ACCOMPLISHMENT REPORT
Central-East Regional Biomass Research Center
May 2014-October, 2015
Submitted by: Rob Mitchell, Coordinator

Locations Reporting

Ames, IA	Peoria, IL
Lincoln, NE	University Park, PA
Madison, WI	Urbana, IL
Mandan, ND	Wyndmoor, PA
Morris, MN	

(1) Feedstock Development

Winter-hardiness can limit high-yielding switchgrass options across the Central Great Plains. ARS scientists in Lincoln, NE and colleagues from the University of San Diego integrated new and published data to develop models that might explain the cellular and molecular events impacting winter-hardiness in switchgrass. These models can be used to test the roles of key genes, and potentially provide genetic markers that can be exploited to improve winter-hardiness in elite, high-yielding switchgrass germplasm. The results will have application to the improvement of winter-hardiness in other warm-season perennial grasses.

Switchgrass biomass yields can be reduced by early leaf senescence. If senescence can be delayed, it could result in increased biomass yields. Many cellular factors that govern senescence are proteins known as transcription factors that can directly bind to the DNA and either increase or decrease gene expression. One major class of plant transcription factors is called WRKY transcription factors which are known to hasten or delay leaf senescence in other plants. Unfortunately almost nothing is known about these WRKY factors in switchgrass. Using bioinformatics tools all of the potential WRKY genes in the switchgrass genome were identified. Using previous data on the global gene expression in switchgrass flag leaf over its natural development, it was possible to assign specific WRKY factors to specific periods of leaf development. Analyses identified 23 WRKY genes that were highly correlated to leaf senescence in field-grown switchgrass plants. Identifying specific WRKYs now provides tools to better understand switchgrass senescence and use these genes as markers in breeding programs.

Understanding switchgrass dormancy using next generation sequencing. Seasonal regrowth of switchgrass occurs from the below-ground crown and rhizome tissues. Insights into molecular processes controlling dormancy and regrowth can be exploited to develop cultivars with greater adaptation to the Central Great Plains. Crown and rhizome tissues obtained in late September from switchgrass cultivars with contrasting winter adaptation (Summer and Kanlow) were analyzed using a combination of protocols. Our data indicates that many molecular signatures associated with dormancy were evident in the well adapted, but lower yielding Summer plants as compared to the higher yielding but less well adapted Kanlow plants. These data will provide information that can be applied in breeding programs to improve this important bioenergy crop.

Promising plant bioengineering targets for enhancing biofuel production in biomass crops. Cell walls in plants are the world's most abundant source of carbohydrates for fermentation into biofuels. Prior to fermentation, these carbohydrates must be liberated from lignin in cell walls by harsh and costly chemical pretreatments. In lab studies, ARS scientists and university colleagues at Madison, WI found that lignin formed in part with natural plant anti-oxidants such as epicatechin gallate, epigallocatechin gallate or pentagalloyl glucose improved the production of fermentable sugars from cell walls following mild alkaline pretreatments. These results provide compelling evidence that genetic engineering of plants to form lignin with these anti-oxidants could reduce the cost of producing biofuels from biomass crops.

Design and validation of chamber to field-label perennial grasses with stable isotopes. Isotope labeling is a technique widely used in research to more readily detect metabolites to obtain an understanding of cellular activities. Since the natural abundance of ^{13}C in plant tissues is quite low, enhanced labeling of plants with external ^{13}C permits measurements across time and plant development processes. Here, we report on the development of a plastic chamber designed to label switchgrass in field or greenhouse environments with ^{13}C. Results indicated that the chamber was well sealed and could maintain a stable concentration of CO_2 when plants were not in the chamber. Switchgrass plants were well labeled with $^{13}CO_2$, and for the first time showed that the box could function effectively for labeling field-grown switchgrass plants. These results should benefit plant researchers working on perennial feedstocks, specifically those focused on understanding carbon allocation patterns in managed feedstock production systems.

Global changes in mineral transporters in tetraploid switchgrasses. Lowering levels of minerals required to grow a unit of biomass is an important factor in long-term sustainable production from perennial bioenergy crops, and biomass with lower mineral content can improve conversion into fuels. Plants absorb minerals from the soil for growth, but some of these minerals are returned to the perennial structures at the end of the growing season. Proteins called transporters regulate the movement of minerals in plants. The tissue specific and growth-stage specific expression of mineral transporters is poorly understood in switchgrass, so a combination of bioinformatics tools and molecular biology were used to identify genes involved in mineral nutrition. A majority of the genes controlling mineral nutrition were discovered and their expression patterns in different tissues were documented. Mineral analysis of below ground structures suggested that some minerals were more likely to be transported from the shoots to the rhizomes at the end of the growing season. This initial study will now enable a detailed analysis of the roles of specific mineral transporters in the flux of individual minerals in switchgrass.

Flag leaf senescence can trigger aerial senescence of the entire plant in switchgrass. ARS scientists at Lincoln, NE produced a detailed molecular and metabolic map of this process. These studies identified key genes that can be manipulated to alter both the onset of leaf senescence in switchgrass as well as the remobilization of nutrients from the aerial tissues to the rhizomes. Greater remobilization of nutrients at the end of the growing season can improve plant health and sustainability of producing switchgrass biomass for biofuels.

Developing concepts to understand switchgrass senescence, dormancy and tillering. An important feature of perennial warm-season grasses is their ability to grow year after year, producing abundant biomass from tiller buds initiated on the below-ground portions of the plant. Although there is increasing knowledge on the agronomy and management of these species as crops, there is limited information on the molecular and cellular machinery that underlie most aspects of plant development. A distinct aspect of these temperate warm-season grasses is their ability to enter into dormancy with the onset of winter, and exit out of dormancy with the onset of spring. In this manuscript the work performed on the molecular aspects of senescence and dormancy in other plants is reviewed as a means to develop models that can be used in the future study of these processes in perennial warm-season grasses such as switchgrass.

Rice ovate transcription factors. All higher organisms possess a number of different regulatory molecules that guide biochemical processes in cells. Transcription factors are one class of such regulatory protein molecules that can bind to specific regions of the cell's DNA and thereby increase or decrease the expression of specific genes. The genes that specifically respond to a transcription factor are part of the factor's regulatory network, and control the changes that occur at the cellular, tissue or even organismal level. There are literally thousands of transcription factors in plants, many occurring as part of large gene families. In this work a specific rice transcription factor, named Ovate Family 2 was studied. Results from this study could be applicable to switchgrass in the future.

Plant peroxidases and aphid resistance in bioenergy grasses. Cereal aphids like greenbug can cause significant economic damage to a number of cultivated grasses. Peroxidases are a class of enzymes frequently enhanced in plants infested by aphids and have been used as markers to assess plant responses to aphid herbivory. A peroxidase enzyme was identified and named SbPx-1 that resulted in enhanced greenbug resistance in sorghum. SbPx-1 was then identified in switchgrass and foxtail millet. When infested with greenbugs several of these peroxidase genes were specifically upregulated in all three grasses suggesting that common modes of regulation and response to insect herbivory might exist in these grasses. It is now possible to determine if specific peroxidases can act as markers for insect resistance in related grasses.

Aphid MicroRNA is a key to understanding plant aphid interactions. MicroRNA (miRNA) is a regulatory molecule that directly affects cell function. Within cells, miRNAs can bind to specific messenger RNA (mRNA) molecules and commit these hybrid molecules for degradation. Lowering mRNA levels by miRNAs can lead to lowered synthesis of key proteins, leading to changed cellular metabolism. With regard to host plant-insect pest interactions, both the plant and the insect can produce miRNAs to influence metabolism of the pest or the host. In the first study of its kind, miRNAs present in two aphids that are major pests of grasses were analyzed using high-throughput sequencing procedures. Bioinformatic pipelines were developed to analyze the miRNA present in greenbugs maintained on sorghum plants and yellow sugarcane aphids maintained on barely plants. In both instances, a number of miRNAs were discovered and could be categorized as originating from the aphids or the host plants. These data will serve as a foundation for studies to evaluate the complex biology of these important plant pests.

Switchgrass can serve as hosts for cereal aphids. One factor that can be expected to play a detrimental role in harvestable yields of biomass is insects. There is limited information on the responses of switchgrass plants to feeding by piercing-sucking insects such as aphids. In this work the relative resistance or susceptibility of several switchgrasses to herbivory by a range of different cereal aphids was evaluated. Results indicate that some switchgrass display broad resistance to several aphids, whereas others are more susceptible to injury from specific aphids such as greenbugs and the yellow sugarcane aphid. These results will be foundational to continuing studies to understand cellular mechanisms underlying differential resistance in switchgrass germplasm that can be utilized for bioenergy.

Switchgrass aphid interactions identify different categories of resistance. Large acreages of switchgrass for bioenergy could become infested by insects resulting in a loss of biomass yield. Aphids are among the most important insect pests. It therefore becomes important to understand the extent of resistance in switchgrass populations to aphids. Three populations of switchgrass were evaluated for their ability to serve as hosts for two insect pests, the greenbug and the yellow sugarcane aphid. Results indicated that switchgrass cultivar Summer could tolerate greenbug infestations, but was susceptible to the yellow sugarcane aphid. The cultivar Kanlow, which has high biomass yields, was mostly resistant to both insects. A population derived from hybridizing Kanlow and Summer plants (K x S) which has high winter survival and high yields was tolerant to feeding by the yellow sugarcane aphid, but was susceptible to feeding by greenbugs. These studies will be foundational to understanding the cellular and molecular mechanisms underpinning these responses and will be used to improve switchgrass germplasm.

Greenbug aphid feeding behavior and host preference among tetraploid switchgrass
Previous research has shown that many switchgrasses are prone to herbivory by aphids, but some cultivars are resistant to aphid feeding. Aphids feed by sucking nutrients required for plant growth. These unique feeding strategies can be exploited to understand both aphid feeding behaviors as well as determining the potential causes underlying plant resistance to aphid herbivory. We used a sophisticated instrument called the Electrical Penetration Graph to monitor aphid feeding on switchgrass. Data show that populations susceptible to aphids have long-feeding times for aphids, whereas aphids do not feed as long in resistant plants. Results indicate that plant resistance is likely based in the phloem. The mechanisms involved in phloem-based resistance can now be investigated and applied to developing more resistant switchgrass.

Switchgrass peroxidases and their roles in plant processes. As the acreage for bioenergy grasses like switchgrass increases, the incidence of insects likely will increase. However, switchgrass defensive mechanisms to insects are not well understood. In this study, literature on cell wall lignin formation, and some specific aspects of plant-insect interactions were reviewed. Data pointed to an important role for a specific class of enzymes, called peroxidases in plant defense against herbivory by piercing-sucking insects. The numbers, types and tissue-expression patterns of genes coding for switchgrass peroxidases has been investigated and described in this paper. It is anticipated that these initial studies will as a foundation for more detailed understanding of the molecular and cellular responses of switchgrass to hemipteran pests.

(2) Feedstock Production

Switchgrass managed for bioenergy is invaded by other grasses. Switchgrass is considered the model perennial grass for bioenergy and has been planted for forage and conservation purposes for more than 75 years. However, there is a concern that switchgrass grown as a biofuel crop could invade other plant communities. ARS scientists in Lincoln, NE demonstrated for the first time that switchgrass managed as a biofuel crop is not invasive, but is invaded by other grasses. After 10 years of different management practices, soil fertility and harvest management appear to dictate which grasses will invade switchgrass. Applying no N fertilizer caused switchgrass to decline and other grasses to increase. Applying at least 60 kg N ha^{-1} was adequate N to meet switchgrass growth demands, limit invasion by other grasses, and maintain quality switchgrass stands. Harvesting switchgrass once each year in August with no N fertilizer caused switchgrass stands to be replaced by other grasses. Harvesting after fall dormancy resulted in fewer invasions by other regionally-aggressive grasses. These results demonstrate that switchgrass is more prone to being invaded by other grasses than becoming an invasive species.

<u>Harvesting corn stover for bioenergy increases importance of nutrient monitoring.</u> A frequently asked question regarding the emerging cellulosic bio-product industries is, "What quantity of nutrients will be removed if I harvest my stover?" Field research in central Iowa determined fertilizer costs to replace nutrients lost by biomass harvesting per ton averaged $22.73 for vegetative material from the ear shank upward, $18.18 for vegetative material from approximately 10 cm above the soil surface to just below the ear, $15.07 for cobs, $24.86 for grain, and $17.60 for machine-harvested stover collected from a single-pass combine harvesting system or stover bales. This information is useful to producers and those responsible for acquiring sufficient feedstock supplies to operate emerging cellulosic biofuel investments in a sustainable manner.

<u>Switchgrass and corn residue can contribute to U.S. energy and environmental goals.</u> ARS scientists in Lincoln, Nebraska collected grain and biomass yield and composition, soil carbon, and production input data to estimate ethanol yield per acre and greenhouse gas emissions from a long-term corn (with and without corn residue harvest) and switchgrass field trial in the western Corn Belt USA. Soil carbon storage in fields planted to corn or switchgrass resulted in large greenhouse gas emission mitigation potential and demonstrated why proper accounting of soil carbon storage will be critical in determining biofuel carbon intensities. Switchgrass, under optimal management, produced higher ethanol yields than the corn grain-only harvests and similar ethanol yields as corn grain with residue removal. Future integration of cellulosic ethanol biorefineries with corn grain ethanol facilities would result in improved energy efficiency for the current corn grain ethanol system.

<u>Dedicated energy crops and crop residues for bioenergy feedstocks in the Central and Eastern US</u>. Perennial grasses and crop residues offer benefits and challenges when grown for biofuels. Feedstocks must have a limited impact on the production of major crops while providing adequate and reliable feedstock supplies. Major advancements have been made in perennial grasses, biomass sorghum, and the management of corn stover for cellulosic biomass. Adding perennial grasses into current agricultural systems may help reduce nutrient escape from fields to surface and ground waters, reduce greenhouse gas emissions, and increase soil carbon sequestration. Dedicated energy crops can increase the production of transportation fuels from recently-fixed plant carbon rather than from fossil fuels. Although there is no "one-size-fits-all" bioenergy feedstock, crop residues like corn stover are the most readily available feedstocks. On marginally-productive cropland, 25 years of research has demonstrated that perennial grasses are profitable, productive and improve the environment.

<u>Corn residue removal affects grain yields between no-till and conventional tillage differently but soil organic carbon stocks are similar</u>. Corn residue demands have increased by the livestock industry for feed, bedding, and grazing. ARS scientists in Lincoln, Nebraska collected grain, biomass yield, total soil N, and soil organic carbon data over 10 growing seasons to determine

the impact of tillage and residue removal rates in an irrigated, continuous corn study. Mean grain yields were lower when corn stover was harvested under conventional tillage while grain yields were higher for no-till when corn stover was harvested compared with no stover removal. Changes in SOC and total soil N occurred at near surface soil depths while cumulative (0 to 150 cm) changes were similar between 2001 and 2010. Results support the need to evaluate SOC cycling processes below near-surface soil layers.

Sub-surface soil carbon changes impact biofuel greenhouse gas emissions. Biofuel feedstocks are being developed and evaluated to partially offset petroleum transport fuels. Accurate accounting of upstream and downstream greenhouse gas (GHG) emissions is necessary to measure the overall carbon intensity of new biofuel feedstocks. Changes in direct soil organic carbon (SOC) can have a major impact on estimating overall GHG emissions from biofuels. In particular, changes in sub-surface soil depths (>30 cm) could have a large impact on GHG emissions from biofuels. ARS scientists in Lincoln, NE showed that corn grain and cellulosic ethanol GHG emissions varied either positively or negatively from baseline GHG emissions depending on SOC changes at near and sub-surface depths. Differences in GHG emissions highlight the importance of accounting for sub-surface SOC changes especially in bioenergy cropping systems with high potential for soil C storage.

Twelve years of annually removing corn stover does not impact grain yield but increases soil erosion potential. Continuous removal of corn stover for livestock or bioenergy could decrease the productivity of agricultural soils. In this long-term bioenergy study in Lincoln, NE, corn stover was removed at a rate of 55% of total non-grain aboveground biomass for 12 consecutive years in a rainfed no-till, continuous corn system. At this marginally productive site, stover removal increased the susceptibility of soils to wind and water erosion. Stover removal, however, did not affect corn grain or stover yields over time. Harvesting corn stover, especially on more sensitive degraded croplands, will likely result in greater erosion risks even if soil organic matter improves and yields are stable. This research will inform and balance management decisions for yield stability, building soil organic matter content, and minimizing soil erosion potential.

Evidence-based agronomic management decisions can support soil health and resilience. Soil microbial diversity is the foundation for soil function. As technology and analytical methodology has improved, ARS scientists from South Dakota, Texas, Maryland, Iowa, Texas, South Carolina, North Dakota, Nebraska, Minnesota, Missouri, Colorado, Washington, and Indiana summarized the state of knowledge regarding the links between soil microbial diversity and ecosystem function, and identified key research questions linking soil health and management decisions, including those commonly used in bioenergy production (e.g. tillage, residue removal, crop rotations, etc.). By identifying quantifiable metrics for soil health, producers will be better equipped to make management decisions to take full advantage of services provided by soil biota while maintaining or improving soil health and resilience.

Soil microbiology plays a key role in reversing soil degradation, improving soil health, and enhancing system sustainability. The challenge for agriculture in the 21st century is to implement more sustainable farming systems that are economically viable and accommodate changing technologies and climate. ARS scientists from South Dakota, Iowa, Indiana, Texas, Colorado, Maryland, Washington, North Dakota, Missouri, South Carolina, and Nebraska outlined critical soil biological questions, identified knowledge gaps, and proposed various strategies for stopping or reversing soil degradation by enhancing soil biological function. Agricultural management decisions critical for supporting food, fiber, and energy needs can be used to manipulate soil biology to increase soil nutrient availability, protect crops from pests/pathogens/weeds, and improve system resilience to climate change.

Soil modeling results match measured changes in soil organic carbon over 10-years in corn stover removal studies. Removing corn stover from fields could negatively affect soil quality. These negative effects may be difficult to measure as they often occur slowly over long time periods. Models may help with assessing management effects on soils but they must be validated using available long-term data. CQESTR is a model that uses management practices and site weather to estimate changes in soil carbon, a major soil quality indicator. Scientists at Lincoln, NE compared CQESTR estimated changes in soil carbon to measured changes in soil carbon in two studies in eastern NE. These studies included rainfed vs. irrigated continuous corn under various tillage, N fertilizer rates, and residue removal rates. Changes in soil carbon estimated with the model agreed very well with changes measured in these studies after ten years. This study demonstrates that CQESTR can predict how bioenergy management practices affect soil carbon storage and could be used to inform long-term management decisions.

Rapeseed to jet fuel life-cycle assessment. Rapeseed is a candidate crop for producing renewable jet fuel, improving soils and reducing greenhouse gas emissions. Scientists at Michigan Technological University, in collaboration with ARS Researchers at Mandan, ND estimated that growing rapeseed in place of fallow in rotation with wheat in 10 Western U.S. states could have positive or negative effects on soil organic carbon depending on production practices and location. Using the best production practices of no-tillage and high residue retention, renewable jet fuel from rapeseed could result in greenhouse gas reductions of 65-96% compared with petroleum jet fuel.

Sustainable bioeconomy. Many steps are needed to successfully build a bioeconomy, which meets energy and economic demands, and safeguards soil and water quality. ARS scientists at Morris, MN, are addressing several steps including determining harvest rates, and safe viable co-products like biochar. An ARS scientist at Morris, MN lead an ARS, multi-site, multi-agency (e.g., USDA, US-DOE, and several Universities)writing team, that noted crop residue harvest rate must be site-specific as one universal target harvest rate or percentage returned is not valid for safeguarding soil and water. Also, researchers at Morris and St. Paul, MN, demonstrated that breakdown and recycling of wheat straw was the same in soils with or without biochar, which suggests this co-product may be safe and useful as a soil amendment. This work provided data to modelers and gave insights necessary for harvest rate guidelines and safe use of a bioenergy co-product. These results are steps along the pathway as producers, industry, and action agencies build a vibrant, sustainable bioeconomy.

Sustainable bioeconomy Life Cycle Analysis (LCA). Companies and regulators use life cycle tools to assist with decision-making and defining policy, but system boundary assumptions can change the predictions. ARS researchers at Morris, Minnesota, and collaborators used life cycle tools that are widely accepted by regulatory agencies to compare four stover and corn grain based ethanol fuel pathways. The results indicated that an integrated corn-based ethanol using a small percentage of corn residue for other uses such as animal feed could result in reduction in greenhouse gas emission relative to the Renewable Fuel Standard 2 gasoline. This information provides an example of how improving life cycle boundaries provides more accurate predictions of environmental benefits and costs. Life cycle analysis provides guidance to the bioenergy industry and policy makers about the benefits and risks associated with plant-based energy, leading to more-informed decisions.

Dual cropping soybean with winter camelina. The debate still looms concerning competition between growing biofuel or food crops on farm lands. Why not produce both on the same land in a single season? ARS researchers at Morris, Minnesota, demonstrated that winter camelina used as a "cash" cover crop for biofuel feedstock can be sustainably dual cropped with soybean in the Corn Belt region. Results showed that inter-seeding (AKA "relay-cropping") soybean into winter camelina in the spring led to combined seed yields that were economically competitive with a single full-season soybean crop. Moreover, a camelina-soybean dual crop system, which adds a cover crop to the rotation, offers environmental benefits such as reduced soil erosion and sequestration of nitrogen and phosphorus that a simple corn-soybean rotation does not.

Optimizing crop management of the new oilseed camelina. Camelina has been touted as a prime biofuel feedstock candidate for the U.S. because it requires fewer inputs than most traditional crops; thus, it is cheaper to produce. However, little is known about how to best manage this relatively new crop to the U.S., and even less is known about how well it performs in the Northern Corn Belt. ARS research in Morris, MN, demonstrated that camelina is well adapted to production in Minnesota producing seed yields as high as 2100 lbs/acre, translating into an oil yield of about 107 gal/acre; much greater than a very good soybean crop, which produces about 60 to 70 gal/acre of oil. The best time to plant camelina in Minnesota was found to be in mid April to early May, about the same time as spring wheat. This information will be used in a growers guide for camelina and will aid farmers, crop consultants, specialty seed companies, and other agriculture professionals involved with producing camelina in the Northern Corn Belt.

Renewable Energy Assessment Project (REAP). Corn stover and other crop residues have been identified as potential energy feedstocks. However, stove left on the soil surface is important to maintaining soil health, and thus it is necessary to understand the implications of this practice and establish stover removal criteria that protect soil health. Therefore, multiple ARS researchers collaborated to provide guidelines for stover removal that can prevent or mitigate associated soil degradation to ensure that the soil resource meets demands for food, feed, fiber and fuel. The ARS multi-location REAP with multi-agency partners, produced publically-available information to support sustainable cellulosic bioenergy and bio-products industries. Residue requirement for soil organic matter maintenance can exceed the residue needed for erosion control. The concept of a minimum residue requirement for protection the soil resource became cornerstone objective component of the USDA-ARS-Renewable Energy Assessment project.

Crop residue management to prevent and mitigate soil degradation. The most important point of the REAP comprehensive SOC and crop residue study is that extreme variability refutes any notion that there is a universal minimum residue requirement for maintaining soil carbon. Instead, the Corn Stover Team unanimously recommends that crop residue harvest decisions must be made at the local level if not the individual field, or better yet, subfield management level. The REAP dataset with 239 site-years is likely one of the most comprehensive research efforts ever conducted to provide replicated field validation for projections such as those in the revised Billion Ton Report. Spatial and temporal variability are tremendous in regards to grain and stover yields and nutrient removal. Impact of crop residue harvest on soil physical indicators assessed. Soil aggregate distribution indicated a shift toward more erodible aggregate class sizes, and measures of soil organic matter were reduced when crop residues were removed without adding another carbon source such as a cover crop. Impact of crop residue harvest on the least limiting water range (LLWR) as an indicator of soil compaction effects determined. Concluded that crop residue removal rates be limited to levels that maintain or even increase SOM levels to avoid degradation of soil physical properties. Yield and nutrient removal information for cereal straws in the Pacific Northwest determined for conventional and no-tillage studies conducted with and without residue harvest. They conclude that substantial tradeoffs must be evaluated on a site-specific basis before making any final decision regarding the sustainability of harvesting crop residues for any purpose. For three levels of stover harvest: none, full, and intermediate showed CO_2 loss from the full removal plots was slightly lower than from the zero removal plots. However, the emission difference between the two treatments was much less than the amount of C removed with the stover. This implies that C was being lost from the full removal plots – a phenomenon confirmed by rigorous soil sampling.

<u>Implications of soil organic carbon maintenance for biofuel production.</u> CQESTR model predicted manure or a cover crop/intensified crop rotation under no-tillage were options to mitigate loss of crop residue carbon from agricultural soils, as using fertilizer alone was insufficient to overcome impact of residue removal on SOC. The CQESTR model based on a sandy soil predicted reductions in soil organic carbon (0-5 cm depth) of 21% under disk tillage and 30% under conservation tillage with 66% residue harvest. Crop residues play a vital role in maintaining SOC, which is not only required for preserving soil functions, but also for ensuring the sustainable long-term production of biofuel feedstock. Impacts of reducing stover return on soil organic matter in the Northern US Corn Belt. Stover harvest and tillage scenarios tested with CQESTR model, found that no-till management is necessary for soil organic matter maintenance. Predicting whether SOM is accumulating or depleting depends upon the soil depth considered. This research contribute to ensuring that the soil resource is protected and can meet the expanding demands for food, feed and fuel.

<u>Data Base and Query Tools for Soil and Crop Management Decisions.</u> REAP database prerelease and publically able version established and populated with data on soil and agronomic response to crop residues harvest facilitating team data exchange and synthesis using the same common data entry template. Rigorous economic analyses are crucial for the successful launch of lignocellulosic bioenergy facilities. The REAP database query tool was used to construct enterprise budget for evaluating short-term economic performance of various biofuel feedstock. Demonstrated usefulness of data query tool for illustrating soil and crop management strategies available for providing sustainable feedstock supplies.

<u>Managing invasion risk in naturalized and pre-commercial Miscanthus populations.</u> Pressure to produce more biomass has led to the introduction of a variety of novel high-yielding bioenergy feedstocks into U.S. cropping systems. The rapid growth, resilience and capacity for spread of many of these crops increases the likelihood that unwanted, invasive escapes will expand into

natural ecosystems. Invasive spread of bioenergy crops can be limited with a three-stage management approach: 1) prevent escapes through careful selection of crops and cultivars; 2) use best management practices, including selection of flat, low-disturbance sites well-away from waterways and surrounding fields with mowed buffer areas, among others; and 3) monitor surrounding areas and eradicate detected populations. This greenhouse study quantified the environmental conditions (including light, soil moisture and plant residue cover of the soil surface) promoting establishment of seeds of a pre-commercial Miscanthus x giganteus bioenergy cultivar and a known invasive genotype of Miscanthus sinensis. Light was the most important factor for establishment success, although Miscanthus seedlings could germinate and survive under all but the lowest light levels. These results indicate that high-light environments in potential receptor areas, such as abandoned fields or canopy gaps in forests, are likely to be the highest priority targets for monitoring of escapes surrounding Miscanthus production fields.

Vegetative dispersal of perennial bioenergy crops as a potential invasion route. Many plant invasions in the U.S. originated from crop introductions. Miscanthus x giganteus, a widely planted biofeedstock, has been considered a low risk for escape because it is does not produce viable seed. Using small scale rhizome fragmentation and movement experiments and literature-based estimates, we parameterized a model to examine the rate of M. x giganteus spread by scouring and flooding. We further evaluated model behavior in response to different buffer widths and monitoring intensities, two key strategies advised for containing biofuel crops. We found that estimates of the vegetative expansion rate alone were sufficient to allow the crop to outgrow setbacks of 3 m or less within 11 to 15 years with low monitoring intensities. Further, models that included the possibility of rhizome dispersal from fields and scouring at field edges support the possibility of long distance dispersal and establishment if management intensities are too low. Our study highlights the importance of considering minimum enforced management guidelines for growers to maintain the ecological integrity of the agricultural landscape.

Reducing the carbon footprint of cellulosic ethanol. After producing ethanol from crop residues such as corn stover and straw, a slowly decomposing byproduct remains which is typically burned for energy recovery, but harvesting crop residues can result in decreased crop yields and soil carbon levels. Scientists with ARS, Drexel University and Colorado State University compared the current practice of burning this residue, to applying it back to the land. They found that although most studies have recommended burning this material to generate electricity for the biorefinery, applying it to the land instead resulted in ethanol with the lowest greenhouse gas (GHG) footprint, highest levels of soil carbon, and the greatest offset of GHG emissions. This finding could help the industry evaluate the different markets for byproducts produced at the biorefinery, considering both the economic and environmental impacts.

(3) Conversion and Co-products Utilization

Enzymes for augmenting commercial biomass hydrolyzing enzymes. Xylan accounts for 30-40% of the carbohydrate present in switchgrass but commercial enzymes are unable to completely digest biomass samples even following extensive pretreatment. ARS scientists at Peoria, IL identified several chemical linkages related to side-branches that are recalcitrant to commercial enzymes and limit the breakdown of xylan. Armed with this information, these scientists were able to identify enzymes that active for cleaving these linkages improving the digestion of xylan. This research demonstrates that xylose yield loss is directly related to deficiencies in commercial xylanases and identifies a pathway to filling in gaps in activities needed for complete xylan hydrolysis.

Napier grass for bioenergy production. Napiergrass is being developed as a bioenergy crop for production in the southeastern U.S. ARS scientists at Peoria, IL and Tifton, GA demonstrated the conversion of Napiergrass to ethanol. The estimated ethanol yield based on laboratory-scale experiment was up to 10,300 liters per hectare. By way of comparison, we estimate a corn field yielding 180 bu/acre would produce 4,640 l/ha.

Effect of switchgrass genotypes on yields of pyrolysis co-products. Biomass offers a means to generate renewable energy with concomitant savings to the economy and the environment. Plant biomass consists largely of carbohydrates, namely cellulose and hemicellulose, and lignin, an energy rich polymer. These compounds can be converted to different forms of fuels using diverse technologies. Among these technologies is a process known as fast pyrolysis, where plant material is rapidly heated in the absence of oxygen to produce a range of compounds that include condensable and non-condensable gases. The condensable gases can be cooled to produce an oil called pyrolysis oil. With proper treatment pyrolysis oil can be used to produce a range of liquid fuels, including jet fuel and boiler oils. This study indicates that switchgrass plants can be bred to improve the yields of specific components. Alternatively, catalysts can be used during pyrolysis to improve the quantity of specific classes of compounds.

Production of fuels and chemicals from guayule feedstock. Guayule is a woody desert shrub cultivated in the southwestern USA as a source of natural rubber, organic resins, and high energy biofuel feedstock. ARS researchers at Wyndmoor, PA used guayule bagasse, the residual biomass after latex extraction as feedstock in a pyrolysis process using conventional hydro-treating with noble metal catalysts and a simple distillation process to synthesize drop-in fuels. The guayule-bagasse tail gas reactive pyrolysis (TGRP) process encompasses pyrolyzing the guayule bagasse without the use of catalyst. This process produced a bio-oil with high energy content and low oxygen content. Further processing followed by a continuous hydro-treatment over a common noble metal resulted in a liquid comprising 66% gasoline range hydrocarbons. This resulted in a patent application filed with the USPTO entitled BIO-OILS AND METHODS OF PRODUCING BIO-OILS FROM GUAYULE BAGASSE AND/OR LEAVES.

Production of biorenewable calcined coke. A pyrolysis-based refinery must produce other valuable chemicals alongside fuel to financially succeed, similar to the model used in the petroleum industry. When heated to very high temperatures (>1200 degrees C), petroleum coke reacts to become nearly pure carbon with many useful physical characteristics (termed "calcined petroleum coke", CPC). Global producers of aluminum and steel rely on CPC for their production, and their demand for better CPC continues to increase, but, increasingly impurities such as sulfur, nickel, and vanadium have interfered with efficient use of CPC. ARS Researchers at Wyndmoor, PA successfully converted the heaviest fractions of renewable pyrolysis bio-oil, recovered after distillation of the volatiles into a product similar to CPC but with improved chemical properties. The technology is the subject of a recently-filed provisional patent application. An MTA has been established with Rio Tinto Alcan, a global consumer of calcined coke, who has expressed continued interest in cooperative research and development with ARS.

Switchgrass biomass quality traits and their effect on fast pyrolysis product yield. Environmental conditions, genotype × environment interactions, and crop management are being evaluated to determine their affects on switchgrass composition and fast pyrolysis conversion. Pyrolysis products were characterized using a pyrolyzer coupled with a GC/MS) as a high-throughput phenotyping method. Key relationships between biomass composition and fast pyrolysis product yield have been identified. Switchgrass biomass characterized for mineral content using ICP and pyrolysis product yield were used to develop new chemometric models for NIR spectroscopy. These data will allow for breeding and selection of biomass traits that will lead to the production of higher quality bio-oil through fast pyrolysis.