High Plains of the Western United States by the combination of drought and heat tolerance with new fatty acid genetics.

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BIOBASED PRODUCTS AND THE CHEMICAL INDUSTRY

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Advances in chemistry have had profound impacts on our everyday lives from the clothes we wear to the cars we drive to the medicines we take. Chemical products and processes have provided us with significant improvements in the overall quality of life. Many of the products that we as consumers depend upon, however, are manufactured through processes that are petroleum based and impacted greatly by the availability and price of oil.

The industrial revolution began the journey of the chemical industry into the fossil fuel era, before that time biomass fueled much of the energy and product needs of the chemical industry. The largest use of oil is for transportation, but industrial uses including the chemical industry account for about 25% of the petroleum consumption. It is becoming accepted that petroleum is not a long term sustainable feedstock for the transportation or chemical industry. This has led to an increasing investigation and use of more sustainable feedstocks such as bio-based resources by the chemical industry. There have been many successful chemical products that are derived from bio-derived feedstocks. These include products such polymers, chemical intermediates, and fine & specialty chemicals. Despite these successes many hurdles to further commercial success exits.

These include:

- Biomass is highly functionalized compared with hydrocarbons so new transformation routes are needed
- New chemistries can pose increased risk because there is a lack of commercial experience and likely require capital investment
- Increased debate about sustainability and environmental impact on the use of bio-based resources
- Variability inherent in bio-derived feedstocks verses petroleum feedstocks

To address these challenges, numerous chemical companies including the Huntsman Corporation have formed sustainability and bio-based product initiatives. In this paper, we will describe in more detail the attractiveness of bio-derived products to the chemical industry, some success stories, and the challenges yet to be overcome.

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USDA Update: The Farm Bill and USDA Competitive Programs

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Public support for research is strongly trending toward competitively awarded grants. While there is strong growth for biobased products (including biofuels) produced from commodity crops, emerging industrial crops remain at the periphery of the vision of Federal programs supporting crop and product development research. One notable exception is the USDA Small Business Innovation Research Program (SBIR). Two of four priority emphasis areas in the USDA SBIR Biofuels and Biobased Products Program target new industrial crops and their products:

New Crops for the Production of Non-food Biobased Products – Identification, agronomic/horticultural testing, and development of new industrial crops that will provide new local or regional economic opportunities for farmers and growers to produce raw materials for the production of non-food biobased products. Focus should be on crops that do not require high inputs of water and fertilizer.

New Non-food Biobased Products from New Industrial Crops – Identification of markets and development of new biobased products and processes for making products from new industrial crops. These products should be economically competitive and have carbon reduction benefits.

The USDA SBIR Biofuels and Biobased Products program has provided over \$2,000,000 in support for small businesses and their university partners to conduct industrial crop development and product research on tobacco, kenaf, camelina, algae, sweet potato, guayule, grain sorghum, switchgrass, and jatropha over the past six years.

The Research Title of the 2008 Farm Bill may impact, if not accelerate, the trend toward competitively awarded

research, education, and extension grants within USDA's Cooperative State Research, Education, and Extension Service (CSREES). CSREES will become The National Institute for Food and Agriculture. The Farm Bill provides other changes that may be relevant to the industrial crop community.

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General Crops

RENEWABLE FUELS TO INCREASE THE SUSTAINABILITY OF AMERICAN AGRICULTURE

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The production of grain ethanol has created public and scientific controversy on the potential impact of diverting croplands for the production of renewable fuels. Our Agricultural Industry has been asked to produce not only the food, feeds, and fiber, but also a portion of our transportation fuels, which presents several ethical and economic issues. Increasingly, it has become necessary to demonstrate that renewable fuel production will focus on the production of non-food crops on marginal lands not well suited to other agricultural commodities. However, the rapidly increasing cost and the politically sensitive supply of petroleum derived fuels threatens both the continued profitability and sustainability of America's mechanized Agricultural Industry. Our hypothesis was that if the production of renewable fuels ensured sustained production of food, feeds and fibers that many of the ethical considerations could be answered.

The objective of this study was to determine the potential impact of farm produced and consumed liquid fuels on the Agricultural Industry of the Texas High Plains.

The three most energy intensive inputs required in crop production in this region are: (1) liquid farm fuels; (2) energy required for irrigation; and (3) fertilizers and agricultural chemicals. Diesel that provides almost all of the liquid fuel utilized directly in the production and harvesting of the crops in this region could be efficiently replaced with biodiesel. However, identifying the renewable resources needed to supply the electricity used for irrigation and the natural gas essential for production of nitrogen fertilizers are more problematic and were not addressed in this study. Our study focused on three locally adapted oilseed crops (castor, safflower, and sunflower) that could be processed into biodiesel using farm scale equipment.

Our study concluded that diversion of approximately 15% to 20% of the cropland to these crops could produce all of the liquid fuels need for crop production.

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EVALUATION OF AGRICULTURAL FEEDSTOCKS FOR THE PRODUCTION OF BIOGAS VIA ANAEROBIC BIODIGESTION

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Traditionally, the majority of the revenue vegetable growers receive is from the sale of their crop to the fresh or processing use markets. Increasingly, competition from both domestic and foreign producers keeps the price of vegetable crops low, and this presents a major economic challenge to many of Ontario's fresh vegetable growers. One possible solution to this is to develop additional sources of revenue from the waste material generated during the growing season. For example, up to 40% of a sweet potato crop may be unsuitable for fresh market sales due to visual appearance or size. Some of these "seconds" may be sold to the food processing industry, but a better use may be as a feedstock for the production of biogas via the process of anaerobic biodigestion. Anaerobic digestion involves the microbial breakdown of organic compounds in the absence of oxygen that results in the production of a biogas consisting primarily of methane and carbon dioxide. After removal of trace impurities (sulphur dioxide and water), biogas can be converted into electrical energy and heat through combustion in a modified generator, burned directly to produce heat, or purified and pumped directly into existing natural gas pipelines.

We are utilizing a mobile, pilot scale (2 m³) anaerobic biodigester to: (1) identify those agricultural by-products most suitable for the production of biogas, (2) identify the parameters that have the biggest impact on biogas production, and (3) assess the nutrient quality of the final digestate effluent. Waste vegetable feedstocks are added daily to a working digestate composed of both swine manure and vegetable matter. The production and quality of the biogas are measured to determine methane, carbon dioxide and sulphur content. Our initial studies using sweet potatoes indicate that gas production is elevated when compared with manure alone, but that the methane content of this gas is lower. We anticipate that methane content will rise as the system equilibrates to the change from manure alone to a mixture of manure and vegetable matter.

Our research indicates that anaerobic digestion has utility as a method for generating "on farm" green energy, but that not all waste vegetable feedstocks are suitable for this system. We have displayed this unit at several agricultural events, and there has been great interest from the farming community in the technology. I will also discuss Ontario's standard offer contract for the purchase of electricity generated from anaerobic digestion systems.

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REVIEW OF ALGAE PRODUCTION FOR BIOFUELS AT TEXAS A&M UNIVERSITY

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Texas AgriLife Research, the agricultural research component of the Texas A&M University System, has partnered with General Atomics as a collaborator for the Strategic Fuel Supply project funded by the Department of Defense. The use of algae for biofuel production has the theoretical capacity to out-perform traditional feedstocks, such as soybeans and palm, 25 to 250 times, respectively, on an area basis.

The Pecos, Texas area, located in the Permian Basin of Texas, famous for its petroleum crude oil and natural gas production, offers a location suitable for algae production research. The Pecos, Texas AgriLife Research Center has available land, infrastructure, and personnel to investigate algae production for biofuels. The brackish groundwater, with an electrical conductivity of 3.85 dS/m (TDS 2781 ppm), provides a select medium for developing strains of high lipid containing algae in a geographic area characterized as an underutilized, desert-like environment. The achievement of symbiosis from the local petroleum industry can lead to a low cost CO₂ sequestration and an energy source for algal growth. This could have a significant economic impact on the West Texas economy within the next 7 to 10 years, assuming the adoption of large areas for biodiesel production. Pilot plant construction is underway in Pecos consisting of 1/16th and ¼ acre test ponds, and 1 acre demonstration ponds, which is the preparation for a 500 to 2000 acre commercial demonstration plant. Additionally, an on-site laboratory is under construction for algae screening, strain selection, and for the optimization of oil extraction.

This research will be instrumental in producing engineering plans for future sites, algae production/separation

systems, the development of agronomic practices for algae production and the logistics for harvesting, transport and storage of algal oil.

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SWEET SORGHUM, A BETTER SOURCE OF ETHANOL

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Increasing oil prices have made alternative fuel crops an exciting and potentially viable option for ethanol production. Diversion of food crops to energy, primarily corn to ethanol, has caused the cost of food to increase. Sweet sorghum [*Sorghum bicolor* (L.) Moench] is a non food crop that currently shows promise as a way to produce large quantities of ethanol with minimal inputs. However, little is known of large scale sweet sorghum production in the Southeastern United States. Knowledge about the basic agronomic practices and ethanol distillation information is needed for the adoption of this crop in Mississippi.

Eight cultivars were planted in 2006 and increased to 12 in 2007 as a half-season crop, intended to follow winter canola. The crops were planted in four-row plots 6.09 m long, with rows 0.91 m apart, and were monitored bimonthly for percent Brix and height. Yield data was taken at the end of the season. Sample canes of each cultivar were crushed to extract juice. The %Brix was measured and samples were charged with yeast. After fermentation, the wort of each cultivar was distilled to determine ethanol output. Mean dry weight was also taken to determine yield of the cultivars. The %Brix values were generally higher for the 2006 growing season, and these values were highest in November. Higher %Brix in 2006 may be attributed to the dryland growing conditions of that cropping season. Mean dry weight yield ranged from 16815 kg/ha to 36993 kg/ha. Preliminary distillation data show that one cultivar, Dale, had the highest ethanol output.

The success of these experiments makes it apparent that sweet sorghum is a potentially viable crop in Mississippi, but more agronomic, economic, and engineering work must be conducted for alternative energy systems to be adopted by the public.

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SWEET SORGHUM AS A POTENTIAL FEEDSTOCK FOR LIQUID BIOFUEL IN ARIZONA

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Sweet sorghum (*Sorghum bicolor* (L.) Moench) is a tropical grass native to Africa, and was introduced into the U.S. in 1854. It was originally cultivated in the southern U.S. for small-scale syrup production, and was developed as a sugar source during World War II. We are interested in using the sugars in sweet sorghum as a direct feedstock (not requiring starch hydrolysis) for ethanol production in Arizona. Sweet sorghum is a low input crop (low water requirement and moderate response to nitrogen) that can be grown on marginal areas not well suited for food production.

The objectives of this work were to: (1) evaluate selected sweet sorghum lines for ethanol production in Arizona; (2) within the selected lines, evaluate the amount of variability for the different components of yield; and (3) identify the major components of yield that will respond most readily to different selection intensities.

In 2006 and 2007, 31 and 24 lines, respectively, were evaluated for lodging, insect damage, field weight, juice weight, and Brix (% soluble solids). Ethanol yield was predicted using the calculated percent sugar per plant and the plot

fresh weight. The six most promising lines were planted both years. Sugar profiles for each line were determined by HPLC.

Lodging and insect pressure were greater in 2006, field weight was greater in 2007, soluble solids slightly more in 2006, with no differences for juice weight and predicted ethanol yield between years.

In 2006, the five highest potential ethanol-yielding lines were Cowley, Topper, M81E, Dale, and Theis. These five lines were also planted in 2007, and again were at the top of the rankings for predicted ethanol yield. Of the six lines planted both years, M81E had the best combination of potential production characteristics. Significant variations were found for all traits evaluated, implying that there is the potential for changing the germplasm through breeding to fit industrial needs. Large variations for sugar profile (amounts of sucrose, glucose, and fructose) were found among lines, implying that selection for sugar profile is also possible. Biomass and juice weight were found to be the two major components of yield (best predictors of ethanol yield), with biomass being the most critical in the yield calculation. Brix % value had essentially no predictive power for ethanol yield, but had a high correlation with sucrose content and the amount of total sugars.

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CELLULAR PATH OF SUCROSE IN SORGHUM STEM

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The sucrose that accumulates in the stem of sorghum (*Sorghum bicolor* (L.) Moench) and other large tropical grasses related to it, such as sugarcane (*Saccharum* spp.) can be of commercial value, can buffer photoassimilate supply during development, and can compete with structural-carbohydrate synthesis for available photoassimilate. Previous study has shown that sucrose can be transferred from phloem to the storage tissue of mature sorghum stem of intact plants without being hydrolyzed.

In this study, stem-infused radiolabelled sucrose was traced between cellular compartments and among related metabolites to determine whether the cellular passage of sucrose during transfer from phloem to storage cells in stem tissue was symplasmic (passing from cell-to-cell through plasmodesmata) or included an apoplasmic (extracellular) step. This cellular pathway was evaluated for immature and mature stem tissues of intact plants of two semidwarf grain sorghums.

On the day after stem infusion of the tracer sucrose, the specific radioactivity of sucrose recovered from the storage compartment of growing axillary branch tissue was significantly greater (nearly twice) than that in the free space (easily rinsed, i.e., extracellular), suggesting that sucrose was preferentially transferred through symplasmic routes. In contrast, the sucrose specific radioactivity in the free space of the mature (ripened) stem tissue was significantly greater (nearly twice) than that in the storage compartment indicating that sucrose was preferentially transferred through apoplasmic routes. Tritium label initially provided in the fructosyl moiety of sucrose molecules was largely (83%) recovered in the fructosyl moiety, confirming that a significant portion of sucrose molecules is not hydrolyzed and resynthesized through the usual pathways during radial transfer of sucrose in the mature stem of sorghum.

During radial transfer of sucrose to the storage cells in intact, mature, ripened sorghum stem, much of the sucrose is transferred intact (without hydrolysis and resynthesis) and primarily through an apoplasmic (i.e., includes an extracellular compartment) pathway. In contrast, much of the sucrose is transferred through a symplasmic pathway in the growing stem tissue. These results indicate that an anatomical blockage of apoplasmic passage of radially transferred sucrose in mature sorghum stem is not present. This contrasts with the apparent cellular pathway of sucrose in mature stem of the closely related species, sugarcane, and suggests flexibility exists in the cellular pathway of radial transfer of sucrose in stems of the grasses in this tribe. We lack information about the cellular and metabolic pathways of sucrose in stems of other grasses with economic importance as lignocellulose- or sugar-feedstock crops.

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GENOTYPE AND ENVIRONMENTAL EFFECTS ON ETHANOL YIELD FROM PEARL MILLET

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In spite of rising feedstock costs and grain-deficit status of the southeast, investors have committed resources to the construction of new ethanol plants in the region. The use of alternative feedstocks will help to alleviate market demand for corn both as a feedgrain and as an ethanol feedstock. As a drought tolerant grain requiring low nitrogen inputs, pearl millet may be a viable supplemental ethanol feedstock for the southeast. Limited information exists concerning genotype and environmental effects on grain yield, starch content, and fermentation efficiency in pearl millet feedstocks.

The objective of this study was to assess experimental pearl millet hybrids for genotype and environment effects on grain yield, starch, fermentation efficiency, and ethanol yield.

Statewide yield trials were established at Moultrie, Tifton, Watkinsville, and Newton GA in 2006 and at Moultrie, Tifton (early and late planted trials), Plains, and Newton GA in 2007. Nine genotypes were evaluated in 2006, and six genotypes in 2007. Fertilizer was applied at 88 kg N/ha. Grain was combine-harvested. Yields were adjusted to 15.5% moisture content. Grain was evaluated for starch content on a dry basis. Fermentation efficiency was determined from the difference between the observed and theoretical ethanol production.

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 the commercial standard Tifgrain 102 in 2006, and 30.3% greater yield in 2007. The hybrid (606 x 2304) had 1.4 % and 2.2% greater starch than Tifgrain 102 in 2006 and 2007, respectively. Across all genotypes, the mean grain yields were highest in Watkinsville in 2006, and in the early Tifton trial in 2007. In both years, grain yields and starch content of the grain were lowest in the trials grown at Newton. Across all entries and locations, the calculated ethanol yield per acre was highly correlated with the grain yield per acre both in 2006 and 2007 ($R^2 > 0.99$).

It will be possible to select hybrids that produce higher levels of ethanol for the developing bioenergy industry in the southeast. Improving grain yield will be the most effective means of improving overall ethanol yield per unit of land.

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TOMATO YIELD RESPONSES TO SOIL-INCORPORATED DRIED DISTILLERS GRAINS

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Dried distiller's grains (DDGs) is a coproduct of dry-grind corn ethanol production, most of which is currently used for animal feed and sold for under \$150/metric ton. Developing higher-value uses for DDGs can increase the profitability of corn-based ethanol. Although DDGs applied directly to a potting medium was phytotoxic to several ornamental plants, preliminary studies with incorporating DDGs into soil and allowing it to decompose for a short period of time (~1 month) indicated that it could promote plant growth of several test species, including tomatoes. In addition, solvent-extractable compounds such as oil, phytosterols, and tocopherols present in DDGs are being evaluated for value-added uses, while the extraction step also eliminates most of the fermentation odor of the DDGs.

The DDGs were obtained from Big River Resources, LLC, Burlington, IA. The DDGs were Soxhlet-extracted with anhydrous ethanol to obtain extracted-DDGs (X-DDGs). The N-P-K values for the DDGs and extracted DDGs were 4.1-0.9-1.4 and 6.0-2.0-1.7, respectively. Cottonseed meal (CSM) was used as an organic control as its N-P-K values were

6-2-1, similar to that of the DDGs. Amendments were added to a silt loam soil at the rate of 1 kg/m into 2 m X 1.5 m blocks and incorporated to a depth of 15 cm on 15 April 2007. The experiment consisted of the three organic amendments and a non-amended control utilizing a Latin Square design with four replicates. Four six-week-old tomato (*Solanum lycopersicum* L. 'Health Kick') plants were planted in each block on 15 May 2007.

Plots treated with all three amendments exhibited no offensive odors at any time during the experiment. Tomato plants treated with both types of DDGs exhibited accelerated growth responses for the initial month compared with either the controls or the cottonseed meal treatments. Initial tomato harvests were on 15 July 2007 and continued until 25 October 2007. The number of fruits per plant averaged 107, 143, 145, and 136 for the controls, CSM, DDGs, and X-DDGs treatments, respectively, with all three treatments being significantly higher than the control. The total yields averaged 8.6, 11.3, 11.6, and 10.6 kg/plant for the controls, CSM, DDGs, and X-DDGs treatments, respectively, with all three treatments being higher than the control, and the DDGs and CSM treatments significantly higher than the X-DDGs. These results indicate that the unextracted DDGs offer excellent potential as an organic fertilizer for tomatoes.

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Medicinals and Nutraceuticals

QUALITY CONTROL AND CHEMICAL COMPOSITION OF ZA'ATAR, A SPICE FROM THE MIDDLE EAST

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Ethnic food and flavoring are becoming increasingly important in the US marketplace. However, the lack of information about the product, uses, chemistry, quality, and origin are factors that limit their commercialization and popularity to the broader base of consumers. The Arabic term za'atar refers to a mixture of various local herbs, such as hyssop, marjoram, oregano, and thyme. In this context, za'atar is a spice mixture that can be composed of dried thyme (*Thymus vulgaris*) or dried Syrian oregano (*Origanum syriacum*), other aromatic plants (*Salvia* spp.) as well as a variety of additional species within this genera plus other flavoring ingredients, such as salt, sesame seeds, and sumac. Thus, za'atar is similar to oregano and refers more to flavoring and seasoning than to a single plant source.

The objectives of this paper were to study the chemistry and the quality of this spice as it is available and being traded in the US marketplace. We hope these results would help in the development of initial standards to provide consumers and end users with more information on this spice.

Four commercial samples (Lebanon, two from Jordan, and Syria) of za'atar were purchased from a Middle Eastern retailer in New Brunswick, NJ and used to conduct all the quality control (color, sieve analysis, moisture, and ash content) and chemical analysis (essential oils and polyphenols).

All the samples of za'atar exhibited low levels of moisture (<10%). The Syrian za'atar had the greatest amount of seeds (58.7%) in the ratio of fine to medium size particles of 26 and 15%, respectively. The za'atar from Lebanon contained the highest amounts of total phenolics (2.5%), and the samples from Jordan (Sample 1) and Syria had lower levels of 0.8 and 0.85%, respectively, and sample 2 from Jordan the lowest level of 0.4%. The sample from Lebanon was also characterized by high contents of essential oils and polyphenols (e.g., rosmarinic acid), whereas the other za'atar samples contained lower levels.

The za'atar samples from Lebanon contained the highest amount of essential oils and total phenols as those components are responsible for the flavor and aroma of the product. The Lebanese Za'atar had three times as much antioxidant activity than the other three samples. These preliminary results showed the variability in za'atar commercial products that could be useful to define initial standards for za'atar.

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A NEW *Flourensia cernua* COMPOUND WITH ANTIFUNGAL ACTIVITY

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Flourensia cernua is an important species because of its secondary metabolites. The chemicals are used for biological and ecological applications with high economical potential. At Antonio Narro University, we have been studying the *F. cernua* plant. The researches developed have focused on the growth, recollection, and extraction of biochemical products with commercial interest of the species in the states of Coahuila, Chihuahua, Durango, Hidalgo, Nuevo León, San Luis Potosí, Sonora, and Zacatecas.

The objectives of this study were to fractionate the ethanol extracts of *F. cernua* to evaluate their effects on *Fusarium oxysporum*, and to characterize the compounds with antifungal activity.

Ethanol extracts were fractionated by column chromatography using silica gel with a gradient system of hexane:ethyl acetate:methanol (10-0:0-10:5-5). The fractions were evaluated over *F. oxysporum* growth on microdilution plates (96 wells) with liquid media RPMI 1640 (BioWhitaker) to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer at concentration fractions of 500, 1000, and 2000 $\mu\text{l l}^{-1}$. The effects of the fractionated extracts on the growth rate of *F. oxysporum* were determined. Measurements were made with a Stat fax 2100 Microplate Reader at 630 to 545 nm absorbance with incubation periods of 0, 24, 48, and 72 h.

The fraction at the higher absorbance decreased in relation to the other fractions eluted with hexane:ethyl acetate (3:7). Antifungal compounds were characterized by FT-IR, Spectra Gas Chromatography with Mass Spectra Detector.

The major constituent of the extracted fraction was a compound with a molecular formula of $\text{C}_{15}\text{H}_{24}\text{O}_2$.

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EDIBLE NUTRACEUTICAL COATING EFFECT ON APPLE FRUITS FOR INCREASING SHELF LIFE

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Edible coatings with biological activity can reduce degradation of some fruits and vegetables, thus increasing their shelf life. The selective permeability of the coatings to gases decreases moisture loss, and therefore, weight losses. Ellagic acid (EA) is a polyphenol antioxidant found in numerous plants that acts as a protection system against diseases, insects, and microbial attack.

The objective of the present work was to evaluate the shelf life of fruits by using edible coatings of candelilla wax mixed with ellagic acid.

“Golden Delicious” apples were used in this research. The treatments consisted of: (1) candelilla with EA coating; (2) candelilla without EA coating; and (3) blank (without coating). The results were analyzed with a completely randomized design. Quality parameters evaluated were: appearance changes, weight loss, color, firmness, solid content, water activity, and pH.

Appearance changes were considerably reduced with the edibles coatings. Weight loss in fruits treated with EA was 56.8% lower than the blank. A similar behavior was present for firmness reduction and total solid content. Color, pH, acidity, and water activity changes were less with the EA-treated apples than the other treatments. The coatings acted as a

barrier, and thus, diminished the natural process of nutrient loss.

Edibles coatings of candelilla with EA retarded significantly the quality decrease of post-harvest fruits.

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BORAGE (*Borago officinalis* L.) RESPONSE TO N, P, K, AND S FERTILIZATION IN SOUTH CENTRAL CHILE

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Borage is an herbaceous new crop grown for its seed oil rich in gamma linolenic acid (GLA). The objective of this study was to determine the response of borage grain yield, oil content, and fatty acid composition to nitrogen, phosphorus, and sulfur fertility treatments.

Three experiments were conducted at Osorno (40°22'45.6'' S, 73°04'13.4''W, and elevation 72 m), Chile, in the 2004 to 2005 and 2005 to 2006 growing seasons. The first experiment was conducted in the 2004 to 2005 growing season. The experimental design was a randomized complete block (RCBD) with a factorial arrangement (4x3x2) with four application rates of N (0, 100, 200, and 300 kg N ha⁻¹), three rates of P (0, 60, and 120 kg P₂O₅ ha⁻¹), and two rates of K (0 and 150 kg K₂O ha⁻¹). The second experiment was conducted in the 2006 to 2007 growing season. Only the effect of N was evaluated because there was no response to P and K on most of the traits evaluated the first season. The experiment was an RCBD with four nitrogen rates (0, 50, 100, and 150 kg N ha⁻¹). The third experiment was conducted in the 2004 to 2005 and 2006 to 2007 growing seasons. The experimental design was an RCBD with two rates 0 and 40 kg S ha⁻¹ and different split-application treatments.

Seed yield was not affected by N, P, K, or their interactions in the first experiment. Both 1000-seed weight and test weight were affected by N fertility levels, and by the interactions N x P and N x K in the first experiment. Seed weight decreased as nitrogen fertility rates were increased. The lowest test weight was for the treatment with 300 kg N ha⁻¹. In the second experiment, nitrogen fertility levels did not have an effect on oil content, oil yield, palmitic acid, stearic acid, oleic acid, and linoleic acid. The only significant response was observed for GLA content. As nitrogen rates increased, the GLA content increased. The effect of sulfur application was significant for seed yield. Seed yields increased 98 kg ha⁻¹ when 40 kg ha⁻¹ of sulfur was applied. There were no yield differences for the split-applications of sulfur treatments.

Results indicate that borage respond to sulfur applications more than nitrogen. Additional research is needed to determine the interactions between N and S applications.

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SEED PROTEIN AND OIL CONTENTS, FATTY ACID COMPOSITION, AND GROWING CYCLE LENGTH OF A SINGLE GENOTYPE OF CHIA (*Salvia hispanica* L.) AS AFFECTED BY ENVIRONMENTAL FACTORS

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Cardiovascular heart disease (CHD), a chronic disease, remains the leading cause of both death and disability in the Western industrialized world, and also is growing rapidly in the unindustrialized countries. Many studies have demonstrated that increased intake of lipids, in particular saturated (SAT), trans-fatty acids, and polyunsaturated ω -6 fatty acids, are closely related with the incidence of CHD. In addition, medical and epidemiological studies have shown that consuming lipids rich in ω -3 fatty acids can reduce the risk of acquiring CHD.

Chia (*Salvia hispanica* L.), along with corn, beans, and amaranth, was a main component in the diet of many pre-Columbian civilizations in America, including the Mayan and Aztec populations. Chia contains the richest botanical oil source of α -linolenic acid known.

The objectives of the present study were to determine the effects of location on the growing cycle length, seed protein content, lipid content, and fatty acid profiles for a single chia genotype. Seeds tested included the chia genotype Tzotzol from eight sites in five different ecosystems of three countries: Semi-Arid Chaco (T₅) of Argentina, Sub-Humid Chaco (T₄) of Bolivia; Coastal Desert (T₃), Tropical Rain Forest (T₂), and Inter-Andean Dry Valley (T₁) of Ecuador.

The growing cycle length of a chia crop in each ecosystem was 150, 130, 120, 105, and 100 days in T₅, T₁, T₄, T₃, and T₂, respectively. The regression coefficient (R^2) showed a positive relation of growing cycle length with ecosystem elevation ($R^2=0.99$, $P<0.0001$). Seeds from plants grown in T₄ and T₃ contained significantly ($P<0.05$) more protein than seeds from the other three ecosystems. No significant ($P<0.05$) differences in protein content were found between T₃ and T₄, and between T₁, T₂, and T₅. In contrast and generally, the seed oil content tended to increase as altitudes increased. Seeds from T₁ and T₅, with 33.5 and 32.2%, respectively, were the highest oil producers. The land elevation was negatively related to protein content ($R^2=0.94$, $P<0.001$), and positively to oil content ($R^2=0.76$, $P<0.001$). Significant ($P<0.05$) differences in palmitic, stearic, oleic, linoleic, and α -linolenic fatty acids between oils from seeds grown in different ecosystems were detected. Polyunsaturated ω -3 α -linolenic fatty acid content was significantly ($P<0.05$) higher in seeds produced in T₁ than in those produced in T₃, T₄, and T₅, but not in those produced in T₂. The α -linolenic fatty acid percentage was negatively related with oleic fatty acid percentage ($R^2=0.73$, $P<0.001$), and with linoleic percentage ($R^2=0.82$, $P<0.001$).

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EFFECT OF PLANT EXTRACTS AGAINST *Fusarium oxysporum* IN TOMATO PLANTS

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Two concentrations of one cropping species (quinua) and four semi-desert species (yuca, lechuguilla, gobernadora, and hojasén) were evaluated on *Fusarium oxysporum* in tomato.

The objectives were: (a) to evaluate the extracts effect *in vitro* of the mycelial growth of *Fusarium oxysporum* (*Fol*) in potato dextrose agar (PDA) infusion on Petri dishes, (b) to determine *Fol* incidence in tomato seedling growth, and (c) to determine *Fol* incidence and severity in tomato yield.

The concentrations of extracts were: 2,000 and 4,000 $\mu\text{l l}^{-1}$ for *Yucca carnerosana* (yuca) and *Agave lechuguilla* (lechuguilla); 1,000 and 2,000 $\mu\text{l l}^{-1}$ for *Larrea tridentata* (governadora) and *Chenopodium quinoa* (quinua); and 500 and 1,000 $\mu\text{l l}^{-1}$ for *Flourensia cernua* (hojasén). A complete random design with four replications was used.

The results were: (a) *L. tridentata* and *F. cernua* extracts inhibited *in vitro* *Fol* mycelial growth, and (b) the extracts did not show *Fol* incidence in the seedling; however, the quinoa extract (2,000 $\mu\text{l l}^{-1}$) had 50% of incidence at 45 days after sowing (DAS), and presented similar incidence as that of the inoculated control. For the growth variables, the *Y. carnerosana* extract (2,000 $\mu\text{l l}^{-1}$) promoted the tallest stem height, *L. tridentata* (1,000 $\mu\text{l l}^{-1}$) the most efficient ($P \leq 0.05$) increase bark thickness and stem central diameter. At 2,000 $\mu\text{l l}^{-1}$, it increased the stem diameter (20.9%) ($P \leq 0.05$) at 45 DAS. The stem central diameter was increased by *A. lechuguilla* at 4,000 $\mu\text{l l}^{-1}$ and also the *L. tridentata* at 2,000 $\mu\text{l l}^{-1}$.

l^{-1} , and *Ch. quinoa* at $1,000 \mu l l^{-1}$ ($P \leq 0.05$). (c) The *F. cernua* extract ($1,000 \mu l l^{-1}$) did not have *Fol* incidence. The *Y. carnerosana* extract ($2,000 \mu l l^{-1}$) had only 25 %. The *Y. carnerosana* ($2,000 \mu l l^{-1}$) and *F. cernua* at $500 \mu l l^{-1}$ extracts increased tomato yield 69.8 and 35.5%, respectively ($P \leq 0.01$), and promoted early harvest.

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***Aloe vera* LEAF GROWTH IN TWO LOCALITIES UNDER SOIL WATER DEFICIT IN NORTHERN MEXICO**

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The objective of this work was to determine the best climatic conditions and soil water deficit in order to establish the *Aloe vera* crop in Northern Mexico.

The experiments were performed in Buenavista, Coahuila (L1) with semiarid mild climate, and in Marín, Nuevo León (L2) with semiarid warm climate. Two soil water deficits were applied to the experiments in both localities: a minimum deficit (T1), irrigation was applied two times per week, and a maximum deficit irrigation was applied when the humidity reached between 8.4 and 1.3%, weight basis (Kg/Kg). At the end of the experiments, three drought cycles were evaluated. Several variables were measured during the period of the experiments.

The ANOVA showed significant differences between localities, soil water deficit, and for the interaction locality x deficit for the variables: weight increase (g) during the three drought cycles; daily growth ($g \text{ plant}^{-1} \text{ day}^{-1}$); harvested leaves; and total yield ($g \text{ plant}^{-1} 225 \text{ days}^{-1}$).

The climatic conditions at Marín, Nuevo León were better than in Buenavista, Coahuila, for the *Aloe vera* development. This could be attributable to the warm climate and higher humidity in the Marín environment, and for this reason the plants had lower transpiration than the Buenavista location.

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Fiber and Cellulosics

UTILIZATION AND VALUE OF SWITCHGRASS IN A DUAL PURPOSE STOCKER CATTLE AND BIOENERGY SYSTEM

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Switchgrass (*Panicum virgatum* L.) may transform agriculture as a dedicated energy crop grown, harvested, and converted to biofuels at industrial refining facilities. In the near and intermediate terms, information is needed on whether switchgrass can be integrated into, rather than require displacement of, present agricultural production situations. Stocker cattle production, which involves the placement of young, lightweight cattle on high quality pastures before finishing on high grain diets, is a viable economic enterprise where switchgrass may have value because of its early spring availability and wide adaptability.

Our objectives were to determine: (1) stocker cattle gain, number of grazing days supported, herbage mass, and herbage nutritional value following early-season grazing of switchgrass; (2) end-of-year biomass yields after grazing

cessation; and (3) the economics of retained stocker cattle ownership on switchgrass.

A 9.72 ha field of 'Alamo' switchgrass was established successfully in May 2007 at the Noble Foundation Red River Farm near Burneyville, OK. Twelve, 0.81-ha paddocks were created within this tract to evaluate effects of early-season stocking density (0, 2, 4, and 6 steers per paddock, grazing from mid April to mid June). Commercial stocker steers were purchased in October, preconditioned, and placed on winter rye pasture to uniformly prepare them to graze switchgrass after winter pasture termination. Animals weighing approximately 306 kg were removed from the winter pasture, shrunk overnight without feed or water, weighed individually, implanted with a growth implant, and randomly assigned to the switchgrass treatments. Herbage mass and nutrient composition were monitored at biweekly intervals during the grazing period and once at the end of the growing season.

Stocking density affected herbage mass and the number of grazing days supported. At the start of grazing, herbage mass and nutrient composition were similar among treatments, ranging from 1500 to 2000 kg DM ha⁻¹, 14% crude protein, and 62% total digestible nutrients. Thereafter, herbage mass increased rapidly in the zero- and two-steer per paddock treatments, with growth rates averaging 113 and 45 kg ha⁻¹ d⁻¹ between 17 April and 6 June for these treatments, respectively. Herbage mass in the four- and six-steer per paddock treatments remained fairly steady from 17 April to 6 June, ranging from 1500 to 2500 kg ha⁻¹. By 6 June, herbage mass averaged 7200 kg ha⁻¹ in the zero-steer, 4200 kg ha⁻¹ in the two-steer, 2200 kg ha⁻¹ in the four-steer, and 2500 kg ha⁻¹ in the six-steer per paddock treatments. The switchgrass paddocks supported 20 days of grazing in the six-steer, 30 days of grazing in the four-steer, and > 60 days of grazing in the two-steer per paddock treatments.

Information on animal daily gain, end-of-season biomass yields, and production economics will be reported as these data become available and should improve understanding of the value of switchgrass as a dual-purpose forage and bioenergy crop and possibly identify an economically viable use of switchgrass today and vehicle for jump-starting the cellulosic industry tomorrow.

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SEED SCIENCE AND TECHNOLOGY OF SWITCHGRASS AS A BIOFUEL CROP

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Many seed crops have been identified as suitable for biofuel production, and thus require high quality seeds that will produce optimum plant stands with the potential for maximum biomass. Seed dormancy, suboptimal soil temperatures, and pathogens often lower seed quality and can negatively impact stand establishment. Seed testing methods provide an overall assessment of the quality of a seed lot; however, variants in the germination test method can have a major influence over final germination rates and percentages. The germination results are greatly influenced by temperature conditions and mold growth can also confound germination test results.

The objective of this preliminary work was to study the seed biology and to apply seed technology to enhance the performance and achieve expected germination of switchgrass (*Panicum virgatum*) seed sources. The initial task was to investigate discrepancies between the labeled germination percentages and the germination results at specific temperature regimes to improve prediction of field emergence and better quantify temperature dependence. Seed coating methods were sought to apply more uniform and adherent seed treatments.

Seeds of the varieties 'Cave-in-Rock' and 'Shawnee' were provided by Ernst Seed Co. (Meadville, PA). Three seed lots were assessed for the effects of 14 day, 5° C stratification, followed by a diurnally alternating 15 to 30° C regime on final germination percentages. A high quality lot was evaluated over a wide range of constant temperatures to investigate the effect on germination. A seed coating method was developed to apply fungicide seed treatments that would decrease mold contamination during germination testing.

Germination at a constant temperature revealed seed lots with high, medium and low dormancy levels. Dormancy was broken with 14-day stratification at 5° C. The optimum constant temperature was 35° C. There was a decline in both the rate of germination and final germination as temperature decreased or increased. Zero germination was recorded after 30 days at 10° C and 45° C, indicating that the maximum temperature is approximately 40° C. A seed coating method was developed including brushing seeds to remove glumes, and then applying a binder and filler to obtain uniform coverage. Seed treatments Captan and Thiram reduced mold growth.

Knowledge of the seed biology of species used for biofuel production is needed to understand factors or conditions limiting germination. Specific technology methods can then be adapted or developed to optimize seed quality and stand establishment.

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PARTITIONING OF CARBON INTO CELL WALL STRUCTURAL CARBOHYDRATES IN SWITCHGRASS – A ¹³C LABELING STUDY

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Switchgrass (*Panicum virgatum* L.) has drawn considerable research efforts in renewable energy production due to its ability to produce significant amounts of biomass when grown on marginal land. Studies, where *in vitro* dry matter digestibility (IVDMD) had been used as a selection criterion to improve its forage quality, have also shown that amongst switchgrass populations differing in IVDMD there were only modest variations in cell wall hemicellulose and cellulose concentration, but a significant change in lignin concentrations.

The purpose of this study is two-fold: (1) to follow carbon partitioning into the cell wall structural carbohydrates and (2) to determine the relative activity of cinnamyl alcohol dehydrogenase, a key enzyme in the lignin biosynthetic pathway in switchgrass plants.

Tissues from both field grown and greenhouse grown plants (Kanlow, a lowland variety and Summer, an upland variety) were used for this study. For isotope abundance studies, five-week-old greenhouse grown Kanlow plants were enclosed in a leak-proof Plexiglas chamber and labeled with 99% ¹³CO₂. Both stover and leaf tissue samples were harvested weekly from both control and labeled plants. A portion of these tissues were frozen immediately in liquid nitrogen and stored at -20° C for enzyme assays. The rest of the tissue samples were oven-dried at 60° C for 72 h for carbon isotope ratio (CIR) mass spectrometry. The ¹³C enrichment (atom % excess) in the sample was calculated as the difference in ¹³C atom% between the sample and its corresponding control tissue. The amount of labeled carbon (A) in the sample was calculated by: ¹³C atom % in the sample - ¹³C atom % in the control * amount of C in the sample. The % total C in tissue samples were determined with a Costech elemental analyzer. Fractionation of cell wall structural carbohydrates into hemicellulose, cellulose, and lignin/ash fractions was performed by the sequential detergent fiber analysis procedures. Cinnamyl Alcohol Dehydrogenase (CAD) activity was assayed spectrophotometrically at 340 nm.

Stover tissues harvested 52 and 81 days after planting (DAP) from plants labeled with ¹³CO₂, had an increase in ¹³C in the lignin/ash fraction per mg lignin than either the hemicellulose or cellulose fractions suggesting an active deposition of lignin in the stover tissue cell wall as plants mature. A gradual increase in the relative activity of CAD was observed in the stover tissue extracts, whereas a gradual decrease in the leaf extracts of switchgrass plants over a 60 day growth period support the results from ¹³C enrichment studies where the stover tissue lignin had an increased ¹³C atom % enrichment than either cellulose or hemicellulose fractions.

Increased CAD activity and increased ¹³C amount mg CWP⁻¹ in lignin fractions of the Kanlow stover tissue suggest an increased carbon partitioning into the lignin fraction. By regulating lignin biosynthesis through conventional or transgenic means, we could increase cellulose content, and thereby, enhance the feedstock of switchgrass biomass.

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EVALUATION OF ENERGYCANE FOR BIOMASS PRODUCTION IN STARKVILLE, MISSISSIPPI

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The current energy crisis has given rise to the development of alternative energy programs that includes the production of ethanol. Energy cane (*Saccharum* spp.) is a domestic feedstock that can be used to reduce the need for foreign oil, and also provide America's farmers with more opportunities to decrease the nation's dependence on fossil fuels. This hybrid formed from sugarcane (*Saccharum* spp.) is well adapted for tropical and subtropical regions due to its efficient conversion of solar energy to biomass, its tolerance to above and below ground plant pests, and its high biomass yields with relatively low soil fertility and moisture requirements. Current cultivars of sugarcane can not be grown in the temperate regions of the U.S. because of winterkill.

Nine cultivars of energy cane were planted at Starkville, MS (33.45° N, 88.82° W, 102 m elev.) in 2006 to determine biomass yields, average height, overwintering success, and sugar content (%Brix). Trials were planted on 26 September 2006 by burying cut stalks (USDA-ARS-SRRC in Houma, LA) 10 to 20 cm deep. The trial consisted of five randomized blocks with nine rows in a block. Overwintering ratings taken in March 2007 and 2008 indicated five of the nine cultivars might be suitable for "northern" growth. Height and %Brix were measured three times during the growing season, and biomass yields were taken at the end of the 2007 growing season.

The tallest average height was attained by US 72-114 cultivar and the highest %Brix the US 02-144 (10.88%). The total yield was achieved by harvesting with a custom Carter flail harvester with the cultivar HO 00-961 having the largest yield at 7544 kg/ha. Over the 2007/2008 winter period, cultivar US 02-144 had the greatest regrowth following post-harvest. The average height, %Brix, and biomass yield measurements for the 2008 growing season have not been completed.

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EVALUATION OF TWO AGRICULTURAL RESIDUES AS LIGNO-CELLULOSIC FILLER IN POLYMER COMPOSITES

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Agricultural residues refer to the waste stream coming from agricultural production and processing operations. These materials are often rich in ligno-cellulosic fibers, but offer no significant value at present. The processing plants usually pay for disposal of these waste streams. However, these waste materials are rich in ligno-cellulosic fibers, and hence may offer value as fillers in ligno-cellulosic polymer composites (LCPC). Wood plastic composites (WPC) are the common type of LCPC, where wood is used as the fiber filler.

The objective of this study was to evaluate the potential of two agricultural residues, cotton gin and guayule wastes as fiber fillers in LCPC.

An experiment was conducted by manufacturing LCPC with five fiber fillers, including cotton burr or carpels (CB), cotton burr mixed with 2% of second cut linters (BL), cotton burr mixed with 30% of guayule whole plant (GW), cotton burr mixed with 30% of guayule bagasse (GB), and oak as control (CL). All materials were ground to a size of 20 to 40 mesh. The formulation of the LCPC was 50% ligno-cellulosic fiber filler, 40% HDPE, 6% lubricant, and 4% mineral filler. The treatments were replicated five times. The oven-dried fiber filler and other ingredients were weighed, hand mixed, and extruded with a twin-screw extruder through a 2.54 x 0.635 cm (1 x ¼ in) die. The extruded samples were water cooled, conditioned for approximately three months, and then tested for physical and mechanical properties.

The LCPC made from cotton and guayule-based fibers showed lower specific gravity and higher water absorption than wood. Strength properties such as MOE, MOR and hardness were lower and nail withdrawal strength was higher for LCPC made from the agricultural residues than wood. They also exhibited much lower coefficient of linear thermal

expansion than the wood-based samples. All the four mixes made from agricultural residues showed good potential as an alternative filler for natural fiber composites. When the four residue-based treatments were compared with each other, the GW had superior physical properties comparable to wood, whereas the GB had better strength properties.

Both cotton burr and guayule fibers have good potential as fiber fillers in LCPC.

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Oilseeds

JATROPHA AND CHINESE TALLOW: POTENTIAL BIOFUELS FOR TEXAS

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Biofuels are primarily produced at this time from corn and soybean, which are staples of food and feed industries, causing some concern about scarcity and pricing of these commodities. *Jatropha* (*Jatropha curcas*) and Chinese tallow (*Sapium sebiferum*) offer an alternative to enhance oil production on lands that may not be suitable for the production of field crops or high yield forages. Chinese tallow grows abundantly in the wild in coastal areas from Southeast Texas to North Carolina and can withstand freezing temperatures that occur in some parts of these areas. *Jatropha* is native to Central America, but could logically be grown in tropical and subtropical regions of Texas and the U.S. where freezing temperatures are unlikely to occur.

The objective of these studies was to establish field experimental sites for both of these crops to elucidate their suitability for seed oil production. Parameters that are being examined include: plant spacing, canopy modification, fertility response, weed management, harvest techniques, plant selection (breeding), and supplemental irrigation.

Research was initiated in Hidalgo County (*Jatropha*) in 2008, where controlled experiments were possible. Transplants of greenhouse-grown *jatropha* were successfully established in field plantings. Studies evaluating plant spacing and herbicide tolerance have been conducted to date and studies on fertility will proceed during 2008. Chinese tallow seedlings are currently growing in a greenhouse and are expected to be established in the field during the summer of 2008. Experiments similar to *jatropha* are planned for 2008.

Jatropha shows remarkable characteristics regarding ease of establishment from greenhouse transplants to the field, with essentially 100% success in our studies. Crop height and canopy development have been measured in the plant spacing study. *Jatropha* tolerance to 11 herbicides has been evaluated.

The suitability of *jatropha* and Chinese tallow for large-scale production will continue to be evaluated at these sites and possibly others. Both species show enormous potential for seed oil production and some related benefits for soil fertility enhancement. More importantly, these crops can likely be grown in soils not conducive to field crop oilseed production.

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PENNYCRESS (*Thlaspi arvense*) AS A BIODIESEL IN A ONE YEAR-TWO CROP ROTATION WITH SOYBEAN

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Increased demand for energy has generated renewed interest in the development of oilseed crops. The short term answer to biodiesel has always been soybean. Unfortunately, soybean oil has several shortcomings for supplying the U.S. market. First and foremost is the fact that if all current soybean oil was converted to biodiesel, it could supply only 12% of the U.S. diesel demand. 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 foods vs. fuel must be considered. New crops that can be grown in rotation with traditional crops, off-season production, and utilization of land not currently under cultivation will also play a role in meeting these industrial needs. Pennycress (*Thlaspi arvense*) has potential as a winter annual in rotation with soybean production throughout the Midwest.

The objective of this study was to evaluate the physical properties of pennycress oil and its methyl esters for suitability as a biodiesel.

Pennycress seeds were obtained from combine harvesting of wild stands using conventional combines. The seeds were cleaned by screening, aspiration and gravity table fractionation. Oil was recovered from whole seed by passing through a screw press and filtration. The oil was converted to methyl esters using a sodium methoxide catalyst in methanol. Pour point, cloud point, viscosity, flash point acid value, copper corrosion, and oxidative stability were determined on both the oil and the methyl esters using the appropriate ASTM method.

The seed was found to contain 36% oil with the major fatty acid as erucic at 38.1%, iodine value of 115. Viscosity index (VI) of the methyl esters was 277 with a 40° C viscosity of 5.0 cSt and pour point and cloud points of -15 and -10° C, respectively. The starting oil had a VI of 222 with a 40° C viscosity of 39.1 cSt and pour point and cloud points of -18 and -10° C, respectively. As expected, the flash point of the methyl esters 136° C was considerably less than the starting oil 234° C. OSI of the oil at 100° C was 39 h and 54.0 h for methyl esters.

The early harvest date of pennycress compared to other winter annual oilseed crops will make it suitable for a two crop rotation with soybeans in most of the Midwestern U.S. In addition, the physical properties of the methyl esters indicate that continued development of the oil as a biodiesel is warranted.

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IDENTIFICATION OF HIGH OIL PEANUT ACCESSIONS FOR A PEANUT BIODIESEL BREEDING PROGRAM

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Recent high energy prices have made fuel production from agricultural products more competitive. Conversion of corn and sorghum seeds into ethanol for gasoline is proceeding on a large scale. Significant price increases in diesel fuel have made a search for alternate diesel fuel sources more important. Also, addition of biodiesel to diesel oil can provide the lubricity lacking as sulfur content is reduced in diesel fuel. Peanut has potential as a source of biofuel. The oil content of the peanut crop is two to three times that of other oilseeds, such as soybean or canola. However, increased oil content in peanut is needed to make peanut competitive as a source of biodiesel. Current cultivars contain approximately 48% oil. There are reports of cultivated accessions containing up to 55% oil and wild species containing up to 65% oil.

Our objective was to screen sources of germplasm to identify peanut accessions with high oil content, which can be used in a breeding program to develop high-oil cultivars.

Several collections of peanut accessions were screened for oil content. A collection of 180 peanut accessions (collectively known as Tarapota lines) collected from Peru and Bolivia were grown in the summer of 2006 and screened for oil content. Additional lines screened included advanced runner breeding lines developed for release as an early-maturing cultivar, several peanut wild species, and additional materials. Oil content in intact seeds was measured

using a Bruker NMR spectrophotometer.

Among the Tarapota materials, we identified four accessions with from 50% to 52% oil in the seed. Two advanced early-maturing breeding lines under consideration for release for the edible market contained from 50% to 53% oil. One Spanish line containing 55% oil and a wild species with up to 59% oil were identified.

Identification of high-oil accessions, and the existence of transgressive segregation in breeding materials suggest that it will be possible to raise the oil content of peanut.

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RENEWABLE FUEL PRODUCTION FROM CASTOR IN THE NORTHEAST

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Castor (*Ricinus communis* L.) is a semitropical perennial plant that has not been explored as a renewable fuel source in the Northeast, but has tremendous potential in this region. Castor is an ideal candidate for fuel production with 150 gallons of oil/acre, which is greater than potential competitors, soybean or canola. Castor is not used for food and can be grown productively on marginal lands of which extensive amounts exist in the region. The extremely high oil content and high yield potential of castor will generate a positive energy balance producing 4 to 8 calories in liquid fuel per calorie invested in production and processing. Castor oil directly offset imported petroleum. Indications are that it can be used “as produced” for #2 heating oil – a vital commodity for winter in the region. It can also meet the “natural” criteria of many cosmetics by replacing petroleum based oils. Castor oil feedstocks in rural communities could stimulate the development of on-farm extraction operations in recently defunct smaller dairy barns many of which have milk tanks for storage. The farmers are experienced at producing, storing, and selling a liquid commodity into local markets. This latter interest has already started with the inclusion of NorthWinds Biodiesel (www.northrenew.com) as a cooperator on this research project.

The primary goal of this rapidly expanding effort is to develop sustainable practices for optimal yields of castor production in the Northeast. Evaluation and selection of germplasm is needed to grow this warm-season crop in the Northeast. Varieties will be identified with good seed quality as well as agronomic traits. Poor germination and stand establishment are major problems associated with producing castors in the Northeast. Moreover, low soil temperatures typically encountered in the spring can exasperate seedling emergence. Research is needed to understand castor seed biology and develop technologies to enhance stand establishment. Sustainable agronomic practices, such as nitrogen rates, are needed to grow castors with high yield potential and maximum oil production.

A very successful initial foray in to Castor production began with plantings of Hale and Brigham in Kinderhook, NY, just south of the capital, Albany. Both hand planting and machine (John Deere Maximerge) were used. Nitrogen trials were established at two planting dates and four nitrogen rates. Hand harvests were made after a killing frost on November 1. Seed yield for the early planting date ranged from 904 to 3175 kg/ha with nitrogen rates from 84 to 252 kg/ha. The later planting date yield ranged from 830 to 2390 kg/ha with nitrogen rates from 26 to 194 kg/ha. The variety Hale yielded considerably higher (1750 vs. 1198 kg/ha) for the earlier planting date than for the later. Brigham yield the same 1267 vs. 1258 kg/ha) regardless of planting date. As Best Management Practices for the Northeast are developed, yields are expected to increase.

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DEGRADATION OF RICIN IN CASTOR SEED MEAL BY TEMPERATURE AND CHEMICAL TREATMENT

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Oil from castor (*Ricinus communis*) has the potential to become an important commodity in the U.S. Unfortunately the seed contain a potent cytotoxin known as ricin, which is cited as the single biggest impediment to the acceptance of large scale castor production in the U.S. Specific heat or chemical treatments may have the potential to enhance the safety of the oil extracted seed meal.

Heat and/or chemical treatments were performed to determine their effects on the denaturation of the ricin.

Whole seed, milled, un-extracted seed, heat extracted, and cold-pressed extracted seed were subjected to such treatments. Boiling and autoclaving showed varying degrees of effectiveness depending upon the sample type. Ricin within the cold-pressed extracted meal was rendered unresponsive to antibody probing after 10 min of boiling or autoclaving. Treatment of cold-pressed, extracted meal with 8 M urea and 6 M guanidine-HCl for 60 min produced no observable reduction in the response of the ricin to the antibody. However, hot-pressing of castor seed produced meal that exhibited no reactivity with the antibody indicating that the ricin has been denatured during the oil extraction. Boiling of the cold-pressed extracted meal in the presence of calcium hydroxide (pH 12.5) produced no detected difference from boiling alone.

Removing the toxic component of the castor seed meal may make this byproduct a new commodity enhancing the overall value of the crop.

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HYDROXYL FATTY ACID PRODUCTION IN OILSEEDS

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The conventional source of hydroxyl fatty acid is from castor oil which contains 90% ricinoleate (C18:1OH). Ricinoleate and its derivatives are used as raw material for numerous industrial products, such as lubricants, plasticizers and bio-diesel. However, the production of castor oil is hampered by the presence of the toxin ricin and hyper-allergenic 2S albumins in its seed. We are developing a safe source of castor oil by two approaches: blocking gene expression of the ricin and 2S albumins in castor seed and engineering *Lesquerella fendleri* to produce ricinoleate. *L. fendleri* is a new oilseed crop whose seed contains lesquerolic acid (C20:1OH) derived from a 2 carbon elongation of ricinoleate. By suppressing the elongation step in *L. fendleri* through genetic engineering, it is possible to generate a *L. fendleri* crop producing ricinoleate.

We conducted a series of seed development studies in castor and *L. fendleri* that included seed morphogenesis, oil and storage protein accumulation, and lipid gene expression. In castor, the entire course of seed development can be divided into eight stages and each stage can be distinguished by seed coat color and volume of cellular endosperm. Synthesis of ricin, 2S albumin and ricinoleate/oil occurred during cellular endosperm development. Concomitantly, we observed increased transcript levels of 12 lipid genes involved in synthesis of ricinoleate/oil, but with various temporal patterns and different maximal induction ranging from 4- to 43,000-fold. These results indicate that gene transcription exerts a primary control in ricinoleate/oil biosyntheses, and there are different transcriptional regulatory mechanisms involved.

The seed development in *L. fendleri* remains largely unknown. We have investigated morphological features and measured physiological changes during seed development in *L. fendleri*. For the period of about 49 days from fertilization to desiccation, the seed development can be divided into seven continuous stages. With each stage, we characterized fresh and dry weight of the whole seed, oil and total protein content, and profiles of the total proteins. The transcript levels of key genes involved in lesquerolic acid synthesis were also quantified. Overall, our series of seed studies are all in parallel with the same time-course, which provides integrative information for understanding their relationships during the seed development in *L. fendleri*.

In order to genetically modify *L. fendleri*, we tested the transformation efficiency of leaf, root and stem tissues using

Agrobacterium-mediated transformation technology. We found that root tissue has the highest efficiency in adventitious shoot formation, whereas the stem has the least. Leaf, because of its abundance, was used to demonstrate stabled transformation. In general, we believe these transformation technology can be used not only to create a ricinoleate-producing crop, but also to improve *L. fendelri* as a superior crop with high yield and disease-resistance.

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FEASIBILITY OF CUPHEA AS A NEW OIL SEED CROP, TO CLIMATE AND SOIL ENVIRONMENTS

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Cuphea, a new oilseed crop rich in medium-chain fatty acids (C8:0 to C14:0) may serve as a renewable, biodegradable source of oil for lubricants, motor oil, and aircraft fuel. Impacts of climate and soil environment on cuphea growth and development are not well understood.

The objective of this study was to evaluate influences of climate and soil characteristics on growth, yield, and seed oil of several cuphea genotypes differing in their fatty acid profiles and agronomic characteristics. Six genotypes [PSR23 (*Cuphea viscosissima* x *C. lanceolata*), HC-10, blizzard, *Cuphea wrightii*, *C. lutea*, and VS-6 (*C. lanceolata*)] were grown at experiment sites in ND, MN, IA, and IL. Air temperature, precipitation, and soil water content were monitored at each site and soil samples (0-15 and 15-60 cm) were taken before planting and after harvest for chemical and physical analysis. Seed oil and fatty acid profiles were analyzed.

Growing season air temperatures and precipitation increased from north to south (ND < MN < IA < IL). PSR23, HC10, and blizzard appeared to be more regionally adapted than VS-6, *wrightii*, and *lutea*. Regardless of genotype, high temperatures affected seed yield. Seed yields were as much as 3 to 4 times greater in MN and ND than at the IA and IL sites. Oil content for PSR23 was 5 to 6% greater in MN and ND than IA and IL. Although oil content was greater at the northerly sites, the content of key fatty acids such as C10, C12, and C8 for various genotypes increased with more southerly latitude.

These results will aid in developing specific regional cuphea genotype management based on site-specific climate changes and temporal and spatial soil environment as well as identifying key plant characteristics for future improvement.

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SEED YIELD COMPONENTS, OIL CONTENT, AND FATTY ACID COMPOSITION OF TWO POPULATIONS OF MORINGA (*Moringa oleifera* Lam.) GROWN IN THE ARID CHACO OF ARGENTINA: DIFFERENCES BETWEEN AND WITHIN POPULATIONS

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Moringa oleifera Lam. (*M. pterygosperma* Gaertn.) [Moringaceae] is a fast-growing, small tree native to the sub-Himalayan tracts of Northern India. The plant has been spread worldwide in tropical and subtropical countries. It was grown in India since ancient times for a number of uses including human food, animal feed, and medicinal. Moringa seed contains 35 to 45% oil that is considered a great natural cosmetic emollient based on its tactile properties, almost total natural absence of color and odor, and fatty acid composition that is more than 73% oleic. The low content of polyunsaturated fatty acids (less than 1%) gives the oil remarkable oxidative stability. Oxidative stability of moringa oil is higher than that of other oils rich in oleic acid such as high-oleic sunflower, meadowfoam, macadamia, hybrid safflower, safflower, almond, and apricot oils.

The objective of this study was to examine the influence of the Arid Chaco of the Central Valley of Catamarca on the seed yield, oil content, and fatty acid composition of moringa.

The experimental trials were conducted in a semi-commercial moringa planting in the subtropical northwestern region of Argentina. The planting was located at 28° 28' S and 65° 46' W at an elevation of 525 m above sea level. This is in the Arid Chaco ecosystem, belonging to the province of Catamarca. Two genotypes designated as PKM-1 and Africa were tested. The completely randomized experimental design with trees as replications was used.

Seed and Oil Yield Components: The results between populations showed that PKM-1 had significantly ($P < 0.05$) higher seed yield and weight (200 seeds) than the African genotype, contributing to oil/tree and oil/ha. The yields of oil/tree and oil/ha were 127 g and 72 g, respectively, for PKM-1, and 212 kg, and 121 kg for the African genotypes. The genotype effect was significantly ($P < 0.05$) different for these two parameters. All other yield data between these two genotypes were not significantly ($P < 0.05$) different.

Fatty Acid Composition: For the results within a population, there was no significant ($P < 0.05$) differences between fatty acid composition when oils of PKM-1 genotype trees were compared regardless of the field or year of seed production.

Fatty Acid Composition: The results between populations showed that both genotypes essentially produced oil with practically identical fatty acid composition. The monounsaturated ω -9 oleic (18:1) fatty acid was the main one for both genotypes. Only the polyunsaturated ω -6 linoleic (18:2) fatty acid of the African genotype trees had a small, but significantly ($P < 0.05$) higher content than PKM-1. All other fatty acid percentages were not significantly ($P < 0.05$) different between oils of the two genotypes.

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SHOOT BRANCHING RELATED TO TEMPERATURE IN THE GROWTH AND DEVELOPMENT OF LESQUERELLA

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Seed yields of *Lesquerella fendleri* plants are most productive when there are a maximum number of branches. This corresponds to increased area for flowering and fruit set. Plants are indeterminate for flowering that occurs along an inflorescence. Once a flower is insect pollinated, a silique (seed pod) forms containing up to 30 seeds. We previously observed that inflorescences branched more with the fall planting the spring planting. This research studied the effects of four temperature regimes on the growth and development of lesquerella.

The night/day temperatures of four growth chambers were set at: (1) 4/13° C; (2) 13/18° C; (3) 21/29° C; and (4) 29/38° C. The entire study was repeated twice. The numbers of branches of 20 individual plants for all four treatments in both studies were counted twice each week. Destructive harvests also took place using between six and nine plants, three times in the first study and four in the second. Counts for the first study lasted until 84 days after planting (DAP) and 105 DAP for the second. Secondary treatments included moving plants from the coldest to the warmer treatments after 44 and 46 DAP to determine whether a vernalization period was required.

In both studies, the two middle temperature treatments, 2 and 3, had significantly more branches and biomass than the coolest and warmest treatments 1 and 4. Biomass in both studies was almost four times greater in the two middle treatments than the coolest and warmest treatments. There were up to seven times more branches in the middle two treatments than in the coolest and warmest treatments. In both studies, the number of siliques in the two middle treatments was up to 44 times greater than the warmest and coolest treatments 1 and 4. Treatment 2 (13/18° C) had a

significantly higher total reproductive parts (buds + flowers + siliques) than treatment 3 even though treatment 3 had more siliques (although not significantly more). There were also more branches containing buds, flowers, or siliques in treatment 2 than the warmer treatment 3.

This study demonstrates that the optimum temperature for branching and seed set is in the range of 13 and 18° C. Thus, the study helps to target lesquerella growing regions for maximum seed production and aids in determining heat units and degree day thresholds for the crop.

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DGAT ACTIVITY IN MICROSOMES FROM SEEDS OF THREE EUPHORBS

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Non-food oilseed crops such as castorbean (*Ricinus communis* L.) or tung (*Aleurites fordii* Hemsl.), and *Jatropha curcas* L. belongs to the family Euphorbiaceae that is being studied for biodiesel production in subtropical areas across the developing countries. Unusual fatty acids: ricinoleate (12-hydroxyoleic acid) in *R. communis* or vernoleate (12, 13-epoxyoleic acid) in *Euphorbia lagascae* L. are desired for industrial uses. Transgenics could be developed for the production of oils with functionalized fatty acids or else to get rid of toxic compounds prior to cultivation. Basic knowledge regarding not only the enzymes involved in the unusual fatty acid biosynthesis as well as the ones responsible for their storage into triacylglycerols (TAG) is required.

The objective of this preliminary work was to study the diacylglycerol acyltransferase (DGAT) activity in microsomes of three euphorbs: *R. communis*, *E. lagascae*, and *Euphorbia lathyris* L. (the latter is rich in oleic acid). The DGAT enzyme catalyzes the final step of transferring a fatty acid moiety to a diacylglycerol (DAG) into TAG.

Plants of the three species were grown in a glasshouse at IMIDA (Murcia, Spain). Endosperms were removed from developing seeds at 30 to 70% filling stage and the tissue was extracted with a buffer. Subsequent ultracentrifugation gave three microsomal preparations for each plant species. In vitro DGAT assays using [¹⁴C]-oleoyl-CoA with or without 1, 2 diolein were carried out. TLC was used to separate lipid by classes and the labelled TAG were recorded using a Molecular Imager and a Scintillation Counter.

The [¹⁴C]-oleoyl incorporation into TAG was greater in *R. communis* and *E. lathyris* (72-89% of total TAG) than in *E. lagascae*. By feeding *E. lagascae* microsomes with exogenous 1, 2 diolein (1mM) as the acyl receptor, the amount of labelled TAG increased to 39% suggesting that other acyl groups were being incorporated as well. In some plates, *R. communis* and *E. lagascae* microsomes gave more polar-labelled TAGs than *E. lathyris* possibly because endogenous DAGs (not 1, 2 diolein) were being used in the reaction.

Although *E. lathyris* microsomes showed specificity towards 1, 2-diolein as a substrate, the preparations from the three euphorbs (*R. communis*, *E. lagascae*, and *E. lathyris*) were able to use the acyl donor and acyl receptor possibly suggesting that the DGAT enzymes would not be a limiting factor to engineer Euphorbiaceae crops with functionalized fatty acids.

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Natural Rubber and Resins

THE REGULATION OF RUBBER BIOSYNTHESIS IN GUAYULE BY LOW TEMPERATURE

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Guayule is indigenous to 300,000 sq. mi. of Chihuahuan Desert in northern Mexico and the Big Bend area of Texas. We have investigated the role of low temperature on the regulation of rubber biosynthesis in the stems of guayule in the fall and winter in the Chihuahuan Desert. The rubber content and activities of the enzymes of the polyisoprenoid pathway were examined throughout the growing season in fields in Pecos County, TX.

The rubber content of the plants transplanted to the fields in May was low in July and August. From October to December, the rubber content increased from 589.0 mg/plant to 4438.0 mg/plant for an 87% increase. This increase was correlated to the accumulated temperature of 5 to 7° C. Electron photomicrographs of the cortical parenchyma of the plants in September showed cells only with vacuoles, mitochondria and chloroplasts, but no rubber particles. In January, photomicrographs of the cortical parenchyma contained an abundance of intact rubber particles in the cytosol. These electron micrographs showed visually the increase in the rubber content of the guayule stems from September to January.

Washed rubber particles (WRP) from guayule bark homogenates were isolated by chromatography on columns of Ultrogel. Nuclear magnetic resonance (NMR) analysis showed no resins were present. Enzymatic assays showed the WRP bound rubber transferase (RT) used an allylic-PP started molecule followed by the polymerization of cis-isopentenyl pyrophosphate (IPP) units in forming long rubber chains. Analysis of the enzymatically formed rubber by gel permeation chromatography on a Waters 150C chromatograph showed the WRP-RT reaction formed high molecular weight rubber similar to guayule rubber. The activity of the RT in guayule bark was 65.5 nmol/h/g fresh weight in September and increased to 375.5 nmol/h/g fresh weight in December.

The activities of the endoplasmic reticulum 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) in the stem bark of the field plants were 21.1 n mol/h/g fresh weight in May decreased to 5.1 nmol MVA/h/g fresh weight in July and increased from 5.0 to 29.9 nmol MVA/h/g fresh weight from October to December. Northern blots with a tomato HMGR-1 cDNA probe showed the HMGR mRNA was high in June and November corresponding to high HMGR activity during seedling growth and rapid increase in rubber biosynthesis.

The guayule plants indigenous to the Chihuahuan Desert have developed a genetic system capable of responding to the low temperatures of the fall and winter of the desert in the biosynthesis of rubber.

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GUAYULINS AS PREDICTORS OF RUBBER YIELD IN GUAYULE

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Guayule (*Parthenium argentatum* Gray) is now a commercial crop for the production of high-quality, hypoallergenic natural rubber latex. Guayule is relatively resistant to both insect and disease pests, and in general its cultivation requires little chemical input. It has been postulated that guayule's chemical defense system is based on terpene derivatives such as guayulins A and B. Guayule breeding programs have increased rubber yield by selecting for higher rubber content, larger biomass, or a combination of the two. If guayulins are guayule's chemical defense system against pests, there is a danger of inadvertently decreasing these compounds unless monitored along with rubber production.

Thus, we evaluated (1) the distribution of rubber, and guayulins A and B among plant parts; (2) the possibility that guayulins might be an appropriate predictor of rubber yield; and (3) whether changes in guayulin content had been inadvertently made through plant breeding.

One- and two-year-old plants from lines 11591, AZ-1, and AZ-3 were separated into eight parts: brown leaves, green leaves, stem tips (the apical meristem), woody stems (with secondary growth) less than 5 mm in diameter, woody stems between 5 and 10 mm, woody stems greater than 10 mm, green stems (primary growth, the current year's growth),

and flower parts (peduncles, inflorescences, and achenes).

The distribution of rubber, guayulin A, and guayulin B among plant parts by plant age and harvest season (spring and fall) was determined. There were no significant differences between plant ages for rubber or guayulin content, or guayulins for harvest season. Rubber percent was higher in spring, which agrees with numerous other investigations.

Guayulins A and B were present in all plant parts, with over 80% of the total guayulin A and between 58 and 80% of guayulin B found in the woody stems. The woody stems make up 56% of the total biomass, and contain 67% of the total rubber when plants are harvested in the fall and 89% of the total rubber for spring harvests. Guayulin A in the woody stems is significantly correlated ($r = 0.66$) with total rubber in the plant, but the rubber in the same woody tissue has a higher correlation ($r = 0.95$) with total rubber. Interestingly, the rubber in the green leaves and green stems are also significantly correlated ($r = 0.61$ and 0.72 , respectively) with the total rubber and could be used as predictors of rubber yield.

Line 11591, an older, relatively unimproved line, had significantly greater guayulin A in the woody stems (greater than 5 mm in diameter), and less guayulin B in all of the woody stems than the two improved lines, AZ-1 and AZ-3.

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TRANSPLANTING DATE AND PLANT POPULATION EFFECTS ON GUAYULE GERMPLASM

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Guayule (*Parthenium argentatum* Gray) is a perennial shrub native to the Chihuahuan Desert. The effects of various agronomic practices, such as transplanting and harvesting dates, plant spacing, cutting height and frequency, irrigation frequency, and herbicide application on newly developed germplasm, and the effects of these agronomic practices on latex concentration and yield have not been reported.

The objectives of this study were to determine the yield and concentration of latex, rubber, and resin of four guayule lines transplanted in the spring and fall at two populations.

Four guayule lines (AZ-1, AZ-3, AZ-5, and 11591) were transplanted at two dates (28 November 2000 and 7 June 2001) and at two plant populations (27,000 and 54,000 plants/ha). Treatments were replicated four times. Each treatment plot was subdivided into six subplots for harvesting at six month intervals beginning one year after transplanting.

Results showed that the transplanting date nor the plant population consistently affected plant size, latex concentration, or yield. Instead, it appeared that time of harvest (fall vs. spring) was more important. The last harvest (sixth) in the fall planting date and the fifth harvest date in the spring planting date were the optimum for plant biomass and latex, rubber and resin concentrations, and yields.

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GUAYULE PRODUCTION ON THE SOUTHERN HIGH PLAINS

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Native guayule populations are scattered throughout 300,000 km² 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 Halfway, 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. Guayule seedlings (60 days-old) were transplanted on 18 May 2006, at the Texas AgriLife Research Station at Halfway. 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, 6 June 2007, 21 October 2007, and 18 June 2008. Guayule cold damage was estimated 6 June 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. Two plants from each plot were harvested by hand on 15 April 2008, weighed, and ground in a Troy-Bilt Chipper/Shredder. Samples were collected for moisture and rubber and resin content, and stored at -20° C. Biomass yields were determined on oven dry weights.

Following the 2006/2007 winter the cold damage index ranged from 1.2 in 11591 to 3.8 in AZ-1. The minimum air temperature recorded was -14° C. Minimal cold damage occurred during the 2007/2008 winter. Biomass of the 24-month-old shrubs harvested in 2008 varied from 9,639 kg/ha in line 11591 to 13,393 kg/ha in line AZ-4. The cold damage index for AZ-4 was 2.1 with 7% of the plants being severely damaged (#4) and 12% completely dead (#5). None of the 11591 shrubs were severely damaged and only 4% was dead.

Lines 11591 and N6-5 had the least cold damage and hold 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 High 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 sprinklers are available for establishing guayule by direct-seeding.

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HEATING TIME AND TEMPERATURE OF GUAYULE TISSUE PRIOR TO EXTRACTION OF RESIN AND RUBBER

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Previous research has shown that pre-extraction sample preparation and handling can have a significant effect on natural rubber extraction. For example, sample particle size, sample settling during subsampling, and proper loading of samples into an extraction cell can have a significant effect on rubber extractions in the Dionex Accelerated Solvent Extractor (ASE) 200. The effect of heating time and temperature on guayule (*Parthenium argentatum*) tissue prior to extraction has come into question.

In this study, we determined the effect of pre-extraction heating time and temperature on resin and rubber yield of different guayule materials determined using the ASE 200.

Guayule plant tissue samples (latex-extracted, ambient air-dried bagasse; chipped, ambient air-dried whole shrub; and defoliated, chopped fresh shrub) were subjected, in air, to six pre-extraction heating times and six temperatures prior to extraction of resin and rubber. The six pre-extraction heating times were: (1) control – no pre-heating, (2) 15 min, (3) 30 min, (4) 1 h, (5) 2 h, and (6) 4 h. The six pre-extraction temperatures were: (1) 50° C, (2) 100° C, (3) 115° C, (4) 135° C, (5) 150° C, and (6) 200° C. Subsequent acetone extractions were at 100° C followed by sequential hexane extractions at 140° C using the ASE 200.

Highest rubber yields were obtained from all three guayule materials when pretreated at 50 and 100° C. At all temperatures above 100° C, rubber yields (hexane extractable fraction) declined progressively with increasing temperature, and with increasing time, until a plateau was reached. Except at the highest temperature of 200° C, the decrease in extractable rubber was matched by a concomitant increase of resin in the acetone extracted fraction. However, at 200° C, resin levels also decreased with time, ending with less resin material than in the control samples.

The striking, deleterious effect of high pre-extraction drying temperatures, especially on rubber yield, was followed by a factorial investigation of the effect of extraction temperature in the ASE method, itself. Extraction temperatures ranged from 40 to 100° C for acetone, and from 40 to 140° C for hexane. Results will be reported.

In conclusion, residual rubber in guayule shrub and bagasse is subject to rapid thermal and oxidative degradation when exposed to heat in the presence of air. However, once degraded sufficiently to become soluble in acetone, the rubber catabolites appear to stabilize. These catabolites, and the original resin fraction, remain stable until subjected to a temperature of 200° C, at which point both fractions are subject to degradation and volatilization leading to a loss of total material.

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BIO-OIL FROM GUAYULE BAGASSE

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Guayule, *Parthenium argentatum*, has recently been commercialized as a domestic source of rubber, and is primarily sold as latex for circum-allergenic medical applications. Latex extraction leads to about 90% of the crop biomass as a finely-divided residue that is a free flowing feedstock suitable for conversion to biofuels. Fast pyrolysis can convert the lignocellulosic biomass into pyrolysis liquids (bio-oils) that can be used as boiler fuel or converted into liquid transportation fuels.

Guayule bagasse and whole shrub material was successfully converted into bio-oil, charcoal, and non-condensable gases by fast pyrolysis at about 500° C in a bench-scale fluidized bed reactor. Bio-oil was produced in the 60% yield range using a sand medium. The bio-oils from guayule pyrolysis had energy content higher than that of typical fast pyrolysis oil, about 30 MJ/kg and 75% the value of heavy fuel oil. Compositional analysis of feedstocks and of the bio-oil suggests that guayule is a very attractive feedstock for fast pyrolysis and subsequent conversion processes.

Thermochemical conversion produces bioenergy while consuming the entire plant, significantly improving the profitability and sustainable growth of domestic rubber in the US.

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ENHANCING EXTRACTION METHOD FOR MEXICAN NATURAL WAX

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Candelilla wax is a biomaterial extracted from *Euphorbia antisyphilitica*, a Mexican desert endemic plant. FAO recommends the use of candelilla wax, which is considered a GRAS substance. We need to find new technology for the traditional extraction process of the farmers called “candelilleros” to diminish the emission contaminants. This extraction process involves treating the plant with sulfuric acid (80 %) in iron kettles.

The objective of the present work was to develop a candelilla wax extraction methodology in order to reduce costs and ecological impacts of the existing procedure.

Non corrosive iron recipients were used. The extracted agents tested were sulfuric acid (20, 40, and 60%), acetic acid, tartaric acid, citric acid, and hexane. A method called “candelilla wax extraction by physical contact” was developed. The procedure was to recover the external wax of stems, following by thermal treatment. Physicochemical characterization was made with NMR (H^+ and ^{13}C), IR, mass spectrometry, scanning electron, and thermogravimetric analysis. A new gravimetric refined method was applied indicating that the majority of contaminants present in extracted wax came from the corrosion of the iron kettle by the strong acid.

The highest extraction yield and quality of candelilla wax were obtained with acid concentrations lower than 20%. The method of “candelilla wax extraction by physical contact” showed better results than that obtained with weak acids and solvents. The gravimetric refined process presented excellent results that avoided the use of conventional techniques such as filtration, H_2O_2 , and activated carbon.

The result of the present research provides the “candelilleros” improved technologies over their traditional process that reduces economic costs with a positive environmental impact.

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DOMESTICATION AND COMMERCIALIZATION OF *Taraxacum kok-saghyz*, A POTENTIAL DOMESTIC SOURCE OF NATURAL RUBBER AND INULIN: PROGRESS AND OUTLOOK

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The Ohio BioProducts Innovation Center and its private- and public-sector collaborators employ a market-pull approach to accelerate the commercialization of renewable specialty chemicals, polymers/plastics, and advanced materials. *Taraxacum kok-saghyz* (TKS) roots contain high levels of inulin and natural rubber, but TKS is undomesticated. Since 2006, a diverse, private-public team has worked to domesticate TKS, the focal point of the WWII-era “Emergency Rubber Project” and a prime candidate to complement *Hevea brasiliensis* as a main source of natural rubber. Still, TKS is relatively absent in the scientific literature.

Our objectives were to improve TKS genetics and extraction methods and to affirm earlier findings that TKS-based rubber meets industry quality standards.

Beginning in Ohio with 39 g (approx. 1500 seeds per gram) of wild-collected TKS seeds, the project team has developed protocols to differentiate *kok-saghyz* from other members of the *Taraxacum*, assessed root rubber and inulin levels, and extracted rubber and inulin using commercial-like methods. The team has also allowed unrestricted crosses and performed controlled crosses among TKS individuals previously testing high for rubber production potential. In addition, various properties of TKS-derived rubber have been analyzed.

To date, in Ohio: (a) a total of 6151 TKS seedlings have been generated and another 9000 *Taraxacum* seedlings await roguing, (b) root rubber levels of 10% or greater (commercialization threshold) have been found in 145 of 1467 individuals tested, (c) the average root rubber levels have increased from 1.4% to 8.9%, (d) root inulin levels have averaged 53.4%, (e) the properties of TKS-derived rubber have been similar to those of *Hevea*-based natural rubber, and

(f) improved *TKS* seeds of 73.5 g have been produced. Ongoing work integrates improvements in *TKS* genetics with others in rubber/inulin extraction methods.

The *TKS* germplasm has been improved and a mechanical extraction method has been used to extract high quality rubber from *TKS* roots.

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GUAYULE LATEX PRODUCTS: PERFORMANCE, STANDARDS, AND REGULATORY UPDATE

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Guayule, *Parthenium argentatum*, latex is commercially available as an alternate rubber source (Yulex[®] Latex) and is currently the sole natural rubber of U.S. domestic origin. Previous commercialization attempts failed largely because of guayule's inability to compete in commodity markets while being produced on a small-scale. Competitiveness and scalability are issues faced by all new crops attempting to become established. It is important to note that guayule is the world's first natural rubber latex that is safe for Type I latex allergy sufferers due to its extremely low protein content and its lack of proteins that cross-react with *Hevea* latex antigenic proteins. These characteristics allow a sufficient premium to permit profitable production during the early and product-limited phases of expansion.

In this paper, the superior product performance and physical properties of guayule latex will be discussed and compared with *Hevea* latex and various synthetic polymers that share similar market space. Sufficient performance is an essential corollary to its circumallergenic[™] characteristics. In addition, the impact of ASTM standards and the recent FDA clearance on guayule latex commercialization will be presented for discussion.

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POSTER PRESENTATIONS (General Crops)

PHOTOSYNTHESIS AND GROWTH RESPONSE OF DIFFERENT SWITCHGRASS ECOTYPES TO FLUCTUATING GROWTH TEMPERATURES

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Switchgrass (*Panicum virgatum* L.), a warm-season C₄ grass that shows good potential as a bioenergy feedstock and conservation crop, is widely adapted throughout North America. However, its productivity tends to decline with increasing latitude. In northern regions where growing season temperatures can fluctuate dramatically, the ability of switchgrass to adjust photosynthetically to maximize carbon assimilation under such conditions, especially exposure to sub-optimal temperatures, may enhance productivity.

To investigate the genetic potential of switchgrass to photosynthetically acclimatize to fluctuating growth temperature, four cultivars, two lowland (Alamo and Kanlow) and two upland (Cave in Rock and Sunburst) ecotypes were grown under day/night temperatures of 32/24° C and later switched to 22/14° C for 15 d. Photosynthesis, growth, and nonstructural carbohydrates were analyzed before and after temperature switching.

Photosynthesis of the lowland cultivars was slightly greater than that of the upland varieties at 32/24° C. After switching to 22/14° C and allowing the newest expanding leaf to mature, photosynthesis at growth temperature was less

than that before the switch, but there was no difference among cultivars. Under the lower growth temperatures, photosynthetic capacity among cultivars slightly decreased or remained similar as compared with that before the temperature switch, although Alamo leaves increased in PEPCase activity and Chl content. While exposed to 22/14° C, Alamo had the greatest dry weight gain (3.8 g plant⁻¹), which was 31, 65, and 153% greater than that of Sunburst, Kanlow, and Cave in Rock, respectively. Soluble sugar and starch contents of Alamo leaves were greater than that of the other cultivars before and after the switch. However, Alamo leaves grown under the lower temperatures contained less soluble sugar and starch than those grown at 32/24° C, whereas the other cultivars responded oppositely. Results indicate that efficient assimilate usage for growth rather than photosynthetic acclimation under sub-optimal growth temperatures may be a key to enhancing switchgrass productivity.

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FLAX: A NEW, OLD CROP FOR TEXAS

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Finding alternative fuel sources has been a topic of major concern in recent times due to the rising fuel prices and environmental concerns. One crop that appears to have promise in South Central Texas for industrial oils and bio-diesel is flax (*Linum usitatissimum* L.). Flax was a popular industrial oil crop in Texas during the 1930s to 1960s, with the peak of 134,000 ha in 1949. Due to the popularity of flax in South Central Texas, Texas A&M University developed and released numerous regionally adapted, cold tolerant flax varieties. However, significant areas of flax have not been grown in South Central Texas since the 1960s due to the competitive economics of petroleum-based products.

The first objective of this study was to evaluate currently available flax varieties for their adaptability, yield, and quality in South Central Texas. The second objective was to obtain and increase seed from flax varieties released from Texas A&M University in the 1950s to 1960s. Based on these initial results, more focused research experiments will be initiated to further enhance oil yields for South Central Texas.

Seventeen varieties of spring flax were collected from various universities in the United States and Canada. The seeds from these varieties were planted with a grain drill in small plots (1.5 m x 4.6 m) in a completely randomized block design with three replications. Flax was planted in the fall (30 October 2007) at a seeding rate of 39 kg/ha. The planting depth, fertility management, and pest management were all based on agronomic experience and current recommendations. Seedling emergence, seedling disease and insect levels, winter hardiness, and yield were quantified for each variety.

At this time, only yields from the College Station location were available. Significant differences (LSD<0.05) were observed in percent emergence and seedling disease (flax rot), but winter kill was not observed in any varieties. The yields ranged from 2072 to 2637 kg/ha for the varieties; however, the yields and test weights did not differ among varieties. The variety Nekoma had the highest yield of 2,340 kg/ha with a test weight of 58.3 kg/bu. The yields demonstrate that potential exists for viable flax production in South Central Texas, especially considering the average yield for flax in the late 1960s was 942 kg/ha.

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VARIABILITY IN ANTHOCYANIN CONTENT AMONG *Abutilon theophrasti*, *Basella alba*, AND *Urena lobata* GENETIC RESOURCES

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Plants contain bioactive phytochemicals and nutraceuticals to be utilized in the pharmaceutical and nutraceutical markets. Sixty-two accessions of *Abutilon theophrasti*, *Basella alba*, and *Urena lobata* 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 seeds. Anthocyanins are not only responsible for leaf, stem, flower, and seed color, but can inhibit LDL oxidation as well as hypertension in humans while the antioxidants, quercetin and kaempferol are known to be anticancer compounds.

The objective of this study was to determine the amount of anthocyanin and related flavonoid compounds from leaves and flowers of *Abutilon theophrasti*, *Basella alba*, and *Urena lobata*.

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, cyanidin, and pelargonidin monoglucosides absorb. Anthocyanin indexes will be recorded from each of three leaves and flowers using this modified anthocyanin meter.

Preliminary analysis revealed that leaf anthocyanin indexes ranged from 7.1 to 8.8 for *A. theophrasti* and 36.4 to 47.9 for *U. lobata*. However, flower anthocyanin indexes from *U. lobata* ranged from 19.6 to 20.9. As of 17 June 2008, *U. lobata* produced a significantly higher anthocyanin index (17.3) than all other species tested. However, *B. alba* produced the second highest anthocyanin index (12.5), which was significantly higher than both *M. uniflorum* and *A. theophrasti*. We will determine the amount of quercetin and kaempferol from these species as well.

A useful, quantitative method employed in the quantification of anthocyanin indexes from *Abutilon theophrasti*, *Basella alba*, and *Urena lobata* will be demonstrated. These species 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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DEVELOPMENT OF SAFFLOWER AS A NEW WINTER CROP FOR THE TEXAS HIGH PLAINS

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Energy and water are the two most essential commodities necessary to keep our agricultural economy operating at optimum efficiency. Consequently, there is a pressing need for crops that produce vegetable oil feedstocks needed for biofuel production that are very water use efficient. Fall planted crops grown on the Texas High Plains during the cooler winter months could reduce water demand on the Ogallala Aquifer.

The purpose of this research was to evaluate several accessions of safflower exhibiting winter hardiness for water use efficiency under variable irrigation rates by determining seed and oil yield.

On 15 September 2007, eight accessions of safflower were planted in a randomized split block design with four replications and five irrigation rates. Irrigation rates were established using Potential Evaporation-Transportation (PET) rates and included 0 (dryland), 20, 40, 60, and 80% PET. Irrigation treatments were applied using subsurface drip tape placed 20 to 25 cm below the root zone on 1 m spacing. Each accession was evaluated for winter survival, maturity date, seed yield, oil content, and fatty acid profile for each irrigation treatment.

Several selected safflower accessions demonstrated high water use efficiency, excellent winter survival, comparable maturity to winter wheat, and seed yields significantly higher than spring planted safflower. However, the oil content of the winter accessions were lower (24-30%) than spring planted safflower cultivars (35-40%) that had been selected for higher oil contents. All eight of the winter hardy accessions also had high levels of linoleic acid.

Use of this unique germplasm will allow farmers in this region to incorporate a new winter crop into their crop rotation that is profitable as well as high in water use efficiency.

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DEVELOPMENT OF A FARM TRACTOR FOR DEMONSTRATION OF MULTIPLE RENEWABLE FUELS

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There is a need to demonstrate the utility and application of renewable fuels to both agricultural and public audiences using existing equipment.

In this study, a John Deere 4310 farm tractor was modified to facilitate evaluation of three different fuels under continuous operation. Auxiliary transparent fuel tanks were attached to the top of the tractor while leaving the original diesel tank as the primary source of fuel. Transparent fuel lines were connected into a selectable 3-way valve leading into the fuel pump. From the pump, the fuel is sent to a selectable 2-way valve isolating the different fuels between a traditional fuel filter and a separate filter for each test fuel. Fuel lines then lead to the fuel rail injector system and into the cylinders. The overflow fuels are re-circulated back into their perspective fuel tanks. This tractor has been used to demonstrate ethyl and methyl esters fuels produced from oil extracted from castor (*Ricinus communis* L.), safflower (*Carthamus tinctorius* L.), and cotton (*Gossypium hirsutum* L.) seeds as well as second generation biofuels.

Demonstrations with this type of tractor will allow isolated experiment stations, extension specialists, and farm mechanic classes to develop consumer confidence in the use of renewable fuel in existing equipment. This grassroots demonstration of renewable fuel will build consumer confidence and encourage taxpayer investment in alternative fuel sources.

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MILKWEED FLOSS BIODIESEL AND NO. 2 HEATING OIL ABSORBENT PROPERTIES

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The Environmental Protection Agency reports 11.8 million gallons of oil are spilled on fresh water every year in the US. The current industry standard for cleaning oil spills utilizes non-reusable polyester sheets and booms. Previous research has shown common milkweed (*Asclepias syriaca*) floss can absorb crude oil under salt water conditions.

The objective of this experiment was to investigate milkweed floss as a potential absorbent for 100% biodiesel and No. 2 home heating oil in fresh water applications. Mesh packets of whole and single floss were filled with approximately one-half gram of floss, agitated in a 200 ml water/50 ml fuel mix for five minutes, and the volume of oil removed was determined. To test re-usability, each packet was rung out with a mechanical wringer and the procedure was repeated four times.

For the biodiesel, whole milkweed floss removed an average of 46.3 g/g, whereas single floss retained 44.5 g/g. For the heating oil, both the whole floss and the single floss absorbed 45.8 g/g. At the conclusion of the experiment, the amount of biodiesel absorbed by whole and single floss decreased to 20.2 g/g and 17.5 g/g, respectively. The amount of heating oil retained by the two flosses decreased to 20.6 g/g and 21.6 g/g. The 40% decrease in absorbency after the

initial use was due to the loss of space between the floss fibers.

Both whole and single milkweed floss could potentially serve as a viable bio-based product for cleaning fresh water oil contamination with the tremendous advantage of re-usability.

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POSTER PRESENTATIONS (Medicinal and Nutraceuticals)

VARIATION IN THE NUTRITIONAL VALUE OF MORINGA (*Moringa oleifera*) FROM RWANDA

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Moringa (*Moringa oleifera*) is an important multipurpose tree, known as “The Miracle Tree” because of its high nutritional value (e.g., the leaves contain high levels of minerals and vitamins). By providing scientific based evidence, discovering new uses and applications, moringa could be further utilized in a manner that can contribute to social, environmental and economic development. While there is a wealth of information on the basic potential nutritional value of moringa, information is far from complete relative to the variation of nutritional quality within and between the many varieties of moringa growing in Sub-Saharan Africa, including Rwanda.

The objective of this study was to assess the nutritional value of *Moringa oleifera* leaves from different regions of Rwanda. This study was also used as a vehicle to involve graduate students in research and development activities.

Three moringa samples were collected from different areas of Rwanda (Gasabo, Kicukiro, and Kibungo) from which different quality (foreign material, moisture, and ash) and chemical analyses (elemental, vitamins, and polyphenols) were conducted.

Foreign matter analysis indicated that the moringa leaves were clean, contained no foreign materials (e.g., insects), and low levels of contamination with sand. The moisture levels of the samples (<7.3%) showed that they were properly dried. The elemental analysis showed that the samples constant levels of potassium (1.8-1.9%), phosphorus (0.4-0.5%), and magnesium (0.3-0.4%). Calcium content varied among the samples (1-2.1%), with the samples from Kicukiro having the highest levels (2.1%). Iron content also had significant variation (100-270mg/100g) among the samples. This study demonstrated that the moringa dried leaves contained very low levels of vitamin C, but were rich in vitamins A (23-46 mg/100g) and E (37-50 mg/100g).

These findings confirm our previous results and further support the use of moringa leaves as an affordable plant-based vehicle that can provide significant sources of elements, proteins, and antioxidants to the diet. Moringa leaves can be an affordable means to improve the health and nutrition in the Sub-Saharan countries, particularly for the undernourished and malnourished children, and also for the pregnant and lactating women.

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POSTER PRESENTATIONS (Oilseeds)

DEVELOPING THE NEXT GENERATION PEANUT – TEXAS AGRILIFE RESEARCH PEANUT BREEDING PROGRAM

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Peanut production acreage in the state of Texas covers a large area and involves vastly different environmental conditions. These environmental differences often lead to regionalized problems associated with diseases and other peanut pests. In the early 1980s, central Texas had an epidemic of *Sclerotinia* blight caused by *Sclerotinia minor* jaggar. In the mid 1980s, south Texas experienced a similar situation with an epidemic of *Tomato spotted wilt virus* (TSWV). Early and late leafspot affect most of the growing regions and must be controlled with costly fungicide applications or else risk losing significant yield potential if the diseases were to go unchecked. Production areas associated with fine sandy land are often infested with peanut root knot nematodes (*Meloidogyne arenaria*). In addition to these pests, acreage shifts from region to region and manufacturer demands are all driving forces that determine the focus of the Texas AgriLife peanut breeding program.

In the past four decades, the program has developed breeding lines and cultivars with individual resistances to *Sclerotinia* blight (Tx901639-3), TSWV (Tamrun 96), nematodes (NemaTAM), and early leafspot (Tx964117). The development of the high oleic trait by the University of Florida created a demand from peanut manufacturers for high oleic peanuts in Texas. The program has since transferred the high oleic trait into the majority of its breeding material and five out of the last six cultivar releases were high oleic.

The current goal of the program is to combine all of the individual pest resistances into a single cultivar with multiple pest resistance while maintaining a high yield potential and high quality attributes such as total sound mature kernels (TSMK), flavor profiles, and favorable fat and sugar contents for the next generation peanut growers and manufacturers.

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OIL CONTENT IN SEEDS OF THE NPGS JOJOBA (*Simmondsia chinensis*) GERMPLASM COLLECTION

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Jojoba, *Simmondsia chinensis*, (Link) Schneider is a shrub native to warm and arid land regions of North and South America. Jojoba seeds contain vegetable oil composed of long (C₂₀₋₂₂), straight-chain liquid wax of non-glyceride esters. Minute amounts of triglycerides in its composition make the oil a liquid and odorless wax. The oil and its derivatives may be utilized in cosmetics, pharmaceuticals, lubricants, electrical insulations, foam controlling agents, plasticizers, and fire retardants. The oil contributes significantly to the seed weight and its content depends on the origin of the germplasm. The USDA-ARS, National Plant Germplasm System (NPGS) maintains a jojoba collection at the National Arid Land Plant Genetics Resource Unit at Parlier, California.

The objective of this study was to evaluate oil content in dry seeds of selected jojoba germplasm accessions maintained in the NPGS collection and in seed samples obtained from a discontinued breeding program at the University of California (UC). The oil content was determined in randomly selected seeds of 390 different accessions (97 from the NPGS and 293 from the UC program), using a nuclear magnetic resonance spectrometer (one seed/replication; four replications).

The oil content varied from 41.9 to 60.7% in the NPGS collection (PI 254487 and W6 10970, respectively) and from 44.3 to 61.2% in the UC material (PARL 530 and PARL 699, respectively). In general, the oil makes up 50% of jojoba seed weight. Accessions with higher than 50% oil content present valuable selection material for crop improvement via traditional breeding or asexual propagation. An analysis of the oil profile is also in progress. Together with available morphological characterization of the accessions, the data will contribute to a consolidation of the UC jojoba accessions, now in NPGS, to a manageable size.

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IDENTIFYING THE YIELD POTENTIAL FOR COOL-SEASON OIL-SEED CROPS FOR SOUTH CENTRAL TEXAS

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Texas AgriLife Extension Service – Texas A&M University System

Establishing significant bio-diesel production in the United States will provide substantial economic and environmental benefits. With about 14 percent of the total United States' capacity, Texas has developed a large bio-diesel production capacity. However, developing dedicated bio-diesel feed-stocks is crucial to sustaining and further expanding the bio-diesel industry. Texas can contribute huge acreage to the bio-fuels production process because it has more than 9.7 million cropland hectares and many more hectares of marginal cropland that can be accessed to help solve our nation's energy-dependency problem.

The objective of our research was evaluate the adaptation of several varieties or breeding lines of the following plants to biotic and abiotic conditions in the Brazos Valley: Safflower, Camelina, Radish, Low Erucic Acid Rapeseed (LEAR), High Erucic Acid Rapeseed (HEAR), and Flax. Based on outcome of the first objective, best management practices will be developed to increase crop yield efficiency and pest-management strategies.

Both spring and winter types of these oil-seed crops were planted on October 30, 2007. The plot size was 6.75 square meter and was replicated three times. The plots were planted with a small-plot grain drill on 19.0 cm centers, except Safflower, which was planted on 38 cm centers. The seeding rate, planting depth, fertility management, and pest management were based on our agronomic experience and current recommendations. Agronomic field notes were taken on pest levels, stand establishment, freeze injury, and other yield-limiting factors on a weekly basis. At the time of this abstract submission, plots have been harvested, but the data have not been processed or analyzed. These results will be presented at the conference.

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SEEDING DATE INFLUENCE ON WINTER CAMELINA PERFORMANCE IN NORTH DAKOTA

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Proper seeding date is important for optimum crop performance that is often related to climatic conditions during reproductive development for spring annuals. Seeding date for winter annuals is important for fall growth and plant size, and subsequent overwintering and survival of plants the following spring. Poor winter survival directly affects stand density and this has a profound influence on crop performance.

The objective of this study was to evaluate the influence of early, mid, and late September seeding dates on winter camelina (*Camelina sativa* L.) performance.

The study was established near Prosper, ND, in the fall of 2007. Seeding date and genotype were main and subplots, respectively, with three replicates sown no-till into standing hard red spring wheat (*Triticum aestivum* L.) stubble approximately 25 cm tall. Traits evaluated included fall stand rating, plant size at freeze-up, winter survival, flowering date, plant height and lodging, seed weight, and seed yield and oil content.

Preliminary results indicate winter survival of stands from all seeding dates exceeding 85%. Flowering and maturity of stands from the fall seeding dates occurred earlier from the earliest seeding date compared with the mid and late seeding dates. Winter camelina yield appears promising as does its potential for double cropping.

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***Thlaspi arvense*, A POTENTIAL BIODIESEL CROP: PRELIMINARY EVALUATION OF THE USDA GERMPLASM COLLECTION**

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As the world moves toward a bio-economy based on the use of renewable plant, animal, and microbial materials, a wide range of species are being evaluated as possible resources, including many taxa within the Brassicaceae plant family. *Thlaspi* is one of 20 oilseed brassicaceae genera with collections in the National Plant Germplasm System (NPGS). The NPGS oilseed brassicaceae collection is maintained by the North Central Regional Plant Introduction Station (NCRPIS) in Ames, IA and is administered by the USDA-ARS Plant Introduction Research Unit in cooperation with the Agricultural Experiment Station at Iowa State University.

The NPGS collection contains 20 *Thlaspi* accessions representing seven different taxa, including 12 accessions of *Thlaspi arvense* collected from within the species' native range in Europe as well as from several locations in the United States and Canada where they occur as a naturalized weed. Oil concentration in *Thlaspi* seed can be as high as 40% and the fatty acid profile of the oil is similar to that observed in other vegetable oils; however, *T. arvense* completes its life cycle by late spring or early summer suggesting a use for this common mid-western weed as a biodiesel component in a double cropping system.

The USDA-ARS New Crops Research Unit in Peoria, IL began to investigate this potential two years ago, using seed from a naturalized population collected near Peoria. In an effort to begin to examine some of the agronomic characteristics of the *Thlaspi* germplasm in the USDA collection and to provide seed for further evaluation, ten *T. arvense* accessions, including the naturalized Peoria population, were direct seeded field in Ames during early September 2007.

Variations in several characteristics including fall flowering, winter survival, and fall versus spring germination rates were observed. Seed lots used in the fall planting as well samples from the non-*Thlaspi arvense* accessions in the collection were analyzed for total oil concentration and fatty acid and glucosinolate profiles using standard pulsed NMR and MS methodologies. Some variation in oil composition was observed. In addition to the populations overwintered in the field, accessions were germinated by direct seeding into peat pellets and maintained in the NCRPIS cool winter greenhouse to ensure 2008 seed production in the absence of winter survival. Winter survival in the field ranged from 0 to 65% and was generally limited to those accessions that did not flower in the fall. Spring germination of fall planted seed was commonly at higher rates than in the fall for those accessions that did not overwinter well, with the result that the final population sizes were as large as or larger than the populations in place before the onset of winter. Processing of the July harvests will be completed by mid-August and yield data will be included on the poster.

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THE COMMON MILKWEED (*Asclepias syriaca*): A NEW INDUSTRIAL CROP

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Asclepias syriaca L. (the common milkweed) is a perennial plant occurring east of the Rockies in the United States but particularly east of the Mississippi River and from Southern Canada to Mexico. The plant has many unsavory given names by frustrated farmers including "the Wheat Farmers Nightmare". The leaves of the plant are the veritable food source for monarch butterfly larvae which store the secondary metabolites of this plant, mainly cardenolides, for protection of the adult monarch butterfly from avian predation. Milkweed seed is adapted for wind dispersal by a fine tuft of hollow, silky fiber (floss). Use of milkweed floss as hypoallergenic filler in high-end pillows and comforters has made milkweed an industrial crop.

To expand utilization of the crop, we have explored different uses for the seed resources.

Milkweed seed contains 20 to 30% oil by weight. This oil is highly unsaturated and we have found it to be cardenolide-free. We, therefore, epoxidized the olefinic bonds of the triglycerides as a platform intermediate to polyhydroxy triglycerides and other products. Both the oxiranes and polyhydroxy triglycerides have also been converted into novel UV absorbing estolides by direct esterification with trans-4-hydroxy-3-methoxycinnamic acid.

The polyhydroxy triglycerides have superior moisturizing capacity that makes them excellent candidates for skin/hair-care products, and the strong UV absorbing derivatives further expand the use of the oil. The seed meal and seed pod hulls on the other hand have shown potential as potent nematocides against *Meloidogyne chitwoodi* as well as pesticide activity against fall army worms.

The unique properties of milkweed oil derivatives have utility in personal care and cosmetics applications, whereas the seed meal and seed hulls have potential uses in crop protection.

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ILLINOIS CUPHEA PROGRESS - 2007

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Cuphea (Lythraceae) is an annual plant that produces a small oil seed rich in saturated medium-chain triacylglycerols. The initial oil characterization of a number of cuphea species was done at the U.S.D.A. Research Center in Peoria, Illinois, in the early 1960s. Cuphea PSR23 (*Cuphea viscosissima* x *Cuphea lanceolata*) is a newer cross of cuphea that contains high amounts of capric (C-10) medium chain fatty acids that are very useful in the formation of lubricants, soaps, and detergents.

During the 2007 season, Cuphea PSR23 was grown at TWO different locations in Illinois, (Morton and Princeville). Our research center has successfully planted and harvested cuphea over the past eight consecutive seasons. Some challenges that cuphea has, as a new crop, are an indeterminate growth and a small seed size. With indeterminate growth,

the plant flowers continuously throughout the growing season, which is a problem because the early maturing seed pods shatter and drop their seed before harvest. In most cases, the plant is harvested “green” with moisture level greater than 50%. Thus, without an effective method to deal with high moisture content of the seed, cuphea can not be a successful new crop.

The objective of this study was to look at different harvest methods and harvest dates and to evaluate the condition and amount of harvested seed. Three different harvest experiments were defined. Direct harvest (6600 John Deere Combine with a bean platform) was made at an early and late date. With the early direct harvest date, a portion of the field was swathed and then harvested (6600 John Deere Combine with a pickup head) at the late direct harvest date.

Comparisons between the swathed and direct combined material were made. In general, the swathed material was drier than the direct harvested material, but depending on the year, the maximum seed yields varied between the different methods and dates. In addition, different problems were present that were associated with the growing and harvesting of cuphea. These challenges are being addressed together with the future planting conditions and locations.

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DEGRADATION OF RICIN IN CASTOR SEED MEAL BY TEMPERATURE AND CHEMICAL TREATMENT

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Oil from castor (*Ricinus communis*) has the potential to become an important commodity in the U.S. Unfortunately the seed contain a potent cytotoxin known as ricin, which is cited as the single biggest impediment to the acceptance of large scale castor production in the U.S. Specific heat or chemical treatments may have the potential to enhance the safety of the oil extracted seed meal.

Heat and/or chemical treatments were performed to determine their effects on the denaturation of the ricin.

Whole seed, milled, un-extracted seed, heat extracted, and cold-pressed extracted seed were subjected to such treatments. Boiling and autoclaving showed varying degrees of effectiveness depending upon the sample type. Ricin within the cold-pressed extracted meal was rendered unresponsive to antibody probing after 10 min of boiling or autoclaving. Treatment of cold-pressed, extracted meal with 8 M urea and 6 M guanidine-HCl for 60 min produced no observable reduction in the response of the ricin to the antibody. However, hot-pressing of castor seed produced meal that exhibited no reactivity with the antibody indicating that the ricin has been denatured during the oil extraction. Boiling of the cold-pressed extracted meal in the presence of calcium hydroxide (pH 12.5) produced no detected difference from boiling alone.

Removing the toxic component of the castor seed meal may make this byproduct a new commodity enhancing the overall value of the crop.

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CULTIVATION TRIAL OF CASTOR (*Ricinus communis* L.) IN EXTREMADURA (SPAIN)

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The European Union Policy is in favour of biofuels and is promoting the use of vegetable oils for biodiesel production; non-food oils are convenient to avoid competition with food uses. Castor (*Ricinus communis* L., Euphorbiaceae) is a non-food industrial oilseed crop cultivated in non EU countries, but with potential to be grown domestically either for industrial oil or for biodiesel. From previous studies at Murcia (Southeastern Spain), it was concluded that castor did well as a spring-summer crop under irrigation.

The objective of this trial was to test field performance of castor at Extremadura (Southwestern Spain) where the availability of land and water for irrigation would allow an extensive production of this crop in the future.

Two varieties of *R. communis*: Castore (indeterminate) and 517 (dwarf) were sown on 20 April 2007 at the Experimental Station “La Orden” in Extremadura to estimate yield potential. The total amount of water (EC=0.8 dS/m) applied in irrigation was 3000 m³/ha and the rainfall during the field trial was 2000 m³/ha.

Two harvests were done: the first on September and the second on November 2007. Total seed yield ranged from 1600 to 3600 kg/ha. The dwarf castor variety (517) had the greatest yield.

The trial indicated good adaptation of the crop to local conditions; however, drought tolerance remains to be tested. The prospects of castorbean uses, whether for industrial oils or biodiesel, are worth considering in the future.

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ROW SPACING AND POPULATION DENSITY AFFECTING CASTOR YIELD

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Castor is an oil seed crop with various uses. Seed oil content of 50% makes it of prime interest, especially as a bioenergy crop. Therefore, optimization of yield is of particular interest. Most planting guidelines arise from studies conducted under rainfed conditions in the Texas, Oklahoma, and Nebraska. In Mississippi, where rainfall is more abundant, it may be possible to plant a denser stand than those recommended by previous researchers.

Population density and row spacing regimes were studied to determine optimal planting densities.

Raceme location and production was monitored to determine the greatest source of yield. Population density and row spacing tests were conducted at Starkville, MS. A randomized complete block with four replications was used. Four-row spacings and four planting densities were evaluated. Racemes were mapped and seed yield harvested by raceme on the 1st through 5th positions on the plant.

Trends indicated that yield increase was present in plant densities from high to low as row spacing increased. The treatment of 40.6 x 101.6 cm produced the highest yield, followed by 20.3 x 101.6 cm, and 30.5 x 101.6 cm. Raceme contribution to yield was highest on the second position.

From these data, it appears that row spacing, not plant density, is the primary determiner of yield. In spite of greater rainfall, the row spacing recommendations made by western researchers apply to Mississippi. The greatest raceme yield was from the 2nd position, indicating that desiccation and early harvest may be a viable option for southern castor.

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BIOACTIVE SUBSTANCES FROM ORIENTAL MUSTARD SEED – BIOREFINERY APPROACH

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Canada is the second largest producer of mustard seed in the world. Three types of mustard seed are produced; yellow (*Sinapis alba*), brown, and oriental (*Brassica juncea*). There is potential for growth in the mustard agribusiness, provided that novel applications for mustard seed are developed. Using a biorefinery approach, new, highly valuable products can be produced for different niche uses.

The objective of this study was to evaluate the use of reverse micelles for the simultaneous extraction of oil, proteins, and glucosinolates from oriental mustard.

The extraction technology evaluated was based on the use of reverse micelles (RMs) made with cetyltrimethylammonium bromide (CTAB) dispersed in isooctane, as per recent paper by Palmieri et al. (J Agric. Food Chem., 2008). The proposed extraction procedure is based on three main steps: seed conditioning; solid-liquid extraction by RM solution; and back-transfer of the RM solution for recovery of the extracted compounds.

It was verified that a 120°C for 5 min treatment was the most effective to deactivate myrosinase. The proposed extraction technology makes it possible to extract about the same amount of oil and glucosinolates as conventional extraction techniques. In comparison with the soluble proteins extracted with phosphate buffer, the RM system at low W_o ($[H_2O]/[Surfactant]$) made it possible to extract only a small part of soluble proteins (ca. 20% at W_o 6), whereas at W_o 50, the total amount of soluble proteins was isolated.

The existing extraction techniques essentially provide only two products, the oil and the remaining protein part, the so-called oilcake, whereas the proposed procedure by Palmieri (J Agric. Food Chem., 2008) is able to extract simultaneously some of the main mustard oilseed components such as oil, soluble and bioactive proteins, and glucosinolates. In addition, a solid protein fraction free of glucosinolate that is exploitable for some food and feed applications is obtained at the end of the extraction procedure.

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BIODIESEL AND SYNGAS FROM HIGH BIOMASS NATIVE SPECIES WITH HIGH LEAF TRIACYLGLYCEROL OIL CONTENT

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A suite of bioenergy feedstocks is needed to accommodate the variable agricultural and industrial requirements needed for a viable agro-industrial biofuel chain. However, important issues must be considered prior to selection of bioenergy species. Plant species selected should: have limited competition with national and international food and fiber needs, use limited water resources, be productive in marginal dry-land saline, or acidic soil conditions, be compatible with conventional agriculture systems, ideally be a perennial and capable of multiple harvests per year, and produce concentrated quantities of low energy extractable high efficiency fuels in the smallest footprint of space.

While much of the current research and industrial infrastructure has focused on ethanol production from seed starch or cellulosic biomass, recent studies have shown that biodiesel yields 3.2 units of fuel product energy for every unit of fossil fuel energy consumed versus 1.34 for bio-ethanol [1]. Diesel engines are also more efficient (45%) than gasoline, and use much less fuel per given power output [2], and the heating value of biodiesel is approximately 120,000 Btu/gallon versus ethanol at only 82,000 Btu/gallon. Biodiesel also has lower emissions than current diesel fuel except for nitrous oxides NO_x [3]. However, current oilseed crops, contribute a limited amount to the diesel consumed by Texas and the United States.

In this context, the objective of this study is to explore the biofuel production potential of highly productive native species from several plant families that we hypothesize can contribute significantly to the energy matrix of Texas, and United States. Some of the families include Asteraceae, Caprifoliaceae, Lamiaceae, Rosaceae, Fabaceae, and Buxaceae. Important attributes that each species have are: a high foliar neutral oil or triacylglycerol content (10-30% of cellular volume), high-biomass, annual and perennial ecotypes, adaptable to the Texas agroecosystems, and drought, saline, acid,

and or heat tolerance.

Triacylglycerol oils are stored in leaves and stems within half unit oil body membranes in the same manner as in seeds of oilseed crop such as canola, castor, corn and soybeans. We *hypothesize* that ecotypes from these and other families/species can be identified and improved to produce even higher concentrations of foliar triacylglycerol oil in a high biomass canopy as a productive source of high-value biodiesel and syngas.

At only 10% cellular volume we, and others in the literature, estimate some high biomass species in these families could yield 40 US barrels/acre from triacylglycerides or latex. If this value were doubled to 20% in identified genotypes the oil production would be equivalent to the highest oil-producing crop, oil palm. When combined with the syngas production from pyrolysis of the remaining cellulosic biomass that can be converted to natural gas, gasoline or biodiesel, the useful fuel potential in an integrated biodiesel/pyrolysis commercial plant is much higher. We believe that leaf triacylglycerol oil bodies and vegetative latex are poorly appreciated yet represent an enormous resource for biofuels.

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POSTER PRESENTATIONS (Natural Rubber and Resins)

DEVELOPMENT OF MICROSATELLITE MARKERS IN PARTHENIUM SSP

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Molecular markers provide the most efficient means to study genetic diversity within and among species of a particular genus. In addition, molecular markers can facilitate breeding efforts by providing tools necessary to reduce the time required to obtain recombinant genotypes with improved agricultural characteristics. To date, only three isozyme markers have been described in guayule (*Parthenium argentatum*, Gray). The development of co-dominant microsatellite markers in guayule could improve the utilization and management of germplasm resources and could be a valuable asset to researchers interested in breeding improved varieties.

The objective of the study was to design microsatellite primers capable of distinguishing among accessions of the same species and between species of the same genus (*Parthenium*).

Three thousand, one hundred and fifty-four expressed sequence tag contig sequences were screened for bi, tri, and tetra tandem repeats using the program TANDEM REPEATS FINDER. Eighty-seven suitable candidates were identified based on the type and location of the repeat sequence within the contig. Primers were designed using the default settings of the program PRIMER3. A standard PCR reaction was used with an annealing temperature generally 3 to 5 degrees below the melting temperature. Fragments were separated and visualized on MetaPhor® high resolution agarose stained with ethidium bromide. Eight accessions of *Parthenium* were screened for polymorphisms (2 *P. incanum*, 5 *P. argentatum*, and 1 *P. tomentosum*).

All primer pairs successfully amplified one or more DNA fragment. Seventy-five percent of the primer pairs produced polymorphism with at least one accession.

The microsatellite markers successfully resolved genetic differences between species and among accessions of the same species.

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POSTER PRESENTATIONS (Fibers and Cellulosics)**MEMBRANE RECOVERY OF PHENOLIC ACID CO-PRODUCTS FROM BIOMASS**R.A. Holser¹, R. Harry O'kuru², S.K. Brandon³, and J.D. Peterson³¹Richard Russell Research Center, USDA-ARS, Athens, GA 30605, USA²NCAUR, USDA-ARS, Peoria, IL 61546, USA³Microbiology Department, University of Georgia, Athens, GA 30605, USA

The technology to convert lignocellulosic biomass to biofuels is progressing with parallel efforts to develop processes to recover valuable natural products and generate additional revenue from these associated co-products. The lignified components of plant tissues contain phenolic acid structures such as ferulic and *p*-coumaric acids that possess bioactive properties and represent potential co-products, e.g., antioxidant and antimicrobial agents. The large-scale separation and recovery of these compounds from biomass prior to the conversion of the cellulosic portion depends upon an economical separation scheme.

The objective of this investigation was to identify a membrane material that could separate ferulic acid from an aqueous extract and demonstrate a feasible approach to recover the acid as a co-product in a biomass conversion facility.

Dilute aqueous solutions of ferulic acid were prepared and tested with five different types of membrane materials in a screening procedure to test the ability of each membrane to selectively retain the analyte, ferulic acid. The tested membrane materials included Teflon, Nylon, regenerated cellulose, polyether sulfone (PES), and polyvinylidene fluoride (PVDF). Test solutions of known ferulic acid concentrations were passed through the different membranes. The membrane permeates were collected and analyzed by HPLC-DAD using a C18 column with isocratic methanol as the mobile phase. Column eluents were monitored at 280 nm and UV spectra were collected by scanning under the detected peaks.

The results of this study demonstrated that a nylon membrane was able to retain 80% of the ferulic acid present in the aqueous solution as determined by analysis of the membrane permeate. Retention of ferulic acid by the other membrane materials was not significant.

The use of a nylon membrane to recover ferulic acid provides an inexpensive separation scheme to obtain ferulic acid as a co-product from an aqueous process stream. Membrane separations are advantageous because they are not capital or energy intensive operations and are environmentally benign.

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