

# 2004 AAIC/NUC Joint Annual Meeting: Industrial Crops & Uses to Diversify Agriculture

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### PLENARY SESSION

#### THE CARBOHYDRATE ECONOMY: MAKING IT HAPPEN

David Morris  
Institute for Local Self-Reliance, Minneapolis, MN 55414 USA

In 2004, we might say we are at the end of the beginning phase of using plants as a fuel and industrial material. Bioproducts, biofuels, and biologically derived energy of all types now compete in multiple markets. Yet they are still very much at the fringes of public awareness and public policy. The next stage, the take off stage, requires a coherent effort to move the biological concept into center stage. That effort is enabled by three powerful contemporary global movements: the movement to fight global warming and toxic pollution; the movement for domestic security in the face of growing unrest in oil producing regions; and the movement to defend and nurture rural economies in the face of increasingly globalized and unrestricted trade in agricultural goods.

*Contact: David Morris, Institute for Local Self-Reliance, 1313 5th St. SE, Minneapolis, MN 55414 USA. Tel.: 612-379-3815. Fax: 612 379 3920. E-mail: <http://www.ilsr.org>*

#### OVERVIEW OF AGRICULTURAL BIOMASS ENERGY PROJECT DEVELOPMENT CONSTRAINTS

Gerald R. (Gary) Elliott

International Applied Engineering, Inc., Marietta, GA, USA; Technical Association of the Pulp and Paper Industries (TAPPI), Steam and Power Committee, New Uses Council (NUC), Washington, D.C., USA

The constraints limiting the development of farm-based agricultural biomass energy projects will be discussed from a strictly commercial basis. These interdependent constraints include site location, conversion technology, feedstock, operating and maintenance, equity, financing, and environmental issues. An overview of all of these critical project development components will be provided from the perspective of the farm community and the farming entrepreneur. Some historical examples of successful projects in the U.S. will be generally presented. A Performance-based critical fault determination approach to the development of potential projects that meet the requirements of the biomass-fueled project financial community will be outlined. The objective is to provide the potential project development entrepreneur with the fundamental tools required to evaluate potential projects, and to estimate the cost of building an investment quality prospectus package.

*Contact: Gerald R. (Gary) Elliott, 1165 Allgood Road, Suite 6, Marietta, GA 30062 USA Tel.: 770-977-4248. Cell: 770-846-2076. Email: [gre@iaeinc.com](mailto:gre@iaeinc.com).*

#### THE ECONOMIC COMPETITIVENESS AND IMPACTS ON THE AGRICULTURAL SECTOR OF SWITCHGRASS AS A BIOENERGY DEDICATED CROP

[Daniel De La Torre Ugarte](#) and Chad Hellwinckel  
Agricultural Policy Analysis Center, University of Tennessee, Knoxville, TN 37996, USA

With crude oil setting new price records, oil corporations readjusting their proven reserves downward, recent bombings of oil infrastructure, and questions about the arrival of the Hubbert peak, there is much discussion about developing a bioenergy sector that is domestic, decentralized, and energy efficient. Recent analyses indicate that switchgrass can be an economically competitive biomass feedstock, which could provide large quantities of biomass, reduce erosion of agricultural lands, and improve the economic viability of the agricultural sector nationally.

Our objective is to estimate a supply curve indicating the quantities of viable switchgrass (*Panicum vergatum*) production by simulating price changes on the 10-year USDA baseline of the agricultural sector using POLYSYS. POLYSYS has been updated to include the 2002 Farm Bill changes in government payments, and the ERS livestock model is fully integrated with the crop sector. Switchgrass yields and operation budgets are estimated for each of the 305 Agricultural Statistical Districts. Switchgrass is then allowed to compete with conventional crops for land resources. Switchgrass production, conventional crop production, and producer net returns are estimated at the regional level. Price changes of conventional crops and livestock are estimated at the national level.

We determined that at the price of \$40 per dry ton, U.S. agricultural lands could place 12.1 million acres into switchgrass and produce 53 million dry tons by 2013. Additionally, land competition would raise traditional commodity prices, reduce government support payments, and increase all regions net returns. If government savings over the entire 10-year period (\$23.2 billion) were used to subsidize switchgrass production, the net cost of feedstock would fall to near zero.

At \$40 per dry ton, U.S. switchgrass production transformed into ethanol could produce the equivalent of 4.1% of domestic gasoline consumption. But after accounting for the life-cycle energy ratio of ethanol production from switchgrass, the amount of domestic gasoline displaced falls to 1.9%.

The supply curve becomes inelastic at prices approaching \$80 per dry ton indicating a limit where food and livestock demands for land resources may interfere with further expansion of switchgrass as a bioenergy crop. At this upper limit, switchgrass can produce 154 million dry tons and displace 5.4% of annual domestic gasoline consumption.

Contact: Chad Hellwinckel, University of Tennessee, 310 Morgan Hall, Knoxville, TN 37996-4519. Tel.: 865-974-5006. E-mail: chellwin@utk.edu.

## **BRINGING A NEW CROP TO MARKET**

Andrew B Hebard  
Technology Crops International, Winston Salem, NC 27116, USA

New crop development is done for many varied reasons. These include attempting to provide industry with better raw materials (functionally superior to existing materials or cheaper than existing raw materials), providing new materials for industry, providing biodiversity for the agricultural sector, providing better alternatives for growers (high returns, crop rotation opportunities), and addressing new drivers such as the need for sustainability. Once a crop is developed, it must be commercialized and brought to the market.

The process of bringing new crops to market is complex and needs to be thoroughly understood to maximize the likelihood of success. The following (non exhaustive) considerations are factored into the process: Is this 'demand pull' or 'supply push'?; What are the economics of production, the cost of goods, cost of competing/alternative raw materials, processing requirements, scale up time and cost, sustainability, legislation and intellectual property issues?

Collectively these fall into a 'grow>make>sell' model and some considerations are specific to either crop production/processing, etc. and some are generic to the entire supply chain (legislation/IP, etc.).

This presentation looks chronologically at these issues and discusses them in detail, citing examples of crops that have been commercialized successfully and some that have failed to deliver the expectations set. It also looks at crops in development and the milestones reached and those still to be realized.

Contact: Andrew Hebard, Technology Crops International. PO Box 11925, Winston Salem, NC 27116, USA. Tel.: +1 336 759 7335 Fax +1 336 759 9406. E-mail: ahebad@techcrops.com. Website: www.techcrops.com

## **THE LINK BETWEEN TECHNOLOGY TRANSFER AND INTELLECTUAL PROPERTY PROTECTION**

Sadiq Shah

Western Illinois Entrepreneurship Center, Western Illinois University, 510 N. Pearl Street, Suite 1800, Macomb, IL 61455, USA

Technology Transfer has become an important implementation mechanism to support commercialization of intellectual property resulting from federally funded research. To support economic growth higher education institutions, independent research institutes, and federal research laboratories are playing important roles to catalyze technology transfer to the private sector. The presentation will focus on the drivers for this growth and the critical steps to facilitate technology transfer.

The first step for the commercialization of a viable intellectual property requires legal protection of the idea. The legal protection of the idea plays an important role in determining the commercial value of the intellectual property, and thereby, it increases the chances for success. The presentation will cover various legal means to protect ideas that include patents, plant variety protection, copyrights, trademarks, and trade secrets. However, legal protection alone is not sufficient for commercialization. Partnership is needed with the private sector to take the idea through the necessary steps to the final launch of the product. These products ultimately improve the quality of life for the society and also create financial return for the inventors and the taxpayers.

*Contact: Sadiq Shah, Director, Western Illinois Entrepreneurship Center, Western Illinois University, 510 N. Street, Suite 1800, Macomb, IL 61455, USA. Tel.: 309-836-2700. E-mail: S-Shah@wiu.edu*

### **OPPORTUNITIES IN THE 2002 FARM BILL**

C.A. Bailey  
USDA-CSREES, Washington, DC 20250-2220, USA

The Farm Security and Rural Investment Act of 2002, also known as the 2002 Farm Bill, describes all the funding authorities and programs under which USDA operates through 2007. The 2002 Farm Bill provides a variety of new funding opportunities for research, development, demonstration, and marketing activities of biobased products and bioenergy.

The Farm Bill also includes a program entitled A Federal Procurement of Biobased Products, which will create a market pull for new products. These opportunities are anticipated to stimulate research and development for new crops and new uses, promote technology transfer, and expand markets for agricultural materials.

The Cooperative State Research, Education, Extension Service supports fundamental, early applied and pre-commercialization research through various programs, [www.csrees.usda.gov](http://www.csrees.usda.gov); in partnership with the Department of Energy Office of Biomass, the Natural Resources Conservation Service administers the Joint Biomass Research and Development Initiative [www.bioproducts-bioenergy.gov](http://www.bioproducts-bioenergy.gov); the Rural Business Cooperative Service offers programs that support value-added processing and renewable energy technologies, [www.rurdev.usda.gov](http://www.rurdev.usda.gov); the Farm Service Agency supports the Bioenergy Program, [www.fsa.usda.gov](http://www.fsa.usda.gov); and the Office of Energy Policy and New Uses maintains a website that describes the status of the program for federal purchasing of biobased products, [www.biobased.oce.usda.gov](http://www.biobased.oce.usda.gov)

*Contact: C.A. Bailey, USDA-CSREES, 800 9th St. SW, Mail Stop 2220, Washington, DC, 20250-2220, USA. Tel.: 202-401-6443. cbailey@csrees.usda.gov*

### **A NEW INITIATIVE FOR LAND GRANT INSTITUTIONS: THE SUN GRANT INITIATIVE**

Kevin Kephart  
South Dakota Agricultural Experiment Station, South Dakota State University, Brookings, SD 57007, USA

Americans benefit from reliable, affordable, safe, and secure food and energy systems. Many generations of Americans have thrived economically, partially because of abundant and affordable food and energy. It is becoming increasingly evident, however, that energy security is a national issue. Many people recognize that agriculture has a role in correcting some of the nation's projected energy problems. Land grant universities were initiated to develop programs in agricultural sciences, including food production, natural resources, and rural communities. The Sun Grant Initiative has been authorized by Congress to broaden the role of land grant universities by implementing new programs on renewable energy and biobased industries. The missions of the Sun Grant Initiative are to (1) enhance national energy security through development, distribution, and implementation of biobased energy technologies, (2) promote diversification and environmental sustainability of America's agriculture through land-grant based research, extension, and education programs in renewable energy and biobased products, and (3) promote opportunities for biobased economic diversification in rural communities.

Regional Sun Grant centers will include South Dakota State University, Oklahoma State University, the University of Tennessee - Knoxville, Cornell University, and Oregon State University. The regions will emphasize integrated research, extension, and educational programs on ag-based renewable energy and biobased industries. Some of the regional priorities include; genetic development of feedstocks for transportation fuels and power; development of enabling conversion technologies to produce a spectrum of products and commodity intermediates; specialty crops and biotechnology for production of non-food products (semi-arid areas of the region may offer unique opportunities for isolation); development of turnkey projects for rapid assistance to communities; utilization of wastes and residues from agricultural industries; and agricultural production and diversification.

Development of new energy markets and implementation of new technologies will also depend on effective partnerships between universities and private industry. Partnerships with private industry will be welcomed to achieve the mission and goals of the Sun Grant Initiative.

Contact: K. Kephart, South Dakota Agric. Exp. Sta., South Dakota State University, Brookings, SD 57007, USA. Tel.: 605-688-4149. E-mail: kevin\_kephart@sdstate.edu

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## NATURAL RUBBER AND RESIN DIVISION

### HARVESTING SMALL PLOTS OF GUAYULE FOR LABORATORY ANALYSES

T. A. Coffelt and F. S. Nakayama

USDA, ARS, U.S. Water Conservation Laboratory, Phoenix, Arizona 85040, USA

Guayule (*Parthenium argentatum* Gray) is the best potential source of hypoallergenic latex to replace latex products made with Hevea (*Hevea brasiliensis* (A. Juss.) Muell.-Arg.) latex that cause Type I allergies. Breeding programs in the past have focused on screening germplasm and developing improved lines with higher rubber and/or resin contents. Thus, previous harvesting protocols have not dealt with the problem of latex loss as plant material is being processed. An improved protocol that allows harvesting of various amounts of plant material in a manner that minimizes latex loss is needed. Furthermore, a standardized procedure will permit reliable comparison of latex analysis among different locations throughout the world.

The objective of this study was to develop a protocol that could be used to harvest various amounts of plant material from agronomic and breeding tests for laboratory analyses. A six-step protocol was developed that could process from 1 kg to over 20 kg of plant material from field plots in a form suitable for laboratory analyses with minimal latex loss. Steps involved were (1) cut the plants in the field as close as possible to ground level ( $\leq 50$  mm); (2) transport the plants from the field to the chipping area as soon as possible in bags to keep plant samples separate; (3) obtain a fresh weight for each sample; (4) process the sample through the chipper and obtain a fresh weight of the chipped material. After weighing and prior to chipping, the bag(s) containing the plant material to be chipped is emptied onto a plastic sheet to minimize sample loss during the chipping process. After the larger plant parts are chipped, the plastic sheet is carefully picked up and the smaller plant materials remaining on the plastic sheet are fed into the chipper/shredder; (5) add antioxidant solution (0.2% sodium sulfite in distilled water at a pH of about 11) so that fresh weight of plant material collected and antioxidant solution are in a 1:1 ratio; (6) thoroughly mix the antioxidant solution with the plant material by stirring the plant material after the antioxidant solution is added. Steps 2 through 6 can be done in less than 3 h to minimize latex loss. The mixture is stored at 4 to 10°C prior to laboratory analysis for latex. While this mixture can be stored under these conditions up to five weeks without loss of latex, samples used in our studies were processed within 3 to 5 days after harvest.

The proposed protocol was evaluated by processing samples harvested for latex content. If samples harvested using the proposed protocol could be processed for latex using the recommended laboratory protocol without additional preparation prior to using the laboratory procedure, then we considered the proposed methodology acceptable. Additionally, the proposed protocol should provide samples that could be used for rubber, resin, and other laboratory analyses.

This basic protocol has been used for over five years to analyze guayule plant material from plants of various ages and sizes for latex, rubber, and resin concentration. This protocol will be useful for other researchers worldwide who are starting to conduct research on guayule.

Contact: T. A. Coffelt, USDA-ARS, U.S. Water Conservation Laboratory, 4331 East Broadway Road, Phoenix, AZ 85040-8832, USA. Tel.: 602-437-1702(X238). E-mail: [tcoffelt@uswcl.ars.ag.gov](mailto:tcoffelt@uswcl.ars.ag.gov)

## DETERMINATION OF RESIN AND RUBBER FROM GUAYULE BY ACCELERATED SOLVENT EXTRACTION (ASE)

V. H. Teetor, and D. T. Ray

University of Arizona, Plant Sciences Department, Tucson, AZ 85721, USA

Accelerated Solvent Extraction (ASE™) is a technique developed by the Dionex Corporation that is designed to streamline sample preparation and analysis. The process is automated, and uses less solvent than a traditional Soxhlet extraction. The homogenizer method currently being utilized for quantification of resin and rubber from guayule has already been compared with several Soxhlet methods, and has been proven to be optimal for a small research laboratory. The ASE seemed to be an attractive alternative to the homogenizer method from a time management perspective. However, the initial cost (around \$47,000) was a bit daunting.

Our objective was to determine whether the ASE was a viable alternative (in terms of savings in time and materials) to the traditional homogenizer method to extract and quantify resin and rubber from guayule.

Finely ground plant material was weighed, mixed with a few grams of Ottawa sand to prevent the sample from clogging the filter, and transferred to an extraction cell fitted with a filter disc. The cell was loaded in the upper tray of the ASE, and a collection vial in the lower tray. The machine was programmed for the extraction and a rinse after each sample. The optimal extraction method was determined to be a 1:1 mixture of acetone and cyclohexane (achieved by use of the solvent controller), with three cycles of 20 min each (using fresh solvent for each), pressure at 1500 psi, and temperature at 140°C. The vials containing the extracts were stored in the freezer until ready to centrifuge, to reduce degradation. Twenty ml of this extract was transferred to a pre-weighed centrifuge tube and an equal volume of methanol was added to coagulate the rubber. Samples were centrifuged for twenty minutes. Most of the supernatant was pipetted into a pre-weighed aluminum pan. Approximately 5 ml was left in the tube. To this, the rest of the extract and a rinse of the vial were added, and an equal volume of methanol. Samples were centrifuged again and all of the supernatant carefully poured into the pan. After drying, the pans and tubes were weighed to determine the weight of the extracts.

Higher percent recovery of resin and rubber can be obtained with the ASE than the homogenizer method, although the ratio of resin to rubber remains the same. An analysis of costs was performed to compare the two methods. The results showed that analyzing 18 samples per day (the current capacity of the ASE, based on the number of cells purchased) by the homogenizer method would cost approximately \$145, versus \$85 to run the same number of samples on the ASE. These figures are based on the cost of consumable materials for both methods and labor (salary). The payback period (the time required for the new method to recover the costs of the initial outlay of the equipment) was calculated to be approximately 5.5 years.

*Contact: V. H. Teetor, University of Arizona, Plant Sciences Department, P.O. Box 210036, Tucson, AZ 85721, USA. Tel.: 520-621-2817. Email: teetor@ag.arizona.edu*

## EFFECT OF POSTEMERGENCE HERBICIDES ON TRANSPLANTED GUAYULE

M. A. Foster<sup>1</sup>, T.A. Coffelt<sup>2</sup>, G. Majeau<sup>3</sup>, and M.M. Main<sup>3</sup>

<sup>1</sup>Texas A&M University Agricultural Research Station, Pecos, TX 79772, USA; <sup>2</sup>U.S. Water Conservation Lab, USDA-ARS, Phoenix, AZ 85040, USA; <sup>3</sup>University of Arizona Maricopa Agricultural Center, Maricopa, AZ 85239, USA

Broad spectrum weed control is essential for successful guayule production. Guayule is a perennial crop, and weeds must not only be controlled preemergence, but also during establishment and after the shrubs have grown too large to permit cultivation.

A study was initiated in April 2003 at the University of Arizona, Maricopa Agricultural Center to test the tolerance of transplanted guayule to four postemergence herbicides. Sixty-day-old guayule seedlings consisting of lines N565, 11591, and AZ-2 were transplanted April 15 on raised beds spaced 1 m apart. The experimental design was a split block with guayule lines as main plots and herbicides as subplots with three replications. The following herbicides were applied on May 22 at ½×, 1× and 2× rates: Cadre (35, 70, 140 g ai/ha); Pursuit (35, 70, 140 g ai/ha); Pursuit Plus (0.5, 1.0, 2.0 kg ai/ha); and Staple (35, 70, 124 g ai/ha). The rates were determined from recommendations for weed control used in other agronomic crops: Cadre (peanuts); Pursuit (soybeans, field corn, peanuts, alfalfa); Pursuit Plus (soybeans, field corn); and Staple (cotton). Evaluations were made weekly by recording the number of plants in each treatment that exhibited chlorosis or were dead. Plant height and width were recorded in March 2004.

One week following spraying, the percentage of guayule plants showing chlorosis ranged from 9 to 100 with no shrub mortality. There was less chlorosis in line N565 than in AZ2 or 11591. The greatest damage occurred with the 2× rates. Four weeks after treatment the plants were actively growing and only plants in the higher rates were showing chlorosis. Plant mortality occurred only in the 2x Pursuit Plus and Cadre treatments in

the three lines. Six weeks after treatment there was no visible chlorosis and no plant mortality since the previous evaluation.

Plant height was significantly lower in all treatments versus the control except in the 1× Pursuit treatment. Plant width was significantly different than the control only in the 2× Pursuit and Cadre treatments when compared to the control. Although significant differences occurred in plant height, latex and rubber and resin production may not be different when the shrubs are harvested at two-years of age in 2005. These harvests will determine whether plant production has been significantly affected by the herbicide treatments.

Contact: Mike Foster, Texas A&M University Agricultural Research Station, Pecos, TX, 79772, USA. Tel.: 432 445-5050. E-mail: mafoster@tamu.edu.

## **BIOMASS, RUBBER AND RESIN POTENTIAL OF MEXICAN GUAYULE CULTIVATED UNDER NATURAL ENVIRONMENTAL CONDITIONS AND EVALUATED AT DIFFERENT PLANT AGES**

D. Jasso de Rodríguez<sup>1</sup>, R. Rodríguez García<sup>1</sup>, and J. L. Angulo Sánchez<sup>2</sup>

<sup>1</sup>Universidad Autónoma Agraria Antonio Narro (UAAAN), Saltillo, Coahuila, 25315, México; <sup>2</sup>Centro de Investigación en Química Aplicada (CIQA), Saltillo, Coahuila, 25100, México

Mexico has large areas of natural stands of guayule distributed in six states in the Northern region. Evaluation of shrubs in these stands shows plants with up to 17% rubber content. Guayule has been studied since the early 1980s with different accessions under natural environmental conditions at the Antonio Narro University experimental field.

The purpose of this study was to evaluate 5-, 10, and 20-year old guayule shrubs cultivated under natural environmental conditions for their biomass, rubber and resin contents, and morphological characteristics.

The 2003 study was conducted on three separate plots cultivated under natural environmental conditions at the University experimental field at Buenavista, Saltillo, Coahuila and the Navidad, Nuevo Leon site located 90 km from the University. The 5- and 10-year old shrubs were in Coahuila, and the 20-year-old in Nuevo Leon. The seeds used in the experiments were collected in Norias de Guadalupe, Zacatecas and Mapimí, Durango. During February 2003, 30 whole plants, randomly selected from each plot were uprooted and transported to the laboratory for dry weight, main stem diameter, height, branch number, and rubber and resin content determination.

The 5-years-old plants weighed 185.5 g/plant, with 1.83 cm main stem diameter, 3.53 cm height, and rubber and resin content of 5.6% and 14.3%, respectively. The 10-year-old shrubs were 600% heavier, 116% taller, with 92.2% larger main stem diameter, and 142% greater rubber content than the 5-year-old shrubs. This plot had plants with up to 19.9% rubber. For the 20-year-old shrubs, the dry weight increased 8.7% with respect to the 10 years old plants, and the rubber content was 25% lower. Surprisingly, the resin content remained constant for all the different shrub ages.

This information is important because natural stands with plant ages ranging from 5, 10, and 20 years and older plants may support the initial shrubs requirements for commercial guayule rubber production.

Contact: D. Jasso de Rodríguez, Universidad Autónoma Agraria Antonio Narro, Buenavista, Saltillo, Coahuila, 25315, México. Tel: 844 4110220. E-mail: dianajassocantu@yahoo.com.mx

## **THE EFFECT OF WATER STRESS ON PHOTOSYNTHESIS AND SECONDARY COMPOUND PRODUCTION IN GREENHOUSE GROWN GUAYULE**

Maren E. Veatch and Dennis T. Ray

Department of Plant Sciences, The University of Arizona, Forbes 303, Tucson, AZ 85721, USA

As a native of the Chihuahuan desert, guayule (*Parthenium argentatum* Gray) has a natural history of exposure to water stress. Guayule's rubber production has been suggested to be triggered in part by environmental stresses. At this time cold night temperatures are considered the best candidate environmental trigger; however, there is evidence from field trials that water stress increases rubber concentration within the plant. It may be that water stress, alone or in combination with cooler temperatures, can trigger rubber production. In order to make rubber the plant needs to have an available pool of photosynthates, and this cannot occur if the photosynthetic pathway has been damaged by water stress.

The objective of this study was to determine the effect of water stress on carbon exchange and how this affects resin and rubber production.

Guayule plants of the lines AZ 101 and AZ-2 were subjected to water stress from June through August 2003. The water stressed plants were

irrigated every 4 days, when the average soil water potential was 6 bars. Plant height was measured weekly and width was measured monthly, in order to evaluate the effect of water stress on growth. Photosynthesis was estimated by measuring carbon exchange rate with the Licor 6200. As there was a difference in leaf expansion before and after irrigation, photosynthesis was measured the day before and the day after irrigation. At harvest not only was the fresh weight of each plant recorded, but also a subset of plants had leaf weight and leaf area measured. Resin and rubber were extracted with acetone and cyclohexane respectively, from dried plant samples. Resin and rubber yields were calculated on a per plant basis.

Although photosynthetic rates were increased after an irrigation in the water-stressed plants, the stressed plants had consistently lower photosynthetic rates than the well-watered plants. In addition to a low photosynthetic rate, water stressed plants also had a low growth rate, low biomass production, and decreased leaf area. Rubber concentration was increased in the water stressed plants, but yields of both rubber and resin were reduced compared with the well-watered plants. In water stressed plants, the leaves also contributed less to overall biomass, and as leaves have low rubber content, the higher rubber concentration in the water stressed plants may due to the increased contribution of the stems to the biomass.

Water stress, which causes a slight increase in rubber concentration in guayule, is not a viable option for increasing rubber production, as the increased rubber concentration cannot compensate for the decreased biomass production.

Contact: Maren E. Veatch, Department of Plant Sciences, University of Arizona, Forbes 303, Tucson, AZ, 85721, USA. Tel.: 520-621-2817. E-mail: veatchm@email.arizona.edu

### **GERMINATION OF GUAYULE (*Parthenium argentatum* GRAY) SEED (ACHENES) BEFORE AND AFTER X-RAY ANALYSIS**

M. H. A. Jorge<sup>1,2</sup> and D. T. Ray<sup>2</sup>

<sup>1</sup>Student sponsored by CAPES, Brazil; <sup>2</sup>Department of Plant Science, The University of Arizona, Tucson, AZ 85721, USA

The quality of guayule seeds used to obtain proper stand establishment is an important element of guayule production. The X-ray technique is a powerful tool that provides information about the presence or absence of essential structures in the seed. The objective of this research is to assess and compare data of germination percentage and rate before and after separation of seed by X-ray analysis.

Seeds were first separated by color using a magnifying scope equipped with fluorescent lights. Gray and opaque seeds were then chosen and used in germination tests after the initial screening. X-ray analysis was performed to detect seeds with well-developed essential structures for germination (filled seed) among gray and opaque black seeds, and the germination tests run again.

For the germination tests, seeds were placed into Petri dishes with two filter papers on the bottom moistened with water equivalent to 2.5 times the weight of the two filter papers and germinated at 25°C with 24 h. fluorescent illumination for 10 days.

Seed size (1/14, 1/16, and 1/18 inches) and pre-conditioning treatment (soaking in water for 6 hours and treatment with 2% sodium hypochlorite - NaOCl - for 2.5 minutes) were previously reported to give the best germination.

The results showed that, before X-ray analysis, germination percentage and rate were not significantly different among the seed sizes studied. Seed pre-soaked in water and treated with NaOCl showed better germination rate than the other treatments. In addition, fungi infestation was higher in the treatments in which NaOCl was not used.

After X-ray analysis, the same patterns of results were found. However, the overall germination percentage and rate for all treatments was higher than seeds tested before the X-ray analysis.

In conclusion, the X-ray analysis was an efficient method to improve the quality of a guayule seed lot. The results showed that pre-conditioning in water and NaOCl gave the highest germination percentage and rate.

Contact: D. T. Ray, Plant Science Department, The University of Arizona, Forbes Building, Tucson, AZ 85721, USA. Tel.: 520-621-7612. E-mail: dtray@u.arizona.edu

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**OILSEED DIVISION****GROWER PRODUCTION OF LESQUERELLA IN ARIZONA**M.A. Foster<sup>1</sup>, D.A. Dierig<sup>2</sup>, and D.T. Ray<sup>3</sup>

<sup>1</sup>Texas A&M University Agricultural Research Station, Pecos, TX, 79772, USA; <sup>2</sup>U.S. Water Conservation Laboratory, USDA-ARS, Phoenix, AZ, 85040, USA; <sup>3</sup>The University of Arizona, Plant Sciences Department, Tucson, AZ, 85721, USA

Research has shown that lesquerella (*Lesquerella fendleri*) can be cultivated in the arid and semiarid Southwestern U.S. Growers need experience in growing lesquerella before this new crop can be commercialized. A reliable supply of seed must be guaranteed before industrial markets can be established. The objectives of this study were to establish large-scale plantings with local growers, to provide seed to industry for testing and evaluating new products unique to lesquerella, and to develop agronomic guidelines for growers.

Lesquerella was planted in three locations in 2003: (1) Jon Chernicky Farm, Stanfield, AZ – seeded October 7 with Brillion seeder at 11 kg seed/ha and drip irrigated; (2) The University of Arizona Maricopa Agricultural Center, Maricopa, AZ – seeded October 9 with Brillion seeder at 11 kg seed/ha and border irrigated; and (3) Larry Rovey Farm, Peoria, AZ – seeded October 10 with Brillion seeder at 11kg seed/ha and border irrigated. Preplant incorporated treatments of Treflan (1.1 kg ai/ha) were applied. Postemergence treatments of Goal (0.6 kg ai/ha) and Fusilade (0.2 kg ai/ha) were applied to control broadleaf and grass weeds. Honeybees (1 hive/0.6 ha) were placed in each field during peak flowering.

Harvests were conducted in June 2004 with conventional combines modified for harvesting alfalfa seed. The Stanfield and Peoria fields were allowed to dry naturally, and the Maricopa field was desiccated with paraquat (0.6 kg ai/ha) before harvesting. Seed yields were 460 kg/ha at Peoria, 2081 kg/ha at Stanfield, and 1350 kg/ha at Maricopa. The low yield at Peoria was probably caused by the combination of weed competition and the resulting impact of large weeds during combining, and plant water stress related to irrigation scheduling.

In the past 10 years, lesquerella seed yields in combine harvested fields averaged 900 to 1000 kg/ha with oil content of 20 to 24%. Our results indicate that the yield traits have improved significantly through improved breeding lines and agronomic practices. A conservative estimate now is 1344 kg seed/ha with 29% oil content. Advances are occurring each year, and within four years, seed yields are expected to reach 2200 kg/ha with oil content of 37%.

Grower experience during 2003 and 2004 helped to update grower guidelines to include seed germination, planting dates, planting methods, irrigation and salinity management, weed control, insect and disease control, pollination, fertilization, harvesting, seed yields and oil content, and crop budgets.

Contact: Mike Foster, Texas A&M University Agricultural Research Station, Pecos, TX, 79772, USA, Tel.: 432 445-5050. E-mail: mafoster@tamu.edu

**PHOTOSYNTHETIC RESPONSES OF *LESQUERELLA* TO ELEVATION AND TEMPERATURE**

N.R. Adam, D.A. Dierig, T.A. Coffelt, and G.W. Wall

U.S. Water Conservation Laboratory, USDA-ARS, Phoenix, AZ 85040, USA

Lesquerella is a new alternative oilseed crop for the southwest United States. A greater understanding of the environmental constraints on photosynthesis is important in determining potential areas for seed production of this crop, especially in estimating growing degree days required for plant production.

The objective of this study was to determine the effects of elevation and temperature on photosynthesis of the various species of lesquerella throughout their life cycle. Field studies were established at four different elevations in Arizona with two species of Lesquerella, *L. fendleri*, and *L. pallida*. These two species were chosen because the traits from *L. pallida* are being introgressed into *L. fendleri* through the breeding program at this Laboratory.

Plots of *L. fendleri* and *L. pallida* were established at four locations in Arizona: Phoenix, elevation 305 m; Tucson, 607 m; Safford, 914 m; and Patagonia, 1219 m. Preliminary analysis indicates accession differences in photosynthesis response patterns at the various locations. Season-long values of photosynthesis for *L. fendleri* were significantly lower in Patagonia than in Phoenix and Tucson, whereas the rates for *L. pallida* were significantly greater in Patagonia than in Phoenix and Tucson. The Phoenix and Tucson locations did not differ in responses of either species. Although survival was poor in Safford, and midseason rates of photosynthesis for *L. pallida* were lower than in the other locations, midseason rates

of photosynthesis for *L. fendleri* were similar to those of the other locations.

These results indicate that the two species responded differently to the four elevation environments. This could be an advantage in that hybrids from the interspecific crosses could have a wider adaptation for seed production areas than either of the parents.

Contact: N.R. Adam, U.S. Water Conservation Laboratory, 4331 East Broadway Road, Phoenix, AZ 85040, USA. Tel.: 602-437-1702 (X247). E-mail: nadam@uswcl.ars.ag.gov

## OIL CONTENT AND SEED YIELD IMPROVEMENT IN *LESQUERELLA FENDLERI*

David Dierig and Gail Dahlquist

U.S. Water Conservation Laboratory, USDA-ARS, Phoenix, AZ 85040, USA

*Lesquerella fendleri* has many agronomic traits that are suitable for commercialization as an oilseed crop. The plant does not shatter excessively, has high seed yields, and contains a high amount of genetic variability. Increasing the oil content of the seeds of this crop would make it more profitable for industry users and growers, enhancing *Lesquerella*'s competitiveness with castor oil as a biodegradable oil without the toxic ricin that is contained in castor meal. Our objective has been to develop new *lesquerella* germplasm with higher oil contents compared with previously released lines using a variable, open pollinated population. The last official germplasm release was made in year 2001 with 29% oil content.

Fifteen hundred individual plants were harvested in years 2001, 2002, and 2003 and analyzed for oil content. The top 15% of the population was selected each year and planted the following fall. The resulting germplasm line, 03LO, was then grown in a yield trial in the 2003-04 growing season at two Arizona locations and compared with a 2001 released line (WCL-LY2) and an unselected germplasm accession (A4042). Plant height, widths, and biomass were measured six times during the growing season beginning in early March.

The oil content was 33% for line 03LO, 30% for WCL-LY2, and 24% for A4042. All three lines were significantly different from each other, but not between locations [this is not too clear]. The seed yield of 03LO was also higher than the other two lines at both locations. 03LO yielded 40g compared with 34 g and 8 g for the other two lines. Biomass production of WCL-LY2 was, in most cases during the growing season and at harvest, higher than 03LO even though 03LO had higher seed yields. Plant height and width were not significantly different between 03LO and WCL-LY2 and both were taller and wider than the unselected accession A4042. After the first week of April, none of the three lines at both locations increased significantly in height or width. The harvest index of line 03LO was 30.15 and 27.7 for the two locations compared with 18.2 and 18.3 for WCL-LY2, and 17.0 and 15.8 for A4042.

The new selection 03LO is improved over the previously released line WCL-LY2 in both oil content and seed yield and should increase the market value of the seed. Our results based on a plant population of 750,000 plants per ha could produce seed yields of 30,000 kg per ha. This population and seed yield possibly may be increased with improved agronomic practices.

Contact: D.A. Dierig, U.S. Water Conservation Laboratory, USDA-ARS, Phoenix, AZ 85040, USA. Tel: 602-437-1702(X265). Email: ddierig@uswcl.ars.ag.gov

## A NEW CYTOTYPE OF *LESQUERELLA PALLIDA*

Darren Ames<sup>1</sup>, Dennis T. Ray<sup>1</sup>, David A. Dierig<sup>2</sup>, and Pernell M. Tomasi<sup>2</sup>

<sup>1</sup>Department of Plant Sciences, The University of Arizona, Tucson, AZ 85721, USA; <sup>2</sup>U.S. Water Conservation Laboratory, USDA-ARS, Phoenix, AZ 85040, USA

The fatty acid seed oil profile of many *Lesquerella* species makes them ideal candidates for use in an assortment of industrial applications. Efforts to introgress higher lesquerolic acid content from *L. pallida* into *L. fendleri* have resulted in hybrids with improved oil profiles, but low levels of fertility. This study was initiated to identify the chromosome numbers and pairing in the parent species and interspecific hybrids.

Meiotic chromosome pairing in *L. pallida*, *L. fendleri* and the interspecific hybrid *L. pallida* × *L. fendleri* was visualized microscopically by staining immature anthers with acetocarmine and isolating meiocytes. Diakinesis or metaphase I chromosome configurations were examined and scored to determine chromosome number and pairing.

The interspecific hybrids of *L. pallida* × *L. fendleri* were found to be sterile, and to enhance fertility, the chromosome number was doubled using colchicine. The doubled interspecific hybrids were all found to have 24 chromosomes (2n = 24, n = 12). This implied that the chromosome number of the *L. pallida* and *L. fendleri* parent plants was 2n = 12 (n = 6). *L. fendleri* was found to be 2n = 12 (n = 6, with 6 bivalents, 6 II). However, 19

*L. pallida* plants were analyzed, and all had  $2n = 34$  ( $n=17$ , with 17 II), rather than  $n=6$ , as was expected and has been previously reported.

The  $n = 17$  *L. pallida* is a never before described cytotype, and is undoubtedly a polyploid (triploid), with aneuploidy ( $6 + 6 + 6 - 1 = 17$ ) of an  $n = 6$  *L. pallida* population. As natural *L. pallida* populations are small and isolated, polyploidy and aneuploidy would not necessarily be uncommon, especially around the periphery of the species range.

It appears that the  $n = 17$  *L. pallida* plants were not used in making the interspecific hybrids with *L. fendleri*, although the  $n = 6$  *L. pallida* has yet to be found. The hybrids do show differing amounts of fertility, and this may be due to differing amounts of chromosome pairing. The most common pairing during meiosis in the hybrids was 11 II + 2 I (11 bivalents + 2 univalents), followed by 10 II + 4 I and 12 II; with anaphase bridges detected at a high frequency in particular plants. Although the data are scant, it appears that there may be a positive correlation between chromosome pairing and fertility (plants with predominately 12 II having the highest fertility, and the fertility decreasing with increased numbers of univalents).

Contact: D. T. Ray, Department of Plant Sciences, The University of Arizona, Tucson, AZ 85721, USA. Tel: 520-621-7612. E-mail: dtray@u.arizona.edu.

### DATA MINING FOR MICROSATELLITES IN EXPRESSED SEQUENCE TAGS (ESTS) FROM ARABIDOPSIS THALIANA AND BRASSICA SPECIES, FOR USE IN LESQUERELLA (BRASSICACEAE)

A. Salywon<sup>1</sup>, M. Barber<sup>2</sup>, N. Herling<sup>2</sup>, W. Stewart<sup>2</sup>, and D.A. Dierig<sup>1</sup>

<sup>1</sup>U.S. Water Conservation Laboratory, USDA-ARS, Phoenix, AZ 85040, USA; <sup>2</sup>Computational Biosciences Program, Arizona State University, Tempe, AZ 85287, USA

*Lesquerella* (Brassicaceae) species are being developed as sources of hydroxy fatty acids with potential for industrial applications. Microsatellite markers can accelerate selection for favorable traits in breeding programs and may be useful in identifying trait loci. Yet, developing microsatellite markers de novo is costly and time-consuming. A cheap and relatively less time-consuming alternative for developing microsatellites entails data-mining public databases for these sequences and designing primers in the flanking regions. For some taxa, the frequency of microsatellites is higher in transcribed regions than in genomic DNA. Therefore, data-mining expressed sequence tags (ESTs) have proven to be an attractive method for many research groups. Because the primers developed for these microsatellite-ESTs are derived from transcribed regions, they have a higher probability of transferring between related species and genera, thereby, significantly extending the usefulness of the primers.

The purpose of this study was to use databases of expressed sequence tags from *Arabidopsis thaliana* L. and *Brassica* crop species to determine the potential for the development of microsatellite markers that amplify across generic boundaries within Brassicaceae and especially in *Lesquerella*.

In April 2004, 347,844 *Arabidopsis thaliana* and 44,851 *Brassica* EST sequences were downloaded from public databases. Sequences containing microsatellites were identified using Perl script and microsatellite-ESTs were then masked using RepeatMasker Program and clustered using StackPACK 2.0 system (with associated d2\_cluster, Phrap, and CRAW programs). The EST database was then again queried with microsatellite containing ESTs clusters to extend the consensus sequences and reduce redundancy by clustering significantly similar ESTs. Information from all stages was stored in a relational database.

Output files from StackPACK after clustering identified 2,058 microsatellite-ESTs for *Arabidopsis* and 540 microsatellite-ESTs for *Brassica* spp. In both *Arabidopsis* and *Brassica*, tri-nucleotide repeat motifs were found to be the most abundant (69 and 59% respectively), followed by di-nucleotide repeat motifs (23 and 36% respectively) and tetra-nucleotide repeat motifs (8 and 5% respectively). Preliminary results from clustering of orthologous microsatellite-ESTs from *Arabidopsis* and *Brassica* indicate that this method can be used to develop microsatellite primers that may amplify across the genera in the family for population or genomic studies although the realized number of orthologous regions for comparison is currently limited by the number of ESTs available.

This study indicates that EST data from the model organism *Arabidopsis thaliana* and from the agriculturally important *Brassica* spp. can potentially benefit molecular genetics studies of other related genera, such as *Lesquerella*, for which little, if any, sequence data may exist.

Contact: A. Salywon, U.S. Water Conservation Laboratory, 4331 E. Broadway Rd., Phoenix, AZ 85040, USA. Tel.: 602-437-1702 (X190). E-mail: asalywon@uswcl.ars.ag.gov

### SUPPLEMENTAL POLLINATION OF JOJOBA

W. Coates<sup>1</sup>, R. Ayerza<sup>1</sup>, and D. Palzkill<sup>2</sup>

<sup>1</sup>Office of Arid Lands Studies, The University of Arizona, Tucson, AZ 85706, USA; <sup>2</sup>Dryland Plants, 261 E. Blackridge Dr., Tucson, AZ 85705, USA

Jojoba (*Simmondsia chinensis* (L.) Schneider) is a perennial evergreen shrub that grows naturally in the Sonoran Desert of the southwestern U.S. and northwestern Mexico. Jojoba is dioecious with small single sex flowers produced on separate plants and is strictly a wind-pollinated plant.

One of the persistent problems confronted during the development of jojoba as a crop has been inconsistent and/or low yields. It is likely that much of this variation is due to pollination problems. Some studies have shown that the male/female ratio in most commercial jojoba plantations around the world today as being too low for maximum seed production.

Supplemental pollination in olives and other tree crops is becoming more and more common, and has been shown to increase yields. To determine whether such a practice would also prove beneficial in jojoba a project was initiated in which pollen was harvested from male plants and then applied to female plants. Due to north/south seasonal differences, pollen was collected in Argentina and then applied in Arizona during two seasons.

Results from the first season showed that the application of supplemental pollen was advantageous, whereas application in the second year did not produce similar results. The second year's testing did show, however, that a dramatic decrease in pollen viability occurred during the collection period. This information indicates that both the pollen application and collection dates are critical.

Contact: W. Coates, Office of Arid Lands Studies, The University of Arizona, 250 E. Valencia Road, Tucson, AZ 85706, USA. Tel.: +1 520-741-0840. Fax: 520-741-1468. E-mail: wcoates@u.arizona.edu.

#### SYNTHESIS OF NOVEL ULTRAVIOLET FILTERS FROM NEW CROP (JOJOBA) OIL

R.E. Harry-O'kuru<sup>1</sup>, A. Mohamed<sup>2</sup>, and T.P. Abbott<sup>3</sup>

<sup>1</sup>New Crops and Processing Technology Research Unit and <sup>2</sup>Cereal Products & Food Science Research Unit, National Center for Agricultural Utilization Research, USDA-ARS, 1815 N. University Street, Peoria, IL 61604, USA; <sup>3</sup>P. O. Box 206, Cle Elum, WA 98922, USA

Jojoba (*Simmondsia chinensis*) is a semiarid perennial shrub native to the Sonoran desert. The seed contains a liquid wax ester comprising 50 to 60% of the seed weight. This oil is uniquely composed primarily of C38:2, C40:2, C42:2, C44:2, and C46:2 species with one double bond in the carboxyl moiety and the other in the alcohol portion of the molecule. This structural feature gives the oil a skin-softening characteristic similar to sperm whale oil. As a result, jojoba oil is already in the market in many cosmetic formulations.

In order to further diversify and expand utilization of the oil, which is presently the only marketable component of the crop, we have chemically modified the carbon-carbon double bonds of the oil to generate new derivatives. Facile epoxidation of the olefinic groups followed by ring-opening in dilute mineral acid gave the tetrahydroxyjojoba intermediate. Esterification of the hydroxyl groups of the intermediate with *trans*-4-hydroxy-3-methoxycinnamic acid (ferulic acid) in the presence of anhydrous ZnCl<sub>2</sub> as catalyst gave jojobacinnamoyl esters.

These ferulates strongly absorb both long and short wavelength ultraviolet radiation at very low concentrations. Their strong UV absorbing property make these materials excellent UV-filter candidates in an assortment of applications. The natural emollience of the native oil amplifies the utilitarian efficiency of the ferulates as sunscreen base materials. The impact of this material would be increased demand for jojoba oil, and consequently increase the area planted to jojoba.

Contact: Rogers E. Harry-O'kuru, New Crops & Processing Technology Research Unit, National Center for Agricultural Utilization Research, USDA-ARS, 1815 N. University Street Peoria, IL 61604. Tel.: 309 681-6341. E-mail: harryore@ncaur.usda.gov

#### CUPHEA SEEDING DEPTH AFFECTS PLANT STANDS

M. T. Berti, B. L. Johnson, and P.J. Petersen

Plant Sciences Department, North Dakota State University, Fargo, ND, 58105, USA

*Cuphea viscosissima* Jacq. × *C. lanceolata* f. *silenooides* W.T. Aiton is a new crop being developed in the northcentral U.S. as an industrial oilseed crop. Cuphea seed has 30 to 35% oil that is rich in medium chain length fatty acids such as capric acid, which is used for chemical manufacturing.

Obtaining a good plant stand is essential for successful commercial production. The objective of this trial was to determine the optimal seeding depth in order to have the highest plant stands.

Seeds of *Cuphea viscosissima* Jacq. × *C. lanceolata* f. *silenoidea* W.T. Aiton PSR23-MN were sown in June 28, 2004, at Prosper, North Dakota (47° 0' N, 97° 7' W) . The experimental design was an RCBD with four treatments and four replications. Treatments were four seeding depths (13, 25, 38, and 51 mm). Seeding rate was 14.6 kg ha<sup>-1</sup>. Each experimental unit had six rows 4.6 m long with a spacing between rows of 31 cm. The trial was fertilized with 89.6 kg ha<sup>-1</sup> of nitrogen as urea fertilizer. Weed control was with preplant incorporation of trifluralin and supplemental hand weeding. Evaluations on plant stands were done on July 21 for final emergence.

Significant differences were found for seeding depth. As seeding depth increased, plant stands were reduced. The best plant stands were obtained with 51 plants m<sup>-2</sup> with seed placement at 13 mm depth. The next seeding depth at 25 mm had significantly lower plant stands of 19 plant m<sup>-2</sup>. However, this stand would still be adequate for high seed yield as the plants branched to compensate for the lower stand density. Few plants emerged from the 38 mm depth and no plants emerged at the 51 mm seeding depth.

Contact: M. Berti, Plant Sciences Department, North Dakota State University, Fargo, ND 58105, USA. Tel.: (701)-231-8158. E-mail: Marisol.beriti@nodak.ndsu.edu

## CULTIVAR AND GROWING LOCATION EFFECTS ON CANOLA SEED COMPOSITION

Harbans L. Bhardwaj and Anwar A. Hamama

Agricultural Research Station, Box 9061, Virginia State University, Petersburg, VA 23806, USA

Extensive research has demonstrated that canola (*Brassica napus* L.) has potential as a source of healthy oil for human consumption. However, substantial amounts of canola seed could be used as feed or as fertilizer for organic production of crops depending upon the composition of whole canola seed. This information is currently unavailable for canola produced in the mid-Atlantic region of United States.

The objectives of our studies were to determine the effects of cultivars and growing locations on the composition of canola seed.

We used data from 10 canola cultivars from the National Winter Canola Variety Trial of 2001-2002 that were grown in Virginia at three locations (Orange, Petersburg, and Suffolk). These cultivars were the top 10 based on mean seed yield at these locations. Whole seeds from two replications at each location were analyzed for seed composition.

Significant variation existed among ten cultivars for seed yield and protein, oil, P, K, Ca, Mg, S, Mn, B, Cu, and Zn contents, but not for fiber, Fe, and Al. Growing locations exhibited significant effects on all traits under consideration. The whole canola seed (% dry weight basis) was composed of N = 3.97, P = 0.83, K = 0.78, Ca = 0.39, Mg = 0.33, and S = 0.50; the elemental composition (ppm) was Al = 12.0, B = 12.9, Cu = 3.7, Fe = 101, Mn = 34.2, and Zn = 43.6; the fiber, oil, and protein contents were (% dry weight basis) 39.2, 24.8, 16.1, respectively. The protein content was positively correlated with P, Ca, S, B, Cu, and Zn contents, whereas a negative correlation existed between protein and oil contents. The oil content of the seed was negatively correlated with P, K, Ca, and Zn contents. The fiber content was positively correlated with protein, Mn, B, and Zn contents. Seed yield was not correlated with any of the seed composition traits. Canola whole seed contained more oil, Mg, Ca, Mn, and Cu, and less protein, P, K, Fe, and Zn than soybean seed.

These results indicate that canola seed may have potential use as a food/feed to replace soybean. Based on mineral composition, whole seed canola may also be useful as a soil amendment.

Contact: H. L. Bhardwaj, Agricultural Research Station, Box 9061, Virginia State University, Petersburg, VA 23806, USA. Tel.: 804-524-6723. E-mail: hbhardwj@vsu.edu

## MODIFYING VEGETABLE OIL MACROMONOMERS FOR OPTIMAL INCORPORATION IN WATERBORNE SYSTEMS

C. Quintero, L. Martinez, S. K. Mendon, and S. F. Thames

The University of Southern Mississippi, School of Polymers and High Performance Materials, 118 College Drive #10037, Hattiesburg, MS, 39406, USA

The Thames Research Group (TRG) proposed the use of vegetable oil macromonomers (VOMMs) as co-monomers in emulsion polymerization to reduce volatile organic compound (VOC) emissions from architectural coatings. The main advantages of VOMMs are the temporary plasticization

of the polymer due to the long chain fatty acids and the potential for crosslinking by oxidative cure. However, VOMM incorporation in emulsion polymerization is challenging as the process relies on diffusion of highly hydrophobic VOMMs through the water phase. Special techniques such as high shear, mini-emulsification and high surfactant levels have been developed to incorporate VOMMs into latex formulations. However, their performance resembles more that of hard and soft polymer blends than that of crosslinked systems. Reducing VOMM hydrophobicity to allow diffusion through the water phase and into the particles is expected to improve incorporation and enhance overall performance.

Ethoxylate groups were chosen to impart hydrophilicity to VOMMs. Ethoxylated fatty acids were used as model molecules to mimic VOMM behavior. Saturated and unsaturated fatty acid surfactants with various ethoxylate lengths were acrylated and characterized by <sup>1</sup>H nuclear magnetic resonance (NMR), Fourier transform infrared (FTIR) spectroscopy, high performance liquid chromatography (HPLC), and photo-differential scanning calorimetry (photo-DSC). Macromonomer diffusion through the water phase was monitored by measuring particle size distribution and residual monomer throughout the polymerization.

Model molecules with lower hydrophobicity proved to incorporate better in conventional emulsion polymerization and no special technique was needed in order to obtain coagulum-free latexes and glossy films. For acrylated fatty acids, an ethoxylate chain of 10-13 units was enough to provide diffusion through the water phase.

It is clear that the hydrophobic nature of macromolecules is of great importance for VOMM technology. Diffusion to the growing particles permits more homogeneous incorporation and potentially maximizing the individual performance of VOMMs. Yet another advantage of using lower hydrophobic molecules is that the use of special techniques such as high shear is avoided. Future work will concentrate on modifying VOMMs to achieve higher unsaturation molecules and to evaluate the effect of size on macromonomer diffusion.

*Contact: C. Quintero, The University of Southern Mississippi, Hattiesburg, MS 39406-10037, USA. Tel.: 601-266-5618. E-mail: Carolina.Q.Quintero@usm.edu*

## **FUNCTIONALIZED SOY OIL DERIVATIVES FOR LATEX AND COATINGS APPLICATIONS**

Sandipan Dutta, Nicholas Alexander Gariano, Shelby F. Thames

The University of Southern Mississippi, School of Polymers and High Performance Materials, 118 College Drive #10037, Hattiesburg, MS 39406, USA

The coatings industry continues to seek the development of technology that reduces volatile organic compounds (VOCs) because of market demand and regulatory pressures from Environmental Protection Agency, South Coast Air Quality Management District, and Ozone Transport Commission. The 2003 global market size of 10 million metric tons of latex annually, worth \$10 billion, speaks for itself and illustrates the importance of this field. Contrary to popular opinion, waterborne coatings continue to possess significant levels of VOCs because they are typically formulated with organic cosolvents, despite technology advancement. These cosolvents, added primarily to facilitate good film formation of higher T<sub>g</sub> polymers, vaporize into the atmosphere after the coatings have been applied, omitting VOCs.

Our group has pioneered the development of novel technology leading to the manufacture of waterborne architectural coatings with little-to-no-odor and zero VOCs. Also, the development of solvent-free and odorless latex polymers has been possible due to the design, synthesis, and utility of a novel class of new vegetable oil macromonomers (VOMMs).

The present work is directed towards functionalizing soy oil derivatives that are useful in latexes and coatings. Typically, an ethylenically unsaturated vegetable oil is modified by the addition of an enophile or dienophile having an acid, ester, or anhydride functionality. The modified vegetable oil is finally reacted with a functional vinyl monomer with a suitable hydroxy, amine, thiol, oxirane, or other functional groups to form the vegetable oil derivative.

Specific to this presentation, soybean oil is reacted with maleic anhydride to form a maleinized soybean oil derivative. This modified renewable product is then further reacted with a suitable functional vinyl monomer to form a VOMMs series.

These soy based VOMMs are then characterized and screened for three main attributes: the ability to perform as a comonomer in free-radical emulsion polymerization, the function as an internal plasticizer, and finally, the role of VOMMs in crosslinking to develop high molecular weight polymers after application as decorative or protective thin films.

*Contact: Dr. Sandipan Dutta, The University of Southern Mississippi, Hattiesburg, MS 39406, USA, Tel.: 601 266 5618. E-mail: Sandipan.D.Dutta@usm.edu.*

[Top of Page](#)[Return](#)**GENERAL CROPS DIVISION****FLAX FIBER IN THE USA: PRODUCTION, PROCESSING, AND EVALUATION**D.E. Akin<sup>1</sup>, J.A. Foulk<sup>2</sup>, R.B. Dodd<sup>3</sup>, and W.H. Morrison III<sup>1</sup><sup>1</sup>Russell Research Center, ARS-USDA, Athens, GA 30604, USA; <sup>2</sup>Cotton Quality Research Station, ARS-USDA, Clemson, SC 29633, USA;<sup>3</sup>Clemson University, Clemson, SC 29634, USA

Bio-based fibers are now sought for a variety of commercial applications, and bast fibers are of particular value in this regard. Bast fibers must be separated from non-fiber fractions of the stem (i.e., retted) and then mechanically cleaned of contaminants. A system of judging fiber properties is needed to determine the fiber quality and the most appropriate application. The objective of this work is to develop and evaluate methods that support a flax fiber industry in the USA. We report recent research on the production of flax for fiber, new retting procedures, the establishment of a flax fiber processing pilot plant, and the work on the development of objective standards through ASTM International.

Flax was produced as a winter crop in the coastal plain of South Carolina and as a traditional summer linseed crop from North Dakota. Flax was dew-retted or retted by a newly developed enzyme-retting system, with various formulations tested and ranked based on cost and fiber yield and quality. Enzyme-retted and dew-retted flax were then mechanically cleaned in a pilot plant, which was recently established in Clemson, SC. This system is based on commercial equipment designed to extract total, non-aligned and non-uniform fiber from stems. Fiber quality was judged based on methods that are currently being documented as test methods in ASTM International through the Flax and Linen Subcommittee D 13.17.

Flax fiber of good yield and quality was produced in the southeast as a winter crop, with traditional farm equipment used to plant, harvest, and bale flax stems. Plants grown to full seed maturity, such as that for linseed production, also provided fibers, but properties differed from those produced in plants grown to optimize fiber yield and quality. Flax from these diverse sources was successfully cleaned through the USDA Flax Fiber Pilot Plant in Clemson, SC. Retting influenced the level of cleanliness and fine fiber yield. Furthermore, the proportion of components in the enzyme-retting formulation influenced yield and properties of the fiber. In similar sources of flax, enzyme-retted fibers were weaker than dew-retted ones, likely due to the presence of cellulases in the commercial enzyme product. In addition to enzyme-retting, modifications in field retting methods resulted in improved fiber quality for samples processed through the pilot plant.

Flax fiber can be a successful new crop for the USA, thus providing a source of natural fibers for multiple uses. In the southeast, flax can be grown as a winter crop, thus supplying income as an additional crop to that from high-value summer crops. Linseed straw from the northern states, which is currently burned for the most part, could supply fibers for a variety of industrial uses. Continued research is needed to determine the optimal applications for these flax sources. Research should focus on optimizing enzyme formulations, based on cost and fiber yield and properties, and integrating the retting and cleaning stages to produce fibers with tailored properties for industrial uses.

Contact: D.E. Akin, Russell Research Center, PO Box 5677, Athens, GA USA. Tel.: 706546-3482. E-mail: [deakin@qaru.ars.usda.gov](mailto:deakin@qaru.ars.usda.gov)

**MORPHOLOGICAL AND CHEMICAL VARIABILITY IN CORIANDER GERmplasm**P. A. Lopez<sup>1</sup> and M. P. Widrlechner<sup>2</sup><sup>1</sup>Graduate Student, Department of Agronomy, Iowa State University, Ames, IA 50011, USA; <sup>2</sup>North Central Regional Plant Introduction Station, USDA-ARS, Ames, IA 50011, USA

Coriander (*Coriandrum sativum* L.) is a member of the umbel family (Apiaceae) that is commonly used as a fresh green herb (from leaves) and as a dry spice (from fruits) from which essential oils are extracted. Studies on the intraspecific classification of coriander are based on morphological, phenological, and chemical traits. Three subspecies and 10 botanical varieties have been distinguished. The objective of this ongoing study is to describe morphological, phenological, and chemical diversity of coriander accessions conserved by the North Central Regional Plant Introduction Station.

In 2002, 139 accessions of coriander were evaluated in the field for morphological and phenological traits. Seed samples were harvested and provided to the Center for Agricultural Utilization Research in Peoria, IL for fatty acid analysis. Based on morphological and phenological traits

and fatty acid analyses, a correlation matrix was computed and highly correlated characteristics eliminated. A cluster analysis was then carried out on the correlation matrix by using PROC CLUSTER from SAS and applying the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm. Based on the results of the initial cluster analysis, 60 diverse accessions were selected for field evaluation with two planting dates in 2003. Samples from the 2003 trial were used for the analysis of volatile compounds from the leaves and for essential oil extraction from the fruits. Analysis of volatile compounds from leaves was performed upon 7 g samples of bulked fresh leaves that were subjected to headspace sampling with solid phase microextraction. Headspace was then analyzed with gas chromatography/mass spectrometry. The essential oils of dried fruits were extracted by hydrodistillation. An analysis of variance was carried out for morphological and phenological data for each year of evaluation; analysis of variance for both fatty-acid composition and essential-oil content was done for only one year.

Nine clusters were revealed by the UPGMA clustering algorithm when an average distance of 0.5 between clusters was applied. Analysis of variance for morphological and phenological traits and for fatty acid and essential oil contents revealed many highly significant differences among accessions and between planting dates, with interaction effects for several traits. The fatty acid content ranged from 4.9 to 28.9%. Volatile compounds produced by the leaves varied little among accessions. The essential oil content ranged from 0.24 to 1.87% and was generally unaffected by planting date.

Preliminary information from this study revealed considerable variation among tested accessions for many morphological, phenological, and chemical traits. Our next step is to apply molecular markers (AFLPs) to complete the germplasm characterization and examine possible relationships between genetic and phenotypic variation.

*Contact: M. P. Widrlechner, North Central Regional Plant Introduction Station, USDA-ARS, Ames, IA 50011-1170, USA. Tel: 515-294-3511. E-mail: isumw@iastate.edu*

### **UPDATE ON COMMON MILKWEED PRODUCTION IN ILLINOIS**

W.B. Phippen

Agriculture Department, Western Illinois University, Macomb, IL 61455, USA

With the decline in commodity prices and the need for crop diversification, research interest has focused on the common milkweed (*Asclepias syriaca*) for the commercial production of industrial fibers, oils, latex, seed, and a potent nematicide. The main driving force behind milkweed development has been the seed floss as a non-allergenic fill to replace imported duck and goose downs in comforters and pillows. Current supplies of seed floss are obtained from hand-harvested, wild milkweed. To sustain the current markets and seek stability in the product stream, a more reliable method of production is needed. Many of the current markets for milkweed products, especially the seed floss, have remained largely undeveloped due to the lack of sufficient quantities of material for product testing.

The overall goal of the research at Western Illinois University is to determine the optimal growing guidelines and efficient harvesting techniques to establish reliable commercial production fields of milkweed in the State of Illinois. With the successful establishment of commercial milkweed fields, reliable and sufficient supplies of milkweed floss and seed can be utilized for the development of new markets and value-added products.

The primary objective of this research has focused on developing planting strategies, field maintenance guidelines, and equipment for the harvesting, drying, and cleaning milkweed pods. Experiments on establishing milkweed have addressed planting methods, crop densities, insect and disease pressures, weed control, pruning, nitrogen requirements, and effects of coal dust on plant establishment. Work has also been initiated on developing and optimizing the harvest and post-harvest handling of milkweed pods.

Results from this research have indicated that the perennial milkweed crop has tremendous potential as an alternative for farmers looking to expanding their production with minimal costs. With the identification of optimal planting densities and pre- and post-emergent herbicides, common milkweed production can offer both crop diversity and sustainable income for Illinois growers. Efforts are now directed towards organizing producers to establish milkweed processing and manufacturing facilities in Illinois.

*Contact: W.B. Phippen, Agriculture Department, Western Illinois University, Macomb, IL 61455, USA. Tel.: 309-298-1251. E-mail: wb-hippen@wiu.edu.*

### **PARTIAL DEHULLING OF MILKWEED SEEDS FOR OIL EXTRACTION**

R. L. Evangelista

New Crops Processing and Technology, National Center for Agricultural Utilization Research, USDA-ARS, 1815 N. University Street, Peoria, IL 61604, USA

Oil-bearing seeds are usually dehulled whenever appropriate. Dehulling increases the throughput of the oil extraction equipment resulting from reduced material to be processed. Removing the hulls reduces the amount of pigments and waxes that get expressed with the oil, thus improving oil quality. The low bulk density of milkweed seeds is largely due to the paper-thin husk (wing) around the edges of the flat seeds. The presence of wings also contributes to the seeds' poor flow characteristics. In addition, the light wings that get detached during handling create dust problems. This study explored the feasibility of removing the wings from milkweed seeds to reduce the material going into oil extraction, to improve material flow, and to minimize dust during processing.

Seeds were hand fractionated to determine the weight fractions and oil contents of the wings, hulls, and cotyledons. One-kg-batches of seeds with 4%, 7% and 10% moisture contents (MC) were processed through an impact type huller at 1250 rpm and 1700 rpm impeller speeds. The material from the huller was screened using standard testing sieves No. 8, 10, 12, 14, 18, and 25. The weight, moisture and oil contents of each fraction were determined. Two 100-kg-batches were processed using the suitable seed moisture and impeller speed combination previously identified.

The wing, hull, and cotyledon accounted for 12.2%, 51.2%, and 36.5% of the seed weight, respectively. Oil contents (dry basis) were 1.9% in wings, 9.0% in hulls, and 41.1% in cotyledons. Seeds with 4% MC and processed at 1250 rpm generated 22% fines fraction (smaller than 14 mesh) that contained 13% of the total oil. At 1700 rpm, the fines fraction increased to 42% and contained 35% of the total oil. Also, seeds with 7% MC cannot withstand higher impeller speed. Nearly 25% fines fraction with 15% of the total oil was produced. Three seed moisture and huller speed combinations (7% - 1250 rpm, 10% - 1250 rpm, and 10% - 1700 rpm) produced the least oil losses (6.4-6.8%) in about 20% of fines.

Two 100-kg batches of whole seeds with 8.5% MC were dewinged at 1250 rpm. Seeds with wings still attached were recycled to the huller until all the seeds were dewinged. After the third pass through the huller, the recycled material was reduced to mostly pod hulls. About 88% of the total material was dewinged seeds with oil content of 24% and bulk density of 276 g/l. The discard fraction (fines and light fractions) had oil content of 7.7%.

Seed wings were effectively removed by using an impact type huller. Suitable seed moisture content and impeller speed combinations were 7% - 1250 rpm and 10% - 1700 rpm. Removing the seed wings reduced the weight by 12%, increased oil content by 11%, increased the bulk density by 52%, and reduced seed volume by 40% while losing less than 5% of the total oil. The significant decrease in seed volume will bring about a large increase in throughput of the oil extraction equipment.

Contact: R.L. Evangelista, National Center for Agricultural Utilization Research, 1815 North University St., Peoria, IL 61604, USA. Tel.: 309-681-6312. E-mail: [evangelr@ncaur.usda.gov](mailto:evangelr@ncaur.usda.gov)

### **ECHIUM (*E. vulgare* AND *E. plantagineum*) WILD POPULATIONS CHARACTERISTICS IN CHILE**

M. Berti<sup>1</sup>, S. Fischer<sup>2</sup>, R. Wilckens<sup>2</sup>, F. Hevia<sup>2</sup>, and R. Araos<sup>3</sup>

<sup>1</sup>Plant Sciences Department, North Dakota State University, Fargo, ND, 58102, USA; <sup>2</sup>Agronomy Faculty, Universidad de Concepción, Casilla 537, Chillan, Chile; <sup>3</sup>LONCOPAN S.A., San Bernardo, Santiago, Chile.

*Echium plantagineum* is a new crop grown commercially in the UK and in Chile. Echium's seed oil is used for cosmetic industry due to properties that reduce skin wrinkles and also as a source of high w-3/w-6 ratio. *Echium plantagineum* and *E. vulgare* are native species in central and southern Chile of South America and are present as weeds in annual and perennial crops.

The objective of this study was to evaluate the characteristics of wild Echium populations existing in Central and Southern Chile in order to determine, low shattering, high seed weight, high oil content, and high stearidonic acid genotypes.

Twenty-seven populations of both species were collected from latitude 35° S to latitude 41° S at high and low altitudes in December 2002. At the collection site, latitude, longitude, altitude and plant characteristics were recorded. Soil samples were taken and brought to the soil and plant analysis laboratory for N-NO<sub>3</sub>, P Olsen, K, pH, and organic matter determination. Seeds from each population collected were sown in May 2003 at the Experimental Station of the University of Concepcion, Chillan, Chile (36° 26 S, 72° 06 W). The experimental design was an RCBD with two replications. Evaluations were days to first flower open, number of black seeds non-shattered, plant height at harvest, seed yield, 1000 seed weight, oil content, g -linolenic acid (GLA) (w-6), a -linolenic acid (w-3) (ALA), and stearidonic acid content (SA) (w-3).

*Echium vulgare* populations had lower oil content than *Echium plantagineum* populations that ranged from 25 to 27% seed oil content. *Echium vulgare* populations did not flower the first season. Plants remained in a low height rosette stage until the following season. This species requires vernalization, and the Chilean mild winters did not provide enough cold for reproduction. In Chile, this species is a biennial plant and would not be of interest for commercial producers because of its delayed seed production.

*Echium plantagineum* seed yields ranged between 115.4 to 617.4 kg ha<sup>-1</sup>. Seed oil content ranged between 27 and 39.5%. No significant differences in seed oil content among populations were observed for ALA and SA, which averaged 38% and 12%, respectively. The GLA content differed significantly among populations with the highest GLA content at 9.9% of the seed oil.

Contact: M. Berti, Plant Sciences Department, North Dakota State University, Fargo, ND 58105, USA. Tel.: (701)-231-8158. E-mail: Marisol.beriti@nodak.ndsu.edu

## AN UPDATE ON GIANT REED AND MIMOSA FOR ENERGY, FIBER, AND OTHER USES

D. I. Bransby<sup>1</sup>, H. Gu<sup>2</sup>, S. R. Duke<sup>2</sup>, G. A. Krishnagopalan<sup>2</sup>, and H. T. Cullinan<sup>2</sup>

<sup>1</sup>Department of Agronomy and Soils and <sup>2</sup>Department of Chemical Engineering, Auburn University, AL 36849, USA

High dry matter yield with low inputs is a desirable trait for both energy and fiber crops. In addition, pulp and paper properties are important traits for fiber crops, and the opportunity to produce high value co-products from either fiber or energy crops can improve their economic viability. Preliminary evaluation of giant reed (*Arundo donax*) and mimosa (*Albizia julibrissin*) indicated that both of these species have demonstrated potential as energy or biomass crops, and for the production of pulp and paper. However, research was needed to further document yields and accelerate establishment of giant reed, and to further evaluate pulping and paper making quality of mimosa. The objective of this paper is to provide an update on research conducted on these two species.

Agronomic studies were carried out on giant reed at the E. V. Smith Research Center located between Auburn and Montgomery in Alabama. Pulping and paper making research on mimosa was conducted at the Department of Chemical Engineering, Auburn University, and research on antioxidant extraction from mimosa foliage has been conducted at the University of Arkansas.

Giant reed can be successfully established by planting cane, as is done with sugar cane. However, establishment is slower than when rhizomes are planted. Tissue culture procedures for establishment have also been successfully developed. Annual yields of tests plots from planting in 1999 to 2004 were 1.4, 8.8, 12.9, 14.3, 14.6, and 19.7 dry tons per acre. No nitrogen fertilizer was applied in 2004. These yields are much better than the typical range of 5 to 8 tons per acre observed for switchgrass (*Panicum virgatum*).

Research on pulp and paper production from mimosa showed some variation in product quality with the age of wood. However, in general, results showed that properties of pulp and paper derived from mimosa were similar to other southern hardwoods, especially when blended with pulp from soft wood. Research at the University of Arkansas has shown that mimosa foliage is high in antioxidants.

Contact: D. I. Bransby, Department of Agronomy and Soils, 202 Funchess Hall, Auburn University, AL 36849, USA. Tel.: 334-844-3935. E-mail: bransdi@auburn.edu

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## POSTERS

### EFFECT OF LONG TERM CUPHEA SEED STORAGE ON GERMINATION

W.B. Phippen and B. Brandt

Agriculture Department, Western Illinois University, Macomb, IL 61455, USA

Cuphea species, rich in unique medium-chain triglycerides, are currently being domesticated as a potential new crop in the US. Recent breeding efforts in these species have focused on the essential agronomic traits for domesticating Cuphea to standard commercial production procedures. Self-pollination, reduced seed shattering, and non-dormancy of seeds have all been addressed in the current breeding lines. Unfortunately, indeterminacy and seed quality relating to germination have proven difficult.

The objective of this study was to investigate various long term storage conditions of Cuphea seeds to improve their viability.

Seed from the advanced breeding line, PSR-23, produced in 2002 in Macomb, Illinois was used throughout this study. Twenty-four sealed plastic vials and paper envelopes were filled with 100 seeds and placed under four storage conditions. Storage conditions included: (a) refrigerator (low temperature, low humidity), (b) room temperature (high temperature, low humidity), (c) protected outdoor environment (extreme temperature and humidity variation), and (d) controlled environment (low temperature, medium humidity). At the beginning of each month, seeds from one plastic vial and paper envelope were collected from each location and placed on germination paper. Seeds were checked for germination at 7, 14, and 21 days. The high and low temperatures and humidity were recorded at each location at the time of collection. Seeds from the vials and envelopes were collected at each location over a 2-year period.

The initial germination rate for PSR-23 seeds in November 2002 was 60% over a 21-day period. For the first 15 months, seeds stored in sealed plastic vials and paper envelopes across all locations had an average germination rate of 53% and 48%, respectively. After 15 months, germination of all seeds began to decline regardless of container type and storage location. However, the most severe decline in germination to less than 20% in the first 8 months occurred with seeds exposed to the varying environmental conditions and stored in paper envelopes.

Storage of PSR-23 Cuphea seed in sealed plastic containers for 18 months maintained a relatively high germination rate. Seeds exposed to outdoor ambient conditions such as grain bins and pole barns would have a dramatic effect on Cuphea seed viability. As Cuphea seed production reaches commercial scale, proper seed storage will be critical for maintaining seed viability and insuring the successful establishment of Cuphea in Midwest agriculture.

Contact: W.B. Phippen, Agriculture Department, Western Illinois University, Macomb, IL 61455, USA. Tel.: 309-298-1251. E-mail: wb-hippen@wiu.edu

## TOTAL SEED OIL AND FATTY ACID METHYL ESTER CONTENT IN WILD CUPHEA ACCESSIONS

W.B. Phippen<sup>1</sup>, T.A. Isbell<sup>2</sup>, and M.E. Phippen<sup>1</sup>

<sup>1</sup>Agriculture Department, Western Illinois University, Macomb, IL 61455, USA; <sup>2</sup>New Crops Processing and Technology Research Unit, National Center for Agricultural Crops Utilization Research, USDA, ARS, Peoria, IL 61604, USA

Cuphea species have been identified as a potential new source of unique fatty acids for both the lubricant and soap and detergent industries. Current breeding programs have mainly focused on improving agronomic traits to make Cuphea suitable for commercial production. Breeding programs will soon begin to focus on altering the total oil and fatty acid content with a special interest in lauric and capric acids. Accessions identified as high in oil content and rich in single fatty acids will be introgressed into the current agronomically sound breeding lines. The objective of this study was to evaluate wild Cuphea accessions for their total oil and fatty acid content. Work focused on developing a reliable and efficient method for determining total oil and fatty acid content in Cuphea seed.

Accessions (185) of Cuphea were obtained from the North Central Regional Plant Introduction Station, Ames, Iowa. The total oil content was determined by nondestructive pulsed NMR on whole Cuphea seed. Previous extraction and derivatization procedures were combined and optimized to minimize time and complexity in extracting medium-chain triglycerides and derivatizing them into fatty acid methyl esters for gas chromatography analysis. The derivatization process was a three step procedure involving (a) extraction of the medium-chain triglycerides from the seed, (b) hydrolysis, and (c) methylation for conversion into the fatty acid methyl esters. The new gas chromatography analysis, extraction, and derivatization procedures were validated for linearity, precision, accuracy, and sample stability.

The total oil content identified by pulsed NMR ranged from 10.1% in *Cuphea llavea* to 39.5% in *Cuphea wrightii* var. *wrightii*. Pulsed NMR results had a 2.5% relative standard deviation. Gas chromatography was used to determine the fatty acid methyl ester content. *Cuphea llavea* contained the highest levels of capric acid at 92.0%. The highest levels of lauric acid were found in *Cuphea wrightii* var. *wrightii* at 72.8%. The results were within the demonstrated linear range of the analysis with a 99.9% confidence level from the linearity studies. Samples were stable for 24 h at room temperature. Recoveries for methyl caprate and methyl laurate were 98%. Relative standard deviations for methyl caprate and methyl laurate were 3.5% and 6.9%, respectively. Validation results demonstrated that the extraction, derivatization, and gas chromatography analysis produced reliable and reproducible results.

The protocol developed for this study can aid breeders to determine accurately the total seed oil and fatty acid content for the various Cuphea species. The Cuphea species identified in this study can serve as potential new sources for high seed oil content and fatty acids to be introduced into the current advanced breeding lines.

Contact: W.B. Phippen, Agriculture Department, Western Illinois University, Macomb, IL 61455, USA. Tel.: 309-298-1251. E-mail: wb-hippen@wiu.edu

## CUPHEA SEED YIELD, BUT NOT SEED OIL CONTENT IS REDUCED BY DROUGHT

Russ Gesch<sup>1</sup>, Brenton Sharratt<sup>2</sup>, and Steven Cermak<sup>3</sup>

<sup>1</sup>USDA-ARS, North Central Soil Conservation Research Lab, Morris, MN 56267, USA; <sup>2</sup>USDA-ARS, Land Management and Water Conservation Research, Pullman, WA 99164, USA; <sup>3</sup>USDA-ARS, National Center for Agricultural Utilization Research, Peoria, IL 61604, USA

Cuphea can potentially serve as a domestic oil replacement for small and medium-chain fatty acids used in chemical manufacturing. Present domesticated lines of cuphea (*Cuphea viscosissima* Jacq. × *C. lanceolata* f. *Silenooides* W.T. Aiton) grow exceptionally well in the upper Midwestern U.S. However, cuphea does not have a deep root system, is not an efficient water user, and therefore, may be susceptible to drought stress.

A field study was conducted in west central Minnesota in 2002 and 2003 to assess cuphea seed yield and seed characteristics under non-limiting soil moisture conditions. In both years, two levels of irrigation were compared with a non-irrigated control treatment. Drip irrigation was used to maintain soil moisture to field capacity (soil matric potential ~ 0.3 bar) for the fully irrigated treatment and at 50% of the available water holding capacity of the soil (soil matric potential ~ 0.7 bar) for the partially irrigated treatment at a 30-cm wetting depth. In 2003, drought conditions were experienced from mid-July and throughout August. During this period, cuphea was flowering and setting seed. Between July 10 and September 9, only 3.2 cm of precipitation occurred at the experimental site.

Severe drought stress symptoms that included dramatically reduced leaf water potential and photosynthetic rates were observed during late summer for the non-irrigated plants. Seed yield of the fully irrigated plants was over 2.5 fold greater than that of the controls. However, biomass production was not as dramatically affected and seed oil content was similar across treatments at about 34 % (w/w). Results indicate that cuphea seed production, but not seed oil content is sensitive to drought. Possibly, the severe drought stress observed in 2003 was partially due to poorly developed roots inherent to present domesticated lines of cuphea. Future, widespread regional success of cuphea may depend on genetic improvement of drought tolerance.

Contact: Russ Gesch, USDA-ARS-NCSCRL, 803 Iowa Ave., Morris, MN 56267, USA. Tel.: (320) 589-3411. E-mail Gesch@morris.ars.usda.gov

## BIO-BASED FLUIDS FROM LESQUERELLA AND CASTOR

Steven C. Cermak and Terry A. Isbell

USDA-ARS-NCAUR, New Crops and Processing Technology Research, 1815 N. University St., Peoria, IL 61604, USA

*Lesquerella fendleri* is a member of the genus *Lesquerella* of the Brassicaceae (mustard) family. *Lesquerella* is a developing oilseed crop currently grown in the southwestern U.S., 40 acres in 2003. The oil is very attractive due to high levels (53%) of the hydroxy fatty acid, 14-hydroxy-cis-11-eicosenoic acid (lesquerolic acid).

Estolides derived from *lesquerella* and castor 2-ethylhexyl esters with different saturated and unsaturated fatty acids were synthesized. The unsaturated fatty acids included both plant and animal sources that demonstrated the potential diversity of fatty acids suitable as starting materials for the synthesis of estolides and their impact on the resulting lubricant physical properties.

The synthesis of lubricants from natural sources will lessen the demand for foreign imported oils. The poor cold temperature property is one of the traditional problems with vegetable oils. However, with the addition of additives the cold pour property can sometimes be improved. These new estolides produced a series of functional fluids with excellent cold temperature properties having pour points <-53°C. These new estolides have been designed to meet the demands of cold weather without the use of additives.

Synthesis of estolides from the fatty esters will be discussed as well as their physical properties, which include pour and cloud points, viscosities, RBOTs and colors. Comparisons will be made with other estolide technologies and commercially available materials.

Contact: Steven C. Cermak, USDA-ARS-NCAUR, New Crops and Processing Technology Research, 1815 N. University St., Peoria, IL 61604, USA. Tel.: 309-681-6233, Fax: 309-681-6524. E-mail: cermaksc@ncaur.usda.gov

## INBREEDING IMPLICATIONS ON REPRODUCTIVE CHARACTERISTICS IN TWO LESQUERELLA POPULATIONS

P.M. Tomasi and D.A. Dierig

U.S. Water Conservation Laboratory, USDA-ARS, Phoenix, AZ 85040, USA

Reproductive characteristics in *Lesquerella* are likely influenced both by environmental and heritable effects. Understanding this relationship would be helpful when selecting for yield related traits in segregating parental and interspecific populations. This study used a controlled greenhouse experiment over two years to examine the implications of inbreeding on reproductive characteristics in *Lesquerella*. Specifically, we produced and characterized two inbred populations of different species of *Lesquerella*, *L. fendleri* and *L. lindheimeri*.

In the first cross season (Fall 2002 to Spring 2003), twelve plants of a high oil germplasm release of *L. fendleri* (WCL-LY2) and twelve plants of an unselected wild accession of *L. lindheimeri* (A2232) were grown in the greenhouse and selfed by bud pollination to produce S1 seed. In the second season (Fall 2003 to Spring 2004), we attempted to bud pollinated three S1 plants from each of the original 12 *L. fendleri* and *L. lindheimeri* populations. A total of 18 *L. fendleri* and 35 *L. lindheimeri* S1 selfs were completed resulting in S2 seed. Reproductive characteristics were recorded for both species and generations, which included: mean petal length, silique swell percentage, ovules per silique, seed per silique, mean seed mass (or 1000 seed weight), and when possible, oil and fatty acid content.

Eleven of twelve plants from each generational species produced enough S1 seed in the first cross season to measure total seed oil percent. Only one *L. fendleri* self from the first cross season did not produce enough S1 seed to analyze oil or fatty acid content. Twenty-five of the 35 *L. lindheimeri* S1 plants (71%) and four *L. fendleri* S1 plants (22%) produced enough S2 seeds in the second cross season to measure total seed oil percent. The results indicate that oil content was variable in both species, but not significantly different. Seed mass was not significantly different between the two *L. fendleri* generations, although it was present between the two *L. lindheimeri* generations. Seeds per silique did not decrease significantly between the S1 and S2 generations in both species, but the number of ovules per silique was different. Both generational species had no significant differences in silique swell percentage and only the mean petal length between the *L. lindheimeri* generations was different.

Other pertinent relationships between these reproductive characteristics and generations will be presented. Quantifying the reproductive characteristics of these species will aid in future genetic breeding studies, specifically the development of molecular markers for selectable traits and targeted production of new interspecific hybrids between promising inbred lines.

Contact: P. M. Tomasi, U.S. Water Conservation Laboratory, 4331 East Broadway Road, Phoenix, AZ 85040, USA. Tel.: 602-437-1702 (X274). E-mail: ptomasi@uswcl.ars.ag.gov

## SESAMIN AND TOCOPHEROL VARIANTS IN SESAME SEEDS FROM GENOTYPES REGENERATED IN GEORGIA, U.S.A.

K.S. Williamson<sup>1</sup>, K. Hensley<sup>1</sup>, and J.B. Morris<sup>2</sup>

<sup>1</sup>Free Radical Biology and Aging Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104, USA; <sup>2</sup>Plant Genetic Resources Conservation Unit, USDA, ARS, Griffin, GA 30223-1797, USA

Sesame (*Sesamum indicum* L.) contains bioactive phytochemicals and nutraceuticals to be utilized in the pharmaceutical and nutraceutical markets. More than 1,000 accessions of this species are preserved at the USDA, ARS, Plant Genetic Resources Conservation Unit, Griffin, GA. Both sesamin and tocopherol congeners such as alpha tocopherol (a-T), delta tocopherol (d-T), and gamma tocopherol (g-T) are present in sesame seed. Sesamin is an antioxidant that can reduce serum cholesterol levels in humans and the antioxidant, tocopherol is known to protect polyunsaturated fatty acids from oxidation.

The primary objective of this study was to determine the amount of both sesamin and tocopherol among 20 eco-geographically diverse sesame genotypes. A secondary objective was to determine the regeneration capability for each sesame genotype.

Sesame seeds from these accessions were solvent extracted with HPLC grade methanol or hexane. Characterization and quantification of sesamin and tocopherol variants consisted of an HPLC elution system and chromatographic separation.

Sesame plant establishment was either by direct seeding or transplanting at the Griffin, GA field site. The regeneration plots consisted of single-row plots, 6.09 m long. Characterization data was recorded for each sesame genotype.

Optimal oxidation potentials from each sesame accession for sesamin and tocopherol homologs were determined. HPLC analysis revealed that sesamin concentrations from all accessions ranged from 1.09 mg/g to 3.31 mg/g; tocopherol congeners such as a-T varied from 0.017 µg/g to 0.575 µg/g; d-T ranged from 0.89 µg/g to 6.11 µg/g; and g-T ranged from 47.7 µg/g to 166.7 µg/g.

Sesame plants regenerated seed numbers ranging from 1,918 to 144,191 per regeneration plot in Griffin, GA. Characterizations showed a wide range in several sesame traits. Capsule length ranged from short to long and capsule axils ranged from 1 to 3. Seed color ranged from white to black and seed size ranged from small to large. Sesame plant maturity ranged from early to late.

A useful, quantitative method employed in the isolation, characterization, and quantification of sesamin and tocopherol homologs from different

sesame accessions has been demonstrated.

Sesame genotypes containing variable amounts of sesamin and tocopherol were identified for current use as a pharmaceutical or nutraceutical crop in the southeastern U.S. Sesame regenerates very well in the Griffin, GA environment with optimum seed production for possible new crop development in the southeastern U.S.

Contact: J.B. Morris, USDA, ARS, Plant Genetic Resources Conservation Unit, 1109 Experiment St., Griffin, GA 30223-1797, USA. Tel.: 770-229-3253. E-mail: bmorris@ars-grin.gov.

## SUNFLOWER PRODUCTION IN NORTHERN MEXICO: BIOMASS, GRAIN YIELD, OIL AND PROTEIN CONTENT

R. Rodríguez García<sup>1</sup>, D. Jasso de Rodríguez<sup>1</sup>, J. L. Angulo Sánchez<sup>2</sup>, and Ernesto Cervantes<sup>3</sup>

<sup>1</sup>Universidad Autónoma Agraria Antonio Narro (UAAAN), Saltillo, Coahuila, 25315, México; <sup>2</sup>Centro de Investigación en Química Aplicada (CIQA), Saltillo, Coahuila, 25100, México; <sup>3</sup>Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP), Campo Experimental Sur de Tamaulipas, Tampico, Tamaulipas, 89339, México

Sunflower is one of the most widely cultivated oil crops in the world due to its oil quality and plant adaptability to different soil and climatic conditions. Presently, sunflower production in Mexico is low. Sunflower must be imported to supply the edible oil market. For 2003, imports were 152,000 ton of grain and 50,000 ton of crude oil. According to these data, sunflower shows promise as a potential alternative crop for the arid and semiarid zones of Mexico, and specifically for the state of Coahuila.

Our primary objective was to evaluate the quality and yield of different sunflower varieties in Northern Mexico for potential commercial production. The biomass and grain yields and other morphological variables were measured. In addition, the oil and protein contents were determined.

The experiment was conducted at the Antonio Narro University experimental field in Buenavista, Saltillo, Coahuila. The seeding date was 22 July 2003 using a random block design with five replications. The four varieties were Primavera 1, Primavera Z, Blend Oil and Compuesto 1 that were supplied by INIFAP. Preplant fertilizer combination was applied at 80 kg N/ha and 60 kg P/ha. The variables measured were plant height, number of leaves, stem and head diameter, leaf area index, biomass production, and oil and protein content. Plant samplings were made at the different phenological stages at budding, flowering, physiological maturity, and harvest.

The results of leaf area index for the different varieties were: Primavera, 3.07; Compuesto 1, 3.88; Primavera Z, 3.97; and Blend Oil, 4.26. Grain yield ranged from 3.57 ton ha<sup>-1</sup> for Primavera Z to 4.59 ton ha<sup>-1</sup> for Compuesto 1. The protein content varied from 24.5% for Blend Oil to 26.4% for Primavera 1. The oil content ranged from 35.7% (Compuesto 1) to 41.6 % (Primavera 1).

The varieties investigated showed excellent adaptability to the environmental conditions of the region. Grain yields were greater than those previously reported for the UAAAN varieties.

Contact: R. Rodríguez García, Universidad Autónoma Agraria Antonio Narro, Buenavista, Saltillo, Coahuila, 25315, México. Tel.: 844 4110353. E-mail: rrodriguez\_uaan@hotmail.com

## DEVELOPMENT OF CAMELINA SATIVA AS A FATTY ACID SOURCE

D.L. Johnson<sup>1</sup>, D. M. Wichman<sup>2</sup>, C. Chen<sup>2</sup>, K.D. Kephart<sup>3</sup>, P.Lamb<sup>4</sup>, G. R. Carlson<sup>4</sup>, and G. D. Jackson<sup>5</sup>

<sup>1</sup>Northwest Agricultural Research Center, Montana State University, Kalispell, MT 59901, USA; <sup>2</sup>Central Agricultural Research Center, Montana State University, Moccasin, MT 59462, USA; <sup>3</sup>Southern Agricultural Research Center, Montana State University, Huntley, MT 59037, USA; <sup>4</sup>Northern Agricultural Research Center, Montana State University, Harve, MT 59501, USA; <sup>5</sup>Western Triangle Agricultural Research Center, Montana State University, Conrad, MT 59425, USA

*Camelina sativa* is a member of the Brassica family that has been used since the Bronze Age as a food and fuel source. It is native to Europe from the Arctic Circle to the Mediterranean Sea. Camelina has been called "Gold of Pleasure" for its flavorful oil, resembling the flavor of almond oil. Camelina has seen a resurgence of interest in Europe. The Montana project was initiated to design a very-low cost production system to produce biodiesel with yields competitive with conventional vegetable oil sources such as canola and sunflower. Camelina oil, however, possesses several potential applications from the manufacture of wax esters to a source of w-3 fatty acids.

The initial objective in this study is to determine the adaptation of camelina to a wide range of production environments.

Camelina was planted in species comparative studies at five agricultural research centers (Kalispell, Havre, Moccasin, Conrad, and Huntley) in Montana. Comparative crops were: canola, rapeseed, crambe, mustard, flax, safflower, sunflower, and soybean. Plantings were made using two or more cultivars when feasible at appropriate dates based on soil temperature. Camelina was seeded early March to early April; canola, mustard, and rapeseed were typically seeded early April to mid-May; flax and safflower were seeded mid-April to mid-May; and sunflower and soybeans early to mid-May. Yields and oil percentages were taken, but not oil quality.

Results varied from location to location. Based upon observations for 2003 and 2004, camelina appears to show excellent tolerance to cold, very good hail resistance and insect resistance. Oil percentages were slightly lower than canola and were similar to mustard at 32-34%. Adaptation appears to be universal for the moderate and low rainfall conditions across Montana. Camelina appears to not have a dormancy requirement, and therefore, is not expected to become an adapted weed, unlike the related species *Camelina microcarpa*.

The project will next initiate industrial development of chemically modified camelina oils for very-low pour point biodiesel and wax ester applications.

Contact: D.L. Johnson, NWARC, 4570 MT Hwy 35, Kalispell, MT 59901, USA. Tel.: 406-755-4303. Email: duanej@montana.edu

## SEEDING DATE EFFECT ON POTENTIAL NEW CROPS FOR NORTH DAKOTA

S. Dash and B.L. Johnson

Department of Plant Sciences, North Dakota State University, Fargo, ND 58105, USA

Seeding date is important for successful crop production and varies among crops and growing regions primarily due to climate. Proper seeding date is essential for maximizing agronomic and economic production. Seeding date for new crops that show potential adaptation in a region is one of the first production factors determined. New crops provide producers economic and crop rotation alternatives, and contribute to new industrial and food products.

The objective of this experiment was to determine the influence of seeding date on new crop performance.

Four new crops along with two adapted crops crambe (*Crambe abyssinica* Hochst.) and flax (*Linum usitatissimum* L.) were sown on two dates at a northern and southern location in eastern North Dakota during 2003. The experiment was a RCBD in a split plot arrangement with date as the whole plot and crop as the subplot. Data were collected for days to emergence, stand, days to flowering, plant height, biomass, seed yield, and harvest index.

Crambe produced greater yield at the northern than the southern location. However, cuphea (*Cuphea viscosissima* Jacq. × *C. lanceolata* f. *silenoides* W.T. Aiton, PSR 23), niger (*Guizotia abyssinica* Cass.), and flax produced similar yields at both locations. Adapted crops crambe and flax produced five to eight times greater yield than the new crops niger and cuphea, respectively. Low yield for cuphea was influenced by poor stands and seed shatter. Yield was greater for crambe and flax at the earlier seeding date. Low yields of the new crops resulted in location by seeding date means not being statistically different. However, yield for niger was reduced 43 and 64% at the northern and southern locations, respectively, when the early and late seeding dates are compared. The same comparison for cuphea showed a 25 and 35% yield reduction between early and late seeding at the northern and southern locations, respectively. Harvest index for crambe, flax, cuphea, and niger were 22.3, 23.7, 4.1, and 2.8%, respectively. Kenaf (*Hibiscus cannabinus* L.) produced approximately 1.4 to 2.8 times greater biomass than sunn hemp (*Crotalaria juncea* L.) at the southern and northern locations, respectively. Seeding date did not influence biomass for kenaf and sunn hemp. At the southern and northern locations, biomass was similar for kenaf, but greater biomass was produced at the southern location for sunn hemp.

Cuphea, niger, kenaf, and sunn hemp show regional adaptation, but additional plant breeding is necessary to increase yields of cuphea and niger, and likely harvest index, so they are economically competitive with regionally grown crops. Earlier seeding benefited yields of crambe, flax, cuphea, and niger, but not biomass of the fiber producing crops kenaf and sunn hemp. Stands were good for niger, kenaf, and sunn hemp, but cuphea needs improvement in seed vigor to produce consistently good stands.

Contact: Snigdharani Dash, Dept. of Plant Sci., North Dakota State Univ., Fargo, ND 58105, USA. Tel.: 701-231-7971. E-mail: snigdharani.dash@ndsu.nodak.edu

## STOMATAL RESISTANCE, TRANSPIRATION, AND LEAF WATER POTENTIAL IN ALOE VERA

R. Rodríguez García, D. Jasso de Rodríguez, A. Zermeño González, and J.A. Gil Marin  
Universidad Autónoma Agraria Antonio Narro (UAAAN), Saltillo, Coahuila, 25315, México

*Aloe vera* is a succulent plant native to the semitropical regions. It belongs to the Liliacea family and is characterized by crassulacean acid metabolism (CAM). The CAM plants normally are developed in desert environments, where the cool night and hot day temperatures favor the stomatal opening at night. Extensive physiological studies of CAM plants have been conducted for the species within the Agavaceae and *Opuntia* genus, but have been limited for *A. vera*.

The objectives of this investigation were to explore the diurnal behavior of the stomatal resistance, transpiration, and leaf water potential of *Aloe vera* and to develop relationships among the stomatal resistance, leaf temperature, and climatic conditions.

The study was conducted in the field and the greenhouse at the Antonio Narro University in Buenavista, Saltillo, Coahuila, Mexico, during the months of March to November 2003. In the field site, the treatments established for promoting a broad range of climatic conditions were: T1, plants with plastic mulch; T2, plants in bare soil; T3, plants under shade net (30% shade); and T4, potted plants in greenhouse. Each treatment had 15 plants.

The plants were irrigated when the soil water tension at 30 cm depth reached 60 centibars. The variables measured were: stomatal resistance, transpiration, leaf water potential, and leaf temperature. Climatic factors monitored were: air temperature, relative humidity, and vapor pressure deficit. Diurnal measurements were taken in two forms: (a) every hour for 24 h, and (b) every hour during the night (8 to 10 h). The data was analyzed by principal component analysis.

Diurnal stomatal resistance values indicated that the stomatal opening in *A. vera* occurred during the day. Leaf temperature did not influence stomatal opening. Stomatal resistance decreased when the air relative humidity increased or when the vapor pressure deficit of the air decreased. This behavior tends to increase the water-use efficiency of the plant without affecting CO<sub>2</sub> exchange.

Contact: R. Rodríguez García, Universidad Autónoma Agraria Antonio Narro, Buenavista, Saltillo, Coahuila, 2531, México. Tel.: 844 4110353.  
E-mail: rrodriguez\_uaan@hotmail.com

## CLADODE AND FLOWERING CHARACTERISTICS OF THE USDA OPUNTIA SP. GERMPLASM COLLECTION

M.M. Jenderek  
National Arid Land Plant Genetic Resource Unit, USDA-ARS, Parlier, CA 93648, USA

Plants of several *Opuntia* species are used as fruit, vegetable, fodder for livestock, and as raw material for cosmetic, pharmaceutical and construction industries. The cactus can be grown in arid and semiarid environments on marginal soils. Various cultivars, particularly in the species *O. ficus-indica*, are already grown commercially in the U.S., Israel, Italy, Mexico, and South Africa. The need for new sources of genetic diversity and subsequent germplasm evaluations is more and more apparent. Until recently, however, no publicly maintained germplasm collection of *Opuntia* existed in the U.S.

The objective of this study was to evaluate the morphological characteristics of selected *Opuntia* accessions maintained in the USDA collection, and to determine the diversity in the collection.

In 2003 and 2004, five plants from 49 different accessions were evaluated for flower and cladode characteristics under the San Joaquin Valley climate conditions. These clones, originated from local wild populations, home gardens, and the Texas A&M University-Kingsville, TX.

The time of initial flowering ranged from the end of March (PARL 200 and 203) to the end of June (PARL 296 and 297). In the second flush of flowering, the number of flowers/plant varied from 0 to 142 (PARL 227) and 150 (PARL 231). The flower petal colors observed were different shades of yellow (15 accessions), orange (8 accessions), and red (8 accessions). Plants of 16 accessions did not flower in either of the evaluation seasons. A cladode size index (length × width × thickness/width at the cladode base) varied from 75.5 (PARL 233) to 472.2 (PARL 228), and the cladode color was from light green to blue-green. The susceptibility of plants to frost and/or mechanical damage varied from 0 (none) (PARL 201, 229, and 234) to 2.8 (PARL 263; rank 3 being the highest). The level of natural infection by scale (*Aspidiotus nerii*) insects ranged from 0 (6 accessions) to 3 (2 accessions). The lowest number of spines/cladode was observed in PARL 235 (1 areole/25 cm<sup>2</sup> of cladode); however, its spines were the longest (119 mm). The shortest spines grew on PARL 242 (1.7 mm) and PARL 261 (1.8 mm). Spines of 32 accessions were ranked as brittle in appearance. The evaluation of *Opuntia* fruit quantity and quality together with the cladode traits and flowering ability continues and ultimately will aid in the selection of germplasm for a future cultivar development.

The first USDA, NPGS *Opuntia* sp. germplasm collection is diverse in regard to flowering time, flowering abundance, cladode size and appearance, and susceptibility to scale insect infestation.

Contact: M.M. Jenderek, USDA, ARS, National Arid Land Plant Genetic Resource Unit, 9611 S. Riverbend, Parlier, CA, USA. Tel.: 559-596-2980. E-mail: mjenderek@fresno.ars.usda.gov.

## QUINOA FOR FORAGE APPLICATION PLANTING WITH DIFFERENT DENSITIES: DRY MATTER YIELD, SAPONINS AND PROTEIN CONTENT

D. Jasso de Rodríguez<sup>1</sup>, R. Rodríguez García<sup>1</sup>, and J. L. Angulo Sánchez<sup>2</sup>

<sup>1</sup>Universidad Autónoma Agraria Antonio Narro (UAAAN), Saltillo, Coahuila, 25315 México; <sup>2</sup>Centro de Investigación en Química Aplicada (CIQA), Saltillo, Coahuila, 25100 México

Quinoa (*Chenopodium quinoa* Willd) is the only member of Chenopodiaceae that makes a significant contribution to food or fodder production. This is due to its cereal-like grain with high protein content and a better balance of amino acids composition than other cereals. This plant is cultivated at elevations over 3000 m under adverse weather and soil conditions. Quinoa may be an alternative crop for the semiarid regions of Northern Mexico.

The objectives this quinoa study was to determine the dry weight, saponins and protein contents in the whole plant and different plant parts under three plant populations and to relate them to their forage potential.

The experiment was carried out at the experimental field of the Antonio Narro University in Saltillo, Coahuila. Seeding was performed on July 22, 2003, under a random block design with five replications. The genetic material was the Chucara variety. The planting density treatments were: T1=12 kg ha<sup>-1</sup>, T2= 9 kg ha<sup>-1</sup> and T3= 6 kg ha<sup>-1</sup> Preplant fertilizer combination was applied at 80 kg N/ha and 60 kg P/ha The evaluated variables were: number of plants per lineal meter, plant density per hectare, number of vegetative and floral branches, plant height, fresh and dry matter yields, and saponins and protein contents.

The analysis of variance showed significant difference (P<0.05) for the number of plants per lineal meter, at the panicle stage where T1 and T2 are different. The average number of plants per lineal meter was 26.4 (T1), 22.7 (T2), and 14.3 (T3). Planting densities at this stage also showed significant difference (P<0.05) between T1 and T3. The average values were: 755,200 plants ha<sup>-1</sup> (T1), 648,740 plants ha<sup>-1</sup> (T2), and 407,770 plants ha<sup>-1</sup> (T3). No difference among treatments was found for dry matter, the average yields were 6,680 kg ha<sup>-1</sup> (T1), 6,070 kg ha<sup>-1</sup> (T2), and 5,490 kg ha<sup>-1</sup> (T3). Protein content was not affected by planting densities although an increase trend was apparent from the stem (10%) to leaf (26.4%) and the glomerules of flowering branches (30.3%) and terminal (32.8%) panicles; the content was lower in the seed (18.53%). Saponin contents were not affected by planting densities and showed similar behavior than protein in the stem (0.26%), leaf (0.29%), glomerules of floral branch (0.52%), and terminal (0.35%) panicles.

The total content of saponins found in this experiment was lower than those reported for the 2000 and 2001 cycles and may be due to the frequent rainfall (362.2 mm) during crop development in this cycle.

Contact: D. Jasso de Rodríguez, Universidad Autónoma Agraria Antonio Narro, Buenavista, Saltillo, Coahuila, 25315, México. Tel.: 844 4110220. E-mail: dianajassocantu@yahoo.com.mx

## CHEMICAL EVALUATION OF POTENTIALLY USEFUL PLANTS FROM THE MEXICAN SEMIARID REGION

J. A. Villarreal Quintanilla<sup>1</sup>, D. Jasso de Rodríguez<sup>1</sup>, R. Rodríguez García<sup>1</sup>, and J. L. Angulo Sánchez<sup>2</sup>

<sup>1</sup>Universidad Autónoma Agraria Antonio Narro (UAAAN), Saltillo, Coahuila, 25315, México; <sup>2</sup>Centro de Investigación en Química Aplicada (CIQA), Saltillo, Coahuila, 25100, México

We have continued and expanded our research program in the selection and evaluation of plants with pharmacological and industrial uses from the Mexican semiarid region. In this work, two additional native species were evaluated: (1) *Ephedra compacta* Rose or "Pitamoreal", a 50-cm tall plant that is usually found on slopes and uplands, from south of Nuevo Leon, Coahuila and Chihuahua, west of Veracruz and North of Oaxaca. It grows in grasslands, chaparral, and pine woodlands usually on gypsy soils; and (2) *Linum lewisii* Pursh var. Lewissi, a 25-cm tall plant that grows in the heavy alluvium soils of mountains, valleys, often with gypsy soils south of Coahuila, west of Nuevo León, and parts of Sierra Madre Oriental.

The objective of this study was to evaluate the chemical content and composition of *Ephedra compacta* and *Linum lewisii*, two Mexican species

growing in the semiarid region, for potential pharmacological and industrial uses.

The plants were collected on February 2004 along the roadside of Saltillo-General Cepeda about 35 km from the UAAAN University. Plant dimensions were measured in the field, and the plants uprooted and transported to the laboratory, and separated into stems, flowers, and seeds. The tissues were dried, milled, and prepared for analysis. The analysis included crude protein and fat using infrared spectroscopic technique.

In this presentation, the chemical potential and possible applications of *E. compacta* and *L. lewisii* will be discussed.

Contact: D. Jasso de Rodríguez, Universidad Autónoma Agraria Antonio Narro. Saltillo, Coahuila. México 25315. Tel.: (844) 4110220. E-mail: dianajassocantu@yahoo.com.mx

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