

**FY 2010 Annual Report**  
**NP 213 Bioenergy**

Congress has set aggressive targets, such as 36 billion gallons by 2022, for U.S. production of renewable fuels. The ARS Bioenergy Program is designed to generate the science and technologies which can ensure that these targets are fulfilled by the sustainable production of herbaceous energy crops and crop residues and by the conversion of these feedstocks into marketable fuels and value-added coproducts. The fundamental structure of ARS Bioenergy Program consists of coordinated thrusts in three areas – feedstock development, sustainable feedstock production systems, and biorefining). Through these three components, the Program strives to ensure 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.*

ARS places a high priority on partnering with stakeholders in the entire bioenergy value-added chain (i.e., production, harvesting, collection, transportation, storage, fractionation, preprocessing, biorefining, and product marketing) so as to ensure that ARS research generates the widest impact in the shortest possible timeframe.

**Component I: Feedstock Development:**

**Enable new varieties and hybrids of bioenergy feedstocks with optimal traits**

Achieving Congressional targets for biofuels production requires new germplasm, parental stocks, and cultivars with value-added traits to enhance biomass yields, conversion efficiencies, and biorefinery co-product value. ARS plays a major role in feedstock development through fundamental research on molecular, biochemical, and genetic control of key plant traits. Component objectives include genetic and genomic research to enhance the value of bioenergy crops, identify molecular markers and functional gene sequences to facilitate selection of desired traits in breeding programs, develop innovative and efficient breeding strategies and evaluation tools, and practical breeding and germplasm development to support the bioenergy and agriculture industries. In most cases, the ARS research which contributes to this component utilizes funding provided through other national programs such as NP 301 (Plant Genetic Resources, Genomics, and Genetic Improvement); NP 302 (Plant Biological and Molecular Processes); NP 303 (Plant Diseases; NP 304 Crop Protection and Quarantine); and NP 215 (Rangeland, Pasture, and Forage Systems). In addition, most projects within this component involve collaborative and coordinated research supported by other governmental agencies, various non-governmental organizations, universities, private industry, and international partners.

## **Selected Accomplishments:**

***Plants with less lignin may be more disease resistant.*** Incorporating lignin biosynthesis mutants (bmr6 or bmr12 – aka brown midrib) into sorghum grain lines reduces lignin content and increases cell wall digestibility. Although lower lignin content can increase biofuel yield, it is commonly believed that low-lignin varieties are more susceptible to disease. In both field and greenhouse studies, however, ARS researchers found that brown midrib lines were actually more *resistant* to infection by the fungus *Fusarium*. In fact, one *Fusarium* species commonly found in wild-type grain was not detected in bmr12 grain. This research shows that crops modified for increased cellulosic biofuel yield are not necessarily more susceptible to pathogens and could even be more resistant.

***Breeding corn stover for higher biofuel yields.*** Corn stover, the most abundant biomass resource today, can be an attractive feedstock for biofuel production. However, research is needed to enable breeding of corn with higher yields of ethanol. ARS and University of Minnesota researchers showed that genetic traits of corn that affect cellulosic ethanol yield (percent/amount? cellulose, percent/amount? lignin, and facility of glucose release) had moderate to high heritability and did not show an accompanying decrease in grain yield. Further, they identified genetic markers for cell wall traits important for cellulosic ethanol production. Their work enables the use of marker-assisted selection to breed corn that exhibits both higher yields of cellulosic ethanol from stover, and higher yields of grain.

In addition, University of Minnesota scientists discovered a corn mutant with reduced ferulate cross-linking in stover; and ARS scientists showed that stover from the mutant variety was more easily digested into fermentable sugars. Research is continuing to isolate the mutated gene in order to breed a superior feedstock for both biofuels production and corn silage production.

***ARS develops switchgrass genetic map.*** ARS scientists working together with the Noble Foundation produced the first published genetic map for switchgrass. This key achievement enables scientists to genetically dissect, identify and assemble genes responsible for many high value traits and enables breeders to better recombine, evaluate and enhance switchgrass germplasm that exhibits desired traits.

***New, improved variety of energy cane.*** ARS researchers developed and released a new high-fiber, low-sucrose energy cane cultivar that is more resistant to smut infection than L 79-1002, another high-fiber variety which ARS developed in the early 1970's. Sugarcane smut appeared in the U.S. in the 1980's and became a major disease which reduced yields of sugarcane,

including L 79-1002, by as much as 50% or more over the crop cycle. The primary method of controlling smut is through the development of resistant varieties of sugarcane.

***New genetic tools for grass breeding.*** Collaborating ARS and Australian scientists used a high-throughput phenotyping platform (phenomics) to characterize over 100 natural accessions of *Brachypodium*, a wild grass. Extensive natural variation was found in several traits relevant to biofuels, including cell wall composition, stem density, and fermentability. ARS researchers also created over 4,000 insertional mutants this year and released over 4,000 mutant lines made in the prior year to the public through a newly established website. These tools will identify and manipulate target genes that affect biofuel production from dedicated energy crops.

***High oleic acid soybeans.*** Biodiesel fuel produced from soybean oil with high levels of oleic acid has significantly better cold-flow properties and higher oxidative and temperature stability. ARS scientists identified and combined mutant alleles of two soybean fatty acid-modifying genes, resulting in beans with high oleic acid content. The researchers also developed molecular markers for these genes, thereby facilitating the breeding of soybean varieties containing this valuable trait.

***Grass genome sequenced.*** The Nation's scientific community chose *Brachypodium* as a simple model for studying grass cell walls and enabling rapid improvements in plant traits for biofuels production. ARS scientists, in collaboration with the Department of Energy (DOE) and other researchers, have annotated the entire *Brachypodium* genome. A paper describing the results was published in *Nature*, and the genomic information is now publically available on several databases. In addition, a project to resequence additional accessions of *Brachypodium* was initiated. To date, four lines have been resequenced and the analysis of the sequences has begun. Knowledge of the genome sequence of *Brachypodium* and the linear order of genes in the genome relative to other grasses will help researchers improve traits in energy crops and grain species.

## **Component II: Sustainable Feedstock Production Systems:**

### **Enable new optimal practices and systems that maximize the sustainable yield of high-quality bioenergy feedstocks**

Agricultural producers, biorefining companies, government agencies, and policy makers across the country need to understand what kinds of bioenergy feedstocks can be produced, how much feedstock can be dependably harvested, and what will be the likely impacts of a large biofuels economy on whole-farm economic return and natural resources quality. The research performed under this component help to ensure that sustainably abundant quantities of

feedstocks will be available for biorefineries and at the same time that the integrity of the Nation's food, feed, and fiber production will be maintained.

ARS researchers contributing to this component enable the creation of optimal production strategies and the development of predictive decision tools, and they utilize funding provided through other national programs: NP-202 (Soil Resource Management); NP-204 (Global Change); NP-206 (Manure and Byproduct Utilization); NP-211 (Water Availability and Watershed Management); NP-215 (Rangeland, Pasture, and Forage Systems); NP-216 (Agricultural System Competitiveness and Sustainability); and NP-305 (Crop Production).

### **Selected Accomplishments:**

***Herbicides to improve switchgrass establishment.*** Weeds limit switchgrass establishment from seed, but few herbicides are labeled for switchgrass establishment. By applying quinclorac, which provides effective control of grassy weeds, plus atrazine, which provides good broadleaf weed control, ARS scientists generated good switchgrass stands for a variety of ecotypes throughout the Great Plains. With good management, including herbicides, switchgrass yields were already half of full in the first year following planting and were at full production by the second year. This research has enabled the labeling of quinclorac for switchgrass establishment in the Great Plains.

***Response of napiergrass to fertilizer.*** Napiergrass, a high-yield perennial, is a promising feedstock for the emerging cellulosic biofuels industry in the Southeast U.S. ARS scientists studied the rain-fed growth of napiergrass under three fertilizer treatments – no fertilizer, poultry litter, and inorganic fertilizer. Relative to the unfertilized control, napiergrass grown with either poultry litter or inorganic fertilizers exhibited yields that were 17% and 48% greater in the second and third year of growth, respectively. These results will contribute to the development of best management practices for viable biomass feedstock production systems in the Southeast.

***Not all biochars make good soil amendments.*** There is a widespread public perception that any type of biochar, produced from pyrolyzing biomass, will improve soil fertility. However, ARS researchers showed that biochar derived from poultry litter was toxic to earthworms, whereas earthworms were unaffected by biochar made from pine chips. Since a healthy earthworm population is a key indicator of productive soils, these results demonstrate that biochars produced from some materials can have detrimental impacts on soil quality, at least in the short-term.

***Northeast CRP lands for biofuel production.*** Growing feedstocks for biofuel production on marginal croplands, such as those often enrolled in the Conservation Reserve Program (CRP), would minimize food-vs-fuel concerns. ARS scientists determined the effects of plant species

composition, diversity, above ground biomass, and chemical composition on potential biofuel yield across major Northeastern ecoregions. Whereas converting CRP land to corn production is unlikely to yield more than 400 gallons of ethanol per acre, this study showed that CRP lands with a high proportion of native warm-season prairie grasses have the potential to produce more than 600 gal-ethanol/ac, while still maintaining the ecological benefits of the perennial grasses.

### **Component III: Biorefining: Enable new commercially-preferred biorefining technologies**

For over 50 years, ARS has been a world leader in the development of technologies for “utilizing” agricultural products. Biorefining and value-added co-products are examples of utilization technologies. ARS research has created out-of-the-box, cutting-edge, disruptive technologies that have changed markets and opened substantial opportunities for agricultural producers and converters.

In particular, ARS has unique R&D capabilities for developing innovative, integrated processes to fractionate, refine and convert agricultural materials into multiple, value-added products. Providing industry with technologies for converting a particular feedstock into multiple products helps minimize business risk. The desired goal is to enable the conversion of all biorefining raw materials into value-added products, eliminate waste streams (close-system biorefining), and allow biorefiners to market multiple value-added products and minimize income risk. ARS research focuses especially on technologies that expand the range of feedstocks which biorefiners can process (feedstock-flexible biorefining) and that enable smaller-scale (at- or near-farm) biorefining. ARS biorefining researchers focus on technologies with strong commercial potential, and they are encouraged to develop technology transfer plans early on in the research cycle.

In addition, ARS has strong capacities in biocatalysis research, and all four ARS Regional Research Centers (Albany, CA; New Orleans, LA; Peoria, IL; Wyndmoor, PA) have pilot plant facilities and skilled engineers for scale-up research on biorefining technologies. ARS also has three cotton ginning laboratories (Las Cruces, NM; Lubbock, TX; Stoneville, MS) where scale-up research for on-farm preprocessing can be done.

Although most of ARS’ biorefining portfolio is focused on 2<sup>nd</sup>-generation cellulosic ethanol, ARS also does significant research on improving efficiencies in 1<sup>st</sup>-generation biorefining and for improving the properties/performance of biodiesel fuels. ARS has unique competencies for research on co-products, and also has world-class in-house resources for doing techno-economic analyses.

ARS biorefining research is closely coordinated with the U.S. Department of Energy, which leads Federal efforts in conversion research.

#### **Selected Accomplishments:**

***New process for producing hydrocarbon fuels from biomass.*** A commercially viable process for converting cellulosic biomass into drop-in replacements for petroleum-derived fuels would be a significant advancement for the biofuels industry. ARS scientists have combined the fermentative production of volatile fatty acids (VFAs) from biomass by ruminal bacteria with subsequent electrolysis of the VFAs to produce propane and other alkanes, which could be subsequently reformed into liquid fuels. The fermentation can be performed on ground biomass without additional pretreatment and without sterilization of the biomass or the culture medium. The electrolysis can be conducted at low voltages with inexpensive graphite electrodes. A U.S. patent application has been filed.

***Increasing energy efficiency of bioethanol production.*** A major concern associated with corn ethanol fuel is the relatively low energy efficiency of its life-cycle production, a situation resulting in large part from the high energy input required to distill ethanol from fermentation broth. In addition, the fermentative conversion of biomass to ethanol involves especially low concentrations of ethanol, so the distillation step requires even larger amounts of energy. ARS scientists invented a new membrane-based ethanol recovery process that exhibits twice the flux of conventional membrane systems. The novel fabrication process lays an ultra-thin, low-permeability layer of an active adsorbent on a very permeable, large-pore rubber support. A patent application has been filed on the technique, which enables an energy-efficient alternative for ethanol production.

***Increasing yield of ethanol from corn stover.*** One reason cellulosic ethanol is more expensive than corn-based ethanol is that biomass contains both six-carbon sugars (hexoses, such as glucose), and five-carbon sugars (pentoses). Corn-based ethanol is produced with brewers yeast, which converts only glucose. Although new recombinant microorganisms have been developed to convert both hexoses and pentoses to ethanol, these organisms ferment glucose preferentially and do not begin to metabolize any pentoses until low glucose concentrations have been reached. As a result, fermentations times are long, and the pentoses are not fully converted. To overcome these hurdles, researchers at ARS and Iowa State University co-developed a two-stage simultaneous saccharification and fermentation process. In the first stage, pentoses are released and fermented to ethanol using an organism capable of highly efficient pentose metabolism; in addition, glucose is released and simultaneously converted to ethanol with brewers yeast. Using this process, an ethanol yield of 85 gal/ton was achieved from corn stover. If the traditional process that ferments only glucose had been used, the yield would have been only 65 gal/ton. This process will enable more efficient and cost-effective production of ethanol from cellulosic biomass.

***New, highly-efficient hemicellulase enzyme.*** Beta-D-xylosidase from the bacterium *Selenomonas ruminantium* is the most efficient enzyme for releasing the sugar xylose from

biomass, but high concentrations of xylose inhibit this enzyme. ARS scientists developed a mutated enzyme that tolerates 3 -fold higher sugar concentrations, thereby lowering the production costs for cellulosic ethanol.

***Increasing the profitability of manure digestion by adding dewatered food waste or switchgrass.*** Methane, the main component of biogas, is currently used as a “clean,” energy-dense transportation fuel. Livestock farmers with anaerobic digesters can increase their income by adding food waste to the digesters and charging fees for accepting the food waste from restaurants and other sources. But how much food waste should a farmer accept? ARS scientists showed that anaerobically digesting mixtures of swine (or dairy) manure and 5% pulped food waste produced about 20% more biogas than mixtures containing only 1% food waste. However, it was found that the ability to achieve higher biogas production required good control of digester pH. ARS scientists also showed that adding switchgrass to a high-solids anaerobic digester increased the biogas yield from dairy manure about four-fold. They also found that the increased yield is the same for green switchgrass (harvested in July) or senescent/brown switchgrass (harvested in January) although the point of harvest changes the timing of optimal biogas production.

***Lowering butanol production costs.*** Butanol is an advanced biofuel more compatible with the Nation’s transportation fuels infrastructure than ethanol. ARS scientists have discovered that furfural and hydroxymethylfurfural, which are produced when biomass is pretreated with dilute acids, stimulate the rate of butanol fermentation by a factor of two or more. This discovery lowers the cost of producing butanol from any plant-derived feedstock.

***Cost-effective ethanol from citrus processing waste.*** Converting pectin- and cellulose-dense citrus processing wastes (such as peels) to ethanol is technically feasible, but the enzyme cocktail typically used to hydrolyze the various polysaccharide fibers is expensive. Using both a new commercially-available, multifunctional cellulase enzyme and a Simultaneous Saccharification and Fermentation (SSF) bioreactor, ARS researchers reduced the number of enzymes required to convert these wastes to ethanol and have thereby made such biorefineries more commercially viable.

***Distillers dried grains with solubles (DDGS) as fish feed.*** Fishmeal, the traditional feed for aquaculture, has become very expensive (~\$1,500/ton) due to declining marine stocks. ARS researchers, in collaboration with South Dakota State University, determined that a feed combination of 40% DDGS (a co-product of corn ethanol production), 9.5% soybean meal and 24% fishmeal resulted in the highest weight gain in yellow perch. Replacing the current diet in

yellow perch aquaculture (40% fishmeal) with the DDGS-based diet could save the industry nearly \$9 million annually.

**Pyrolysis economics.** Unlike other biomass conversion technologies, pyrolysis may enable biorefining processes on or near the farm at a relatively small scale, thereby minimizing the costs associated with transporting large quantities of low-density biomass. By developing and using a process cost and simulation model for biomass fast-pyrolysis, ARS researchers found that a 200 tons-per-day plant is the smallest size that would be competitive with \$85/bbl petroleum. This information provides a valuable guideline for designing small-scale biorefining operations.

**Distillers dried grains with solubles (DDGS) as a high-protein, high-fiber food.** ARS researchers, in collaboration with South Dakota State University, conducted studies using various blends of DDGS (a co-product of corn ethanol production) in Asian flat breads (naan and barbari). DDGS, which cost only \$0.05/lb, could replace corn-based food ingredients, which cost ~\$3/lb, at levels up to 20% with only minimal reductions in bread performance and consumer acceptability,

**Using ethanol to lower energy costs for food refining processors.** ARS researchers determined that ethanol can be used to dehydrate food processing or refining products such as wheat gluten, resulting in up to 60% lower capital cost and up to 60% lower energy consumption relative to standard industry practices such as fluidized bed, flash or rotary drying. The study considered total system costs, including capital costs for ethanol recovery (via distillation), and also showed that dehydrating with ethanol can improve product quality. Ethanol could thus be a cost-saving addition to the food refining process.