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Solving Problems with Industrial Crops

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Program Abstracts

PLENARY SESSION (BIOENERGY/BIOPRODUCTS)

RESPECTIVE ROLES OF AAIC, NUC, AND BMA IN COMMERCIALIZING BIOBASED PRODUCTS AND BIOENERGY

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The New Uses Council (NUC) and the Association for the Advancement of Industrial Crops (AAIC) should be positioned to play a cooperative and major role in the advancement of the biobased economy/carbohydrate economy. This partnership will be strengthened by including the Biobased Manufactures Association (BMA), the marketing arm for products produced through the joint efforts of AAIC and NUC.

We should see this extended partnership as three distinct industries working synergistically to bring a new and wide range of renewable products into the American and world marketplace: several biofuels, including ethanol, biomethanol, biodiesel, bio-oil, biogas, and biohydrogen; biopower and cogenerated thermal energy; and, a plethora of biobased products. The AAIC will focus on industrial crops for the more profitable production of these bioproducts; the NUC will assist by encouraging support for development of these crops that will not only increase productivity and offer value-added options, but also enhance the environment while improving the soil.

The NUC will also work for supportive public policy, heightened interest by governments at all levels, and the advancement of industries to refine crops, residues and waste streams into products. The BMA will market these products which, in the end, is the principal determinant of success in this overall process.

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INTEGRATED BIOREFINING SYSTEMS FOR BIOBASED PRODUCTS AND BIOENERGY

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The likely outlines of integrated biorefining systems are now beginning to emerge. Integrated biorefining systems will produce a wide variety of chemicals, materials, food, animal feed, and fuels from many different plant sources. We will discuss features of biorefining systems on which there are both substantial agreements, and also much less agreement, or at least certainty. Life cycle analysis will also be described in terms of its value to help optimize our choices and pathways to a more biomass-dependent future.

Some features of biomass refining systems on which there are substantial agreements include: 1) their inherent advantages conferred by low cost, widely available and diverse biomass raw materials, 2) their increasing future competitiveness due to relative technological immaturity, particularly the relatively immature state of bioprocessing technology, 3) the probable continuing diversification of biobased products, 4) the impact that advances in the life sciences will have on biomass production and biorefining, and 5) the effect that increasing efficiency of biomass raw material utilization over time will have on the system (National Research Council, 2000). The consequences of these and other high consensus features will be briefly discussed.

Other features about which there are fewer consensuses, and which, therefore will be treated in more depth, include, among others: 1) possible competition of biomass derived chemicals (including fuels) and materials with food production (in the context of possible land use constraints), 2) whether large scale fuel production from biomass is possible, or even desirable (in a life cycle context), 3) the likely trajectories of the biorefining industry (will it diversify like the petroleum refining industry, from high volume toward high value products, or will it take the other direction?), and 4) how biorefineries will or might integrate with the agricultural production sector.

It is crucial to develop this new biobased products industry in an environmentally sustainable manner. The tools of life cycle analysis are available to help guide the development of biorefining systems. In many ways we have a unique opportunity to choose whether or not we would like to inhabit a world based more on renewable plant sources - and then to construct sustainable pathways that will take us to such a future.

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U.S. DEPARTMENT OF ENERGY'S FEEDSTOCK RESEARCH PROGRAM

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The "Vision for Bioenergy and Biobased Products in the United States" sets forth the goal that by 2030, 5% of the nation's power, 20% of transportation fuels, and 25% of the chemicals will be derived from biomass. This production of fuel, power, and chemicals is about 15 quadrillion Btus (quads) annually and will require approximately 1 billion tons of biomass feedstock per year.

Achieving the 1 billion tons/year goal by 2030 is a significant undertaking and will require a focused research and technology development program. This program will have four critical objectives as follows:

Biomass Availability - Current U.S. availability of biomass is estimated to be approximately 400 million tons/year. Therefore biomass production must be more than doubled, which will require significant changes in the U.S. agricultural system.

Sustainability - Currently, the U.S. food, fiber, and feed production system produces about 1 billion tons of product annually. A viable lignocellulosic biorefining industry will double this production demand, whose doubling can only be sustainably achieved through enhanced utilization of natural resources and reduced environmental impacts at both the enterprise, national and global levels.

Infrastructure - The current biomass harvest, collection, transport and storage infrastructure system is designed primarily to meet the low volume dispersed demands of the diary and livestock industries. This system is inadequate to meet the high volume, centralized demand of the biorefinery concept, and hence, new technology and infrastructure are needed.

System Profitability - The economics of fuels, power and chemicals productions from biomass dictate that the delivered lignocellulosic biomass feedstocks costs (including preprocessing) must approach $35 per ton. Within this price target growers, brokers/transporters, equipment suppliers, and every other involved industry sector must realize a self-sustaining profitable business venture to ensure an economically viable feedstock supply chain.

A series of colloquies has been held involving growers, equipment suppliers, harvesters, transporters, processors, and other involved stakeholders to define the high priority research and technology development needs. From this input, a feedstock supply roadmap has been developed to guide the biorefinery feedstock research and technology development program.

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EXPANDING BIOBASED MARKET SHARE

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Conventional wisdom among academics and bioscience laboratories is that further research is the most important contributing factor to the successful development of the biobased market. University and National Laboratories have certainly provided the needed technologies upon which a multitude of excellent biobased products are based. However, with biobased manufacturers struggling financially in virtually all 20 major product categories, their overwhelming concern is for increased sales. Biobased manufacturers now needs support not only in science and technology, but in marketing and finance as well. As a representative of biobased manufacturers, I would like to recommend ways in which Universities, Federal and State governments can help to promote the growth of the biobased economy.

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BIOENERGY FEEDSTOCK ISSUES: A GOVERNMENT PERSPECTIVE

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The energy demand of the United States is huge and current use of fossil fuel to meet that demand creates security and environmental concerns. Bioenergy crops have the potential to address these concerns, while also benefitting the rural economy. However, there are several challenges to be overcome before the potential of growing crops for use as energy feedstocks can be realized.

Issues such as economics, production, logistics, conversion, and sustainability must be addressed in order to achieve significant development of energy crops and their commercial conversion into liquid fuels and other forms of energy. Research and development are needed for improving the genetics and production of bioenergy crops; for gathering, handling, storage, and delivery of large quantities of energy-crop biomass with appropriate characteristics; and for converting such biomass into more useful energy forms. The Agricultural Research Service and other agencies of the U.S. Department of Agriculture have research underway to solve these technical problems.

Though technical challenges are great, other challenges exist that may be even greater. Currently, the cost of fossil feedstocks is, and will for some time likely remain lower than the cost of bioenergy feedstocks and as a result fossil-based energy is cheaper than biobased energy. Therefore, because comparative cost is of such importance in making purchasing decisions, bioenergy has a significant marketing disadvantage. For bioenergy to be competitive, there is a need for implementation of policies that cause the true overall cost (including energy security, the environment, and the rural economy) of each energy option to be reflected at the point of sale.

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Top of Page

Return

NAKAYAMA SYMPOSIUM

BIOCHEMICAL REGULATION OF RUBBER BIOSYNTHESIS IN GUAYULE

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Natural rubber is an irreplaceable raw material vital to industry, transportation, medicine, and defense. At present, most of this rubber is produced from clonal plantations of Hevea brasiliensis in southeastern Asia. Temperate-zone rubber-producing crops are greatly desirable to increase the biodiversity, protect supplies, and provide a safe natural-rubber alternative for the large number of people suffering from Type I latex allergy to proteins in existing Hevea latex products.

We have used a combination of basic and applied research approaches, from biochemistry and metabolic engineering to process chemistry and performance testing, to make the production of latex from Parthenium argentatum (guayule) a commercial reality. However, new guayule lines are still needed that have high latex yields, improved agronomic characteristics, and broader environmental cultivation ranges. Understanding the biochemical regulation of rubber yield (principally rate) and quality (principally molecular weight) in guayule is an essential preliminary step to the identification and manipulation of the key regulatory steps in rubber synthesis.

In this presentation, the biochemical regulation of guayule rubber biosynthesis will be discussed, and features unique to guayule highlighted.

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BREEDING GUAYULE FOR COMMERCIAL PRODUCTION

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Breeding a new industrial crop, such as guayule, is not appreciably different from enhancement and breeding of conventional crops. In both instances, plant breeders take the extant germplasm and search for genetic variability in the desired traits. The major differences are that in new crops plant breeders are often working with an unfamiliar species that is not yet fully domesticated and the available germplasm is often limited.

The main objective of the guayule breeding program is to facilitate successful commercialization by developing higher yielding cultivars. Improvement has been accomplished, with newer lines yielding up to 250% more rubber than lines developed in the 1940s and 1950s. This is surprising because the genetic base from which improvement has been made appears to be very narrow, and because guayule reproduces predominately by apomixis (asexual reproduction by seed).

Improvement through plant breeding is dependent upon having genetic diversity within the available germplasm, and being able to identify different genotypes. Our measurements have shown that the available guayule germplasm exhibits extreme variability both within and between lines for morphological traits such as height, width, and biomass, chemical constituents such as rubber, resin, and latex contents, and genetic (isozymes), chromosomal, and molecular (RAPD) markers.

The measured variation is due partly to the facultative nature of apomixis in guayule (asexual reproduction and sexuality coexisting), which periodically releases genetic variation among progeny. It has also been shown that a great amount of this measured variation is due to environment, and selections, to take advantage of only the genetic differences, must be made within the first two-years of growth.

There have been relatively few individuals involved in guayule breeding. Thus, with limited resources and time, most of the improvement has been made by single-plant selections from within populations. Although this method has the potential for only modest long-term gains, it requires a relatively short time period to realize improvements. To facilitate the selection process, indirect measures have been developed so that many more plants can be evaluated by relatively few individuals. For instance, most selections are made for plant height, width and biomass because they have been found to be highly correlated with rubber yield. As pointed out by Dr. Francis Nakayama, by making selections in this manner, we may be inadvertently changing the plant in ways that are not necessarily desirable, such as the increased resin to rubber ratio found in some newer lines. To facilitate guayule improvement, a breeding scheme is being suggested that combines recurrent crosses between sexual and apomictic genotypes, which will allow the release of more genetic diversity from which selections can be made.

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GUAYULE AGRONOMICS: IRRIGATED PRODUCTION, ESTABLISHMENT, AND WEED CONTROL

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Irrigation is a critical factor influencing guayule establishment and production. Standards were developed during the Emergency Rubber Project that included annual water requirements for guayule production, and irrigation water quality. Early investigations confirmed that stress played an important role in rubber production. Approaches to controlling water stress focused on the theory that plant stress, caused by soil water deficits, could increase rubber production. A simple, reliable method was needed for following water stress so that the stress/rubber production interrelationship could be clearly defined. The Crop Water Stress Index (CWSI), developed for other economic crops, was adapted to transplanted guayule in the early 1980s by Dr. Francis Nakayama and co-workers at the USDA-ARS, U.S. Water Conservation Laboratory (USWCL). The CWSI was computed from the plant and air temperature difference, versus the water vapor pressure deficit of the atmosphere. The CWSI/rubber yield correlation revealed that under low stress rubber yields averaged 930 kg/ha, and only 540 kg/ha under high stress. The index has become a valuable tool for monitoring and managing water stress and irrigation scheduling.

Understanding plant stress and the water requirements of guayule led to successful stand establishment by direct-seeding. Dr. Nakayama and others at the USWCL found that conditioned seed could be planted 10 mm deep and its germination was superior to untreated, raw seed. They reported that under drip irrigation, intermediate water levels were best for stand establishment (two irrigations per week during the first five weeks following planting, and a single irrigation per week between weeks six and ten). These studies led to successful establisment trials by Texas A&M University and New Mexico State University.

Extensive weed control research has been conducted since the 1970s, but no treatments are currently labeled by the U.S. Environmental Protection Agency for guayule production. Investigations by New Mexico State University revealed that DCPA (9.0 kg ai/ha) and Prowl (1.1 kg ai/ha) demonstrated adequate selectivity for preemergence weed control in direct-seeded guayule. Studies by Texas A&M University confirmed these results. Herbicide injury is decreased in transplanted stands because the older, larger plants are more tolerant to herbicides, and are also more competitive against weeds. Field experiments in New Mexico indicated that Treflan, Prowl, and Surflan applied as preeemergence treatments were safe for transplant establishment. Texas A&M University scientists reported that Barricade (2.2 kg ai/ha), Gallery (0.6 kg ai/ha), Prowl (1.1 kg ai/ha), and Treflan (1.1 kg ai/ha) were all adequate for controlling annual broadleaf weeds and grasses. Presently, no herbicides are safe for use as postemergence, over-the-top sprays in either direct-seeded or transplanted situations.

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GUAYULE PROCESSING: BULK RUBBER AND LATEX

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Guayule rubber (GR) has not been an article of commerce for more than 60 years. Nevertheless, the production of bulk rubber or latex from guayule has been pursued at various times throughout this period. We describe here the most recent efforts in this area, with particular consideration given to the properties of guayule cis-1,4-polyisoprene that influence process development.

As recently as 1990, multi-ton lots of bulk GR have been produced for evaluation in tires and other rubber goods. At a prototype processing plant operated by Bridgestone/Firestone, Inc., 8.8 t of GR were produced by simultaneous extraction of rubber and resin with a mixed organic solvent. The rubber component of the resulting miscella was fractionated to yield material meeting standard specifications established for bulk rubber from Hevea (NR). The nameplate capacity of the Sacaton, Arizona, facility was 152 t (150 long tons) of GR/y. Running at capacity, the pilot plant would have consumed an estimated 860 kg/h of baled shrubs.

More than 3.3 t (37%) of the rubber product met the specification for TSR20 NR. This material was tested as a direct replacement for NR in aircraft tires. Another 2.1 t (24%) met the FEMA specification for bulk GR and was used to fabricate light truck tires.

The need for a fractionation step arose from the fact that GR has lower bulk viscosity than NR, the result of a lower Mn and a broader molecular weight distribution. GR bulk viscosity varies with the cultivar, the date of harvest, and the level of entrained resin (non-rubber extractables). Because GR lacks the protein components of NR, GR must be stabilized from both thermal and oxidative degradation.

On the threshold of pilot-scale production, guayule latex has been prepared to date in 25- to 50-kg batches. The latex is washed from ground shrubs, and then concentrated by a combination of multistage centrifugation and creaming. Development of a commercially-viable latex process will have to take into account several factors that distinguish GR latex from Hevea (NR) latex, among them a greater sensitivity to changes in temperature and solids concentration, a lower protein content, and a higher resin content.

In terms of its cure characteristics, the resulting product behaves more like synthetic polyisoprene (IR) latex than NR latex. Most importantly, dipped goods prepared from GR latex are free of the protein allergens that elicit a Type I allergic response in individuals sensitized to NR latex.

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GUAYULE FUTURE DEVELOPMENT

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Successful commercial development of guayule (Parthenium argentatum Gray) will depend on using as much of the plant as possible. At present, latex is the plant's primary product. Utilization of the remaining components that include resin and biomass can greatly improve the economics of guayule. The purpose of this presentation is to review new and possible alternate products that can be made from guayule "waste" material that can enhance the commercialization of guayule.

Coproduct development can augment the commercialization of natural, renewable resources. For guayule, about 90% of the plant material to be grown in several hundred thousand hectares would be available for coproduct development. Fortunately, guayule synthesizes many potentially useful compounds for industrial and commercial applications. These include fatty acid triglycerides, flavonoids, polyphenols, terpenes, sesquiterpenes, and waxes that make up about 10% of the whole plant. Also, we cannot neglect the other 80% of the cellulosic material. The resinous material is of special interest because of its antitermitic and wood-rot resistance properties.

Because of the water-based process used to extract latex, the residual plant material or bagasse will still contain the resin. This resin-containing bagasse without additional chemical processing has been fabricated into high-density, construction-grade, composite boards that are resistant to attack by termite and wood-rot fungi. In the future, bagasse could be blended with many other wood sources with different densities and physical proerties that have insect control properties. The bagasse or the resinous extract could be incorporated into wood putty or caulking, for example, to make such repair material insect resistant.

The resin extracted from the latex-processed bagasse with a polar organic solvent can be used without purification. Such resins when impregnated into wood can provide protection against other wood destroying organisms such as marine borers. Possibly, this resin extract can be used to protect wood and trees against wood attacking insects such as carpenter ant and bark beetle. In addition, the resin can be used in paint primers and varnishes with similar insect control properties. The resin has been incorporated with epoxy polymers to produce coatings that are readily strippable, a useful property for storage-protection of aircrafts, ships, and other industrial equipment undergoing environmental exposure.

Other potential uses for the bagasse or resin is in the area of energy production. The bagasse can be formed into fire logs, briquettes, and pellets. Such combustible material has higher energy value than other wood sources because of the resin, which can make up about 10% of the dry mass. The bagasse has been converted into gaseous and liquid fuel, and with improved pyrolysis technology, could become a source of diesel-type fuel. De-resinated bagasse could be converted into a source of alcohol and other type of chemical entities for liquid fuel or solvents.

Ongoing attempts to increase the rubber content and biomass of the plant, have increase the resin to rubber ratio to 2:1 from that of 1:1. Although unintentional, this crop improving development may be fortuitous because the resin fraction appears to be just as valuable as the latex component. The Parthenium genus, consists of numerous species that can grow faster than guayule with larger biomass, but consists mostly of resinous material instead of rubber. The future commercial development of these other Parthenium genus also appears promising.

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Top of Page

Return

NATURAL RUBBER AND RESINS - SESSION 1

GUAYULE NATURAL RUBBER LATEX - COMMERCIALIZATION UPDATE

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Type I latex allergy has continued to be a critical problem for the health care community since the late 1980s when the increased use of tropical latex medical devices, especially medical gloves, precipitated an epidemic of latex allergy in the United States. Studies of serum antibodies from the general population conducted by the USDA in 1994 identified approximately 20 million Americans who have developed some level of sensitivity to tropical proteins. Although synthetic lattices are now utilized for manufacturing latex medical devices, performance attributes are inferior to natural latex while material costs are significantly higher.

Yulex Corporation ("The Company"), through an exclusive licensing agreement with the USDA-ARS, has embarked on an ambitious commercialization project to meet the critical demands for nonallergenic natural rubber latex. The pilot project is privately financed and focused on three key areas: 1) agricultural expansion of the guayule plant (*Parthenium argentatum*, Gray), 2) scale-up of the latex bioprocessing methods developed by Dr. Katrina Cornish and her team at the USDA-ARS laboratories, and 3) product development relating both guayule latex products and various co-products derived from the guayule plant.

The Company announced in July 2003 the completion of the first guayule natural rubber latex pilot plant facility. The pilot facility is located in Maricopa, Arizona with the desinged capacity to process approximately 750 metric tons per annum of biomass (green weight). This pilot facility is located in close proximity to the company's mature guayule crops, which will be harvested and utilized as feedstock for the pilot facility.

With the completion of the pilot facility, Yulex has entered into agreements with medical device manufacturers who are assisting Yulex with process scale-up and product development. The Company believes guayule natural rubber latex will be competitive in the short-term against high-priced synthetic latex for the premium medical device sectors while projecting competitiveness against tropical latex with the successful commercialization of co-products currently under development.

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QUALITY OF LATEX EXTRACTED AND PURIFIED FROM GUAYULE AGRONOMIC TRIALS WITH DIFFERENT SPACINGS AND DATES OF PLANTING

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Current guayule commercialization efforts are based upon the production of hypoallergenic latex. However, little is known about the optimal agronomic condition for maximum latex production. In this study, we tested the effect of planting density and date of planting on the yield and quality of latex in four guayule lines harvested at three times.

We found differences in latex content among lines, planting dates, planting densities, and harvest times. Line AZ-5 had the highest latex concentrations followed by AZ-1, 11591 and AZ-3. Latex concentrations were lower in all lines harvested in October 2002, compared with harvests in April of 2002 and 2003, reflecting plant growth and low latex production between April and October. Three of the four lines (AZ-3 being the exception) had higher latex concentrations at a density of 54,000 plants/ha, than at 27,200 plants/ha. Also, the date of planting did affect the latex content, but in a line-specific manner. Most strikingly, AZ-5 shrubs planted in June 2001 had a much higher latex content in April and October 2002 than the older AZ-3 shrubs planted in November 2000. This difference was no longer apparent in April 2003. Variation between shrubs from the two dates of planting was large in April 2002, but had substantially decrease by April 2003.

Rubber particle size also varied slightly. At 27,200 plants/ha, the older plants had a larger particle size than the younger plants, but this was only seen in AZ-5 at 54,400 plants/ha. In the older plants, particle size was consistently greater in shrubs grown at the lower plant density. Little difference was found in particle size among younger plants regardless of planting density. Protein concentrations in April 2002 followed a similar trend as the latex concentrations - protein concentrations were higher in the latex of younger plants and also in the latex of plants for the higher field densities, with 11591 and AZ-3 showing the largest differences. Data from the later harvests are not yet available.

The data indicate that the older plants (based on date of planting) at lower planting density make less rubber in fewer, but larger particles than the

older plants at higher density or the younger plants in general. No information is yet available on plant size at the different harvests so that extrapolations to the actual latex yield/ha are not yet possible.

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EFFECT OF NIGHT TEMPERATURE ON GROWTH AND PHOTOSYNTHETIC RATE IN GUAYULE

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Guayule (*Parthenium argentatum* Gray) produces rubber primarily during the winter months. There is evidence from growth chamber studies that cold night temperatures are responsible for this increase. The objective of this preliminary study was to determine what physiological changes occur in guayule when exposed to cold night temperatures that might account for the rubber accumulation during the winter months.

Plants from the lines N6-5, AZ6, and 11591 were placed in open containers inside an unheated greenhouse so that all plants were exposed to the same daytime temperatures. At night, covers were placed on all of the containers and heat provided to one-half of the containers to maintain the average nighttime temperatures above 20°C. The unheated containers were exposed to ambient night temperatures, which averaged less than 10°C.

Plant heights were recorded every 15 days. Photosynthetic rate estimated by CO2 exchange was measured three times in January and February 2003 within one hour of solar noon. Photosynthesis measurements were also taken once 4 h before solar noon and 3 h after solar noon. On 19 March 2003, all plants were harvested by cutting the plants at the soil surface and roots were removed from the soil and washed.

Those plants exposed to cold night temperatures were significantly smaller in both height and fresh weight than those plants subjected to heated conditions during the night. Photosynthetic rates were not significantly different between cold- and warm-night-treated plants in the early morning and late afternoon, but were significantly higher for the cold-night treated plants when measured around solar noon.

The higher photosynthetic rate at solar noon in plants exposed to cold night temperatures could account for some of the increased rubber production during the winter months. These plants have low growth rates. Therefore, rubber production could be a sink for the photosynthates being produced.

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NEW GUAYULE (*Parthenium argentatum* Gray) VARIETY TRIALS

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Complementary data on guayule varieties along with guidelines on suitable locations for its growth and management practices are needed. Nine locations were chosen for a breeding yield trial, Maricopa (AZ), Marana (AZ), Yuma (AZ) (2 sites), Pecos (TX), Tucson (AZ), Safford (AZ), Los Cruces (NM), and Saltillo (Mexico). The different locations were chosen for their differences in elevation, annual rainfall, frost-free season, and soil type. The following summary only concerns the first 5 locations, i.e., the Maricopa Agricultural Center, Marana Agricultural Center, and two at the Yuma Agricultural Center, which are all part of the University of Arizona and the Texas A&M Research Station, Pecos, TX.

A total of 14 different lines (11591; AZ-1; AZ-2; AZ-3; AZ-4; AZ-5; AZ-6; AZ-101; AZ-R2; N565; N6-5; N9-3; N13-1; G1-16) were planted in a completely randomized complete block design with 4 replicates in Maricopa on 27 and 28 November 2001, in Marana on 16 May 2002, at both sites in Yuma (the first 13 lines only) on 29 and 30 May 2002, and in Pecos (the first 13 lines only) on 2 May 2002. Measurements taken at each site included: stand count after planting; plant height and width (twice a year, late October-early November and late April-early May); latex, rubber and resin content; and plant biomass (yearly for 3 years starting 2 years after planting).

After only 2 sets of plant measurements (height and width), four lines (AZ-1, AZ-2, AZ-3, and AZ-101), common to all locations, have a higher growth rate. Although these lines do better overall, differences in plant height among the various locations for these 4 lines are quite different. For instance, even with 6 months difference in the planting date, the plant heights from Marana are comparable with the ones from Maricopa for the fall 2002 measurement (Pr - 0.0001).

Early in the trials, and with only one year's data, no conclusions can be made at this time. However, environmental factors such as elevation, annual rainfall, frost-free season, soil type, and also the field management (irrigation, fertilization and weed control) may be responsible for the differences among locations. These trials will help establish the lines that have the best commercial potential for growers in different locations where guayule will be grown. It can also provide useful guideline information for future guayule growers.

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GUAYULE PRODUCTION IN NATURAL STANDS IN MEXICO, RECENT DATA

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Guayule (*Parthenium argentatum* Gray) remains a potential commercial source of hypoallergenic natural rubber latex that may be used for the production of different products. Currently, there are no guayule plantations in México. Guayule grows wild in the arid and semiarid regions as natural stands. These stands are exclusive to the country. Evaluation of the plant natural spreading is important allowing the identification and selection of high yielding varieties in different environmental zones.

The present objective was to determine the morphological characteristics as well as rubber and resin contents of wild guayule plants at four sites over a period of three years.

The study sites were defined within the localities of Rocamontes (Coahuila), Norias de Guadalupe (Zacatecas), and two at Gomez Farías (Coahuila). We had evaluated the sites previously during 1997. The present study was carried out from January 1999 to February 2002 with samples collected generally every month. Ten plants per site that were representative of the population were measured in the field for height and spread, and then uprooted and transported to the laboratory for biomass, main stem diameter, rubber and resin content measurements.

The results showed plant spreading and development during the three years study causing an apparent reduction in the average biomass production, but an increase in rubber and resin content. The present global average biomass value (242 g) is greater than that (52 g) found in our previous evaluation of the sites. Rubber content increased from 8.9% to 10.9% in the three years, whereas resin content changed from 12% to 11.2%. The average rubber content in the sites is approaching the highest average value (11.7%) found in the native stands at Mapimi, Durango. Accordingly, plant natural reestablishment is noticeable and will create a good source of raw material and seeds that may be able to support and develop commercial plantations.

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NATURAL RUBBER AND RESINS - SESSION 2

POST-HARVEST GUAYULE STORAGE TESTS

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Guayule (*Parthenium argentatum* Gray) is a perennial shrub native to the Chihuahuan Desert of northern Mexico and southern Texas. New

germplasm has shortened harvest time from 3 to 5 years to 2 to 3 years. Biomass yields of newer lines approach 22 t ha^{-1} within 2 years. One of the most valuable products from guayule is its hypoallergenic latex. However, little research has been done on methods to handle the shrub from the time it is harvested in the field until it is processed for latex extraction. Past results have shown that extractable latex yields from shrubs stored under ambient conditions are almost zero within 24 h following harvest. The development of storage and handling systems to maintain latex yields during the critical period from shrub harvest until latex extraction would be very beneficial for growers and processors.

The objective of this study was to determine the effects of various storage treatments on the latex content of harvested guayule. The treatments consisted of storing the shrub dry in the shade, and with various moisture treatments in the shade.

Results indicate that storage under dry conditions results in almost zero latex being extracted. These results confirmed our previous observations. However, storage under several different moist conditions maintained latex yields equal to freshly harvested guayule latex yields for periods of up to four weeks. Results from these tests indicate that harvested guayule shrub can be stored and processed following field harvest without losing latex yield.

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LATEX YIELD AND QUALITY DURING STORAGE OF GUAYULE (*Parthenium Argentatum* GRAY) HOMOGENATES

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Extraction and purification of latex from guayule (*Parthenium argentatum* Gray) require that harvested shrub first be homogenized in an alkaline aqueous buffer. We have extended an earlier investigation into the stability of the latex in homogenates made in different ways and stored under different conditions.

Neither the length of post-harvest storage (up to five weeks) nor the diameter of the branch affected the concentration of the latex in homogenates. Latex concentration was not affected by the length of grinding used to make the homogenate. However, latex concentration declined at acidic pH, and after one month at 24°C in one experiment, and after six months in another when the initial latex concentration in homogenate prepared from defoliated shrub was below 5 mg/ml. This decline was less apparent in homogenates made from leafy shrub, which suggests a protective effect derived from the leaves. Storage at 4°C prevented latex loss under all treatments.

The quality of the rubber polymers in the latex fraction was investigated using size exclusion chromatography/multiangle laser light scattering detection. Polymer molecular weight and molecular radius declined in parallel, but declined faster in the dilute homogenate generated from the second grind of the guayule bagasse than in the more concentrated homogenate from once-ground shrub. Degradation was greatly slowed in all treatments when the homogenates were stored refrigerated. Polydispersity values were low in all treatments, and only slightly increasing over time, with the exception of homogenate generated by the second grinding of shrub in the presence of leaves and stored at 24°C. The relatively rapid polymer degradation of the latex fraction in this homogenate led to an increase in polydispersity followed by a decrease as the latex fraction was degraded to below detection levels.

We conclude that guayule homogenate provides a stable environment for latex yield and quality, even at room temperature, for at least 13 to 16 weeks provided that the pH is basic and the concentration of rubber particles is at least 5 mg/ml. This is in contrast to the extractable latex content of harvested branches, which is prone to rapid coagulation and degradation in situ unless the branches are stored hydrated and refrigerated.

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X-RAY AS A TECHNIQUE TO STUDY GUAYULE (*Parthenium argentatum* Gray) SEEDS (ACHENES)

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In guayule production, seed quality is important for stand establishment. The X-ray technique is a nondestructive procedure that can provide

information about internal structures and seed quality. The objective of this work was to evaluate the physical and physiological quality of guayule seeds by correlating X-ray radiographs, seed coat color, weight, and percentage germination.

Three lines were studied: 11591, N13-1, and AZ1. Seeds were classified by width using a 1/14 (1.8 mm) rounded sieve, and separated by color using a magnifying scope equipped with fluorescent lights. The seeds were separated into four grades, yellow, gray, opaque black, and bright black. These same seeds were further separated by weight using a precision balance. For X-ray analysis, the seeds were placed on a plastic film and X-rays taken with adjustments in the radiation level and time for better quality of the images that could characterize the seeds as filled, partially filled, and empty.

Before the germination tests, seeds were pre-soaked in water for 6 h, treated with 1.5% sodium hypochlorite (3 min) and rinsed with water. Standard germination tests were performed with four 25-seed replicates for each seed lot. Seeds were placed in Petri dishes with two filter papers on the bottom moistened with water equivalent to 2.5 times the substratum weight (the two filter papers) and germinated at 25oC and constant fluorescent lighting for 10 days.

There was a high correlation between X-ray pattern, seed weight, seed coat color, and percentage germination. Filled-gray and filled-opaque black seeds had statistically higher percentage germination than the empty, filled, partially filled, and bright black and yellow seeds.

According to the results, the X-ray technique can be used for seed analysis of guayule to identify the quality of seed lots and for information about the presence or absence of internal structures.

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CONCENTRATION OF RESIN, RUBBER, AND GUAYULINS IN VARIOUS PARTS OF GUAYULE PLANTS

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Guayulins A and B are esters of the sesquiterpene alcohol partheniol present in the resin, or acetone-extractable fraction of guayule (*Parthenium argentatum*). Guayulin A has been identified as a potent elicitor of contact dermatitis. It is quantified by reverse-phase HPLC in samples that have been analyzed for resin and rubber. A potential problem was discovered with this analysis, however. Some duplicate samples with small deviations in percent resin were found to have large deviations in percent guayulin. Our hypothesis was that guayulin content varied significantly among plant parts and that the sub-samples contained different ratios of these plant parts. The objective of this study was to determine whether there are differences in resin, rubber, and guayulin concentrations among the various plant parts.

One- and two-year-old guayule plants of three lines (11591, AZ-1, and AZ-3) were harvested from the U.S. Water Conservation Laboratory in Phoenix, AZ in October 2002. A total of 12 plants (two of each age and line) were separated into eight parts: brown leaves, green leaves, stem tips (including several immature leaves), stems less than 5 mm in diameter, stems between 5 and 10 mm, stems greater than 10 mm, green stems (where multiple green leaves were attached), and flower (inflorescence) parts. The samples were dried and sequentially extracted by the homogenizer method with acetone and cyclohexane to remove resin and rubber, respectively. The resin fraction was analyzed by reverse-phase HPLC to quantify guayulins.

In all ages and lines, resin content was highest in green stems. Rubber and guayulin A were most prevalent in stems larger than 10 mm in diameter, and a rank test showed a high correlation. This can be explained by the fact that both compounds have a common precursor. Flowers contained the least amount of both resin and rubber. When yields are calculated, resin content is highest in stems less than 5 mm, whereas rubber content is highest in stems greater than10 mm for all ages and lines. Resin and rubber yields are lower overall in 11591 than the two AZ lines, which are newer and have been selected for higher yields. The ratio of resin to rubber is lowest in the largest stems, meaning that those stems have the most amount of rubber with the least amount of resin. They can also be assumed to be the oldest tissue. This experiment will be repeated with another 12 plants harvested in April 2003 to determine whether the time of harvest is a factor.

In reviewing other literature in which rubber was determined in each plant part, it was discovered that most authors assumed that there was no rubber in the leaves of guayule, and state that plants should be defoliated as a first step in any procedure to determine rubber content. The results of our study show that there is indeed rubber in the leaves, with an average of 1.7 % in the brown leaves and 2.0% in the green leaves. This amounts to a total yield of 52.9 g in all leaves in all the 12 plants, which is 25.8% of the total rubber recovered.

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GROWTH, RUBBER, AND RESIN EVALUATION OF TWO-YEAR-OLD TRANSGENIC GUAYULE

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Guayule (*Parthenium argentatum* Gray) is a natural source of high-quality latex and rubber. Improvement through conventional selection techniques has been made and further improvement is being attempted by transforming guayule with one of three genes in the rubber biosynthesis pathway. The objective of this study was to evaluate the effect of these transgenes on growth and rubber and resin production in field grown guayule.

Tissue culture generated transgenic plants, provided by Katrina Cornish, for lines AZ 101, G7-11, and N6-5 were placed into field plots for two consecutive years. Terry Coffelt planted the initial field and we planted the second field. In both plots, plant height and widths were measured monthly. Resin, rubber, and guayulin production were sampled every four months starting at one year of growth. Resin and rubber were sequentially extracted by the homogenizer method using acetone and cyclohexane, respectively. Guayulin production was quantified using HPLC. The first plot was harvested at the end of two years of growth, whereas the second plot has just completed the first year of growth and will remain in the field for an additional year before harvest.

Transformation had no significant effect on growth in G7-11 and N6-5 in both plots. In the first plot, transformation appeared to have a drastic effect on the height and width of transformed AZ 101 compared with its empty vector control. However, the first plot was not randomized and lacked the non-transformed controls. In the second plot, which was randomized and contained both positive and negative controls, the AZ 101 transformants were significantly larger than the empty vector AZ 101 control, but were not significantly different from the non-transformed controls.

Resin content increased throughout the year up to January 2003, but decreased by the time of harvest in March. Rubber content, on the other hand, was high in May 2002, but decreased throughout the summer, before steadily increasing during the winter months. Guayulin production was low overall, especially Guayulin A production, compared with the conventional lines. Guayulin A production was particularly low in the AZ 101 transformants compared with their empty vector controls.

Insertion of genes for precursors in the rubber biosynthetic pathway did not appear to have any affect on overall growth. However, due to the significantly lower growth in the empty vector control of AZ 101, the gene may have been inserted into the genome in an area important for growth. Although transformation did not affect growth, it did appear to have an effect on Guayulin A production. The low levels of Guayulin A, a contact allergen, may be a beneficial effect of transformation and is worthy of further investigation.

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WHAT IS THE CHANCE FOR GRINDELIA BECOMING A RESIN CROP?

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In the last 15 years, the pine rosin industry in Argentina has moved toward the production of goods of higher value such as printing inks. While the value of extracted gum rosin is around 0.45 $US/kg and that of a manufactured resin can be as high as 1.80 $US/kg. The main product is resin-based. Inks are mostly exported to the U.S. and Europe. The industry in Brazil exports larger volumes than Argentina although of an unmodified resin. Chile is the third country producing resin in South America with sensibly lower export values.

Economic changes in the region in the last two years, especially the currency devaluation in Argentina (and of lesser degree in Brazil) has greatly improved the conditions to produce and manufacture exportable goods. Added to this general condition, resins from Argentina have been included in the U.S. Generalized System of Preferences (GSP) with no import taxes. On the other hand, higher wood prices threaten to reduce the availability of pine rosin creating a situation of high demand by the industry and low availability of raw materials. These and other changes have broadened the niche for alternative resins to enter the naval stores industry. Grindelia resin is one of these alternatives. After the discovery of the

resins by Joseph Hoffmann and Steve McLaughlin, at the University of Arizona, it was originally conceived as a substitute for pine rosin. The source of Grindelia resin was G. camporum although, particularly due to low resin content (high cost of biomass production, manipulation and extraction) it was concluded that it could not compete with pine-rosin. The idea was "resurrected" a few years ago, with the finding that one South-American Grindelia species, G. chiloensis had resin contents up to 40% D.W. Since 1995, we have developed a cropping system, based on eco-physiological responses both at the plant and at the crop levels as well as selected populations and clonal material with resin yields greater than 0.1 kg/plant, resulting in yields that could reach 6,600 kg of resin/ha. At these production levels, there is enough margin to grow, harvest and extract the resin for a value close to 0.30 $US/kg.

A central theme of our current research is resin extraction and processing. We are testing three alternative methods intended for different scale processing facilities. Because all three methods are based on solvent extraction, the different polarities of the solvents result in different residues (non-diterpene) extracts that have to be removed in the purification process as they reduce resin quality. Alternative uses, such as disinfectant solutions and concentrates, are also under investigation.

Our presentation will expand on the economics prospects of new resins in South America, on the development of Grindelia as a crop, and on the current status of extraction, purification, and characterization of the resin, keys to the successful cultivation of this species.

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YIELD AND RESIN PRODUCTION OF *Grindelia camporum* AND *G. chiloensis* ACCESSIONS GROWN IN PATAGONIA, ARGENTINA, AND OREGON, USA

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Worldwide demand for resins continues to increase due to their use in manufacturing high quality copier and laserjet paper, as well as specialty chemicals, ester gums, and rubber. The traditional raw material for these resins is pine rosin, although the supply of such materialsfluctuates dramatically with wood and pulp prices. *Grindelia camporum*, a native of California, and *Grindelia chiloensis*, a native of Argentina, both produce significant quantities of grindelic acid and related resins on the surfaces of their leaves, flowers, and stems, although the proportions vary dramatically depending on species. Previous studies in Oregon have shown that *G. camporum* grows well and can produce large amounts of biomass and crude resin. Studies in Argentina have shown similar results for *G. chiloensis*.

This study was done to compare biomass yield, resin production, survival, and related physiological observations for several accessions of *G. chiloensis* and *G. camporum* in southwestern Oregon and Patagonia, Argentina, in order to better understand the growth and resin production characteristics of these selected accessions as well as to determine likely production sites.

Replicated field plots were established near Medford, Oregon, USA, and near Trelew, Chubut, Argentina. One *G. camporum* accession collected in California and six *G. chiloensis* accessions collected in Argentina were grown in the glasshouse and transplanted to the field in the spring at both locations. Observations on growth and flowering were made during the season, and whole plants were harvested for biomass yield in the fall (approximately a 7-month cycle). Plant components were separated, and then resin was extracted with dicloromethane and refined following standard protocol.

At both locations, total biomass of G. camporum was more than double that of any *G. chiloensis* accession, with most of the difference due to much greater stem and flower biomass in the *G. camporum*. *G. camporum* biomass in Oregon was almost twice that in Argentina. Among the *G. chiloensis* accessions, 533 and 576 had much greater total biomass in Oregon, 555 and 575 had greater biomass in Argentina, and 561 and 569 were similar at both locations. On a whole-plant basis, resin content was greater for all six *G. chiloensis* accessions than the *G. camporum* at both locations, ranging from 9.2% to 14.4% in Oregon (compared with 6.7% for *G. camporum*), and from 10.6% to 19.0% in Argentina (compared with 7.0% for *G. camporum*). However, due to the much greater biomass of *G. camporum* in Oregon, its resin yield of 71.2 g/plant was greater than all *G. chiloensis* accessions, which ranged form 7.9 g/plant for 561 to 51.1 g/plant for 533. This contrasted with the result in Argentina, where the resin yield of *G. camporum* (39.7 g/plant) was less than accession 555 (54.2 g/plant), but greater than the others, which ranged from 5.5 to 35.1 g/plant. Flowering and maturity patterns were similar between the two locations, although the *G. chiloensis* accession 533 did not flower by the time of killing frost in Oregon. Winter survival was best for *G. camporum* at both locations (nearly 100%), whereas no plant of accession 576 survived the winter at either location. Other accessions of *G. chiloensis* had intermediate survival rates at both locations. Accession 575 survived better than the others in Oregon (69%), but its survival was very poor in Argentina, a difference that is not yet understood.

Similar field trials continued for several years, although only first year results are included here. Based on these results it appears that both *G. camporum* and *G. chiloensis* are good semiarid, temperate zone crop candidates to fill industry's need for commercial resin production. Differences

in response to climate and management will dictate which accession is best suited for a particular location.

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Top of Page

Return

GENERAL CROPS

DEVELOPMENT OF A NEW CROP, *Alyssum murale*, FOR PHYTOMINING NICKEL FROM CONTAMINATED OR MINERALIZED SOILS

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Certain rare plant species called Ni-hyperaccumulators contain more than 1% Ni in the dry shoots when grown on Ni-mineralized or contaminated soils. Because Ni metal sells for about $8.75/kg and plants could phytomine about 400 kg Ni/ha-y, it seemed possible to develop a new commercial crop for northwestern U.S. serpentine soils naturally rich in Ni. Co in the soils could be phytomined after Ni has been largely removed. This presentaiton will summarize the development of the crop and management practices, obtaining patents, and commercialization of the technology in Oregon and Canada.

After examining all species reported to be Ni hyperaccumulators, the team selected several species for development. Seeds were collected for some germplasm and initial work completed, which justified a Utility Patent for Ni phytomining. Then diverse germplasm was collected to breed improved cultivars for production on serpentine soils in OR and CA, and separately in Ontario, Canada. All nutrient requirements were established by experiments. The species Alyssum murale was developed the most. This species occurs naturally across southern Europe wherever serpentine soils occur. By collecting seed from different eco-niches and testing their growth and Ni accumulation potentials in replicated field plots in Josephine Co., Oregon, we were able to demonstrate the probability that a commercial crop could be introduced. The plant is highly adapted (endemic) to serpentine soils that are very low in Ca and P fertility, and contain high Mg and Ni levels. The plants accumulate Ni to defend themselves against diseases and chewing insects. Because the plants were adapted to serpentine soils, they were able to obtain adequate phosphate for sufficient yields with only small P fertilizer applications. One of the most surprising outcomes in our research was that acidification of the soil, which increased soil Ni solubility, decreased Ni uptake to the shoots; and for some soils lower in Fe, liming increased Ni uptake even though solution Ni decreased. Existing hay-making equipment could be used to harvest the crop. Baled biomass can be burned to produce a Ni-rich ash that can be sold as a high grade Ni ore and as an energy source. A test for Ni recovery from the biomass ash was conducted at the Inco Ltd. smelter in Sudbury, Ontario, Canada, with easy recovery of the Ni for sale.

Based on the experience to date, Viridian LLC initiated commercial contracts for production of Alyssum murale on serpentine soils in several locations in Oregon, California, and smelter-contaminated soils near Port Colborne, Ontario. Especially cold tolerant germplasm was used in the Canadian production location with tillage adapted to the poorly drained soils by ridge-tilling the field to provide adequate drainage. A recurrent-selection breeding program has been conducted for about five years with clear improvement in the plant for this use.

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RESEARCH AND COMMERCIALIZATION OF *Alyssum murale* AND *Alyssum corsicum* AS POTENTIAL NEW NICKEL PHYTOMINING CROPS

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Nickel is used in large quantities for production of stainless steel, rechargeable batteries, etc. The U.S. currently imports all the Ni it uses. Traditional strip mining is very costly and can be environmentally damaging. However, certain rare plants, called hyperaccumulators, accumulate more than 1% Ni dry weight in their aboveground biomass, and thus have the potential to phytoextract Ni from enriched soil, avoiding the cost and problems of traditional mining methods. Alyssum murale and A. corsicum were selected by the USDA and University of Maryland researchers as potential temperate zone Ni phytoextraction crops due to their significant biomass production and Ni uptake as observed in the wild. Because little is known about the crop production requirements of these wild species, a series of agronomic and genetic improvement studies were done in southwestern Oregon in the largest area of Ni-rich serpentine soils in the U.S.

Seed from wild collections made in southern Europe was used in these studies, conducted near Cave Junction, OR on a classic serpentine soil containing more than 4000 mg Ni/kg. The area was prepared using standard farming and plot research equipment appropriate to each experiment. Herbicides used for canola controlled weeds in Alyssum. Crop biomass was harvested by hand and weighed, and plant Ni content was measured using ICP-atomic emission spectroscopy..

In fertilizer tests, only small amounts of added P were required to obtain full yields. Plant density and biomass yield were improved with added gypsum, but not with lime. Plant density was significantly reduced when the soil was acidified. In the planting date tests, the yield from a spring planting date harvested one year later was just as good or better than yields from fall plantings made 1.5 years before harvest, regardless of whether the fall plantings were also harvested on one intermediate harvest date or not. Planting in April, May, or June did not have a significant effect on yield when harvested the following June. Seedbed preparation tests showed that emergence and density tended to be better when seeds were planted on the surface after minimal disturbance compared with those planted at 6 mm depth following more extensive preparation with a rotary tiller. Tests in rocky soil showed that Alyssum germinates readily given minimal soil disturbance. Although early growth is favored by some irrigation, plants that germinate in the spring can persist through the summer and the following winter without irrigation, although the resulting yield will be less than those that have at least some irrigation through the first summer. Fall seeding will result in a greater number of plants that persist through the following summer if no irrigation is available.

Alyssum tends to concentrate the stored Ni more in the leaves than stems. Leaves senesce and rapidly fall off the plant as it moves into its flowering phase, so harvest is done at early flowering. Plants that germinate in the late fall are less likely to be vernalized than those planted earlier, and thus will persist in the vegetative state longer the following summer, resulting in improved biomass and Ni yield the first year, although flowering behavior and biomass yield will be the same in subsequent summers. Ni uptake was not affected by any of the planting date treatments as long as harvest was made before flowering occurred. A plant breeding program was begun starting with more than 160 wild accessions using traditional cross pollination and recurrent selection methods. This has resulted in genotypes with a wide variety of growth habits and Ni uptake rates. Subsequent rounds of selection are ongoing.

Based on limited agronomic tests, the first commercial fields of Alyssum have been planted in Josephine Co., Oregon. Although early results have been encouraging, many factors are not well understood, making it difficult to consistently and confidently produce predictable quantities of seed and biomass for a given situation.

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SUN GRANT INITIATIVE: STATUS REPORT

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Many people have recognized that agriculture has an important role to play in solving some of the nation's projected energy problems. A concept, named the Sun Grant Initiative, has been developed to broaden the role that land grant universities play in their unique approach to higher education by implementation of a new program that will focus the efforts of the land grant universities on renewable energy and biobased industries. A network of five land-grant universities serving as regional Sun Grant centers has been proposed. The universities include South Dakota State University, Oklahoma State University, the University of Tennessee - Knoxville, Cornell University, and Oregon State University. The regional centers will emphasize integrated research, extension, and educational programs on renewable energy and biobased industries based in rural communities. Each center will receive base federal funding to solidly establish them as leading research, extension, and higher education institutions for the biobased economy. In addition to the five centers, significant resources and expertise exists at other land grant institutions throughout the nation. The Sun Grant centers will engage with them as a synergistic mechanism for technology transfer and higher education for the benefit of a rural biobased economy. These programs will embrace the multistate, multifunction, multidisciplinary integrated approach that is at the heart of the land-grant method of addressing problems. Moreover, the centers will interface their activities with DOE research laboratories. The

mission of the Sun Grant Initiative will be to (1) enhance national energy security through development, distribution and implementation of biobased energy technologies, (2) promote diversification and environmental sustainability of America's agriculture through land grant-based research, Extension, and education programs in renewable energy and biobased products, and (3) promote opportunities for biobased economic diversification in rural communities.

The Western region held two planning meetings in July and November of 2002. A list of "principles to follow" was established for the Western Center that includes an inventory of regional biobased activity, and a list of planned goals, objectives criteria, and priorities. Through these meetings, we learned that the Western region has many similarities than differences when compared with the other regions of the U.S. This Western region already has established research efforts in the utilization/engineering of biobased products, plant genetics, and biotechnology programs for the production and development of new biobased feedstock materials, and small-scale energy systems consistent with the needs of isolated and remotely located communities. Planning activities for 2003 to 2004 will continue to refine this model. The activities will include developing a road map and establishing administrative, technical, and stakeholder committees. Oregon State University will begin routine communications within the region about the status of the Sun Grant Initiative and relevant activities that occur throughout the region.

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GROWING YOUR OWN FUEL

Alan W. Teel[1] and Keith Booth[2]

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The cost of hydrocarbon-based energy in rural areas to heat homes, shops, and other work areas as well as to dry grain continues to escalate. People living in rural areas have sufficient space (acres) to raise enough fuel to supply their energy needs for heating and grain drying. This capability has been in place for a number of years. What has been missing is the right technology to burn the biomass as well as applying the correct manufacturing process to produce an acceptable fuel source in the right form.

The objective of this project is to bring together the correct burn technologies and a manufacturing process that will allow people in rural areas to grow their own fuel for creating heat energy as well as electrical power. This project will be designed to create a model that may be used in any rural area of our country

Our fuel source will be Switchgrass (*Panicum vergatum*), a native warm season grass that grows well in North America from Canada to Texas and from Nebraska to the Atlantic Ocean. We will pelletize the Switchgrass using low-cost technology to size the material and a standard, but small, pellet mill setup. This facility will serve a 4 to 6 county area and will have a low capital cost. Eventually, a Coop will be formed to own the facility. Three burn technologies will be demonstrated. A forced air unit that can be attached to a standard forced air home heating system, a hot water boiler system that can be used for a hot water system, and a gasifier that can produce hot gases which in turn can be burned for heat generation or combusted in an internal combustion engine attached to a generator to produce electricity.

The various parts of this project have been demonstrated over the past few months. We have manufactured the pellets using known technology in a different way and reducing the capital cost significantly. We have burned our pellets in the three burn technologies described with great success. What remains, is to bring all the parts together in a structure that will be acceptable to people living in a rural area. It is estimated that one acre of Switchgrass will produce enough BTUs to heat a normal-size rural home in the Midwest. This can be accomplished with considerable cost savings while utilizing a completely renewable fuel source. In the process, we will create a new rural enterprise.

The successful completion of this project will be a step forward in reducing fuel cost in rural America and demonstrating how rural communities can be active participants in the goal of becoming less dependant on foreign energy and the use of an environmentally friendly and renewable fuel source.

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SINGLE-PASS HARVESTING OF CORN FOR GRAIN AND THE CLEAN STOVER FOR INDUSTRIAL PURPOSES

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The dispersed, diverse, and low density nature of biomass feedstock is a major constraint on the wider commercial utilization of biomass. For low specific costs, improved or alternative methods are needed to collect, handle, and store biomass such as corn stover. So far, biomass collection has depended on baling, initially with small rectangular balers, then with large round balers. But round-baling of corn stover is a two- or three-pass operation. Also, balers pick up dirt and most of the cobs are left in the field. There is a strict limit in stover moisture if bales - whether round or square - are to be stored.

The objectives of this program at Iowa State University were to design, develop, and evaluate single-pass harvesting systems capable of simultaneously collecting the grain along with clean stover and facilitate densification of the stover to reduce transportation costs.

Several prototype machine systems were developed and evaluated that addressed what was perceived as the most likely clientele for such harvesting approaches. They were successfully operated at several sites for Iowa corn crops and preliminary economic assessments were made.

Stover cost with the single-pass harvesting of the whole plant in two streams was well below $10 per ton, whereas the method used to date to collect stover costs from $12 up to $30 per ton. The stover was free of any dirt picked up from the ground. Harvesting of corn for grain need not be significantly delayed, provided that suitable stover haul-out equipment is available. The equipment can be adjusted to leave behind sufficient residue in accord with local soil conservation requirements.

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PRODUCTION VARIABLES AFFECTING POD AND BIOMASS DEVELOPMENT IN COMMON MILKWEED

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With the reduced profitability of corn and soybeans, growers are looking for ways to supplement their businesses. An attractive option for these growers involves the introduction of new crops into their farming practices. One crop of particular interest is common milkweed (Asclepias syriaca) for the production of industrial fibers, oils, latex, and a potent nematicide.

The primary objective of this study was to examine numerous production variables affecting pod and biomass development in common milkweed production fields. Experiments from 2001 addressed the effects of intra-row plant spacing on pod formation and the effects of coal dust on plant establishment. The 2002 studies focused on planting times, inter-row spacing, and nitrogen requirements.

Milkweed transplants were planted in replicated 2.5 m by 2.5 m plots in late May in 2001 and 2002. Information on pod and stalk biomass was collected on individual plants after two and three years of growth.

Milkweed growth indicated pod weight and total number of pods per plant increased with row spacing. The dry stalk biomass per plant also increased with row spacing. However, the total dry biomass per hectare decreased with wider row spacing. Nitrogen and coal applications do not appear to have any effect on either pod or stalk biomass. First year transplants produced pods late in the season. However, they were not of sufficient quality or maturity for either floss or seed material.

Milkweed being a perennial crop with several market opportunities offers a tremendous advantage for farmers looking toward expanding their production with minimal costs.

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FORMALDEHYDE-FREE SOYBEAN PROTEIN-BASED ADHESIVES FOR WOOD COMPOSITES

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In recent years, wood composites have grown in popularity due to limited lumber resources. Fiberboard and particleboard are increasingly used in

the manufacture of furniture and cabinets as a more economical alternative to natural wood. Current industrial composite manufacturing methods employ formaldehyde-based resins such as urea-formaldehyde (UF) as the adhesive. Formaldehyde emissions from such materials are a health concern, as formaldehyde is a probable human carcinogen. Moreover, formaldehyde-based resins are derived from petrochemicals, a non-renewable resource. Increasingly stringent environmental regulations and the growing awareness of "green chemistry" have caused researchers to reconsider natural resources for composites. Furthermore, utilization of soybeans and soybean byproducts is a growing area of interest.

The objective of this study was to prepare wood composites using formaldehyde-free soybean protein-based adhesives that demonstrate performance properties comparable with commercial particleboards manufactured with UF resin.

Adhesives were made from soy protein isolate (SPI), defatted soy flour (DSF), and SPI/DSF blends. The composition of DSF is approximately 53% protein, 30% carbohydrates, 18% fiber, and 9% moisture. Further purification of soybean meal results in SPI that consists of more than 90% soybean protein and 6% moisture. The adhesive and wood furnish were blended and then heated in an oven at 50°C to remove excess moisture. The mixture was compressed at 160°C and 1.65 to 1.79 MPa (240-260 psi) for 5 min. After cure, modulus of rupture, modulus of elasticity, internal bond, and face pull of the particleboards were determined. Moisture content and water absorption tests were also conducted. All tests were performed according to ASTM D 1037-96a and the results were compared with ANSI A208.1-1999.

The mechanical properties of SPI boards exceeded ANSI standards by 15% and were equal to or exceeded the properties of control boards manufactured with UF resin. The mechanical properties of DSF and SPI/DSF blend boards exceeded ANSI standards with the exception of modulus of rupture. It is notable that soybean protein boards have much higher modulus of elasticity than the UF control boards. SPI and SPI/DSF boards exhibited decreased water resistance compared with UF control boards. DSF boards, however, had outstanding water resistance.

The Thames Research Group has demonstrated that soybean protein-based adhesives can be used to make wood composites. The adhesives were made almost entirely from natural products and are completely formaldehyde-free. The particleboard properties compared favorably with that of commercially available products made from UF resin. The development of soybean protein-based adhesive and utilization on a commercial scale will not only reduce formaldehyde emissions, but also benefit soybean farmers in the United States.

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Top of Page

Return

MEADOWFOAM

MEADOWFOAM SUCCESS AND RESEARCH IN 2003

Jerry Hatteberg

OMG Meadowfoam Oil Seed Growers Cooperative, Inc. & Natural Plant Products, Inc., 2767 19th Street SE, Salem, OR 97302, USA

Meadowfoam enjoyed another financially successful business year filled with new opportunities. The 9+ % growth in cosmetic oil sales provided revenues to eliminate all loans and retire the largest grower payment pool six months ahead of schedule. This trend is expected to continue in 2004. Current oil research should provide several new meadowfoam seed oil products to the worldwide cosmetic market in the near future. Distributors universally are confirming new products and the reformulation of existing products using meadowfoam seed oil (MFSO).

Meadowfoam research efforts have seen many positive results in a wide range of areas:

Biobased hydraulic fluid has been successfully formulated and test results confirmed.

Biobased lubricant products containing MFSO are currently being formulated.

Pharmaceutical uses of MFSO are currently in the research stage.

Pesticide and herbicide research is ongoing with both MFSO and meal.

Meadowfoam meal sales in nursery market remain slow, but use in organic production areas is showing increasing interest.

Researchers continue to explore the growth enhancement potential of MF meal.

Current estimates indicate demand for new meadowfoam seed production will be required in 2004 as oil inventory declines. Future outlooks for establishing meadowfoam as stable crop are beginning to take shape.

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MEADOWFOAM SEEDMEAL INHIBITION OF SOILBORNE PATHOGENIC AND MYCORRHIZAL FUNGI

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Meadowfoam (*Limnanthes alba*) is grown in Oregon for its high quality seed oil. Meadowfoam seedmeal (MSM) remaining after oil extraction contains chemicals, such as glucosinolates, that may degrade to release byproducts inhibitory to weeds, insects, and soilborne pathogens. MSM in soil also has been shown to stimulate plant growth.

The objectives of our studies were (1) to determine if MSM, alone or in combination with the oil, would have any effect on pathogenic soilborne fungi in vitro, and (2) to determine if there were any adverse effects on non-target beneficial arbuscular mycorrhizal (AM) fungi in soil.

Combinations of meadowfoam oil and MSM, or MSM alone, were incorporated into agar media in Petri plates, and the pathogens inoculated to the agar surface. Colony diameter after 72 h was compared with the non-amended control plates. In tests for volatile effects, MSM alone or incorporated into soil (4% by volume) was placed on one side of the divided Petri plates and the pathogen inoculated on the opposite side. Colony diameter and sporulation of test pathogens were measured after several days of incubation. For tests on the effects on mycorrhiza formation, MSM was amended at three rates into an organic soil mix, into which marigold or onion plants, inoculated or not with the AM fungus Glomus intraradices, were planted and grown in the greenhouse for 10 weeks before assessing plant biomass and level of AM colonization.

Mycelial growth of most fungal pathogens was inhibited by combinations of oil and MSM, as was sporulation by some *Pythium* and *Phytophthora* species. Amendment of soil with MSM enhanced growth of marigolds at 0.594 or 1.188 kg m^{-3}, but inhibited growth at 1.782 kg m^{-3}. AM formation on marigold or onion was inhibited at all rates tested, but additional tests indicated that the AM fungal inoculum was inhibited, not killed. Ground MSM alone incorporated into agar did not inhibit pathogen mycelial growth, but did inhibit production of oospores by *Pythium irregulare* and *sporangia*, but not chlamydospores of *Phytophthora ramorum*. In vitro tests for volatile effects with MSM alone or mixed into soil showed that the volatiles released from the MSM inhibited sporulation of *P. ramorum*.

These results indicate that the volatile or non-volatile compounds released from MSM could inhibit soilborne fungal pathogens, at least in vitro, but tests on plants in MSM-amended soils are needed to determine whether MSM can suppress the diseases they cause. The adverse effects of MSM on non-target mycorrhizae and the mode of action against AM fungi in soil remain problematic. Nonetheless, the potential for MSM amendment to soil or potting media for disease control deserves further research.

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IMPROVED BIO-BASED HYDRAULIC FLUID USING MEADOWFOAM OIL

B.N. Rhodes

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This presentation will review the evaluation of vegetable-oil-based hydraulic fluid containing Meadowfoam oil. The improvements in performance properties over conventional hydraulic fluids due to the presence of the Meadowfoam oil will be highlighted. These improvements occur in the high- and low-temperature properties of the hydraulic fluid. The properties of a demonstration formula will be compared with the specifications of the U.S. Navy for bio-based hydraulic fluid.

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Top of Page

Return

OILSEEDS

MORPHO-PHYSIOLOGICAL DETERMINANTS OF SEED-YIELD IN ANNUAL AND PERENNIAL SPECIES OF LESQUERELLA

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Although *L. fendleri* has been chosen as the prime candidate for domestication in fall-sown crops in the southwestern U.S., the best alternative for cool, Mediterranean climates is still not clear. In the process of selecting our best cropping system, we have taken an approach that includes the understanding of eco-physiological responses associated with growth and development of the different candidate species and planting dates. Our objective was to evaluate seed-yield for five species of Lesquerella in fall and spring sowings and to characterize the relationships between seed-yield and phenology, biomass accumulation and partition.

Three plots each of the *L. fendleri*, *L. angustifolia*, *L. gracilis* (annuals), and *L. pinetorum* and *L. mendocina* (perennials) were established by direct seeding in March (fall) and September (spring) in Patagonia (43° 16" S, 65° 21" W) in a completely randomized design. Plant phenology was recorded, and growth and partition were measured harvesting whole plants when fruits were ripen, which occurred at different times during the summer.

Average yield per plant was strongly influenced by species and planting date ($P < 0.05$). Yields were (fall and spring sown, respectively): 1.58 g/pl and 0.33 g/pl for *L. angustifolia*, 1.02 g/pl and 1.74 g/pl for *L. pinetorum*, 0.99 g/pl and 0.60 g/pl for *L. gracilis*, 0.63 g/pl and 0.61 g/pl for *L. fendleri*, and 0.29 g/pl and 1.24g/pl for *L. mendocina*. *L. angustifolia* and *L. gracilis* accumulated significantly more total biomass in fall than in spring sowings ($P < 0.05$), whereas the harvest index did not change. The same pattern of biomass accumulation was found for *L. fendleri* although seed-yield did not drop on spring planting dates, due to a significant increase in harvest index (15.5% and 18.4% for fall and spring dates, respectively with $P < 0.05$). Both perennial species did not flower the first summer when sown in spring, and completed their first growth cycle one whole year after the annual species. This pattern resulted in more total biomass for spring planting dates ($P < 0.05$). In contrast for *L. pinetorum*, the partition to roots increased from 2.5% (fall) to 8% (spring) and the harvest index remained unchanged (ca. 20%, for both planting dates), both partition to roots (10% vs. 13%) and harvest index (7% vs. 15%; $P < 0.05$) increased in *L. mendocina* when planted in the spring. For all species tested, there was a significant correlation between the length of the growth cycle and total biomass, probably related to more total accumulated radiation.

This experiment confirms previous results and supports the idea of developing *L. angustifolia* for fall plants as an alternate hydroxy-fatty acid crop for Patagonia and similar cool, Mediterranean climates. On the other hand, *L. pinetorum* and *L. mendocina* combine adequate seed yields and perennial growth habit, an important characteristic in erosion-prone environments and for crops such as *Lesquerella*, which have very low initial growth rates and poor competitive ability against weeds.

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ANNUAL AND PERENNIAL Lesquerella SUBJECTED TO WATER STRESS DURING SEED FILLING

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Lesquerella fendleri is an annual oilseed crop under the process of domestication for irrigated arid lands. *L. mendocina* is a perennial species native from Argentina. Some of the anatomical, physiological, and developmental traits present in *L. mendocina* and related to the perennial habit could

be exploited in breeding programs oriented to nonirrigated systems, with frequent water stress. The objective of this work was to analyze the impact of water stress during seed-filling for yields of both *L. fendleri* and *L. mendocina*.

A field experiment was conducted in Buenos Aires, Argentina. Plants were sown in late autumn of 2001 in 2.0 m x 1.5 m plots under a rain shelter. Crop density was 30 plants/m^2, 0.2 m between plants, spaced 0.3 m apart. Water-stress conditions were set by withholding water from visible floral-bud stage during 75 days (this is ca. 40 days before harvesting). Yields of stressed plants were compared with those of plants subjected to irrigation during the overall cycle.

Similar yields were found for both species under high water availability conditions, reaching 1g $plant^{-1}$ (average of both species). Water stress drastically reduced yield up to 80% respect to that of irrigated plants in L. mendocina, whereas no reductions attributed to water stress were observed in L. fendleri. Differences between species responses were positively associated with leaf area compensations after the end of water stress period until maturity.

The results clearly indicate that the annual *L. fendleri* is capable of great yield-compensation after being released from a severe water stress. Although similar yields were found in both *L. fendleri* and *L. mendocina* under irrigation, yield stability would be greater in the former. Hence, promising yields would be expected in *L. fendleri* grown under nonirrigated systems when early crop establishment is assured.

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MEASUREMENTS OF LESQUERELLA INTERSPECIFIC HYBRIDS AND PARENTS

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Hydroxy fatty acids (HFA) are the major components of the seed oil profile of *Lesquerella* species. One of three different HFA predominates in the seed oils of species of plants from this genus. Lesquerolic acid (C20:1OH) is the primary HFA found in species from the western and southwestern U.S. The range of quantity varies depending on the species. *Lesquerella fendleri* is being developed for commercialization because of its productivity and adaptability to farm management practices. However, the range of natural variability for this trait is limited in this species. Lesquerolic acid accounts for only around 60% of the total fatty acids compared with other species that have up to 85% of this acid, but they lack other important yield-related traits.

Our objective has been to introgress the trait for high HFA content into *L. fendleri* from other species. We measured various characters to follow the pattern of inheritance between crosses. *L. pallida* and *L. lindheimeri* both have high HFA amounts and were used along with *L. fendleri*, crossed among each other and also self-pollinated. It was necessary to double the chromosome number of plants using colchicine to overcome sterility and obtain seed from hybrids. Ovule culture was used in cases when seed did not develop to produce plants of the next generation. The traits measured were petal length, number of ovules per silique, number of seeds per silique, and weight of 1000 seeds. Fatty acid compositions were also measured, and flower color and fertility were scored. Parent plants were diploids, n = 6, and hybrids were amphidiploids, n = 4x = 24.

L. pallida has a white flower color and the other two species have yellow flowers. Petal length was smaller on *L. pallida* and *L. lindheimeri* than *L. fendleri*. The 1000 seed weights and the number of ovules per silique were different among all three species. *L. lindheimeri* had the highest seed weight and lowest ovules per silique. *L. fendleri* had the lowest seed weight and highest number of seeds per silique. Seeds per silique counts indicated that autofertility occurred in *L. pallida* but not in the other two species. HFA oil content of L. fendleri seeds was 50.5% compared with 84.1% for *L. lindheimeri*. F1 hybrids indicated maternal influence over flower color and petal length when reciprocal crosses were examined. White flowers were expressed on hybrids when the female parent had yellow flowers (*L. fendleri*). Petals were smaller in this case than the reciprocal cross but still intermediate of both parents. Pale yellow flowers were expressed and petals were longer than both parents when the female parent had white flowers (*L. pallida*).

These measurements will help predict the value of the different types of interspecific crosses for breeding. Segregation for various yield-related traits should allow selection for favorable improvements in the HFA trait and seed yield.

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CROSS-SPECIES AMPLIFICATION OF SIMPLE SEQUENCE REPEATS (SSRs) BETWEEN *Arabidopsis thaliana* L., *Brassica* SPECIES, and *Lesquerella* AND *Physaria* (BRASSICACEAE)

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Lesquerella and *Physaria* species (Brassicaceae) are sources for three types of hydroxy fatty acids (HFA), auricolic, densipolic, and lesquerolic, with potential for industrial applications including lubricants, novel plastics, protective coatings, cosmetics, surfactants, drying agents, and pharmaceuticals. Although identified as possible new crops in the 1950s, a breeding and selection program has been developed for them only within the last 15 years. While much progress has been made within this time using traditional selection methods for increased seed size, yield, oil content, oil composition, and improved agronomic characteristics, DNA-based markers should accelerate the selection time frame and should be useful in precisely identifying trait loci.

Many DNA-based markers are used for crop improvement. However, simple-sequence repeat (SSR) loci, or microsatellites are becoming the molecular marker of choice in marker-assisted plant breeding and marker-based genetic analysis because they are easy to use, abundant, dispersed throughout the genome, usually co-dominant, and generally more polymorphic than other genetic markers. However, developing these markers de novo is costly and time-consuming. Several studies have shown the ability of SSRs developed for one species to be amplified in related species or genera, thus saving time and money. Hundreds of SSR primers have been developed for related species in Brassicaceae, particularly from *Arabidopsis thaliana* L. and *Brassica* crop species, so that a great potential exists to use these SSR primers in our breeding program with Lesquerella and Physaria.

The goal of this study was to test 24 SSR primer pairs developed for *A. thaliana*, *Brassica napa* L., and *B. rap*a L. for their transferability to loci in *Lesquerella* and *Physaria*, with the long term aim of using these SSR loci to construct a genetic linkage map for the several species of *Lesquerella* and *Physaria*.

Sixteen primer pairs developed for *A. thaliana* that cross amplified with *Brassica* spp. These were shown to contain micro satellite repeat regions. Eight additional primer pairs for *Brassica napa* and *B. rapa* (4 from each species) were chosen for this study. Preliminary results indicate that a majority of the primer pairs amplify fragments in both *Lesquerella* and *Physaria*. DNA sequencing and probe hybridization assays are underway to determine whether the loci contain SSRs.

These SSR markers are the first DNA markers to be developed in our breeding program and the first step toward developing genetic maps for *Lesquerella* and *Physaria*. Once constructed, the genetic map will allow us to investigate genes influencing important traits and their location along the chromosomes. Also, it will help us implement marker-assisted selection for improvement of specific phenotypes.

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GRAIN YIELD, AGRONOMIC RESPONSE AND FATTY ACID COMPOSITION OF CANADIAN CANOLA VARIETIES IN SOUTHERN SONORA, MEXICO

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México imports four million tons of oilseed per year, The most important crops are soybean, canola, sunflower, and cotton. Until 2002, the best variety of canola (Brassica sp,) for southern Sonora, México, has been Hyola 401 from Advanta Seeds of Canada. Its highest grain yield has been 3610 kg/ha under commercial conditions. The Asociación Nacional de Industriales de Aceites y Mantecas Comestibles,A.C?.-ANIAME of Mexico supports canola grown in central Mexico, utilizing Hyola 401 Variety. Nonetheless, it is important for producers to obtain other varieties with better yield and quality grain than Hyola 401 in order to increase the commercial area. The objectives of this study were to evaluate yield, agronomic characteristics, and oil quality of InterMountain-Cargill canola varieties grown in southern Sonora, México.

The cultivar evaluation trial was conducted in the Experimental Field of the Instituto Tecnológico Agropecuario No. 21 (Ita 21) during 2002 and 2003 growing season. The experimental design was a randomized complete blocks with four replications. Plots of four rows 8 m wide and 5 m long. Treatments consisted of six InterMountain-Cargill Company canola varieties from East Canada (IMC 104, IMC 105, IMC 108, IMC 204,

IMC 205, and IMC 207); two genotypes of Advanta Seeds from Canada (Hyola 60 and Hyola 401 Local Check); and four open pollinator varieties from Australia (Monty, Karoo, Eureka and Charlton). Hand planting was made on December 16, 2002 in a clay soil with irrigation. Harvest was made on April-May, 2002. Test weight, vigour, plant density, days to beginning bloom, plant height, days to physiological maturity, lodging, specific grain weight were measured. Chemical analyses were made on the grain.

There were highly significant yield differences among the cultivars with 6.1 % of Variation Coefficient. The best genotype was Hyola 401 (3040 kg/ha) according to Tukey.05 test. However, IMC 204 (2270 kg/ha), and IMC 205 (2268 kg/ha) had high chemical quality. Oil contents were from 29.9% (IMC 104) to 39.8% (IMC 108), and protein contents from 20.6% (IMC 104) to 25.4 %(Karoo). The fatty acid composition of the oil is genetically more variable than any other major vegetable oil. However, a high content of oleic acid was present in IMC 204 (76.1%) and IMC 205 (77.3%), whereas the local check Hyola 401 had an average of 66.7%.

Experimental results indicated that InterMountain-Cargill ?IMC- canola varieties can be grown in Yaqui and Mayo Valleys of southern Sonora, México, in order to the high quality and oil content. However, it is necessary to establish an appropriate commercialization system in order to increase the cultivated area.

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VEGETABLE OIL MACROMONOMERS AS COPOLYMERIZABLE HYDROPHOBES IN MINI-EMULSION POLYMERIZATION

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Vegetable Oil Macromonomers (VOMMs) have been shown to be an attractive intermediate for volatile organic compound (VOC) reduction in architectural coatings. VOMMs are long-chain acrylated triglyceride derivatives used as co-monomers in latex formulations to lower the polymer glass transition temperature (T_g). The main advantage of the VOMM technology is the temporary plasticization effect of the fatty acid chains with the potential for crosslinking after the film is formed. However, VOMM incorporation in conventional emulsions is challenging because its hydrophobicity restricts its diffusion through the aqueous phase. While special techniques have been developed to incorporate VOMMs successfully in conventional emulsion polymerization, incorporating large amounts can be difficult.

Mini-emulsion polymerization has been widely used to incorporate highly hydrophobic monomers. Diffusion limitations are avoided by polymerizing inside the monomer droplets, and to ensure this, droplet stabilization is required. Hydrophobic molecules (hydrophobes), oligomers, or polymers are commonly used to stabilize monomer droplets. However, because they are not covalently bonded to the polymer, they are prone to leach from the coating or phase separate.

VOMMs were evaluated as hydrophobes in mini-emulsion polymerization. Unlike other hydrophobes, the acrylate functionality allows them to bond covalently to the polymer and ideally crosslink upon film formation. Mini-emulsion polymerization of BA/MMA was performed using a soybean oil-based VOMM as a copolymerizable hydrophobe. Monomer droplets were properly stabilized prior to polymerization, and a coagulum-free mini-emulsion was obtained. Gel content studies indicated that a crosslinked network was formed upon drying of the film. This method permitted 35% VOMM to be incorporated into the polymer backbone.

Mini-emulsion polymerization of VOMMs permits the production of waterborne crosslinkable polymers. Unlike other mini-emulsions that crosslink by in situ grafting, the crosslinking in VOMM mini-emulsions mechanism takes place upon film formation, and enables good flow and leveling. The incorporation of higher levels of VOMM will facilitate increased commercialization of alternative crops in coatings and replacement of non-renewable resources.

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EXPRESSION OF INDEHISCENCE AND CAPSULE ANATOMY IN VERNOLA

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Euphorbia lagascae L. is a new industrial crop with a seed oil content ranging from 49 to 52% and vernolic acid comprising between 58% and 67% of total oil content. The complete dehiscence of capsules of the wild E. lagascae types was overcome by developing indehiscent genotypes (cultivar vernola). In the early field experiments with vernola, it was observed that environment influenced the expression of the character.

The objectives of this work were to study: 1) the degree of indehiscence of vernola grown in different seasons in southern Europe, and 2) the histological basis of partial dehiscence in capsules.

Field experiments were established in the spring and autumn of 2001 through 2002 in Murcia (Spain) and Osimo (Italy) to observe the influence of relative humidity and temperature during capsule development in the expression of the character under study. Besides, indehiscent and dehiscent capsules were sampled and conditioned by historesin inclusion techniques, then longitudinal and transversal cuts were made to identify the presence and thickness of the carpele wall layers.

A combination of higher environmental moisture and lower temperatures during capsule development is negative for indehiscence expression. For example, a decrease from 87% to 68% retained capsules per plant was obtained in Spain. Also, the autumn sowing (37% indehiscent capsules per plant) was not favourable compared with the spring sowing (77.4%).

In Italy, when plants were sown in the spring, they fall within the 70 to 80% interval of retained capsules, whereas when sown in the autumn, the retention was more frequently found in the 50 to 70% indehiscence range.

Partial dehiscence occurs from the bottom and along the dorsal suture in the carpeles and is explained by the anatomical features, namely, the mesocarp remains at the carpele dehisces in transversal cuts than the thicker layer in the upper part in longitudinal cuts.

The nonseed shattering nature of vernola represents a great advantage for E. lagascae cultivation although the genotype x environment influence in the expression of the character has to be taken into account for agricultural production.

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SUPPLEMENTAL POLLINATION OF JOJOBA

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

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

Supplemental pollination in olives and other tree crops is becoming more and more common, and has been shown to increase yields. To determine whether such a practice would also prove beneficial in jojoba, a project was initiated in which pollen was harvested from male plants and then applied to female plants. Due to seasonal differences between the northern and southern hemispheres, pollen was initially collected in Argentina in South America and then applied in Arizona in North America.

Equipment to collect and apply the pollen was fabricated and both pieces performed well. Low viability of the pollen collected limited the size of the test plots. However, the first year's results show that application of supplemental pollen can increase yields. More extensive trials will be undertaken during the second year of the project with plans calling for the application of pollen in semi-commercially sized fields in both Argentina and Arizona. Additionally, to improve the efficiency of future pollen harvests, male clones with improved pollen production characteristics are being selected from existing plantings.

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EVALUATION OF CONDITIONING AND SCREW PRESSING PARAMETERS OF LESQUERELLA OILSEEDS

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Lesquerella fendleri, a winter annual oilseed crop native to the desert southwestern United States, is currently being developed for commercial industrial applications. The seed contains 25 to 30% oil, of which 55 to 60% is lesquerolic acid, a hydroxy fatty acid (HFA) analogous to recinoleic acid from castor oil. The current source of HFA is recinoleic acid, which is used in the manufacture of lubricants, plastics, coatings and cosmetics, and imported primarily from Brazil and India. The seed coat contains a unique gum, which will be potentially useful as coating, binder and thickener.

Mechanical extraction of oil followed by solvent extraction of the press cake will provide the solvent-free oil preferred by the cosmetic industry and the solvent-extracted oil for other applications. Large quantities of Lesquerella seeds are now available for pilot-scale processing and product development and testing. We are currently evaluating seed conditioning and screw press parameters to determine the conditions for optimum oil yield and quality. The effects of the seed moisture content, heating/cooking temperature, heating/cooking time, pressing temperature, pressing rate and press discharge back pressure on oil yield and quality screw press efficiency are being investigated and will be presented.

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Top of Page

Return

POSTERS

GENERAL CROPS

EFFECT OF FERTILIZATION ON OIL PRODUCTION OF *Agathosma betulina*

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Buchu (*Agathosma* spp., family Rutaceae) is an indigenous, odoriferous shrub found mainly in the fynbos biome of the southwestern Cape. The valuable essential oil synthesized in the leaves of A. betulina is not only used as a food flavor enhancer, but also in cosmetic products, perfumes, aromatic oils, and traditional medicine. The high market demand for buchu has resulted in the over exploitation of buchu in the wild. The sustainable cultivation of buchu is definitely needed to secure the future of this valuable crop, but very little is known about its cultivation requirements, including fertilization.

The objective of this study was not only to determine the influence of fertilization on the vegetative growth of the plants, but also the influence on the quality and quantity of the oil yield.

A randomized block design, with four replications, was used to evaluate the influence of a chemical vs. an organic fertilizer on three-year-old plants, in the presence or absence of straw mulching. The fertilizers had a N:P:K ratio of 3:1:5. After seven months, the treatments were evaluated from the fresh weight of the vegetative growth, the oil quantity and its quality.

Chemical fertilizer had a major influence on vegetative plant growth, resulting in a significantly higher vegetative yield. Results using organic fertilizer, organic fertilizer and mulch, and mulch alone did not differ significantly from the control. Of significance was that the amount of oil extracted through steam distillation did not differ significantly between treatments. Chromatographs did not indicate a significant difference in oil composition and quality between the treatments either.

It can be concluded that it is possible to fertilize A. betulina with a general 3:1:5 fertilizer to increase the vegetative yield of the plants, and still

maintain the required oil yield and quality.

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DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI ASSOCIATED WITH *Agathosma betulina* (BUCHU)

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A group of soil fungi, of which the biotechnological potential is widely recognized, is the arbuscular mycorrhizal (AM) fungi. AM fungi are known to form mutualistic symbiosis with the roots of herbaceous plants. The significance of this type of symbiotic fungal infection for plant nutrition makes it one of the potentially more efficient biological means of assuring crop profitability with a minimum input of chemicals such as fertilizers.

The objective of this preliminary study was to estimate diversity and relative abundance of AM fungi associated with Agathosma betulina (buchu) as part of a project aimed at developing this plant as a commercial crop. A. betulina is a valuable indigenous shrub found in the fynbos biome of the southwestern Cape.

Plant roots and rhizosphere soils were collected at a pristine fynbos site in the Western Cape. AM fungal spores in rhizosphere soil samples were extracted by means of wet sieving and decanting in combination with sucrose density gradient centrifugation. The spores were subsequently enumerated using dissecting microscopy and stained with Melzer's reagent. Clearing and staining of Agathosma root samples with aniline blue confirmed that AM colonization of Agathosma roots did occur in fynbos. Additionally, molecular techniques were used to complement identification procedures based on morphology. DNA was extracted from Agathosma root samples and subjected to nested PCR with universal fungal primers (NS5, ITS1, ITS4) and Glomalean specific primers (GLOM1310, ACAU1660, GIGA5.8R).

AM spore numbers ranged from 1000 to 4000 spores/50 g rhizosphere soil and according to spore wall characteristics and spore colour, as obtained by staining with Melzer's reagent, the most prevalent genera were Glomus and Acaulospora. Fungal structures characteristic of Glomus and Acaulospora were also observed in the roots of Agathosma. The percentage AM infection ranged from 10 to 60%. Quantification of nested PCR products revealed multiple bands obtained with the primer pair NS5 and GIGA5.8R. These products will be cloned for sequence analysis.

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SAPONINS CHANGES IN STEMS AND LEAVES OF QUINOA PLANTS SOWN AT DIFFERENT DATES

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Quinoa (*Chenopodium quinoa* Willd) is a pseudocereal that is tolerant to drought and frost that has potential use as a forage crop because the grain, stems, and leaves have high protein content. However, the saponins level in the grain has prevented its use due to its bitterness and potential toxicity when ingested in large quantity. The limited reports on saponin content in the plant do not provide information about or composition changes during the crop development.

In this work, the determination of saponin content and composition was carried out in plants sown at different dates.

Two quinoa varieties (Sajama and Chucara) were sown in the University experimental field during 2001, on 13 March, 11 June, and 03 September. The experiment was established under a split plot design with four replications for the March and June dates. The main plot included two soil water deficits (SWD), D1 and D2. For D1 SWD, water was applied when the soil reached 50 to 60% of soil water depletion, whereas in D2, water was applied at 80 to 90% of soil water depletion. The subplots corresponded to varieties. In the third sown date the Cachoeira variety was sown under a randomized block design with four replications and the two SWDs. Plant sampling was performed during panicle and flowering stages, and saponin content (dry weight basis) determined by Soxhlet extraction; the extracts were analyzed by FTIR.

The analysis of variance of the results for dates showed differences ($P<0.05$) in SWD for saponins in the March and June sown. The mean values for D1 SWD were 0.64% (panicle) and 0.77% (flowering). The values for D2 SWD were 0.72% and 0.96% for the mentioned stages. Saponin contents were 0.66% in March and 0.53% in June for the Sajama variety under D2 SWD at the panicle stage, whereas at flowering stage they were 0.96% and 0.76%. The Chucara variety at the panicle stage show saponin of 0.79% (March), 0.53% (June), and 0.69% (September) and 0.96%, 0.92%, and 0.77% at the flowering stage. Under D1 SWD, the contents of saponins were similar. For the composition monitored by FTIR, we found variation in the relative absorbance of the signals related to saponin structures (-OH, -COOR, -COOH and -CH) as a function of sowing date, development stages, and SWD. The saponin content was minimum (0.49%) for D1 SWD, Sajama variety, panicle stage, in the June seeding, whereas the highest value (0.96%) was found at the D2 SWD, in both varieties, flowering stage, in the March seeding.

Our results are similar to those reported (0.1 to 0.73%) for 17 cultivars of quinoa and indicate that saponin content is affected by soil water deficit and environmental conditions.

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GUAR: A POTENTIAL NEW CROP IN GEORGIA, USA

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The southeastern state of Georgia provides an excellent environment for quality seed regeneration and potential new crop production for such crops as guar (*Cyamopsis tetragonoloba* L.) Taub. Guar is currently grown in the state of Texas with quality seed yields primarily for use in the food additive industry. However, recently various high value uses of guar seed as nutraceuticals have been discovered and these products are in the U.S. market. Another potential market exists in the fresh vegetable market because immature guar pods are used as a vegetable in the Asian populations.

The objective of this preliminary study was to determine whether guar can be successfully grown and regenerated in two different locations within the state of Georgia.

More than 200 guar accessions were planted in each of one 6-m row plot at both Griffin, GA and Ashburn, GA between 01 May and 01 June in 2001 and 2002. Guar accessions were characterized for height, date of maturity, plant surface pubescence, pod length, seed production, seed weight, and stem type.

High quality plants regenerated from most of the accessions. Guar accessions exhibited variability for all traits characterized especially for seed production with seed numbers per plot ranging from 24 to 8,792. Guar could be produced for the immature pods as a fresh vegetable, mature seeds as nutraceuticals, and as a cover crop because it is a legume and could provide adequate nitrogen for subsequent non-leguminous crops such as grasses or vegetables.

Guar's drought tolerance, minimum pest problems, nutraceutical, vegetable, and cover crop properties are all attractive traits plus the fact that it can be successfully grown in Georgia.

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EFFECT OF PLASTIC MULCH ON *Aloe vera* GEL AND JUICE YIELD AND SOLID CONTENT

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The *Aloe vera* gel and juice are widely demanded worldwide for different cosmetic and medical uses. In Mexico, the plant is cultivated mainly in the states of Yucatan and Tamaulipas. Cultivated area was 2846 ha during 2000, with 843 ha irrigated and 2003 ha under natural environmental

conditions. No report on the use of plastic mulch on *A. vera* was found. There is insufficient information on plant management or yield. There is a great demand of the dried material, and hence information about the solid content variation during the plant harvest is required.

In this study, we evaluated the yield and solid content of juice and gel of *A. vera* during the year as well as the solid content in both products in plants developed under mulch and bare soil treatments.

The study was carried out in the experimental field of the University in February 2001 using a split plot design with four replications. The main plot consisted of the treatments (mulched and bare soil) and the subplot with three fertilization levels (150, 100, and 0 kg/ha of nitrogen). Two harvests were performed, on 28 August and 06 December. From each subplot, 10 plants were sampled by cutting four external leaves that were weighed in the field and transported to the laboratory where the gel and juice were extracted by means of a laboratory roll processor and quantified. The solid content was measured gravimetrically, drying at 80°C for 24 h.

The ANOVA results and the mean test for the leaves, gel, and juice showed differences in gel yield between the mulched and bare soil treatments for the August harvest. The yield of gel was 4.17 ton/ha for bare soil and 3.37 ton/ha for the mulched treatment, whereas the juice yields were 8.39 ton/ha and 6.99 ton/ha. The average yield of leaves was similar for the August (19.3 ton/ha) and December (19.8 ton/ha) harvests. No effect was observed for fertilizer levels in the experiment. The gel solid contents were lower in the December samples than the August despite the treatment. The juice solid content was lower than that of the gel. In the mulched treatment, there was difference between a harvest date and among fertilizer levels, but the results were very similar for the bare soil treatment.

We may conclude that the use of plastic mulch has a small effect on the gel solid content, but not on the juice solid content. In addition, the August result showed that the gel yield was lower for the mulched treatment.

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INFLUENCE OF NON-STRUCTURAL CARBOHYDRATE LEVELS ON FRUIT PRODUCTION IN *Prosopis denudans* (MIMOSACEAE)

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Prosopis pods are an important source of livestock and human food in arid environments. Additionally, fruit pericarp and seeds are a source of commercial gum (mainly galactomannan). One of the major problems for their potential utilization as a source of gum is the variation between years in fruit production. This variation can be explained either through ecological constrains (insufficient water, nutrients, etc.) or through internal resource limitation (carbohydrate reserves).

The objective of this study was to determine the influence of reserves in the reproductive output of Prosopis denudans. Plants allocate significant amounts of resources, including carbohydrates, to produce inflorescences. In that sense, patterns observed in perennials show that plants making a large investment in reproduction in one year may have a lower probability of flowering in the subsequent year or years because stored resources are depleted by the large reproductive effort. For experimental purposes, depletion of carbohydrates from belowground organs can be obtained by removing, partially or totally, the source of photoassimilates (leaves).

A field experiment with one factor (leaf removal) and four levels (0% -control, 33%, 66%, and 100% of leaf removal; 10 reps/treatment) was carried out in Chubut, Patagonia, Argentina. Inflorescence biomass, and fruit production was measured in the 2001 to 2002 and the 2002 to 2003 growing seasons. Total non-structural carbohydrates (TNC) were determined by the anthrone method.

TNC content prior to budburst (September 2001) and the biomass of inflorescences produced in the 2001 to 2002 period did not differ among defoliation treatments. A significant ($P<0.01$) lineal and positive correlation was found between inflorescence biomass and total TNC content of the shrub aboveground biomass, prior to bud sprout. Control plants fruited at a higher proportion (60% vs. 23% for the other treatments together) than those under treatments of leaf removal. After the first source removal, the shoot TNC content of plants under 100% defoliation treatment was significantly lower ($P<0.01$) than that found in plants under other treatments. The root TNC content at the end of 2001-2002 growing season was significantly higher in control individuals than in those under other treatments. No significant relationship was found between fruit biomass and total TNC content of the shrub aerial biomass, prior to bud sprout (September 2001; $P=0.9$; $R=0.007$) and post-fruit maturation (April 2002; $P=0.16$; $R=0.11$). The lowest proportion of flowering individuals during the next season (2002-2003) was found in the 100% leaf removal treatment (20% vs. 87% for the other treatments together). The number of inflorescences produced by control plants was significantly higher ($P<0.01$) than that found for plants under other treatments (309 vs. 46.3 infl.m-2 for the other treatments together).

Conclusions: 1) the number of flowers produced in a given year depends on the amount of carbohydrates stored prior to flowering and 2) fruit production is not related to carbohydrate reserves. Other hypothesis (pollinator limitation, resource limitation, etc.) should be tested.

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DESERT MEXICAN PLANTS: CHEMICAL CONTENT AND COMPOSITION FOR POTENTIAL USE

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Research on desert plants has been largely developed due to the importance of its metabolites for pharmacological or industrial uses. In this study, six species growing in the Mexican desert are evaluated: *Acalypha hederaceae* Torr. (*A. monostachya* Cav.) or *hierba del cancer* is common on the grasslands and scrubs from Coahuila, Nuevo Le¢n, Durango, Zacatecas, San Luis Potos¡, Tamaulipas, Oaxaca, Puebla, Hidalgo, and Texas; Jatropha dioica Cerv. or *sangre de drago* inhabits the arid scrubs and grasslands from Texas, Coahuila, Nuevo Le¢n, Tamaulipas, Durango, Zacatecas, San Luis Potos¡, Guerrero, Hidalgo, Oaxaca, and Puebla; *Stillingia sanguinolenta* Muell. Arg. is common on the piedmont scrubs and intermittent streams from Nuevo León, Coahuila, San Luis Potosi, and Veracruz; *Mentzelia multiflora* (Nutt.) A. Gray or *pegaropa* grows on the desert scrub from Coahuila, Chihuahua, California, and Wyoming; *Thymophylla setifolia* or *parraleía* inhabits south of Texas, Coahuila, Chihuahua, Nuevo Leon, Zacatecas, San Luis Potosi, and Hidalgo; and Parthenium lozanianum grows on the lower slopes in the Western and Central Nuevo Le¢n at the Sierra Madre Oriental.

The objective of this work was to study the chemical content and composition for potential use of six Mexican species growing in the desert region.

The plants were collected on September 2001 in Sierra de San Jos, in the State of Nuevo Leon, 47 km from the University. The samples were taken to the laboratory and separated into stems, leaves and flowers, dried, milled, and prepared for analysis. The analyses included crude protein, fat, ash, fiber, and infrared spectroscopy (FTIR).

The flowers of the species had the largest chemical content, followed by the leaves and stems. The protein content in the flowers and leaves varied from 11.1% (*M. multiflora*) to 22.1% (*S. sanguinolenta*) and from 8.7% (*M. multiflora*) to 18.3% (*S. sanguinolenta*), respectively. In the stems the protein content varied from 0.51 (*M. multiflora*) to 7.8% (*J. dioica*). The fat content was similar, ranging in flowers from 4.3% (P. *lozanianum*) to 9.6% (*S. sanguinolenta*); for leaves varied from 0.81% (*M. multiflora*) to 5.9% (*S. sanguinolenta*), and for stems ranged from 1.23% (*M. multiflora*) to 2.66% (*J. dioica*).

The results showed differences among the different species and their plant parts. These plants had been used in folk medicine for the inhabitants of the desert regions. Also, some of them could be used for industrial purposes as antimicrobial products.

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NATURAL RUBBER AND RESINS

EFFECT OF BIOMASS STORAGE CONDITIONS ON *Grindelia chiloensis* RESIN STABILITY

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Resin produced by arid adapted species *Grindelia chiloensis* is a promising raw material for the naval stores industry. Resin contents up to 46% on a dry-weight basis have been obtained. The diterpene resin-acids that comprise the resin are oxidized derivatives of grindelic acid, the target component. Although similar to other terpenes, the synthesis is based on the Mevalonic Acid Pathway. The synthesis process of grindelic acid and its oxidized derivatives is still unknown. One of the possibilities is that the oxidation occurs after resin deposition on leaves and stems, a process that would be dependent on environmental conditions such as temperature, oxygen level, radiation and the length of the exposure to these

conditions.

The aim of this work was to determine the best conditions for biomass and resin storage that minimize oxidation of grindelic acid into its derivatives.

Two experiments were conducted using plant biomass collected at Isla Escondida, Chubut, Argentina in February 2001. In the first one, plant material was divided into four samples and they were stored at four different temperatures in the dark (-10°C, 4°C, 18°C, and 25°C). Sub-samples were taken every three months and crude resin was extracted using dichloromethane in a Soxhlet apparatus. The solution was concentrated at reduced pressure and oven-dried at 60°C. The residue was dissolved in methanol and filtered through filter paper. The solution obtained was treated with activated charcoal and then vacuum-filtered through a glass filter funnel. Refined resin was concentrated at reduced pressure and re-dissolved in dichloromethane. Analytical silica gel plates were used for chromatographic analysis with chloroform-methanol (8:2) and dichloromethane-methanol (9:1) as solvents. Spots were revealed using $CeSO_4$. The same procedure was followed to extract resins in the second experiment in which extracted resin was stored under different conditions. Refined resin was dissolved in ethanol and stored under 4°C in the dark or 18°C in the dark and light. Subsamples were extracted every three months and analyzed through silica gel chromatography.

Results showed no variation in the number of spots present in their form or color of different samples during a period of over two years. Also, the Rf's remained unchanged. These results provide supporting evidence on the stability of the resin of *G. chiloensis* stored in the dark or light at very different temperatures, before and after extraction from the plant material. More conclusive evidence will be obtained after gas chromatographic analyses are completed.

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EVALUATION OF GUAYULE GROWTH POTENTIAL IN SEVEN CLIMATIC AREAS OF SOUTH AFRICA

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Large parts of southern Africa are arid, and therefore guayule (*Parthenium argentatum* Gray), a semidesert plant, has the potential to be successfully commercialized in this region. New crop development suitable for cultivation by rural farmers is needed to develop agribusiness and contribute to the economic improvement of the regions and be self sufficient in the demand for natural rubber.

The objective is to determine the economic viability of guayule by means of its growth and biomass. Evaluation of the latex production in the different areas will determine whether production will be economically viable as preliminary results indicate that latex production in certain areas is viable

To investigate the viability of this industrial crop, field trials were established in seven different climatic regions of South Africa to determine the growth potential and biomass. Growth (stand count, height, canopy diameter and stem diameter) and biomass (wet and dry weight) were recorded. Analysis of variance was done to determine the mean growth and biomass for the different areas and cultivars.

Results show significant interaction between location and cultivar for growth and biomass. The greatest growth potential was produced by cultivars AZ-2 and AZ-3 (both bulkier and broad-leaved shrubs), compared with N565 and 11591 (slender, slim-leaved shrubs). Cultivars produced similar results for biomass production, but were significantly different in the different sites. Soil composition correlated with these findings as sandy soils provided a higher biomass index than the loam structures. Dry land establishments relied on rainfall patterns and temperature regimes. Field mortality was correlated with initial poor establishment of transplanted seedlings.

The growing potential of guayule under South African environmental conditions have been identified in suitable locations. Partnership formed between the private sector and government in South Africa to commercialize guayule could play an important role in ensuring that the rural farmers become part of commercial agriculture of this crop in South Africa.

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QUALITY AND PERFORMANCE OF LATEX PURIFIED FROM DIFFERENT PLANT PARTS OF MATURE GUAYULE SHRUBS

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Commercial development of guayule latex for the medical products market requires the ability to consistently produced high quality latex. Although we have previously shown that guayule latex is of high quality, little information was available about the effects of harvest times, branch age or differences between lines. Also, it is expected that some harvests may result from pollarding and some from digging the entire shrub, which would include latex from the roots and stem bases. Again, little was known about the quality of this latex fraction.

In this study, we quantified, purified, characterized and tested latex produced from four subfractions of five-year-old guayule plants of three lines from New Mexico, including small, medium and large branches as well as a root and stem base fraction, and included a comparison with harvest time for one line.

Analysis of the yield data shows that line AZ-R2 had a higher yield overall than the guayule lines 11605 and Cal-6, and older tissues had more latex than the youngest ones. The youngest stems had a similar latex content among the lines, and the overall yield difference was attributable to differences among the larger branches. In all lines, purified latex from the roots and stem bases had a lower protein concentration than latex from the stems. In addition, line 11605 latex generally seemed lower in protein than latex from Cal-6 and AZ-R2.

The rubber particle size was similar among lines, but was age dependent with particle size slightly decreasing as stem size increased in all three lines.

Molecular characterization of the latex rubber molecules indicated that latex from all lines and all parts of the guayule plant had high molecular weight, low polydispersity, and similarly configured as random coils.

Physical testing of films made from the different latex samples, and compared with films made from different samples of *Hevea brasiliensis* latex, indicated that high quality properties could be achieved from all samples.

In conclusion, this study suggests that latex is quite uniform among lines and throughout the plant in healthy guayule plants. Also, high quality latex should be obtainable whether the shrub is harvested by pollarding or digging.

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TRANSFORMATION OF GUAYULE LEAF SECTIONS

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Guayule (*Parthenium argentatum*, Gray) is a new crop candidate for the rubber industry. Transformation of guayule with genes may lead to new lines with improved rubber yields and agronomic traits. However, the existing transformation method using shoot nodes is technically difficult, time consuming, and tedious so that more rapid methods are desirable. An earlier report of successful leaf disk transformation by this laboratory proved to be unreliable.

We have developed a new, reproducible, method of guayule transformation using leaf sections as explants for guayule transformation. A BAR selective marker and an intron containing the GUS reporter gene have been introduced into the guayule. Transgenic guayule plants with this method show resistance to the herbicide glufosinate and positive GUS staining. Southern blots confirmed the transformation.

This new method should greatly speed the creation, development, field-testing, and release of improved guayule lines.

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RESIN PRODUCTION IN *Grindelia chiloensis* IS AFFECTED BY PHOTOPERIOD AND TEMPERATURE

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The elucidation of the function of photoperiod and temperature in resin production of *Grindelia chiloensis* is of special relevance for the definition of a latitudinal range and the potential sites for cultivation of this species. In this study, we have taken an integral approach to the understanding of the ecophysiology of resin production in *G. chiloensis*. We examined the effects of daylength, temperature and their interaction on the resin content of leaves of different age. We also attempted to describe the role of the trichomes on such responses.

Four groups of plants were grown under two temperatures (35/25°C and 19/13°C day/night) and two photoperiods (15 h and 9 h) in controlled-environment growth cabinets. At equal phenological stage, four partially expanded leaves (PEL) from each plant and four fully expanded leaves (FEL) were removed and resin extracted with an ethanol protocol. Refined-resin yields were determined as the percentage of dry biomass and as the ratio resin mass: leaf area. For specific leaf weight (SLW) determinations, the area of partially expanded leaves was estimated by digitalizing the leaves. Leaves were then oven-dried and dry mass was determined. Trichomes density was evaluated on partially expanded leaves. Treatment effects were tested by two-way ANOVA. The significance of differences between means was tested by Duncan's Multiple Range test.

Resin content (g/g) on PEL was significantly higher under low temperature treatments and was not affected by photoperiod. Resin content on FEL was significantly higher under short photoperiod but was not affected by temperature. When expressed as the ratio resin mass:leaf area (g/cm^2), the resin content on FEL was significantly higher for plants grown under low temperature and short photoperiod treatments. In general, resin content on PEL expressed as the ratio resin mass:leaf area, was higher for plants grown under low temperature or short photoperiod treatments, but the magnitude of the increase varied with photoperiod x temperature interaction. SLW was higher in plants grown under low temperature and short photoperiod treatments. Trichome density on both sides of the leaves was significantly higher under low temperature and long photoperiod treatments. The effect of temperature and photoperiod on resin content (leaf area basis) was attained through an important effect on SLW and through a not so strong effect on resin production on a leaf weight basis. Regarding the role of trichomes on such responses, a positive relationship was found between resin content and trichome density when considering the effect of temperature. In contrast, an inverse relationship was found the effect of photoperiod was considered.

The responses to temperature and photoperiod found in *G. chiloensis* would restrict the cultivation of this species to locations with cold and dry autumn, conditions that should promote resin accumulation before harvest.

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SINGLE OR MULTIPLE HARVEST FOR *Grindelia chiloensis* GROWN IN ONE-YEAR CYCLES?

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Grindelia chiloensis (Asteraceae) is under investigation as a diterpene resin source for cold arid environments, such as Patagonia. The knowledge of the effects of the harvest regime, and its interaction with temperature on resin production is of special interest for the definition of some agronomic practices such as date of transplanting and opportunity of harvesting. It has been established that for *G. camporum* a dual harvest scheme (Summer and Fall) increased total resin yield compared with a single harvest, and that re-growth biomass harvested in the Fall had a higher resin content than Summer-harvested biomass.

The objective of the present study was to characterize the influence of biomass harvest and temperature on resin yields.

Plants of *G. chiloensis* were grown at two temperatures (35/25°C and 25/15°C, day/night) and three harvest regimes in two greenhouses. Harvest regimes consisted of: (A) a single harvest 13 months after sowing (July), (B) two harvests: 9 months after sowing (March) and a final harvest 13 months after sowing (July), and (C) two harvests: 10 months after sowing (April) and a final harvest 13 months after sowing (July). Four partially expanded leaves (PEL) from each plant were removed every 30 days since the date of the first harvest (March, April, May, and June), and resin content evaluated. Refined-resin yields were determined as the percentage of dry biomass and as the ratio resin mass : leaf area. For the specific leaf weight (SLW) determinations, the area of partially expanded leaves was estimated by digitalizing the leaves. Leaves were then oven-dried and the dry mass was determined. Treatment effects within each harvest date were tested by two-way ANOVA. The significance of differences between means was tested by Duncan's Multiple Range test.

In March, resin content (g/g) was not affected by temperature regime. In April, resin content (g/g) was significantly higher for plants grown under low temperature and there were no significant differences on resin content between harvest regimes (A versus B). In May and June, resin content (g/g) was higher under low temperature and no previous harvest treatment, but the magnitude of the effect varied with temperature x harvest interaction. When expressed as the ratio resin mass:leaf area (g/cm^2), resin content was significantly higher under low temperature (all harvests). In April, May and June resin content (area basis) was significantly higher under regime A (no previous harvest). Resin content (g/cm^2) was: A>B>C for the May harvest and A>C>B for the June harvest. The effect of low temperatures and absence of previous cutting on the promotion resin content (leaf area basis), was attained through an important effect on SLW and through a not so strong effect on resin production on a leaf-weight basis.

The responses to temperature and harvest regime found in *G. chiloensis* would indicate the convenience of a single harvest in the autumn, when temperature and photoperiod are promoting conditions that maximize resin yields.

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EFFECT OF PLANT DENSITY ON RESIN PRODUCTION IN *Grindelia chiloensis*

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Grindelia chiloensis (Corn.) Cabr. is a potential resin crop for arid and semiarid environments. The traditional plant density used in Argentina for agronomic experiments have been 33,000 plants/ha although the incomplete soil cover described for these experiments suggests a sub-optimal plant density. Sub-optimal plant density should not affect individual plant growth, but would decrease resin content. On the other hand, supra-optimal plant density would reduce plant growth through competition for nutrients, water, or radiation. The resultant lower availability of water and nitrogen (in this case due to intraspecific competition) is known to increase resin content.

The objective of this work was to assess the performance of *G. chiloensis* under three different plant densities of 33,000, 47,000, and 66,000 plants/ha (three plots/density; 24 plants/plot) during one year (05/2001-06/2002). Plots had 4 rows of 6 plants each, at 0.30 m between plants. Different plant densities were obtained through changes in the separation between rows (1, 0.7, and 0.5 m, respectively). Plots were irrigated every three weeks during the growth cycle. In June 2002, 4 plants per plot were harvested for a total of 12 plants per treatment. Dry weight, biomass partition (roots (R), shoots (S), senescent leaves (SL), green leaves (GL), inflorescences (I)), resin and nitrogen contents were determined.

No significant differences were found in biomass production and partition, resin and nitrogen contents for individual plants under the different treatments, whereas at the crop level, total plot biomass increased with plant density (12.1, 20.4, and 28.8 ton/ha). This increase in biomass resulted in an increase in resin production (2.0, 2.8, and 3.9 ton/ha), whereas no significant differences were found in the total nitrogen uptake among treatments (133, 183, and 207 kg/ha)

For a one year crop cycle, plant density of *Grindelia chiloensis* can be doubled to 66,000 plants/ha, duplicating the amount of resin produced per unit area without affecting biomass production and partition at the individual plant level. Although the increase in total nitrogen uptake was non-significant among density treatments, a clear tendency was found. These results also suggest that the highest density used in this experiment is still supra-optimal for this cropping system.

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EFFECT OF SEEDING DEPTH, PRETREATMENT, AND GERMPLASM ON *Grindelia chiloensis* SEED GERMINATION AND ESTABLISHMENT

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Direct seeding has not been thoroughly tested to establish *Grindelia chiloensis*, mainly because of the lack of enough selected seed and the use of clones. One characteristic of *Grindelia* seed of importance for this type of planting scheme is that freshly harvested seeds can have high levels of dormancy that can be overcome with time, by washing the seeds or with stratification. The degree of seed dormancy varies among accessions.

The objective of this work was to test the possibility of establishing a crop by direct-seeding *G. chiloensis*, with the aid of pretreatments to break seed dormancy.

Seeds were harvested in January 2003 from plants of four accessions grown in a common garden in Trelew, Patagonia. Two pretreatments intended to break seed-dormancy were applied to the seeds: (A) seeds were washed with running tap water for 48 h, and seeded immediately, and (B) seeds were washed for 48 h and allowed to air-dry for five days before seeding (priming). A third group of untreated seeds was used as a control. A total of 192 plots was established including four factors: accession (four levels), pretreatment (three levels), seeding depth (four levels: 0.5cm, 1 cm, 2 cm, and 4 cm), with four replicates each. Each replicate consisted of 20 seeds placed in a 1-m seeding-bed. The plots were seeded on 31 March and weekly watered by furrow irrigation. Seed germination and seedling establishment were recorded every-other day for two weeks and a final count was made on 25 May. Maximum germination rate of these accessions was tested in a lab experiment with a pretreatment of 7-day stratification at 4°C. Maximum germination rates for the four accessions tested in the lab were 87%, 58%, 53%, and 69%.

Overall germination and establishment rates in the field experiment ranged between 66% and 2%. The highest germination and establishment were found both for seeds placed at 0.5 cm and at 1 cm depth (between 50% for accession 750 and 24% for accessions 547), with those at 2 cm and 4 cm having a lower establishment rate (between 28% and 10%; $P < 0.05$). Accession 750 had a higher germination rate (39%, all pretreatments and depths averaged) than the other three accessions (18%, 21%, and 28%, for 547, 775, and 734, respectively; $P < 0.05$). Washing and drying the seeds resulted in lower germination rates than washing alone or untreated controls ($P < 0.05$; 30%, 32%, and 19%, for controls, washed, and washed and dry, respectively, all accessions averaged).

Direct seeding of *Grindelia chiloensis* could be a low-cost alternative to currently used transplants, if enough seeds from selected germplasm are secured. Although emergence was reduced beyond 2 cm below the soil-surface, Grindelia seed could tolerate a wide range of seeding depths.

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TERMITE RESISTANCE OF KENAF COMPOSITION BOARDS TREATED WITH GUAYULE RESIN

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Composition boards resistant to insect damage are a desirable feature for their use in industrial and home construction. Such material should greatly reduce the costly replacement of termite-damaged wood and insure the structural integrity of the building. Acceptance of such products would be further enhanced by using raw materials that are cheap and environmentally acceptable. We propose to combine fibers from kenaf (*Hibiscus cannabinus* L.) and resinous material of guayule (*Parthenium argentatum* Gray), both of which can be considered as secondary products from the primary ones, i.e., paper from kenaf and latex from guayule. Thus, the objective of this investigation was to fabricate termite-resistance, medium density kenaf composition boards.

Kenaf stalks consisting of four different cultivars were used to make the boards. "Flake-board" was made by cutting the stalks into 7.62-cm sections and flaking with a ring-flaker. The flaked material was dried (8 to 10% moisture content) and screened to remove the fines. Phenol formaldehyde (PF) thermosetting resin (7%) and wax (2%) were mixed with the kenaf flakes and the fiberboard made using 6.0 MPA pressure at 185°C for 4 min. Flake-boards with an average thickness of 11.1 mm and specific gravity of 0.70 were produced. "Fiberboard" made from fiberized stalks that were steam pressurized in a single rotating disk and dried to 5% moisture content. The prepared stalk was mixed with 7% PF and 1% wax and the board formed at 6.0 MPA pressure at 185°C and 4 min press duration. Sections (2.54 × 2.54 cm square) of flake-board and fiberboard were treated with de-rubberized guayule resin in a treatment chamber. The chamber with the sample was first evacuated for 5 to 10 min and then the resin solution was introduced into the chamber to impregnate the blocks. Pressure in the chamber was maintained for 30 min at 690 kPa. After exposure, the excess resin was wiped off the treated blocks, and the blocks dried in a vacuum oven to remove the acetone carrier solvent. The resin-treated and untreated blocks were exposed to the Eastern termite (*Reticulitermes* spp.) following ASTM procedures.

The resin-treated kenaf flake-board and fiberboard were found to be resistant to termite damage. Termite survival was zero after a 2-week exposure.

A combination based on two new crops, kenaf and guayule, provided the means of producing medium-density flake-board and fiberboard that were resistant to termites. When the raw materials are considered as waste or by-product sources, the fabrication of termite-resistant, medium-density flake-board and fiberboard appears to be economically feasible. Also, the possibility exists of blending guayule bagasse that still contains the resin with kenaf fiber to produce higher density composition boards.

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PELLET FUEL FROM GUAYULE PLANT MATERIAL

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Agricultural plant wastes when properly processed into useful commodities can become an economic asset for the particular crop. The extraction of latex rubber from the guayule plant (*Parthenium argentatum* Gray) will involve about 10% of the harvested material. Uses must be found for the remaining 90% plant residue or bagasse. One application is to process the bagasse into renewable, compact pellet-type fuel that can be used at the site or transported to the consumer, whichever will prove the most profitable. Furnace and water heaters that can burn pelletized plant materials have become popular and their safety, low pollution, and reasonable operational costs have been demonstrated. Also, the drastic increases in the price of liquified fuel and its uncertain supply place a premium for finding and using alternate, low-cost, cellulose-based fuels.

The objectives of our study were to fabricate pellet fuel from guayule bagasse and to determine its physical properties and burning characteristics.

Guayule bagasse remaining from the latex extraction process and byproducts from a local cotton gin were collected and air-dried in a greenhouse to approximately 6% water content. A blend of 75% guayule bagasse and 25% processed cotton gin byproducts were mixed and pelleted to a density of 534 kg/m^3 (33.34 lb/ft^3).

Stable pellets (6 mm diameter × 25 mm long) were produced with a commercial pellet mill without the use of additional binding agents. The processed cotton gin material provided the lubricant and some of the binding agent. The resinous material that was still in the guayule bagasse also contributed to the binding. The energy equivalent was about 22.49 Mj/kg (9670 BTU/lb) and approximately 10% higher than presently available pellet fuels. The guayule resin is reported to have an energy value of about 38.0 Mj/kg (16,400 BTU/lb), and because it makes about 10% of the bagasse material, this fraction contributed to the energy value of the pellet. The ash content was about 5.0% and the sodium oxide 4.6%. The sodium ash was high because sodium sulfite is included in the latex extraction solution as an antioxidant. However, this sodium fraction could be readily removed by washing the bagasse after the last step in the latex extraction procedure. The pellets were fed and burned easily in a commercially available pellet stove. The average combustion efficiency of the guayule/gin byproduct pellets was 90.9% that compared favorably with the 96.8% obtained from burning premium-grade wood pellets.

A blend of guayule bagasse with cotton gin byproducts provided the raw materials needed to make pellet fuel that has economic potential. These waste materials can be considered as useful and renewable bioenergy sources.

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EFFECTS OF HARVEST INTENSITY ON SURVIVORSHIP, LEAF RESIN CONTENT, AND BIOMASS PRODUCTION IN *Grindelia chiloensis* (ASTERACEAE)

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Grindelia chiloensis Corn.(Cabr.), a perennial shrub native from Patagonia and Monte in Argentina, is a candidate crop as a source of diterpenic resins. The resin is secreted on leaves, stems, and capitula, which implies that all aboveground biomass needs to be harvested. The objective of this

work was to determine the effects of three harvest intensities on plant survival and on plant productivity for a multiseason crop system.

Plants were generated from seed collected in Playa Union, Cabbed (accession DAR 575) and were grown in experimental plots located in Trelew, Patagonia (43° 16" S, 65° 21" W). Harvest treatments started 17 months after transplanting (first harvest: 29 March 2003). Treatments were evaluated at the plant level so that neighboring plants were removed to avoid interference. The first harvest treatment (H1) was done by cutting plants at 0.25%, 0.50%, and 0.75% of their total height, whereas the control treatment was uncut plants. Fourteen months later (30 May 2001), a second harvest cycle (H2) was done by cutting all plants 3 cm above the first cut, and 20 months later (16 June 2002) a third harvest cycle (H3) was done by cutting plants 3 cm above the last cut. Biomass production and partition (leaves, stems, and capitula) were determined and resin content was characterized through Soxhlet extraction. Student-Newman-Keuls test with $n \geq 5$ per treatment was used for means separation at a significance level of $P<0.05$.

Plant survival was not affected by harvest intensity. Thirty-four months after the first harvest (19 January 2003), survival for all treatments was around 60%. At H1, both the aboveground biomass and leaf biomass were higher with harvest intensities of 0.75, 0.5 than in the control treatment. At H2, we found an inverse response where plants originally harvested with low intensity (25%) showed significantly higher regrowth than plants harvested at 50% and 75%. At H3, no significant differences for regrowth biomass production among treatments were found. The accumulated biomass production during the experiment (H1+H2+H3) was also compared among treatments. The highest aboveground and leaf biomass production at the end of this experiment was found in the intact plants. Regrowth biomass partition was different in each harvest date. At H1, the higher proportion of leaves was found in 25% (75% of leaf biomass), whereas the lowest proportion was found for 75% (44% of leaf biomass). For the second and third harvests, no differences were found in biomass partition among treatments. No significant differences were found among harvest treatments for leaf resin content.

A major effect of harvest intensity was found on post-harvest biomass production. Regrowth capacity of plants exposed to the different harvest intensities was not enough to compensate for the biomass production of intact plants, which implies no compensatory growth in G. chiloensis. Cutting intensities had no impact on leaf resin content, whereas plant mortality did not change among treatments, but seems high (around 40%), a response that requires more detailed experiments.

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OILSEEDS

MINIMUM GERMINATION TEMPERATURE FOR LESQUERELLA AND GUAYULE

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Lesquerella (*Lesquerella fendleri*) and guayule (*Parthenium argentatum*) are potential alternative crops that are being studied for commercial production. Understanding the minimum temperatures for seed germination and seedling growth are important in determining the potential areas for seed production of these crops. This information is useful in estimating growing degree days required for plant production. Climatic data can then be used to determine the potential areas for production.

The objectives of this study were to determine the minimum temperature of germination and seedling growth, and to screen ecotypes for germination and growth characteristics. We will then conduct field studies at various elevations to assess temperature differences in the field, and to see how the field data compare with laboratory results.

A temperature gradient table was constructed to allow germination of ecotypes over a range of temperatures in the laboratory. A 2.54 cm-thick aluminum block 0.61 m wide and 1.22 m long was insulated on all sides with Styrofoam board insulation. Water baths were connected to each end of the aluminum block and used to control the temperature gradient. Seeds were germinated in Petri dishes placed on the table and time to germination was observed. Time to 5 mm root length and time to 5 mm shoot length were also measured to assess the minimum temperature for seedling survival and growth.

Preliminary laboratory data have shown varietal differences in time to germination, as well as in seedling growth characteristics. Further investigation is being carried out to assess adequately the temperature effects on germination and growth variables.

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POPULATION DYNAMICS AND SEED YIELD OF A NATIVE STAND OF *Lesquerella mendocina* IN PATAGONIA, ARGENTINA

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Lesquerella mendocina is a perennial species native to the Monte and northern Patagonia regions in Argentina. Natural stands are few, small, and inconspicuous. On more than 10,000 km taken on collection trips, our group has only been able to locate four populations of this species. The material collected on these trips has proven of value for the selection of a perennial *Lesquerella* crop. The objective of this work was to characterize plant density, plant size, and seed yields of a native stand of *Lesquerella mendocina* during a three-year period.

A native stand located at the Parque Nacional Lihue Calel (La Pampa, Argentina) was evaluated in December 2000, 2001, and 2002, at the peak flowering stage. Plant density was measured with 10 9-m transects. All plants encountered were measured (diameter and height), and divided into four diameter groups (<2 cm; <2 cm-4 cm>; <4 cm-6 cm>; and 6 cm>). A sample of 30 plants per diameter group was uprooted, oven-dried, and separated into roots, stems, leaves, and fruits (including seeds). Each fraction was weighed and the biomass partition, seed yield, and harvest index were calculated. Data were analysed using one-way ANOVA and correlation. The significance of differences between means was tested by Duncan's Multiple Range test.

No differences among years were found in total plant density that varied between 9.5 and 7.2 plants m^{-2}. Patches with higher plant density had smaller plants than those with fewer plants, indicating intra-specific competition. Plant size (diameter) and biomass were linearly related and the relationship was similar for the three years of the study.

Average seed yield/plant was significantly greater in 2001 and 2002 than in 2000, mainly because of differences in productivity for plants in the 2 cm to 4 cm diameter group. Seed yield/plant was directly dependent on plant size: 0.36 g/plant, 0.17 g/plant, 0.06 g/plant, and 0.02 g/plant for plants in the > 6 cm, 4 cm-6 cm, 2 cm-4 cm, and < 2 cm groups, respectively (average of all three years). Harvest indexes varied between 4% and 6% and were significantly greater in the years 2000 and 2001 than in 2002.

During the three years of this study, the population at Lihue Calel was stable, although no attempt was made to determine plant establishment or mortality. Seed yield within size groups was also stable among years, except for the plants in the intermediate size range of 2 cm^{-4} cm. The comparison of these results with biomass partition and seed-yields obtained in cultivated stands (normally lower partition to roots, and higher harvest index) showed a high degree of phenotypic plasticity in *L. mendocina*.

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CUPHEA INJURY RATES WITH PREEMERGENCE HERBICIDES

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Cuphea seed oil, a medium-chain triglyceride rich in lauric acid, is essential for the soap and detergent industry. Many agronomic issues relating to commercial Cuphea production as a new oil seed crop are currently under investigation. As with any new crop, a reliable weed control method is necessary. This study focuses in identifying the effects of preemergence herbicides on Cuphea germination and growth rates.

A 2001 preliminary greenhouse study screened 12 potential herbicides to identify six promising herbicides for future field trials. Replicated field trials in 2002 and 2003 were conducted to investigate weed control and Cuphea injury rates of six soil-applied herbicides at 1-, ½-, and ¼-times the labeled application rates. Isoxaflutole and imazethapyr displayed good weed control and little Cuphea damage and were later assessed at 8-, 4-, 2-, 1-, and ½-times the labeled rates to observe plant injury. Both studies were conducted in randomized complete block design with plant height, plant density, and stage of development. Measurements were taken at ¼-m^2 transect.

Our results suggest that isoxaflutole at eight times the labeled rate does not damage Cuphea. The identification of a promising preemergence herbicide program will not only enable commercial Cuphea production to become successfully established, but will help farmers diversify their

crops and expand their markets.

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VARIATION IN MORPHOLOGICAL AND PHENOLOGICAL CHARACTERISTICS IN SELECTED POPULATIONS OF THE USDA LIMNANTHES GERMPLASM COLLECTION

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Limnanthes (Limnanthaceae; meadowfoam) seed is a source of long-chain fatty acids with very high oxidative stability. The seed oil may be used in lubricants, inks, rubber additives, and plastics. Germplasm with short vegetation cycles, uniform and high seed yield is needed for cultivar improvement.

Seeds of 10 different *Limnanthes* accessions were sown in nursery flats in a screenhouse, and four months later transplants were planted in the field in a randomized complete block design (4 replications) with 50 plants per replication. The trial was carried out at the National Arid Land Plant Genetic Resource Unit, Parlier, California. The characteristics evaluated were plant survival and vigor, number of plants without flowers, time of flowering of the first and 50% plants, and mass of seeds maturing at different time within each population.

Within individual populations, the number of viable plants able to grow in the field from transplants varied from 94.3 (PI 283728, *L. striata*) to 26.8% (PI 283721, *L. floccosa* ssp. *pumila*). The largest fraction of vigorous plants (97.6%) was observed for L. gracilis (PI 283722) and the lowest (65.3%) for *L. flocossa* ssp. *bellingeriana* (PI 420132). The ability to develop flowers on each plant varied between populations from 100 (*L. alba* ssp. *alba*, PI 283703, and *L. alba*, PI 283728) to 73.2% (*L. gracillis*, PI 283722). The number of days from sowing to first bloom was 142 (*L. gracilis*, PI 283722 and *L. flocossa*, PI 420130) to 163 days (*L. alba*, PI 374793). The shortest duration of flowering (days from first to 50% bloom) was observed in *L. striata*, PI 283728 and *L. alba*, PI 374791 (3 days) and the longest was in *L. alba*, PI 374800 (41 days). The time from sowing to first seed maturity varied from 182 (*L. striata*, PI 283728, and *L. alba*, PI 374791) to 215 days (*L. alba*, PI 374800). The span between maturity of seeds on the first and last plants was from 19 (*L. alba*, PI 374800) to 39 days (*L. gracillis* 283722). Seed yields varied within the populations for different seed maturity dates, and among the 10 populations evaluated.

The wide range of variation for the characteristics evaluated indicated that the 10 germplasm accessions are a valuable source for development of improved *Limnanthes* cultivars.

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DEVELOPMENT OF HYOLA 401 CANOLA HYBRID ON FOUR SEEDING DATE AND PLANT DENSITY IN THE YAQUI VALLEY, SONORA, MEXICO

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The Yaqui Valley of southern Sonora, Mexico suffers from water scarcity, poor soil conditions, and biological problems such as white fly (*Bemisia argentifolii*) damage that eliminated cultivation of soybeans (*Glycine max*) and sesame (*Sesamum indicum*). Since 1994, nontraditional crops have been tested. Canola (*Brassica* sp) shows promise because it is adaptable to the local environment as an economical water-efficient crop, and its performance has been acceptable. However, the canola research now is based on introduction, varietal tests, and some agronomic trials. The objective of this study was to evaluate Hyola 401 canola hybrid from Canada with different seeding dates and plant densities under the Yaqui Valley, Sonora, México conditions.

The seeding date evaluation trial was conducted in the Experimental Field of the Instituto Tecnol¢gico Agropecuario No. 21 (Ita 21) during the

2002 and 2003 growing seasons. The experimental design was a randomized complete block with three replications, and plots of four rows 8 m wide and 5 m long. Treatments consisted of: four plant densities (5, 10, 20, and 30 plants per meter) of Hyla 401 canola hybrid at four different seeding dates (November, December, January, and February) during the first week each month. Seeds were hand-planted in a clay soil with irrigation. Harvests were made in April and May 2003. Test weight, vigor, plant density, days to beginning of bloom, plant height, days to physiological maturity, lodging, grain weight measurements and chemical analysis of the seed were made.

There were highly significant yield differences among the planting dates, and plant densities. The better two planting dates were November (2849 kg/ha) and December (2878 kg/ha). However, the canola planted in January (2175 kg/ha) and February (1733 kg/ha) decreased in grain yield due to the environmental influence and damage caused by the insects Bemisia argentifolii and Nysius ericae. The canola responded when planted at high seed density during December, January, and February. However, the low plant density was better in November planting date. Oil content was 43.4% in December and decreased to less than 30% in February. However the linoleic acid increased (71.4%) in late planting compared with the normal values for November (64.6%) and December (66.4%). The Yaqui and Mayo Valleys of southern Sonora, México from November to December. However, it is necessary to establish yield trials with other genotypes at different seeding dates to validate the establishment of commercial planting in different environments.

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ACCUMULATION OF PROTEIN AND OIL DURING SUNFLOWER SEED DEVELOPMENT

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Sunflower (*Helianthus annuus* L.) is an industrial crop with seeds high in protein content (15% to 24%) and edible oil (30% to 50%) according to the fatty acids composition. The meal obtained after oil extraction is used in food rations for livestock and poultry. The plant can also be used as forage due to its high protein content. The sunflower is resistant to drought and adapts to saline soils and low temperature. In Mexico, it is cultivated in the states of Tamaulipas, Zacatecas, Guanajuato, and Durango. It constitutes an alternative crop for the northern arid and semiarid lands.

The objectives of this work were to study the accumulation of protein and oil in the sunflower grain at four development stages, to look for correlation between protein and oil and its relation with the morphological traits and grain yield, and to carry out a regression analysis for protein and oil content in the grain as a function of time.

Seeding was carried out at the University experimental field in May 1998. Five genotypes were sown: SAN-3C, SANE 23578, SANE 1278, Gordis, and CIANOC (check variety) under a random block design with four replications. Fertilizer application was a 180-20-00 (N-P-K) formulation, and water was supplied four times (13, 28, 48, and 70 DAS) during crop development. Samples and data were collected every 10 to 15 days. The evaluated variables were: leaf area, dry weight, plant height, leaf number, stem diameter, stem head, grain yield, nitrogen, protein, and oil content.

The results showed that the protein content in the grain during flowering varied from 17.5% to 25.3% and increased to 25% and 30.6% at harvest for the genotypes. The highest oil content was 13.5% at flowering and 32.8% at harvest. There was a positive correlation between protein content and oil at the stages of end of flowering and beginning of physiological maturity, whereas at harvest the correlation was negative. Protein and oil content increased as the grain developed and the protein accumulation was a function of genotype. Our results provide information for genotype selection based on the defined final product as well as the possibility to increase yield by crop management techniques at specific stages.

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PHOSPHOLIPIDS FROM *Simmondsia chinensis*

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The fruits of *Simmondsia chinensis* contain 50% of a wax mainly used for cosmetic and dermatological applications along with 2.5 to 5% of appetite suppressing components (simmondsin and simmonsin 2'-ferulate). The fruits contain also other not yet identified components with e.a. hair restoring activity and activity on oviduct development in chickens. For those reasons, a thorough study was started for the isolation of new products from jojoba.

Jojoba oil and jojoba meal samples were supplied by T. Abbott (New Crops and Process Research, USDA-ARS-NCAUR, Peoria, IL). Methanol extracts prepared from jojoba oil and jojoba meal, were fractionated on silica gel columns with different solvents to isolate residual oil, simmondsin-ferulates, simmondsins, carbohydrates, and a fraction that reacted positively on TLC for the presence of e.a. phosphorous and nitrogen. This last fraction was examined by HDLC with a Chromolith RP-C18 column with a gradient of methanol and water from 96/4 to 85/15 (v/v); eluants were monitored by a diode array detector. Baseline separated peaks were collected and after elimination of the solvents were examined by TLC with specific detection reagents, MS, bidimensional ^{1}H and ^{13}C NMR, GC and GC-MS.

Twelve peaks were detected and collected separately. MS, mono and bidimensional ^{1}H and ^{13}C NMR of the two major peaks allowed the assignment of a specific phosphatidylcholine (C18:1, C18:1 fatty acids) and a specific lysophospatidylcholine (C18:1 fatty acid). Minor fractions could be identified by correlation of the obtained mass spectra with the mass spectra of the identified phosphatidylcholine and lysophospatidylcholine. GC-MS analysis after transmethylation of the different isolated products confirmed the identity of the different acyl chains (C16:0, C18:1, C18:2, C18:3, and C20:1 fatty acids) of the major and minor phosphatidylcholines and lysophosphatidyl-cholines present in jojoba meal and jojoba oil.

Identical phosphatidylcholines and lysophosphatidylcholines were present in both jojoba meal and jojoba oil although in different relative concentrations.

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MICROSPORE CULTURE IN GENUS *Lesquerella*

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In the cultivated species of Lesquerella, *L. fendleri*, the variability of the hydroxy fatty acid (HFA) trait is limited to approximately 60% lesquerolic acid. Seeds from other Lesquerella spp. contain elevated lesquerolic acid quantity. Introgression of these wild variability is currently being attempted by production of interspecific amphidiploids within the genus. Another potential breeding option to increase HFA variability is to induce point mutations within cultured microspores via chemical mutagenesis. If successful, the labor-intensive alternative of mutagenizing diploid seed embryos and selfing them for several generations to express the mutant phenotypes would be avoided. Haploid plants obtained through microspore culture and doubled with the use of colchicine (dihaploids) are homozygous in one generation compared with the several generations required to produce nearly isogenic lines. Dihaploid plants would also aid in the development of genetic mapping populations. Therefore, the objective of this preliminary study was to evaluate the factors affecting microspore culture in order to determine whether haploid embryos could efficiently be produced from several different species of Lesquerella.

In the cultivated species, *L. fendleri*, microspores from 12 plants grown at 20/15 24°C were isolated from four classes of bud sizes (2-3, 3-4, 4-5, and 5-6 mm) and plated at three densities (25,000 50,000, and 100,000) in four different NLN carbohydrate media (sucrose, glucose, maltose, and trehalose) and two different NLN media containing 25% PEG instead of the carbohydrate source. Floral buds less than 4 mm long from four other wild species including *L. auriculata*, *L. densipila*, *L. lindheimeri*, and *L. pallida* were also cultured at the three different densities in the same four carbohydrate media. For the species *L. pallida*, *L. auriculata*, and *L. densipila* the floral buds were sampled from two, five, and six plants, respectively. They were grown in two different temperature regimes of 20/15°C and 15/10°C. Only two plants of *L. lindheimeri* from the warmer chamber were used to sample buds. Depending on the number of plants available and the buds available from each species, the microspores were suspended in either 2.5 ml of media in 60 × 15 mm or 10 ml of media in 150 ×15 mm Petri plates. All cultures were dark-incubated at 32°C for 3 days.

After the initial heat shock treatment, the cultures were placed into a 24°C incubator in the dark and were scored for embryo development. The amount of embryogenesis and the efficiency of each microspore culture treatment on this limited number of selected Lesquerella species will be presented.

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MEDICINAL

DEVELOPMENT OF SCAR MARKERS FOR AUTHENTICATION OF *Atractylodes japonica* AND *Atractylodes macrocephala*

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Packchul, *Aractylodes japonica* or *A. macrocephala*, is a very important Chinese herbal medicine in Korea. It is also called Sabju in Korean botanical nomenclature. The levels of the active components are different between these two species, but these medicines are sold in Korean herbal markets without discrimination. This study was carried out to develop a method that could be used to discriminate between *A. japonica* and *A. macrocephala* by the application of the development of molecular markers.

Development of a convenient method to discriminate between the two species *A. japonica* and *A. macrocephala* was performed based on RAPD, AFLP, and SCAR molecular markers. Eighteen species-specific RAPD bands were obtained from 52 OPERON and URP primer sets. Three of the 18 RAPD bands were cloned into pGEM-T-Easy vectors and then subjected to the nucleotide sequence analysis and designated as AjR1 (1,117 bp), AjR2 (619 bp) and AmR1 (1,325 bp). As a result of the AFLP analysis, 74 AFLP clones were obtained and two clones, AjA1 (168 bp) specific to *A. japonica*, and AmA1 (138 bp) specific to *A. macrocephala*, were further analyzed. Two SCAR markers were developed only from RAPD clones: SAjR2 (600 bp) from AjR2 and SAmR1 (1,200 bp) from AmR1. These two markers were enough to discriminate between *A. japonica* and *A. macrocephala*, as well as between Korean and Chinese herbal medicines obtained from Korean herbal markets.

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COMPARISON OF ESSENTIAL OIL COMPOSITION BETWEEN *Angelica gigas* AND *Angelica acutiloba*

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Two kinds of Angelica belong to Umbelliferae collected, the one is *Angelica gigas* that is an inhabitant in Korean and the other is *Angelica acutiloba* that is indigenous in Japan. Plants grown at the Snyder Research and Extension Farm Rutgers University, New Jersey were analyzed by GC and GC/MS. The composition of the essential oil of the different aerial parts of Angelica has been studied. The oil yields obtained upon hydrodistillation were 0.18% (v/w) in Korean Angelica and 0.44% (v/w) in Japanese Angelica on dry root weight basis.

By the growing stage in the Rutgers greenhouse condition, leaf and root of essential oil content a little decreased on 9 months later than 4 months later except for *Angelica gigas* leaf. Both of Angelica showed that the quantity of the essential oil content present was in the order leaf > petiole > root according to different plant part.

The analysis of the essential oil from the Angelica root led to the identification of 14 constituents totaling 64% in Korean Angelica and 13 constituents totaling 68% in Japanese Angelica. The major constituents of the Angelica root essential oil were ligustilide (47%) and gamma terpene (14%) in the Korean Angelica, and alpha pinene (32%) and nonane (25%) in the Japanese Angelica

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CONSERVATION, CULTIVATION, AND UTILIZATION OF MEDICINAL CROP IN KOREA

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For hundreds years before the introduction of Western medicine, Koreans have used medicinal herbs for health maintenance and treatment of the illness. At present, Western medicines are mainly used for medical treatment. More recently, the importance of natural drugs has been emphasized due to possible side effects of synthetic drugs. Therefore, all countries in the world are extensively making efforts to develop new medicines containing natural substances. The functional effects of natural substances have been investigated for the production of medicines and health maintenance, cosmetics, food, and food additives.

At one time, Korea produced medicinal herbs of high quality in sufficient quantities for export. However, with increasing needs of the raw materials for oriental medicine and utilization for food additives, its domestic production has become inadequate. Now, more than 50% of market demand depends upon import from other countries. Thus, we suggest here a plan for developing industries related to medicinal herbs based on the recognition of their importance with economic analysis for the current situations, agricultural production, and market demands of medicinal plant resources.

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Top of Page

Return

ADDENDUM

THE INSTITUTE FOR BIOBASED PRODUCTS

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The Institute for Biobased Products was established by line item funding from the Montana State Legislature in 2001. Additional funding has been provided by USDA-CSREES in 2003 to allow the Institute to develop into a series of research and commercialization efforts to benefit the rural communities of Montana, the Pacific Northwest, and the Northern High Plains. The Institute provides coordination among university faculty, staff, and students on identified research projects and provides a commercialization avenue through its Montana Agricultural Innovation Centers. The Innovation Centers are currently in the planning stage but will transfer technology to rural communities by providing feasibility, market analyses, business strategies, business planning, and marketing recommendations.

Projects currently under development by the Institute include biobased fuels and lubricants, equine care products and nutrition, food safety, meat products, bio-pathogens, and gluten-free cereals. The research involves scientists at the Montana State University campus and the University of Montana as well as faculty at six of the state's research centers. The administrative center is located at the Northwest Ag Research Center in Kalispell, Montana. The Montana Agricultural Innovation Center will also be a virtual center with the administration being conducted by the Mission Mountain Market in Ronan, Montana. The Innovation Center will be comprised of six satellite centers, each affiliated with one of the agricultural research centers. This will allow a maximum of information transfer between research and application. In 2004, the Institute will be expanded to encompass additional local needs such as malting grains, beef products, vegetables, and energy. For a project to be approved by the Institute, it must undergo a market analysis prior to research or development.

Research funded by the Institute requires a market feasibility be completed before research can be initiated. Basically the Institute asks the researcher (or the marketing specialist at the Institute) to identify the market, who the competitors are, the growth of the market, and the market

value. Upon approval by the Institute's Board of Directors, the researcher can develop research consistent with commercialization including timelines. The end product of the project must include enterprise budgeting and an implementation plan. This document is then circulated to the Innovation centers for commercial development. Research will be encouraged also to come from the Innovation centers to the research centers. This two- way interaction is seen as important to developing good research-commercial interactions.

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Top of Page

Return