GERMPLASM IMPROVEMENT AND AGRONOMIC DEVELOPMENT OF NEW ALTERNATIVE CROPS

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PROJECT SUMMARY

Agricultural diversification is important for achieving economic stability and future growth of agriculture. One way to achieve diversification is the development of new crops. New/alternative crops must complement instead of compete with existing traditional crops. In addition, the new crops must be able to conserve water and nutrients and help in improving the environment. The objectives of this project are to (1) acquire and characterize germplasm of promising new/alternative crops; (2) evaluate and enhance new crop germplasm for industrial materials; (3) develop basic knowledge of floral biology, seed production, and plant responses to environmental stresses; (4) develop economical production systems for new crops under various environmental and management conditions; and (5) develop methods for efficient guayule latex extraction and seed oil analyses for characterizing latex, resin, and oil properties. This research will result in scientific and popular publications on the basic biology, characteristics, production systems, and methodology for evaluating, enhancing, and growing new crops. The long-term goal is to provide high yielding germplasm of new crops that is adaptable to a variety of environments and has materials needed for industrial uses. This CRIS is the lead USDA-ARS project for breeding, genetics, germplasm collection, germplasm evaluation, and germplasm enhancement of new crops, and is the major source of the raw materials needed for pursuing related work on product development and utilization. The development of guayule as a new crop would provide relief to the 6 % of the US population with allergies to Hevea latex products. This includes 40 % of the medical workers and 60 % of multiple surgery cases. It would also develop a domestic source of latex, reducing our dependence on imported rubber. Development of additional products from the bagasse could result in additional products such as insulation, termite and wood rot resistant wood products, and a new natural gum base to use in chewing gums. Development of lesquerella as a new crop would result in a domestic source of hydroxy fatty acid, replacing castor oil imports that cost over $100 million annually. The oil would serve as base for renewable based lubricating oils.

OBJECTIVES

1. Acquire and characterize germplasm of guayule, lesquerella, vernonia, and other promising new/alternative crops.

2. Evaluate and enhance germplasm of new crops for industrial materials.

3. Develop basic knowledge of floral biology, seed production and plant responses to environmental stresses.

4. Develop economical production systems for new crops under various environmental and management conditions.

5. Develop methods for efficient guayule latex extraction and seed oil analyses for characterizing latex, resin, and oil properties.

NEED FOR RESEARCH

Description of the Problem to be Solved

The need exists for improving the economic status of the U.S. farmer and reducing the costs associated with surplus crops. In addition, improving this country's balance of payment and decreasing its vulnerability to imports of strategic industrial raw materials cannot be readily dismissed. Successful commercialization of the new crops may even lead to the export of the raw and finished products. Agricultural diversification is important for achieving economic stability and future growth of agriculture. New/alternative crops must complement instead of compete with existing traditional crops. In addition, the new crops must be able to conserve water and nutrients and help in improving the environment.

The U.S. spends over one billion dollars annually importing Hevea rubber - the only source of natural rubber for use in industry and commerce. More recently, it was discovered that a large portion of the world population has become allergic to the Hevea rubber hygienic products. These allergies can sometimes be life-threatening. Guayule (*Parthenium argentatum*) synthesizes latex rubber, which has been found to be hypoallergenic and offers an alternative to Hevea latex. Since the plant is native to the southwestern United States, cultivation of this crop could mean additional economic sources for the farmers in this region. Successful commercialization of guayule, however, depends on the identification and development of acceptable production practices and processing methods. While much is presently known about maximizing solid rubber production, little is known about maximizing latex production.

The U.S. imports over 40 million dollars of castor oil, a strategic raw material, annually for use in lubricants, cosmetics, plasticizers, protective coatings, surfactants, and pharmaceuticals. Production of castor in the U.S. is restricted because of its high level of allergic reactions and seed toxicity. Lesquerella (*Lesquerella fendleri*), a plant native to the U.S., produces a hydroxy fatty acid, which is an acceptable alternative to castor oil. Successful commercialization of lesquerella depends on the identification and development of enhanced germplasm with high seed yields, high oil content, high lesquerolic acid content, autofertility, and acceptable production practices. A large germplasm collection is being developed and evaluated for desirable characteristics at the U.S. Water Conservation Laboratory. Much work remains to be done to finish the evaluation of the collection and to transfer the desired traits into commercially acceptable lines. Information is also needed on optimizing production practices.

The oil-based paint and pesticide industries are looking for ways to reduce emissions of volatile organic compounds (VOC), which contribute to the pollution of the atmosphere. One alternative to correct this problem is to use vegetable oils high in epoxy fatty acids. Vernonia (*Vernonia galamansis*) is one of the few plants that naturally synthesizes an epoxy fatty acid, which has low volatility and good solvent properties needed in paints. Other industrial uses for the oil are in epoxy-alkyd paints, toughened epoxy resins, dibasic acids, lubricants, pesticides, and adhesives. Successful commercialization of vernonia depends on the development of germplasm with high yield and oil content, high vernolic acid content, good seed retention, uniform maturity, day neutral flower induction as well as acceptable crop production features.

Information available on the cultural management of these new crops is incomplete. Thus, additional work must be done to obtain answers before wide-scale commercial production is possible. Some examples of areas needing work are dates of planting for maximum stand establishment and yield,
seeding rates that are economical, seed treatments to ensure stands and break dormancy, planting methods that result in acceptable stands and result in maximum yields, dates of harvest for maximum yield and quality, harvesting methods that result in minimum losses, water use data for scheduling irrigations, nutrient requirements that minimize pollution and result in high yields, pest control measures for insect, disease, and weed problems, post-harvest and preprocessing studies to maximize yields and quality.

Relevance to ARS National Program Action Plan

This research involves collecting, evaluating, and enhancing germplasm of new crops, while developing planting, growing, and harvesting systems for producing a profitable crop, which contributes to the Plant Germplasm Conservation and Development National Program. Cooperative research with other scientists leads to commercial and industrial applications for new crops and new analytical methods necessary for making progress in a breeding program. Besides the primary uses of these crops, additional products such as gums, bagasse, resins, and seed meals for animal feed contribute to the New Uses National Program 306.

Potential Benefits

The development of guayule as a new crop would provide relief to the 6 % of the US population with allergies to Hevea latex products. This includes 40 % of the medical workers and 60 % of multiple surgery cases. It would also develop a domestic source of latex, reducing our dependence on imported rubber. Development of additional products from the bagasse could result in additional products such as insulation, termite and wood rot resistant wood products, and a new natural gum base to use in chewing gums. Development of lesquerella as a new crop would result in a domestic source of hydroxy fatty acid, replacing castor oil imports that cost over $100 million annually. The oil would serve as base for renewable based lubricating oils.

Anticipated Products

This research will result in scientific and popular publications on the basic biology, characteristics, production systems, and methodology for evaluating, enhancing, and growing new crops. The long-term goal is to provide high yielding germplasm of new crops that is adaptable to a variety of environments and has materials needed for industrial uses.

Customers

Customers of this research include other scientists, cooperative state extension personnel, regulatory agencies, growers, users of the GRIN system, other federal agencies, and industry.

SCIENTIFIC BACKGROUND

Germplasm improvement and varietal trials have identified lines that have almost double the rubber yield of older lines. Estimates of the amount of genetic diversity within a facultative apomictic populations have shown high amounts of variation from one generation to the next. This variation is due to the facultative (apomixis and sexuality coexisting) nature of guayule. Various breeding strategies have been devised to accommodate this and improve yield characteristics. Reports relative to the cultural management and production of guayule have been completed. In light of the great impact of the hypoallergenic guayule latex, research has been done to determine the extractability and post-harvest handling of the shrubs prior to processing. Specific Cooperative Agreements, based primarily on DOD-Advanced Materials from Renewable Resources, have been established with the University of Arizona and University of Akron to work on latex physical and chemical properties, and analysis. Cooperative work has been maintained with the ARS Western Regional Laboratory on the biological aspects of the latex.

Several new populations of *L. fendleri* have been collected for use in improving seed yields and other related plant characteristics. These populations had not previously been available through the National Plant Germplasm System. New collections are necessary for plant breeding since they may have improved traits over the present germplasm being used. Other species of *Lesquerella* and a closely related genus *Physaria* also have been collected. These species contain seed oil rich in hydroxy fatty acids that could be developed for lesquerella production in different environments. Preliminary evaluations of some of this new material have been completed. This work is partly funded through a grant form USDA, ARS, Plant Exploration Office and was partially funded in the past through a USDA, Alternative Agricultural Research and Commercialization (AARC) Center grant. A feature of this project is the creation of a network of relationships among government and private sector organization The USWCL has been responsible for defining cultural management practices including irrigation timing and amounts, planting and harvesting techniques, as well as the initial research and development that will eventually result in an alternative crop for the U.S. farmers in a relatively short amount of time.

Hybrids have been produced between a variety of *Vernonia galamensis 'petitina'*, that has a day-neutral flowering response, and germplasm with other desirable characteristics. The initial phase of successfully combining these two germplasm sources has been accomplished. These hybrids have been evaluated at this location and at a number other sites across the U.S. Selections better adapted to specific locations have been made. These hybrids are continuing to be developed through breeding and agronomic studies. Grants have also allowed us to evaluate seeds for oil content and the profile of the fatty acid distribution by the purchases of NMR and gas chromatographic equipment.

Short- and long-term goals are variable depending upon the new crop and its stage of development. Thus, each of the three crops will have certain priorities peculiar to that crop. Short term goals are: (1) development and release of higher yielding guayule, lesquerella, and vernonia germplasm; (2) development of production, harvest, post-harvest, and storage guidelines and recommendations for growers and industry; (3) evaluation and collection of new lesquerella germplasm from the US and Mexico; and (4) develop new uses for primary and secondary products in cooperation with others. The long-term goal for all crops is to provide high yielding germplasm adaptable to a variety of environments with raw materials useful for industry in order to successfully commercialize these new crops. All work will be closely coordinated within the CRIS unit, and between cooperators and industrial users.

Literature Review

A CRIS search was conducted for projects conducting research in the areas of Guayule, Lesqueralla, and Vernonia. Of the 55 projects received, 26 were for projects that have been discontinued before January 1995, 12 were for projects that will expire in CY 1995, and 17 were for projects expiring after January 1996. Twelve of the 17 will expire in CY 1996, in which two are support for the existing CRIS, one is a cooperative project with this project, and the other two are not related to research proposed in this CRIS. These findings indicate the importance of continuing this project for successful development of lesquerella, guayule, and vernonia as new industrial crops. This CRIS is the lead USDA-ARS project for breeding, genetics, germplasm collection, germplasm evaluation, and germplasm enhancement of new crops, and is the major source of the raw materials needed for pursuing related work on product development and utilization.

<u>Guayule</u>

The United States is totally dependent upon Hevea natural rubber from southeast Asia for commerce and defense. The annual importing cost of this critical industrial material is about $1 billion. Guayule, a native to north-central Mexico and southwestern Texas, is capable of synthesizing rubber with properties equivalent to the Hevea plant (Hammond and Polhamus, 1965). In the early 1990s, the wild shrub was harvested and processed for rubber in Mexico. The rubber was used in the manufacture of automobile tires. The rubber was replaced by Hevea because it could be produced cheaper than guayule. However, no reasons exist that would prevent this plant from being grown commercially in the United States. The economics for cultivating guayule have not been good in the past, but the petroleum crisis of the 1970s and the large increase in natural rubber prices in the mid 1990s have greatly improved the possibility for commercialization of the plant.

The recent discovery by an ARS scientist that the rubber from the guayule plant is hypoallergenic has further enhanced the potential for commercializing this crop (Cornish, 1995; Siler and Cornish, 1994). Because of the widespread use of latex products and poor quality control, many people have become allergic to Hevea latex. The allergy can be mild to life threatening and special meetings have been convened to discuss Hevea allergies (European Rubber Journal and Rubber Consultant meeting, 1993). There appears to be no solution to the allergy problem with Hevea, since the allergy causing proteins present cannot be completely removed without affecting the quality of the ruber products. Thus, there is a tremendous market for guayule latex for the production of medical products such as catheters, surgical gloves, contraceptives, balloons, and toys. The use of guayule latex could generate a larger economic impact than that of guayule rubber for tire production. Resins, another major natural product of the guayule plant, hold promise for developing co-products such as adhesives, coatings, and biological control agents, which may have an economic value equal to or greater than the natural rubber (Bultman et al., 1991; Thames and Kaleem, 1991; Nakayama et al., 1992).

Guayule rubber extraction to obtain and maintain the latex form has not been seriously considered, since the ultimate goal of removing the guayule rubber was to get it in the solid form. Unlike Hevea, the guayule rubber particles reside in individual cells and must be physically removed from the cells. For getting solid or bulk rubber, the procedure is to grind the shrub and treat it with an organic solvent to dissolve out the rubber from the ground material. In the case of latex, which is an emulsion of suspended rubber particles, a different procedure must be used. Thus, methods must be developed to extract and preserve the emulsified form of rubber. The latex extraction process is less drastic than organic solvent extraction of bulk rubber removal since it is water based. Actually, a procedure for extracting latex with water was made in the mid 1940s, but the object at that time was primarily to remove rubber from the plant and get it into the solid rather than the latex form (Jones, 1948). The solid rubber produced in this manner contained resin impurities, which could be removed by solvent extraction/washing. However, the introduction of an organic solvent into a rubber latex emulsion to remove the resin material would immediately cause coagulation of the latex and formation of solid rubber. This cannot be allowed in the production of latex-based products such as gloves that depend on the formation of a film from the latex.

The resinous impurities in rubber are known to lower the physical quality of solid rubber and would be expected to be true also for latex rubber products (Winkler and Stephens, 1978). Thus, these must be removed but must be done with procedures which would avoid the use of organic solvents. Some of the water soluble and extractable resin-related materials have been identified (Schloman and Hilton, 1991), but those directly related to the rubber particles of the latex have not and must be determined. Industrial standards for guayule latex quality have not been established, but probably would closely follow those for Hevea latex.

Guayule cultivation has never been done on a large and continuous scale, but knowledge on its culture has been developed through the years. Information on its latest management practices has been compiled in a book edited by Whitworth and Whitehead (1991). However, little is known about growing guayule for latex production. Because the principles of latex and solid rubber extractions are different, information is needed about the harvesting, handling, and storage of the shrub. For example, we found that the drying of the harvested shrub can greatly affect the extractability of the latex (Nakayama, 1995). Also, data are needed on the handling and storage of the latex material after extraction. Formulations needed to construct the final latex products must be developed, but the manufacturer at this time would be best able to handle this aspect of product fabrication.

The plant breeding and genetic efforts on guayule for the 1942-1959 period have been summarized by Hammond and Polhamus (1965). Conventional breeding of guayule is hampered by the presence of apomixis in most polyploid material and by self-incompatibility in most sexual diploids. These constraints also have limited the amount of current knowledge on the heritability of economically important characters. Thompson and Ray (1988) and Estilai and Ray (1991) have thoroughly reviewed many of the aspects for improving guayule through breeding and management practices.

Numerous researchers have found a high degree of variability within and between guayule lines (Ray, 1983, 1989; Benitez and Kuruvadi, 1985; Naqvi, 1985; Thompson et al., 1988; Dierig et al., 1989a, 1989b; Thompson et al., 1990b; Estilai and Ray, 1991). Several of these studies have also shown that plant dry weight is generally a better predictor of final rubber or resin yield than either the rubber or resin percentage. These results indicate that significant improvements in plant dry weight, rubber yield, and latex yield should be possible.

The better rubber yielding lines in the Uniform Regional Varietal Trials have been selected and replanted for additional testing and seed increase. Because of the facultative apomictic nature of reproduction in guayule improvement of germplasm through traditional breeding methods is difficult. Tissue culture technology has been developed for clonal propagation of guayule (Radin et al, 1982, 1985). Initial studies been made on the characterization of isozymes in guayule (Estiali et al., 1990; Ray et al., 1993). Development of isozyme patterns in guayule would be helpful for the identification of sexual hybrids in crosses among apomictic parents.

Lesquerella

Domestication of *Lesquerella* species as a new crop for arid lands and a domestic source of hydroxy fatty acids has been discussed (Hinman, 1984, 1986; Princen, 1979, 1982, 1983; Princen and Rothfus, 1984; Senft, 1988; Thompson, 1985, 1988, 1989, 1990a; Thompson and Dierig, 1988; Thompson et al., 1989; Thompson, 1990). Rapid progress is being made toward full commercialization with cooperation between industry and government agencies (Dierig, et al., 1993). Lesquerella is a new World genus of annual, biennial, and perennial herbs. Over 90 species have been described (Barclay et al, 1962; Rollins and Shaw, 1973). The basic chromosome numbers for species within the genus are n=5, 6, 8, and 9 (Rollins and Shaw, 1973). Rollins and Solbrig (1973) demonstrated that interspecific hybridization occurs in nature among some taxa of Lesquerella. This indicated that controlled crossing among selected species many permit transfer of desirable genes and result in genetic recombinations that may be amenable to breeding and selection. However, a sporophytic multiple allele incompatibility system was found to be operative in lesquerella, which may place constraints on transfer of genetic factors (Rollins and Shaw, 1973; Rollins and Solbrig, 1973; Sampson, 1958). Several putative male sterile plants of *L. fendleri* have been observed.

The chemical composition of lesquerolic acid in lesquerella seed oil is very similar to that of ricinoleic acid in castor oil, and is seen as a viable replacement for imported castor oil. Because the carbon chain length of lesquerolic acid is longer than that of ricinoleic acid (C20 vs C18), the possibility exists that lesquerella oil may also prove to be more useful than castor oil in the formulation of new industrial products. About 95% of lipstick is currently being made from castor oil and part of this can be readily replaced with lesquerella oil. Research indicates that when lesquerella seed oil containing lesquerolic acid is polymerized to form polyesters or polyurethanes in the presence of polystyrene, a new class of tough plastics (interpenetrating polymer networks) is formed (Sperling and Manson, 1983). The two other types of hydroxy fatty acids, auricolic and densipolic acid, found in various lesquerella species, may also prove to be valuable industrial feedstocks for the development of new, unique products.

As with other members of the Brassica family, glucosinolates are present in lesquerella seed meals, but none were found that give rise to goitrogenic substances (Daxenbichler et al., 1962). Seed meals resemble those of other Brassicaceae such as rapeseed and crambe. Lysine contents were found to be significantly higher than those of 41 other species of Brassicaceae, which suggest that they may serve as good protein supplements for feed grains (Miller et al., 1962). A specific glucosinolate (methylsulfinylpropyl isothiocyanate) found in *L. flendleri*, *L. gordonii*, and other species is believed to have anti-cancerous tumor activity, and is currently under investigation at the University of Minnesota (Dr. L. W. Wattenberg, personal communication).

One species native to the arid Southwest, *L. fendleri*, is considered to be the prime candidate for domestication (Gentry and Barclay, 1962). This species is a perennial grown as a winter annual at elevations between 600 and 1800 meters in areas of annual precipitation ranging from 250 to 400 mm. Germplasm evaluations by Thompson (1988) and Dierig et al. (1995) have confirmed the prediction of Gentry and Barclay (1962) that *L. fendleri* contains the best germplasm for development of a new industrial oilseed crop. Fortunately, germination of seeds of *L. fendleri* appears not to be hampered by dormancy as has been found with other species (Bass et al., 1956; Sharir and Gelmond, 1971). A breeding and selection program and initial agronomic research was initiated by USDA-ARS in 1984 in Arizona (Thompson and Dierig, 1994). Thompson and Dierig (1988) and Thompson et al (1989) reported on the yield potential of *L. fendleri*, preliminary results on the effects of plant population, and water usage. Plant populations of around 1 million/ha appear to be optimal. Seasonal water use of about 640 mm has been found to produce good yields of 1700 kg/ha of seed, 30% oil, and 50% of lesquerolic acid. Lesquerella can be produced in a cropping system very similar to wheat or other small grains grown as a winter crop in areas such as central Arizona (Thompson, 1988). Harvest of lesquerella has been successfully accomplished with a standard combine equipped with small-sized screens (Dierig et al, 1993).

<u>Vernonia</u>

Vernolic acid was first discovered in seed oil of *Vernonia anthelminitica* by Gunstone (1954). This species also was identified in the USDA-ARS plant screening program at NCAUR, Peoria, IL, where oil contents of 26.5% were determined (Earle et al., 1960), and the presence of vernolic acid in the amount of 67% was identified (Smith et al., 1959). Substantial research was conducted to develop this plant into a new crop source of epoxy fatty acids used as industrial feedstocks for the coatings and plastic industries (Princen, 1979, 1982, 1983; Princen and Rothfus, 1984). However, there were major constraints to the domestication of this species that forced termination of the research effort.

Vernonia belongs to the Asteraceae (Compositae) family. There are about 1,000 species in the Vernonia genus (Jones, 1977). Plant exploration and collections were attempted in 1966-67 by Smith (1971) for other potential *Vernonia* sp. in Ethiopia, Kenya, Uganda, Tanzania, and South Africa.

Prior to this exploration, collections of *V. galamensis* (formerly known as *V. pauciflora*) were made in Ethiopia by Perdue while on another mission in 1964 (Perdue et al., 1986, and Gilbert, 1986). Seeds of this new germplasm contained 42% oil and 73% vernolic acid, which is considerably higher than *V. anthelmintica*.

The important discovery of this germplasm led to a revived interest in the domestication of vernonia as a source of epoxy fatty acids. Additional germplasm has been collected from Malawi, Ghana, Nigeria, and Kenya. Six subspecies are taxonomically recognized, with one containing four distinct botanical varieties (Gilbert, 1986; Jeffrey, 1988). Currently, there are 33 accessions of the taxa available for crop improvement. In 1989, USDA-ARS initiated a major germplasm development program at the U.S. Water Conservation laboratory. Available accessions of *V. galamensis* were characterized in regard to seed oil content, fatty acid composition, seed weights, and chromosome numbers (Thompson et al., 1994a) Germplasm also was grown at other locations in Texas, Louisiana, Arizona, Virginia, Oregon, and Iowa, to determine the extent of variation under different environmental and geographic conditions (Thompson et al., 1994b).

Vernonia can be grown in most areas of the U.S. only in warm seasons, since it is frost sensitive. The germplasm being evaluated flowered only under short day (cool season) conditions, except in one accession, *V. galamensis* ssp. *galamensis* var. *petitiana*. This accession flowered under any photoperiod condition (Phatak et al., 1989). Unfortunately, our evaluations demonstrated that this variety lacked important agronomic characters present in other subspecies and varieties.

To overcome day-length restrictions, a hybridization program to recombine day-neutral flowering with the other desirable growth characteristic was attempted. Thompson et al. (1994c) outlined the successful progress of these crosses. The main emphasis of the present breeding program at USWCL is to further develop yield characteristics of these day-neutral hybrids. Other plant characteristics being investigated include autofertility, non-dormant seed germination, seed retention, and increased uniformity of seed maturity (Dierig and Thompson, 1993). Lipase activity in the seed, capable of hydrolyzing the triglyceride, is also an industry concern (Ayorinde et al., 1993).

Interest also has been revived in vernonia utilization research (Afolabi et al., 1989; Ayorinde et al., 1988, 1989; Carlson et al., 1981; Carlson and Chang, 1985). A pilot plant for extraction of seed oil produced in Zimbabwe has been completed (K. D. Carlson, personal communication), which will provide both oil and meal for further research and evaluation by industry. The low viscosity of vernonia oil may permit it to be used as a solvent or additive to alkyd-resin paints with the expectation that emissions of volatile organic compounds (VOC) will be greatly reduced. VOCs react with nitrogen oxides in the presence of sunlight to crate ground-level ozone, a deleterious component of smog. The more promising uses of vernonia oil for industrial products are baked coatings on metal panels (Carlson et al., 1981), and the synthesis of dibasic acids and interpenetrating polymer networks (Afolabi et al., 1989; Ayorinde et al., 1988, 1989).

APPROACH AND RESEARCH PROCEDURES

The evaluation and development of new-crop germplasm that leads to useful agronomic cultivars with concurrent development of appropriate crop production practices are long-term research activities. Timetables are difficult to construct and frequently inaccurate or misleading even with the most conservative estimates of the problem. Progress toward achieving the objectives will be constantly reviewed and changes made as necessary to maximize efficient use of personnel and resources.

Objective 1 - Acquire and characterize germplasm of guayule, lesquerella, vernonia, and other promising new/alternative crops.

Experimental Design

The success of this breeding program is based on a diverse, well characterized germplasm base. Once this diversity is established, necessary breeding strategies will be implemented to further develop and improve the specific crop. The Germplasm Resources Information System (GRIN) database of the National Plant Germplasm System (NPGS) will be used as a source to identify potential plant diversity. However, in many cases, the available species accessions found in the NPGS do not adequately represent the amount of genetic diversity available in nature. In these cases, new seed collections will be acquired, evaluated, and enhanced. An extensive database has been established for *Lesquerella* and *Physaria* species, which includes extensive locality information from many U.S. herbaria. This database now has over 1400 different entries for collection use.

The goal of our germplasm collections is to obtain genetically diverse plant populations of the potential crop. Related species will also be utilized in crop development and will also need to be collected. Seed will be increased following collection from native areas. Some initial evaluation information will be obtained from original native population sites, and when the seed is increased. Additional evaluation information will be obtained from replicated yield trials when adequate planting seed becomes available. Another important aspect of this objective will be to incorporate these new acquisitions into NPGS. Once accessions are evaluated and characterized, the desired traits will be exploited through breeding strategies.

Contingencies

The successful completion of this object will depend on the continued support by outside funding for collection trips and evaluation and seed increase of germplasm collected. Funding levels will determine whether all three crops can be studied or if studies will be limited to areas that are the most promising. Adequate student and technical help is critical to successful completion of this objective.

Collaborations

Collections and evaluation are coordinated with the Plant Germplasm Introduction and Testing Research Unit, Pullman WA; Regional Plant Introduction Station, Ames IA; and National Seed Storage Laboratory, Fort Collins CO.

Objective 2 - Evaluate and Enhance Germplasm of New Crops for Industrial Raw Materials.

Experimental Design

Standard breeding techniques will be used to develop improved germplasm of each potential new crop. Depending on the floral structure and biology of the crop, different breeding strategies are employed for cop development. Some of these strategies include: (1) improvement of germplasm by half-sib family recurrent selections in open-pollinated populations; (2) incorporation of specific traits, such as day-neutral flowering, from donor plants through backcross breeding; (3) development of lines with natural or chemically inducted mutations for altered seed-oil fatty acid profiles; (4) development of lines through mass, family or single plant selection, as appropriate or wide crosses through intra- and interspecific hybridization to obtain desired recombinations. Lines or cultivars will then evaluated in yield trials at single or multiple locations.

The U.S. Water Conservation Laboratory (USWCL) is equipped to participate in various types of screening and evaluations needed for conducting a breeding program. Seeds can be non-destructively analyzed for oil content by NMR and fatty acid profiles by GC. Assays will be developed for rapid screening for amounts of glucosinolates and seed-coat gums. Work has been done on seed conditioning. Light and dissecting microscopy is routinely performed for cyrological and floral biology information. Haploid production through anther culture or microsporogenesis will be attempted for mutation breeding and generating homozygotes for various genetic studies. Isozyme markers have been identified for various crops and will continue to be used as co-dominant genetic markers. Our laboratory is also equipped for DNA marker analyses, such as RAPD's, utilizing Polymerase Chain Reaction (PCR) techniques.

Contingencies

Successful completion of this objective will depend on being able to achieve the desired crosses between divergent parents within and between species. Funding levels will determine whether all three crops can be studied or if studies will be limited to areas that are the most promising. Adequate student and technical help is critical to successful completion of this objective.

Collaborations

Analyses of other seed oil constituents are at times sent to ARS, USDA, National Center for Agricultural Utilization Research (NCAUR), Peoria IL. We also collaborate with the University of Arizona and the ARS, USDA, Western Regional Research Lab, Albany CA to develop methods to analyze latex, rubber, and resin content of single guayule plants. Collaborations also exist with the National Forest Products Lab, University of Illinois, The University of Arizona, and others in developing uses for guayule bagasse following latex extraction.

Objective 3 - Develop knowledge of floral biology/seed Production and Plant Responses to Stresses

Experimental Design

In order to make hybrids and develop enhanced germplasm, basic knowledge of floral biology such as time of anthesis, degree of autofertility, sterility, day length requirements, seed retention traits, and terminal flowering habits will be developed. Basic studies utilizing accepted and new methodologies and experimental procedures will be used to determine: (1) the amount of autofertility present in the lesquerella germplasm collection; (2) the types and of causes of male sterility, including cytoplasmic, in lesquerella; (3) the day length flowering requirements in the vernonia germplasm collection and hybrid populations; (4) the seed retention traits of guayule, lesquerella, and vernonia; (5) the relationship between head size and yield of vernonia; and (6) the development of terminal flowering habits in vernonia for uniform maturity at harvest.

Successful production of new crops also required knowledge of the crop's response to stress. While the effects of water stress on guayule for rubber production have been determined, similar studies will be conducted to determine the effects on latex production, and seed production in lesquerella and vernonia. Susceptibility to insects and diseases will be evaluated as the need arises utilizing accepted procedures or modifications as necessary. Since guayule and lesquerella are proposed for production in semiarid regions, water requirements and related management studies will be conducted.

Contingencies

Funding levels will determine whether all three crops can be studied or if studies will be limited to areas that are the most promising. Adequate student and technical help is critical to successful completion of this objective.

Collaborations

Cooperative work with the Irrigation and Water Quality group at USWCL, the Remote Sensing unit of the Environmental and Plant Dynamics group, and The University of Arizona will be needed to accomplish this objective.

Objective 4 - Develop Economical Cultural Practices and Production Systems for New Crops under Various Environmental and Management Conditions

Experimental Design

For new crops to become successful, economical cultural practices and production systems must be adequately defined and established. Similarly for the breeding program to be successful these practices and systems must also be specified so that the desired traits can be selected and incorporated into advanced lines. Production practices for solid rubber production for guayule have been developed. However, the effects of these practices on latex production are unknown.

Therefore, tests will be conducted to compare the advantages and disadvantages of direct seeding versus transplants. If transplants are found to be the more desirable practice, the newest field transplanting techniques developed for other crops will be adapted. Concurrently, studies with multiple germplasm lines will be conducted to determine the effects of stem size, time of harvest, and frequency of harvest on latex content. Appropriate field designs and statistical analyses will be used in all experiments. Basic tests will also be conducted to develop production systems for lesquerella and vernonia. Information needed includes fertility levels, optimum plants populations, weed and pest control methods, and planting and harvesting methods. Information developed from these studies will be utilized in the breeding program when selecting parents for developing new populations.

Contingencies

Funding levels will determine whether all three crops can be studied or if studies will be limited to areas that are the most promising. Adequate student and technical help is critical to successful completion of this objective.

Collaborations

Cooperative work with the Irrigation and Water Quality group at USWCL, the Remote Sensing unit of the Environmental and Plant Dynamics group, and The University of Arizona will be needed to accomplish this objective.

Objective 5 - Develop Methods for Efficient Guayule Latex Extraction and /Seed Oil Analysis for Characterizing Latex and Oil Properties

Experimental Design

The development of new crops requires specific extraction and analytical procedures that are not presently available. In addition, the germplasm improvement program will need a vast number of samples to be analyzed and processed rapidly in time for selection and preparation for the next planting cycle. Areas that will need special attention will be sample preparation prior to analysis and automation of the analysis and data reduction. New developments in instrumentation also will be incorporated into the analytical scheme.

A reliable procedure for extracting latex from the guayule shrub is not available at present. Thus, several methods for obtaining latex only will be investigated. The primary procedure being used at present is a water-based process where the shrub is ground with a mixture of antioxidant, resin absorber, and water. The crude latex will be initially cleaned of other plant residue by centrifugation, and the resultant latex will be used for various types of physical and chemical property testing. Preliminary results indicate that the latex must be further purified to remove more of the resin material. The resin material will be analyzed and characterized. Several possible routes to attain acceptable latex purity will be followed. These include solid phase extraction and selective solvent extraction techniques. All the steps taken from shrub preparation, extraction, and purification of the latex will be carefully evaluated for achieving maximum yields. Latex preparation also will be coordinated with cultural practices, such as time of harvest and methods of shrub storage, in order to maximize latex production.

Contingencies

Funding levels will determine whether studies will be limited to areas that are the most promising or if other possible areas can also be investigated. Adequate student and technical help is critical to successful completion of this objective.

Collaborations

We will collaborate with The University of Arizona and the ARS, USDA, Western Regional Research Lab, Albany CA to develop methods to analyze latex, rubber, and resin content of guayule plants. Collaborations also exist with the National Forest Products Lab, University of Illinois, The University of Arizona, and others in developing uses for guayule bagasse following latex extraction.

Milestones and Expected Outcomes

This project is scheduled for formal review in 2002, thus only three year milestones and expected outcomes are listed.

Date	Objective 1	Objective 2	Objective 3	Objective 4	Objective 5
January 2002	New lesquerella germplasm from Mexico will be obtained and seed increased	Guayule germplasm lines will be evaluated for latex and growth	Environmental effects on guayule will be determined	New studies on water use for guayule will be started	Effects of different surfactants on latex extraction will be established
January 2003	New lesquerella germplasm from the US and Mexico will be obtained, evaluated, and seed increased for GRIN system	New lesquerella germplasm lines will be released with higher oil yields	Production system guidelines for lesquerella will be released to growers	Harvesting guidelines for lesquerella will be developed and made available to growers	New products for lesquerella oil will be developed and tested in cooperation with a commercial partner
January 2004	New vernonia germplasm will be released	New higher yielding and faster growing guayule germplasm lines will be released	Production system guidelines for guayule will be released to growers	Production system guidelines for guayule will be developed and made available to growers	New products from guayule bagasse will be developed in cooperation with industry

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