

# 2005 AAIC Annual Meeting: International Conference on Industrial Crops and Rural Development

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## Program Abstracts

[Plenary Session](#)

[Bioenergy](#)

[Soil Bioremediation and Wastewater Treatment](#)

[Poster Session 1:](#)

Bioenergy; Soil Bioremediation and Wastewater Treatment

[Bioproducts](#)

[Poster Session 2:](#)

Bioproducts

[Natural Rubber and Resins](#)

[Oils: Part 1](#)

[Oils: Part 2](#)

[Fibres, Proteins, and Carbohydrates](#)

[Poster Session 3:](#)

Natural Rubber & Resins; Oils; Fibres, Proteins, and Carbohydrates

[Networking and Panel Discussion](#)

[Top of Page](#)

[Return](#)

## PLENARY SESSION

### PROSPECTS AND USE OF RENEWABLE RAW MATERIALS IN THE USA

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Biomass crops, such as willow, poplar, and switch grass, and agricultural waste systems are positioned to become the new feedstocks for electric power, liquid fuel, and chemical production. Advances in technology in farm production and processing offer tremendous market opportunities for the nation's producers and hold the potential of transforming a significant portion of our fossil fuel based economy to a biobased economy for the

21st Century. The objectives for this continuing transition are many and will affect every phase of a new paradigm that includes directed and competitive research, formalization of coordination and cooperation between state and federal agencies, and greater inclusion of the private sector in developing the strategic plan for future implementation of all the component parts for this agenda. The discussion for advancement of these concepts related to bioproducts will focus on research-based approaches that include the private sector in making these products economically viable. Farmers and processors will need to be involved in practical and successful demonstration projects. Demonstration activities will include (a) programs for biofuels and lubricants; (b) agricultural utilization of new crops and new products; (c) biomass initiatives; and (d) biobased industrial product research, development, and commercialization. The development and expansion of a renewable fuel and biobased products industry founded on a strong and viable agricultural and natural resource sector can play an increasingly critical role in enhancing energy security, contributing to a cleaner environment, and promoting farm and rural economic growth and stability. Petroleum and fossil fuel prices, feedstock costs, co-product markets, energy and environmental policies, and advances in science and technology are critical determinants of market growth for biofuels and biobased products from agricultural commodities.

## **A PERSPECTIVE ON THE USE OF BIOPOLYMERS WITHIN THE EUROPEAN UNION**

J. Tomkinson

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## **INDUSTRIAL CROPS AND RURAL DEVELOPMENT: IMPACT OF THE COMMON AGRICULTURAL POLICY REFORM IN THE MEDITERRANEAN AGRICULTURE**

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After some thoughts on the genesis and nature of the development processes that are taking place in the rural areas of Mediterranean Europe and the role that agricultural systems are playing in these processes, the paper considers the need for a multifunctional approach to analyse what role agriculture should play in rural development. From a multifunctional viewpoint, it is necessary to bear in mind that agricultural systems fulfill other sociocultural, territorial and environmental functions, apart from production. These functions are often related to the externalities that are generated from farming activities.

Within this new context, not only does agriculture have to produce foods, fibres, energy, etc., efficiently, but it also has to do this by occupying land naturally and aesthetically, and contributing to the sustainable economic, social and environmental development of rural communities.

As source of renewable resources, industrial non-food crops offer specific opportunities for integration in the development processes of many rural areas. These specificities range from productive and agronomic questions to land occupation and the internalisation of some externalities of agricultural systems. Within the context of the CAP Reform, the trends in WTO agreements and the environmental problems induced by global climate change, this paper analyses what features could determine the interest on non-food crops of being better or worse suited for inclusion in sustainable rural development processes.

## **ALTERNATIVE CROPS FOR INDUSTRY AND RURAL DEVELOPMENT IN SPAIN**

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The prospects of growing crops to provide industrial feedstocks in Spain are assessed in terms of the benefit to rural communities by creation of new jobs in the agricultural sector and by developing small business activities. Industry has shifted interest to plant-derived natural materials for crop protection, energy, food preservation, fragrances, and pharmaceuticals.

Current conventional industrial crops in Spain are: sugar beet (*Beta vulgaris* L.), cotton (*Gossypium herbaceum* L.), tobacco (*Nicotiana tabacum* L.), olive (*Olea europaea* L.), and sunflower (*Helianthus annuus* L.). European agricultural policies have undergone a period of considerable change that threaten in one-way or another the future viability of these agricultural sectors.

The objective of this paper is to report on the scope for possible alternative industrial crops in Spain by using a combination of literature reviews,

European project results, and authors' experiences.

In the Mediterranean region, the potential for crops suited to dryland conditions should be greater, for example oilseeds such as safflower (*Carthamus tinctorius* L.), castorbean (*Ricinus communis* L.), and Ethiopian mustard (*Brassica carinata* A. Braun); or those providing unique specialty oils: marigold (*Calendula officinalis* L.), vernola (*Euphorbia lagascae* L.) and borage (*Borago officinalis* L.), for new applications in place of established oleochemicals.

Cardoon (*Cynara cardunculus* L.) which has been extensively researched in many Spanish regions is the most promising biomass energy crop in the Iberian Peninsula. New processing facilities close to the production sites should promote rural development.

Novel fibre crops to Spain such as kenaf (*Hibiscus cannabinus* L.) as well as better known esparto grass (*Stipa tenacissima* L.), hemp (*Cannabis sativa* L.), and flax (*Linum usitatissimum* L.) have potential in paper and board industries or outlets in niche markets: high value hand made carpets, textiles or handicrafts. Development is facilitated if multipurpose crops can be developed, for example, using stems for fibre, the seeds for oil, and the by products for cosmetics or pharmaceutical use.

Popular condiments in Spanish cuisine such as paprika (*Capsicum annum* L.), saffron (*Crocus sativus* L.), thyme (*Thymus vulgaris* L.), have worldwide acceptance. Such crops require only a small arable land area, but have good prospects if marketed with a guarantee of country of origin or if organically produced.

Cosmetic products derived from jojoba (*Simmondsia chinensis* L.) and aloe (*Aloe vera* L.) are in demand by European consumers. In Southeast Spain, suitable soil and climatic conditions exist for these alternative crops.

Alternative crops also have to be viewed in terms of their benefit to the environment. Plant species useful for: soil biofumigation or phytoremediation (Cruciferae, Compositae), erosion control (Chenopodiaceae), building soil fertility (Leguminosae), biological control of pest enhancement (Umbelliferae), or wastewater treatment (macrophytes) can be used to improve the sustainability of areas of intensive agriculture in Spain.

Waste management is an important problem in the Mediterranean countries. In future, it is likely that greater use and recycling of by-products from the agro-food industries will occur to obtain, for example, biomass for biofuels, organic soil amendments, active antioxidant or medicinal compounds, dietary fibre, and various raw materials for novel bioproducts.

[Top of Page](#)

[Return](#)

## **BIOENERGY**

### **REGENERATIVE ENERGY PRODUCTION USING ENERGY CROPS**

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As a result of shrinking fossil fuels regenerative energies gain importance. Besides hydro power and wind power, biomass is one of the most important regenerative energy sources. Due to the increasing meaning of biomass for energy generation it is essential to investigate convenient thermal procedures. On this evidence an analysis and evaluation of diverse conversion technologies for biomass usage with different boundary conditions is indispensable.

Kind and form of the biomass as well as the type of the plant cause different qualities of energy output as well as compositions of emissions. Conversion plants show differing characteristics concerning the biomass and the produced quality of gas and energy generation, depending on reactor type, kind of heat supply, and the pressure ratio in the gasification reactor. Conversion technology for the combustion of wood is extensively well-engineered, whereas the usage of other biomass like straw or sewage sludge, especially for gasification is insufficiently approved. According to this it is necessary to investigate an appropriate procedure for the use of biomass.

### **CHARACTERISTICS OF PELLET FUEL FROM COTTON GIN BYPRODUCTS UTILIZING VARIOUS PROCESSING TREATMENTS**

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Agricultural plant wastes when properly processed into useful commodities can become an economic asset. It has been estimated that in the United States, there are over 2.04 million Mg of cotton byproducts generated each year. On average, the disposal of these byproducts costs the cotton gin approximately \$1.65 (U.S.) per Mg. One means of changing a financial liability into a potential revenue generator is to process the byproducts into renewable, compact pellet-type fuel that can be used at the site or transported to the consumer. 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 liquefied 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 cotton gin byproducts using various processing techniques, determine its physical properties, and measure the emissions when fired in a commercial pellet stove used for residential heating.

Byproducts from two cotton gins were collected and processed into fuel pellets. Seven different treatments were evaluated. The treatments resulted from using different material streams from the ginning process as well as varying quantities of starch and/or crude cottonseed oil during the fuel pellet manufacturing process. The fuel pellet density from the various treatments ranged from 488 to 678 kg/m<sup>3</sup>. The various treatments were burned in a conventional pellet stove (four replications) and the gaseous and particulate emissions measured.

The average calorific value of the pellets ranged from 17.9 to 20.9 MJ/kg (HHV). The ash content for the various treatments ranged from a low of 4.9% to a high of 9.8%. The sodium content indicated concentration ranges from 91 to 282 ppm depending on the treatment.

The emissions from the cotton gin byproduct pellets were higher than for a premium grade wood pellet. The emissions measured during testing were CO, NO, NO<sub>2</sub>, SO<sub>2</sub>, and particulates. The pellet stove was setup following the manufacturer's recommendation to burn wood pellets, but was not adjusted for the cotton gin fuel pellets.

By utilizing various additives and processing techniques, cotton gin byproducts could be used to manufacture a pellet fuel that has economic potential. However, work remains to minimize the ash content and determine the optimal settings for maximizing combustion.

## OVERVIEW OF USDA-CSREES SUPPORT FOR BIOBASED PRODUCTS AND BIOENERGY

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Industrial crops provide raw materials for the production of numerous industrial and consumer products such as textile fibers, coatings, adhesives, personal care products, and pharmaceuticals. In addition, industrial crops are a plentiful source of fuels that can lessen U.S. dependence on imported energy supplies. Use of agricultural biomaterials for fuels or products provides a renewable alternative to petroleum-based feedstocks along with the potential for reduced emissions and by-products and improved biodegradability of end products.

The National Research Initiative's Biobased Products and Bioenergy Research Program supports innovative research to improve the conversion of biobased materials into value-added industrial products and biofuels. The interplay of various interests include characterizing feedstock, improving/developing catalyst, and improving/designing more efficient processes/reactors for biomass conversion.

Several projects that have served to advance the scientific knowledge base for the production of value added biobased products will be highlighted. Eligibility for NRI support is limited to U.S. citizens; however opportunities for international collaborations are highly encouraged.

## BIOREFINERIES FOR THE CHEMICAL INDUSTRY – A DUTCH POINT OF VIEW

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The Dutch government has defined very ambitious policy targets for biomass in the longer term (2040), namely 30% fossil fuel substitution in the power and transportation sector and 20 to 45% fossil-based raw material substitution in the industrial sector. Biomass is expected to be a major contributor with an anticipated share of more than 50%. Presently, biomass in the Netherlands that is not used for food applications is mainly utilised as animal feed and fuel for power (and heat) production. Biomass is converted mainly by means of direct/indirect co-firing in conventional coal-fired power plants and also by stand-alone combustion plants (Cuijk, Lelystad). To meet the longer-term policy ambitions, biomass has to be

applied in additional market sectors of the Dutch economy, using new thermochemical and (bio) chemical conversion/production processes, such as advanced gasification and fermentation technologies. Within this framework, biorefineries are believed to play a major role in the transition to a more sustainable Dutch economy. Realisation of high-efficient biorefining processes at places where biomass can be gathered, grown and/or imported and where the “green” products can be sold to a cluster of chemical and material industries are believed to be key technologies to meet the longer-term policy goals.

The paper will first provide the societal and institutional context for the transition to sustainability in evolving the chemical industry. Second, it reviews various perspectives on the future of a modified chemical industry, partly resulting from emerging technological opportunities. Third, by taking into account these emerging technological opportunities, this presentation will discuss the potential of the use of biomass in the chemical industry of today and tomorrow. The following aspects related towards biomass will be addressed – sustainability issues related towards biomass for the chemical industry, the potentials of biomass refining and pretreatment. The enormous prospective of thermal, chemical and bioconversion technologies, partly as a result of the ever increasing knowledge on thermochemical and biotechnological pathways, for the conversion of biomass into chemical products will be shown. The effect of chemicals and materials design on the chosen processes will be presented. Special emphasis will be given towards the potential that “biorefineries” offer the chemical industries.

### **IRRIGATION AND N-FERTILIZATION EFFECTS ON THE GROWTH AND PRODUCTIVITY OF THREE SUNFLOWER HYBRIDS IN AN AQUIC SOIL IN CENTRAL GREECE**

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The growth and the biomass productivity of the kenaf variety Tainnung 2 were investigated under optimal and sub-optimal irrigation and N-fertilization inputs in western Thessaly, Greece, in 2004. A 3×4 factorial completely randomized split-plot design was used in three blocks with the main plots comprising three irrigation treatments, and the subplots comprising four nitrogen dressings. The study soil was a deep, calcareous, fertile clayloam, classified as Aquic Xerofluvent, that represents large areas in the extensive Karditsa plain in central Greece. The crop was harvested periodically during the growing period and in each harvest, plant height, stem diameter, leaf area index, total dry, and stem dry biomass were measured. It was found that fertilization within the studied rates did not affect growth and biomass productivity of the crop, apparently due to the high fertility status of the study soil. Contrary to fertilization, a significant (P=0.05) effect of irrigation was found, with the fully irrigated plants (500 mm) reaching maximum growth rates in excess of 270 kg ha<sup>-1</sup>d<sup>-1</sup> and dry biomass reaching 17.5 t ha<sup>-1</sup> and by 9% and 21% lower productivity for irrigation inputs equal to 50% and 25% of the potential evapotranspiration, respectively. Stem biomass contributed to about 90% of the total dry biomass for all treatments by the end of the growing period. The leaf area index reached 4.3 (full irrigation), and remained above 3 (in all treatments) for large parts of the cropping period. Maximum height and stem diameter of the fully irrigated plants were 337 cm, and 2.5 cm, respectively.

### **BIOETHANOL FROM CORN FIBRE: ADVANTAGES OF THREE STEP HYDROLYSIS**

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**Author(s) not present.**

### **DILUTED ACID HYDROLYSIS PRETREATMENT OF AGRO-FOOD WASTES FOR BIOETHANOL PRODUCTION**

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Due to its environmental benefits, bioethanol is considered a promising biofuel for substituting gasoline fuel in transport sector (Portrait, 1999). In order to make it competitive against fossil fuels it is necessary to reduce its production costs by using new alternative biomass feedstocks. Due to its high agricultural potential, Spain has a strong food processing industry that produces approximately 450,000 tons of organic waste per year. The canning industry has to be taken into account because of its increasing development and capacity in Spain. Despite its development, increasing amounts of wastes are being generated by this kind of industries, and solutions have to be developed in order to diminish its environmental effects.

For this study wastes from fresh and processed vegetables have been used as feedstocks for a diluted acid hydrolysis process using sulphuric acid as catalytic agent.

The results obtained from the dilute acid hydrolysis assays regarding simple sugar solubilisation in the liquid fraction have been very successful reaching values of 3.61 and 7.83 g/L for tomato and red pepper residues, respectively. The suitability of this pretreatment has also been evaluated by the low levels of sugar degradation compounds detected in the samples which are significantly lower than the concentrations reported in the literature (Larsson, 1999; Oliva, 2003). In the case of legume residues a subsequent saccharification step is required to maximise sugar solubilisation.

In conclusion, due to the easy accessibility of sugars in fresh and processed vegetable wastes, they can be considered as potential feedstocks for bioethanol production.

## INDUSTRIAL APPLICATIONS OF *CYNARA CARDUNCULUS* FOR ENERGY AND OTHER USES

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In this work a review of *Cynara cardunculus* L. is presented. The species can be cultivated as an energy crop and be used for industrial purposes. As an energy crop it is grown according a perennial cultivation system especially developed for producing biomass. For that purpose the economic crop produce is the whole above-ground biomass. As an energy crop two categories of products are cropped: lignocellulosic biomass and oil seeds. The lignocellulosic biomass is regarded as a solid biofuel that can be directly used for generating electricity or for domestic heating. The seeds are also suitable for energy applications since they contain oil that can be used as a raw material of biodiesel. In addition to the energy applications of the crop other applications are reported: green forage, paper-pulp, pharmaceutical compounds and rennet.

[Top of Page](#)

[Return](#)

## SOIL BIOREMEDIATION AND WASTEWATER TREATMENT

### BIOFUMIGATION:

#### A NEW TECHNOLOGY BASED ON THE USE OF SULFUR CONTAINING METABOLITES IN *BRASSICA* PLANTS

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Over the last decade, the demand is for sustainable, nonpolluting agricultural techniques that are able to provide healthful products safe for the end user. This requirement is crucial also in view of new international regulations that will progressively reduce any agricultural and food treatments that are still being allowed. In this context, possible less dangerous alternative procedures to control soil-borne pathogens, nematodes, and post-harvest storage diseases of fruits and vegetables will become critical in the near future. Biofumigation based on the use of cruciferous-derived materials appears to be suitable as an alternative technology as it shows great promise for improving human health and reducing environmental problems. Thus, in recent years, an increasing interest has developed for natural compounds with high biocidal activities as possible substitute for methyl bromide and other synthetic fungicides. In contrast, conventional chemical treatments to control similar pathogens have become less acceptable by the public as some treatments have become ineffective. One of the most promising emerging technologies makes use of biobased compounds and products derived from plant materials containing the myrosinase-glucosinolate system. This is due to the strong biocidal activity of the biofumigant isothiocyanate-glucosinolate-derived products. To exploit this enzymatic system to control soil-borne and post-harvest fruit pathogens, several vitro and in vivo trails were carried out, including an original and economically sustainable oilseed extraction procedure able to provide an oil with improved protein meal and bioactive molecules. The bioactive, sulfur-containing metabolites were tested against post-harvest pathogens. A pilot-plant facility for fruit treatment and storage was constructed to run the tests. Some important findings and a general overview on the use of biofumigation technology in different fields of application for controlling different plant diseases under real conditions will be presented.

## UPTAKE OF LEAD AND ZINC BY WILD PLANTS GROWING ON CONTAMINATED SOILS

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On 25 April 1998, toxic water and tailings from a pyrite mine in Aznalcollar (Southern Spain) spilled into the Agrio and Guadiamar River basins affecting an area of about 55 km<sup>2</sup>. Immediately after the spill, the Autonomous Council of Andalusia began soil reclamation activities in order to minimize the potential impact caused by leaching of the toxic heavy metals in the affected area. Even after physically removing sediments, the soils remained polluted with trace elements such as Pb, Cu, Zn, Cd, Tl, Sb, and As. In studies performed in the Aznalcollar area, several plant species have been identified as not only being able to grow on these polluted soils, but also accumulate high amounts of the pollutants in their shoots. Our objective was to assess the performance of the different wild plant species to extract Pb and Zn as an economically viable alternative to other physical on site methods for detoxifying polluted soils. The implications of these results for phytoremediation are discussed.

### PHYSIOLOGICAL CHARACTERIZATION OF A METALLIFEROUS FLORA: IDENTIFICATION OF PROMISING SPECIES FOR PHYTOREMEDIATION PURPOSES

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Current technologies for soil remediation are very expensive, especially in the case of heavy metal contamination. Some plants are able to accumulate high concentrations of toxic ions, but most of them have very low growth rates, and therefore are not suitable for phytoextraction purposes. Plants species that exhibit high growth rates and can spontaneously colonize heavy metal-contaminated areas would be likely candidates for soil remediation.

Mature seeds were collected from plants growing on a contaminated site from the Sierra Minera of Cartagena ("Peña del Aguila", Llano del Beal) in the southeast of Spain. Four species were considered: *Atriplex halimus* L., *Zygophyllum fabago* L., *Dorycnium pentaphyllum* Scop., *Piptatherum miliaceum* (L.) Cosson. Seedlings from the collection were grown on Hoagland-modified nutrient solution in a fully controlled environment and exposed to various doses of Zn (0-1000 µM), Cd (0-100 µM), Pb (0-50 µM) or to a mixture of those contaminants. Growth kinetics, plant water status, ion accumulation, photosynthesis, and carbon isotope discrimination were quantified for various duration of exposure to the heavy metals (up to 8 months).

Among the analyzed species, both *Atriplex halimus* and *Zygophyllum fabago* were able to survive in the presence of high concentrations of heavy metals and to accumulate significant amounts of Cd and Zn in their shoots with only a limited impact on plant metabolism. Heavy metal tolerance was linked to the stimulation of antioxidative defense and to the over-synthesis of osmoprotecting compounds such as proline, glycinebetaine, and polyamines. Both species, however, displayed a high level of intraspecific variability for growth and for heavy metal accumulation. Moreover, salinity may interfere with cadmium absorption and reduce it, probably as a consequence of CdCl<sup>+</sup> complex formation in the nutrient solution. In contrast, *Dorycnium pentaphyllum* was able to resist high levels of external contamination, but did not translocate heavy metals to the shoot. *Piptatherum miliaceum* was sensitive to Cd and Zn, but highly resistant to Pb.

It is concluded that species from this metalliferous flora may be used either for phytoextraction (*Atriplex halimus* and *Zygophyllum fabago*) or phytostabilization (*Dorycnium pentaphyllum* and *Piptatherum miliaceum*). These species also constitute an interesting material to identify physiological properties linked with heavy metal resistance in plants.

### ATRIPLEX HALIMUS AND BITUMINARIA BITUMINOSA: UTILISATION OF THE INTRASPECIFIC VARIATION OF THESE MULTI-PURPOSE SPECIES

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Two species of particular interest to us are *Atriplex halimus* L. (Chenopodiaceae) (Saltbush) and *Bituminaria bituminosa* (L.) C.H. Stirton (Fabaceae) (= *Psoralea bituminosa* L.). *A. halimus* grows throughout the Mediterranean basin and is tolerant to drought, heat, and salinity. It is used to provide forage in the arid and semiarid areas. Its extensive root system can stabilise degraded soils. However, its geographical distribution is limited by low winter temperatures. It also has potential use in phytoremediation of metal-contaminated sites because of its high tolerance to and accumulation of heavy metals. Interest is also directed to its saponin content with possible biocidal activity and utilisation as a herbal medicine. *B. bituminosa* is a nitrogen-fixing perennial legume used (after cutting and drying) as a forage plant in the Canary Islands. However, its productivity is limited by cold winters and summer droughts. The plant is also of interest due to its ability to stabilise degraded soils, its possible role in phytoremediation of heavy metal-contaminated soils, its furanocoumarins content (used in cosmetics and photochemotherapy), and its iso-flavanoid derivatives, which have anti-clastogenic activity.

At IMIDA, we have collections of *A. halimus* and *B. bituminaria* from a wide geographical range, spanning the Mediterranean Basin countries and the Canary Islands. We wish to select and characterise populations that are tolerant to the prevailing edapho-climatic conditions in the region of Murcia (Southern Spain) with poor soil quality of low organic matter content, low and irregular rainfall (200-500 mm per year, with a summer drought), high potential evapotranspiration (900-1300 mm per year), high summer temperatures, and sub-zero winter temperatures in the upland areas.

We found a clear relationship between the freezing tolerance of the plants in the field and that of the leaves in the laboratory. Initial results show that, for *A. halimus*, the tested populations from Spain were more cold-tolerant than those from North Africa. The tolerance seemed to be related to osmotic adjustment and salt accumulation in leaves. For *B. bituminosa*, the plants from the sites with colder winters (high-altitude zones in Tenerife and Murcia), were more cold-tolerant, possibly due to their greater ability to decrease their relative water content in winter.

Based on our observation on cold-tolerance, the intraspecific variability within the two chosen species should allow selection of populations tolerant to the semiarid conditions of Southern Spain. Tolerant lines that can be grown in low- (or no-) input systems will be suitable not only for sustainable livestock rearing and soil stabilisation, but also for other novel uses.

## **AQUATIC MACROPHYTES FOR WASTEWATER TREATMENT**

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This work focuses on the use of aquatic macrophytes for the treatment of wastewaters in constructed wetlands. Types of macrophytes, mechanisms of contaminant removal and systems of wastewater treatment with macrophytes are reviewed. Recently-developed system Floating Macrophytes Filter (FMF) is described. Performance of FMF at a pilot wastewater treatment plant is presented. Finally, EU Life-Environmental demonstration project of the FMF system is reported.

## **PIG SLURRIES SUSTAINABLE REUTILIZATION:**

### **POTENTIAL APPLICATION FOR CROP PRODUCTION AND SOIL REMEDIATION, WATER RECYCLING AND DISPOSAL RISK ASSESSMENT**

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Pig slurry constitutes at the moment a very serious environmental issue mainly due to the change of pig production to a productive system of intensive type what means that slurry doesn't have a territorial area where it can be applied or poured. We show in this paper the results of some projects to determine the sustainable revalorization of the pig slurries for crop production, polluted soil remediation and water recycling. As a consequence, we propose different alternatives of reutilization of those slurries minimising also disposal risks. Those alternatives should be considered among others as solutions to manage safely pig slurries.

[Top of Page](#)

[Return](#)

## **BIOPRODUCTS**

### **CHEMICAL PRODUCTS FROM TEMPERATE FOREST TREE SPECIES**

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Forest trees are an integral part of the landscape and rural economy. Unfortunately, the economic returns from timber production have declined



significantly in parts of Europe. In some cases it is no longer economically viable to harvest trees for timber or pulping. The challenge is to find alternative uses for, and to add value to the forest resource. Only 25% of felled wood is converted to timber, the remaining material is a rich composite of primary and secondary metabolites and plant fibres, which represents a relatively unexplored and unexploited resource for potentially novel products that could complement revenue from traditional market outlets. Wood from temperate forest trees has traditionally been used as a source of tannins, terpenes, rosins, and aromatic phenolic compounds. This project set out to collate existing information on such chemical groups to identify the most suitable routes for further research and development.

Information was collated on the primary and secondary metabolites of alder (*Alnus glutinosa*), ash (*Fraxinus excelsior*), aspen (*Populus tremula*), beech (*Fagus sylvatica*), birch (*Betula pendula*, *Betula pubescens*), cherry (*Prunus avium*), corsican pine (*Pinus nigra*), Douglas fir (*Pseudotsuga menziesii*), larch (*Larix decidua*, *Larix kaempferi*), oak (*Quercus robur*, *Quercus petraea*), poplar (*Populus nigra*, *Populus gileadensis*, *Populus alba*, *Populus trichocarpa*), Scots pine (*Pinus sylvestris*), sitka spruce (*Picea sitchensis*), and willow (*Salix alba*, *Salix fragilis*).

Information was extracted from commercial and in-house databases, phytochemical databases, research papers, conference proceedings, books, unpublished reports and company literature. In total, over 37,000 records published over the last three decades were interrogated. Data extracted included, identified metabolites, the tissues from which they were extracted (e.g., bark, leaves, heartwood, roots), reported yields, reported properties, and hazards. Very little data currently exists on the yield or variability in yield of individual metabolites, which currently limits the ability to assess economic potential.

Traditional and new markets for exploitation of tree metabolites were also reviewed along with possible methods of extraction. Computer-aided Quantitative-Structure Activity Relationship Modeling (QSAR) augmented the search for novel applications for the tree metabolites. This tool was then used to model and predict the potency of tree metabolites as anti-microbials. Some monoterpenes with useful anti-microbial properties were identified.

All the information presented and the metabolite database has been made available via the worldwide web at [www.tree-chemicals.csl.gov.uk](http://www.tree-chemicals.csl.gov.uk).

### **BLACK COHOSH (*ACTAEA RACEMOSA* NUTT.) PROPAGATION, ROOT GROWTH, LEAF AREA, AND PHOTOSYNTHESIS RATE**

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Black cohosh (*Actaea racemosa* Nutt., Syn. *Cimicifuga racemosa*) roots were traditionally used by Native Americans to treat female complaints and today are used to treat menopausal problems. Most roots come from wild harvest in the eastern U.S. of which 95% is exported. Wild populations are declining and there is an urgent need to conduct research in the propagation and cultivation of this specie to supply the increasing demand. The plant is not difficult to grow, but very little information is published for those who want to grow it artificially. The objectives of this research were (i) to characterize black cohosh growth and development under artificial shading, (ii) to determine the minimum root size that can be used for vegetative propagation, and (iii) to determine photosynthetic assimilation and the rates of carbohydrates accumulation during one season of growth. The experiment was conducted in Chillán, Chile. Roots for propagation were imported from North Carolina, U.S. in 2001. They were planted, at 30 × 30 cm distance between plants and 5-cm deep, and shaded with PVC with 80% light absorption in July 2003. The experimental design was a randomized complete block design with four replicates. Different root sizes and root ages were used to obtain seven treatments, that included the whole root (T1), root portion of one-year-old (T2), root portion from the last growing season (T3), one-half of the T2 portion (T4), one-half of the T3 portion (T5), stolon with four buds (T6), and root bud alone (T7). The evaluations included the number of buds and stems, plant height, total leaf area and leaf area index, leaf nitrogen content, CO<sub>2</sub> assimilation rate (AR) and stomatal conductance (SC), root weight and root relative growth rate (RGR). Plants derived from the whole roots (T1) and root portions from the last growing season (T3) had greater number of initial buds and produced more stems. A close relationship was found between initial root weight and plant height, total leaf area and root growth. All treatments showed similar AR and SC (6.01 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> and 1.54 cm s<sup>-1</sup>, respectively), and leaf nitrogen content. The RGR of roots was significantly different among treatments with the highest value for T5. Vegetative propagation of black cohosh is possible using fragments of rhizomes that include the root portion of one-year-old (T2) or one half of the last growing season roots (T4).

### **MEDICINAL PLANTS WITH HYPOGLYCEMIC/ANTI-HYPERGLYCEMIC PROPERTIES: A REVIEW**

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Diabetes mellitus is one of the major diseases currently affecting an estimated 143 million people worldwide and the number is growing rapidly. In the USA, about 18.2 million or 6.3% of the population suffer from diabetes or related complications. Diabetes is an epidemic among African Americans in general and Native Americans in particular. The estimated direct and indirect costs of diabetes exceed \$132 billion annually. Some of the major reasons for the increasing rate of Type 2 diabetes also called non-insulin dependent diabetes are stress, and lack of proper diet and physical exercise.

Plant-based medicinal products have been known since ancient times. About 800 plant species have been reported to possess antidiabetic properties. Several plant species have been used for prevention or managing diabetes by the Native Americans, Chinese, South Americans, and Asian Indians.

A limited number of medicinal plant species have been studied and validated for their hypoglycemic properties using laboratory diabetic animal models and in clinical studies using human subjects. Several medicinal plants and their products (active, natural principles, and crude extracts) have been reported in the literature as having been used to control diabetes in the Indian traditional system of medicine called 'Ayurveda'. Among these species, *Allium cepa*, *Allium sativum*, *Aloe vera*, *Coccinia indica*, *Caesalpinia bonducella*, *Eugenia jambolana*, *Ficus bengalensis*, *Gymnema sylvestre*, *Momordica charantia*, *Mucuna pruriens*, *Ocimum sanctum* syn. *tenuiflorum*, *Pterocarpus marsupium*, *Swertia chirayita*, *Syzygium cumini*, *Tinospora cordifolia*, and *Trigonella foenum-graecum* are considered the more effective and more extensively studied in relation to diabetes and their complications. Plant species adapted to North America, such as prickly pear (*Opuntia robusta*), *Rosemarinus officinalis*, *Ocimum gratissimum*, and noni (*Morinda citrifolia*) have also been evaluated for their hypoglycemic properties using laboratory animal models in western countries.

Several oral hypoglycemic agents are the primary forms of treatment for diabetes. However, prominent side-effects of such drugs are the main reason for an increasing number of people seeking alternative therapies that may have less severe or no side-effects. Thus, plant based herbal drugs or botanicals are emerging as the primary components of holistic approaches to diabetes management. In this review, selected species that have been validated for their hypoglycemic or antihyperglycemic properties using laboratory diabetic animal models and in clinical trials using human subjects, and published in refereed journals are presented.

## FRUIT AND VEGETABLE PROCESSING BYPRODUCTS AS SOURCES OF FUNCTIONAL FOOD INGREDIENTS

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Epidemiological studies have related the dietary consumption of plant-derived food products, and particularly fruits and vegetables, with a decrease in the incidence of some cancer types and cardiovascular disease mortality. This diet-related health benefit has been associated with the phytochemicals intake as one of the key factors. Clinical and recently gene expression studies support the role of plant food phytochemicals as health-promoting food constituents. The phytochemicals are preferentially biosynthesised in the external plant tissues. These external parts are the main waste material during handling and processing of fruits and vegetables in the food industry and constitute a good source for extraction of phytochemicals.

Some plant-food industrial residues from production of juices, musts, wine, oli and cider are currently used for the extraction of phytochemicals from oranges, grapes, apples, and industrial residues from olives. In addition, the use of tomato and berry residues for phytochemical recovery (lycopene and anthocyanins) has been recently developed.

In a recent project, we have explored the use of vegetables residues for the preparation of phytochemical extracts and dietary fibre. This study included cauliflower, artichoke, lettuce, broccoli, celery, and onion residues. The amount of wastes generated by the vegetable industry can reach up to 70% of the harvested plant material as in the case of artichokes. A simple extraction method by boiling the fresh plant material with water or methanol-water mixtures, extracting and purifying with filtration through non-ionic polymeric Amberlite resin columns can yield extracts containing large amounts of the polyphenolic antioxidants. The extracts can be used after freeze-drying or spray-drying for the preparation of dietary supplements or ingredients in various types of food products (juices, soups, etc.). The extraction of specific phytochemicals such as folates from leafy vegetable residues, sulphur compounds from onion wastes, and glucosinolates from Brassicaceae residues is also possible. Research on the metabolism, bioavailability, and physiological activities of the phytochemicals present in these extracts in humans are necessary to understand their health-promoting properties, and to support their use as ingredients of functional foods. In addition, it is necessary to evaluate the risk of pesticide residue concentration during the extraction and concentration processes.

## DEVELOPING ESSENTIAL OIL RESEARCH AND PROCESSING INDUSTRY IN WESTERN CANADA

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Essential oils are primarily used in the preparation of fragrances such as soap and perfume. However, there is a growing interest in their application for the food and nutraceutical industries. Hydro-distillates and extracts from aromatic plants are valuable ingredients that might be incorporated into bioproducts to improve their functionality (sensory, shelf-life, nutraceutical value).

Factors such as plant varieties, growing conditions, harvesting, and processing influence the quality and quantity of essential oil production. The Olds College School of Innovation (OCSI) laboratory is conducting a study on the quality of essential oils produced in Western Canada. Selected essential oils from herbs, medicinal plants, and conifers were analyzed for their components. The GC and GC/MS systems were used for the analyses. Two columns of different polarities, polyethylene glycol (DB-Wax) and 5% phenyl 95% polydimethylsiloxane (HP-5), were utilized to enable the separation of several co-eluting components. Up to 127 compounds were identified that accounted for 78 to 97.6% of the oils.

Red clover extracts were analyzed for isoflavones by HPLC equipped with a laser light scattering and ion trap detectors.

Data for representative samples from commercial producers, farmer associations, and research organizations were entered into a database. The constructed database will contain information on the geographic location, agricultural practices, as well as the chemical composition of the aromatic and medicinal plants grown in Western Canada.

Such a database will be a crucial tool for the development of specifications, trade standards, and quality control processes for the herbal and medicinal plant industry.

Five of the researched plants (juniper, artemisia, chamomile, flax, and red clover) have been selected for a pilot commercialization study that includes test production of cosmetics (soaps, lotions, creams) containing their oils and/or extracts.

#### SEASONAL VARIATION OF *THYMUS HYEMALIS* LANGE AND SPANISH *THYMUS VULGARIS* L. ESSENTIAL OILS COMPOSITION

M.J. Jordán,<sup>1</sup> R.M. Martínez,<sup>1</sup> K.L. Goodner,<sup>2</sup> E.A. Baldwin<sup>2</sup> and J.A. Sotomoayor<sup>1</sup>

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*Thymus hyemalis* L. and Spanish *Thymus vulgaris* L. shrubs are characterized by a large chemical intraspecific variability among the plants. This fact makes it difficult to detect real changes occurring in their essential oil composition during the vegetative cycle. Based on this, the clones of *T. hyemalis* and Spanish *T. vulgaris* were used in this present work to monitor seasonal variations in the composition of the essential oil. Shrubs were harvested at five different phenological stages during the vegetative cycle. The volatile profile of the essential oil samples was determined by capillary GC/MS analyses. This technique identified 99 and 98 components in *T. hyemalis* and *T. vulgaris* essential oils, respectively.

For the Spanish *T. vulgaris* essential oil, the major components quantified were cineol, followed by terpenyl acetate, borneol, linalool,  $\beta$ -pinene,  $\alpha$ -terpineol, and camphor. With respect to the concentrations of some of the most abundant components, the vegetative stage seems to be the most appropriate harvesting time for this species. Cineol, borneol, monoterpene hydrocarbons, and camphor exhibited their maximum concentrations at this phenological stage. In contrast, terpenyl acetate,  $\alpha$ -terpineol, and linalool, probably components that are associated with the fresh aroma in the oil, were mostly concentrated from full bloom to advanced fruit formation. Correlations were detected among the concentrations of the most abundant components in this essential oil. Thus, terpenyl acetate and cineol concentrations varied during the entire vegetative cycle. The same behaviour was observed between sabinene and linalool.

For the *T. hyemalis*, the thymol, which defines the chemical type and the essential oil quality, and its precursors *g*-terpinene and *p*-cymene, showed synchronized patterns of variation during the entire vegetative cycle. In this way, the maximum relative concentration of *g*-terpinene, a precursor of *p*-cymene, was achieved at the full bloom (FB) phenological stage that coincided with the minimum concentration detected for *p*-cymene, a precursor of thymol. However, the maximum relative concentration of thymol was detected at full bloom/at the beginning of fruit maturation (FB-FR). From these observations, we can conclude that between FB and FB-FR stages could be the period beyond which the sequence *g*-terpinene @ *p*-cymene @ thymol begins. On the other hand, the essential oil exhibited the highest amount in alcohols, ketones, and esters at the vegetative stage.

#### AROMATIC PROFILES OF *THYMUS HYEMALIS* AND SPANISH *THYMUS VULGARIS* ESSENTIAL OILS BY GC-MS/GC-O

1 1 2 2 2

K. L. Goodner, K. Mahattanatawee, A. Plotto, M.J. Jordán, and J.A. Sotomayor

<sup>1</sup>USDA Citrus and Subtropical Products Laboratory, Winter Haven, FL, USA; <sup>2</sup>IMIDA, Murcia, Spain;

*Thymus hyemalis* L. and Spanish *Thymus vulgaris* L. shrubs are characterized by a great chemical intraspecific variability among plants. This property makes it difficult to detect real changes occurring in their essential oil composition during the vegetative cycle.

Based on this, clones of *T. hyemalis* and *T. vulgaris* were used to monitor seasonal variations in the composition of the essential oil. Shrubs were harvested at five different phenological stages during the plant vegetative cycle. Capillary GC/MS with olfactometry analysis will be used to determine the volatile profiles of the essential oil samples.

GC-Olfactometry analysis will be conducted on each phenological stage for *T. hyemalis* and *T. vulgaris*. Aroma extraction dilution analysis (AEDA) will be used to determine the aroma activities of the volatile constituents of the oils. Additionally, the Osme GC-O technique will be used at each dilution step to obtain more subtle information.

## **AROMATIC AND MEDICINAL PLANTS (MAP) PRODUCTION AS A RURAL DEVELOPMENT STRATEGY**

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The Non-Wood Products Department of the Forest Technology Centre of Catalonia has been contributing to the rural development in Catalonia (north Spain) with different projects and tools dealing with MAP production systems in different scales (from home-made to large-scale) and taking into account commercialisation and processing aspects. MAP production systems include both cultivation and sustainable wild harvesting.

The socio-economic situation of rural areas is heterogeneous and changeable and MAP crops are not a traditional activity. Otherwise, the industry demand of MAP raw material is increasing. This situation is a challenge for us to establish these crops in the primary sector.

The presentation of different studies and their analyses would help us to design new strategies to develop the MAP sector.

## **LESSER KNOWN HERBAL SPIRITS OF THE VALENCIA AND ALICANTE PROVINCES (EAST-SOUTHERN SPAIN)**

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Universidad de Alicante, Spain

Spirit art and its improvement, overtime and with different cultures, has allowed the development of many alcoholic drinks. In the South of Valencia Community (Spain), it has made characteristic herbal liqueurs by a variable number of plants soaked in sweet or dry anis liquor.

The complexity of the formulation and the diversity of plants used means there is practically one formula for each distiller family.

Yearly, several litres of this liqueur are prepared for their own family use. But, there are not many people who want to buy it and even in this case, the market is very limited.

Local well-known spirits are called “herbero” or “herberet”. Furthermore, the percentage of native species is higher (greater than 80 %), and the foreign species used are mostly old and traditional crops.

The number of species is different in each “herbero”, we can find recipes with about 8 to 27 species (mean 13). This is a substantial number if we compare this traditional liqueur with others which are available in the industrial market.

The quantities of each plant used in these spirits are small, between 10 g and 30 g of dry weight of plant per litre. The taste and flavour in each liqueur depends on the proportions of different essential oils present in the plants and the quantity of plant added. All these plants have traditional uses as local aromatic and medicinal flora. In these formulas some species could be considered a pattern, and they appear in a high percentage of the samples recorded (50-80%). The others can be found only in one or two samples, but these minor groups of species do not seem to be chosen randomly. Generally they are substitutions of similar species that belong to the same botanical families or even to the same genus. Often the local “herbero” makers are highly aware of plant constituents that are able to either enhance flavours in other plants added or even act as antidotes to toxins that other added plants contain.

The objective of the present work is to check and identify the botanical composition and ethnobotany subject related to the traditional making of

“herbero” spirits. The promotion and commercialisation of this local product could improve the economy of these little villages, but firstly requires good regulation and management for the long term use of the plants, mainly endemics, to prevent their overexploitation in the absence of controls.

[Top of Page](#)

[Return](#)

## NATURAL RUBBER AND RESINS

### DETERMINING OPTIMUM HARVEST TIME FOR GUAYULE LATEX AND BIOMASS

T.A. Coffelt and F. S. Nakayama

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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 period from 3 to 5 years to 2 to 3 years. One of the most valuable products from guayule is its hypoallergenic latex. However, little research has been done on the optimum harvest time for latex concentration and yield. The objective of this study was to determine the optimum harvest time during the growth cycle for latex content, plant biomass, and latex yield of guayule.

Treatments consisted of three guayule lines (Cal 6, AZR2, and AZ 101) harvested monthly for two years. Plants were transplanted on October 13-14, 1999 at the U.S. Water Conservation Laboratory in Phoenix, Arizona. Harvesting began in February 2002 and continued each month through January 2004. Samples were analyzed for latex concentration and latex yields were calculated based on the latex concentration and the dry plant biomass. The experimental design was a randomized complete block with four replications.

Results varied among lines. During the first year, peaks in latex concentration generally occurred in March/April, September, and again in December/January. During the second year peaks occurred in February/April, June/July, October, and again in January. Plant biomass was less variable and generally was highest in late summer and fall. Latex yield which is a function of both latex concentration and plant biomass was highest the first year in September and December, and the second year in June and October. These results indicate that for maximum latex a late spring (May/June) or fall (October/November) harvest may be best.

More research must be done to determine whether specific environmental factors can be associated with the optimum harvest time. There appears to be enough differences among lines that planting lines selected for different optimum harvest dates would allow growers to spread the optimum harvest time throughout most of the year.

### ABSENCE OF CROSS-REACTIVITY BETWEEN NATURAL RUBBER LATEX FROM PRODUCTION LOTS OF *HEVEA BRASILIENSIS* (HEV-B) AND *PARTHENIUM ARGENTATUM* (GUAYULE)

K. Cornish, W. Xie, D.K. Shintani and R.G. Hamilton

Yulex Corporation, Carlsbad, CA, USA

Natural rubber is a valuable raw material that is important to industry, transportation, medicine, and defense. It is largely produced from *Hevea brasiliensis* (Hev-b) plantations in Southeast Asia. Alternative rubber-producing crops are desired to increase biodiversity, protect supplies, and provide a safe rubber alternative for individuals suffering from life-threatening Type I Hev-b latex allergy.

Guayule latex is currently under commercial development as a source of latex that is non-allergenic to Hev-b sensitized individuals. It was initially targeted for use in medical products, for which synthetic polymers with acceptable physical properties and cost have not been identified. Earlier research demonstrated that guayule latex is low in protein and that its proteins are not recognized by mouse, rabbit, and human antibodies specific for Hev-b latex proteins. However, as production scales up, it remains important to confirm that the guayule latex continues to be safe for use by Hev-b latex sensitive people.

No Hev-b cross-reactive proteins were detected by the ImmunoCAP Inhibition Assay in ammoniated guayule latex using either the adult and pediatric IgE anti-Hev-b latex serum pools. The degree of inhibition was not significantly different ( $P = NS$ , Student t-test) from the neoprene negative control extract ( $< 1 \text{ AU ml}^{-1}$ ). This indicated an absence of detectable cross-reactive allergenic protein in the ammoniated guayule preparations. In contrast, the Redline and Triflex (a 100-fold concentrate) powdered latex examination glove positive controls produced 1,812 and 1,283,900  $\text{AU ml}^{-1}$  of detectable allergen, respectively. A low level of IgE anti-Hev-b latex reactive protein ( $3.9 + 4.2 \text{ AU ml}^{-1}$ ) was, however, detected in the non-ammoniated guayule latex, but only using the pediatric IgE anti-Hev-b latex serum pool. These data indicate that any potentially Hev-b cross-reactive protein in guayule is denatured following treatment with ammonia. Moreover, these low levels of Hev-b cross-

reactive protein in the non-ammoniated guayule latex contrast with the high levels of Hev-b allergen that are extractable from finished powdered Hev-b latex examination gloves (600-1800 AU ml<sup>-1</sup>).

Moreover, the polyclonal rabbit IgG anti-guayule raised against non-ammoniated latex (buffered-washed, purified rubber particles) reacted strongly against non-ammoniated guayule latex and more weakly against proteins from ammoniated guayule latex. However, these antibodies detected no proteins on either the ammoniated or non-ammoniated Hev-b latex allergosorbent, further suggesting an absence of structural similarity between Hev-b latex and guayule latex proteins.

These results indicate that proteins present in production lots of ammoniated guayule latex are not detected by human IgE, and thus are not cross-reactive with Hev-b latex allergens. This suggests that devices manufactured from guayule latex as an alternative natural rubber source should be safe for use by Type I Hev-b latex allergic individuals.

## POST-HARVEST STORAGE CONDITIONS ON GUAYULE LATEX QUALITY FROM AGRONOMIC TRIALS

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Current guayule commercialization efforts are based upon the production of hypoallergenic latex. However, little is known about the optimal agronomic conditions for maximum latex production. A series of agronomic trials were carried out over four years to investigate latex yield and quality in different lines of guayule planted at different times of year, and at different densities. Sub-samples of plants were harvested several times to determine the effect of shrub age on yield and quality. The effects of storage conditions on the harvested shrubs were also investigated. In this presentation, we report on the effects of the agronomic and post-harvest storage trials on the quality of latex extracted and purified from chipped shrubs from the agronomic trials. The latex quality parameters measured include rubber particle size distribution, rubber molecular weight, latex protein content, and other physical properties.

## AN OVERVIEW OF GUAYULE RESEARCH AND DEVELOPMENT IN MEXICO

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Guayule (*Parthenium argentatum* Gray, Asteraceae) is native to the semiarid lands in Mexico where it grows wild and associated with other species such as *Euphorbia antisyphillytica*, *Larrea tridentata*, and *Agave lechuguilla* in the states of the so called guayule region. Studies were carried out starting in the 1970s, related to: genetics, plant breeding, identification of high yielding plants, rubber and resin contents, rubber molecular characteristics (structure and molecular weight), environment and age effect on rubber production, agriculture management, and products development. Also, monitor of the rubber content as a function of age has been performed. The present paper presents an overview of work carried out in Mexico, particularly those accomplished by our research group. The results allowed the identification of plants and native sites with high rubber content that may be related to genetics, environment, agriculture management, and rubber accumulation as a function of plant age. Molecular weight determination showed that multimodal curves may be observed when the shrub is synthesizing rubber and the existence of synthesis cycles triggered by environmental factors. Irrigation management did not affect rubber content in the plant, but drastically increased the yield due to biomass accumulation. Significant findings were: shrubs with rubber content up to 21%, diploid plants at the Mapimi site, and total increment of rubber and resin yield was raised from 18 to 105 g plant<sup>-1</sup> by controlling the number of irrigation during the dry season.

## WATER STRESS EFFECTS ON RUBBER CONCENTRATION AND RUBBER DISTRIBUTION IN GUAYULE

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Guayule (*Parthenium argentatum* Gray) is a native of the Chihuahuan desert and is naturally subjected to periods of low water availability. Agronomic studies have shown that rubber yields increase with increasing irrigation, but rubber concentration per plant decreases. This study was conducted to examine how water stress affects rubber concentration and rubber distribution throughout the plant. One-year-old guayule plants of lines AZ-2 and AZ 101 were grown in a greenhouse and subjected to water stress from June through August 2003 and 2004. The well-watered

plants were irrigated daily and the water-stressed plants were irrigated when the average soil water potential reached 0.6 or 0.3 MPa. Plant responses to the different irrigation treatments were monitored by measuring the height, width, stem diameter, and photosynthesis. Fresh weight of stems and roots was recorded at harvest and a subset of plants was defoliated for leaf weight and leaf area measurements. Resin and rubber extracted from dried and ground plant samples with the leaves and stems from the subset of plants were analyzed separately. Water-stressed plants had lower photosynthesis, growth, leaf weight, and leaf-to-stem ratio than the well-watered plants. Rubber concentration, but not resin concentration, was higher in the water-stressed than well-watered plants, but had lower overall yield. Resin and rubber concentrations were approximately equal in leaves of the different treatments, but were significantly higher in the stems of the water-stressed plants. Rubber is deposited mainly in the bark parenchyma of guayule stems, and to a much lesser extent in other parts of the plant. The greater contribution of stem biomass to overall biomass in the water-stressed plants could result in increased overall rubber concentration. The water-stressed plants also had much smaller stem diameter than the well-watered treatment. The small stem diameter would cause the water-stressed plants to have a greater bark to wood ratio than the well-watered plants, which would also contribute to a higher rubber concentration in the water-stressed plants. Commercial processing is more efficient when guayule plants have small biomass and high rubber concentration. Water stress could be used as a management strategy to increase rubber concentration while decreasing the size of the plants. Plants could also be planted at a higher density to offset the loss of yield per plant that is the result of decreased biomass in water-stressed plants.

## **DEVELOPING A COMMERCIALY VIABLE SYSTEM TO THRESH, CLEAN AND CONDITION GUAYULE SEED**

W. Coates

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One of the problems in the commercialization of guayule (*Parthenium argentatum* Gray, Asteraceae) has been crop establishment. Transplanting has been shown to work well, but the costs are high. Direct seeding is cheaper, but in order for this system to work well the seed must be conditioned. To keep conditioning costs low and to allow the seed to be singulated in the planters the seed must be threshed and cleaned. To date a commercially viable system to do this has not been available. The objective of this project was to develop a commercially viable system to thresh, clean and condition guayule seed. The project investigated both a burr clover huller and a parallel belt system for threshing the seed. Cleaning systems investigated were: various combinations of screens, a gravity table, an air classifier and an indented cylinder. Modifications to the equipment were made to improve performance and prevent seed damage. Additionally a wide range of operating variables were examined for each piece of equipment in order to optimize performance. A seed conditioning system was developed. This allows large quantities of seed to be conditioned in a relatively compact area.

## **MAGNESIUM REGULATION OF IN VITRO RUBBER SYNTHESIS BY *PARTHENIUM ARGENTATUM***

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Natural rubber is an important resource due to its unique properties. It is a strategic raw material that cannot be replaced entirely by synthetic rubber. Currently, most of the natural rubber is produced in Southeast Asia by tapping of latex from *Hevea brasiliensis*.

The higher the molecular weight of rubber the higher is the quality of the material. Natural rubber is produced by *cis*-prenyl transferase (rubber transferase). The rubber transferase uses allylic pyrophosphate to initiate the rubber molecule and isopentenyl pyrophosphate (IPP) to elongate the molecule. Rubber biosynthesis also requires a divalent metallic ion. Very little is known about the rubber molecular weight regulation. Understanding the rubber transferase regulatory mechanism will allow the manipulation of plants to produce greater yields of high molecular weight rubber molecules. This would diversify natural rubber production, thus reducing global dependence on *H. brasiliensis*, which is susceptible to diseases, such as leaf blight. A potential crop for natural rubber production is guayule (*Parthenium argentatum*), a plant native to semiarid regions of the United States and Mexico, which is known to produce a high molecular weight rubber. If the regulatory mechanism of rubber transferase in *P. argentatum* is known, its yield may be improved without affecting the high quality of the rubber it produces.

In this study, we purified enzymatically-active rubber particles from *P. argentatum* latex and characterized the effects of magnesium on the biosynthetic rate of rubber in vitro and related them to the effects of magnesium on the in vitro rubber production by *H. brasiliensis*.

Magnesium is required for the activation of the rubber transferase, leading to an increase in the biosynthetic rate. However, excess magnesium inhibits the enzyme, thus decreasing the biosynthetic rate of the rubber molecule. The observed effects in *P. argentatum* are similar to *H. brasiliensis*, where the affinity of rubber transferase for IPP was shown to depend on magnesium concentration.

## RUBBER BEARING PLANTS OF CENTRAL ASIA

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[Top of Page](#)

[Return](#)

## OILS PART 1

### RESEARCH AND DEVELOPMENT OF BIOBASED INDUSTRIAL LUBRICANTS FOR THE TRUCKING AND RAIL INDUSTRIES

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Biobased products are broadly defined as nonfood, nonfeed industrial products derived from agricultural and forestry raw materials and wastes. Many biobased products are successfully entering the marketplace and adding value to agricultural materials beyond the traditional food, feed and fiber markets.

The University of Northern Iowa Ag-Based Industrial Lubricants program (ABIL) develops new products from soybean oil with funding, in part, from the U.S. Department of Agriculture. Twenty-eight lubricants have been commercialized to date. The program also addresses sustainable markets that integrate economic, environmental, and rural development considerations.

This presentation describes ABIL's research, development, and commercialization of two soybean oil products, semi-truck fifth wheel grease and rail flange grease. Major U.S. companies in the trucking and rail industries are now using soybean oil-based lubricants exclusively because of documented economic and environmental advantages. Advantages include better performance than the petroleum-based counterparts because of better adherence to metals, and higher lubricity and viscosity index. Human health and safety advantages include low toxicity, high flash and fire points. These products are total loss lubricants, i.e., they are lost to the environment during use, and thus, biodegradability is an important feature. Production of the greases in rural northern Iowa has resulted in new farming and business opportunities.

Because biobased lubricants can compete successfully with traditional petroleum products, they are catalyzing the emerging biobased industry in the U.S.

### OPPORTUNITIES FOR THE USE OF VEGETABLE OIL ESTERS IN THE PRINTING INDUSTRY

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Many organic hydrocarbon solvents traditionally used in industry contain high levels of volatile organic compounds (VOCs) associated with ozone depletion. As such, there is legislative pressure to curb their use and reduce emissions in the workplace. In 1999, the European Commission introduced the Solvent Directive (1999/13/EC), designed to reduce the emissions of VOCs from industrial processes by approximately 66% by 2007 (compared with baseline in 1990).

The UK is a significant centre for printing in the EU and the UK printing industry is the second largest user of solvents in the UK that are used in both ink formulations and washes to clean print machinery. The UK printing industry accounted for 14% of manufacturing solvent demand in 2000 and about 40,000 tonnes per annum of hydrocarbon-based solvent is used to clean print machinery. As a result of pressure from both new legislation and health and safety demands in the workplace, the printing industry is currently looking for alternative low VOC, low-hazard solvents as replacements for conventional petroleum-based solvents such as isopropyl alcohol and alkyl aromatics.

Vegetable oils and their fatty acid ester derivatives have a number of characteristics that suit their use in the solvent sector, which include good solvating properties combined with high boiling point and low VOC emissions. Vegetable oil based solvents have been widely used in ink formulations. However, uptake in print-wash solutions has been limited. Although vegetable-based print wash solvents have been used in



continental Europe (e.g., Germany and Denmark) they have not been taken up in the UK. The aim of the work was to address concerns and barriers in the UK industry to wider adoption. The limited experiences in the UK to date relate to experiences gained in, and publicity arising from, the EU SUBSPRINT project, which highlighted a few case studies in the UK and the issues raised by industry in response, many of which demonstrated a lack of understanding or reluctance to change working practices. In this new demonstration project funded by the UK Government (Defra), representatives of the UK printing industry and the UK Health and Safety Executive were approached to ascertain their attitudes and concerns relating to the use of conventional and vegetable derived solvent and to address these with practical demonstrations and evaluation of vegetable solvents in a commercial setting.

A key objective of the project was to work with the printing industry to provide a comparative assessment of vegetable-based and conventional (traditional and new low-boiling point hydrocarbon solvents) print blanket and roller washes used by the lithographic industry, to provide an independent appraisal of cost, performance, and hazards associated with vegetable-based solvents.

Three vegetable-based print wash solutions were sourced and evaluated against conventional print wash solvents on two lithographic printing presses, the Komori Sprint S228 (manually cleaned) and the four-colour Heidelberg QM46.4DI (automatic cleaning system). Cleaning performance and impacts on the subsequent print image, time and labour required to clean and volumes of solvent used were recorded over three print runs. Issues arising included the level of oily residue left on machinery by different solvents (which increased time to clean) and dealing with spills/oil contamination of equipment, and the need to match solvent formulation to print machinery wash system. The vegetable based cleaning agents proved to be as effective as the petrochemically derived cleaning agents, and costs per wash were comparable due to the lower volumes of solvent used with vegetable-oil based washes.

### **EARLY EMERGENCE AS AN INDICATOR OF COLD RESISTANCE AND PRODUCTIVITY IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)**

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As with other crops in rain-fed conditions, sunflower (*Helianthus annuus* L.) yield is affected by the amount of soil water and weather conditions. In the Mediterranean area, where adequate water is available in the winter and spring, but drought conditions prevail during seed filling, the potential yield is often related to the amount of water stored in the soil. This storage can be maximised if the crop grows in the first part of the year when there is reduced evapotranspiration. A genotype able to grow under low temperatures, and consequently reduced evapotranspiration, could save water for use during seed filling. Moreover, the water used when there is a higher frequency of rainfall could be restored, would allow higher yields later. In addition, early growth permits seed filling to occur before the drought period begins.

To determine whether early emergence in suboptimal conditions can be used to select for cold resistance and productivity in sunflower in East-Central Italian conditions, two F3 populations derived from two commercial hybrids (Dolia and Aurore) were sown in early February 2002 at Osimo (43° 29' N; 13° 28' E). Plants emerged within 15 days. These were divided into 5 classes of 3 days each. Many plants of each class were cross tested with a narrow genetic-based cytoplasmic male sterility line tester with good general combining ability. Test cross plant progenies from each population were evaluated together with three commercial controls in a completely randomised block design replicated three times in 2003 at Osimo.

The analysis of variance of the test crosses showed significant differences between groups for seed yield and oil content in both populations, and proved the importance of early emergence with regard to achene and oil production.

Higher achene as well as oil yields were obtained with group 1 (early emergence) in the Dolia population, whereas the third group gave better oil production than the fourth and the fifth groups.

In the Aurore population, however, the earliest emerged group showed no productive advantage either in terms of achenes or oil. In this population, the second and the third groups performed better than the respective remaining groups that emerged later, indicating that also for this population, the later emerging plants were less productive than the earlier ones.

Early emerged plant in suboptimal temperature conditions have a high potential that can be used in the selection of sunflower.

### **BREEDING STRATEGIES FOR IMPROVEMENT OF *LESQUERELLA FENDLERI* (BRASSICACEAE)**

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The obvious goal of a new crop breeding program is to facilitate commercialization at a profit for large-scale production. Plant breeding can have a significant impact on, achieving this goal, although improvements in agronomics, water management, mineral nutrition, and planting and harvesting technology will also help to improve this potential. The primary objectives for our breeding program for *Lesquerella fendleri* are to develop varieties with higher seed oil quantities, higher amounts of hydroxy fatty acids (HFAs), especially lesquerolic acid, and to improve seed yields. These traits are seen as having the greatest potential for reducing the cost of the seed-oil. Our breeding program has focused on the natural genetic variability contained within *L. fendleri* and on the introgression of traits from other *Lesquerella* species as the two main sources of improvements for new germplasm and varieties. The extensive germplasm collection that our laboratory has acquired over the past 10 years has provided an extensive source of materials. The objective here is to outline the breeding progress and strategies we have employed. The discussion will focus on the results from interspecific crosses, segregating populations, parental self pollinations, and on selected populations for various traits. *Lesquerella pallida* has been used as a source of traits to introgress into *L. fendleri*. This species was chosen because of its elevated HFA content compared with *L. fendleri* and because both species have the same  $n=6$  chromosome number. Plants were selected in the A2, A3, and A4 generations (designated as such instead of F2, F3, and F4 because the chromosome number was doubled using colchicine to produce amphidiploids) and some were able to produce seed without ovule culture. Transgressive segregates for the lesquerolic acid content trait were found in A3 and A4 generations where the parental values were approximately 50% and 80%. However, the seeds produced per silique were less than 1. The normal number of seeds per silique for *L. fendleri* is approximately 10 and for *L. pallida*, 5. This indicates that although the HFA content trait was successfully introgressed, seed yields have not recovered and will require more generations to restore full seed production, as was the case with canola (*Brassica napus* L.), which required five generation cycles. We examined the variability for lesquerolic acid content by half-seed analysis within a population of *L. fendleri* selected for high oil content (33%) and high seed yields (above 40 g/plant). We found lesquerolic acid to range from 46 to 70% in a population of over 900 seeds. We then selected those seeds greater than 62 and less than 53% lesquerolic acid by planting the remaining half-seed of the selected individuals, and then self pollinating. Traits being improved through these breeding strategies will make lesquerella more profitable for industry users and growers, thus enhancing its competitiveness with castor oil (*Ricinus communis* L.) as a biodegradable oil without the toxic ricin contained in castor.

## POSTEMERGENCE HERBICIDE TREATMENT EFFECTIVENESS FOR *LESQUERELLA*

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Weed control is critical for establishing lesquerella (*Lesquerella fendleri*). Lesquerella seedlings grow slowly after emergence, and offer little competition against broadleaf weeds. Several herbicides are registered under Special Local Needs (24c) in Arizona and Texas for the control of annual broadleaf weeds: (a) trifluralin provides adequate preemergence weed control, (b) fluzifop controls grasses postemergence, and (c) oxyfluorfen for postemergence control of annual broadleaf weeds. Alternate postemergence treatments for broadleaf weed control that have a broader spectrum of control and are more cost effective than oxyfluorfen must be investigated. The objective of this research was to examine the tolerance of lesquerella to six herbicides labeled for use in other field crops.

Our study was initiated 13 October 2004 at The University of Arizona Maricopa Agricultural Center. Advanced generation lesquerella seed was planted on level basins with a Brillion Seeder at 11 kg/ha and flood irrigated. Herbicide treatments consisted of plots (8 m long and 2.5 m wide) arranged in a randomized complete block design with four replications. The following postemergence herbicides were applied 23 November 2004 when the lesquerella seedlings were in the 8 to 10 true leaf stage: clopyralid (0.3, 0.6, 1.1 kg ai/ha), ethametsulfuron (15, 20, 30 g ai/ha), imazamox (35, 45, 56 g ai/ha), oxyfluorfen (0.3, 0.6, 1.1 kg ai/ha), thifensulfuron (15, 20, 30 g ai/ha), and thifensulfuron+tribenuron (15, 20, 30 g ai/ha). Treatments were applied using a CO<sub>2</sub>-powered backpack sprayer with a three-nozzle boom (Teejet 8003 flat fan nozzles) delivering 225 L/ha at 187 kPa.

Lesquerella stand density was determined in each plot by counting the seedlings in a 0.25 m<sup>2</sup> quadrant. Ten lesquerella seedlings in the 2 to 4 leaf stage were marked in each plot to monitor the effect of the herbicides on this growth stage. Overall lesquerella injury and weed control were estimated visually from 0 to 100% with 0 = no visible effect and 100 = complete crop death or weed control. Ratings were made weekly for 60 days by comparing each treatment with the untreated control within the same replication. Plant biomass and seed production were determined in June 2005 by harvesting an 8-m by 2-m area in each treatment with a Hege 180 combine. Seed oil production and oil content was determined at the USDA-ARS, U.S. Water Conservation Laboratory in Phoenix, AZ. Results from this study will identify postemergence herbicides that may be safe for use in lesquerella production.

## BIOLUBRICANTS FOR THE TEXTILE AND TANNERY INDUSTRIES AS AN ALTERNATIVE TO CONVENTIONAL MINERAL OILS: AN APPLICATION EXPERIENCE IN THE TUSCANY PROVINCE

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The use of chemical compounds of vegetable origin in industrial production shows several environmentally beneficial effects (renewable, biodegradable, low toxicity, no impact on CO<sub>2</sub> level) over synthetic products presently being widely used.

In this presentation, results will be reported and discussed on some pre-industrial studies on the potential of substituting some mineral lubricants (mainly alkylbenzene) widely used in textile (spinning phase of wool) and tannery (both in dry and damp phase) processing in two Tuscan industrial districts with formulations based on High Oleic Sunflower Oil (HOSO). This research was carried out by all the private companies that depend on seed production (farmers and their main associations, Seed Company), oil extraction, refining, and formulation. The new, low environmental impact formulations were tested at the industrial level to verify their application potential from the technical, environmental, and economic viewpoints.

The results confirmed that HOSO oil could substitute for mineral oils in textile and tannery industrial productions without any technical problem and without any facility modifications. In some cases such as crust leather production, the HOSO treatment improved the softness of the finished product. The environmental impact evaluation carried out by the DPSIR model confirmed how a higher sustainability could be obtained by the utilisation of new oils with a significantly higher biodegradability. Finally, the economic analysis showed a higher production cost and price of vegetable oils, but at the same time, indicated the possibility of reducing the required amount due to a lower utilisation rate. This decrease in usage in some applications cancelled the price difference between the mineral and vegetable-based oils. Based on our results, we conclude that HOSO can represent an efficient alternative to mineral oils, and that HOSO can be utilised in other industrial applications. Following these studies, a new commercial product called BIOVIT based on HOSO was developed and commercialised.

## AGRICULTURAL MANAGEMENT OF CUPHEA AND ITS POTENTIAL FOR COMMERCIAL PRODUCTION IN THE NORTHERN UNITED STATES

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Cuphea (*Cuphea* sp.) is a new oilseed crop that has undergone agricultural domestication for about the past 20 years. Its seed is rich in small- and medium-chain fatty acids that are highly valued for manufacturing soaps, detergents, personal care products, and industrial lubricants. Since 1999, our research group has focused on developing an agricultural management strategy for cuphea production utilizing conventional technologies to minimize the need for specialized equipment. Our long-range goal is to provide an economically viable crop that can be rotated with maize and soybean in a region of the U.S. predominated by these two crops. The semi-domesticated genotype PSR23 that was developed through the interspecific hybridization of *Cuphea viscosissima* Jacq. (native to the U.S.) × *C. lanceolata* W.T. Aiton (native to Mexico) performs well in temperate, short growing-season climates. PSR23 is an annual plant that has a relatively shallow root system, a high water requirement for growth, and prefers mild temperatures, particularly during its reproductive phase. By using the best management practices developed by our team, we have obtained seed yields as high as 1400 kg ha<sup>-1</sup>.

The summer of 2004 marked the first year for an experimental commercialization of cuphea. Technology Crops International, in cooperation with the USDA Agricultural Research Service, contracted six farmers within a 32 km radius of Morris, Minnesota (45.35°N, 95.53°W) to produce from 2 to 4 ha each of cuphea for a total of 18.6 ha. Some of the crop (about 2.6 ha) was lost to severe weather and herbicide drift from other nearby crops, but the harvestable plantings produced seed yields ranging from approximately 78 to 744 kg ha<sup>-1</sup> at 12% moisture. Valuable knowledge was learned through this experience that might not have been gained by plot-scale experiments alone. For instance, post-harvest management of seed on a large-scale (e.g., drying, cleaning, and storing) was problematic, indicating a further need for research and development in this area. Overall, the 2004 commercialization project made considerable progress in advancing cuphea towards large-scale production. This paper summarizes some of our research results regarding the best agronomic practices for cuphea production and reports on results obtained from the 2004 commercialization project.

## CUPHEA SEED OIL CONTENT AND FATTY ACID COMPOSITION CHANGES DURING SEED DEVELOPMENT

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Cuphea (*Cuphea viscosissima* Jacq. × *C. lanceolata* f. *silenooides* W.T. Aiton, line PSR23) is a new crop being developed in the north central U.S. as an industrial oilseed crop. Cuphea PSR23 seed has 300 to 350 g kg<sup>-1</sup> oil that is rich in medium-length fatty acids such as capric acid used to manufacture soaps and detergents. Cuphea also has the potential to replace coconut (*Cocos nucifera* L.) and palm kernel (*Elaeis guineensis* Jacq.) imports. The objective of this research was to determine when physiological maturity of an individual cuphea seed is reached and how seed development affects seed moisture, weight, oil content, fatty acid content, germination, and vigor. To evaluate seed development, 2000 cuphea flowers were tagged at anthesis in the field at Prosper, North Dakota in 2004. Each flower was tagged with the date of anthesis and position on the main stem or branch. Two hundred capsules from the tagged flowers were harvested at 3- to 4-d intervals from 5 to 48 d post anthesis. Seed weight increased as a function of growing degree days (GDD) (base temperature of 10°C) and the days from anthesis. Physiological maturity was estimated to occur when maximum dry seed weight was attained. Seed weight increases followed the Gompertz function with a R<sup>2</sup> = 0.90. All flowers, independent of stem position, statistically followed the same growth function for seed weight. The maximum seed weight estimated by Gompertz fluctuated between 3.22 to 3.97 mg seed<sup>-1</sup>. Physiological maturity estimated with a quadratic function occurred between 35 and 42 d post anthesis or 229 and 275 GDD. Seed moisture decreased from 900 g kg<sup>-1</sup> at 5 d post anthesis to 450 g kg<sup>-1</sup> at 48 d post anthesis. Seed germination was 83% when harvested 32 d post anthesis. Oil content increased from 98 g kg<sup>-1</sup> at 5 d post anthesis to 279 g kg<sup>-1</sup> 48 d post anthesis. Fatty acid composition varied throughout seed development. Immature seeds prior to 11 d of development had linoleic acid (18:2) and palmitic acid (16:0) as the major fatty acids. Seed developed for 15 d and greater had greater than 66% of capric acid (10:0). Cuphea should be harvested 32 d post anthesis when most capsules on the main stem have attained maximum seed weight (physiological maturity), germination, and oil content, but because, cuphea has indeterminate growth seed maturity varies within and among plants.

[Top of Page](#)

[Return](#)

## OILS PART 2

### THE USE OF NEAR-INFRARED SPECTROSCOPY (NIRS) IN THE STUDY OF SEED QUALITY COMPONENTS IN PLANT BREEDING PROGRAMS

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The standard methods of analysis traditionally used for the study of seed quality components in plant breeding programs (fiber, oil, protein, fatty acid, glucosinolate, etc.) are expensive, time consuming, need trained staff, and may use hazardous chemicals. In addition, most of the procedures involve sample destruction to conduct the analysis, which could be a handicap in the case of valuable and scarce materials. In contrast, the use of fast analytical techniques such as Near Infrared Reflectance Spectroscopy (NIRS) has many advantages over existing standard techniques. NIRS analysis is rapid, low-cost, and does not use hazardous chemicals. Furthermore, samples can be analysed in their natural state without destruction and with simultaneous analyses of several traits.

Our research group at the Institute of Sustainable Agriculture (IAS, CSIC) has been applying NIRS analysis for many years in the agricultural field. The research efforts of our group are focused in breeding for multiple seed quality components of different plant species of agronomic interest for semiarid conditions, such as *Vicia faba*, *Cicer arietinum*, and oilseed Brassicas.

In this paper, we review the main features and results obtained in using NIRS for the non-destructive analysis of different seed storage components, such as protein, oil, fiber, fatty acid, and glucosinolate in the different species noted previously.

The NIR analytical results indicate that the use of this technique is a valuable tool in the routine analysis of various seed quality components in many different plant species for our plant breeding programs. NIRS can be considered as an alternative to the standard techniques when a large number of analyses have to be performed for germplasm screenings, mutagenesis programs, hybridization programs, etc. In addition, seed samples can be analyzed non-destructively, which is of crucial importance in the case of scarce or valuable seeds.

### BIOBASED LUBRICANTS: IMPROVEMENT IN OXIDATION AND LOW TEMPERATURE STABILITY

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The search for environmentally friendly materials that has potential to substitute mineral oil in various industrial applications is currently being considered a top priority research in the fuel and energy sector. This emphasis is largely due to the rapid depletion of world fossil fuel reserves and increasing concern for environmental pollution from excessive petroleum use and their disposal especially in loss lubrication, military applications, and in outdoor activities such as forestry, mining, railroads, dredging, fishing, and agriculture hydraulic systems. Vegetable oils can contribute towards the goal of energy independence and security due to their naturally renewable resource. Vegetable oils are promising candidates as base fluid for eco-friendly lubricants because of their excellent lubricity, biodegradability, better viscosity-temperature characteristics, and low evaporation loss. Their use, however, is restricted due to low thermo-oxidative stability and poor cold-flow behavior. This paper presents a systematic approach to improve the oxidation and cold-flow behavior of vegetable oil derivatives and the study of antioxidant/antiwear additive synergism in vegetable oils using pressure differential scanning calorimetry (PDSC) and Rotary Bomb Oxidation Test (RBOT). Synergism was investigated on a set of one antioxidant and three antiwear additives. Among the various possible avenues available, the combination of chemical additives and high-oleic vegetable oils offer the best option for achieving the ultimate goal. Vegetable oil-based lubricants formulated using the preceding approach exhibit superior oxidative stability, and improved low temperature properties such as pour points compared to some of the commercially available industrial oils such as bio-based hydraulic fluids, biodegradable oils for heavy equipment, and biobased drip fluid for agriculture equipments. These vegetable oil-based formulations are comparable with petroleum-based lubricants for use in high-temperature and high-pressure applications, and often outperform the competition in some of their properties.

### **ENRICHMENT OF ANIMAL PRODUCTS WITH OMEGA-3 FATTY ACIDS USING CHIA SEED**

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There is considerable evidence suggesting that regular consumption of omega-3 fatty acids prevents cardiovascular diseases, including atherosclerosis and thrombosis. Most studies, however, have been carried out with fish products. However, in many countries such as the U.S., annual per capita consumption of marine products is low. In addition, for many people, a strong limitation for the use of fish as a food is that fish has been recognized as a potent allergen, both in food and occupational allergies. An alternative is to enrich products of animal origin most consumed and the goal of this project was to obtain an alternative to fish products using chia seed-based feed ingredient. Methodology included dietary different levels of chia seed (whole and grown), and chia oil. The research and development showed the feasibility of enriching animal products like eggs, poultry meat, cow's milk and pork meat with omega-3 fatty acids, and suggested that none of the current levels of omega-3 fatty acids that can be produced by the incorporation of chia in animal diets can be reached using flax, fish oil or algae-based diets without strongly affecting animal performance and/or one or more of the intrinsic characteristics of the final product. In all cases, the limiting factor for utilization of high percentages of available omega-3 sources, with the exception of chia, is flavor, smell and/or atypical textures transmitted by these sources to the products. Also, in the case of flax, animal production would be negatively affected.

### **THE EFFECTS OF HUMIC ACID FERTILIZER ON SAFFLOWER PRODUCTION**

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Although Turkey has high production potential of oilseed, it must now import 50% of the oilseed needs. In 2003, 1.3 million tons of oil seed and 300,000 tons of crude oil were imported in which the import cost was \$400 to 500 million. The major oilseeds grown in Turkey are cottonseed, sunflower, groundnut, and soybean. In 2003, the productions were 1.4 million tons for cottonseed, 220,000 tons for sunflower, 90,000 tons for groundnut and 75,000 tons for soybean. In contrast, safflower production was the lowest at only 25 tons. The total production of oilseed in Turkey is 2.5 million tons and refined oil is 735,000 tons. Edirne in northwest Turkey is one of three provinces of the Thrace peninsula region. More than one-half of vegetable oil factories of Turkey are in this region that provides employment for 3000 people. Because of the shortage of vegetable oil in Turkey, there is low consumption of oil per capita yearly (17 kg) and a need exists for oilseed factories in this region. Safflower (*Carthamus tinctorius* L.) has a high nutrient value and an alternative production of the crop is being tried. Furthermore, because safflower is resistant to arid conditions, it would be adaptable to the climatic and land conditions of the Thrace Region and Edirne. In general, there is little organic matter (1%) in the soil of both Turkey and the Thrace Region. For this reason, the yield and the quality of the plant are decreasing. To solve this problem, the use of humic acid has become widespread among the producers in recent years. For the producers to increase safflower production, we investigated the appropriate application method, the rate of humic acid fertilizer application, and the effects of this fertilizer on yield and quality.

This research was established on the Experimental Field of Trakya Agricultural Research Institute in Edirne, Turkey. The experiment was based on the Split-Split Plot on Randomized Complete Block design with three replications in 2004. Two safflower varieties, Dincer and 5-154, were obtained from the Eskisehir Agricultural Research Institute. Mechanical planting was made on 4 April 2004 on four-row, 0.9-m wide and 5-m long plots. Hand harvest was made on 6 August 2004. Nitrogen fertilizer was applied to the plots after planting and in addition three different doses of humic acid fertilizer (Control, 3000, 6000, and 9000 mL/ha) were applied to the leaves as potassium humate before flowering. Plant height,

number of branch achene's, number of seed per achene's, 1000 seed weight, seed yield, oil content, and fatty acid composition of the oil were determined.

The highest seed yield (1824 kg/ha) and the highest oil content (28.9%) were obtained for the 5-154 variety with 6000 mL/ha. The highest linoleic acid content (84.1 %) was obtained for the Dincer variety with 9000 mL/ha. The highest oleic acid content (40.8 %) was obtained for the 5-154 variety with 9000 mL/ha.

Our results show that the safflower varieties Dincer and 5-154 can be grown in Edirne, Turkey because of their high yield, quality, and oil content. In addition, 6000 and 9000 mL/ha of humic acid fertilizer can be applied to the 5-154 and Dincer varieties, respectively. Thus, the growth disorders that occur in safflower because of low humic acid content in the soil can be corrected.

## **EXPANDED USES OF CASTOR (*RICINUS COMMUNIS*) AS AN INDUSTRIAL CROP**

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The castor seed has evolved biochemical mechanisms that allow it to produce large amounts of ricinoleate without affecting membrane function. To elucidate the flexibility of the castor oil biosynthetic pathway, we have initiated the cloning of the genes for enzymes in the pathway, expressing these genes in active form, and characterizing their enzyme activity and specificity. We have cloned the diacylglycerol acyltransferase type 1 (RcDGAT1), a key gene involved in castor oil biosynthesis, carrying out the final step that converts diacylglycerol to triacylglycerol. The cloned gene has been expressed in yeast under the control of an inducible promoter, and yeast microsomal DGAT activity is enhanced seven-fold under induction conditions. Using lipase digestion to produce the substrate 1,2 diricinolein, we have compared the preference of the RcDGAT1 with that of DGAT type 1 from *Arabidopsis thaliana* (AtDGAT1). In this comparison, the RcDGAT has a preference for diricinoleoyl glycerol as a substrate, while displaying no preference for diolein or dipalmitolein substrates. We are currently characterizing the specificity of RcDGAT1 using substrates containing other polar fatty acids to determine the flexibility of castor in producing oil containing other useful fatty acids.

[Top of Page](#)  
[Return](#)

## **FIBRES, PROTEINS AND CARBOHYDRATES**

### **ADVANCED FIBER PLANT DECORTICATION - MECHANICAL AND THERMAL PROPERTIES OF THE FIBERS**

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Bast fibers of flax, linseed, and hemp have excellent mechanical properties, which predestine these fibers for diverse industrial applications, e.g., at the manufacturing of compound formed fabrics or fiber reinforced composites. Agriculture can provide such natural fibers as renewable raw material for many industrial applications at competitive prices.

The knowledge of the mechanical and thermal behavior of the bast fibers, however, is essential for the development, calculation, and manufacture of new materials and products.

Conventional retting of the bast fiber plants on the fields often causes a wide variation in the range of the fiber parameters by uncontrolled and frequently changing influence of moisture and temperature.

In order to limit this variation range, a new technology has been developed by the Institute of Agricultural Engineering, Potsdam, which is suitable for processing non-retted bast fiber plants, such as hemp, flax, linseed, and others. The decortication is accomplished by the impact stress of the beaters of a swing hammer mill directly on the surface of the stalks.

The machine line includes all process stages from pick-up and cutting of the straw bales up to the cleaning of the final products, which are the fibers and shives.

The processed fibers have a fineness in the range from 2.5 to 15 tex.

The fiber length varies in an adjustable range from 50 to 200 mm after the decortication. This length meets the requirements of many industrial

applications. The optimal fiber length for the production of composites is only 2 to 4 mm. Such lengths are cut after the fiber cleaning using special fiber cutting machines or cutting mills. Normally, the fiber length distribution is spread in the range from 1 to 13 mm after such a cutting.

The parameters of processed bast fibers such as flax, hemp, and linseed were measured and compared with sisal, jute, or kenaf. The average values are mean diameter of fiber bundles 80  $\mu\text{m}$  tensile strength 620 N/mm<sup>2</sup>, modulus of elasticity 50 kN/mm<sup>2</sup>, breaking elongation 1.6 %, remaining shives content < 2 %.

Thermogravimetric and Differential Thermal Analyses show that moisture desorption occurs in the temperature range from 30 to 110°C. The fibers are thermally stable in the range from 110 to 175°C. Thermal degradation starts above 180°C.

The bast fibers produced can substitute for synthetic, glass, and carbon fibers over a wide range of applications. The advantages of the bast fibers in contrast to glass and synthetic fibers are low density, high specific strength – comparable with steel, excellent adsorption of water and solvents, low power consumption for their production, biodegradable and lower price.

A capacity of 3 t/h of fiber plant input and a reduced investment permit the manufacture of natural fibers at competitive prices while meeting the requirements of many industrial applications.

### BIOMASS YIELDS OF KENAF IN SOUTH EUROPE

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Kenaf (*Hibiscus cannabinus* L.) is an annual crop of great interest for both the production of industrial raw materials and as bio-fuel under the pedoclimatic conditions of south Europe. Although kenaf is being cultivated worldwide mainly for fiber production, in Europe there is no much data concerning the adaptability, growth and biomass yields of the crop. There are only few references regarding the agronomic aspects of the crop at European pedoclimatic conditions. The aim of this work was to determine the sustainable yielding potential of kenaf as an energy crop in Southern Europe (Greece, Italy, Spain, Portugal and France), and to quantitatively assess the effects of the important cultivating techniques (irrigation, fertilization, sowing date and plant density) on crop performance. It should be pointed out that this work is part of a European project entitled "Biomass Production Chain and Growth Simulation Model" that funded in the 5th Framework Programme and started in March 2003 and will be ended in August 2006. In the view of this work a total number of thirteen field trials have been carried out in 2003 to determine the adaptability and productivity of kenaf in southern EU countries. In these trials the tested factors were: two late-maturity varieties (Tainung 2, Everglades 41), two plant populations (200,000 and 400,000 plants/ha), two sowing dates (early and end of May), four irrigation rates (0, 25, 50 and 100% of PET) and four nitrogen rates (0, 50, 75 and 150 kg N/ha). The dry stems yields came up to 20 t/ha (Greece and Spain) with a mean value between 13-15 t/ha, averaged overall sites and treatments. The main conclusions derived from the field trials were: a) the high plant population increase the biomass yields but this increase was not statistically significant, b) between the two sowing dates it was found that significant higher yields were achieved when the sowing took place in the beginning of May, c) in all sites, especially in the dry ones, the applied water was very critical factor for yielding maximization, while d) the increase of nitrogen application was not always increase the yields.

### BOLTING IN ROOT CHICORY: CHILLING ACTION

V. Debavelaere,<sup>1</sup> V. Dielen,<sup>2</sup> S. Lutts,<sup>1</sup> C. Notté,<sup>2</sup> C. Van Herck<sup>2</sup> and J.M. Kinet<sup>1</sup>

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Inulin is a fructan consisting of 2 to 60 fructose units with a terminal glucose unit. This molecule is used in the food industry especially for people suffering from diabetes as well as for non-food purposes. Root chicory (*Cichorium intybus* L. var. *sativum*) is the most efficient crop for inulin extraction. It is a biannual plant with vegetative growth during the first year and flowering during the second one, but is cultivated as an annual crop. Early bolting and flowering induced especially by low temperatures (vernalization) drastically decrease yield. However, to increase the inulin content in the roots, sowing is made very early in the year at the end of March or the beginning of April. This practice may expose seedlings to endure a cold period, which causes plants to bolt during the following summer, although the existence of a juvenile phase that is insensitive to vernalization is sometimes reported. The objective of this study consisted of developing a better understanding of the relationship between flowering and environmental conditions by determining (1) the effect of the sowing date, (2) the vernalization treatment, and (3) the influence of plant age on vernalization efficiency.

The experiments were performed during two growth seasons, in 2002 and 2004, using three root chicory varieties: (a) "Fredonia" (SAREA, Austria), which is considered to be sensitive to bolting, (b) "Melci" (Chicoline, Belgium), which is classified as moderately sensitive, and (c) "Orchies" (Desprez, France), which is classified as resistant. All experiments, except the one evaluating sowing date effect, started in growth rooms to control precisely the duration of the vernalization treatments. After the vernalization period (exposure at 4°C), the plants were transferred to the field.

Several distinct behaviours were observed. (1) The percentage of bolting increased with early sowing. The effectiveness of the chilling period depended on the variety. (2) Moreover, a positive quantitative relationship was observed between the duration of vernalization and the percentage of flowering. (3) Finally, the sensitivity to cold in terms of flowering induction evolved with plant age in a complex manner with the earliest growth stages (imbibed seed and early germination stages) being the most sensitive to cold. Furthermore, no juvenile phase of insensitivity to vernalization was found. Fluctuating temperatures (16 h at 4°C followed by 8 h at 15°C) were also able to cause bolting. Vernalization effectiveness was affected by climatic variations where high temperatures and drought could be among the environmental factors that interact with low temperatures to control the bolting of root chicory. The present work demonstrates that the efficiency of a vernalizing treatment is modulated by environmental conditions prevailing after its occurrence. The strategy consisting in advancing the sowing date to increase the duration of growth and yield has to be complemented by cultural practices and variety improvement.

## ON THE USE OF THE STALKS OF *HELIANTHUS TUBEROSUS* L. FOR BIO-ETHANOL PRODUCTION

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This work deals with the potential of *Helianthus tuberosus* L. for the production of sugars from the stalks, instead of the tubers, as a raw material of bio-ethanol. Final objectives of this proposal are to decrease the crop costs and to develop a perennial cultivation system. A field experiment was conducted to study the stalk sugar production of twelve clones of *H. tuberosus*. Biomass production, biomass partitioning and soluble carbohydrates content of the whole stalks were determined at three phenological stages: floral buds, blossom and head senescence. Results of the first year experiment showed that the peak stalk content in total soluble carbohydrates (TSC, 43.9% on dry weight) coincided with the phenological stage of floral buds for most of the clones. At that stage, the yield of the early clones (52.2 g TSC plant<sup>-1</sup>) was lower than the yield of the mid-season clones (158.8 g TSC plant<sup>-1</sup>), as a result of the lower stalk biomass production. The highest production was recorded for the clone Boniches but, as a drawback, it exhibited a low unitary tuber weight. The late clones China and Violette de Rennes showed the most balanced results in terms of stalk TSC yield (143.4 g TSC plant<sup>-1</sup>) and unitary tuber weight (5.1 g dry matter tuber<sup>-1</sup>).

[Top of Page](#)  
[Return](#)

## NETWORKING AND PANEL DISCUSSION

### EUROPEAN NETWORK ON SUSTAINABLE MULTIFUNCTIONAL UTILISATION OF UNDER UTILISED CROPS

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Recent paradigm shifts in European agriculture have led to heightened interest in changing production strategies. Reforms in land use and have stimulated new interest in the potential role for alternative crops including underutilised crops and commodities (UCCs). European researchers active in sustainable agricultural development within Europe are keen to apply underutilised crops and commodities to diversify existing production by encouraging local production and consumption and wider scale commercialisation. Many public and private organisations in Europe have expertise in underutilised crops' production and commodity development. Specialists in sustainable natural resources management, genetic resources management and utilisation, crop production, post-harvest handling and processing of products, market development and in socio-economic impacts on human livelihoods are working in universities, research organizations, and industry. However, their work on UCCs is often fragmented and uncoordinated to the point that at no single location is there a sufficient critical mass of effort for outputs to be delivered efficiently. Several underutilised crops and commodities can make significant contribution to several non-food uses, such as pharmaceutical and functional food uses that appeal to the markets in Europe (i.e., renewable energy, fibre, industrial products, oil seeds, herbs, and spices).



By considering the whole technology chain, from producer to market, European network of utilisation of multifunctional underutilised crops (ENUC) brought together specialists from all the major areas of concern. Through such an organization, a critical mass of targeted institutions was established, acting as virtual centre of excellence for longer integration. The objective of the network is to contribute to the development of durable structures based on common research interests in the area of underutilised crops and addresses the constraints hindering the development of their use. The network enables partners in Europe to integrate their activities for collaborative research and implementation of research programmes jointly for the development and utilisation of UCCs. This partnership with common objectives to develop research on commodity groups and their crops and to demonstrate its effectiveness in pursuing the goal of the network for diversification and sustainable development is based on consumer awareness and willingness to use specific products.

## **IENICA - AN INTERACTIVE EUROPEAN NETWORK FOR INDUSTRIAL CROPS AND APPLICATIONS**

C. A. Holmes

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Crop-derived products can provide novel raw materials, as well as technical, environmental and waste disposal advantages over synthetics derived from fossil sources, whilst offering alternative options for agriculture.

Utilization of renewable raw materials, however, is uneven and fragmented throughout Europe. In recognition of this, the EU in 1997 commissioned the Interactive European Network for Industrial Crops and Applications (IENICA) project. IENICA provides an overall, pan-European facility, involving 26 countries, linking all EU industrial crop activities.

IENICA has identified significant potentials for renewable raw materials in Europe through a series of integrated activities, including the production of a novel agronomic crop booklet, raw material specification sheets, newsletters, individual state reports, and an overall European Summary report, and the organisation of conferences and seminars. The project has also identified a number of barriers and constraints to the development of the industry, which must be overcome before true market exploitation of renewable raw materials can occur.

Of particular significance in this project is the introduction, in 2000, of 10 of the European Union's accession and associated states. Bulgaria, Cyprus, the Czech Republic, Estonia, Hungary, Lithuania, Poland, Romania, Israel, and Switzerland have joined EU-15 (except Luxembourg) in this work and the industrial crop activities and potentials in these countries have been studied for the first time. This approach allowed a comprehensive review of the nonfood crop industry in Europe, including the changes that will develop through a much-enlarged EU.

This paper outlines the major findings of the IENICA project, based primarily upon the European Summary compiled from information provided by all partner countries. This summary studies the fibre, oil, carbohydrate, and specialty market sectors and identifies future applications with the best potential for European-grown crops. It gives actual figures for current and potential markets and changes in the industry over recent years are discussed. Barriers and constraints for development are identified and legislation, regulations, and policies are analysed.

## **THE MEDITERRANEAN AGRONOMIC INSTITUTE OF ZARAGOZA (IAMZ) COOPERATIVE RESEARCH AND NETWORKING ACTIVITIES**

A. López-Francos

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The Mediterranean Agronomic Institute of Zaragoza (IAMZ) is one of the four institutes of the International Centre of Advanced Mediterranean Agronomic Studies (CIHEAM), an intergovernmental organization that groups 13 Mediterranean countries to develop cooperation through post-graduate training and promotion of cooperative research in agriculture and natural resources. IAMZ promotes and coordinates cooperative research programmes in order to facilitate the collaboration among experts belonging to research teams from CIHEAM member countries. The general objective is to analyse the problems affecting Mediterranean agriculture and the environment, carrying out a joint search for results and enabling the exchange of results by coordinating methodology and applied techniques. Structures to develop cooperative research programmes are research networks, working groups and research projects. Several of these structures are jointly coordinated with other international organizations, namely, FAO, EC, ICARDA, World Bank, EAAP, etc., where case experts from other geographical regions participate.

The research networks are structured groups of researchers, covering topics of wide scope, with a stable coordination structure and a large and consolidated participation. The working groups have more limited objectives than the networks and their participation is smaller.

The research projects arise from the networks and working groups and always have a fixed duration (normally 3 to 4 years) determined by their financing, generally external, mainly from the EC. Until 1989, a number of IAMZ networks collaborated closely with the AGRIMED Programme

of the Directorate General of Agriculture (D.G. VI) of the EC. In the 1990s, the CAMAR Programme of the EC-D.G. VI, financed eight projects which strongly consolidated the working groups of the EU Mediterranean countries. CIHEAM financed the participation in these projects of experts from the South and East of the Mediterranean. CIHEAM decided later to submit proposals only at the calls opened for its member countries and not just EU countries. In recent years, more than 20 projects have been submitted to the INCO and MEDA programmes, five of which have been financed. Currently, IAMZ is coordinating 3 EC funded projects, while one is in the final phase of negotiation, and a last one is in the submission process. IAMZ is also a partner in charge of the training activities in three more projects and concerted actions within the FP5 and FP6.

Activities generally fall into the following categories: (i) exchange of information and material, (ii) direct technology transfer through study missions, (iii) organization of seminars and courses, (iv) publications in scientific journals, (v) Mediterranean-scope research work using common methodologies, and (vi) preparation and development of joint research projects

The thematic orientations of IAMZ research activities are (i) Plant production: study, conservation and use of plant resources (nuts, apricots, other fruit trees, forest species, cereals, and legumes) adapted to arid Mediterranean conditions. (ii) Animal production: improvement of sheep and goat production systems adapted to arid Mediterranean zones, Mediterranean pasture and forage crops, Mediterranean aquaculture, both its technical and its socio-economic aspects, rabbit meat and Mediterranean pig production. (iii) Environment: forest restoration in the Mediterranean basin to combat desertification, climate change and its impact on agriculture and the environment, drought effects prevention and mitigation strategies and the evaluation and management of fishing resources. (iv) Agro-food marketing: the largest activity is focused on marketing nuts.

Scientific and technical publications are the most frequent outputs of research networks and projects. In 2003-04, eight issues of the "Options Méditerranéennes" journal and three other books were published related to these activities. Other outputs are the direct technology transfer through seminars, courses and study missions, and the previously mentioned submission of research projects to donors.

## **RURAL DEVELOPMENT IN THE REGION OF MURCIA 2000-2006, FINANCED BY FEOGA – ORIENTACIÓN**

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Director General de Regadíos y Desarrollo Rural, Consejería de Agricultura y Agua, Murcia

[Top of Page](#)

[Return](#)

### **POSTER SESSION 1**

#### **Bioenergy**

#### **ALTERNATIVE CROPS FOR THE PRODUCTION OF LIQUID BIOFUELS**

J. Fernández

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The adoption of Directive 2003/30/EC on the promotion in the use of biofuels and other renewable fuels for transport in Member States has encouraged the production of biofuels, thus becoming an emerging sector. As a reference target, 2% minimum share of biofuels is set by 2005 that should gradually rise to 5.75% by the year 2010. According to the Directive, biofuels are any liquid or gaseous fuel for transport produced from biomass. Therefore, the key point of the production chain of biofuels is the steady production of biomass.

There are two routes traditionally considered for the development of liquid biofuels: bioethanol and biodiesel. Bioethanol is presently produced from sugar- and starch-based biomass. Steps used in the process are: juice extraction, ethanol fermentation, distillation, and dehydration. Technologies for producing bioethanol from lignocellulosic materials are available, but they are not economically feasible yet. Current crops used in Europe for producing bioethanol are sugar beet, wheat, barley, and maize in the USA and sugarcane in countries such as Brazil with tropical climates. All of the biomass sources are conventional crops bred for human food. In this work, sweet sorghum (*Sorghum bicolor* (L.) Moench.) and Jerusalem artichoke (*Helianthus tuberosus* L.) are presented as alternative crops for bioethanol production, which produce a more economical raw material than conventional crops.

The other common route of the biofuels is biodiesel, a term applied to methyl esters produced from vegetable oils. The process of production of

biodiesel involves: oil extraction, fatty acids transesterification, esters separation, and purification. Rape and sunflower are the current crops used in Europe for biodiesel production. At present, biodiesel produced from these crops is not competitive with fossil diesel oil, so that subsidies and tax exemption are needed. In this work, the alternative crops: castor oil plant (*Ricinus communis* L.), oil palm (*Elaeis guineensis* Jacq) and jatropha (*Jatropha curcas* L.) for tropical and sub-tropical areas and cardoon (*Cynara cardunculus* L.) for the Mediterranean areas are presented.

### **TECHNO-LEGAL ASPECTS OF THE JATROPHA BASED BIODIESEL SYSTEM IN INDIA: AN ANALYSIS**

M.P.R. Mohan and N.V.L. Kumar  
TERI, New Delhi, India

**Author not available for presentation**

### **EVALUATION OF CARDOON (*CYNARA CARDUNCULUS* L.) BIOMASS PRODUCTIVITY IN SOUTHWESTERN SPAIN**

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Junta de Extremadura, Spain

*Cynara cardunculus* L. is a herbaceous perennial plant originally from the Mediterranean area with high lignocelulosic biomass yield. The main goal of the study has been the development of the cardoon crop in dry conditions to use its lignocelulosic biomass. For this aim trials have been carried out in Extremadura region. The characteristic studied over a seven-year period has been the aerial biomass productivity.

### **COMPARISON OF METHYL ESTERS FROM GERMAN, BRAZILIAN, AND SPANISH WASTE OILS**

M.P. Dorado, Universidad de Jaén  
EUP Linares, Spain

Vegetable oil used for deep frying processes becomes a contaminating waste product after use. Brazil, Germany, and Spain are some of the large producers of used frying oil in the world. Among the possibilities for recycling used vegetable oil is its conversion into either soap or biofuel of which the latter is the most interesting. The conversion of waste oil into biofuel for diesel engines is the most promising alternative due to its benefits to the environment. In this work, biofuels from Brazil, Germany and Spain were compared. The main objective was to investigate the implications of the factors involved in the transesterification of the selected used oils. The raw materials used were the waste oils from Brazil (palm oil, soybean oil and hydrogenated fat), Spain (olive oil), and Germany (mixture of several vegetable oils of unknown origin). The transesterification process was carried out in two steps using a stoichiometric amount of alcohol, the necessary amount of catalyst, and supplemented with the exact amount of catalyst to neutralize acidity. Several parameters related to their performance as fuel were analyzed. Results revealed that free fatty acid (FFA) content is a key factor to determine the viability of the waste vegetable oil transesterification process. The higher the acidity of the oil, the lower was the conversion efficiency. All methyl esters had adequate densities, but slightly higher viscosities, which could contribute to incomplete combustion. The Higher Heat Value (HHV) was slightly lower for the biodiesel than for the diesel fuel, which can be tolerated. The transesterification reaction did not require any additional purification. However, the high acidity that was present in some of the samples produced corrosion problems to the engine. This was considered to be a key factor to take into account. Finally, we conclude that the two-step transesterification process without any costly purification step is an economical method for biofuel production using the waste vegetable oils from different sources.

### **Soil bioremediation and wastewater treatment**

#### **POSSIBLE USE OF *ALLIUM* BY-PRODUCTS FOR SOIL FUMIGATION**

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CRITT Innophyt, Tours, France

The pesticide properties of *Allium* spp. (garlic, onion, leek, shallot...) are attributed to sulphur compounds that are produced by the degradation of the plants tissues. The active molecules against insects, nematods and pathogens are mainly disulfides and more precisely dimethyl disulfide

(DMDS), diallyl disulfide (DADS) and dipropyl disulfide (DPDS).

The objective of this study was to investigate the possibility of using *Allium* spp as biofumigant by incorporating into soil onion and leek by-products.

The fungicide potential of *Allium* by-products (onion and leek) on *Pythium ultimum*, a pathogenic fungi that is a sign of the soil health, was studied thoroughly. Moreover the behaviour of the disulfides emanating from the by-products incorporated in soil was investigated in vitro during several weeks.

The disulfides show a different efficacy and DMDS, being the most active compound, produce a stimulant effect on the plant growth. The onion and leek by-products are proved to be effective in vivo on *Pythium ultimum*. After incorporation of onion or leek in soil, the only persistent metabolite is DPDS, detected during more than one month.

thus *Allium* spp present a biofumigant potential as a possible alternative of methyl bromide as it is recently developed with Brassica spp. The choice of *Allium* spp must be done in relation with the composition of DMDS because it is the most active sulphur compound.

### **EFFECTS OF BIOFUMIGATION PLUS SOLARIZATION ON CROP PRODUCTION**

M.M. Guerrero, C. Ros, M.A. Martínez, M.C. Martínez and A. Lacasa  
IMIDA, Murcia, Spain

In more than 45 ha of sweet pepper organic crop in Murcia Region, biofumigation plus solarization is used to pathogens control and for its low environmental effect.

In one experimental greenhouse infested by *Meloidogyne incognita*, the effect of the reiteration of this method has been measured through the yield. Disinfection began in August and treatments were compared with Methyl Bromide and untreated soil in a complete block design with three replicates per treatment.

Since the second year of reiteration of biofumigation plus solarization, *M. incognita* control levels were similar than MB; plant growth was lower or the same as MB and finally, marketable and total yield were higher or the same as MB according to the year of reiteration, but it was always superior to the untreated soil.

Biofumigation plus solarization is shown as an effective and stable method for soil disinfection.

### **EFFECTS OF BIOFUMIGATION ON SOIL FERTILITY**

P. Fernández, M.M. Guerrero, M.A. Martínez, C. Ros, A. Lacasa, A. García and A. Bello  
IMIDA, Murcia, Spain

In the Southeast of Spain, sweet pepper crop grown under greenhouse conditions, is a monoculture which is disinfected because of two reasons: soil pathogens *Phytophthora capsici* and *Meloidogyne incognita* and soil fatigue due to the reiteration of the crop in the same soil. The last phenomenon makes a decrease in yield. Biofumigation plus solarization technique is an alternative to Methyl Bromide that is carried out in experimental greenhouses in Murcia. The measure of application effects of organic amendment with a reiterated use over soil fertility and environmental sustainability, its sustainability have been the aims of this work

First results reveal that there are significative differences in physical parameters in biofumigated and solarized soils versus MB, with a 5% of apparent density and a decrease in the instantaneous and accumulated infiltration in a 50%. This process improves the mobility of water into soil, a great aspect to take into account since sweet pepper crop is affected by root asphyxia.

The application of organic amendment has raised macro and micronutrients in assimilated way. This fact has an outstanding consequence in the crop and it is an important characteristic for soil fertirrigation and organic agriculture.

### **EFFECTS OF BIOFUMIGATION PLUS SOLARIZATION ON SOIL MICROBIOLOGY**

M.A. Martínez, M.M. Guerrero, C. Ros, M.C. Martínez, A. Lacasa and J. Tello  
IMIDA, Murcia, Spain

In greenhouses in Murcia Region, sweet pepper is grown as an organic crop where biofumigation plus solarization is used as a soil disinfectant. The efficacy of this method over soil pathogens increases when the process is repeated in the same soil every year. The effects of different treatments and different years of reiteration (several quantities of fresh sheep manure + chicken manure) over soil microbiology have been studied. Biofumigation plus solarization treatments were compared with Methyl Bromide and one untreated soil.

Both non disinfected soils and biofumigated and solarized soils for the first time had a higher fungi density: *Fusarium* genera, *Aspergillus*, *Penicillium*,... than soils disinfected with MB or with the reiteration of B+S. Along the growing season, *Fusarium solani* and *Aspergillus* spp. density were increasing at the same time that sweet pepper crop in soils disinfected with MB. The increase was lightly lower in soils disinfected with the reiteration of B+S. Results reveals that soil fatigue is reduced with the reiteration of biofumigation plus solarization, so this disinfection method has a low environmental effect in soil biodiversity.

## **REUSE OF TREATED WASTE WATERS FROM THE FRUIT AND VEGETABLE PROCESSING INDUSTRY IN AGRICULTURAL IRRIGATION**

L. Rincón, A. Abadía, A. Pérez, J. Sáez, J.A. Linares and C. Pellicer  
IMIDA, Murcia, Spain

In this work, some aspects of the modernisation of the irrigation projects used by the Community of Irrigation Users of the Campos del Río (Murcia) are analysed, a modernisation based on the possibility of re-using treated waste waters produced by the fruit and vegetable processing company Halcón Foods. The study focuses on the problems arising when the water arrived at the crop fields, namely the choice of irrigation system, the irrigation management, the control of the irrigation and fertilisation dosages, the maintenance of traditional crops and the introduction of new crops. The main objective is the evaluation of the effect of irrigation with treated waste waters on the physico-chemical equilibrium of the crop soils and the plant growth.

## **ENVIRONMENTAL IMPACT OF MINING ACTIVITIES IN TWO SOILS FROM SE SPAIN – REMEDIATION ACTIONS**

A. Zanuzzi and A. Faz  
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The Sierra Minera of Cartagena - La Unión (Murcia, SE-Spain) has been subjected to intense mining activities which lead to high heavy metals (Pb, Zn, Cd and Cu) accumulation in soils. As a consequence of the arid climate of the region, strong water and wind erosion transport polluted soils to the surrounding landscape, producing great risks for the environment and human health. Analysis carried out in two representative mining ponds from the mining activity, showed potential risks associated to the study areas and that remedial measures are urgently required. Therefore phytostabilization and amendments addition are recommended as a suitable remediation action in these zones.

[Top of Page](#)

[Return](#)

## **POSTER SESSION 2**

### **Bioproducts**

#### **ANTIFUNGAL ACTIVITY *IN VITRO* OF FLOURENSIA EXTRACTS ON *FUSARIUM OXYSPORUM*, *RHIZOCTONIA SOLANI*, AND *ALTERNARIA* SP.**

D. Jasso de Rodríguez, F.D. Hernández Castillo, J.A. Villarreal Quintanilla, R. Rodríguez García and J.L. Angulo-Sánchez  
UAAAN, Saltillo, Coahuila, México

The *Flourensia* genus is important because it synthesizes a large amount of secondary metabolites that are widely used for biological and ecological applications. However, the bioactivity of extracts from three species endemic to the Chihuahuan desert region, namely (1) *Flourensia microphylla* (A. Gray) S.F. Blake, (2) *F. cernua* DC., and (3) *F. retinophylla* S.F. Blake, against fungi affecting crops of commercial importance is

not known.

The objectives of this study were to evaluate the inhibitory effects of *Flourensia* extracts on the mycelial growth of three phytopathogenic fungi and to determine the extract concentrations that can inhibit mycelial development.

The *F. microphylla* was collected at the Carneros rail station by highway 54 Saltillo-Zacatecas (25° 07' 13" N, 101° 07' 24" W, 2383 m asl) on 11 March 2004, *F. cernua* on the same day (24° 56' 49" N, 101° 05' 01" W, 1922 m asl), and *F. retinophylla* at Sierra de Paila (25° 59' N, 101° 28' W) on 20 March 2004.

For extraction, the fresh leaves from the three *Flourensia* species were placed separately into dark flasks with ethanol for 36 h with mechanical agitation at room temperature. The solutions were filtered, evaporated, and weighed for yield determination.

Antifungal activity of the *Flourensia* extracts was evaluated on mycelium development. *Fusarium oxysporum* and *Alternaria sp.* were isolated from a potato crop by monospore technique. Testing was also performed on the mycelium development of *Rhizoctonia solani* isolated from a potato crop by the hyphae point technique. Plant extract concentrations were 0, 10, 100, 500, 1000, and 1500 ppm. Fungal plugs 0.5 mm in diameter were placed in Petri dishes with potato-dextrose-agar culture media, and treated with the various extract concentrations as previously indicated. The cultures were incubated at 24 °C and the mycelial radial growth was measured five days after exposure. The antifungal effect was determined using a completely randomized design with four replications.

The three *Flourensia* species showed antifungal effect against *F. oxysporum*, *Alternaria sp.*, and *R. solani*. The extracts inhibited more than 50% of the mycelial growth of the three pathogens at concentrations greater than 100 ppm. The *F. cernua* and *F. retinophylla* inhibited 100% *R. solani* at 1000 ppm. All three *Flourensia* species inhibited completely *F. oxysporum* growth at 1500 ppm and approximately 90% of *Alternaria sp.*

As far as we know, this is the first report of antifungal activity for *F. retinophylla* and *F. microphylla* against fungi that affect crops of commercial importance.

#### LEAF RESISTANCE, TRANSPIRATION, AND GROWTH OF *ALOE VERA* UNDER SOIL WATER STRESS

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*Aloe vera* (sábila) is a specie used in folklore medicine in many countries. However, the information about the plant as a crop is scarce, particularly water management (irrigation frequency, amount of water application, influence on plant development, etc.). The plant has a crassulacean acid metabolism (CAM) that allows water conservation within the tissue, and thus resistance to high water stress.

*A. vera* plants were submitted to water soil stress in an experiment carried out in a greenhouse, to evaluate the response of physiologic processes such as stomatal resistance and transpiration as well as on leaf growth and yield. The experiment consisted of three irrigation regimes under a completely randomized design. The results showed no initial effect on stomata resistance or transpiration but as time elapsed, changes in these variables were noted. We suggest that high water content in the parenchyma maintains stomata open despite the water stress. The changes detected in stomatal resistance and transpiration were attributed to non-soil water stress factors. Factor analysis established that air temperature, leaf temperature, and vapor pressure deficit affect transpiration directly, but resistance inversely. This result is opposite to those reported for other CAM plants.

#### FIELD ESTABLISHMENT OF *URGINEA MARITIMA* BULBS FROM IN VITRO PROPAGATION

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*Urginea maritima* (L.) Baker (Liliaceae) is a plant species native to the countries of the Mediterranean Basin. The plants grow well in calcareous soils under dry climates. Plant products isolated from the bulbs have been used as rodenticides and medicinals, and other new applications are also being investigated for pest control. For this potential new crop to be developed, a rapid and reliable propagation method is needed. Both seed and vegetative (bulb division) propagations are feasible. Successful in vitro culture has been reported in the literature that is important to improve the rate of increase for this species. The objective of this work was to study the survival of in vitro propagated *U. maritima* plants (obtained from the Institute of Pharmacognosy in Vienna) after being transplanted in the field in southeast Spain.

Field plantings were made in the autumn of 1997 (with plant materials immediately after their receipt) and 1998 (with plants grown in the nursery

for one year). Monitoring of the number of plants and their growth (number and leaf size) was done every winter for seven years.

Our findings demonstrated for the first time that in vitro propagated bulbs could be successfully established in the field for long periods. The plants were able to develop, to grow following their usual natural cycle, and to flower once the bulbs were adequately developed.

The sooner the plants were transplanted onto the field, the better was their succeeding growth. It is important that the bulblets are at least 0.8 cm diameter to obtain a high survival rate. Alternately, a conditioning growing period in the nursery is recommended. The average survival rate was 34.6% at the end of the seventh year. Maximum survival rate of 60.5% could be achieved in some instances.

The first three years were critical for *U. maritima* establishment because the plants grew very slowly. However, by the succeeding fourth or fifth year, the plants grew faster, produced larger leaves, and were able to flower. With appropriate agronomic practices, it should be possible to optimise field establishment of plants derived from in vitro cultures of *U. maritima*.

## INSECT ANTIFEEDANT ACTIVITY AND PHYTOCHEMICAL STUDY OF *SALVIA HISPANICA* L.

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The species belonging to Labiatae family are well known for their special secondary metabolites such as sterols, flavonoids, and terpenoids. Pure products have been obtained from an important number of plants belonging to this family, genus *Salvia*. Several of these products have shown antifeedant activity against some lepidopteran larvae.

The aim of this project was to carry out a phytochemical study of *Salvia hispanica* L., a labiatae belonging to the American subgenus **Calosphace**. This plant is native to the Pacific-American coast and its importance is due to the high content of omega-3-linolenic acid in the seed. Extracts and pure products were obtained and their antifeedant activity was assessed against the Lepidoptera *Spodoptera littoralis* (Boisduval, 1833).

Phytochemical studies were based on silica column chromatographic (CC) separations. Once the pure products were isolated, they were characterized using appropriate analysis and spectroscopic techniques. Antifeedant activities of the extracts and pure products were evaluated in the larvae of *S. littoralis*' final stage by choice bioassays with glass fibre discs and by calculating the Antifeedant Index (AI).

Crude extracts, fractions, and pure products were obtained. Most of the pure products isolated such as b-sitosterol, a mixture of saturated and unsaturated acids, a mixture of ursolic and oleanolic acids, and two flavones, gardenin B and 5-O-desmetilnobiletin, were very common in superior plants. These flavones were isolated from *S. hispanica* for the first time. Antifeedant activity studies showed that the acetone extract (AE) and F7 fraction of *S. hispanica* were active at 10,000 ppm and 1,000 ppm respectively. The activity of the F7 fraction was thought to be due to a synergistic effect among the components. The isolated pure products were not active.

Dose/response studies of acetone extract and F7 fraction were undertaken. The effective doses obtained in 50% of the insects (ED<sub>50</sub>) were: for the AE (ED<sub>50</sub><sub>AI>0</sub> = 13 362 ppm; ED<sub>50</sub><sub>AI≥50</sub> = 21 239 ppm), and for the F7 (ED<sub>50</sub><sub>AI>0</sub> = 1 313 ppm; ED<sub>50</sub><sub>AI≥50</sub> = 1 928 ppm). The obtained results indicate that the potential use of these extracts in integrated pest control.

## BIOFUMIGATION WITH GLUCOSINOLATE DERIVED PRODUCTS FROM *BRASSICA* MEAL FOR CONTROLLING POST-HARVEST FRUIT PATHOGENS

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Allyl isothiocyanate (from sinigrin) is one of the best isothiocyanates to be employed in fruit fumigation mainly due to its favourable vapour pressure. Its efficacy was confirmed on fruits inoculated with *P. expansum* conidial suspension and treated with vapours of commercial allyl-isothiocyanate or allyl isothiocyanate produced by the endogenous sinigrin-myrosinase system of *Brassica carinata* defatted meals. The treatment was able to reduce over 85% of blue mould infections on pears with an average concentration of 0.7 mg L<sup>-1</sup> and a treatment time of 8 hours. No phytotoxic effects on fruits were detected under these treatment conditions. Considering these results, post-harvest fruit biofumigation with isothiocyanates produced by the Brassica myrosinase-glucosinolate system seems to be a reliable alternative to fungicide post-harvest treatments. This is reinforced by the positive result of a first trial of pear biofumigation in a pilot plant, where we are now developing the results of this study to verify the possible industrial application of this technique to control other post harvest pathogens.

**GERMPLASM RESOURCES FOR NEW CROPS RESEARCH:  
NORTH CENTRAL REGIONAL PLANT INTRODUCTION STATION**

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The North Central Regional Plant Introduction Station in Ames, Iowa, is part of the United States National Plant Germplasm System. The objectives are to conserve the genetically diverse crop germplasm and associated information, to conduct germplasm-related research, and to distribute this germplasm to researchers and educators worldwide. We manage numerous industrial, nutritional, and medicinal germplasms including species of *Amaranthus*, *Chenopodium*, *Cuphea*, *Echinacea*, *Euphorbia*, *Helianthus*, *Hypericum*, *Linum*, *Perilla*, *Vernonia*, and diverse Umbelliferae.

The *Cuphea* collection includes 651 accessions (64 of the estimated 260 species in this genus). Accessions from 10 of these species have been used in the development of *Cuphea* lines for domestic production of industrially important medium length chain fatty acids (caprylic, capric, lauric, and myristic) for which the only other known plant sources are coconut and palm oils. As part of a national team made up of public and private sector groups, our station is establishing the most reliable germination conditions for the wild germplasm that provides the reserve of genetic diversity for *Cuphea*.

We maintain 151 accessions of *Echinacea* (all nine species in the genus) and 92 accessions of *Hypericum* (48 species) as part of a focus on collection, regeneration, and evaluation of genetically diverse medicinal plant species. In collaboration with Iowa State University, the University of Iowa, and the National Institutes of Health, we provide germplasm for in-depth study of the phytopharmaceutical properties of *Echinacea* and *Hypericum*. Research projects associated with our medicinal plant collections include studies of genetic and chemical diversity, bioactivity, proteomics, determination of mechanisms of action, and enhancement breeding.

The *Amaranthus* collection includes 3,329 accessions (40 of the estimated 60 species). The germplasm originated in 85 countries. A representation of both wild and cultivated sources make the collection an important resource for taxonomic studies as well as for baseline herbicide resistance, nutritional, and molecular research. The world's record tallest amaranth (461 cm) was grown in Ames in 2004 from seeds of *Amaranthus australis*, accession PI 553076. The large plants of PI 553076 are potentially useful for biomass production.

Further information about our station and links to information about our holdings, availability, and germplasm requests is on line at:  
<http://www.ars-grin.gov/nc7>.

**ANTIOXIDANT CAPACITY OF PEPPERS CULTIVATED WITH ORGANIC AMENDMENT AND SUPPLEMENTAL ADDITIONS  
OF MINERAL FERTILIZERS**

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A greenhouse experiment was conducted to examine the effect of using an organic amendment combined with supplementary additions of mineral fertilizer on the antioxidant activity of pepper. Treatments were imposed under identical cultivation conditions and included combined organic amendment with synthetic fertilizer, applied at the rates recommended for the organic (T1), low-input (T2), and conventional (T3) practices. Peppers were harvested at three stages of ripening (green, turning and fully-mature red) and antioxidant activities in the hydrophilic (HAA) and lipophilic (LAA) fractions were determined. Treatments had no significant effect on HAA or LAA, but did affect the mean weight and water content of fruits. Antioxidant activity of both the hydrophilic and lipophilic fractions increased from the green to the red stage of ripening. HAA contributed about 80% of the total antioxidant activity.

**SCREENING ANTIOXIDANT ACTIVITY IN TABLE GRAPE HYBRIDS**

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The presence of phytochemical compounds with antioxidant capacity in fruits and vegetables has a considerable importance in the prevention of chronic diseases related to oxidative stress in the human body. In grape, many of these compounds have been found, that provide a much stronger antioxidant activity than traditional antioxidant vitamins. These compounds are mainly polyphenols, most of which are proanthocyanidins and phenolic acids that have been identified as proanthocyanidin metabolites.



The principal aim of this study was to verify the possibility of correlating the levels of phenolic acids, the anthocyanin content and the external colour to the antioxidant capacity of berries.

Table grape hybrid cultivars (Moscatuel × Ruby Seedless) of different colour gradation, from green-yellow to dark-red, were harvested in Murcia (Spain). External colour was measured with a reflectance spectrophotometer and the CIRG colour index was calculated. Antioxidant capacity, anthocyanic and phenolic compounds content and anthocyanin and polyphenols profiles were determined in hydrophilic and lyophilic fractions extracted from skins and berry pulp.

The hydrophilic fraction extracted from grape skin showed the highest antioxidant capacity (>70 % of total) and this increased with the value of CIRG. The results showed that anthocyanic and phenolic content was closely related with berry external colour and their antioxidant capacity. Correlation between anthocyanin and polyphenols profiles and the antioxidant capacity was also studied.

## **TUMORICIDE AND ANTIGENOTOXIC EFFECTS OF OLIVE OIL, SEED OILS AND FRESH PLANT OF *BORAGO OFFICINALIS* AND *BRASSICA CARINATA***

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Many of the recent palliative and prevention therapies for cancer diseases include the use of traditional herbal preparations. Although some of the herbal components can be bioactivated resulting in cancer, most of the commonly used are considered as healthy commercial products. Epidemiological data relate the origin of cancer with persistent inflammatory processes which is associated to an oxygen and nitrogen radical excess. Many vegetable compounds are protecting against xenobiotics, either inducing detoxifying enzymes or inhibiting oxidative enzymes. Although knowledge of the specific mechanisms of action of many phytochemicals is still poor, due to the great variety of potential carcinogens contained in diet, they can only be avoided theoretically. An alternative strategy is taking place at present by consuming anticarcinogens/antimutagens that can prevent or revert some of the effects produced by carcinogens.

The aim of our study is to assess on the safety of the use of *Borago* and other by-products and the possibility of their use as nutraceuticals to help cancer prevention.

Antigenotoxicity assays. The Somatic Mutation And Recombination Test on imaginal discs of *Drosophila melanogaster* has been used to assess on the lack of genotoxicity (DNA safety) and the antigenotoxicity of the *Borago*, *Brassica* and olive oil whole mixtures and some components of them. Tests have been carried feeding larvae either with different concentrations of different oil or lyophilized fresh plants, combined with different concentrations of hydrogen peroxide as a potent mutagen.

Citotoxicity assays: HL60 human leukaemia cells have been used to determine the tumoricide activity of the different oils, fresh plants and single compounds. The policy of the cultures was for seven days. Dose response curves are obtained from 72 h data of survival.

Results obtained from antigenotoxicity assays agreed with those of the citotoxicity. Lyophilized Fresh plant of *Borago officinalis* (blue flowered), *Borago officinalis* (white flowered) and *Brassica carinata* were antimutagenic against hydrogen peroxide and tumoricide. The phenol rosmarinic acid contained in *Borago* plants showed a strong antiproliferative capacity, but not other components like syringic and sinapic acid. Olive, *Borago* seed, triolein and the main phenol contained in the olive oil hydroxytyrosol were antigenotoxic against the hydrogen peroxide and all of them showed antiproliferative activity although in a lesser extent than any fresh plant. Flax, sunflower and soy oils showed different levels of tumoricide effects.

Our preliminary conclusions are that fresh plants and olive oil show more antigenotoxic and antiproliferative capacities than the seed oils.

## **NEW VARIETIES OF ÑORA SWEET PEPPER FOR PAPRIKA**

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The cultivation of paprika pepper is one of the oldest established horticultural activities in Murcia with great economic importance. However, the cultivated area has decreased during the past years due to damage caused by a new Tomato Spotted Wilt virus and increasing labour costs.

The local variety “Ñora” or “Bola” used for paprika is well adapted to the soil and climate conditions in Murcia. This variety is non-pungent (absence of capsaicin) and its rounded shape is suitable for processing. Unfortunately, the extractable colour of the “Ñora” is very low (90-120

ASTA) and the harvesting must be repeated several times over its long ripening period. Consequently, it is not suitable for a single hand or mechanical harvesting.

Considering these facts, we started a breeding programme intended to produce more grouped ripening varieties and to improve the extractable colour of our local variety.

Crosses between the Spanish cultivars “Negral”, “Datler”, “Ocal”, “Belrubí”, and “Ñora”, with some differences in shape, fruit orientation, and colour quality were performed. A continuous processing of selfings, backcrossing, and repeated selection cycles yielded several promising lines.

A randomized block design with four replications was carried out at Totana (Murcia) in 2002. Four experimental lines were included in the test, “Inboro” and “Rosarito” with their rounded fruit shape, “Costal” and “Rubicón” with their elongated fruit shape, and a selection of the local variety “Ñora” as the control.

Agronomic and commercial features of lines were evaluated based on vegetative vigour, plant morphology, and total yield. A sample of 40 fully ripe fruits of each line was evaluated for size, fresh and dried weight, and total extractable pigments of the pericarp.

All experimental lines matured earlier than the “Ñora” variety. The lines “Rubicón” and “Costal” with elongated fruits achieved a profitable grouped ripeness.

The analysis of variance of production data showed a statistically significant difference only between the local varieties “Ñora” (0.44 kg per plant) and “Rubicón” (0.50 kg per plant). In contrast, an analysis of variance of colour data indicated statistically significant differences among all varieties. “Ñora” had the least colour (206.55) and “Rubicón” the best colour (328.60).

The new experimental lines “Costal” and “Rubicón”, with good colour and grouped ripeness, seem to be better adapted for a single hand or mechanical harvesting than the rest of lines, and also possible potential for cultural cost reduction.

The lines “Inboro” and “Rosarito” had better colour than “Ñora” and could replace this variety in the future.

## **COLOUR STABILITY IN BREEDING LINES OF PAPRIKA PEPPER**

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The mature fruits of red pepper (*Capsicum annum* L.) are considered one of the richest sources of natural pigments. The colour of red peppers is due to over 27 carotenoid pigments identified in the pericarp. Some of pigments (capsanthin, capsorubin, and capsanthin 5, 6-epoxide) are considered to belong almost exclusively to the genus *Capsicum*.

Ever since pepper was introduced to Spain and spread to the rest of the world, growers have been selecting and breeding different pepper cultivars for the most interesting properties and characteristics directed toward different purposes. The result is a great number of very different cultivars that show a wide range of morphological and organoleptic characteristics including colour and pungency that determine their use. These materials represent an invaluable source of potential genetic variation useful in plant breeding.

The most highly valued characteristic for paprika is its high carotenoid content that directly depends on the relative pigment richness. However, the initial colour and colour retention in the dry powder of the paprika are important and desirable for commercial purposes.

The pigments are extracted from dehydrated ground peppers with solvents and determined spectrophotometrically. Although this method is not specific for individual pigments, it provides a quantitative measure of the total concentration of pigments. This method is readily applicable to the study of deterioration of these pigments during processing and storage. In processing, the deterioration of the extractable colour pigments in paprika is affected by different factors, such as drying temperature and drying rate. During storage, the moisture content of the atmosphere and temperature are also critical factors.

This study reports on the relative colour stability of several experimental paprika varieties and the deterioration of pigments during storage. Fruits of the local variety “Ñora” and the experimental varieties “Inboro”, “Rosarito”, “Costal”, and “Rubicón” obtained through a breeding programme were used.

Following harvest, fruits were surface-washed and dried to constant weight at 65°C. The seeds and stems were removed from the pods and the remaining pepper walls were ground. A subsample was immediately analyzed for colour and another subsample was stored in plastic vials in the dark at laboratory temperature until the colour was determined. Colour was measured on the stored powder at 4-week intervals over a 3-month

period.

A regression analysis of colour deterioration was computed on data for length of storage for each variety. The results showed the lines were different. The variety "Rubicón" had the highest colour stability with colour losses after the 3 months of 9.51%. At the same time, the variety "Ñora" ranked second with 16.24% followed by the varieties "Inboro" (17.82%) and "Costal" (18.61%). The variety "Rosarito" had the lowest colour stability (20.61%). The cause for such variation may be due to compositional differences in carotenoids mixtures, levels of naturally occurring antioxidants, or other factors.

### **EXTRACTION OF INDIGO FROM *ISATIS TINCTORIA* L. AND *POLYGONUM TINCTORIUM* AIT AS A BASIS FOR LARGE SCALE PRODUCTION**

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Indigo is the most important blue component in the class of natural dyes for textile dyeing. Nowadays there is a growing interest to replace the dyes obtained by chemical synthesis with bio-based products. In the Mediterranean European climate woad (*Isatis tinctoria* L.) and dyer's knotweed (*Polygonum tinctorium* Ait.) could be interesting sources for natural indigo. Woad had been cultivated in Europe since the Middle Ages. Indigo was produced by woad balls made from crushed harvested fresh leaves which contain two indigo precursors: Indican (indoxyl b-D-glucoside) and Isatan B (indoxyl-5-ketogluconate). By this method indigo formed at the cut surfaces of the leaf material, by oxidation. The balls were allowed to dry for over 4 - 6 weeks, then crushed, moistened with water and allowed to ferment aerobically over a period of some weeks. The woad was then ready to be used by the dyer. Dyer's knotweed had been grown in Japan and China to obtain natural indigo since ancient time. The Japanese did not mill the leaves but they chopped them to separate them out from the stems and let them dry. The leaf mass was spread deep in sheds to be treated and turned into sukumo, the equivalent of woad balls. The unique indigo precursors of dyer's knotweed is indican that is water-extracted from leaves and it is degraded to indoxyl and glucose by the action of a native b -glucosidase. Dimerization of indoxyl by air oxidation will follow leading to the formation of indigo.

At present to re-introduce these species as alternative crops for indigo production it is of crucial importance to define new and sustainable on-farm technologies for indigo extraction. The amount of indigo was usually determined by HPLC analysis of precursors substances, but for an evaluation of the overall indigo production from the crop, losses during the chemical transformation of the precursors into indigo have to be considered. Therefore the aim of this work was to optimise the extraction process from laboratory to farm-scale, including methods for standardisation and quality control of the product. Following the cultivation of woad and dyer's knotweed on a field scale at Pisa University, simple procedures for the extraction of the indigo precursors by water were investigated with regard to crops and quality of the dye obtained. A set of data was collected for indigo extraction. From the results of different extraction experiments and on the basis of the analytical data a final protocol was set up to optimise indigo yield from fresh leaves.

Furthermore a prototype machine for indigo extraction has been tested at farm level during two summer seasons. Experiments on large-scale indigo extraction allowed us to define the extraction conditions for woad and dyer's knotweed from fresh leaves directly on farm, with the help of an appropriate workstation, water supply and heavy equipment to move fresh and then spent leaves. The crude indigo dye obtained was analysed for its indigo content spectrophotometrically. The yield was lower than the theoretical maximum predicted by laboratory studies, but that is to be expected for the first farm scale trials, as problems of scale-up are encountered and overcome. The disposal of spent leaves does not represent an ecological problem because they can be recycled as organic manure for their considerable amount of macronutrient and in particular for high content of nitrogen.

### **EFFECT OF DIFFERENT SOWING DATES ON LEAF YIELD AND INDIGO PRODUCTION OF WOAD (*ISATIS TINCTORIA* L.) IN THE MEDITERRANEAN ENVIRONMENT**

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Woad (*Isatis tinctoria* L. Family Cruciferae) is a biennial herbaceous plant and one of the earliest known sources of natural indigo. The influence of seeding date on indigo production in Italy have been studied for the first time in this region during 2001, 2002 and 2003. Four/five sowing dates in the Spring-Summer period from March to August at monthly intervals during each growing season were compared. Crops were harvested when the plant's rosette had reached a height of 25 cm and subsequent harvests were taken when this height had been regained. Leaf fresh and dry yield, as well as indigo production, were determined. Indigo content was determined by spectrophotometric analysis.

Woad showed a high flexibility to fit into different sowing dates. Seeding date significantly influenced seasonal crop production and indigo yield.

The spring sowings showed higher leaves and indigo yield allowing to extend the vegetative crop cycle and to carry out three or four harvests during the growing season. On the other hand the warm and dry weather conditions prevailing from late spring onwards caused a shortening of the growing cycle of summer crops with negative consequence on leaf and indigo yield. With summer sowing dates no more than two harvests a season were usually possible. With delaying sowing, yield generally tended to decrease being the total indigo yield in spring sowing dates higher than in the summer sowings ( $63 \text{ kg ha}^{-1}$  and  $40 \text{ kg ha}^{-1}$  respectively). Furthermore the summer crops showed a greater instability in yield and increasing risks of crop failure. Averaged across the sowing dates a fresh leaf yield of 31, 52,  $54 \text{ t ha}^{-1}$  corresponding to a dry yield of 4.4, 5.4,  $6.3 \text{ t ha}^{-1}$  respectively in 2001, 2002 and 2003 was observed. The mean seasonal indigo yield was 39, 73,  $57 \text{ kg ha}^{-1}$  and the high range of variation was due to the great yield instability of the crops sown in summer dates. Results showed that in central Italy it is possible to sown woad from March until the end of July with important advantages for this crop even if the summer crops are characterised by wide yield variation.

## CROPS AND WILD HOST PLANTS FOR PREDATORY PLANT BUGS IN THE SOUTHEAST OF SPAIN

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Prospecting for predator mirids with interest in biological pest control on horticultural crops was carried out during 2002, 2003 and 2004 in the province of Murcia (Southeast Spain) to determine species composition and abundance, and wild host plants. Tomato, pepper, squash and eggplant were among the crops more frequently sampled. The most abundant mirids on crops were *Macrolophus* sp., *Nesidiocoris tenuis*, *Dicyphus cerastii*. *Dereaocoris punctulatus* was frequently found but at a lower number. *Dicyphus tamanii* was occasionally found. About 100 wild plants belonging to 30 families were sampled in the surrounding of the crops. *Macrolophus* sp. was very abundant on *Dittrichia viscosa*, *Marrubium vulgare* and *Ononis natrix*. *Dicyphus* was found at a low number on *Withania frutescens* and *Ononis natrix*.

## A BIOMETRIC MONITORING OF PLANT GROWTH AFFECTED BY NITRATE TREATMENTS

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Different methods of sweet pepper cultivation (organic, integrated and conventional farming) were evaluated under greenhouse conditions. During the crop cycle, plant growth parameters, including plant height, leaf width and length, were monitored to determine the effects of cultivation methods. Total nitrogen concentration was also analysed in each organ. Biometric determinations were expressed as relative growth rate. Our data show that relative growth rates were significantly affected by the nitrogen concentration in each organ and were directly related with the cultivation method. Biometric monitoring of this crop was a useful tool to follow the nutrient status at the different phenological stages.

[Top of Page](#)

[Return](#)

## POSTER SESSION 3

### Natural Rubber and Resins

## CHEMICAL CONSTITUENTS AND PHYSICAL PROPERTIES OF GUAYULE WOOD AND BARK

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About 15 *Parthenium* species grow in the North American continent. Of these, *P. argentatum* (guayule) is the only species with harvestable amounts of the rubber latex. The predicted commercialization of the guayule plant for its hypoallergenic latex will result in a significant amount of waste fiber or bagasse biomass that can also be put to use for making wood, paper, and other chemical products, as well as in energy production. Thus, the guayule wood and bark fibers can be considered a new source of plant biomass that may be used as a direct substitute for forest-based wood fiber. However, little information is available on the chemical composition of the wood and bark tissues of guayule (*Parthenium argentatum*) and its cultivars.

The objectives of this study were to determine the chemical and fiber composition of guayule and to compare it with other wood fiber sources.

Three lines of mature *P. argentatum* (guayule, Cal-6, AZ-101, G7-15) and another species of *Parthenium*, (*P. tomentosum*, PT), juvenile soft maple (*Acer* spp.), a deciduous tree, and milkweed (*Asclepias syriaca* L) that has long fibers were the plant sources. Separate wood and bark tissues were analyzed for hot water, 1% sodium hydroxide, and alcohol-toluene extracts. In addition, the lignin, holocellulose, alpha cellulose, and pentosan contents were determined.

All the chemical components in the wood fibers for the *Partheniums* were equal to or greater than the juvenile maple tree. Milkweed had higher alpha-cellulose and lower alcohol-toluene extract contents than both the guayule and soft maple. The guayule bark fibers had more chemical extracts than the wood fibers. The specific gravity of guayule wood was greater than most of the deciduous wood species. However, the fiber lengths of soft maple wood, guayule wood, and milkweed woody part are similar.

Based on the chemical composition, the *P. argentatum* and *P. tomentosum* could serve as raw materials for the paper and chemical industries as well as for energy production.

## Oils

### STRATEGIES FOR NEW CROPS BREEDING AND DEVELOPMENT: VERNONIA GALAMENSIS AS A MODEL

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### LESQUERELLA FENDLERI RESPONSE TO DIFFERENT SOWING DATES IN NORTHERN MEXICO

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*Lesquerella fendleri* (Gray) Wats., is a Brassicaceae native to northern Mexico and southern United States. The seeds contain an oil similar to castor oil that may be used for industrial applications. This crop may be an option for Mexican producers in the semiarid lands of the U. S. market imports 41,000 t of castor oil per year. This paper reports on the effect of sowing dates on seed yield and oil content in *Lesquerella* grown at Saltillo, northern Mexico. The plant was sown at three different dates (23 October 2003, 16 December 2003, and 17 March 2004) and harvested in 2004 on 15 May, 24 June, and 27 August, respectively. Irrigation was applied for germination and seedling establishment and to maintain soil moisture. The experimental design was a complete randomized block design with eight replications. Plant morphological variables as well as yield characteristics were measured and related to sowing dates and climatic conditions. Plant densities depended upon sowing date although within the reported for high yield. The best date for sowing was in December according to yield data. The oil content increased as a function of temperature. Infrared spectra of the seeds crude extracts showed different functional groups that varied with climatic conditions.

### HYDROTIME ANALYSIS OF LESQUERELLA FENDLERI SEED GERMINATION RESPONSES TO PRIMING TREATMENTS

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*Lesquerella fendleri* seeds contain industrial oil, which is increased under arid environments. In such environments the water needed for germination is available for only a short-time and, consequently, successful crop establishment depends not only on fast and uniform germination of the seed lot, but also on its aptitude to germinate under low water availability. All of these attributes of the seed lot can be analyzed through the hydrotime model (HT). A methodology to improve the speed and uniformity of germination is the priming "P". This methodology reduces the value of the hydrotime constant ( $Q_H$ ) and, sometimes, displaces the mean base water potential ( $Y_o(50)$ ). The latter would result in an increase in the capacity of the seed to germinate under low water availability.

The aim of this work was to improve i) the velocity and uniformity of germination, and ii) the ability to germinate under situations of low water availability, in seeds of *L. fendleri*. We also intended to analyze and model through the HT model, changes in the physiological behaviour of the seed lot as a result of the application of the priming treatment, with seeds sown either in Petri dishes or in the field.

Seeds were subjected to priming in Petri dishes with a solution of polyethylene glycol at 5 (P5) or 20°C (P20). One half of the seed lot was used for determination of HT parameters through incubation in Petri dishes at 10, 20 and 30°C, and in water or PEG's solutions calibrated to obtain different  $Y_a$ . The remaining one half of the seed lot was sown in boxes. Different treatments (3) of water availability were imposed: field capacity or control, 75%, and 50% of the field capacity. In each of the boxes were sown with P5 seeds, with P20 ones and with untreated seeds. Both under controlled and field conditions, P20 and P5 seeds germinated faster and more uniformly than untreated (control) seeds. The HT model analysis revealed that in both P20 and P5 seed lots, the  $Q_H$  constant had been reduced and  $Y_o(50)$ , had been shifted towards more negative values. The latter was consistent with the higher germination percentage attained by P20 and P50 seeds in the field experiment under reduced water availability (i.e., 75 and 50 % of field capacity) compared with that attained by untreated seeds under the same situations.

## **WILD ANNUAL *HELIANTHUS ANOMALUS* AND *H. DESERTICOLA* AS POTENTIAL SOURCES OF IMPROVED OIL CONTENT AND QUALITY IN CULTIVATED SUNFLOWER**

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The genus *Helianthus* is composed of 51 species and 19 subspecies with 14 annual and 37 perennial species. The narrow genetic base of cultivated sunflower has been broadened by the infusion of genes from the wild species, which have provided a continuous source of unique agronomic traits. Interest in using wild species in breeding programs has increased, but concerns about the introgression of low oil concentration and quality from the wild species persist. *Helianthus anomalus* Blake and *H. deserticola* Heiser are excellent candidates for oil concentration and quality improvement based on their desert environment. Unfortunately, due to the demand for achenes of these species and the difficulties of regenerating the original populations, achenes have not been available for research for almost 25 years. This report documents an exploration to the desert southwest USA to collect achenes of the two desert species and the initial assessment of their potential for improving oil content and quality in cultivated sunflower. The sunflower exploration took place from 16 to 23 September 2000. The exploration covered 4100 kilometers in three states, Utah, Arizona, and Nevada. The *H. deserticola* population had an average oil concentration of 330 g/kg, whereas the populations of *H. anomalus* had very high oil contents of 430 and 460 g/kg, respectively, the highest ever recorded in any wild sunflower species. The linoleic fatty acid concentration in the oil of *H. anomalus* populations was uncharacteristically high for a desert environment, approaching 700 g/kg. A linoleic acid concentration of 540 g/kg in *H. deserticola* was more typical for that fatty acid in a desert environment. *Helianthus anomalus* has the largest achenes and the highest oil concentration of any of the wild sunflower species, and the same chromosome number ( $2n=34$ ) as cultivated sunflower. These features will facilitate the introduction of genes from this wild annual progenitor into cultivated sunflower. The lower saturated fatty acid profile in this species is also a desirable trait offering the potential to reduce saturated fatty acids in cultivated sunflower. Further research will be needed to determine the inheritance of the fatty acids and oil content. Other agronomic traits will need to be monitored during the introgression of these traits into cultivated sunflower.

## **THE EFFECTS OF ZINC SULFATE FERTILIZER ON YIELD, YIELD PROPERTIES AND FATTY ACID COMPOSITION OF SAFFLOWER (*CARTHAMUS TINCTORIUS* L.) IN EDIRNE, TURKEY**

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The total production of oilseed is 2.5 million tons and refined oil is 735,000 tons in Turkey. Turkey imported 1.3 million tons of oil seed and 300,000 tons of crude oil. Imports provide one-half of the oilseed need. Edirne is in northwest of Turkey and one of three provinces of the Thrace peninsula. This region has a production potential of oilseeds such as rapeseed and safflower. More than one-half of vegetable oil factories of Turkey are in this region that provides employment for 3000 people. Because of shortage of vegetable oil in Turkey, there is low consumption of oil per capita yearly (17 kg) and a need exists for oilseed factories in this region. Safflower (*Carthamus tinctorius* L.) has a high nutrient value and an alternative production of the crop is being tried. Furthermore, because safflower is resistant to arid conditions, it would be adaptable to the climatic and land conditions of the Thrace Region and Edirne. However, problems exist with the nutrient status of the soils. One of the problems is zinc deficiency observed with safflower and other crops, which has been caused by the excessive applications of phosphorus fertilizer that has gone on for a long time. As a result, the yield and quality of safflower has been adversely affected. In the past decade, producers have used zinc fertilizer especially in rice and wheat and other plants, and also found the practice necessary for safflower production. To develop guidelines for the producers and to increase the production of safflower, investigations were conducted on the application methods of zinc fertilizers, rate of application, and the effect of this fertilizer on the yield and quality of safflower.

This research was established at the Experimental Field of Trakya Agricultural Research Institute in Edirne, Turkey. The experiment was based on a Split-Split Plot design with a Randomized Complete Block with three replications in 2004. Two safflower varieties, Dincer and 5-154, were obtained from the Eskisehir Agricultural Research Institute. Mechanical planting was made on 4 April 2004 on four-row, 0.9-m wide and 5-m long plots. Hand-harvest was made on 6 August 2004. Nitrogen fertilizer was applied to the plots after planting at three rates of zinc sulfate

fertilizer (Control, 1500, 3000 and 4500 mL/ha. The nutrient was applied to the leaves as zinc sulfate before flowering. Measurements were made on plant height, number of branch and achene, number of seed per achene, 1000 seed weight, seed yield, oil content, and fatty acid composition of the oil.

The highest seed yield (2037 kg/ha) and the highest oil content (29.7%) were obtained with the 5-154 variety at 1500 mL/ha. The highest linoleic acid content (84.1%) was obtained with Dincer variety with 3000 mL/ha. The highest oleic acid content (40.8%) was obtained with the 5-154 variety at 3000 mL/ha.

Our results show that the safflower varieties Dincer and 5-154 can be grown in Edirne, Turkey because of their high yield, quality, and oil content. In addition, 1500 and 3000 mL/ha. rates of zinc sulfate fertilizer can be used for the 5-154 and Dincer varieties, respectively. Thus, the growth disorders that occur in safflower because of low zinc content in the soil can be corrected.

## **SCREENING OF JOJOBA COMMERCIAL GENOTYPES TO *FUSARIUM OXYSPORUM***

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Fusarium wilt of jojoba (*Simmondsia chinensis*) caused by *Fusarium oxysporum* is a major disease causing economical damage in plantations in Israel. Chlorosis and wilting of the plants appear usually in the third or fourth year after planting, and may affect 50 to 70% of the plants within a few months, and eventually cause plant death. The objective of the study was to develop a rapid assay to select *F. oxysporum*-resistant jojoba genotypes.

Sixteen isolates of *F. oxysporum* originating from jojoba were tested for their aggressiveness against jojoba, and the most aggressive one was used for screening jojoba genotypes. Tolerance or susceptibility of 11 jojoba genotypes was screened in three systems: (1) plantlets in tissue culture, (2) young plants in pots in a greenhouse, and (3) stem cuttings collected from 10-year-old plants growing in a commercial plantation. In each system, plant material was inoculated with conidial suspension. Susceptibility was evaluated by scoring wilt and senescence symptoms, and levels of fungal colonization.

None of the tested jojoba genotypes was detected as resistant to *F. oxysporum*. However, different levels of tolerance were observed. Some genotypes tested in the three systems showed similar response to *F. oxysporum*. This was usually correlated with the symptoms and colonization of the fungus in mature plants in a commercial plantation. Genotype 64 was observed as the most susceptible to the fungus, and genotype Benzioni was one of the most tolerant. The other genotypes were intermediate. Wilt and senescence symptoms were not always correlated with the genotype susceptibility. The methodologies developed must be improved before being used for systematic evaluation of jojoba genotypes response to *F. oxysporum*.

## **IMPACT OF FEEDING HIGH OMEGA-3 OILS TO TROUT**

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Crops that can be used to produce both low cost vegetable oils for biodiesel production and additional secondary products provide increased opportunities for the agricultural community. The crop we selected as being the most adaptable to Montana conditions is camelina (*Camelina sativa*). Camelina is considered as a low production cost source of oil. The residual meal has a market in livestock feeds due to its high protein content (40%) and soluble fiber. Furthermore, the oil is high in polyunsaturates, ranging from 46 to 64%. The oil also contains eicosenoic, eicosadienoic, and eicosatrienoic acids. These oils are composed predominantly with omega-3 oils that have nutraceutical properties known to reduce inflammation in humans. Such oils are known to lower the risk of diabetes, cancer, infertility, and other related human disorders. The common sources of these oils are from fish, canola (low erucic rapeseed), chia, and flax oils. Camelina contains unique antioxidants that are effective in reducing rancidity much better than those in canola or flax. The resulting product is far easier to process than flax or canola oil and requires nominal care in shipping and use for quality preservation. Processing the meal either by cold pressing or reintroducing the oil into the solvent-extracted meal at 8 to 10% by volume provides high-energy livestock rations with the potential of introducing the omega-3 oils into the fat of the animal. Of particular interest is the conversion of vegetable omega-3 oils to eicopentaenoic acids (EPA) and docosahexaenoic acids (DHA) by the fish or the microbes associated with the fish. EPA and DHA are the most effective anti-inflammatory constituents of the oils.

Rainbow trout fingerlings (*Oncorhynchus mykiss*), 7 to 9 cm long, were fed with camelina-based pelletized feed ration and compared with fish fed the conventional meal. At the end of the test period, the fingerlings were harvested, frozen, and analyzed for fatty acid content. Homogenized fish were blended with warm hexane to extract the oil. The extracted oil was then converted to methyl esters and analyzed for fatty acid, omega-3, and

particularly the DHA and EPA contents.

The fingerlings showed no aversion to consuming the camelina-enriched meal compared with the conventional trout feeds. The fingerlings fed with the camelina meal had three times the omega-3 oil content than those on the conventional diet. Similarly, the EPA and DHA levels were three to four times greater in the trout fed the camelina meal than those fed the conventional meal.

### **Fibres, proteins and carbohydrates**

#### **KENAF BIOMASS PRODUCTION IN EXTREMADURA (SPAIN)**

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Kenaf (*Hibiscus cannabinus* L., Malvaceae) is a warm season annual fiber crop closely related to cotton (*Gossypium hirsutum* L., Malvaceae). Kenaf has been accepted by the European Union for the "non-food set-aside" and designated for utilization in the production of industrial fiber. The objective of the study has been the determination of the sustainable kenaf yielding potential as a non-food crop in Extremadura. For this aim, trials have been carried out in order to determine the biomass yields of three varieties.

#### **EFFECTS OF IRRIGATION AND NITROGEN FERTILIZATION ON GROWTH AND YIELD RESPONSES OF KENAF IN SPAIN**

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Kenaf (*Hibiscus cannabinus* L.) is an annual plant that can be useful as a source of low cost natural fibre. It is a fast-growing plant, and can be used in the industry for a wide range of products, especially for his fibre content useful for the paper production industry. To the farming point of view this crop can be also seen as an useful alternative to the irrigated summer crops in our zone. However, there are few data about kenaf agronomy during the last decade for our continental climate.

The objective of this work was to study the effect of irrigation and nitrogen fertilization on plant kenaf yield, in central plateau of the Iberian Peninsula.

A field study was carried out in two years, 2003 and 2004, on a sandy clay loam soil (Calcic Haploxeralfs) at the experimental farm "La Canaleja" from INIA (Alcalá de Henares, Madrid) in order to determine the appropriate irrigation and nitrogen fertilization requirements. The kenaf ("Tainung 2" variety) was cultivated at four irrigation levels (I<sub>0</sub>, without irrigation; I<sub>25</sub>, 25 % PET; I<sub>50</sub>, 50 % PET; I<sub>100</sub>, 100% PET) and three nitrogen fertilization levels (1st year: N<sub>0</sub>, 0 kg N/ha; N<sub>75</sub>, 75 kg N/ha; N<sub>100</sub>, 100 kg N/ha; and 2nd year: N<sub>0</sub>, 0 kg N/ha; N<sub>75</sub>, 75 kg N/ha; N<sub>150</sub>, 150 kg N/ha;). The plants were grown in 7×5 m<sup>2</sup> in a randomised block design with three replicates in each essays. The crop was harvested 7 times during the growing period, on samples of one-meter row for plant growth (plant height and basal stem diameter) and plant yielding components (dry biomass of leaf, bark and core) assessment.

The maximum biomass production of kenaf was reached 130-140 days after sowing. Nitrogen fertilization was not significant effect in the range of 0-150 kg N/ha on kenaf plant growth and plant yield. We have found that the irrigation was the principal factor in the plant growth and plant yield of the kenaf crop for the two essayed years, and it affect significantly to the: plant height, stem diameter and also to the others yielding components (leaf, bark and core). The I100 was the best treatment, and dry yields of 15 t/ha in total biomass and 10 t/ha in stem biomass may be obtainable under optimal conditions in central plateau of Spain.

In the central region of Spain, were the kenaf trials were located irrigation practices should be performed at 100 % PET. We have found that the reduction of water amount decrease the production of plant kenaf biomass in our climatic conditions. We have found also that in our climatic and crop rotation conditions it is not necessary the application of nitrogen fertilization.

#### **SOWING TIME AND PLANT DENSITY EFFECTS ON GROWTH AND BIOMASS PRODUCTIVITY OF TWO KENAF VARIETIES IN CENTRAL GREECE**

N.G. Danalatos and S.V. Archontoulis



Growth and biomass productivity of two promising, late-maturing kenaf varieties (Tainnung 2 and Everglades 41) were investigated under two different sowing times (1/6 and 1/7/04) and two plants densities (20 and 40 pl m<sup>-2</sup>). A field experiment using a 2×2×2 factorial completely randomized block design was carried out on a deep, fertile soil in central Greece in 2004. The growth characteristics (plant height, stem diameter, leaf area index) and the biomass productivity (leaves, stems, and storage organs) of the crop were measured in subsequent harvests throughout the growing period. Leaf photosynthesis was measured for different radiation and temperature conditions. A significant effect of sowing time on all growth indices was found with the earlier crop attaining much higher values by end of the growing period. Plant height, stem diameter and leaf area index reached maximum values in excess of 300 cm, 2.57 cm, 4.32 in case of early crop and 305 cm, 2.27 cm, 3.44 for the late crop, respectively. The total dry biomass reached 16.52 t ha<sup>-1</sup> (dry stems 14.81 t ha<sup>-1</sup>) for the early crop, and 13.34 t ha<sup>-1</sup> (dry stems 10.81 t ha<sup>-1</sup>) for the late crop. Considering that maximum growth rates exceeding 240 kg ha<sup>-1</sup>d<sup>-1</sup> were measured, the large difference in biomass production was attributed to the longer period available for the early crop for maximum growth. A slight, but non-statistically significant superiority on biomass productivity of variety Tainnung 2 vs. Everglades 41 was found.

## IRRIGATION AND N-FERTILIZATION EFFECTS ON KENAF GROWTH AND BIOMASS PRODUCTIVITY IN CENTRAL GREECE

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The growth and the biomass productivity of the kenaf variety Tainnung 2 were investigated under optimal and sub-optimal irrigation and N-fertilization inputs in western Thessaly, Greece, in 2004. A 3×4 factorial completely randomized split-plot design was used in three blocks with the main plots comprising three irrigation treatments, and the subplots comprising four nitrogen dressings. The study soil was a deep, calcareous, fertile clayloam, classified as Aquic Xerofluent, that represents large areas in the extensive Karditsa plain in central Greece. The crop was harvested periodically during the growing period and in each harvest, plant height, stem diameter, leaf area index, total dry, and stem dry biomass were measured. It was found that fertilization within the studied rates did not affect growth and biomass productivity of the crop, apparently due to the high fertility status of the study soil. Contrary to fertilization, a significant (P=0.05) effect of irrigation was found, with the fully irrigated plants (500 mm) reaching maximum growth rates in excess of 270 kg ha<sup>-1</sup>d<sup>-1</sup> and dry biomass reaching 17.5 t ha<sup>-1</sup> and by 9% and 21% lower productivity for irrigation inputs equal to 50% and 25% of the potential evapotranspiration, respectively. Stem biomass contributed to about 90% of the total dry biomass for all treatments by the end of the growing period. The leaf area index reached 4.3 (full irrigation), and remained above 3 (in all treatments) for large parts of the cropping period. Maximum height and stem diameter of the fully irrigated plants were 337 cm, and 2.5 cm, respectively.

## QUINOA STARCH PROPERTIES AS INFLUENCED BY NITROGEN FERTILIZATION IN CHILE

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Quinoa (*Chenopodium quinoa* Willd.) is a plant native to the Andes. It was used as a staple food for ancient civilizations in South America. Recently, there has been a great interest to develop quinoa due to its potential as a nutritious food that can be used to make gluten-free bread, soups, salads, and infant formulas. The protein content in quinoa varies between 10 to 22% and its biological value is similar to that of casein. In Germany, researchers have been trying to incorporate quinoa as a daily food. In the U.S., quinoa starch is being studied as a raw material to produce biodegradable polyethylene.

The objective of this study was to evaluate the protein content, thermal, and physical properties of two quinoa genotypes ('Faro' and 'UDEC10') fertilized with different nitrogen rates in Chillán, Chile.

Quinoa seeds were sown in Chillán (36°56'S, 72°3'W, 124 m asl), Chile. The soil analysis before starting the experiment was a silty loam texture with 7.8 mg kg<sup>-1</sup> N-NO<sub>3</sub>, 25.1 mg kg<sup>-1</sup> P, and 6.17% organic matter. The experiment was a randomized complete block design with a factorial arrangement, with four replications, two genotypes, four rates of nitrogen (0, 75, 150, and 225 kg N ha<sup>-1</sup>) applied in two applications as sodium nitrate. All experimental units were fertilized with 100 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> (phosphate) and 50 kg ha<sup>-1</sup> of K<sub>2</sub>O (potassium sulfate). Raw and cooked seeds previously washed to eliminate saponins were analyzed. Seeds were milled (Retsch mill) and the protein content was determined by the Kjeldahl method (%N×6.25). For starch characterization, water absorption index (WAI), swelling power (SP), and water solubility index (WSI) were measured. Calorimetric differential scanning (CDS) was determined for the aqueous suspension (60% p/v), which was heated to 240°C at a

rate of 10°C min<sup>-1</sup>. Enthalpy (H) and the temperatures of gelatinization (start (T<sub>0</sub>), maximum (T<sub>p</sub>) and end (T<sub>e</sub>)) were obtained from the thermogram. The degree of gelatinization of the starch (DGS) was obtained from the H's from the cooked and raw samples with the equation (%) =  $1 - (H_{\text{cooked}} / H_{\text{raw}}) \times 100$ .

The average protein content ranged between 13.5 and 15.0% and was significantly different with the application of 225 kg N ha<sup>-1</sup>. The capacity of absorption and retention of water of quinoa flour was not affected by soil nitrogen application rates. There was significant interaction between genotypes and nitrogen rates for the WSI, where the maximum value was observed for 'Faro' with an application of 225 kg N ha<sup>-1</sup>. The lowest nitrogen content was for the 'UDEC10' with an application rate of 75 kg N ha<sup>-1</sup>. These results indicate the highest and lowest levels of starch degradation and modification, respectively. There were no significant differences for the degree of gelatinization of the starch.

Nitrogen fertilization did not affect the water absorption index, swelling power, and degree of gelatinization of starch. However, the water solubility index and the thermal properties of quinoa were affected by the nitrogen rates.

### **THE EFFECT OF SOWING DATE ON THE FIBRE QUALITY OF FLAX (*LINUM USITATISSIMUM* L.) AT BUENOS AIRES, ARGENTINA**

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### **THE WICKER-WILLOW (*SALIX* SP.): IMPORTANCE OF ITS CULTIVATION FOR THE RURAL DEVELOPMENT OF A POOR AREA IN CASTILLA-LA MANCHA**

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Wicker is the name commonly given to the one-year sticks that result from the willow's prune, a hybrid plant between *Salix viminalis* L. × *Salix purpurea* L. It is grown in the cool fertile irrigated lands because it requires large quantities of water, even though it can withstand cold winters (frost). The cultivated areas in Castilla-La Mancha are concentrated in two provinces, basically in the regions of the Alcarria in Cuenca, the Alcarria in Guadalajara, and the Serranía in Cuenca. The characteristics of these regions make them especially suitable for this crop because the willow is a good option for an area that does not allow the profitable growth of other species due to the inclement weather. These areas have frosts for eight months a year with temperatures sometimes below -18°C, and thus limit the choice and variability of crops for the irrigated lands. For this reason, more than 96% of the national production of wicker is obtained from these three regions in the provinces of Cuenca and Guadalajara.

At present, the region is suffering from an alarming diminution of willow-cultivated areas that can lead to the disappearance of this crop unless measures are immediately taken. This deterioration has diverse causes, among which are the following:

- The gradual population withdrawal from the rural areas. The low population density, together with the need for workers to grow willow results in willow cultivation to be complicated and unfeasible. This forces farmers to choose other more convenient crops.
- The competition with other similar products, mainly from Asia, such as the marrow or the "ratán", which come into the market at lower prices than the wicker.
- The excessive dispersion of willow growers, which makes them fall into the dealers hands to manipulate prices, so that they can be doubled or devalued to one-half the price that the farmer can get from one season to another.

In order to solve these problems, the Willow Growers Association called APROMICU was created in Cuenca where most of the willow production occurs. This Association has developed a Strategic Plan for this sector. One of the conclusions of this study is the need to concentrate the supply of the whole area by creating willow growers cooperative. This cooperative would carry out different actions for the development of this sector, such as wicker promotion, search of new markets and products, supply organization, etc. The "Centro de Investigación Agraria de Albadalejito, Consejería de Agricultura, Junta de Comunidades de Castilla-La Mancha" in cooperation with APROMICU launched a new research project on willow growing in 2003. This project was called "Estudio de la problemática del cultivo del mimbre en Casilla-La Mancha y creación de una colección de cultivares en el C.I.A. de Albadalejito" (Nº REG.: PT/54-CL03) (Study on the problems of willow growing in Castilla-La Mancha and creation of a collection of cultivars in the C.I.A. in Albadalejito). The aims of the project are:

-The exploration, collection, and conservation of different willow materials from willow grown in Castilla-La Mancha. Create a collection of willow cultivars.

-Agronomic study of willow growing. Analyse the willow situation in Cuenca.

-Study and identify the insects and pathogens associated with willow cultivation.

### **QUINOA: DRY MATTER, PROTEIN, AND SAPONIN YIELD RESPONSE TO ENVIRONMENTAL CONDITIONS**

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The state of Coahuila in northern Mexico is characterized by arid and semiarid regions with scarce and irregular rainfall. The forage crops in this region are oat, barley, corn, sorghum, wheat, and triticale. Yields in 2002 fluctuated from 8.3 t ha<sup>-1</sup> to 13.5 t ha<sup>-1</sup> for oats in the non-irrigated dryland areas, whereas the irrigated yields were 25.0 t ha<sup>-1</sup> for barley and 43.4 t ha<sup>-1</sup> for corn. Quinoa (*Chenopodium quinoa* Willd) is a high protein content specie growing in adverse climatic and soil conditions. This makes it a possible alternative among the forage producing species.

The objective of this work was to evaluate the adaptability of quinoa to the environmental conditions of northern Mexico. Information was obtained for the four cultural cycles and analyzed by factor analysis that allows the grouping and identification among the response variables.

Four experiments were carried out during 2000 and 2001. A randomized block design was used with two soil water deficit treatments and four replications. Two cuttings were made during crop development. The evaluated variables included dry matter, protein, and saponin content to carry out a global analysis of the four experiments and quantify the relations among the variables to determine the effect of irrigation, rainfall, evaporation, and accumulated heat units on dry matter yield, protein and saponin contents.

The highest dry matter yield was 16,962 kg ha<sup>-1</sup> obtained during flowering with a 13.8% protein and 0.70% saponin content. The lowest yield was 9,894 kg ha<sup>-1</sup> during flowering with a 15.4% protein and 0.67% saponin content.

### **INFLUENCE OF PLANT GROWTH REGULATORS AND NITROGEN ON TOTAL SUGAR, PROTEIN, AND CRUDE PROTEIN CONTENTS OF BABY CORN - THE NEW INDUSTRIAL CROP OF INDIA**

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[Top of Page](#)

[Return](#)