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## 2009 *The Next Generation of Industrial Crops, Processes, and Products*



November 14 - 19, 2009

Chillán, Chile

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### ABSTRACTS

### Keynote Speaker

#### THE NEXT GENERATION OF BIOFUELS

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Ethanol and biodiesel have established themselves as viable commercial biofuel products. These first generation fuels have been successful despite limitations with each fuel type compared to the petroleum-based transportation fuel supplies, gasoline, and diesel they supplement. But new generations of biofuels have reached the development stage of the RD&C process and should show up as commercial products soon. These new fuels have better properties and are produced more efficiently than ethanol and biodiesel. Most of these fuels are derived from one of three basic pathways: the sugar platform, the thermochemical platform, and the crop oil platform. It is not just enough to produce one of the three primary transportation fuels (gasoline, diesel, jet fuel), renewable options must be commercialized for all three to avoid economic disruptions as these products are introduced into the marketplace. Jet fuel is particularly challenging as this fuel must have a very low freeze point and very high energy density. Currently, the only viable renewable jet fuel processes utilize crop oils as their primary feedstocks. The SUNRISE research group in North Dakota is one of the groups that have been developing renewable fuels via the crop oil platform. These include a drop-in compatible JP-8/Jet A aviation fuel, a cold flow diesel fuel, and a high octane blendstock for aviation gasoline.

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## Plenary Session

### DEVELOPMENT OF INDUSTRIAL CROPS IN CHILE

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Agricultural land in Chile is limited due to the country's complex physiography. The area where annual crops are grown has a temperate or rainy Mediterranean climate with a marked 5 month rainy season from May through September and a dry season from October through April. Crops planted in April and May are grown in dryland areas and those planted from August through November are usually irrigated.

Chile has about 100,000 ha planted with industrial annual crops. The major crops grown are sugarbeet (*Beta vulgaris* var. *saccharifera*), chicory (*Chicorium intybus*), canola (*Brassica napus* L.), lupins (*Lupinus* spp.), and tobacco (*Nicotiana tabacum*). Many alternative industrial crops have been evaluated in Chile, including biofuel types with potential to reduce fossil fuels imports. The Chilean government, through several agencies, has supported the evaluation and development of alternative crops used for pharmaceutical, nutraceutical, food, and biofuel feedstocks. Also many native plants have been evaluated as sources of metabolites for pharmaceuticals and sources of metabolites for natural pesticides.

There have been a few new crops successes; however, many crops are still being evaluated or waiting for better market opportunities. New crops such as chicory, lupin, borage (*Borago officinalis* L.), flax (*Linum usitatissimum* L.), and quinoa (*Chenopodium quinoa* L.) have been successfully commercialized. Those awaiting better markets for commercialization as food source, nutraceutical, or a feedstock for biofuels include boldo (*Peumus boldus*), canelo (*Drymis winterii*), piretro (*Chrysanthemum cinerifolium*), calendula (*Calendula officinalis*), meadowfoam (*Limnanthes alba*), evening primrose (*Oenothera biennis*), camelina (*Camelina sativa*), oriental mustard (*Brassica juncea*), and jathropha (*Jathropha curcas*).

Chile imports 68% of all energy consumed and 98% of all fossil fuels for transportation, industry, and agriculture. Production of renewable fuels is in the government's plan for the future. In the last 5 years, there has been more support for initiatives on clean energy production. Research grants have been funded in the following areas: biodiesel production from canola, jathropha, camelina, and algae; biogas production and utilization from manure and agricultural residues; and the use of agricultural and forestry residues for energy production.

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### DEVELOPMENT OF INDUSTRIAL CROPS FOR ARID LANDS IN ARGENTINA

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The economic crisis of 2001 reshaped agricultural production in Argentina. Low internal product prices and production costs set at international levels, as well as increasing taxes, resulted in extremely low revenues for traditional products such as corn, wheat, sunflower, and soybeans, the four major crops of the country. Even stronger constraints worked their way into the meat, dairy, and wool production. Regional products and regional economies were also hit hard. While the revenue was at its lowest, land prices kept increasing, driven by speculation. Large investing pools shifted their goals towards marginal land, in search of lower land values. These lands had been largely overlooked and mostly underdeveloped because structural and functional constraints limit their potential productivity. In addition, the lack of alternative crops prevented new developments. Such as in previous attempts, investors appeared to be ready, but alternative crops were not.

Here, I will describe past and present efforts to develop new industrial crops for arid lands in Argentina, and evaluate public and private constraints to this process. I will focus mainly on crops for medium to large size markets and not on boutique or niche products. The most successful story is the introduction of Jojoba in the early 1990's, although after a lag face the crop seems to be facing a potential decline, similar to what happened two decades ago in the U.S. and Mexico. The causes of this decline can be tracked to the lack of basic research and fluctuations in public policy. Attempts for the commercial introduction of guayule, kenaf, and guar have failed. Ongoing efforts on resins, specialty seed oils and fibers, for which the commercialization has not started yet, will be described.

Despite some sporadic efforts, local domestication and the introduction of new crops will continue to be depressed due to the lack of incentives for farmers. The use of marginal lands will not grow until the country establishes a comprehensive program to support attempts of crop diversification.

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## LESQUERELLA, A POSSIBLE MODEL FOR NEW CROP DEVELOPMENT AND COMMERCIALIZATION

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*Lesquerella fendleri* was first discovered and published as a potential industrial oilseed plant by the U.S. Department of Agriculture in 1960. The unique seed oil is predominately composed of a hydroxy fatty acid, lesquerolic acid (C20:1-OH), that is similar to ricinoleic acid (C18:1OH) found in castor oil. Uses include lubricants, greases, coatings, and most recently, as an additive to biodiesel fluid to improve lubricity. There is also value in the water-soluble gums located on the seed surface, and in the seed meal. Improvements in agronomics, breeding, genetics, and the expansion of new markets started in the 1980's, and has made lesquerella a viable potential crop that could utilize thousands of hectares in arid climates of the world.

While lesquerella is not yet a commercial crop, its history serves as a model for new crop development. The most important characteristic is the absence of any biological barriers to commercialization. Other potential crops may have valuable, high-demand products but possess difficult traits to overcome such as seed shattering or poor yield capacity. Lesquerella has a distinctive plant architecture that is conducive to seed productivity under a variety of conditions, and the trait can be further exploited. The plant also has high amounts of within-species genetic diversity allowing breeding improvements. Also, other species of *Lesquerella* have desirable traits that can be introgressed through interspecific hybridization. *Lesquerella* belongs to the Brassicaceae family and benefits from genetic information gained from the closely related model plant, *Arabidopsis*. There is great potential for mining genes discovered in *Arabidopsis* studies and *Lesquerella* is amenable to genetic transformation. This could include genes for herbicide tolerance or information on the biosynthetic pathways for oil production.

Considering these crop traits may help in the decision management of other new crop programs. Often in the past only the end products and markets have been the focus without consideration of the biological traits, genetic diversity, yield potential and trait development.

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**Oilseeds**

## CALENDULA: A POTENTIAL NEW OILSEED CROP FOR THE NORTHERN U.S.

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Seeds of calendula (*Calendula officinalis*) are a rich source of calendic acid (conjugated C18:3), a highly oxidative fatty acid that can be used to replace volatile organic compounds (VOC) as a drying agent in many industrial chemicals including paints and adhesives. Calendula flourishes in temperate climates, and its residues have been shown to have nematicidal properties. Therefore, it may be a beneficial rotational crop for the Corn Belt region of the U.S. where crop diversity is lacking and nematodes are a substantial problem in corn (*Zea mays*) and soybean (*Glycine max*) production.

Little is known about calendula's agronomic potential in the northern U.S. or best management practices for its production. The objective of this initial study was to determine optimum seeding date and yield potential of three calendula genotypes in west central Minnesota on a Barnes loam soil. Carola, an open pollinating cultivar, and two hybrids, 1557-3 and 99276-3, were drill-seeded at a depth of 6.5 mm and a rate of 11.2 kg ha<sup>-1</sup> in mid-May, early-June, and mid-June in 2008 at approximately two-week intervals.

Seedling emergence was greatest for the earliest sowing, averaging 139 plants m<sup>-2</sup> across cultivars. Time to 50% flowering ranged from 52 to 56 days after planting (DAP) for the latest and earliest sowing, respectively. Because of its indeterminate flowering, calendula was harvested when 80% of seed heads had matured. The time from planting to harvest ranged from 104 to 112 DAP, corresponding to 970 to 1015°C d accumulated growing degree days (using 10°C as the base temperature for calculation). No differences were noted among cultivars for either time to 50% flowering or harvest. Moreover, seed yield did not differ among cultivars, but was greatest for the earliest sowing (mid-May), which averaged 1197 kg ha<sup>-1</sup> as compared to 729 and 785 kg ha<sup>-1</sup> for the early- and mid-June sowing dates, respectively.

Calendula grows well in the northern Corn Belt of the U.S. and yields greatest when sown as early as soybean in this region. Owing to its relatively short life cycle, when sown in May, calendula can be harvested long before corn and soybean, making it an attractive new crop for this region. Additionally, we are examining the potential of calendula residues to inhibit soybean cyst nematode proliferation. Further research will also be needed to optimize agronomic practices for calendula in the northern U.S. and to determine its rotational effects on traditional crops in this region.

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## HERBICIDES FOR CALENDULA

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The rising need for replacements for volatile organic compounds (VOC), which are used in the manufacture of paints, plastics, pesticides, etc., has placed demands on drying oils that may not be met by current sources. The primary source is eleostearic acid, or tung oil, which is derived from the seeds of the tung oil tree, *Aleurites fordii*. Calendic acid is an alternative drying oil, and it is produced by seeds of calendula or pot marigold (*Calendula officinalis*). Few other plant-based drying oils polymerize as quickly as do eleostearic and calendic acids. Importantly, calendula grows well in temperate climates, but the tropical tung oil tree does not.

The objective of this preliminary study was to identify herbicides tolerated by calendula (var. 'Carola'). Efficacious herbicides are needed to enable large-scale testing and cultivation of this new crop unencumbered by broadleaf weed infestations. (Grass weeds are controlled easily in calendula with any of several grass-specific herbicides.)

Preliminary experiments in a greenhouse examined calendula injury and growth responses to 21 herbicides applied in a logarithmic dilution series. This series was equivalent to 0.01, 0.1, 0.5, 1.0, and 10.0 times normal field rates for labeled crops, as well as a check (0) treatment. Products were accompanied by adjuvants where necessary. All were applied using a specially constructed spray chamber. Approximately equal numbers of soil-applied (SA) and postemergence (POST) herbicides were tested. Herbicides that appeared promising were field-tested in small replicated plots on a research farm and a commercial farm. SA and POST products were applied and examined separately and sequentially.

Three SA herbicides (metolachlor, pendimethalin, and trifluralin) were safe for use on calendula at 1.0X rates.

Two POST herbicides also appeared useful. Calendula exhibited no injury symptoms with imazamethabenz at the 1.0X rate. Imazamethabenz appears to be safe even when applied following any of the three SA herbicides. The second useful POST herbicide was a tank mix of desmedipham and phenmedipham, which traditionally is used on sugar beet and is known commercially as Betamix. Betamix killed calendula if applied at the cotyledon to 3-leaf stage of growth. However, if a 1.0X rate was applied at the 4- to 5-leaf stage, older leaves were burned but the plant recovered and subsequently grew vigorously. Despite severe leaf burning caused by Betamix, this short-term affliction to the crop is more than compensated for by the high level of weed control afforded by the herbicide.

In conclusion, five herbicides tentatively were identified for use in calendula. Additional research must thoroughly document the safety of these herbicides as well as identify additional useful products. Nevertheless, a sufficiently broad spectrum of weed species can be controlled by these five herbicides to facilitate large-scale testing of calendula in temperate zones and, ultimately, calendic acid production and VOC replacement.

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## **RELATIVE KINETICS OF BIODIESEL FORMATION IN A JET FLOW STIRRED REACTOR**

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Biodiesel production occurs throughout an alcoholysis reaction known as transesterification, where tryglicerides with an alcohol and a catalyzer react to generate esters of the alcohol called biodiesel, with glycerol as a by-product. Commercial production of biodiesel requires developing transesterification methods to accomplish this reaction in economical and efficient way from the standpoint of energy consumption and processing time.

To study the rate of conversion from vegetable oil to methyl esters in a jet flow reactor under different stirring hydraulic regimes.

The rate of biodiesel formation was assessed in a transesterification reactor stirred with a dual jet flow close loop. A blend of 85% of soybean and 15% of sunflower oil, methanol and NaOH were used to study the rate of conversion from vegetable oil to methyl esters. A nine liters cylindrical reactor with conical bottom discharge connected to two centrifugal pumps for fluid recirculation through dual opposite radial jet flows in its upper part was developed for the experiment. Four different diameters of ejector corresponding to initial Reynolds Number (Re) ranging between 1300 and 6470 were tested to assess the reactor heating profile along with the kinetics of biodiesel formation.

Clear performance differences among ejector diameters and Reynolds

Number values were observed showing that higher Re result in low relative conversion times along with higher final temperature in the reactor. For 98% of relative conversion to biodiesel, the ejector with Re=6470 showed 88% reduction in transesterification time with respect to an ejector delivering a Re=1300 and without requiring any external heat source.

Jet flow stirring with high Re Number is a fast process to produce biodiesel, which permits self heating of the reactants and high conversion rates.

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## **INDIRECT MORPHO-PHYSIOLOGICAL CHANGES ASSOCIATED WITH TRADITIONAL SELECTION PROGRAMS FOR INCREASED YIELD IN WILD SPECIES OF LESQUERELLA**

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Higher yield of improved cultivars generally comes from the re-partitioning of photo-assimilates towards an increase in harvest index (H.I.). Thus, selection based on seed-yield tends to be linked to reductions in drought tolerance, plant defenses, and plant longevity. As a result, the improvement of new crops (particularly targeted for arid lands) carries an implicit increase in sensitivity to changes in the environmental quality similar to what has been found in conventional crops. *Lesquerella* is considered a promising seed-oil crop, annual, and perennial species are included in domestication programs. Ideally, breeding programs of crops targeted for arid lands should strive for cultivars with lower but more stable yields than traditional crops, and retain some of the basic traits found in their wild relatives that provide adaptation for growth and survival in low-resource environments. A better understanding of the relationship between seed-yield and drought-tolerance related traits is key to defining new criteria of selection in crops specifically targeted to arid land.

This field study was conducted to assess the effects of breeding for increased seed-yield on traits related to drought tolerance and growth, in annual (*L. gracilis* y *L. angustifolia*) and perennial (*L. pinetorum* y *L. mendocina*) species of *Lesquerella*.

In a completely aleatorized design with two factors (species \*selection), we compared growth, morpho-physiological and allocation traits, and seed-yield in selected (three selection cycles for seed-yield) and wild (bulk collections from native stands) germplasm.

We found a change in the seasonal pattern of growth in the selected accessions (SA) of *Lesquerella*. The SA of the two annual species and *L. mendocina*, had a lower relative growth rate in autumn and winter (coincident with the vegetative phase, RGRveg) compared to non-selected accessions (NSA), which was linked to a decrease assimilation rate (A,  $p < 0.01$ ), and a lower allocation to leaves (LMR,  $p < 0.05$ ) and leaf area ratio (LAR,  $p < 0.05$ ). In all four species proportional allocation to storage (TNC) during the vegetative stage was lower in SA ( $p < 0.01$ ), with no changes in allocations to roots (RMR). When we compared SA vs. NSA (all species) in the reproductive phase (spring-summer) we found higher RGR in SA, related to higher SLA ( $p < 0.01$ ), A ( $p < 0.05$ ), stomatal conductance (gs;  $p < 0.05$ ) and WUE ( $p < 0.01$ ), and lower proportional TNC at maturity in the SA of perennials ( $p < 0.01$ ). The higher total biomass of SA of *L. pinetorum* was consequence of a longer growth cycle (RGRtotal was not changed). In *L. angustifolia* and *L. pinetorum* the higher seed-yield of SA was linked to higher H.I.

Selection for seed-yield resulted in changes in structural traits (lower RMR and TNC, higher SLA) which explain the increase in seed-yield but should result in lower yield stability in drought prone environments. On the other hand, the higher seed-yield was linked to changes in functional traits such as increased A without a changes in E, which resulted in higher water use efficiency (WUE) in the reproductive phase. The lower TNC at maturity of SA in perennials could compromise the longevity of these species.

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## THE PERFORMANCE OF *EUPHORBIA LAGASCAE* IN SOUTHERN SPAIN (ALBACETE)

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Cultivation trials of *Euphorbia lagascae* L. (Euphorbiaceae), an oilseed species rich in vernolic acid, have been done at small scale and on an experimental basis (usually linked to the funds of research projects) since the 1990's. The main agronomical problem found, with the wild accessions of the species, was seed dehiscence. In spite of this characteristic, seed yields from 400 to 1600 kg/ha were obtained in Spain (in a winter mild coastal area with spring or autumn sowing and irrigation) and also 1100 kg/ha on average were estimated in Southern England.

Indehiscent genotypes of *E. lagascae* (vernola) have been tested in Germany and Portugal giving seed yields within the range 274 to 1574 kg/ha with spring sowing and irrigation. There is a scarcity of information on how this plant will perform under less favourable conditions. The objective of this work was the evaluation of production potential of 3 indehiscent genotypes in a cold winter inland area with semi-arid Mediterranean climate. A randomized block design with 3 replications was sown (density 90 seeds/m<sup>2</sup>) on November 2008 under rainfed conditions and on February under

irrigation at “Las Tiesas” Experimental Station belonging to ITAP and located in Albacete (Spain).

The investigations conducted were to field evaluate the potential of vernola as an alternative to winter barley (November-June) or rapeseed (January-September) in crop rotations. *E. lagascae* establishment was below the target due to uneven germination particularly for the M24-3-7 genotype. The plants withstand cool temperatures (below -6°C) and are able to grow successfully without irrigation in the November plantings although with slow growth during the winter months. *E. lagascae* was susceptible to weed competition in the irrigated trial.

At harvesting we will estimate the potential of the species under the agricultural system tested and will identify the needs in plant breeding and/or agronomic practices to advance in the commercial introduction of this crop.

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## SAFFLOWER (*CARTHAMUS TINCTORIUS* L.) ADAPTATION IN CENTRAL-SOUTH CHILE

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Several safflower research and agronomic studies as well as preliminary commercial production efforts have been developed in Chile as early the 1950's and continue to date. The crop has shown good adaptation and seed yields but so far not enough to compete with more profitable crops.

Most recently, studies on the potential of new improved safflower lines were initiated in 2002. Safflower is now being used as storage of transgenic proteins mainly for pharmaceuticals use under the leadership of SemBioSys Genetics Inc., a biotechnology company using its proprietary oilbody/oleosin-based protein production technology to develop high-value proteins including protein-based pharmaceuticals, industrial reagents, food additives, and nutritional oils. Currently, SemBioSys is using the safflower plant to develop efficient and low cost production of high-value pharmaceutical proteins, biosimilar insulin and ApoA-1 Milano.

Data on AgroSearch agronomy and adaptation studies performed in Chile with non-GMO safflower will be presented, and the future research to be developed in the next three seasons under a project headed by Fundación Chile will be described.

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## SEEDING DATE INFLUENCE ON CAMELINA SEED YIELD, YIELD COMPONENTS, AND OIL CONTENT IN CHILE

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Camelina (*Camelina sativa* L.) was introduced for the first time in Chile in 2008 as a potential feedstock for biodiesel and also as high omega-3-containing seed oil for the salmon feed industry. The objective of this study was to determine the optimum seeding date to maximize seed yield in South Central Chile. The experiment was conducted, under dryland conditions, in Chillán, El Carmen, Los Angeles, Gorbea, and Osorno in the 2008-2009 seasons. The experimental design was an RCBD with a split-plot arrangement with 4 replicates, where the main plot was the seeding date (five dates) and the sub-plot the cultivar (Gold of Pleasure, Suneson, and Blaine Creek). Experimental units consisted of 6 rows, 5 m long and spaced 30 cm apart. Seeding dates at each environment were targeted to 30 April, 15 May, 30 May, 30 June, and 30 July of 2008. Seed yield was taken from the center-four rows of each experimental unit discarding 0.5 m of plants from row ends. Seed yield components evaluated were: number of plants m<sup>-2</sup>, number of siliques plant<sup>-1</sup>, number

of seeds silique, and 1000-seed weight. Seed oil content was determined on a 40 mL sample of each experimental unit with a Newport Nuclear Magnetic Resonance (NMR) analyzer. Each location-year combination was defined as an “environment” and was considered a random effect in the statistical analysis. The combined analysis of variance indicated no cultivar main effects or interactions with cultivar. The date by environment interaction was significant for seed yield. There were no seed yield differences among seeding dates in Chillan and El Carmen. The first three seeding dates were the highest yielding in Los Angeles and Osorno and only the first seeding date was significantly higher in seed yield than the other four in Gorbea. Highest seed yield at Los Angeles, Gorbea, and Osorno were 1995, 1310, and 2314 kg ha<sup>-1</sup>. Seed yield differences observed were due to the differences in number of siliques plant<sup>-1</sup> and seed weight. Seed oil content was not different among seeding dates in Chillan and El Carmen. Highest seed oil content occurred at Osorno and Gorbea on the first three seeding dates and ranged from 420 and 457 g kg<sup>-1</sup>. Camelina is well adapted to South Central Chile as a winter annual crop when seeded before 30 May. When seeded before 30 May temperatures are lower during seed filling compared with planting 30 June and 30 July. This same response was observed in canola planted after 30 May.

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## EVALUATION OF SPRING CANOLA AS POTENTIAL ALTERNATIVE CROP IN THE CENTRAL GREAT PLAINS OF THE USA

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To determine adaptability of spring canola (*Brassica napus* L.) to the High Plains as oil seed crop, 26 trials were conducted from 2005 to 2008. Trials were divided into five regions: 1. 36-37N 108W, 2. 39-40N 101-103W, 3. 41-42N 102-103W, 4. 41-42N104W, and 5. 44N 106-108W. Cultural practices were based on site-specific protocols. Four standard cultivars, Hyola 357 Magnum, Hyola 401, SW Marksman, and SW Patriot, were planted in replicated plots in April or May under standard irrigation and harvested in July to September. Hyola 401 and Hyola 357 Magnum were the highest yielding cultivars across the five regions and within Regions 1, 2, 3, and 5. Regions 1, 2 and 3 yielded significantly greater than did Regions 4 and 5. Except in Region 2, the four cultivars had greater than 35% oil content, and SW Marksman and SW Patriot had the highest. The highest oil content was achieved in Regions 1, 4 and 5. Samples from 18 trials were examined for their fatty acid distribution. Canola oil is high in oleic (C18:1) and linoleic acids (C18:2), which are commonly used for food and industrial purposes. Across and within regions, the percent of oleic acid did not differ for the four cultivars. The mean content of C18:1 oils increased going north from Region 1 to Region 5, inversely to yield in the High Plains. Considering yield and oil quality together growing spring canola in the Nebraska Panhandle may be the best location in the High Plains.

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## SEEDING DATE INFLUENCE ON FIELD PENNYCRESS IN NORTH DAKOTA

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Seeding date is important for spring annuals for optimizing climatic conditions during reproductive development. Seeding date for winter annuals is important for fall growth and plant size, and subsequent overwintering and survival of plants in the following spring. Low winter survival affects stand density and this can have a major impact on crop performance.

The objective of this study was to evaluate the influence of mid and late September and early October seeding dates on field pennycress (*Thlaspi arvense* L.) performance.

The study was established near Prosper, ND, in the fall of 2007 and 2008. Seeding date and genotype were main and subplots, respectively, with three replicates sown no-till into standing hard red spring wheat (*Triticum aestivum* L.) stubble approximately 25 cm tall. Traits evaluated included fall stand rating, plant size at freeze-up, winter survival, flowering date, plant height and lodging, seed weight, and biomass and seed yield, harvest index, and seed oil content.

First year results indicated winter survival of stands from all seeding dates exceeding 85%. Flowering and maturity occurred earlier from the earliest seeding date compared with the mid and late seeding dates. Seed yield was similar from mid and late seeding dates, but 30% less than the early seeding date. Harvest index was approximately 30% and considerably higher than many new and alternative crops. Earliness of field pennycress harvest indicates potential for double cropping.

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## ESTOLIDES FROM CORIANDER FATTY ACIDS

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Coriander (*Coriandrum sativum* L.) is new crop that is currently being investigated for cultivation in Central Illinois by USDA-NCAUR. Coriander is an annual herb belonging to the Apiaceae (Umbelliferae) family and indigenous to the Mediterranean basin areas and the Near East. This plant is widely distributed and mainly cultivated for its seeds which are used for different purposes (food, drugs, and cosmetics). Coriander seed oil is rich in an unusual fatty acid, the petroselinic acid (C18:1, n-12), which composes as much as 85% of the total fatty acid. The fresh green leaves of coriander, commonly known as cilantro are widely featured on the menu in China, Mexico, South America, India and Southeast Asia. The development of a new crop often depends on the synthesis of novel compounds. Estolides are one such derivative of new crop oils which show promise in industrial applications.

Estolides are formed when the carboxylic acid functionality of one fatty acid links to the site of unsaturation of another fatty acid to form esters. Estolides were derived from coriander fatty acids and various other fatty acids in the presence of an acid catalyst at 60°C for 24 hrs. The free acid estolides were then esterified to the 2-ethylhexyl esters under standard conditions.

These new estolide esters were converted to their corresponding hydroxy fatty acid and the degrees of polymerization were determined by GC analysis. Physical properties (pour points, cloud points and viscosities) of the coriander estolide esters were compared to previously synthesized homo-estolides and coco-estolides, which have current industrial applications. These new estolides show promise as a cheap alternative biobased material.

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## CASTOR AS A RENEWABLE FUEL IN THE NORTHEAST USA

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Castor (*Ricinus communis* L.) is a semitropical perennial plant that has been successfully grown for three years as a renewable fuel source in the Northeast USA. Field results indicate we are just beginning to realize the potential for this crop. Castor, an ideal candidate for bio-oil production with 1,200 liters/hectare, is higher yielding than soybean or canola. Castor is not used for food and can be grown productively on the extensive underutilized marginal uplands of the region. The extremely high oil content and yield potential of castor will generate a positive energy balance producing 4 to 8 Calories in liquid fuel per Calorie invested in production and processing. Castor oil directly offsets imported petroleum. It can be used "as produced" for heating oil – a vital product for winter in the region. It has greater value as a premier lubricant replacing petroleum based oils. There is a market for every gallon produced. NorthWinds Biodiesel and Alnor Oil have both expressed high interest in purchasing local product. On-farm production/extraction operations are targeted for the many recently defunct smaller dairy farms, most of which have milk tanks for storage. The farmers are experienced at producing, storing, and selling a liquid commodity into local markets

The objective of a two year New York Farm Viability Institute research grant is to develop sustainable practices for optimal castor oil yields in the Northeast. Problems being addressed are: 1- evaluation and selection of best germplasm for high latitudes yet possessing good seed quality as well as agronomic traits; 2 - understand and correct the biology producing poor germination and stand establishment issues in castors in the Northeast; 3- develop germination profiles for low soil temperatures typically encountered in the spring; 4 – develop basic agronomic practices of row width, in-row spacing, and the control of growth/reproductive characteristics by plant growth regulators. Dr. Taylor is researching castor seed biology and developing technologies to enhance stand establishment. Mr. Kilcer is developing agronomic practices. The goal is to grow castors with high yield potential and maximum oil production in manageable plant size.

2008 completed the second year of production of castors Hale and Brigham, just south of Albany, New York. Deep zone tillage (43 cm) had a significant beneficial impact on increasing root and top growth. A John Deere corn planter was used to plant on 76 and 38 cm. rows. The narrow rows maximized intercepted light in the short season and had a major effect in keeping late season weed pressure to a minimum. In-row spacing of 30, 45, and 60 cm was used to determine optimum plant spacing. After a killing frost at the third week in October, the plots were hand harvested on Oct 31. Each harvested block was two meters long. The 38 cm spacing out yielded the 76 cm row space in both varieties. The 45 cm in-row spacing in both Hale and Brigham gave the highest average yields. A test of 36 varieties found two lines that yielded 60% and 75% higher than the standard dwarf variety Hale. This indicates considerable upside yield potential for this already high yielding oil crop.

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## CASTOR BREEDING IN TEXAS: CHALLENGES AND OPPORTUNITIES

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The United States is a lucrative market for oil derived from the castor plant (*Ricinus communis*). The state of Texas has vast areas of land not well-suited for most food crops; however, castor would be a viable alternative in these areas. Since castor research in the United States has been largely neglected for more than 30 years, production systems and germplasm are in need of development. In addition, producers and marketers of other commodities have expressed concern over potential co-mingling of castor beans and food crops.

In response to these challenges, projects are ongoing throughout Texas to improve castor germplasm and to create safeguards that would mitigate health and market concerns associated with ricin.

Castor breeding lines are being screened and developed with high-salt tolerance, drought resistance, disease resistance, plant morphology traits compatible with mechanized harvesting, and early maturity habits. Initial screening trials have identified several lines with good drought and salt tolerance. The mutating agent, EMS, has been used on two elite breeding lines in an effort to identify plants with reduced ricin content, which would improve the safety of castor. So far one cultivar, Brigham, has been developed with reduced levels of the toxins ricin and RCA. Advocates of castor

production in Texas are working closely with other commodity groups to develop public policies and strategies that protect food supply chains from ricin contamination.

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## ENGINEERING *LESQUERELLA* FOR SAFE CASTOR OIL PRODUCTION

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Castor oil contains 90% ricinoleate (C18:1OH) which is the conventional source of hydroxyl fatty acid. Ricinoleate and its derivatives are used as raw materials for numerous industrial products, such as lubricants, plasticizers and surfactants. The production of castor oil however, is hampered by the presence of the toxin ricin and hyper-allergic 2S albumins in castor seed. *Lesquerella fendleri* (L.) (*Brassicaceae*), being developed as a new industrial oilseed crop in the southwestern region of U.S., is valued for its unusual hydroxy fatty acid (HFA). Lesquerolic acid (C20:1OH), the HFA in *L. fendleri*, is derived by a 2 carbon elongation of ricinoleate. By suppressing the elongation step in *L. fendleri* through genetic engineering, it is possible to generate a *L. fendleri* crop producing ricinoleate.

As a part of genetic approach to engineering ricinoleate synthesis, we investigated the seed development in *L. fendleri*. The morphological, physiological and biochemical changes during seed development were characterized from 7 days after pollination (DAP) to desiccation. The entire course of seed development lasted about 49 days and it can be divided into seven sequential stages (I to VII). During the early stages (I to III, 7 to 21 DAP), seed grew rapidly, showing dramatic increase in size and fresh weight. During mid-maturation stages (IV to V, 28 to 35 DAP), storage lipids, proteins and other components of dry weights accumulated at maximum rates. The accumulation curves followed a sigmoidal pattern during seed development. When seed progressed to late-maturation/desiccation stages (VI to VII, 42 to 49 DAP), the size of the seed decreased slightly and the color changed from green to orange-brown. Seed proteins were also analyzed using SDS-PAGE. Proteins with high molecular weights were prominent in developing seed at early stages (I to III). At stage IV (28 DAP), proteins with low molecular weight appeared while the high molecular weight proteins decreased in proportion. These low molecular weight proteins became predominant throughout the remaining stages of seed development. Forty-seven percent of freshly harvested seed at 35 DAP were able to germinate after 7 days incubation. The germination rate increased to a maximum of 95% at 42 DAP. These results provided integrative information for understanding the seed development in *L. fendleri*, which is critical to the development and implementation of a genetic approach for crop improvement.

To genetically modify *L. fendleri*, we have successfully developed a protocol to transform *L. fendleri*. We have demonstrated a GUS reporter gene that was incorporated into the genome and inherited to the next generation of transgenic *L. fendleri*. The transformation protocol provides means not only to engineer a ricinoleate-producing *L. fendleri* for safe castor oil production, but also to make *L. fendleri* a superior crop by improving yield and disease-resistance.

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## Natural Rubber and Resins

### A SET OF DESCRIPTORS FOR EVALUATING GUAYULE GERMPLASM

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Commercialization of guayule (*Parthenium argentatum* Gray) as a source of rubber is receiving world-wide attention as an alternative to Hevea in order to meet increasing demand for natural rubber. As more breeders, agronomists, botanists, and other scientists become involved in evaluating and developing guayule germplasm, it is imperative that a uniform set of germplasm descriptors is available. These descriptors are also necessary for use in obtaining plant variety protection certificates.

The objectives of this study were to develop a set of descriptors that could be used for identifying and describing germplasm lines and cultivars of guayule. A set of descriptors was developed and used to evaluate breeding nurseries of both traditionally developed and transgenically developed guayule plants. The descriptors were easy to use and required a minimum amount of time per plant, so that a large number of lines could easily be evaluated. The descriptors adequately covered the range of diversity observed in the nurseries evaluated. Of the descriptors used, a subset is proposed as a minimum to use in evaluating lines. For germplasm protection uses, the full set of descriptors should be used.

The descriptors will provide uniformity in comparing germplasm performance across environments. The descriptors will also be valuable to regulatory agencies in granting various plant protection certificates.

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## DEVELOPMENT AND EVALUATION OF A GUAYULE DEBARKER

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Guayule (*Parthenium argentatum* Gray) is the only species other than *Hevea* with the potential to produce natural rubber. Unlike *Hevea*, which bears rubber latex in a system of easily tapped ducts, guayule stores its rubber primarily within the cells of its bark. The present rubber extraction method involves rupturing of these cells by grinding the whole plant. Guayule bark constitutes only 30% of the dry weight of the whole guayule shrub. Debarking guayule soon after harvesting at the crop site would substantially improve the efficiency of latex extraction technology by reducing transport costs to the processing plant and increasing the processing capacity of the latex extraction plant.

Main objectives of this study were to develop an efficient guayule debarking system, evaluate the effectiveness of the technology and provide appropriate recommendations as to how it can be used to advance commercialization of guayule.

Following literature review and preliminary laboratory investigations, a guayule debarking system consisting of bark removal and separation mechanisms was designed and developed. The bark removal unit consists of a pair of grooved rubber rollers installed one on top of the other. The bottom roller was offset by 100 mm to the feed side to catch and guide cut stems into the machine. The rollers rotate in opposite directions at different speeds, drawing and crushing the cut stems without chipping the core. The speed difference between the rollers created a shearing action required for peeling the bark. The clearance between the rollers is adjustable and springs are fitted so that the rollers can handle different stem sizes ranging from 6 to 45 mm.

The separation unit consists of a 4 kW fan attached to a 6 m long metal tubing 150 mm in diameter. A discrimination chamber 1.4 m long and 30 cm wide was designed and attached to an air supply system for grading processed material by density. The other end of the chamber leads to a 40 litre capacity rectangular water trough where lighter material is removed by flotation.

The debarking system was tested to determine the bark removing performance as well as separation of bark from other processed plant material. A debarking efficiency of up to 95% and maximum separation efficiency of 75% were found from the evaluation test. The prototype debarking machine produced a throughput capacity of up to 450 kg/h, which can be further increased by scaling up and modification of the current design.

The findings of this study have demonstrated that it is possible to significantly reduce transport costs and increase the throughput capacity of a latex extraction plant by retaining 70% of plant material in the field. Further work on up scaling the prototype to a full-size field machine will be of great commercial benefit to the guayule industry.

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## PLASTIC MULCH AS AN AID TO DIRECT SEED GUAYULE

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Guayule (*Parthenium argentatum* Gray) is a natural source of rubber with great untapped potential. Guayule is already being grown for the lower volume, higher value, niche market of medical and commercial grade hypoallergenic latex. In addition, Guayule has the potential to meet the much larger demand for the multitude of other uses of natural rubber as well as producing resin and biomass. Commercial guayule fields are established through transplanting. This is an effective and low risk method for crop establishment and works well for low and medium sized plantings. However, transplanting adds significant cost to crop establishment and is not amenable to large scale up. Establishing a stand using direct seeding has been successful under many conditions, but is risky. Even with successful emergence, survival of the newly emerged seedling is often poor or variable. The ability to reliably establish good stands with direct seeding is essential.

The objective of this study was to determine if plastic mulch could be used to facilitate seedling emergence, survival, and growth on furrow irrigated direct seeded guayule.

Preliminary pilot studies suggested that temperatures are too high for seedling survival under clear or black plastic. Temperatures under white plastic with 50 % light transmittance were still higher than ambient, but were acceptable in the cooler part of the traditional seeding season. Pilot studies also showed that plastic mulch clearly enhanced emergence, survival, and growth as compared to direct seeding without mulch. Pollination netting mulch was even superior to plastic mulch in emergence and survival, but not in growth. However, netting costs are prohibitive for large plantings.

In the main study, we direct seeded guayule into a shallow (5 cm deep) furrow on top of shaped beds that were 1m from center to center. The beds were covered with plastic mulch, and the crop was furrow irrigated. The plastic was vented by adding holes at various stages after emergence. The plastic was removed at various times after venting. Temperature in the seeded furrow below the plastic was measured before and after venting and compared with ambient temperature.

Venting the plastic with holes was necessary soon after emergence. Seedlings in plots with non vented plastic did not survive well. Survival after plastic removal was best with plastic that was vented at least two weeks prior to removal. Final stands were best in plots that were vented at four weeks after planting with plastic removal anywhere from six to ten weeks after planting. Issues still to resolve to remove the risk from this method of stand establishment are weed and insect control under the plastic mulch.

Direct seeding guayule under plastic mulch shows promise. However, some details remain to be worked out before this is an economical and low risk alternative to transplanting.

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## RAPID FIELD MEASUREMENT OF RUBBER CONTENT IN RUSSIAN DANDELION

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Natural rubber is a critical and strategic raw material for industrial manufacturing and national defense. In 2008,

10 million tons of NR were produced for commercial use, most of it from *Hevea brasiliensis* in tropical countries. The annual US import deficit for NR is approximately \$1 billion. Development of a US-based supply of NR is recognized in the Critical Agricultural Materials Act of 1984 (Laws 95-592 & 98-284) and is the subject of expanding research and development in the public and private sectors.

*Taraxacum kok-saghyz* (TKS), Russian dandelion, produces natural rubber, cis-1,4-polyisoprene, in its roots and has been used commercially during times of short supply, especially in Eastern Europe. Evaluation of Russian dandelion as an alternative rubber-producing crop for cultivation in the U.S. requires high-rubber producing lines, as raw material for breeding, processing, and agronomic development. The objective of this work was to develop a fast field method for quantification of natural rubber in fresh TKS roots, to accelerate development of research and development TKS lines. Near-infrared (NIR) spectroscopy is used to measure chemical composition and physical properties of biomass between 750 and 2500 nm, and has been used to quantify rubber content in guayule (ground shrub, bagasse, and latex solutions).

Freshly-harvest TKS roots from field plantings in Wooster, Ohio, and Corvallis, Oregon (USA), were shipped overnight to the USDA/ARS QARU Quality and Safety Assessment Laboratory (Athens, Georgia). An axial cut was made across the root and near-infrared spectra acquired using 3 instruments varying in sensitivity. The Polychromix Phazir (MEMS system at 8 nm resolution with 20 co-added scans over range of ~1600-2400 nm) is a handheld instrument that could be used in the field on as is samples. Root tissue were then dried, ground, and rubber content measured by Accelerated Solvent Extraction (ASE). The inulin content of the rubber-extracted roots tissue was determined by colorimetric assay.

The rubber content of the roots tested varied from 0-17% dry weight root (dwr), inulin from 0-43% dwr. Results showed no correlation between rubber and inulin levels.

NIR chemometric analysis can provide a fast, reproducible method for nondestructive screening of Tks germplasm in the field. The model developed from over 100 plants grown in Ohio and Oregon predicts rubber to within 7-9mg/g, depending on parameters used.

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## A NEW METHOD FOR *EUPHORBIA ANTISYPHILITICA* WAX EXTRACTION IN A PILOT PLANT IN MEXICO

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*Euphorbia antisiphilitica* Zucc (candelilla) is a species from the Mexican semi-desert that produces a lot of wax with high quality. This wax has several uses in pharmaceuticals, cosmetic and nutritional industry, especially on the elaboration of edible covers for preservation of fruits. However the traditional extraction wax process has been use by the “ejidatarios” for more than 100 hundred years. The plants must be extracted in a sulphuric acid solution and to keep it until boiling, but the purity of the acid is unknown and it can cause health problems, as well as environment pollution, and wax production of low quality.

The objective of the present work was to evaluate the traditional candelilla wax extraction method and to compare with an improve methodology of wax extraction in a pilot plant, and also to modify the refine wax process.

A field trip was carried out to ejido La Vega in Cuatrociénegas, Coahuila, to observe the traditional wax extraction process, and also a wax extraction was made with the new proposed method, using 250 kg of candelilla plants.

The wax yield obtained with the traditional method was 2.4 percent (5.9 kg), meanwhile with the new method the yield reached 1.2 percent (2.9 Kg), half of the traditional (commercial) process. However in order to achieve a friendly methodology with the environment, it is necessary not only to consider the yield, but also other conditions such as the health benefits for the ejidatarios. Due to the low yield obtained we recommended to continue with the research in order to reach a wax yield of 2 to 8 percent.

On the other hand, we found that it's not required to melt the wax again in a sulphuric acid solution, it is only

necessary to melt the wax in water.

We can conclude that it is possible to change the extraction solvent to improve the traditional process in the candelilla wax extraction.

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## **Bioenergy Symposium**

### **COMPARISON OF FIVE SECOND GENERATION BIOFUEL PATHWAYS**

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The 5 major pathways to a biomass biorefinery are outlined. The author has been involved or has intensely studied each. The techno-economic strengths and weaknesses of each are presented. Key metrics are developed so that a new project can be compared to its pathway or various pathways can be compared to opportunities.

Biofuels are liquids or gases made from renewable sources. Petroleum represent the largest segment of imported energy in the United States. The technology is here for liquid biofuels to supplement petroleum-based transportation fuels. Activity is increasing on this needed energy source.

The consensus forecast on world oil availability is changing because, for the first time, the annual increase in oil demand exceeds the annual discovery rate of new oil. The U.S. has established renewable fuel standards that go well beyond the capability of corn-based ethanol. Many analysts forecast the need for “second generation” biofuels to meet demands. Commercial activities show that renewable fuels can be cost-effectively produced on a commercial scale.

The presentation will also discuss:

- Current process pathways to produce “second generation” biofuels.
- A comprehensive summary of commercial North American activities in “second generation” biofuels and the biorefinery.
- A comparison of the 2007 U.S. Department of Energy (DOE) funded projects with “Section 932” grants.

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### **BIOENERGY AND BIOMATERIALS FROM THE CHILEAN FORESTRY INDUSTRY**

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Chile has built a very active forest industry which is based on *Pinus radiata* as well as *Eucalyptus globulus* and *nitens*. A principal contributing factor to this development has been the outstanding growth conditions present in the region. The industry which deals with the plantation, harvesting and processing of forest biomass has strived for efficient and profitable operations. The products of the forestry sector are principally lumber, pulp, wood based panels, fire logs in addition to electricity and industrial heat. The supply forecast – based on harvesting of industrial residuals and sustainable operations – of forest biomass indicates an increase of raw material availability by at least half.

Chile’s forest industry has seen large growth over the past 40 years, and today it successfully competes in the global market. Nevertheless, its lean management organization needs to be complemented with a strategy that includes technology innovation if it intends to enjoy continued success. Among other things, this means that new products have to be developed, use of biomass needs to be widened and technology companies need to be created.

Forest biomass will play a central role in replacing, non-renewable, fossil raw materials. New materials,

biomaterials and forest based chemicals can be produced from both technical and economic considerations. Overall, this represents a great challenge, which highlights the urgency of improving collaborations between industry, R&D institutions and government.

Up to now, the key competing factor for Chile's forestry industry has been low production cost. The strategy while effective should now be complemented by technology innovation in order to be able to introduce new products with higher added value into the market. The transformation of the traditional wood processing industry into forest biorefineries has already begun.

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## U.S. DEPT. OF ENERGY REGIONAL BIOMASS FEEDSTOCKS PARTNERSHIP

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The Regional Biomass Feedstocks Partnership (RBFP) is a collaborative association of production-oriented researchers at the Land Grant universities and the U.S. Dept. of Agriculture, with scientists at the U.S. Dept. of Energy (DoE). For the RBFP, the DoE settled on four crop species (switchgrass, *Panicum virgatum*; giant miscanthus, *Miscanthus x giganteus*; sorghum, *Sorghum bicolor*; and energycane, *Saccharum* sp.) and Conservation Reserve Program (CRP) land. CRP land is composed of a mixture of plant species, native or naturalized at each location. Energycane testing is limited to locations south of 33°N latitude, while switchgrass and sorghum are more broadly adapted.

The objectives of this partnership are: 1. To investigate critical methods to maximize each crops' production across the environmental clines of the USA, 2. Evaluated cultivars of each crop and CRP land for sustainable production, 3. Determine potential expansion of crops into new locations.

Larger-than-plot-size tests were established in 2008 for each of the domestic crops. Research plots were imposed on existing CRP land, across the US. Fertility, genotype adaptation and harvest timing are being assessed for their impact on yield. Biotic (weeds, insects and pathogens) and abiotic (drought, cold, fertilizer) stresses are being monitored. Data is being uploaded from 29 sites around the USA to a national database for use by researchers and industry.

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## Fiber and Cellulosics

### EVALUATION OF CELLULOSIC ENERGY FEEDSTOCKS FOR PRODUCTION IN NORTH CENTRAL MISSISSIPPI, USA

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The objective of this study was to compare the end of season biomass yields between four potential cellulosic energy crops. Energycane (*Saccharum* spp.), sweet sorghum (*Sorghum bicolor*), switchgrass (*Panicum virgatum*), and giant miscanthus (*Miscanthus x giganteus*) were evaluated.

Replicated field trials were conducted on each species on the campus of Mississippi State University in Starkville (33.45° N, 102 m elev). End of season harvests took place during the dormant season when total biomass accumulation had already taken place. Harvesting was completed using a custom Carter<sup>®</sup> flail harvester with an on-board scale. End of season harvest yields indicate energycane, specifically the cultivar US 02-147, as having the greatest biomass accumulation (58 Mg/ha). Following energycane was sweet sorghum (cv. Della) with 51.56 Mg/ha., giant miscanthus with 44.84 Mg/ha, and switchgrass (cv. Alamo) with 24.66 Mg/ha.

For biomass crops to transition into modern farming practices planting, weed control, harvesting, and transportation must be easily accomplished. The availability of local markets must be in place for these crops to be economically successful. Decisions a producer needs to make are whether to plant an annual (sorghum) or perennial (energycane, miscanthus, and switchgrass), by seed (switchgrass and sorghum) or by vegetative propagation (energycane and miscanthus). Biomass crops could be viable options for the future of agriculture in Mississippi, but additional research is needed.

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## A COMPARISON OF HARVEST REGIMES FOR MISCANTHUS AND SWITCHGRASS

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Cellulosic ethanol production has been at the forefront of the biofuels industry in recent years. Feedstocks that produce large yields of cellulose, while limiting lignin are of prime interest with respect to ethanol production. Giant miscanthus (*Miscanthus x giganteus*) and switchgrass (*Panicum virgatum*) have been identified as two bioenergy crops that have these desirable characteristics. Multiple harvest regimes imposed on perennial crops often result in increased total annual yield.

The objective of this study was to determine how harvest regime influence biomass yield and nutrient removal of ‘Alamo’ switchgrass and ‘Freedom’ giant miscanthus.

Alamo switchgrass and Freedom giant miscanthus were planted May 15, 2002 in four row plots with four replications in a RCB design. They were subjected to two different harvest regimes beginning the following year. Plots were designated to be harvested twice at 90 day intervals or once at 180 days. Plots were harvested using a Carter forage harvester. Yield and mineral content were determined.

Mean yield during the first three years of this study were: 35.5 Mg/ha for giant miscanthus twice-harvested, and 34.3 Mg/ha for switchgrass twice-harvested. Single-harvested plots had a mean yield of: 32 Mg/ha for giant miscanthus and 29.3 Mg/ha for switchgrass. Ash content of the twice-harvested plot samples indicated significant mineral removal was occurring from the two harvest plots. During the fourth year of this study (2006), a fortuitous series of events occurred. Rainfall was 40cm less than average. The twice harvested plots were mistakenly harvested only once at the end of the season (180 days). The limited rainfall had significant impact on the twice-harvested plots, resulting in a 50% yield reduction. Giant miscanthus yield of the formerly twice-harvested plots was 50.2 Mg/ha; 25.2 kg/ha less than the previous year. Switchgrass yield of the same treatments was 40.8 Mg/ha, 19.1 Mg/ha less than the previous year. Interestingly, the single-harvest (180 day) plots were relatively unaffected by lack of rainfall.

Giant miscanthus and switchgrass typically have greater yield potential when harvested twice a season compared to once a season until inadequate rainfall conditions are encountered. Drought conditions significantly decrease biomass yield of switchgrass and giant miscanthus when harvested twice a year, while a single-harvest has no significant impacts on yield.

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## EFFECTS OF PLANTING DATE ON SUGAR AND ETHANOL YIELD OF SWEET SORGHUM GROWN IN ARIZONA

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Sweet sorghum (*Sorghum bicolor* (L.) Moench) is an annual crop currently being investigated for biofuel production in the arid southwest US. Sweet sorghum is an ideal candidate because it can be grown on marginal land, with few inputs (water, fertilizer), so that valuable cropland is not being displaced. Many varieties have been bred for high sugar, for syrup and forage production, but much biodiversity still remains to be utilized.

In previous studies, we found that high biomass and percent juice were the best predictors of potential ethanol yield per area. Our objective in this investigation was to plant several high-biomass varieties at different dates to determine what effects planting and harvest dates have on overall sugar yield and predicted ethanol yield.

Four varieties (Dale, M81E, Theis, and Topper) were planted in April, May, June, and July of 2008. They were harvested at physiological maturity (30 days after 50% of the plot was flowering); dates ranged from August 26 to December 2. Fifteen stalks from each replication and treatment were weighed, stripped of leaves and panicles, and weighed again before juicing. Juice weight and Brix were recorded. Samples were analyzed in the laboratory by HPLC for fructose, glucose, and sucrose.

All of the plots planted in July germinated poorly and subsequently had low stand counts. Theis was the earliest to mature, and Topper the latest. All lines increased in total sugar over time except M81E, which had a sharp drop in sucrose concentration at the last harvest (planted in July). Levels of glucose and fructose in each variety remained fairly constant at each harvest.

Theoretical ethanol yields were calculated based on biomass, juice weight, and percent sugar. These were compared to actual yields obtained from laboratory-scale fermentations of the harvested juice, which ranged from 7.4 to 11.2 percent (58.1 to 88.6 g/L). Since our predictive model uses the maximum conversion rate of sugar to ethanol and this was not reached in the lab, the predicted yields were always higher than the actual yields. However, the model can be a useful tool for estimating ethanol yield per area.

When planting sweet sorghum in arid climates, earlier in the spring is better in terms of germination, stand development, and, in some varieties, sugar yield. Sweet sorghum juice has been successfully fermented into ethanol, which indicates this crop may be able to play a transitory role in the emerging biofuel market.

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## ANALYSIS OF BIOMASS SUPPLY FOR BIOGAS PRODUCTION IN BIO BIO VALLEY, CHILE

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With a national potential production of 1,000 millions of Nm<sup>3</sup>/year, biogas from residual biomass could substitute up to 18% current consumption of gasoline in Chile, or a percentage equivalent to 25% of natural gas consumption. According to this, it is going to pay every time higher attention on biogas as a suitable alternative for energetic

diversification, due to it seems as an efficient and sustainable energetic option.

Notwithstanding the promising amount of biogas that could be obtained from different sources located in Bio Bio Valley, the first assessments has indicated a lack a reliable information concerning cost of transport, storage and preparation of biomass as well as location analysis for installing biogas plant with specifics purposes.

In the present work the problem of logistic analysis for biomass supply is carried out considering the resources already estimated by different methodologies, which also include a comparative assessment and its validation. And systematic approach is developed in order to compare the best use of biogas under the current economic framework, dealing with this approach as optimization problem under different economical and environmental restrictions.

The research presented shows a complete analysis of an alternative of biogas use, which does not exist at the moment. The cost analysis offers the first approximation with a market oriented point of view and provides useful information to stakeholder and the public sector.

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## UNDERSTANDING THE PHYSIOLOGY AND MECHANISMS OF SEED DORMANCY IN SWITCHGRASS (*Panicum virgatum* L.)

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Switchgrass (*Panicum virgatum* L.) is a perennial warm-season grass established by seed and native to most of North America. Switchgrass has been used as ground cover, forage for livestock, soil and water conservation, and wildlife habitat. In the United States, the Department of Energy (DOE) recommended switchgrass as a model herbaceous biofuel crop, as switchgrass produces high yield, contains high levels of cellulose, requires low energy input for production, grows in marginal lands, and can be a dedicated biofuel crop. However, as in many other perennial grasses, neoteric seeds exhibit dormancy, resulting in delayed and sporadic germination and emergence, jeopardizing establishment of a good stand. In nature, seed dormancy can be imposed by morphological, physical, and physiological properties, or by combinations of these properties. In switchgrass, dormancy has been alleviated by mechanical or chemical scarification and by stratification, suggesting physical and/or physiological dormancy; however, the causes, mechanisms, and physiology of dormancy are not well understood.

This work investigated the contribution of the different switchgrass seed structures to dormancy in 'Cave-in-Rock', removing one layer at a time under a dissecting microscope. Hormonal response to exogenous application of abscisic acid (ABA), gibberellins (GA), and fluridone (FLU) was also examined. Research was conducted to understand the effect of stratification (pre-chilled period at 5°C for 14 days) on seed germination.

Lemma, palea, and pericarp interfered with germination of seed with high dormancy. Germination at 30°C of intact seeds was 1% while the same lot with glumes, lemma, and palea removed had 50% germination. In addition, cutting of the pericarp and removal of the endosperm resulted in faster and higher germination (96%), indicating no morphological dormancy, characterized by underdeveloped embryos. Results showed that the lemma, palea and pericarp did not contain inhibitory compounds, but rather acted as a barrier for the diffusion of compounds through these layers as seeds with intact lemma and pericarp decreased the response to exogenous ABA and FLU. Data suggested that embryos were not only sensitive to ABA, and GA, but also synthesized *de novo* ABA during imbibition. In stratified seeds from a high dormancy seed lot, lemma and pericarp increased permeability to exogenous ABA.

Understanding the physiology and mechanisms involved in switchgrass dormancy will provide important and valuable information for future breeding programs focused on the development of low-dormant cultivars, and for the improvement of dormancy-breaking treatments.

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## COMMERCIAL PRODUCTION OF SHORT-FIBRE FLAX FOR INDUSTRIAL AND TEXTILE USES

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Fibre flax (*Linum usitatissimum*) is cultivated for the production long fibres (known as “line flax-fibre”) used in the manufacture of linen yarns. Short flax fibres (known as “tow”), are also produced as a by-product during the extraction of line-fibres from flax straw. Traditionally flax tow fibres are used in the production of paper, but they are now used for the manufacture of non-woven products, such as insulation materials or automotive panels. Flax tow fibres may also be used as a feedstock for the production of textiles, for example when ‘cottonised’ for use in apparel yarns, or when blended and spun with wool for use in upholstery fabrics. However, as the production of flax tow is as a by-product of the production of long fibres for linen yarns the quantity of short flax fibre produced globally per annum is relatively small (<250,000t). In order to exploit many of these developing end uses for flax fibre the production of fibre-flax crops for a whole yield of short-fibres is a potential way of targeting a supply of short-fibre flax for specific end uses.

The objective of the work here was to investigate the production and processing of short fibre-flax crops as a source of fibre for use in high-value cottonised yarns and for the production of fibre for industrial end-uses. Specifically, the objective of the work reported here was to investigate the mechanical extraction and cleaning of flax fibre suitable for the production of a whole yield of short fibres for textile and industrial end uses. Fibre flax crops were grown in field scale trials in 2006-08 to examine the impact of cultivar and plant density on yield per ha and fibre quality. The flax crops were retted using the method of stand retting. Harvested straw was decorticated using a laboratory scale decorticator and the fibre extracted to calculate the yield. The fibre was then assessed for fibre fineness, fibre length, fibre strength and texture. These results were then compared to those obtained from commercial scale decortication and fibre extraction trials conducted in a short-fibre flax processing facility in the UK.

Fibre-flax specifically grown for short fibres produced a high yield of fibre (up to 1.25 t/ha) from the modern flax cultivars trialled and when extracted through a high throughput decortication and fibre-cleaning production line. Satisfactorily fine flax-fibre (mean fibre diameters of 14-25 microns) was obtained from the stand retted flax crop after mechanical separation, without reducing staple fibre length significantly. As a result the flax fibre extracted was found to be suitable for cottonisation. In addition, coarser fibres were also effectively decorticated for use in industrial end uses, in composites and insulation materials. However, fibre cleanliness was essential to the determination of fibre value in any end use.

It was concluded that fibre-flax can be successfully used in yarns with other fibres produced on the woollen spinning system, while suitably fine flax fibres can be used in yarns produced on the cotton system; however, it is necessary to ensure that adequate fibre separation has occurred.

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### Medicinal and Aromatic Plants Symposium

#### ISSUES AND TRENDS IN THE GLOBAL BOTANICAL MARKET

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The growth of the use of herbs and medicinal plant products for health purposes is a global phenomenon. Market

statistics suggest that despite the recent world economic downturn consumers and health professionals are still purchasing, using, and/or recommending herbal products, either as the so-called "dietary supplements" as they are called in the United States, or as "traditional medicines", over-the-counter and/or prescription medications, depending on how they are regulated in various areas. This presentation will review recent economic trend data from the United States and other countries, as well as how herbal medicines are regulated in different countries, and various quality control and other relevant issues that face industry and regulators. Finally, the presentation will also discuss growing concerns about climate change on future availability of medicinal and aromatic plants (MAPs) and various organizations and programs dedicated to conservation of MAPs.

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## Medicinals and Nutraceuticals

### A SCIENCE-BASED PROGRAM TO SUPPORT THE ESSENTIAL OIL INDUSTRY IN RWANDA

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Natural plant products are receiving increased attention due to their potential to contribute to rural economic development. Essential oils, which have applications in the cosmetic, flavor and pharmaceutical industry, are considered high value crops since they can provide higher returns to farmers as compared with traditional food crops. In 2002, Rwanda began to rebuild their essential oil industry, by producing geranium (*Pelargonium graveolens*) and eucalyptus (*Eucalyptus globulus*) which are important aromatic crops that yield high value essential oils for international markets.

The objective of this study was to conduct chemical analysis on essential oils of geranium and eucalyptus from Rwanda to assess their quality and develop trade standards to support the commercialization of these essential oils in international markets.

The essential oils extracted from geranium (*P. graveolens*) varieties locally available, an improved geranium strain, and different varieties of *E. globulus* were used in this study. All the essential oils were characterized based on their sensory (color and aroma), physicochemical (refractive index) and chemical properties (essential oil components).

The essential oils of the locally available varieties that were introduced into cultivation showed that the sensory and chemical profile of these essential oils were not suitable for international markets but had application for local and regional markets. New geranium plantlets of the Bourbon type, considered to be the highest quality, were obtained from South Africa. The newly introduced cultivar yielded essential oils with a suitable sensory and chemical profile that matched the international specifications of a Bourbon geranium oil. Several naturalized Eucalyptus varieties were also analyzed and many were found to have the desired chemical profile (high in 1,8 cineole and low in  $\alpha$ -phellandrene) for international markets. To support the essential oil industry in Rwanda, there also needs to be a mechanism to evaluate essential oil quality locally and in this manner we partnered with IKIREZI in the establishment of a quality control lab, and conducted trials to optimize the production of essential oils.

These results have demonstrated the importance of the selection and introduction of most appropriate germplasm or chemotype with the acceptable organoleptic and chemical profiles to generate the greatest value and profitability. These features are among the key factors needed to obtain high quality essential oils that will meet international expectations and standards. This work was funded by the USAID via the Global Development Alliance and the Partnership in Food Industry Development for Natural Products ([www.pfidnp.org](http://www.pfidnp.org)).

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## **OBTAINING A MOUTHWASH WITH ANTI-INFLAMMATORY PROPERTIES AND ANTIBACTERIAL FROM *MATRICARIA RECUTITA* L, TYPE CHAMOMILE PUELCHÉ SPRING WITH DENTAL CLINIC USE POTENTIAL**

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The most prevalent oral diseases in Chile are dental caries and periodontal diseases that can be facilitated by malocclusions. These conditions usually affect mostly the lower economic stratum of the population. The most important cause of these diseases is the micro-organisms in plaque. The application of mouthwashes with antibacterial activities, can certainly help to reduce the burden of oral microorganisms, both in children and in adults. The objective of this work were to evaluate the antimicrobial effect of a mouthwash using an alcoholic extract of chamomile (*Matricaria recutita* L, chamomile type spring Puelche) in an *in vitro* and *in vivo* study, as an alternative application for the prevention of caries and periodontal disease in school children of 10 to 12 years of age.

The mouthwash was made with an alcoholic extract of chamomile, mixed with glycerine, water and ascorbic acid. The *in vitro* tests were performed by measuring the growth of aerobic and anaerobic bacteria (antibiograms) that are usually found in the oral cavity. The clinical study (*in vivo*) was conducted in 98 schoolchildren (10 to 12 years of age) from the “Marina de Chile” School, Lorenzo Arenas, Concepcion. School children were randomly separated in three groups: treatment, positive (chlorhexidine 0.12%) and negative control (placebo). The children, supervised by an adult, were instructed to make two mouth rinses a day (30 seconds each) with 5 mL of the mouthwash. The clinical samples were taken from the tooth surface and oral mucosa at the beginning of the experiment (time 0) and then after 24 hours, one, three, four and five weeks.

The *in vitro* tests showed an increased susceptibility of anaerobic and facultative anaerobic bacteria, the most affected were *Streptococcus mutans* and *Bacillus* sp., while for aerobic bacteria, the most affected strain was *Pseudomonas intermedia*. The *in vivo* tests showed a decreased in bacterial load over time for those who used the chamomile mouthwash and for the control group, particularly after the third week. In the clinical trials, we did not observe any adverse effects of the mouthwash on the oral cavity. The results of the *in vitro* and *in vivo* test showed that the chamomile based mouthwash was effective in reducing the bacterial load of the oral cavity.

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## **PREVENTING ADD (ATTENTION DEFICIT DISORDER) IN PRESCHOOLERS**

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In the past years, children with Attention Deficit Disorder (ADD) diagnosis in Chile has increased. At present, the percentage of Chilean population with this diagnosis under 7 years old fluctuates between 3 to 7%. However, children without clinical diagnosis, but with different problems at school (lack of concentration, motivation, aggressiveness, learning and emotional problems) bring this rate up to an astonishing 20-30%. At least 75% of consultations to children neurologists, psychologists, and psychopedagogists are due to the same reason.

The objective of this work is to discuss the prevention of ADD, a clinical problem that 30 years ago was unheard-of in Chile and in other countries as well.

Work on a preventive ADD model based on a worldwide necessity to stop medicating children due to side effects, high costs and consequences for the family, when the base of the problem is the lack of appropriate brain nutrients, will power and training.

The preventive ADD model is based on merging nutrition and training. The focus of nutrition is to enable the

brain to build new neuronal connections and thicken or strengthen previous ones. Raw material for building up intelligence is based on essential fatty acids extracted from oilseeds (specialty oils) with active ingredients supplemented with omega-3 fatty acids obtained from fish oil or micro algae. The balanced formulation of fish oil and antioxidants according to age and sex, provide the necessary requirements for the functioning of the Central Nervous System when consumption is low in daily diet. With proper nutrition, training will be more effective and allow a solid base for future learning processes.

Through 20 years of experience working with children diagnosed with ADD and their families we have come to the conclusion that parents are lost and confused what their children need to eat and do in order to build their learning structures. They feel guilty because they are not around most of the time and do not want to frustrate their children and say "no", on the other hand, children are not receiving sufficient repetitive training in order to acquire skills to have certain competence at school because their families do not demand them to repeat because exercising is boring.

Giving tools and knowledge to parents about the benefits of using essential fatty acids, appropriate training and facing boredom for their children under 6 years old can improve family quality of life and prevent future learning problems. ADD treatment causes high investment in Health and Education Departments in order to face this problem and its consequences.

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## FLAXSEED (*Linum usisatisimum*) CULTIVATED IN CHILE: POTENTIAL TO PRODUCE NEW RAW MATERIAL FOR HUMAN NUTRITION

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Flaxseed is an ancient crop known for its high content of oil, mainly  $\alpha$ -linolenic acid (ALA; an omega-3 fatty acid). The seed contains other valuable compounds such as lignans and phenolic compounds (cancer-preventing agents and antioxidants), as well as mucilages, which correspond to the so called 'soluble dietary fibre'. The above mentioned bioactive compounds make this crop important for human nutrition. However, the main use of flaxseed is for oil production. The 'cake', the residue after oil pressing, is a by-product and has a limited use in animal feed, but it has a potential for human nutrition as a source of lignans and soluble dietary fibre.

The objective of this study was to identify flaxseed varieties suitable for production in Chile, and to design the strategy for the development of new varieties for industrial flaxseed production.

We have determined the chemical composition of seeds as well as their genetic identity. Genetic variability of the collection was evaluated by using 10 polymorphic microsatellites and the data was subjected to clustering analysis using software PAUP 4.0 and the neighbor-joining method.

In our study, four local accessions (Chilean landraces) and twelve introduced varieties of flaxseed were cultivated during the 2006/2007 season. We found that the oil content varied between 43 to 48% and four accessions presented values over 47%. The ALA was found in a range from 55 to 60% of total fatty acids, with six accessions containing over 57%. Total phenolic compounds were found in the range of 301-641 mg/100 g and three accessions were over 500mg/100g. Mucilages, representing the soluble fibre, ranged from 6.3 to 10.3% of seed weight and five accessions were over 9%.

The analysis of genetic diversity revealed that our collection is composed by three different groups. Chilean accessions grouped separately among them in each main cluster. This results suggest that Chilean landraces are genetically different compared with the introduced varieties.

The chemical profile allows the identification of some accessions suitable to be cultivated for human nutrition purposes. Based on the genetic and chemical results, some Chilean landraces appeared potentially useful for hybridization to improve the seed quality. To our knowledge, this is the first time that native and introduced accessions of flaxseed are

chemically and genetically characterized in Chile.

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## ANTIFUNGAL ACTIVITY OF MEXICAN CHIHUAHUAN SEMI-DESERT PLANTS EXTRACTS ON POSTHARVEST FRUIT FUNGI

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Chemical fungicides have been intensively used for the control of postharvest fruit diseases. However, their prolonged use has caused the development of resistance by the phytopathogens. But also, their use is causing different environmental problems, as well as health related issues such as the contamination of foods.

The objectives of this study were evaluate the *in vitro* antifungal activity of hexane and ethanol based extracts of *Lippia graveolens*, *Yucca filifera*, *Yucca carnerosana* and *Agave lechuguilla* against *Rhizopus* sp., *Colletotrichum* sp., and *Penicillium* sp., fungi that cause postharvest fruit diseases.

Plants were collected near the cities of Saltillo and Torreón in the South region of Coahuila State in Northern Mexico. The dried plants were extracted with ethanol and hexane and then concentrated in a rotavapor. Fungi strains were isolated from apple, mango and orange fruits grown in Potato-Dextrose-Agar (PDA) in Petri dishes. The fungal growth parameters were mycelial growth, and spore number. A completely randomized design was used.

The results showed that *L. graveolens* extracts showed a 100% fungicidal activity against *Rhizopus* sp., *Colletotrichum* sp., and *Penicillium* sp. *Agave lechuguilla* was also highly active (100%) against *Rhizopus* sp., and *Colletotrichum* sp. While the extracts of *Yucca filifera* showed also a complete inhibiton of *Rhizopus* sp.

The ethanol based extracts showed higher antifungal activity than the hexane extracts, both *L. graveolens* extracts showed a total antifungal activity with lower doses against *Rhizopus* sp., *Colletotrichum* sp. and *Penicillium* sp. *Agave lechuguilla* extracts also showed antifungal activity toward *Rhizopus* sp. and *Colletotrichum* sp. Both extracts of *Yucca carnerosana* and *Yucca filifera* inhibited the mycelial growth of *Rhizopus* sp. However ethanolic extract of *Yucca carnerosana* enhanced the sporulation of *Colletotrichum* sp. It is concluded that all plants extracts showed inhibitory activity against the growth of these three fungi.

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## IN VITRO ANTIFUNGAL ACTIVITY OF COWANIA PPLICATA AND PISTACIA LENTISCUS AGAINST TWO PHYTOPATHOGENIC FUNGI

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The use of synthetic fungicides is causing different environmental problems and health concerns in both humans and animals. In recent years, there has been an increased interest in the search for natural fungicidal derived from plants. This interest is in part due to the fact the use of many synthetic pesticides is being restricted.

The objectives of these study were a) to determine the biological activity of the methanolic extracts of *Cowania*



*plicata*, (known as “alejandría”), and *Pistacia lentiscus* (mastic tree), on the *in vitro* growth of *Fusarium oxysporum* and *Colletotrichum coccodes*, b) to determine the part of plant (leaf, root, flower or fruit) with the highest antifungal activity, and c) to identify the bioactive chemical compounds responsible for this activity.

The Inhibitory Concentration (IC) of methanolic extracts was determined in Potato Dextrose Agar (PDA) infusion on Petri dishes. The characterization of chemical compounds was performed using a spectrophotometer and HPLC.

The results showed that the inhibitory concentration (IC<sub>50</sub>) of *P. lentiscus* leaf extract against *C. coccodes* was reached at 2,000 ppm while the IC<sub>90</sub> at 16,000 ppm. However, the IC<sub>50</sub> of *C. plicata* extracts against *F. oxysporum* was obtained at 3,000 ppm and the IC<sub>90</sub> at 28,000 ppm.

The leaves of *P. lentiscus* methanolic extracts showed the best inhibitory concentration IC<sub>50</sub> and IC<sub>90</sub> against this phytopathogenic fungi. The quantity of substances with biocides properties varied in the different plant parts. Many research studies has reported some chemical components responsible for this activity such as copper, phenolics compounds (hydrolysables and condensate) tannins, gallic acid and terpene-derived compounds such as pinene, myrcene, and germancene. The chemical analysis suggested the presence of some of these components that may be responsible for the antifungal activity of these plant extracts. These results demonstrated the antifungal activity of *C. plicata* and *P. lentiscus* extracts.

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## ANTIFUNGAL ACTIVITY OF *LARREA TRIDENTATA* AND *FLUORENSIA CERNUA* EXTRACTS AGAINST *PHYTOPHTHORA CINNAMOMI*

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Several crops of commercial importance in Mexico are affected by *Phytophthora cinnamomi* fungus, causing the root rot disease. At the present time, the control of this disease is made by mean of chemical products, due to the environment concerns, more research is required to obtain natural bioactive products for the control of *P. cinnamomi*.

This research was carried out to evaluate *in vitro* effect of *Larrea tridentata* and *Flourensia cernua* extracts on the mycelial growth inhibition of *P. cinnamomi*.

*L. tridentata* and *F. cernua* were collected in the southeast of Saltillo, Mexico, October 2008. The dry leaves were extracted with water and lanolin in a ratio of 1:6 (plant:dissolvent), during 7 h at 60°C. The concentration of hydrolysable and condensate tannins were determined spectrophotometrically. The antifungal *in vitro* activity was determined by the method of mycelial growth of *P. cinnamomi* in Potato Dextrose Agar (PDA) infusion on Petri dishes. A complete random design with four repetitions was used. Also a PROBIT analysis was carried out to determine the concentration of inhibition for each extract (at 50% and 90%).

The results showed that the inhibition of mycelial growth varied from 10% in *L. tridentate* at 200ppm extracted in water to 100% in *F. cernua* at 500 ppm in water. The best antifungal activity was observed in *F. cernua* extracted in water at 300, 400 and 500 ppm that inhibited the mycelial growth of *P. cinnamomi* by 98 to 100%.

The inhibition of growth of *P. cinnamomi* by *L. tridentata* at 1500 ppm extracted with lanolin was 93%, this treatment showed the highest antifungal activity for this species. The extract of *F. cernua* in water presented higher antifungal activity when compared with *L. tridentata* extract. This is the first research report of the *in vitro* activity of the extract of against *P. cinnamomi*.

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## ANTIFUNGAL ACTIVITY OF *LARREA TRIDENTATA* AND *FLOURENSIA CERNUA* EXTRACTS AGAINST *RHIZOCTONIA SOLANI*

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*Rhizoctonia solani* is a fungus that causes diseases in plant roots and tubercles in several economic important crops of Mexico. The use of chemical products for controlling these diseases increases the crop production cost. Recently, plant extracts have been tested in search for natural antifungals, as an alternative to synthetic products.

The objective of this work was to evaluate the antifungal *in vitro* activity of *Larrea tridentata* and *Flourensia cernua* extracts on the inhibition of *R. solani* mycelial growth.

*L. tridentata*, known as “gobernadora” and *F. cernua* as “hojasén” were collected in the southeast of Coahuila, Mexico. The dry leaves were extracted with water and lanolin in the ratio of 1:6 (plant:dissolvent). The tannin concentration was determined by the Folin-Ciocalteu technique. The studies of the *in vitro* activity of the extracts were determined by measuring the growth inhibition of *R. solani* mycelia in the Potato Dextrose Agar (PDA) infusion method on Petri dishes. A complete random design with four repetitions was used. For each extract, the PROBIT analysis was carried out to determine the inhibited concentration at 50% and 90%.

The water extract of *L. tridentata* at 500 ppm, showed no inhibition of mycelia growth. Higher inhibition of growth was observed in the lanoling extracts of *L. tridentata*. Thus, a concentration of 1500 ppm inhibited the fungal growth by 96.5%. High concentrations (2000-3000ppm) resulted in a complete inhibition of the mycelia growth. While, the extracts of *F. cernua* (1500ppm) in water were also found to significantly inhibit (94.2%) *R. solani* growth. These results have shown the potential of plant extracts of *L. tridentata* and *F. cernua* to control fungal diseases that cause important economic damage to important Mexican crops.

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## LIPPIA GRAVEOLENS POLYPHENOLIC EXTRACTS ACTIVITY AND CHARACTERIZATION, TOWARD *PENICILLIUM* SP.

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The postharvest damage of fruits and vegetables cause economics loss to producers, due to the shelf life diseases that reduce the production in 30 to 50 %. *Lippia graveolens* (orégano) is a specie native from the Chihuahuan Semidesert and is used for its flavor in traditional food of the region, and also as natural remedy to heal respiratories, intestinal and foot fungi diseases. There are reports about microbicidal and antioxidant properties of *Lippia graveolens* extracts attributed to the essential oils (thymol and carvacrol). Nowadays *L. graveolens* extracts can inhibit the development of mycelial fungi in very low doses.

The objectives of this study were to evaluate the antifungic activity *in vitro* of the *L. graveolens* polyphenolic fraction against fungi that attack fruits, as well as, to determine the chemical characterization of the active compounds.

The *L. graveolens* plants were collected at the southern of Coahuila State in Mexico. The ethanolic extracts were carried out in the ratio 1:2 (m/v), plant/solvent, during 48 h at room temperature. The polyphenolic fraction was obtained in a column chromatographic and the chemical compounds were characterized by HPLC. The polyphenolic extracts inhibitory effect was evaluated *in vitro*, by means of the mycelial growth of *Penicillium* sp. in Potato Dextrose Agar

(PDA) infusion on Petri dishes.

We consider obtain a high inhibitory effect of the polyphenolic extract due the nature of the chemical compounds of this specie and also to the results reported for the *L. graveolens* crude extracts, toward different microorganism.

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## BERRIES AS A VALUABLE SOURCE OF BIOACTIVE POLYPHENOLS

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Studies have recently proven that berries display a wide range of biological activities including antioxidant, anti-inflammatory, anti-diabetic, antimicrobial, anti-carcinogenic and neuro-protective effects. Health related properties of berries are linked to their high concentration of polyphenols (flavonoids, tannins, phenolic acids).

The objective of this study was to identify and quantify the bioactive components in wild and cultivated Canadian berries. Additionally, the stability of polyphenol compounds in cultivated berries (Saskatoon berries and black currants) during a 9 month storage period at -20°C was evaluated. Wild and cultivated berries were collected in Western Canada. Polyphenols were extracted with aqueous methanol (containing 0.1% formic acid) and analyzed by spectrophotometry. The identification of phenolic compounds was performed using a LC/MS. The antioxidant activity of extracts was determined using the Trolox equivalent antioxidant capacity (TEAC) assay with ABTS and DPPH radicals.

Twelve berries native to Canada were analyzed for their total anthocyanin (TA) and polyphenol (TP) contents. Among them honeysuckle berries contained the highest amount of polyphenol compounds (1111 mg/100g of TP and 1081 mg/100g of TA). Wild strawberries contained the lowest level of TA (43 mg/100g) and TP (438 mg/100g). The 17 varieties of Saskatoon berries and 5 varieties of black currants were analyzed for their polyphenol contents and antioxidant activities. The variety 'Nelson' represents Saskatoon berry cultivar with highest total polyphenol and anthocyanin contents (801 and 382 mg/100 g fresh weight, respectively) while 'Ben Alder' was the best among black currant cultivars (682 and 368 mg/100g, of polyphenols and anthocyanins respectively). Those cultivars were characterized also by the highest antioxidant potential measured with ABTS radical (5.0 and 4.6 mM/100 g FW, respectively).

Cultivar-dependent changes in polyphenol content after storage in freezer were observed. The 'Nelson' and 'Lee 2' among Saskatoon berries and 'Ben Alder' among black currants were the most stable cultivars during storage. The high polyphenol content and antioxidant activity of the 'Nelson' and 'Ben Alder' cultivars and their good storage stability would make these cultivars the optimal material for fruit growers and functional food producers.

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## ANTHOCYANINS IN BERRIES: BIOLOGICAL ACTIVITIES AND PROCESSING

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Anthocyanins are the largest water-soluble pigments in the plant kingdom. They are widely distributed in the human diet through crops, fruits, beans, vegetables, and red wine. Humans ingest a significant amount of anthocyanins from plant-based daily diets. In general, anthocyanin pigments are stable under acidic conditions, but are unstable in

neutral or basic conditions. Therefore, anthocyanins have not been recognized as a physiological functional food factor. However, in recent years, numerous studies have shown that anthocyanins display a wide range of biological activities including antioxidant, antimicrobial and anti-carcinogenic activities; improvement of vision; induction of apoptosis; and neuroprotective effects. Attention has been focused on anti-inflammatory activities of anthocyanins.

Although, anthocyanins have been shown to act as antioxidants, they may exert a wide range of health benefits through other mechanisms. Many potential chemopreventing polyphenols may interrupt or reverse the carcinogenesis process by acting on intracellular signaling molecules involved in the initiation and/or promotion progress of cancer. The effects of the anthocyanins seem to be cell type- and dose-dependent. Depending on their specific structures, anthocyanins affect different cellular signaling elements that are crucial for the regulation of cell proliferation. The cyclooxygenase isoform COX-2 is inducible and contributes to pathological processes such as inflammation and abnormal cell proliferation, is overexpressed in human colon carcinomas.

In our study, anthocyanin extracts obtained from saskatoon berries and black currants grown in Western Canada have been shown to be powerful antioxidants. The black currant anthocyanin extract was also shown to be a potential COX-1 and COX-2 inhibitors. The purple corn polyphenols reduced blood glucose level and reversed the decrease of pancreatic beta cells more efficiently than glimepiride, a known insulin secretion agent. Dietary intake of purple corn polyphenol-rich food might be beneficial in preventing the onset of type 2 diabetes mellitus. These diverse protective effects of anthocyanins, have contributed towards a growing interest in the application of these polyphenols as dietary supplements and cosmetic agents. The presentation will show some perspectives for development of technologies for the incorporation of anthocyanins into functional foods.

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## VARIATION IN BREEDING SYSTEMS IN *HYPERICUM PERFORATUM* AND *PRUNELLA VULGARIS*

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The effective conservation of new crop germplasm and its efficient use in new-crop development both rely on a clear understanding of the crop's reproductive biology. *Hypericum perforatum* (St. John's wort) and *Prunella vulgaris* (Common selfheal) are two medicinal plant species with potential for crop improvement and increased cultivation. The North Central Regional Plant Introduction Station in Ames, Iowa serves as the active curation site for *Hypericum* and *Prunella* germplasm within the U.S. National Plant Germplasm System. Published reports indicate that *H. perforatum* is typically tetraploid and a facultative apomict. However, other ploidy levels are known, and the degree of sexual reproduction can vary widely among populations. Past research has noted that the floral morphology of *P. vulgaris* can vary from forms that are strongly inbreeding to those that can promote outcrossing.

The objectives of our studies were to (1) develop protocols to use flow cytometry of individual seeds and plants to determine ploidy levels and the degree of apomixis in germplasm accessions of *H. perforatum* and (2) examine floral parts and conduct bagging experiments to determine the degree of self-pollination in *P. vulgaris*.

Flow cytometry was used to evaluate individually ground seed and leaf samples of three accessions of *H. perforatum*. Using seed samples, we were able to confirm that all accessions were facultative apomicts with tetraploidy the predominant cytotype (85-91%), relatively few hexaploids (10-14%), and diploids (5%) detected in only one accession (Ames 27490). The proportion of seeds with sexually produced embryos varied among accessions from 17% in Ames 28292 to 43% in Ames 24790, with 6-34% resulting from the union of reduced (diploid) gametes. Leaf samples from seedlings were tetraploid (87-97%) and hexaploid (3-13%).

In *P. vulgaris*, we found that pollen was often shed before the flowers opened. Accessions varied in the degree of separation between anthers and the stigmatic surface. Evaluation of seed production from bagged inflorescences revealed that plants with relatively small flowers from the Republic of Georgia (Ames 29156 and 29157) displayed the most selfing; bagged flowers had similar rates of seed set (ca. 80%) to those of open-pollinated flowers. Accessions collected from Oregon set 31% (Ames 29049) and 48% (Ames 29047) selfed seed, respectively, while an accession originally collected in Japan (Ames 29995) produced only 4% seed set when bagged.

Information will be posted on the Germplasm Resources Information Network (GRIN) database about ploidy-level

variation and the frequency of apomixis in *H. perforatum* accessions, and on variation in the degree of self-pollination in *P. vulgaris*. This will allow users to know which accessions may be expected to breed true from open-pollinated seed and which ones might be best suited for breeding and crop improvement through hybridization.

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## CHARACTERIZATION AND PROPAGATION OF SOME MEDICINAL PLANTS IN THE CENTRAL-SOUTH REGION OF CHILE

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Medicinal plants are those that contain active ingredients with proven therapeutic properties that benefit health. In the last years, there have been increased interests in the use of medicinal plants for health purposes, which have encouraged the search for new active compounds from plants from countries like Chile, where the 85% of vascular flora is native and over 50% is endemic. Additionally, approximately 13% of the vascular flora has a current or potential use.

This study aimed to rescue, propagate and characterize both *in situ* and *ex situ* genetic material of some native medicinal species that grow in the Bío Bío Region, in the South-Central Area of Chile in order to contribute to the maintenance of regional genetic heritage. The species selected for this project were: *Buddleja globosa*, *Geum queylyon*, *Adesmia emarginata*, *Psoralea glandulosa*, *Lomatia hirsuta*, *Chenopodium ambrosioides*, *Luma apiculata*, *Cheilantes glauca*, *Adiantum chilensis*, *Centaurium cachalahuén*, *Ugni molinae*, *Linum chamissonis*, *Sophora macrocarpa*, *Fabiana imbricata* and *Haplopappus* sp.

The species were collected considering longitudinal and transverse transects (36 ° 00'-38 ° 30 'S 71 ° 00' W) up to the Pacific Ocean, 37062.6 km<sup>2</sup>. Morphology, habitat for growth, type of soil in which plants grew (chemical and physical characteristics) and phenology were evaluated. Seeds, cuttings, roots or whole plants were collected and sexually propagated, using light and temperature, and / or asexually propagated in order to determine the most appropriate method of propagation for each of them. The main active ingredient associated with medicinal use was also determined. It was possible to observe different behavior for the same species *in situ*. A 2500-m<sup>2</sup> plot of medicinal plants was established in El Nogal Experimental Station of the University of Concepcion in Chillan where each species was characterized *ex situ*. The domestication of all species was carried out. *Adesmia emarginata*, *Buddleja globosa*, *Chenopodium ambrosioides*, *Centaurium cachalahuén*, *Geum queylyon*, *Haplopappus glutinosus*, *Linum chamissonis*, *Lomatia hirsuta*, *Luma apiculata*, *Otholobium glandulosa*, *Sophora macrocarpa* and *Ugni molinae* can be multiplied by seeds, whereas *Adiantum chilense* and *Cheilantes glauca* by rhizome division and *Buddleja globosa*, *Fabiana imbricata*, *Luma apiculata*, *Ugni molinae* and *Otholobium glandulosa* by cuttings. *Cheilantes glauca* and *Adiantum chilensis* had to be grown under Roshel mesh. Annual or perennial species, such as *Adesmia emarginata*, *Buddleja globosa*, *Chenopodium ambrosioides*, *Centaurium cachalahuén*, *Geum queylyon*, *Haplopappus glutinosus*, *Linum chamissonis*, *Fabiana imbricata* and *Otholobium glandulosa* bloomed the first or second year of establishment. The identification and quantification of the marking molecule content was carried out in most species.

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### General Crops and Products

GRIN-GLOBAL: AN INTERNATIONAL PROJECT TO DEVELOP A GLOBAL PLANT GENE BANK AND

## INFORMATION MANAGEMENT SYSTEM

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Many of the world's national genebanks, responsible for the safeguarding and availability of their country's Plant Genetic Resource (PGR) collections, have lacked access to high quality IT needed to document and manage their collections electronically. The Germplasm Resource Information System (GRIN), developed by the USDA-ARS National Plant Germplasm System, is widely recognized as a superior genebank management system, largely because it has been continuously developed and enhanced during the past 22 years. As system complexity has grown, so has the importance of its information content and delivery systems to researchers and genebank personnel. International genebanks interested in adopting GRIN have been challenged by technology licensing fees and GRIN's complexity. The Global Crop Diversity Trust recognized the common needs of the world's genebanks and the resources being expended by many genebanks or consortia independently and initiated the GRIN-Global (G-G) Project.

The mission of the GRIN-Global Project is to create a new, scalable version of GRIN to provide the world's crop genebanks with a powerful, flexible, easy-to-use PGR information management system. The system will help safeguard PGR and information vital to global food security, and encourage PGR use. Developed jointly by the USDA Agricultural Research Service, Bioversity International and the Global Crop Diversity Trust, G-G will be deployed in selected plant genebanks worldwide in 2010.

The .NET Framework and Visual Studio development environment were chosen for the project. A core set of web services, enterprise services or other technologies will update data stored locally or on networks, distribute centralized data to off-site systems, and enable third party data sharing. The database and interfaces will accommodate commercial and open-source programming tools, be database-flexible, and require no licensing fees. The database will be deployable on stand-alone computers or networked systems.

A demonstration of the GRIN-Global system's public interface will be presented.

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## SEQ CHAPTER \h \r 1NEW OPPORTUNITIES FOR INDUSTRIAL CROPS

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The Farm Security and Rural Investment Act of 2008, also known as the 2008 Farm Bill, describes all the funding authorities and programs under which USDA operates through 2012. The 2008 Farm Bill provides a variety of funding opportunities for research, development, and demonstration of industrial crops with a focus on energy crops. The Cooperative State Research, Education, Extension Service (CSREES) supports fundamental, early applied research and pre-commercialization research through various programs, [www.csrees.usda.gov](http://www.csrees.usda.gov). CSREES, in partnership with the U.S. Department of Energy Office of Biomass, administers the Biomass Research and Development Initiative, [www.brdisolutions.com](http://www.brdisolutions.com). All projects funded through this initiative are required to include a life cycle perspective. This presentation briefly describes funding opportunities and describes a project that addresses sustainable crop development and conversion technology to produce biofuels and biobased products.

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## COMMERCIAL FRUITS SHELF LIFE INCREASE USING A COVER OF CANDELILLA WAX WITH LIPPIA GRAVEOLENS ANTIFUNGAL EXTRACTS

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Several management practices on fruit production and commercialization in order to protect them of the post harvest maladies are carried out in Mexico. Microorganisms as *Rhizopus sp.* and *Penicillium sp.*, attack the damage fruits due that they have a broad spectrum effect during the period of shelf life.

The objective of the present study was: a) to evaluate the cover effect of candelilla wax with *Lippia graveolens* extracts on the fruit shelf life increase and b) to determine the extracts antifungal activity.

Apples fruits var. Golden Delicious and tomato var Floradade were used. The study was carried out under a completely randomized design with six treatments and fifteenth repetitions: a) fruits without cover (control); b) fruits cover with wax; c) fruits cover with wax and ethanolic extract at 3000 ppm; d) fruits cover with wax and hexanic extract at 4000 ppm; e) fruits cover with etanolic extracts at 3000 ppm in water and f) fruits cover with hexanic extracts at 4000 ppm in water. The evaluate variables were: weight loss, changes in appearance, firmness, pH and solids content. The study was carried out during six weeks in an isolated room at ambient temperature. Data analysis was carried out using the software SAS 9.0.

Antifungal activity evaluation was carried out using plastic transparent boxes as humid chambers. Fruits were injured longitudinally and transversely with a sterile pin. Inoculation was carried out by aspersion using spores suspension of *Penicillium sp.* and *Rhizopus sp.* over apples and only *Rhizopus sp.* over tomatoes. As positive and negative response were considered infected ad non-infected fruits, respectively, compared at the same time with the blank.

Results showed that applying candelilla wax edible covers with *L. graveolens* extracts over the fruits color and firmness parameters where higher than the observed with the blank.

*Rhizopus sp.* in apples showed the highest growth delay effect, being the most susceptible pathogen to extracts presence, but over tomatoes the effect was not the same.

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## LCA AND SUSTAINABILITY METHODOLOGIES FOR ASSESSING INDUSTRIAL CROPS, PROCESSES AND END PRODUCTS

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Providing food, energy and materials for the increasing global population is a challenge which is compounded by decreasing availability of natural resources such as land, water and fossil sources of raw materials. Greenhouse gas emissions from human activities have increased with industrial development and population increase, and it is anticipated that resulting climate change phenomena might further limit agricultural productivity through changes to weather patterns and agriculturally productive land. Growing crops for industrial uses is seen as a way of balancing greenhouse gas emissions and as an option for reducing dependency on fossil sources of raw materials. However, in order to assess the extent to which processed crops will affect the greenhouse balance and provide more energy efficient systems, the use of methodologies to make these calculations is required.

Studies carried out at the Porter Alliance have identified over 250 different scenarios for commodity crops and biofuel production. In order to rationalise this, a modular approach to Life Cycle Analysis (LCA) and sustainability has been taken. This allows assessment of discrete sections of the supply chain and comparisons to be made between different crop production systems, different process systems and different end product use.

LCA profiles of a number of important biofuel production technologies have been devised. As an example, the presentation will review a biomass crop, such as miscanthus or willow, linked to lignocellulosic technologies as conversion systems, to produce bioethanol for liquid road transport fuel as the end product. Genetic variation, growing conditions and harvest conditions in the crop production system are probed and related to biomass properties in process systems using existing and developing technologies for the conversion of biomass to bioethanol.

The results of this work show the variation which exists in growing and process systems and that the way LCA methodological approaches deal with variations and uncertainties can affect the outcomes of energy and greenhouse gas balances.

LCA methodologies provide data to inform governments and industry of the potential a specific supply chain may have for energy and greenhouse gas saving and the negative impacts which may occur as the result of changing land use. It is critical that data continue to be accessed and reviewed to ensure that the most appropriate crops are grown and processed for the most appropriate end use.

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## DEVELOPMENT OF *HELIANTHUS TUBEROSUS* L. AS A NOVEL CROP PLATFORM FOR PRODUCTION OF FUNCTIONAL FOOD AND BIOPOLYMERS

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Jerusalem artichoke (*Helianthus tuberosus* L.) is a perennial plant native to the North American plains. Although closely related to common sunflower, Jerusalem artichoke develops below-ground tubers. In the Canadian province of Alberta, yields of above-ground biomass range between 15-20 dry tones per hectare, while yield of tubers ranges between 18-25 tonnes per hectare, depending on cultivar and soil quality. Fresh tubers contain up to 20% of water soluble carbohydrates (predominantly inulin), while high volumes of dry residual aerial biomass left after tuber harvest contain 40 to 50% sugars, mostly fructans and fructose.

The objective of this study was to evaluate the agronomic performance of ten cultivars of Jerusalem artichoke and their suitability for production of inulin and biopolymers.

Inulin from tubers of the three highest-yielding cultivars in the field was extracted in a laboratory/pilot scale set-up. Tubers were subjected to hot water extraction. Purification and final processing steps included ion-exchange chromatography, membrane separation and spray-drying. Using both commercial and proprietary microorganisms, we were able to ferment water soluble sugars from the residual stalks to lactic acid, which was then polymerized to bioplastic resins (PLA) in our labs.

Quality of inulin obtained throughout our process matched industry standards. Based on our laboratory data we have designed a commercial scale inulin production facility in Alberta and evaluated its technical and economic feasibility. The PLA resins were combined with industrial hemp fibre (generated by another ARC research program) as a reinforcing agent to develop complete biocomposites. Testing of the physical properties of the bioplastics is currently underway.

Jerusalem artichoke produced 3-4 times more dry matter than other field crops typically grown in Alberta, and appears to be an excellent raw material for production of inulin, bioplastics and other bioproducts (i.e. bioethanol) on the Canadian prairies. We are currently in a process of securing commercial partners to construct a pilot plant biorefining facility.

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## GROWING CHICORY IN CHILE AS A CHALLENGE FOR INVESTIGATION

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The BENEIO group, with its origin in Belgium (Europe), decided to build a second factory for processing chicory in the southern hemisphere in order to achieve Inulin production for almost the whole year. After examining the potential of various countries to grow chicory, the decision was taken to build the plant in Chile.

The objective of the Orafti investigation team was to develop the optimum manual for the crop in combination with defining the optimum processing period.

Various trials have been established from 2004 onwards to determine recommendations for seeding date, fertilization, weed and disease control, irrigation and harvesting machinery. The yield and the quality of the chicory grown under various conditions have been measured and analyzed.

Some European-based recommendations for chicory production had to be changed due to the different soil and weather conditions, weed, and disease pressure in Chile. The high temperatures in March and April prohibited storage in the yard, which led to recommending a “just in time” delivery schedule. The enormous weed pressure forced up to 7 applications of herbicides. Some Trumao soils showed a high phosphorus (P) fixing potential, requiring P fertilizer to be banded during seeding. Daily evapotranspiration rates of 10 mm during summer need to be compensated by continuous irrigation. Insects, leaf, and soil borne diseases have to be controlled. The Inulin quality in general was higher than typically seen in Europe, and would allow the production of premium products for the functional foods market.

It was possible to establish the crop under Chilean conditions and to take advantage of the special climatic situation which would lead to high quality products.

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### POSTER PRESENTATIONS (Oilseeds)

#### BIODIESEL FROM CAPER SPURGE, CASTOR OIL PLANT AND RAPESEED

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The current problems generated by high oil prices and its derivatives, as well as growing environmental concerns are pushing the exploration of new sources of energy. These energy sources must be renewable and low in CO<sub>2</sub> emissions. This is the case of the energy crops which can produce biofuels such as bioethanol and biodiesel. Biodiesel is low cost, environmentally friendly and can be produced from various vegetable oils and animal fats. The most common oilseed crops that can be used for this purpose are jatropha, castor, rapeseed, camelina, sunflower, soybean and palm. Many other oilseeds have also been used for biodiesel production. Caper spurge (*Euphorbia lathyris*) and castor (*Ricinus communis*) are naturalized weeds in Chile. They grow on marginal soils with low nutrient availability and under arid conditions. Due to their high oil content and favorable lipid profile, it is interesting to consider the suitability of these species for biodiesel production in Chile. The objective of this study was to make biodiesel in laboratory scale, using oil extracted from caper spurge, castor and rapeseed, and compare their properties as biofuels. Also their oil cakes were analyzed to determine their potential as biofertilizer.

The oil was extracted by mechanical expelling seeds in a screw press followed by solvent n-hexane extraction. The biodiesel was obtained by basic transesterification in a small reactor (500 ml), with agitation (1500 rpm) at 50°C for 2 hours. The quality of the biodiesels was evaluated according to the international standards ASTM D-6751 and EN 14214.

Biodiesel from rapeseed and caper spurge oil meets the quality standards specified in ASTM D-6751 and EN

14214. On the other hand, the biodiesel obtained from castor oil had higher kinematic viscosity (14.77 mm<sup>2</sup>/s) than the maximum allowed, and lower cetane number (29.75) than the minimum required in both standards. Therefore, caper spurge could be considered as another good crop for biodiesel production. The three oil cakes show low C/N ratios (caper spurge 13.5, castor seed 11.6 and rapeseed 16.4). Also, the three oil cakes presented N-P-K concentrations higher than 0.36%.

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## **DEVELOPMENT MANIPULATION DURING DIFFERENT PHASES IN OILSEED RAPE (*BRASSICA NAPUS L.*): ITS IMPACT ON YIELD AND YIELD COMPONENTS**

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Oilseed rape yield potential could be improved increasing the duration of the late reproductive phase by a higher number of grains per unit area. Photoperiod sensitivity could be used as a tool to manipulate the reproductive phase and thereby the number of number. The aim of the present study was to assess (i) the effects of different combinations of photoperiod on the duration of different phases and (ii) analyze how the changes in the duration of that phase affects yield and its components in oilseed rape.

Field experiments were conducted in a factorial combination of three cultivars and three photoperiod regimes (control, emergence-visible flower buds-visible flower buds-VFB- and VFB-maturity) arranged in a completely randomized block design with three replicates, using different sowing dates during two growing seasons.

Results showed differences among genotypes in their photoperiod sensitivity, in both vegetative and reproductive phases. The positive relationship between grain number per m<sup>2</sup> and the duration of the late reproductive phase (VFB-Maturity) suggest that yield could be increased lengthening the duration of that phase. Thus, the photoperiod sensitivity found in the reproductive phase, regardless the effect on the previous phase, opens the possibility to manipulate the relative durations of vegetative a reproductive phases, increasing the length of the reproductive phase at the expenses of a reduction in the duration of vegetative phase, without changing the whole duration of the crop cycle as a future strategy to increasing yield in oilseed rape.

Variations in yield were mostly explaining by changes in the grain number per unit area without significant association with grain weight. Since oil concentration showed to be a more conservative attribute, increase in crop yield through a higher grain number per unit area would be a suitable avenue for increasing oil yield since no reductions in oil concentration can be expected.

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## **SEEDING DATE INFLUENCE ON MUSTARD (*BRASSICA JUNCEA L.*) SEED YIELD, YIELD COMPONENTS, AND OIL CONTENT IN CHILE**

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Mustard is a promising alternative crop as a feedstock for biodiesel production. The objective of this study was to determine the best seeding date of mustard in South Central Chile. The experiment was conducted in Chillán, El Carmen,

Los Angeles, and Osorno in the 2008-2009 seasons. The experimental design was an RCBD with a split-plot arrangement with 4 replicates, where the main plot was the seeding date (five dates) and the sub-plot three different experimental mustard lines (J05Z-014556, J05Z-07993, and J05Z-07146). Experimental units consisted of 6 rows, 5 m long and spaced 30 cm apart. Seed yield was taken from the center-four rows of each experimental unit discarding 0.5 m of plants from row ends. Seed yield components evaluated were: number of plants  $m^{-2}$ , number of siliques  $plant^{-1}$ , number of seeds  $silique^{-1}$ , and 1000-seed weight. Plants were counted on 1-m row of the two center rows, the number of siliques was counted on three plants from each experimental unit, and a sample of 20 siliques was taken from plants in the two center plot-rows for determining the number of seed  $silique^{-1}$ . Seed oil content was determined on a 40 mL sample of each experimental unit with a Newport Nuclear Magnetic Resonance (NMR) analyzer. Each combination of location-year was defined as an environment and considered a random effect in the ANOVA. Significant differences were determined for the seeding dates, lines, and seeding dates x lines effects, and their interaction with the environment. Seed yield was highest at the first and second seeding dates at Chillan, Los Angeles, and Osorno. Two of the lines evaluated were high yielding J05Z-014556 and J05Z-07146 while the remaining line yielded less because of poor stand establishment at all environments. Reduction in seed yield at later seeding dates was due to frost heaving, during the winter, which caused stand loss. Also plants from the later seeding dates were shorter and had less siliques plant. Highest seed yields were 762, 1256, 1278, and 1842  $kg\ ha^{-1}$  in Chillán, El Carmen, Los Angeles, and Osorno, respectively, and were from the first two seeding dates. The seed yield differences observed occurred mainly to differences in number of siliques  $plant^{-1}$  and seed weight. Highest seed oil content was obtained with the first seeding date at all environments. Seed oil content was 410, 414, 412, and 434  $g\ kg^{-1}$  in Chillán, El Carmen, Los Angeles, and Osorno, respectively. Oriental mustard looks promising as an alternative oilseed crop for south central Chile when seeded before 15 May.

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## **ORIENTAL MUSTARD (*BRASSICA JUNCEA* L.) SEED YIELD, AND PLANT NITROGEN ABSORPTION RESPONSE TO NITROGEN, SULFUR, AND PHOSPHORUS FERTILIZER IN CHILE**

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Oriental mustard is used in many countries of the world and currently is of interest for biodiesel production. The objective was to evaluate the effect of different nitrogen (N), sulfur (S), and phosphorus (P) rates on seed yield, yield components, oil yield, oil content, and N absorption in mustard. The experiment was conducted in Los Ángeles and Osorno, Chile, in 2008 and 2009. The experiment was a RCBD with a factorial arrangement of 4 rates of N (0, 75, 150 and 300  $kg\ N\ ha^{-1}$ ), and two rates of S (0 and 40  $kg\ S\ ha^{-1}$ ) with 4 replications in Los Ángeles. In Osorno, in addition to the 4 rates of N, and 2 rates of S, three rates of P (0, 50, 100  $kg\ P_2O_5\ ha^{-1}$ ) were added to the experiment. Experiments were planted between 21 April and 7 May, 2008. Results indicated a seed yield response to N applied at both locations. The highest seed yield 1275  $kg\ ha^{-1}$  was obtained with 198  $kg\ N\ ha^{-1}$  in Los Ángeles. In Osorno the highest seed yield was 2519  $kg\ ha^{-1}$  with the 300  $kg\ N\ ha^{-1}$  rate. Oil yield also responded to N with a maximum of 532  $kg\ ha^{-1}$  of oil with 195  $kg\ N\ ha^{-1}$  in Los Ángeles while in Osorno the maximum oil yield was 914  $kg\ ha^{-1}$  with 300  $kg\ N\ ha^{-1}$ . The total N absorbed by the plant was much less in Los Ángeles than in Osorno. Nitrogen absorption was less in Los Angeles because seed development occurred during high temperatures and dry soil conditions. Seed oil content decreased from 417 to 414  $g\ kg^{-1}$  with increasing N rates in Osorno. Sulfur addition had a positive effect on seed oil content increasing it from 404 to 415  $g\ kg^{-1}$  when 40  $kg\ ha^{-1}$  of S was applied. There was not a response to P application in Osorno where soil P levels were adequate. In our study, oriental mustard showed a seed and oil yield response to N only; oil content decreased with increasing N rates and increased with the addition of sulfur.

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## CAMELINA (*CAMELINA SATIVA* L.) SEED YIELD, RESPONSE TO NITROGEN, SULFUR, AND PHOSPHORUS FERTILIZER IN SOUTH CENTRAL CHILE

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Camelina (*Camelina sativa* L.), Brassicaceae, is a new oilseed crop, with potential as a low cost feedstock for biodiesel in many regions around the world. The objective of this study was to evaluate seed and oil yield response of camelina to nitrogen (N), phosphorus (P), and sulfur (S) nutrition in South Central Chile. Experiments were conducted in Chillan, El Carmen, Los Angeles, Gorbea, and Osorno, Chile, in 2008 and 2009. The experiment at Chillan, Los Angeles, and Gorbea was a RCBD, with 4 replicates, in a factorial arrangement of 4 rates of N (0, 75, 150 and 300 kg N ha<sup>-1</sup>) and two rates of S (0 and 40 kg S ha<sup>-1</sup>). The experiment at El Carmen and Osorno was also a RCBD with 4 replicates in a factorial arrangement where, in addition to the 4 rates of N, and 2 rates of S, three rates of P (0, 50, 100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) were included. Seed yield was taken from the center four rows of each experimental unit discarding 0.5 m of plants from row ends. Seed yield components evaluated were i.) number of plants m<sup>-2</sup>, ii.) number of siliques plant<sup>-1</sup>, iii.) number of seeds silique<sup>-1</sup>, and iv.) 1000-seed weight. Seed oil content was determined on a 40 mL seed sample, from each experimental unit, with a Newport Nuclear Magnetic Resonance (NMR) analyzer. Each location-year combination was defined as an environment and was considered a random effect in the statistical analysis. The combined analysis of variance indicated the N rate by environment interaction was significant for seed yield. A positive seed yield response with N additions was observed at all environments except in Gorbea. Maximum seed yield was obtained with the 300 kg N ha<sup>-1</sup> rate in Los Angeles, El Carmen, and Chillan, and was 2390, 1766, and 1104 kg ha<sup>-1</sup>, respectively. In Osorno, the seed yield increased to 1917 kg ha<sup>-1</sup> with the 150 kg N ha<sup>-1</sup> rate. Seed yield components affected by N were number of siliques plant<sup>-1</sup> and seed weight. The addition of P or S did not increase seed yield at any of the environments. Nitrogen fertilization increased N content in plant tissue and seed and also increased the seed protein content. The highest N rate applied decreased the oil content from 436 to 416 g kg<sup>-1</sup> averaged all environments. Rates higher than 75 kg N ha<sup>-1</sup> caused serious plant lodging in Osorno. Camelina N requirement is below 100 kg N ha<sup>-1</sup>, less than that of canola grown in the same area. These results confirm that camelina is a low input crop although it may have a high response to applied N in soils with low levels of N.

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## DOUBLE CROPPING SUNFLOWER AFTER FIELD PENNYCRESS IN NORTH DAKOTA

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Double cropping offers producers potentially greater economic returns than growing a single crop. In temperate regions short growing seasons, temperature and rainfall patterns, and soil moisture often limit or prevent double cropping. Early harvested winter annuals may provide a sufficient residual growing season for crops such as sunflower (*Helianthus annuus* L.) that exhibit good performance when seeded before mid-July in northern regions.

The objective of this study was to evaluate sunflower performance following field pennycress (*Thlaspi arvense* L.) production in North Dakota.

The study was established near Prosper, ND, in the 2008 and 2009 growing seasons. The experimental design was a RCB with two early maturity sunflower hybrids seeded as soon as possible after field pennycress harvest. The field pennycress experiment was RCBD with two genotypes and three replicates. Traits evaluated included flowering date, seed weight, seed yield, and seed oil content.

First year results indicated field pennycress yields of 1213 kg ha<sup>-1</sup> and sunflower yields of 1100 kg ha<sup>-1</sup> with a 21 July planting date. Late planted sunflower in an adjacent study yielded 1900 kg ha<sup>-1</sup> when planted on 14 July in 2008. Seed oil content was lower from double cropped sunflower compared with sunflower sown before mid-July. Greater sunflower yields are expected in 2009 from an earlier field pennycress harvest and a sunflower planting date of 13 July.

Double cropping field pennycress and sunflower shows potential if sunflower can be planted before mid-July and occurrence of killing frosts is later in October. Production economics will also be an important factor in the feasibility of this double crop combination.

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### **BRASSICA NAPUS (CANOLA) GROWN ON A HEAVY METAL CONTAMINATED SOIL**

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Soil and sediment contaminated with heavy metals are frequently found nearby industrial parks in most big cities. Several species within the Brassicaceae family have been identified as metal accumulators and for being tolerant to high concentrations of heavy metals in shoot tissues. With the objective of improving the use of the contaminated areas through the application of organic materials to the soil, a greenhouse trial was carried out with canola (*Brassica napus*) grown on soils affected by dredged sediments contaminated with lead (Pb), nickel (Ni) and cadmium (Cd), amended with two different vermicomposts of cattle ruminal content (VCR1 and VCR2). The following treatments were carried out with five replicates in a completely randomized design: T0 as control treatment, with soil affected by dredged sediments, and T1 and T2 with mixtures (20% w/w) of soil and VCR1 and VCR2 respectively. The effect of the humified materials on seed germination and their influence on the availability of heavy metals to the plants were compared. Yield (fresh and dry weight) and metal content in aerial biomass were measured. In an initial bioassay the presence of phytotoxic compounds in the aqueous extracts of soil and mixtures was tested through the Global Germination Index, a factor of relative seed germination and relative root elongation. It showed no phytotoxicity risk for all treatments, and significantly larger values for treatments with VCR. Fresh and dry matter productions, as well as the yield of fruits were significantly higher in treatments with addition of VCR. Metal concentrations in leaves of canola were higher in the control treatment with the exception of Cd. In treatments with VCR no significant levels of Pb, Cd or Ni were detected in fruits. The addition of organic amendments as VCR resulted in improving the production and lowering the content of metals in the biomass especially in fruits of canola. No harmful health effects to humans are expected from the use of canola oil from plants cultivated on soils contaminated with Pb, Cd or Ni amended with *high doses of VCR as those tested in this experiment*.

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### **GENOTYPE X ENVIRONMENT INTERACTION OF CANOLA (*BRASSICA NAPUS* L.) IN SOUTH CENTRAL CHILE**

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Farmer selection of canola cultivars to grow in Chile is based solely on information provided by seed companies. Field studies to determine which canola cultivars have better seed yield potential have not been conducted at different locations in South Central Chile, and consequently genotype stability among environments is unknown. Determination of genotype adaptation and the genotype x environment (GxE) interaction of canola genotypes was performed with the SREG (Sites Regression) model. Experiments were conducted, in Chile, at 5 environments (Chillan, Los Angeles, El Carmen, Gorbea, and Osorno) in the 2008-2009 seasons. Seed yield data collected by the seed company Agresearch Ltda. in 8 environments (Mulchén, Cañete, Collipulli, Victoria, Lautaro, Gorbea-B, Mafil, and Paillaco) was also used to analyze the GxE interaction. The experimental design at each environment was an RCBD with four replicates and 26 open-pollinated or hybrid canola genotypes. Studies conducted by Agresearch Ltda. were a RCBD with 3 replicates and 17 genotypes. Each experimental unit consisted of 6 rows 5 m in length and separated 30 cm apart. Canola was seeded between 21 April and 7 May at all environments and plots were harvested between 15 December, 2008, and 7 January, 2009. An ANOVA was used to determine the significance of the GxE interaction and biplots were used to graphically interpret and determine the superior genotypes at each environment and determine the corresponding mega environments. Genotype x environment interaction was significant for seed yield. Most seed yield variation determined by the analysis was due to environment and GxE effects. Principal components (PC1 and PC2) of the SREG model with 5 and 8 environments accumulated 74.5% and 61.1% of the total variation, respectively. Two mega environments were formed one represented by the Chillan environment and the second included the remaining environments. Six of the genotypes evaluated were superior and all were hybrids (Monalisa, Hornet, Rohan, Exagone, and Hammer) except for Goya. The mean vs. stability analysis indicated the hybrid Monalisa was the highest yielding and most stable genotype evaluated across all the environments. The information obtained from this study will be useful to select the best genotypes for the different canola producing areas in South Central Chile.

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## ARTHROPOD COMMUNITIES RELATED TO DIFFERENT MIXTURES OF OIL (*Glycine max*) AND ESSENTIAL OIL (*Artemisia annua*) CROPS

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Plants can host many herbivores even natural enemies during their growth cycles. Thus, changes in the relative abundance of the different plant components in crop fields, such as crop and weeds in a monocropping system or different crops in an intercropping system, may produce great bottom up impacts in the specific and functional structure of spontaneous communities of arthropods. The combination of two contrasting species (*i.e.* *Glycine max*, soybean, Fabacea, N<sub>2</sub> fixing plant and *Artemisia annua*, annual wormwood, Asteracea, VOCS plant) may lead to greater overall biological productivity than their monocultures because the mixture can use resources more effectively or because one species attracts natural enemies and thus facilitates the escape of the second species from pest attack. The hypothesis of this study was soybean-wormwood mixtures would be related to different spontaneous communities of arthropods depending on the proportion of soybean and wormwood in the mixture.

The objective of the study was to analyze the structure of spontaneous communities of arthropods related to different soybean (S) – annual wormwood (W) mixtures using standard crop management for S production in Argentina (*i.e.* GMO soybean).

Factorial CRBD field experiments with 3 replications were carried out during 2006 and 2007. Soybean of MG IV (DM 4800) was sown in 8m x 2m plots with 0.35m inter- row spacing on 10 January. S density was kept constant (40 plants m<sup>-2</sup>) and different W densities (plants m<sup>-2</sup>) were added. Treatments were pure S, S+2W, S+4W, S+8W and pure W (8 plants m<sup>-2</sup>).

Arthropods were sampled at soybean full flowering. Sampling was done using a sweep net with fixed net sizes (30

cm diameter) and sweeping patterns (2 net sweepings/ plot) and similar climatic conditions and time (10.00am-15.00pm). Arthropod morphospecies were classified in functional groups as herbivores and non-herbivores.

S and W total dry matter and W essential oil content from leaves and inflorescences were determined. Arthropods morphospecies abundance (frequency of a given species in each treatment) and richness (number of species occurring in each treatment) were determined. Data was analyzed using uni and multivariate techniques.

Arthropods belonging to the same 7 orders in both years presented a total richness of 48 in 2006 and 34 in 2007, while arthropod total abundance was 380 in 2006 and 317 in 2007. The proportion of non-herbivores was higher (70%) than the proportion of herbivores (30%). Five different communities were determined according to each treatment. No differences were found among treatments in S+W and S total biomass production and S yield, while W total biomass and essential oil yield were different among treatments ( $P < 0.05$ ).

Annual wormwood could be used as an accompanying essential oil crop or left as a weed in the densities tested in this work favoring biodiversity and, eventually, pest management without compromising soybean crop yield.

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## OILSEED GENOMIC RESOURCES: A *LESQUERELLA FENDLERI* EST COLLECTION

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Castor bean (*Ricinus communis* L., Euphorbiaceae) produces a seed oil in which the predominant fatty acid (>89%) is the hydroxy fatty acid ricinoleic acid (12-hydroxy oleic acid; 12-OH 18:1). Due to its unique composition, castor oil trades at prices well above those of other commodity oils, and is used in applications ranging from paints and cosmetics to high performance lubricants and polymers. Castor oil is the only commercially available source of ricinoleic acid, but the plant is poorly suited for commercial production as the seeds contain high levels of allergens, alkaloids and toxic proteins (ricin and *Ricinus communis* agglutinin). A number of strategies have been suggested to provide alternative sources of hydroxy fatty acids, these include the development of new crops that naturally produce hydroxy fatty acids, and the engineering of hydroxy fatty acids in existing oilseed species by gene transfer.

*Lesquerella fendleri* A. Gray, Brassicaceae) produces a seed oil containing Lesquerolic acid (14-hydroxy eicosenoic acid: 14-hydroxy eicosenoic acid; 14-OH 20:1). This species is a promising new oilseed crop plant, but also offers an alternative to castor as a model to understanding hydroxy fatty acid production, and as a source of genes encoding enzymes with specificity towards hydroxy fatty acids. In order to identify and clone genes encoding enzymes of seed oil biosynthesis, we have taken an EST sequencing approach.

Messenger RNA was prepared from developing seeds of *L. fendleri* and used to construct a plasmid cDNA library. Subsequently, 30,048 randomly chosen cDNAs were sequenced from the 5' end to create an EST sequence collection. Sequences were analysed using the FIESTA-2 Bioinformatics software system developed at NRC-PBI and clustered to give 4380 contigs and 10637 singletons. Annotation via BLAST revealed sequences encoding seed storage proteins and ribosomal proteins to be highly abundant. ESTs encoding the FAD3 fatty acid desaturase (0.77% of all ESTs) and the oleate hydroxylase/desaturase (FAH12, 0.22% of all ESTs) were also abundant. Comparison of the LFAH12 ESTs to a previously published genomic sequence (AF016103) indicated the presence of a small 81 base pair intron in the 5'UTR of this gene. Gene Ontology (GO) annotation identified 376 unigene sequences associated with "Lipid metabolic process". These included sequences encoding oleosins and enzymes of fatty acid biosynthesis and glycerolipid assembly and represent a significant genomic resource for this species.

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## OXYGENATED FATTY ACIDS IN THE SEEDS OF *PLANTAGO* SPECIES

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Unusual hydroxy and oxo fatty acids have previously been reported from the seed oil of members of the genus *Plantago*. An isomer of ricinoleic acid, 9-hydroxy-cis-11-octadecenoic acid, was observed as a minor component (1.5%) of oil from *Plantago major* L., Plantaginaceae, and two oxygenated fatty acids, 9-hydroxy-cis-12-octadecenoic acid (iso-ricinoleic acid) and 9-oxo-12-octadecenoic acid were reported from *Plantago ovata* L., Plantaginaceae seed oil.

We have examined the fatty acid composition of seeds of 12 species of *Plantago*. The predominant fatty acids were palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic acid (18:3). Oxygenated fatty acids were present in the oil from 5 species with levels ranging from approximately 1% of total seed fatty acids, to more than 16%. These were identified by Gas Chromatography/Mass Spectrometry to be iso-ricinoleic acid and 9-oxo-octadecenoic acid. Iso-ricinoleic acid was the predominant oxygenated fatty acid in all 5 species. No other oxygenated fatty acids were detected.

These fatty acids may be a useful taxonomic marker for identification of *Plantago* species.

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## RECRUITMENT OF VOLUNTEERS, AN APPROACH TO CROP ESTABLISHMENT FOR LESQUERELLA IN LOW RESOURCE ENVIRONMENTS

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Traditional crop management practices (such as sowing) are not always suitable in arid lands, because they may lead to losses of organic matter of the top soil and increase the risk of erosion and desertification. An alternative way to seeding is to reestablish the crop using volunteer recruitment. We present a study in four *Lesquerella* (Brassicaceae) species with an incipient degree of domestication including high levels fruit dehiscence. This dehiscence generates an important loss of seeds, which enhance the seed-bank and in turn germinate and generate new seedlings which can be used to re-establish the crop. Seed dispersal represents a link between seed production and seedling establishment and determines the spatial arrangement. Emergence and survival of seedlings are mainly controlled by water availability in the top soil layer and the micro-environment close to the soil surface.

The goal of this study was to characterize volunteer recruitment in *Lesquerella* as a new tool to reestablish the crop. We studied annuals (*L. gracilis* and *L. angustifolia*) and perennials (*L. pinetorum* and *L. mendocina*), and determined seed dispersal, emergence and survival of seedlings and the effect of water and density as modulators of final density of the new stand.

A split-plot field experiment was conducted in Chubut, Argentina with three factors: specie, water availability and density. Seed dispersal was evaluated at physiological maturity, using seed-traps. We marked six plots per treatment after the first germination pulse. On each plot the number of seedlings was recorded once a week. At physiological maturity all plants were harvested to determine total biomass, allocation, and seed -yield and components.

Seed dispersal was higher in *L. pinetorum* and *L. mendocina* (4.7 Kg/Ha) than in *L. gracilis* and *L. angustifolia* (3.4Kg/Ha). No significant differences were found between water availability treatments ( $p = 0.92$ ). Only *L. pinetorum*'s seeds germinated in autumn, which coincided with the sowing date proposed for the establishment of this crop in Chubut. There was no interaction  $\delta_i$  \* water availability ( $p = 0.62$ ) for the emergence and survival of seedlings. Emergence and survival was different among  $\delta_i$  ( $p < 0.0001$ ): the density of seedlings was stable in time for the low and medium  $\delta_i$ , and decrease only at the highest  $\delta_i$ . Low water availability resulted in a reduction in seedling density ( $p < 0.05$ ). The seeds of *L. angustifolia* and *L. gracilis* germinated in spring, and the plants completed their life-cycle in just three months. The seed of *L. mendocina* remained dormant in the seed-bank.

The amount of seed dispersal was adequate for the reestablishment of the crop in the perennial species, with similar seeding-rate than reported for *L. fendleri* (4.5 Kg/Ha). Our results show that the survival of seedling is modulated



by a density dependent mechanism, and seems to be independent of the water availability. Of the four species evaluated, only *L. pinetorum* showed adequate germination timing and growth cycle for the target environment although a larger field experiment is needed to corroborate our results. Volunteer recruitment could be considered as a way to reestablish the crop in other oil-seed crops in arid and semi arid lands.

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## RELATIONSHIP BETWEEN SEED-YIELD AND MORPHOLOGICAL AND FUNCTIONAL TRAITS RELATED TO STRESS-TOLERANCE IN WILD AND COMMERCIAL ACCESSIONS OF EVENING PRIMROSE *OENOTHERA* L. (ONAGRACEAE)

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Evening primrose (*Oenothera* spp.) is an oilseed crop rich in d-linolenic acid, a relatively rare fatty acid, which is used both as a nutritional and medicinal supplement. Wild species of *Oenothera*, native to Argentina, have been suggested as a new crop in irrigated valleys of semi-arid Patagonia. Although for most grain crops, seed-yield is the principal breeding objective, stress-tolerance traits are also key characters to be selected for new crops in resource-limited environments. Wild species native to these environments exhibit several morphological and physiological traits that could contribute to increase ecological and economical sustainability.

Our purpose was to establish the relationship between seed-yield and morphological and functional traits related to stress-tolerance in wild and commercial accessions of *Oenothera*. A two factor (species and density) field experiment with a completely randomized design was performed in the Chubut River valley, Patagonia Argentina (43° 21' 31'' S; 65° 38' 39'' W). In this area the mean annual precipitation is 179 mm and the mean low temperature of the coldest months (June and July) is 1°C. Two commercial (*O. biennis* and *O. lamarckiana*), and two native accessions (*O. mendocinensis* and *O. odorata* x *O. mendocinensis*, a natural occurring hybrid) were grown in a common garden at three plant densities. Plant density treatments generated a range of resource availability. Biomass allocation, morphological traits related to stress-tolerance (SLA and root allocation) and seed-yield were recorded. The effect of plant density on these traits was described and integrated in a principal component analysis.

Commercial accessions were characterized by traits associated with a poor performance under stress conditions (high specific leaf area -SLA- and low root allocation) and by allocation of resources preferentially to growth (high leaf area and leaf mass ratio) and reproduction (high seed-yield). Native accessions were characterized by traits related to stress-tolerance (high root allocation and low SLA). Seed-yield was lower at high plant densities for the commercial accessions suggesting seed-yield instability in response to changes in the environmental quality. No changes in seed-yield in response to plant density were recorded for native species. SLA was positively related to plant density for commercial accessions while total biomass decrease with increasing plant density. Seed-yield was negatively correlated with SLA and positively correlated with total biomass.

Traits characterizing commercial accessions would result, under stressful conditions, in a poor performance compared to that of their native counterparts. Our data indicate that germplasm co-selection for stress-tolerance traits and yield could result in higher seed-yields stability, particularly in resource-limited environments.

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## DIVERSITY IN OIL CONTENT AND OIL PROFILE IN SEEDS OF *LIMNANTHES* ACCESSIONS MAINTAINED BY THE U.S. NATIONAL PLANT GERMPLASM SYSTEM

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*Limnanthes* (Limnanthaceae; meadowfoam) seeds contain long chain fatty acids which are stable under metabolic and environmental conditions. The fatty acid composition makes the oil valuable for use in cosmetics, lubricants, rubber additives and plastics. The oil content in seeds vary; however, 95% of the oil is comprised of four predominant acids: *cis*-5-Eicosenoic (C20:1d5); *cis*-5-Docosenoic (C22:1d5); *cis*-13-Docosenoic (Erucic acid, C22:1d13); and *cis, cis*-5-*cis*, 13-Docosadienoic (C22:2d5, 13) acids. Wild meadowfoam plants typically grow in vernal pools and valley grasslands of California and Oregon; however, a few cultivars are grown on a production scale in Willamette Valley, Oregon. The amount of variation in oil quality has not been examined in these wild accessions.

The purpose of this study was to evaluate the oil content and profile in seeds of *Limnanthes* accessions maintained in the National Plant Germplasm System (NPGS) collection. Ethyl ester samples extracted from seeds of 67 accessions were analyzed for the percentage of eight fatty acids.

The total oil content varied from 11.1 to 30.0% and was the highest in PI 374792. The highest content of *cis*-5-Eicosenoic acid was in PI 283716 (66.8%), *cis*-5-Docosenoic acid and *cis*-13-Docosenoic acid was in PI 283725 (6.2% and 23.2% respectively) and *cis, cis*-5-*cis*, 13-Docosadienoic in PI 367900 (20.5%). The total oil content and its composition depended on the accession, species and harvest year.

The data may be useful for breeders and researchers involved in meadowfoam crop improvement and will be entered in the GRIN (Germplasm Resource Information System) database of the NPGS.

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## THE EFFECT OF RADIATION ON BIOMASS PARTITION AND NON-STRUCTURAL CARBOHYDRATES ACCUMULATION IN *Oenothera mendocinensis* Gillies ex Hooker et Arnott (Onagraceae)

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Evening primrose (*Oenothera* spp.) seed oil contains gamma-linolenic acid (GLA), an essential fatty acid with applications as a nutritional and a medicinal supplement. Although there are other sources of GLA, evening primrose oil appears to be clinically the most effective, and it is the most common commercial source. Wild species of *Oenothera*, native to Argentina, such as *O. mendocinensis*, have been suggested as a new crop for semi-arid Patagonia.

Low light intensities stress plants because irradiance limits CO<sub>2</sub> assimilation, and thus net carbon gain and plant growth. Plants that grow under low levels of irradiance change their pattern of biomass partition and certain morphological attributes, particularly those related to leaf structure and allocation, such as specific leaf area and leaf mass ratio.

The objective of this work was to determine the influence of radiation on allocation patterns of *O. mendocinensis* in four phenological stages. We performed a factorial field experiment with one factor (time of shading) in a completely randomized design in the Chubut River valley, Patagonia Argentina (43° 21' 31'' S; 65° 38' 39'' W). Plants (30 plants per plot; 3 plots per treatment) were randomly assigned to one of four light environments: 1) direct solar radiation; 2) 50% reduction in solar radiation during rosette stage; 3) 50% reduction in solar radiation from bolting to the anthesis of the third flower and 4) 50% reduction in solar radiation from anthesis of the 3rd flower to [ripened or mature] fruit. Full radiation was 1726 mmol seg<sup>-1</sup> m<sup>-2</sup>. Black 100% polypropylene fabric provided the shade; this fabric did not alter the red/infrared ratio of the incoming radiation.

Time of shading did not affect total biomass (g plant<sup>-1</sup>), root biomass (g\*plant<sup>-1</sup>) or root mass ratio (g root biomass\*g total plant biomass<sup>-1</sup>). Reduced radiation availability after bolting (S2) significantly increased stem allocation (stem mass ratio; g stem \* g total plant biomass<sup>-1</sup>) and decreased leaf mass ratio (g leaf \* g total plant biomass<sup>-1</sup>). Neither carbohydrate reserves accumulation, nor reproductive attributes (reproductive effort, reproductive output, number of fruits per plant and harvest index) were affected by light environment.

In our experiment, plants growing under reduced light availability during bolting enhanced stem allocation, as expected for plants growing in shade. Growth, reproduction and storage were not affected by low irradiance. These results indicate that a 50% reduction in radiation availability did not provoke a severe source limitation. Most likely, in *Oenothera* species, photosynthesis becomes light-saturated at irradiance higher than  $850 \text{ mmol seg}^{-1} \text{ m}^{-2}$ . High irradiance in Patagonia causes problems in many plants as *Mulinum* and *Grindelia*, which present structural and/or chemical mechanisms to avoid excessive radiation.

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## EFFECTS OF SOURCE-SINK VARIATION ON THE REPRODUCTIVE ASSIGNATION OF ANNUAL AND PERENNIAL SPECIES OF LESQUERELLA (BRASSICACEAE)

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The development of perennial oil-seed crops offers an interesting alternate to alleviate problems associated with agricultural practices needed to produce on fragile environments such as extra-andean Patagonia. Although the annual *Lesquerella fendleri* is the prime candidate for the development of a lesquerolic rich oil-seed crop, within this genus there are several perennial species available to breeders some of which are perennials when cultivated. Still the feasibility of a perennial crop of *Lesquerella* is not clear because seed-yield in perennials is usually limited by low harvest indexes resultant from a trade-off between reproductive output and biomass allocated to organs devoted to perpetuation. Increases in seed-yield tend to reduce perenniality. Assimilated carbon is assigned to different organs (leaves, stems, roots, reproductive organs, etc) as the result of a set of metabolic and transport processes that govern the flux of energy through a system of sources and sinks. Within this context, one of the strategies used to evaluate variations in reproductive allocation is to alter source-sink relations of the plant.

The aim of the present study is to evaluate the effect of source-sink ratio on the reproductive allocation of four *Lesquerella*'s species with different life-cycles.

A field experiment was carried-on in Chubut, Patagonia Argentina (43° 14'S, 65° 18'W). We used a completely randomized design with twelve treatments in which we varied source-sink relationships on two annuals (*L. angustifolia*, *L. gracilis*) and two perennials (*L. pinetorum*, *L. mendocina*). We used a combination of shading timing (reduction of source) and removal of flower-buds (reduction of sink) to develop a range of source-sink relationships. Reproductive allocation was evaluated as the number of fruits per plant. Data was analysed using two-way ANOVA and post-hoc Tukey's Multiple Range test.

All four species had a similar response to source/sink treatments ( $p < 0.05$ ). The number of fruits was lowest for plants shaded during the vegetative (rosette) stage and highest for plants which had half their flower-buds removed at flowering. Plants shaded during the reproductive stage and control plants had a similar, intermediate number of fruits/plant. Species differed in the total number of fruits/plant ( $p < 0.05$ ). *L. pinetorum* was the species with the highest number of fruits/plant and *L. mendocina* and *L. angustifolia* had the lowest. Our preliminary results show that carbon-gain during the rosette stage may be relatively more important than actual assimilation during reproduction, which means that carbon storage also plays a key role in reproduction both in annual and perennial *Lesquerella*. The increase in fruit-set found with bud removal (compensatory fruiting) could potentially reduce longevity in perennial species, although it may also come from an increase in carbon gain, induced by sink enlargement.

The mechanisms behind these responses should be further evaluated, especially the understanding of the relative importance of carbon reserves, current photosynthesis and carbon partition in determining yield components, and future yield and plant longevity.

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## THE CHALLENGE OF JATROPHA IN CHILE

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The interest in using *Jatropha curcas* L. as a feedstock for the production of bio-diesel is rapidly growing. The properties of the crop and its oil have persuaded investors, policy makers and clean development mechanism project developers to consider *jatropha* as a substitute for fossil fuels to reduce greenhouse gas emissions. However, *jatropha* is still a wild plant of which basic agronomic properties are not thoroughly understood and the environmental effects have not been investigated yet.

Despite the interest that is being shown in the large-scale cultivation of *jatropha*, genetic resources remain poorly characterized and conserved as there has been very little plant breeding for improved traits. The aim of this study is to determine the potential of the plant for the production of biodiesel as a renewable energy source and to use the meal obtained after the extraction of oil as a source of animal food

We present the studies carried out to introduce the crop in Chile which include the identification of potential sites of adaptation, development of in vitro propagation methods and establishment of 10 pilot areas for the selection of elite clones.

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### POSTER PRESENTATIONS (Natural Rubber and Resins)

#### EFFECT OF CROP DENSITY AND NITROGEN AVAILABILITY ON BIOMASS AND RESIN FOR TWO *Grindelia chiloensis* (Asteraceae) CONTRASTING GENOTYPES

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*Grindelia chiloensis* (Corn.) Cabr. is a shrub native to arid environments in Argentina, being developed as a new resin crop. Diterpene resin acids are deposited externally on leaves, stem and inflorescences have industrial applications in the production of paints, lacquers, varnishes and glues. The plant's genotype sets the limit for potential resin content although realized resin production is under strong environmental influence and has been found to conform to the Growth-Differentiation –Balance Hypothesis.

The objective of this study was to assess physiological and morphological responses to changes in crop density and nitrogen availability in two *G. chiloensis* genotypes which differ in potential resin production. We predict that the genotype with higher resin content will be associated with traits that reflect a more conservative resource use strategy.

A field experiment was carried out in a completely randomized design with three factors: two levels of nitrogen availability, four densities (4.7, 6.7, 11.0 and 16.7 plants. m<sup>-2</sup>) and two genotypes of *Grindelia chiloensis* (accessions 743 and 775), with four replicates (plots) per treatment. We report preliminary data after one and a half years of growth on aboveground biomass, specific leaf area (SLA), and leaf resin content.

Aboveground biomass was significantly affected by plant density. The highest individual plant-biomass was found at the lowest density and the lowest biomass at the highest density. Soil nitrogen content had no effect on individual biomass production. Plot biomass production was significantly affected by plant density for both accessions.

We found differences between genotypes in leaf resin concentration. Accession 775 had significantly higher leaf

resin concentration than accession 743, while SLA was significantly lower for 775 than for 743. Resin content per unit leaf-area showed no significant differences between accessions.

We propose that these differences in SLA and resin concentration should be related to differences in resource use (*sensu* Grime 1979). The lower leaf resin concentration ( $\text{g}\cdot\text{g}^{-2}$ ) together with the higher SLA in accession 743 would be associated with a greater potential for acquiring resources and higher biomass production, while accession 775 showed a more conservative strategy. At the crop level these different strategies of resource acquisition and partition should produce two contrasting crops, one with more stable resin content, adequate for lower resource environments (accession 775) and one with high resin production potential (at the whole plant level) but resource demanding (accession 743).

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## TERMITE RESISTANCE AND MECHANICAL PROPERTIES OF BIOBASED COMPOSITION BOARDS MADE FROM COTTON GIN BYPRODUCTS AND GUAYULE BAGASSE

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Vast quantities of cotton gin byproducts (CGB), also known as cotton gin trash or cotton gin waste, are being produced across the cotton belt of the United States annually. Similarly, guayule wastes after rubber latex production, also known as guayule bagasse (GB), is expected to increase as this industry begins to expand. Use of these waste materials in value-added products can help the economics of the crops, and importantly, aid in alleviating waste management and environmental problems. Conventional wood preservatives used to protect wood from insect and microbial damage are presently of major concern to human health and the environment. Finding alternative and economical preservatives is of interest to manufactures of composite building materials.

The objectives of this research were to evaluate the physical properties and termite resistance of experimental composition boards made from CGB and GB.

Composition boards were made from five different ratios of CGB and GB: 100:0, 75:25, 50:50, 25:75 and 0:100 (C100, C75G25, C50G50, C25G75, and G100). Board composition was homogenous with no orientation of fibers. In addition, three-layered boards (3-layer) consisting of 25 % GB (upper layer), 50 % CGB (middle layer), and 25 % GB (bottom layer) were made. For comparison, a commercial southern pine lumber (SPL) board, a commercial oriented strandboard (OSB), and a commercial preservative treated medium density fiberboard (MDF) were included in the testing. In addition to the termite testing, mechanical properties of the CGB and GB composition boards were measured and compared with various standards of other commercial boards. Five specimens (1.1cm thick x 2.5cm x 2.5cm) were cut from each of the nine different board materials and tested using Eastern subterranean termites. More than 400 active termites were placed into a specimen bottle consisting of sand and the material block to be tested. Weight loss, termite survival days, and visual grade of each specimen at the end of the test were determined. The approximate termite mortality in each bottle after one week was estimated according to the testing standards. Test results confirmed good termite control quality of boards made from GB alone. Boards containing CGB to guayule wastes ratios of 75:25 and 50:50 obtained similar termite resistance to that of the commercial OSB based on the average values of total weight loss and one-week termite mortality rate. In visual grading of tested specimens after the test, all six CGB and GB composition boards and the treated MDF showed better than average rating compared with the commercial OSB and SPL. No difference was found among the average total termite surviving days for all six groups of boards made from CGB and GB. Overall, the biobased CGB and GB boards showed some potential. Results indicate the CGB possesses some inheritable quality of termite resistance.

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[Top of Page](#)**POSTER PRESENTATIONS (Fibers and Cellulosics)****BIOETHANOL PRODUCTION FROM ORGANOSOLV PULPS OF TENSION WOOD OF *EUCALYPTUS GLOBULUS***C. Muñoz<sup>1</sup>, R.T. Mendonça<sup>1,2</sup>, J. Baeza<sup>1,3</sup>, J. Freer<sup>1,3</sup><sup>1</sup>Laboratorio Recursos Renovables, Centro de Biotecnología<sup>2</sup>Facultad de Ciencias Forestales<sup>3</sup>Facultad de Ciencias Químicas, Universidad de Concepción, Concepción, Chile

The global energy crisis and Chile dependence on external sources of energy supply, force the country to seek different alternative to produce new and clean fuels that can contribute to resolve this problem. Bioethanol fuel, as one of the clean fuels for environmental protection, has been used as substitute of fuel or fuel additive. Chile has in the forest resources exploitation, a surplus of raw material that can be used in ethanol production with comparative advantages. Actually in Chile, there are approximately 400,000 ha of eucalyptus plantations, with *Eucalyptus globulus* covering about 74% of this area. Some special feature of hardwoods (including Eucalyptus species) is the presence of tension wood, characterized by the presence of a gelatinous layer of almost pure and high crystalline cellulose in the secondary wall, the G-layer. Other frequent characteristics of tension wood are the lower lignin amount, thicker cell wall, longer fiber and higher coarseness as compared with normal and opposite wood. These characteristics might be favourable for ethanol yield, due to the high abundance of cellulose that could be saccharified in glucose and further fermented into bioethanol.

In the present study, chemical characteristics of tension wood of *E. globulus* and its effect in the bioethanol production were evaluated. Tension wood was pretreated with aqueous ethanol organosolv process, for ethanol production through simultaneous saccharification and fermentation (SSF). This pretreatment method is highly effective in delignification and in hemicellulose solubilization of lignocellulosic biomass.

The carbohydrate composition of tension and normal wood of *E. globulus* was ~46% cellulose, ~20% hemicellulose, ~26% lignin and ~2.6% extractives. The organosolv pretreatment of tension and normal wood of *E. globulus* was performed with ethanol 60%, liquor/wood ratio 6/1, cooking time (30, 60, 120 and 240 min) and temperature (180° and 200°C). Mild cooking conditions produces pulps with high amount of rejects (uncooked wood chips) high lignin content and low glucose amount. Organosolv delignification of tension wood at 200°C and 30 min (H-factor ~6500) produced pulps with high pulp yield (~59%) and low rejects (~0,5%) in tension wood. In addition, the organosolv pretreatment of tension wood produced a favourable effect in the pulps composition with high cellulose and lower lignin content. The high cellulose content in tension wood may have significant effect on the ethanol yield from organosolv pulps.

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**A PINE BARK BIOREFINERY**A. Berg, C. Fuentealba, [J.C. Carrasco](#)

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Pine bark is widely available in Chile. Indeed, it represents one tenth of the round wood processes for industrial use, aprox. one million tons per year. This material is currently combusted in industrial boilers for electrical generation and heat supply, with a very low added value. On the other hand, pine bark contains commercial interesting phenolic

components, specially flavonoids and catechins. But also waxes and pectins which are potentially valuable.

The objective of this paper is to establish a process to use pine bark in an integral way to produce biomaterials, fine chemicals and standardized fuel.

*Pinus radiata* D. Don bark produced in sawmills in Southern Chile was processed at laboratory and semi-industrial scale to obtain valuable products. The phenolic components were extracted with concentrated methanol and thereafter fractioned in water soluble and water insoluble parts; the production of wood adhesives and thermoplastic material for extrusion was studied with these fractions. Hexane and water soluble components were also analyzed.

It was observed that the best extraction conditions to separate polyphenolic components from pine bark was a methanolic solution at a concentration of 75% (w/w), a temperature of 120°C and a extraction time of 120 min. A yield of 14 to 18% was achieved, 40% water soluble and 60% water insoluble. The former, of lower molecular weight, were used very successfully as a phenol replacement in wood adhesive resins; the latter was processed as a thermoplastic material to produce construction profiles. The possibility to use also waxes and pectins is currently being studied. The remaining solids can also be used as a standardized solid fuel (briquettes or pellets), as a gardening material or as a raw material for a thermochemical conversion.

Pine bark is a very interesting raw material to obtain valuable products, based on its huge availability and specific composition. The most interesting possibility is to extract different type of components using solvents of different polarities.

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## ACETIC ACID AS PULPING MEDIA

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Crude oil can be partially replaced as a raw material for fuels, materials and chemicals by lignocellulosics. Forest biomass is certainly the most important source but also cereal straw is an important alternative. In Chile aprox. 1.5 Million ton of wheat straw is burnt in the field causing big damage to the environment. New technologies are needed to use commercially this raw material because the traditional pulping methods based on inorganic chemicals make it very difficult to implement recovery systems. Also, the use of all lignocellulosic components - cellulose as well as lignin and hemicelluloses – is not possible.

The objective of this paper is to develop a technology to separate lignocellulosic raw materials in its main components: cellulose, lignin and hemicelluloses, to evaluate in a later step commercial applications for these intermediate materials.

*Pinus radiata* and *Eucalyptus globulus* wood as well as heat straw were digested in concentrated acetic acid. The influence of the acetic acid concentration and the temperature was studied. In a second stage, an oxidative nitric acid treatment in acetic acid media was applied to dissolve the remaining lignin in the lignocellulosic fibers. The resulting solution was concentrated and separated in water soluble (carbohydrates) and water insoluble (lignin fragments) components.

The optimal conditions for the delignification of the three raw materials are: An acetic acid concentration of 87 % (w/w), and a digestion time of 2 hours at maximal temperature. Only the temperature differs, being lower in the case of eucalyptus and wheat straw (160°C) and higher with pine (180°C). A pulp yield between 40% and 50% a Kappa Number of 16 to 18 were achieved. A further treatment with 8% nitric acid (based on dry pulp) makes it possible to obtain completely delignified fibers. The viscosity as well as the mechanical properties was measured and a balance of sugars was made. The inorganic components of the straw remained principally in the pulp.

Acetic acid is a very interesting solvent for the separation of lignocellulosics into its main components. The delignification proceeds in a very selective way. Based on this fact, a process can be conceived to obtain cellulose, lignin and solubilized hemicelluloses, intermediate products which have different commercial applications.

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## NEW COMMERCIAL APPLICATIONS FOR WOOD COMPONENTS

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Kraft and Sulphite processes for chemical pulping are well established practices. Unfortunately, the yield of these pulping processes is less than 50%. Therefore, more than half of the processed material is in the end destined to combustion, in order to recover inorganic chemicals and energy. The Organosolv process allows to separate the 3 main components of lignocellulosic materials (pulp, hemicelluloses and lignin), with a yield of about 90% in useful materials, through an energetically more efficient operation. The development of new applications for hemicelluloses and lignin becomes both attractive and necessary, due to the expected raise in their availability.

The objective of this work is to characterize hemicelluloses and lignin obtained from wood and wheat straw, and to identify commercial applications for these components that are massive and achievable in the short or medium term, in the Chilean market.

Recent developments in the use of lignin as support material for the elaboration of controlled release formulations show that this component can have a positive environmental impact in field application of pesticides and other agrochemicals, which is a priority for Chilean growers and their destination markets. Also, 30.000 tons of sugar beet molasses are expected to be used as animal feedstuff this season in Chile. This volume is expected to grow, considering the positive effect of molasses on ruminal metabolism and animal performance; therefore, an attractive market is open for wood molasses.

*Pinus radiata*, *Eucalyptus globulus* and wheat straw hemicelluloses and lignin were produced by pulping the raw materials with hot concentrated acetic acid, concentrating the dissolved lignocellulosic components under vacuum conditions, and separating the water soluble fraction (carbohydrates) from the water insoluble fraction (lignin). Complete chemical characterization was conducted for both carbohydrates and lignin.

The potential use of these carbohydrates as feedstuff for beef and dairy cattle is addressed, and well as its use as ingredient of swine, broilers, hens and rabbits diets. The rationale of the use of this lignin in controlled release formulations for the Chilean agricultural industry is established.

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### POSTER PRESENTATIONS (Medicinal and Nutraceuticals)

#### **BOLDO (*PEUMUS BOLDUS*) CULTIVATED UNDER DIFFERENT LIGHT CONDITIONS, SOIL HUMIDITY AND PLANTATION DENSITY**

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Boldo (*Peumus boldus* Mol.) is a medicinal plant native to the central zone of Chile and part of the sclerophyll forest. The 1500 tons of dried leaves exported annually are all recollected from the wild. To assure a long-term production of a homogeneous medicinal product without interfering the fragile ecosystems habited by boldo, different light and soil humidity conditions were studied to propose a successful cultivation system for this slow-growing species. Besides, the low market prices induced us to assess high plantation densities for mechanized harvest techniques.



The effect of light conditions was assessed by shading part of the plants in a split-plot design to simulate an intercropping system. As the natural habitat is characterized by hot and dry summers the effects of water supply monitored by tensiometers on the growth, leaf yield and concentration of chemical compounds were studied. Essential oil concentration of the shade dried leaves was quantified by hydro distillation, whereas the alkaloid concentration was determined spectrophotometrically.

Plants subject to different treatments were evaluated during three growing seasons. Those grown under shade only showed higher stem percentage in the harvested product when compared with plants exposed to full sun, but the leaf yield per plant as well as the alkaloid and essential oil content were the same for both treatments. From this result we can conclude that the intercropping of boldo in forest plantations could be a rentable alternative to the leaf-collection of single plants or monocultures without affecting the essential oil or alkaloid content of the leaves.

High plantation densities with 8 and 16 plants/m<sup>2</sup> led to low-yielding individuals, but leaf yield per area increased with the density, while percentage of leaves in the harvested product and plant height were the same for both treatments.

The excellent adaptation to dry conditions could be confirmed, as the different treatments of soil humidity led to similar leaf yields and plant heights. Water stressed individuals produced less but longer shoots and showed lower essential oil contents. With exception of the latter characteristic none of the treatments affected the medicinal quality requirements of alkaloid or essential oil concentration in the leaves. This study suggests that boldo is a species suitable for cultivation.

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## ***BUDDLEJA GLOBOSA* HOPE: VARIABILITY IN NATURAL POPULATIONS AND CULTIVATED PLANTS OF DIFFERENT PROVENANCE**

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*Buddleja globosa* Hope is a medicinal shrub native to Chile, known as “matico”. Its leaves have been traditionally used for wound and ulcer healing. Different medicinal properties, such as outstanding antioxidant, anti-inflammatory, cicatrising and analgesic activities have been reported. Today, all raw materials are coming from wild collection or recently established crops from unselected plants. There are no previous studies of the genetic or environmental variability of the species that would allow the optimization of yield factors or chemical compounds through the selection of genetic material or suitable production sites and management techniques. In the framework of domestication we studied different morphological characters including those related to the leaf yield, as well as flavonoid and tannin contents.

To assess which traits are determined genetically some morphological characteristics of three wild populations and of cultivated plants from the same provenance were compared. Yield characteristics, such as leaf yield, portion of leaves in the aerial part, and shoot number per plant, as well as the contents of flavonoids and tannins were assessed in cultivated plants of different provenances: three coming from home gardens and three from wild populations. For the cultivation studies individual plants, were arranged in a randomised block design, and those for the irrigation studies in split plots treated with 20% and 65% field capacity.

Plant characteristics such as height and width of the plant, their ratio, stem diameter and leaf density differed significantly among natural populations. In leaf characteristics only the presence of stipules showed significant differences. The cultivated progenies or clones may give an idea if these differences among populations are due to environmental or to genetic effects. Compared with other provenances, plants from the coastal provenance Los Ruiles are tallest in both natural populations and cultivated clones and also showed superior leaf yields in spite of their lower leaf density.

The pilosity of the leaves is characteristic of the species. At the moment of harvest the trichomes may cause allergic reactions. It's interesting that wild plants growing in Los Queñes and their cultivated progenies have abundant pilosity only on the lower side of the leaf, not on the upper side. This characteristic is not influenced by different irrigation treatments and shows no significant differences between cultivated and wild plants.

While provenances coming from home garden plants showed significantly higher leaf yields and more shoots per plant, flavonoid and tannin concentrations in the leaves were similar to wild plants. We assume that formerly plants with

a higher leaf production were selected for cultivation in rural home gardens.

Irrigation intensity showed significant higher leaf yields for plants with a higher water disposal. Nearly all water stressed plants presented resinous exudation. The flavonoid and tannin contents were not significantly affected by the irrigation treatments.

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## **OPTIMIZATION OF DIURNAL HARVEST REGIME AND WILTING CONDITIONS DURING PRODUCTION OF IKIREZI GERANIUM ESSENTIAL OIL IN RWANDA**

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Geranium (*Pelargonium graveolens*) is an important essential oil bearing industrial crop. Because of its strong rose-like odor, is one of the main ingredients in perfumery and aromatherapy. Additionally, geranium possesses high antiseptic and astringent properties and for this reason finds many applications in medicine. The terpenoid concentration and composition of this oil is affected by many factors, such as age of the leaves, intensity of light, temperature, season and time of harvesting. Thus, even with the correct chemotype, the best management practices and good agricultural practices are needed to maximize the yield and quality of the essential oil.

The objective of this study was to conduct series of field experiments to investigate the effect of wilting period and time of harvest on essential oil yield and composition of the major constituents in the geranium essential oil from commercial field-grown plants in rural Rwanda.

Aerial parts of the plants were collected manually from the commercial fields of a single rose-geranium clone at each harvest time, while the plant was actively growing and in a vegetative stage of development and prior to flowering. Fresh geranium biomass was dried under shade for an indicated time and then distilled. To investigate the effect of diurnal variation on the oil composition and yield, geranium plants were harvested in six-hour intervals. The essential oil was isolated from the biomass by steam-distillation and the chemical composition of the oil was determined by gas chromatography-mass spectroscopy. Physicochemical properties, such as refractive index, optical rotation and assessment by the private sector were also assessed.

The results showed that the maximum essential oil yield was obtained from geranium that underwent post-harvest wilting under shade for 17 hours and from the plants harvested in the early afternoon. Wilting period and time of harvest did not significantly affect the relative percentage or ratio of the oil major constituents. The chemical profile further showed that the IKIREZI geranium oil exhibits the chemical composition and quality acceptable to the international markets. The optimization of the time harvest during the day and the length of time to condition the harvested biomass to maximize oil recovery can be incorporated into the quality assurance procedures in the commercial production of Rwandan geranium oil.

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## **A MODEL FOR THE DOMESTICATION OF AN ESSENTIAL OIL BEARING PLANT**

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Medicinal and aromatic plants constitute an important group among the economic plants. In Argentina, most medicinal plant species are collected from the wild to supply the increasing demand of the pharmaceutical, food and flavor industries. As a result, the natural populations of medicinal plants have seriously declined and in some cases become endangered. Domestication and cultivation of aromatic plants allows the production of uniform and high quality raw material compared to the collection from the wild. Plant tissue culture offers the advantage of mass propagation of endangered species or valuable mother plants, to accelerate plant introduction into cultivation. Studies on the introduction of aromatic plant into cultivation are scarce. Therefore, the movement toward domestication as a vehicle for genetic preservation and conservation is an important strategy.

The aim of this work was to introduce a native aromatic plant from Argentina, *Lippia junelliana* into cultivation, and to compare the aerial biomass production (leaves, stem and inflorescences) and essential oil accumulation between cultivated and wild plants. This work also sought to re-introduced the domesticated plants to enrich local population and thus contribute to their conservation.

Seeds of *L. junelliana* were collected from wild plants growing in the hilly region of San Luis province, and propagated through tissue culture as previously described. The micropropagated plantlets were then introduced into field plots. Simultaneously, these plants were re-introduced into their original natural habitats as part of an enrichment program. The aerial biomass and essential oil yield and composition were compared between the cultivated, wild plants and the re-introduced plants.

The leaves and inflorescences of cultivated plants accumulated, on a dry weight basis, higher quantities of essential oil than their wild counterparts. Cultivated plants also produced more biomass in the leaves (43%) than the wild and re-introduced plants (31-34%). As a result, total essential oil accumulation of the different cultivated plant parts (leaf and inflorescence) was significantly higher than in the wild plants. In relation to total biomass, there were no significant differences between cultivated and wild plants though the biomass of re-introduced plant was significantly lower. This work demonstrates that cultivation, through micropropagation, can be a more efficient vehicle to both preserve and exploit medicinal plants than collection from the wild, because higher yields of leaf biomass and oil accumulation can be achieved, while essential oil composition is unaffected.

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## THE EFFECTS OF LIGHT AND NITROGEN FERTILIZATION ON BIOMASS AND ESSENTIAL OIL ACCUMULATION IN LIPPIA JUNELLIANA

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Many factors are recognized to affect the production of aromatic plants and their essential oils. Nitrogen fertilization can influence different aspects of growth and development, thus nitrogen is usually the most important nutritional factor in crop production. The effect of nitrogen on productivity is mainly exerted through the absorption of light and production of carbon skeletons. Since glandular trichomes, where essential oil components (e.g. terpenes) are synthesized and accumulated, are not photosynthetic, the availability of carbon skeletons can limit the synthesis of essential oil components.

The aim of this work is to evaluate the combine effect of light intensity and nitrogen fertilization on the biomass and essential oils accumulation in micropropagated plants of *Lippia junelliana*.

The plants grew in an inert media watered weekly with the nutrient medium, consisting of the Murashige and Skoog (1962) medium supplemented with four concentrations of nitrogen (total nitrogen 10, 20, 30 and 50 mM). In a

second experiment, four treatments were designed to combine two nitrogen concentrations (10 and 30 mM) with two light intensities (1000 y 200  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). At the end of the experiment (60 days), the plants were harvested and dried to calculate yield of aerial parts and measure essential oil content and composition.

The nitrogen fertilization increased both the biomass and the essential oil content. At 50 mM, it was observed a decreased accumulation of biomass, and also in essential oil accumulation. Regarding the combine effects of light and nitrogen, the highest accumulation of biomass (leaves, stems, and roots biomass) was observed in the plants fertilized with 30mM of nitrogen growing at the highest light intensity. Although the light intensity and fertilization produced significant changes in the main components of essential oils (limonene, citral and piperitenone), the essential oil profile was quite similar in all the treatments.

It was observed a close relationship between the accumulation of biomass and essential oils, suggesting that nitrogen fertilization increased the photosynthetic capacity of the leaves. Thus the increased biosynthetic capacity of the leaves seems to enhance the carbon flux to the biosynthesis of carbon-based secondary metabolites, such as the essential oils.

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## THE QUALITY AND CHEMISTRY OF SHEA BUTTER FROM THE BUIPE AREA (NOTHERN GHANA)

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The Shea (*Vitellaria paradoxa*) tree is a medium size and evergreen tree, with multiple uses in Western and Eastern Africa. The fruit of the tree contains a nut, which is shelled to obtain the kernel from where the shea butter is extracted. Shea butter is an important natural ingredient in cosmetic and personal care products and can be found in other confectionary products such as chocolate. In Northern Ghana, shea butter is impacting more than 600,000 women who rely on shea butter as an additional source of income, often one of their only income generating activities. One of the major challenges facing the shea industry is in contamination and quality degradation. Thus, the assessment of shea butter chemistry and quality is one of the first steps to contribute to quality improvements and the procurement of a consistent product.

The objective of this work was to set up quality control procedures to assess the general quality and chemistry of shea butter produced in the town of Buipe in Northern Ghana. Different procedures were set up to determine the chemical profile of shea butter including density, refractive index, fatty acid composition and total amount of unsaponifiables. Other procedures were tested to evaluate the level of degradation of shea, including free fatty acids, peroxide value, moisture and insoluble impurities.

This study which was done in concert with the Pure Company, a private sector partner and processor in northern Ghana which provided the shea materials from the 2009 harvest showed that the physical and chemical profiles of shea butter samples produced in Buipe Ghana were quite stable. The colors of the shea samples were light-yellow, with a cocoa butter like aroma. The density and refractive indexes showed low variation suggesting a similar fatty acid profile. The samples were high in stearic acid (42-45%), and oleic acid (41-43%), with minor amounts of palmitic, linoleic and linolenic acids. Palmitoleic and myristic acids were found in traces amounts. The levels of unsaponifiables (4-5%) showed low variation. The peroxide value of the samples were very low (<2%) while the free fatty acids showed varying levels (1-3%).

A range of quality control procedures were set up to evaluate the quality of shea butter locally, Results showed that for this initial set of samples produced in Buipe, the samples showed a low variation in their physical and chemical parameters. Further studies are now examining the storage and temperature conditions that would allow for this product to remain chemically stable over time for export purposes.

This work was funded by the USAID via the Global Development Alliance and the Partnership in Food Industry Development for Natural Products ([www.pfidnp.org](http://www.pfidnp.org)) in partnership with Rutgers University, and the Agri-Business in Sustainable African Natural Plant Products Program and the Pure Company, Accra, Ghana.

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## EVALUATION OF GENETIC DIVERSITY OF ESSENTIAL OIL AND SELECTION OF HIGH CARVACROL YIELDING LINES

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*Origanum* species, commonly known as oregano, are perennial herbaceous plants of the *Lamiaceae* family. *Origanum* species, particularly *O. vulgare*, have been traditionally used in food preparations to increase aroma and flavor and also used as spice. Oregano is also a source of essential oils, which is important for its antibacterial properties. As industrial crop, oregano has been used as a source of carvacrol for the veterinary industry.

A wide collection of *Origanum* species were evaluated to assess the genetic diversity in both essential oil yield and the accumulation of carvacrol, and to identify an efficient and rapid extraction method of carvacrol extraction and quantification.

Essential oils from 72 commercial and non-commercial sources of oregano were greenhouse-grown under identical conditions. Diethyl ether solvent extraction resulted in the highest recovery of carvacrol. From all these accessions, four European Oregano varieties were identified to have high absolute carvacrol content of 0.22g, 0.24g, 0.3g and 0.29g per 100gram of dry weight, respectively.

Six selected oregano accessions (four high and two low relative to their respective carvacrol content) were further screened using hydrodistillation as a second oil extraction method. The high carvacrol containing European Oregano varieties showed high relative percent of carvacrol, 77%, 75%, 72% and 75%, respectively. The low carvacrol containing accessions were also found to have low relative percent of carvacrol, 1.2% and 1.4 % respectively. This comparison validated the solvent extraction method and the data showed that either procedure can be used to determine carvacrol content.

The study shows that the solvent extraction method is a suitable method to quantitate carvacrol and may offer advantages above the traditional hydrodistillation method for the rapid determination of high yielding carvacrol lines.

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## ENCAPSULATION OF ALLYL ISOTHIOCYANATE (MUSTARD FLAVOR) USING $\beta$ -CYCLODEXTRIN

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Canada is the second largest producer of mustard seed in the world and the dominant exporter. Three types of mustard seed are produced: yellow, brown and oriental, which are used primarily as a condiment. Allyl isothiocyanate (AIT) is known both as a pungent mustard flavoring agent and a potent inhibitor of a large number of pathogenic microorganisms. Several studies have shown the potential of AIT as a natural antimicrobial in various food matrices. Recently, intense research has been conducted to develop antimicrobial active packaging containing AIT. Also, AIT has been demonstrated to inhibit tumor growth. However, new applications for AIT are limited because of its low solubility in

aqueous systems. Among the various flavor encapsulation techniques used so far, molecular inclusion in a  $\beta$ -cyclodextrin ( $\beta$ -CD) molecule is the most effective one and has the advantage of increasing the solubility of lipophilic flavor compounds in aqueous systems and improving their stability against oxidation, heat, and volatilization.

The objective of this study was to investigate the inclusion capacity of AIT in  $\beta$ -CD and the powder recovery using three encapsulation methods: co-precipitation in water, co-precipitation in ethanol-water solution, and paste methods.

Five starting ratios of core material (mustard essential oil containing 83.4% AIT) to  $\beta$ -CD were used: 5:95, 6.5:93.5, 7.5:92.5, 10:90, and 15:85. The parameters investigated were total AIT, surface AIT and powder recovery. Pilot plant encapsulation was performed using the paste method. The dough mixture was prepared in Thermomix and the suspension containing 20% of solids spray dried using a Niro Mobile Minor 2000 unit.

The main factors that affected the formation of the inclusion complex were the encapsulation method chosen, time, temperature, the molar ratio of guest to host, and the addition of ethanol. All encapsulation experiments conducted for prolonged time (paste method, 3 h or co-precipitation method, 72 hours) resulted in a loss of AIT. The results obtained using co-precipitation and paste methods conducted for 1h (paste method) and 24 hour (co-precipitation method) were then selected for evaluation.

It was verified that the encapsulation procedure using ethanol-water solution resulted in lower encapsulation efficiency. Ethanol might dissolve AIT and make it unavailable for interaction with  $\beta$ -CD. It was also observed that the amount of surface AIT in powders obtained using co-precipitation methods was very low. At higher AIT:  $\beta$ -CD molar ratios, the encapsulated AIT content was higher for powders obtained by co-precipitation method using water in comparison to paste method.

The paste method was chosen for the microencapsulation of AIT in  $\beta$ -CD because of the highest powder recovery and inclusion capacity at low molar ratios of AIT:  $\beta$ -CD. The recovery of AIT powder was 84.3% in pilot plant scale trial.

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## EVALUATION OF PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY IN GRAPES AND POMACE

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The pomace is generated during the process of winemaking after maceration and press of grapes. Often it is discarded or used as fertilizer for growing vines. Grapes phenolic compounds are transferred to wine during the process, however, these compounds are mainly found in skins and seeds. There is evidence that certain amount of them are not extracted during the winemaking process and remains in the pomace, so this could be used as raw material for manufacturing products having antioxidant activity.

The purpose of this study was the systematic evaluation of phenolic compounds profile and antioxidant activity in grapes and pomaces after winemaking process at industrial scale. The profiles of flavonoids (anthocyanins, flavonols, flavan-3-ols) and stilbenes were evaluated by reverse phase HPLC. The total phenols were determined by the absorbance at 280 nm and the Folin-Ciocalteu index and the antioxidant activity was studied by TEAC method.

The concentration of flavonoids, stilbens and antioxidant activity in grapes and pomaces was compared for Cabernet Sauvignon, Carmenere and Pinot Noir varieties harvests in 2008 and 2009. The results shown that pomaces presented significant concentrations of phenolic compounds (anthocyanins 33-56 mg/100g, flavonols 2,1-3,0 mg/100g and flavan-3-ols 4,4-11,8 mg/100g) being the anthocyanins the compounds with higher concentrations. However, during the maceration, flavonols were proportionally less extracted than anthocyanins. Residual levels of these compounds were 12-29% for flavonols and 7-11% for anthocyanins, in comparison with the original concentrations in grapes. The stilbenes were quantified as free resveratrol after acidic hydrolysis. They concentration in grapes was low (0.4-1.0 mg/100g), appearing only trace in pomaces. The concentration of total phenols was higher in grapes than pomaces. The same trend was observed with the antioxidant capacity.

These results showed that pomaces produced after industrial scale vinification constitute an interesting source of pigments, so it is possible that this product could be used as raw material to obtain phenolic compounds such as anthocyanins, which have potential use as natural colorants in foods.

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## CHARACTERIZATION OF SOME MEDICINAL PLANTS FROM THE BIO-BIO REGION IN CHILE

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Due to the increase of land areas used for cultivation and forestation in South Central Chile, and the increasing wild crafting of medicinal plants, plant population density of many native and endemic medicinal plants is diminishing. There are no regulations to limit wild crafting activities and there are only a few attempts to domesticate and cultivate them. Danger of extinction of some endemic plants is possible if no action is taken.

The objective of this study was to collect genetic material and characterize the environment conditions in which twelve native medicinal plants grow on the Bio-Bio Region, Chile, and to determine the content of specific bioactive molecules. Ten accessions from each species (*Adesmia emarginata*, *Buddleja globosa*, *Cheliantes glauca*, *Centaurium cachenlahuen*, *Fabiana imbricata*, *Geum quellyon*, *Haplopappus glutinosus*, *Linum chamissonis*, *Luma apiculata*, *Otholobium glandulosa*, *Sophora macrocarpa*, and *Ugni molinae*) were collected in 2003, 2004 and 2005 on a longitudinal and transversal transect of the Bio-Bio Region (36°00' -38°30' S; 71° W). On the collecting site, altitude, light conditions, associated flora, and chemical and physical characteristics of the soil were recorded.

*A. emarginata*, *Ch. glauca*, and *H. glutinosus* grow only on the Andes mountains area above 1500 m; *C. cachenlahuen* on the foothills of Andes, *F. imbricata*, *L. apiculata*, *O. glandulosa*, and *S. macrocarpa* in the foothills, valley, and coastal mountains. *B. globosa* and *G. quellyon* grow in both mountain ranges Andes and coastal. *L. chamissonis* in the oriental slope of the coastal mountain range and, *U. molinae* only on the west side of the coastal mountains. In general, all species are adapted to grow on poor soils, at a different range of pH, with low organic matter and nitrogen content, and variable levels of P and K. Some species require more humid soils and lower radiation than others. Flavonoids were determined in the leaves of *A. emarginata* (0.6-1 %), *B. globosa* (9.7-13.9 %), *H. glutinosus* (2.4-3.9 %), *O. glandulosa* (1.2-3 %), and *U. molinae* (5.5-8.9 %). Rutin was determined in *Ch. glauca* ferns (1.3-2.2 %), *F. imbricata* leaves and stems (1.3-5.3 %). Phenols as gallic acid equivalents were also measured in *G. quellyon* roots (32-40 mg/100 g<sup>-1</sup>) and *L. apiculata* leaves (4.2-34.1%).

A high variation in the content of active principles with medicinal activity was observed, suggesting a corresponding high quality variation in the raw materials for the pharmaceutical industry. This work provides a foundation to conduct more research on Chilean medicinal plants, since the information on propagation, cultivation and agronomic practices is scarce to assure a good pharmaceutical quality.

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## POSTER PRESENTATIONS (General Crops)

### EFFECTS OF WATER AVAILABILITY ON GROWTH, DRY MATTER ALLOCATION AND RESIN IN

## SEEDLINGS OF *LARREA DIVARICATA*

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Although usually more expensive than their synthetic equivalents, there is still interest in natural antioxidants considered best suited for human consumption. One potent antioxidant is nordihydroguaiaretic acid (NDGA), commercially extracted in the past from the North American *Larrea tridentata* plant. The success of this plant has triggered a renewed effort to develop a source of NDGA from South American species of the genus *Larrea*. One of the reasons for this interest is the widespread distribution of *L. divaricata* and *L. nitida* throughout about one-fourth of Argentina's continental territory.

The aim of this preliminary study was to establish if moisture availability could differentially affect total biomass, its partition, and the production of phenolic compounds (external phenolic resins as well as total phenols) in seedlings of *L. divaricata*.

A greenhouse experiment was carried out in which one-month old seedlings growing in 2.25 L pots were subjected to five soil moisture contents as a percentage of dry weight: 13%, 11%, 9%, 7% or 5% (water content at field capacity for this soil is 15%). After 150 days, seedlings were harvested and oven-dried at 45° C for 48 hours. Each seedling was then divided into roots, stems and leaves. Since resins are moderately lipophilic, they were extracted with ethanol by soaking the green leaves of each plant for one hour, filtering the extracts and evaporating until dryness.

Total biomass, its partition into plant parts, and the production of phenolic resin and total phenols were not affected by water availability. On average, leaf dry-weight was  $0.71 \pm 0.03$  g per seedling and their resin content was  $20.9 \pm 1.0$  %. These results demonstrated that seedlings of *L. divaricata* have low sensitivity to water availability at least for the range of water supplied in this study. Resin content was high although the content of NDGA from this fraction has not been evaluated yet.

This preliminary evaluation of biomass and resin content shows *L. divaricata* is a good candidate for continuing research towards developing a local source of antioxidants. A stable production of resin, independent of water availability, is considered a promising characteristic encouraging the use of natural stands of *L. divaricata* from the Patagonia and Monte regions in Argentina.

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## COMPARISON OF BARLEY AND RYE PRODUCTIVITY AND QUALITY AS A WINTER COVER CROP FOR BIOENERGY AT DIFFERENT HARVESTING STAGE

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In mono rice cultivation system in Korea, winter cover crop cultivation (barley, Chinese milk vetch, hairy vetch, rye, etc.) is strongly recommended for improving soil quality and carbon sequestration, but current cover crop cultivation is limited to less than 10% of crop lands. As interest in growing crops for bioenergy increases, the criteria to choose cover crops might change from crops that supply higher nutrients to crops that produce high quality, high-yielding biomass production. Of the commonly recommended winter crops, barley and rye are most likely biomass candidates in a mono rice paddy soil system, due to their relatively high productivity and better quality (high levels of fibre) for bioenergy.

The objective of this study was to select the winter cover crop having the best bioenergy potential in terms of biomass yield and quality (fibre content), and to determine its optimum harvest timing.

Biomass productivity and quality of rye and barley were investigated in two-year field tests. In each year, the two



cover crops were seeded into dry soil in late October after the rice crop was harvested. On the basis of the recommended rice transplanting day (June 20) in the investigation region, the above ground biomass of cover crops were harvested on the 1st, 2nd, 3rd and 4th week before rice transplanting. For each harvest date, the biomass quality was evaluated by measuring acid detergent fibre (ADF) and neutral digestible fibre (NDF) concentrations.

Above-ground dry matter biomass yield was slightly higher in barley than in rye, but differences were not statistically significant. The highest biomass yield of both cover crops was achieved at the end of May (on the 3rd week before rice transplanting). ADF and NDF of barley increased significantly for each successive harvest date, and did not reach a peak point within the harvesting time frame. In contrast, ADF and NDF for rye was highest on the 3rd week before rice transplanting, and thereafter slightly decreased during the remaining harvest dates. For both crops, the maximum yields of NDF and ADF, calculated from above ground biomass yield and each fibre concentration, were observed on the 3rd week before rice cultivation, due to the highest biomass productivity observed on that date.

The maximum fibre productivity was higher in rye (ADF 6.0 Mg ha<sup>-1</sup> and NDF 10.2 Mg ha<sup>-1</sup>) than in barley (ADF 4.3 Mg ha<sup>-1</sup> and NDF 8.2 Mg ha<sup>-1</sup>). Therefore, rye might be a reasonable winter cover crop for bioenergy production, and should be harvested at the end of May (the 3rd week before rice transplanting) for the best quality.

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## BASELINE FOR PRODUCTION OF CROPS FOR BIOGAS AND BIODIESEL UNDER MARGINAL CONDITIONS IN THE ATACAMA DESERT OF CHILE

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We have undertaken the establishment of an experimental farm for production of biogas and biodiesel from crops, including Prickle pear cactus (*Opuntia ficus-indica*), Castor bean (*Ricinus communis*), and alternatives. A reliable conversion of crop biomass to methane gas (CH<sub>4</sub>) was previously obtained by our group using cladode biomass of *O. ficus-indica*. The experimental farm is near the historical copper mining town of El Salvador, located at 2200 meters above sea level, in Northern Chile in the Atacama Desert. The irrigation system includes drip and micro sprinkler emitters, using municipal waste water of the town.

Our main objective was two-fold: a) Find viable crop production alternatives for the renewable energy market; and b) Test a production system that uses marginal soil and water resources. Study methodology included a characterization of the site in comparison with a traditional production zone (as reference) and the selection of test species based on their ability to cope with water and salt stress to some degree. Examining traditional Prickle pear cactus production zones (as reference), showed that the crop is normally produced in soils that are heavier, contain more organic matter, have greater water holding capacity and available phosphorus, and are less salty and alkaline than soils at this study's experimental farm. Water analysis showed a high salt content in El Salvador's municipal waste water, with an electrical conductivity of 4620 µSiemens•m<sup>-1</sup>. Among tested water parameters, there were high levels of arsenic, boron, chloride, fecal coliforms, lithium, sodium and sulfate.

Crops selected for this study included species likely to produce good biomass and biodiesel production: Prickle pear cactus, , Castor bean, eight putative salt tolerant accessions of Jojoba (*Simmondsia chinensis*), Safflower (*Carthamus tinctorius*), *Lesquerella fendleri*, Switchgrass (*Paspalum vaginatum*), and wild local populations of tarweed (*Madia sativa*), Brea (*Tessaria absinthioides*) and *Aptenia cordifolia*.

For Prickle pear cactus, where vegetative biomass is preferred over reproductive growth for energy production, major constraints have been associated with the lack of commercial genotypes that favour cladode over flower promotion, or genotypes with a reduced blossom. In addition, salt tolerant genotypes would be desirable. For castor bean and the other tested species, water use efficiency and salt tolerance traits are key evaluation criteria to determine feasible candidates for the non-conventional renewable energy market when produced under stress conditions. Further development and interactions of the selected crops are discussed in terms of stress tolerance and water use efficiency.

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## FUNGISTATIC ACTIVITY OF CRUSHED BARK, N-HEXANE EXTRACT AND ESSENTIAL OIL FROM *DRIMYS WINTERI* AGAINST SOILBORNE PATHOGENS

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Because of public concern, and the development of microorganisms that are resistant to chemicals applied to crops, is necessary to find safer alternatives for plant disease control. Botanical fungicides have long been promoted as attractive alternatives to synthetic chemical fungicides for disease management because botanicals reputedly pose little threat to the environment or to human health. *Drimys winteri* [J.R. Forster & G. Forster (*Winteraceae*)] is a perennial tree, native of Chile, that is used traditionally as a curative plant by indigenous people. A variety of secondary metabolites with medical applications have been reported from this plant, but little has been published regarding its interactions with phytopathogens.

The objective of this study was to evaluate *in vitro* fungistatic potential of *D. winteri* bark and bark extracts on important soilborne pathogens such as *Phytophthora cinnamomi* Rands, *Phytophthora capsici* Leonian, *Phytophthora nicotianae* Breda de Haan, *Sclerotinia sclerotiorum* (Liberty) De Bary and *Sclerotium cepivorum* Berk.

At the first screening all pathogens were exposed to crushed bark at concentrations of 500, 1000, 2000 and 4000 mg L<sup>-1</sup>. Then the most sensitive pathogens were tested to n-hexane extract (100, 200, 400 and 800 mg L<sup>-1</sup>) and essential oil (100, 200, 400 and 800 mg L<sup>-1</sup>) both extracted from *D. winteri* bark. Petri dishes containing PDA amended medium were inoculated with a mycelia disk of the pathogens and were incubated at 24°C. The radial growth of mycelium was measured daily, and the Growth Inhibition Index (% GII) was calculated.

*Sclerotium cepivorum* and *P. cinnamomi* were the most sensitive strains to crushed bark, at 4000 mg L<sup>-1</sup> their GII were 94% and 98%, respectively. On the contrary, *S. sclerotiorum* was the most resistant (23% GII). *S. cepivorum* was more sensitive to n-hexane extract and essential oil than *P. cinnamomi* at the majority of concentrations tested.

*Drimys winteri* has potential to be a good source of natural fungicide, and it could be used as part of future strategies for controlling soil borne pathogens.

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## WHEAT FLOUR PENTOSANS AND THEIR EFFECT ON DOUGH RHEOLOGICAL PROPERTIES

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Wheat flour and its products are the main source of human diet calories in South America. In Uruguay they are the foundation of the main agroindustrial chain. Wheat flour is able to form doughs with unique rheological properties that allow breadmaking. Much is known about wheat protein content's effect on dough rheological properties, but very little evidence exists concerning the role of other minor constituents. Pentosans, a group of non-starch branched polysaccharides present in wheat flour, are closely related to gluten-forming proteins, and clearly contribute to their functional properties and thereby to bread quality. Pentosans are formed mainly by a xylopiranosyl linear chain branched with arabinofuranosyl residues. The spatial distribution and branching pattern of pentosans can affect the interaction

among starch, gluten and other flour components, so they can also affect the dough rheological properties and bread quality. Many authors hypothesize about their role, but currently there is no theoretical model that can explain the experimental data.

The aim of this study was to investigate the chemical and physical properties of endogenous pentosans in flours milled from Uruguayan wheat varieties with diverse rheological and bread-making properties.

The extractable and non-extractable pentosans were isolated from wheat flour after enzymatic degradation of starch. Size exclusion chromatography was used to isolate the water-soluble pentosans while non-soluble pentosans were determined by difference between total and soluble pentosans. Free monosaccharides were determined by gas chromatography of adequate derivatives in both flour samples and isolated pentosans samples. The amount of total and water- extractable pentosans was determined by the orcinol-HCl method. An improved method for the isolation of pentoses either for extractable and non-extractable polymers, and a simple colorimetric method to estimate soluble and total pentosans directly on flour were developed.

No free pentoses were found in flour, being them found only as constituents of the flour endogenous pentosans polymers. Total and soluble pentosans were determined in both flour and pentosans enriched fractions. Their relationship with dough rheological parameters will be presented.

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## **ROOT YIELD RESPONSE OF JERUSALEM ARTICHOKE (*HELIANTHUS TUBEROSUS* L.) TO NITROGEN FERTILIZATION AND HARVEST DATES**

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Jerusalem artichoke (*Helianthus tuberosus* L.) is an herbaceous plant in which stems, leaves, tubers, and flowers are used. Inulin, fructose, and flour are derived from the roots for food uses such as high fructose syrup, sweeteners, and animal feed. Nitrogen fertilizers are one of the main costs in production. There is a need to know the response of Jerusalem artichoke to different levels of N availability under the agro climatic conditions of the Bio-Bio Region of Chile.

The aim of this study was to evaluate the effect of N fertilization and harvest dates on aerial biomass, and root yield and quality.

The experiment began in 2008-2009 season at "El Nogal" Experimental Station, Universidad de Concepcion, Chillan, Chile. The design was a randomized complete block with a split-plot arrangement where the main plot was the harvest date. The first, second, and third harvests were made in April, May, and June 2009, respectively. The sub-plot treatments consisted of applying 0, 50, 100, 150 and 200 kg N ha<sup>-1</sup> applied as CAN27 (27% of N, 4% of Mg, and 6% of Ca). Seeding date was 19 September, 2008 with a plant density of approximately 1400 kg roots ha<sup>-1</sup>. The experimental units were each 12.5 m<sup>2</sup> in size.

There was no significant interaction between N rates and harvest dates. The highest root yield was obtained with the 150 and 200 kg N ha<sup>-1</sup> rate; however, the difference between these two rates was not significant. No significant differences were observed in aerial biomass moisture between the April and May harvest dates. There was a significant difference in biomass fresh weight among harvest dates. Highest fresh aerial biomass yield was observed in the first harvest date. This is explained by the observed loss of foliage as stand age increased. There was no significant difference for harvest index among harvest dates that could be used for making harvest timing decisions.

Results indicate that N rate and harvest date influence root yield and quality. Because of Jerusalem artichoke's use as functional food, it is necessary to know its response in order to obtain a high quality product.

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