FY 2009 Annual Report
NP 213 – Bioenergy

The ARS Bioenergy Program is a flexible, holistic, long-term research effort involving coordinated thrusts in feedstock development (FD), sustainable feedstock production systems (SFPS), and biorefining (B). The holistic nature of ARS bioenergy research ensures that bioenergy production is integrated into existing agriculture in ways that…

- *provide consistent, attractive returns to producers*,
- *minimize adverse impacts on existing markets for food, feed and fiber,* and
- *demonstrate good stewardship of soil, water and air resources*.

Given the ARS mission and the breadth of ARS' research capabilities…

- ➢ *in all three major bioenergy research areas (**FD**, **SFPS**, **B**),*
    - ❖ most notably spanning all aspects of **FD** & **SFPS**
- ➢ *for solving complex technical problems involving multiple agricultural industries (food, feed, fiber and fuels),*
- ➢ *in agriculture-associated natural resources, including carbon cycling and water utilization,*
- ➢ *which can be targeted at any agricultural region in the Nation,*

…ARS has a unique ability to implement this integrated approach and enable the Nation to optimize bioenergy production as soon as possible.

Selected Accomplishments

Component I: Feedstock Development

Hi-Yield Switchgrass Strain. ARS scientists developed a strain of switchgrass that, when grown in Nebraska, produced a potential ethanol yield of 355 gallons per acre – 20 gallons per acre greater than that of the previous best cultivar. This is the first publicized example of a switchgrass strain specifically bred for improved conversion to ethanol. (NP 307, Component 1, P.S. 1a, Performance Measure 1.1.3) [Vogel]

Cell Wall Genomics. The plant cell wall is a complex composite of polysaccharide polymers, phenolic compounds and proteins; and the genes controlling cell wall composition are poorly understood. In collaboration with U.S. Department of Energy (DOE), ARS scientists sequenced the entire genome of the grass *Brachypodium*; and a complete draft of the genome was released through the website www.brachypodium.org. In addition, ARS researchers developed over 200 inbred *Brachypodium* varieties which are freely available to researchers, and have created over 4,000 T-DNA lines which will soon be released to the public. Using near infrared spectroscopy (NIR), ARS scientists have identified 27 mutants of *Brachypodium* with altered cell wall composition. This work will enable research to improve cell wall properties for biofuel production. (NP 307, Component 1, P.S. 1b, Performance Measure 1.1.3) [Tobias]

Rapid Predictive Method for Ethanol Yield from Biomass Feedstocks. Conventional wet chemistry analyses of biomass composition and tests for biomass conversion to ethanol are time-consuming and expensive. ARS scientists developed Near-infrared Reflectance Spectrometry (NIRS) calibrations for predicting biomass ethanol yield (per ton) for switchgrass. The NIRS measurements provide data on cell wall composition, cell wall sugars, soluble sugars, lignin, released and fermented glucose from cell wall cellulose, released cell wall pentoses, and other biomass quality attributes. The calibrations enable rapid and accurate estimation of theoretical ethanol yield from hexose sugars, theoretical ethanol yield from pentose sugars, total ethanol yield per ton, total ethanol yield per acre, total theoretical ethanol yield per ton, and total theoretical ethanol yield per acre. These calibrations will be useful for feedstock breeding, genetics, and management research, and can also be used by biorefiners to determine ethanol yield from a particular biomass feedstock. (NP 307, Component 1, P.S. 1b, Performance Measure 1.1.3) [Vogel]

Lignin Gene Mutation Increases Cellulosic Ethanol Yield. ARS scientists found that a low lignin sorghum mutant can produce significantly more ethanol (gal/ton) than wild-type sorghum. Incorporating this gene mutation into energy crops should enable the development of new varieties with superior traits for 2nd generation (cellulosic ethanol) biorefining. (NP301; Component 2, P.S. 2C; 1275-21000-263-00D; PM 2.2.3)

New Tools for Sorghum Breeding. The genes which enable a plant to sense the length of daylight must be incorporated into tropical sorghum lines so that they will flower and produce seed in temperate locations, such as the U.S., where the day-length is longer during the growing season. ARS researchers developed molecular markers and new genomic methods to efficiently breed varieties having these photoperiod-response genes. In addition, ARS scientists developed a quick and low-cost fluorescent screening assay to identify drought-tolerant sorghum germplasm. The fluorescent method is as effective as conventional selection techniques which required multi-year field trials at multiple locations. These new sorghum breeding tools will accelerate the development of new sorghum varieties with superior traits for bioenergy feedstock production. (NP301; Component 2, P.S. 2C; 6202-32000-021-00D and 1275-21000-263-00D; PM 2.2.3)

Component II: Sustainable Feedstock Production Systems

On-farm pretreatment of biomass. Pretreatment of cellulosic biomass is necessary in order to obtain reasonable yields of ethanol, but it requires expensive equipment and so is a major cost component in cellulosic ethanol production. In addition, the narrow window for crop harvesting requires long-term storage of biomass feedstocks, which can lead to significant losses from spoilage. ARS scientists developed simple, yet novel methods to combine these two steps --storage and pretreatment -- on-farm. They found that biomass can be stored with sulfuric acid or lime for one to six months in sealed plastic bags typically used for silage production. On-farm pretreatment/storage resulted in ethanol yields comparable to those obtained when pretreatment methods were done at a biorefinery, while avoiding spoilage losses, reducing overall costs, and providing farmers with opportunities to capture more return from their biomass crop. (NP 307, Component 3a, P.S. 3a1, Performance Measure 1.1.3) [Weimer]

CRP for Energy Crops. It has been suggested that land under Conservation Reserve Program (CRP) contracts can be used productively for growing perennial grasses as energy crops. ARS scientists showed that Old World bluestem grown on CRP land in central Oklahoma produced an average of only 1.7 DryTons/acre and that a native mix produced 0.9 DT/acre. Maximum yields were obtained at the October harvest for both Old World bluestem (1.86 DT/acre) and the native mixed species (1.0 DT/acre). Although soil characteristics were not altered by three years of annual harvest, biomass production consistently declined at all sites over the three harvest years. This study shows that some CRP land may not be attractive for biomass feedstock production. (NP 307, Component 2, P.S. 2b, Performance Measure 1.1.3) [Venuto]

Simple Method for Estimating Switchgrass Biomass Volumes. Efficient and accurate methods to estimate the amount of switchgrass biomass feedstock within a production area will help decision makers, and biorefiners in particular, plan their operations. ARS scientists evaluated the effectiveness of various indirect methods for on-field estimation of switchgrass yields in a multi-year study. Visual obstruction (as measured horizontally through a stand of switchgrass) was the best method for estimating yield on switchgrass fields with low to variable stand densities, while elongated leaf height measurements should be used on switchgrass fields with high, uniform stand densities. Twenty to 30 elongated leaf height measurements in a field could predict switchgrass biomass yield within 10% with 95% confidence. These procedures can be used by biorefiners to estimate feedstock supply in a production area, and also by the USDA National Agricultural Statistics Service (NASS) to estimate national bioenergy supplies from switchgrass. (NP 307, Component 2, P.S. 2a, Performance Measure 1.1.3) [Vogel]

Incorporating Perennials in Corn-Soybean Cropping Systems. Combining annual and perennial crop species in rotational cropping systems – termed "living mulch" cropping systems – could enable the production of both food and bioenergy on the same land and thereby minimize the displacement of food crops by cellulosic energy crop production. Concurrent management of food/feed crops such as corn or soybeans with perennial crops (forages) requires that the forages be suppressed during row-crop production. ARS scientists investigated combinations of reed canarygrass or orchardgrass with leguminous forages such as alfalfa, kura clover, and birdsfoot trefoil in a corn-soybean-forage rotation. The cover crops were managed by harvesting four times during the forage year and by suppressing with a 10 inch glyphosate band over the row during the corn and soybean years. They found that a combination of alfalfa, kura clover, and reed canarygrass resulted in the highest forage yields and lowest weed densities. They also found that seeding an unadapted alfalfa in the spring of the forage year supplemented yield and suppressed weeds in the former crop row. Use of the cover crops also allows producers to remove more corn stover (for bioenergy) and still maintain long-term productivity of the soil. In short, this study showed that producers can produce both food and bioenergy crops on the same land, diversify their cropping systems, obtain high yields of forages for livestock or bioenergy, eliminate the lower yields usually encountered in the first (establishment) year for perennials, and improve ecosystem function of corn production systems. (NP 216, Component 1A and NP 307, Component 2, P.S. 2B) [Singer]

Optimal Peanut Varieties for Biodiesel Production. Peanut oil could be an excellent feedstock for biodiesel production, but no data currently exist about which cultivars might be best suited for this market. ARS scientists evaluated the economic and agronomic performance (under both low and high input management strategies) and biodiesel engine performance of over 40 different cultivars. The research identified five peanut cultivars which exhibit superior production performance and oil characteristics and will help to enable on-farm biodiesel production. (NP 216, Component 1B and NP 307, Component 2, P.S. 2B) [Lamb]

Component III: Biorefining

Increased energy efficiency of corn biorefining. A major criticism of corn-based ethanol is its relatively low life-cycle energy efficiency, and that the bulk of the energy used in bioethanol production is consumed by the corn biorefinery. In turn, almost half of the total energy usage in a corn biorefinery goes into drying of the distillers grain co-product. ARS scientists discovered that cell wall degrading enzymes dramatically decrease the water binding capacity of the by-product grains and so make them much easier to dry. A plant trial of the new technology reduced natural gas usage in the distillers grains dryer by almost 15%, and increased the amount of water recycle in the biorefinery. The technology can easily be adopted by existing ethanol plants to reduce their energy usage and operating costs. (NP 307, Component 3a, P.S. 3a3, Performance Measure 1.1.3) [Johnston]

High-productivity bioreactor for cellulosic ethanol. Biorefining cellulosic biomass to ethanol is relatively expensive for a number of reasons. For instance, cellulosic biomass contains significant amounts of 5-carbon sugar, but yeast ferment only 6-carbon sugars. In addition, most ethanol-producing microorganisms are significantly inhibited by sugar degradation byproducts formed by acid pretreatment. Also, cellulosic biorefining as traditionally envisioned requires multiple steps and so more pieces of equipment. By using a combination of…

- a recombinant ethanol-producing microbe capable of fermenting both 5-carbon and 6-carbon sugars,
- an aerobic fermentation step to remove inhibitors typically produced by pretreatment, and
- a fed-batch, simultaneous saccharification and fermentation (SSF) process to consolidate equipment,

ARS scientists were able to convert wheat straw into ethanol in a manner that…

- increased the final ethanol concentration by 75% (to 4.5%),
- decreased the total processing time by 20%, and
- decreased the amount of hydrolysis enzymes by 50%.

These advances significantly increase the commercial viability of producing ethanol from cellulosic biomass. (NP 307, Component 3a, P.S. 3a1, Performance Measure 1.1.3) [Saha]

Corn oil from ethanol biorefineries. Most corn oil is extracted from corn in wet mill refineries because they are large enough to justify the use of expensive solvent extraction. In contrast, most bioethanol plants use dry grind process and do not produce edible corn oil as a co-product. ARS scientists developed a process called "aqueous enzymatic oil extraction" (AEOE) to separate corn oil from the germ produced at a dry-grind biorefinery, but the AEOE process needed a pretreatment step to increase oil yields. Consequently, ARS scientists developed an enzyme-based pretreatment step that increased oil yields to 90%. Preliminary cost estimates indicate that with the pretreatment step, the AEOE process will allow ethanol biorefineries to produce edible corn oil economically. The process may even replace hexane extraction in wet mill refineries. This advancement will help corn ethanol refineries produce a valuable co-product (corn oil), and be more economically resilient to volatile corn and ethanol prices. (NP 307, Component 3a, P.S. 3a3 and 3a4, Performance Measure 1.1.3) [Moreau]

Corn Ethanol Co-products as Fish Feed. A major feed component in fish farming is fishmeal, which is produced from marine stocks. Because of the rising cost and declining availability of marine stocks (and fishmeal), ARS scientists investigated the use of distillers dried grains (DDGS) as an alternative to fishmeal in aquaculture feeds. By optimizing extrusion conditions, starch binders, and DDGS levels, it was determined that the optimum DDGS level in feed for either catfish or tilapia was 15-20%. Since feeds for these species currently contain about 15% fishmeal (now $1,000/ton), replacing all the fishmeal they consume with DDGS (at $150/ton) could result in both a potential savings of $66 million for the U.S. aquaculture industry and a new 77,000 ton/yr market for DDGS producers. (NP 307, Component 3a, P.S. 3a4, Performance Measure 1.1.3) [Rosentrater]

Bioplastics from cellulosics. Today's major commercial bioplastic is polylactic acid (PLA), and the lactic acid bacteria which produce the L-lactic acid monomer for PLA can only ferment 6-carbon sugars such as those in sugar cane and corn starch. ARS scientists discovered a heat-tolerant, 5-carbon-fermenting lactic acid bacterium in dairy manure compost. This new strain uses all the sugars in biomass feedstocks such as corn fiber, switchgrass and wood; and it tolerates inhibitors typically produced by biomass pretreatment. This discovery may enable the commercial production of PLA plastics from cellulosic biomass. (NP 307, Component 3a, P.S. 3a4, Performance Measure 1.1.3) [Bischoff]

Improving Cold-Flow Performance of Biodiesel. Biodiesel typically thickens in cold temperatures, a phenomenon that can lower engine performance and may even clog engine fuel filters. ARS scientists determined that adding low levels (2.5%) of ethyl levulinate, a biobased material, improves cold flow performance of the resulting biodiesel fuel. ARS scientists also determined that enriching the vegetable oil feedstock in certain types of fatty acids (e.g., decanoic acid) results in biodiesel fuels with better cold flow properties. This knowledge can be used by crop breeders to produce vegetable oils better suited for biodiesel production. (NP 307, Component 3c, P.S. 3c3, Performance Measure 1.1.3) [Knothe]