SOUTHEAST REGIONAL BIOMASS RESEARCH CENTER
Progress Report, May 15, 2014 for 2010-2014

Temple, TX: Grassland, Soil and Water Research Unit (GSWRL), (NP211, 212, 215)

Accomplishments: The accomplishments listed below summarize the GWSRL research for bioenergy. Since 1993 we have been evaluating switchgrass and other bioenergy crops with the aim to being able to predict their productivity and to evaluate relevant environmental impacts of their production with simulation models. We have conducted research on biomass (switchgrass, giant miscanthus, and other warm season grasses; energycane, sugarcane, and biofuel sorghum), and oilseed (canola/rapeseed, camelina and sunflower), deriving plant parameters to enable their simulation. Below are the accomplishments from our ARS Research Unit.

Accomplishments (the first five are funded by the Office of Naval Research)

Cellulosic Crop Simulation Modeling Research.
Four key databases are needed to run the ALMANAC model: ***Weather, Soils, Crop parameter Management.*** We have collected key ALMANAC model parameter data: *Weather* – We have complied annual weather data files for 18 weather stations on Maui. *Soils*- Eight representative soils on Maui have been downloaded from the NRCS website. *Crop parameters*- These have already been compiled for sugarcane, energycane, Napiergrass and energy sorghum based on data collected from field trials established on Maui and at Temple, TX. These parameters are also valuable for use with other models. *Management* - Processing of field management practices and preliminary model testing have already been conducted. Model simulations outputs have shown a very agreement between model predicted and actual measurements of biomass yields, soil organic carbon storage and greenhouse gas emissions.

Additional data currently being collected by cooperators include: crop parameters for the ratoon crops, soil characterization of representative fields targeted for model simulations of *"What If"* management scenarios, greenhouse gas emissions, evapotranspiration and bioenergy crop responses to water stress will be used to parameterize and test the ALMANAC model's capability to accurately simulate spatial and temporal biomass yields, water and nutrient use efficiencies, and the associated environmental impacts.

Oilseed Crop (for Hydrotreated Renewable Jet Fuel in the Wheat Belt) Simulation Modeling Research.
The overall objective is to determine the production potential and environmental impacts of jet fuel produced from oilseed crops grown in rotation with wheat in the Pacific Northwest and Great Plains. We are collecting field data to parameterize the ALMANAC model for four candidate oilseed crops (rapeseed, yellow mustard, brown mustard, and camelina) across the wheat belt. Field trials have been planted at seven locations (Temple, TX; Ames, IA; Morris, MN; Pendleton, OR; Sidney, MT: Akron, CO; Moscow, ID) in the winter of 2012 & 2013 (containing five rapeseed varieties and one camelina variety) and spring of 2013 & 2014 (containing five rapeseed varieties, two brown mustards, two yellow mustards, two Ethiopian

mustards, and one camelina variety) growing seasons by USDA-ARS collaborators. Plant measurements are being taken throughout the growing seasons on light interception, leaf area index, plant height, developmental stage, and leaf tissue nutrients. At the end of each growing season seed yield and above ground biomass is measured. This data is being analyzed and used to develop and refine the ALMANAC/EPIC crop parameters for rapeseed, yellow mustard, brown mustard, and camelina.

In addition we are creating spatial databases of weather, soils, management, and long-term yields necessary to validate large-area spatial estimates of the production potential for each oilseed crop. These data are being used to run the ALMANAC model across the Pacific Northwest and Great Plains will be developed. As part of this effort with this simulation model, we are making spatial production estimates and determine the environmental impacts of oilseed crop production. The oilseed crop parameters and spatial production estimates will be a key input for several subsequent analyses performed by our collaborators. The oilseed crop parameters developed will be provided for use in EPIC to estimate greenhouse gas emission and soil organic carbon storage for life cycle analysis. The spatial oilseed production estimates will be an input for the break-even production price analysis and POLYSIS economic analysis. The production estimates will be a key input for the transportation network analyses.

Application of the SWAT Model to Sugarcane Cropping Systems in the Hawaiian Island of Maui.

A Soil and Water Assessment Tool (SWAT) model has been set up to represent Hawaiian sugarcane cropping system. The model was calibrated and validated for sugarcane in Hawaii. The model set up is currently used to simulate water stress and water usage for the plantation as well as the viability of alternative biofuel feedstock (Napier grass and energy cane). The current set up also allows analysis of water intake and transport though the ditch system. The watershed subdivision was compared to datasets for the island and accounts for the diversion of water through the ditch system. Another deliverable is that the SWAT model was implemented as an alternative decision support tool to provide irrigation managers with comprehensive water balance information by integrating soil water transport processes with crop growth. The web interface is under testing at Temple and will be reviewed by the Hawaiian sugarcane company (HC&S) before being transferred to the company.

Modeling Differential Growth in Switchgrass Cultivars Across the Central and Southern Great Plains.

Switchgrass is an important biofuel crop that is adapted to a wide range of environmental and climatic conditions. Zones of adaptation for many switchgrass cultivars are well documented and attributed to local adaptation to the temperature and day length at the locations that they originated. In our study we developed values to describe growth of four switchgrass varieties so we could simulate them with the ALMANAC model. These values (parameters) are based on where each variety was developed. We then used these to predict production of two lowland varieties and two upland varieties in the central and southern Great Plains. Five plant parameters were adjusted to reflect the temperature and daylength at each variety's location of origin. Average simulated and measured yields differed by less than 0.5 Mg per ha across all seven field locations for each cultivar. The parameters do a reasonable job of estimating the average yield of each cultivar for most of the field locations. In addition, regional simulations of the four cultivars each show realistic spatial variation in yield across the central and southern Great

Plains. The parameters derived in this project for the ALMANAC model provide a tool for optimizing choice of switchgrass cultivar on different soils, in different climates, and with different management. As such, these results are valuable for future investigations modeling switchgrass production across large geographic regions.

Adaptations Between Ecotypes and Along Environmental Gradients in Switchgrass.
Patterns and mechanisms of adaptation to different habitats across the natural landscape are important for understanding the differentiation of populations and the evolution of new species. Recent studies of natural selection have focused on ecotype pairs that occur in differing environments. In contrast, field studies of switchgrass, have involved patterns of climatic adaptations across eastern North America and local ecotype adaptations between adjacent habitats. We reviewed field experiments of both levels of variation in switchgrass. We then analyzed several recent field trials to explore the adaptation of several switchgrass cultivars to climate. Finally, we examined the population structure of switchgrass and discussed the emerging view of switchgrass as a species complex containing multiple locally adapted populations and ecotypes. With the recent genome sequencing efforts of switchgrass, it is poised to become a model system for understanding the adaptation of grassland species across eastern North America. The identification of genes involved in climatic adaptations will help to understand the mechanisms of adaptation to the natural landscape and provide useful information for the breeding of high yielding cultivars of switchgrass for different regions.

Invaded Grassland Communities Have Altered Stability-Maintenance Mechanisms but Equal Stability Compared to Native Communities.
Plant communities are considered to be stable when biomass production varies little among years. Stability often is greater in communities with many than few plant species, but community stability is disproportionately influenced by the properties and growth patterns of dominant species. We compared stability of species-rich native plant communities with that of species-poor communities of non-native or exotic plants. We tested whether stability is being reduced by the decline in species richness that is occurring as native species are replaced by exotics. Native combinations of species exhibited many of the characteristics typically associated with stable communities – species grew at different times of years and were more productive when growing with other native species than with individuals of the same species. However, native and exotic communities did not differ in biomass stability over five years because the species that strongly dominated exotic assemblages were more stable than other exotic species. Native and exotic communities exhibited similar stability but were stabilized by different mechanisms. Exotic communities may be particularly vulnerable to herbivores, pests, or other agents that severely damage dominant species.

Species Richness and the Temporal Stability of Biomass Production: An Analysis of Recent Biodiversity Experiments.
The amount of growth achieved by groups of plant or algal species (communities) varies among years as a result of year to year differences in weather. Among-year variation in growth may be smaller in communities that contain many than few species, but the question of why this 'species richness effect' occurs remains to be resolved. We studied effects of increasing the number of species on among-year variation in growth of grassland and freshwater algal communities using data from 27 recent experiments. Among-year variability in growth was smaller in grassland

communities with many than few plant species because different species were favored by the differences in weather conditions among years. Conditions that were favorable for growth of some species were not favorable for others. In contrast, the number of species had little effect on variability in growth of algal communities. Our results indicate the importance of maintaining or increasing the number of plant species in grasslands as a means to increase the year-to-year reliability of these systems to produce vegetation to support the livestock upon which a growing human population depends for food.

Predicting Ecosystem Stability from Community Composition and Biodiversity.
Year-to-year variability in biomass production of plant communities can be reduced by increasing the number of plant species in the community. The mechanisms involved in stabilizing production remain contentious, however. We use theory and information from monocultures of the species present in plant communities to mathematically test the relative roles of three mechanisms in stabilizing production: (1) differences in species' responses to environmental variation, (2) the general tendency for production to be greater in species polycultures or communities than monocultures, and (3) decreased observation error. Calculations were tested using field measurements from four long-term grassland diversity experiments. Our mathematical estimate explains 21-53% of the observed variability in community production in these experiments. The relative importance of the three mechanisms in stabilizing production differed among experiments at different locations. Differences in species' responses to environmental variation contributed more to stabilizing production at locations where the environment varied widely among years than was relatively constant, for example. Our approach could be used to predict the stability of plant production and other functional aspects of ecosystems from knowledge of the responses of individual species to environmental variation.

Spatial Forecasting of Switchgrass Productivity Under Current and Future Climate Change Scenarios.
Evaluating the potential of alternative energy crops across large geographic regions and over time is necessary to determine if feedstock production is feasible and sustainable in the face of growing production demands and climatic change. Switchgrass, a perennial grass, is a promising candidate for biofuel. ALMANAC, a process-based computer simulation model that simulates growth over time, has been modified for some representative switchgrass varieties and used to realistically simulate switchgrass yields in 11 states. In this study, we show how ALMANAC can be used to forecast the current and future productivity of switchgrass across the central and eastern U.S. under predicted climate change scenarios. Our results reveal that there is substantial variation in switchgrass yield within regions and over time. Areas that currently have and maintain high yields under both future climate scenarios should be targeted for long-term growth of switchgrass to minimize land conversion and loss of biodiversity. Florida and the Texas and Louisiana Gulf Coasts have the highest long-term productivity potential but contain critical habitat for biodiversity. Marginal agricultural lands in the Northern Great Plains have variable yields under current climate conditions, but are expected to experience large increases in productivity with climate change. In general, larger future yields are expected in regions where future temperature and precipitation are predicted to increase, whereas regions that experience a future decrease in precipitation will produce smaller yields. Climate alone does not explain all future yields. For example, future increases in temperature and precipitation for the interior

southeast are not expected to have a large effect on yield. Other factors, such as nutrient limitations or soil texture, may be the limiting factor in these regions.

Spatial Trade-offs Between Land Use for Biofuel, Agriculture, and Maintenance of Biodiversity.
By 2022, 16 billion gallons of cellulosic biofuel produced in the U.S. is required to come from alternative energy crops such as switchgrass. Most of the best agricultural land in the U.S. is used for food/feed/fiber production, forestry, urban development, and biodiversity conservation. We used computer-based optimization routines to analyze land use trade-offs for biofuel production in the central and eastern U.S. We looked at reducing competition with the agriculture industry and biodiversity by using Conservation Reserve Program (CRP) land for producing biofuel crops. First, we limited our analysis to land enrolled in the CRP, and we analyzed the trade-off between biofuel production on CRP farmland and conservation of wildlife diversity. Current CRP farmland can potentially produce 62.4 million Mg of switchgrass biomass per year. However, including biodiversity trade-offs did not reduce the negative impact to biodiversity on CRP land. Second, we expanded our analysis to include all land and we analyzed the trade-offs between agriculture, biodiversity, and biofuel. The amount of land required for biofuel increased 84% when trade-offs with biodiversity and/or agricultural lands are considered. Minimizing conversion of agricultural land to switchgrass reduces the farmland and forest converted to biofuel. Whereas, minimizing the impact on biodiversity increased the farmland and decreases the amount of forest land converted to biofuel. Reptiles and amphibians could lose twice as much range area when trade-offs with biodiversity are not considered. Our results indicated a need for methodical land use planning in the face of increasing demand for biofuel production.

Energy Sorghum Biomass Harvest Thresholds and Tillage Effects on Soil Organic Carbon and Bulk Density.
Bioenergy feedstock production systems face many challenges, among which is the lack of guidelines on sustainable biomass harvest thresholds and tillage cropping systems that minimize the potential cumulative effects of fresh biomass harvesting equipment-induced soil compaction. We used the ALMANAC model to evaluate four biomass removal rates, 0%, 50%, 75% and 100%, and four tillage cropping systems, No Till (NT), Conventional Till (CT), and periodically plowing NT (NTCT), or subsoiling NT (NTSS) lands to alleviate compaction at Shorter, AL. Overall, biomass removal resulted in reduced total biomass, left-over residues, roots and total soil biomass inputs. Reduced biomass inputs to the soil resulted in N nutrient pool depletion, which were reflected as increased N stress days suffered by the crop. Whole profile soil organic carbon (SOC) storage was directly proportional to the soil biomass inputs. Given the importance of SOC as a soil quality indicator, we premised sustainability upon the maintenance of SOC at or above the initial SOC levels. For the current study, the 75% biomass removal rate can be applied on continuous NT energy sorghum production systems, assuming annual biomass inputs to the soil of 13.4 Mg per ha. Compared to plowing, subsoiling would be more effective in offsetting the potential cumulative effects of harvesting equipment-induced soil compaction on NT systems, with less impact on SOC storage, but at the reduced biomass removal threshold of 50%. There is an urgent need for guidelines on biomass harvest thresholds and biomass feedstock production systems that sustain productivity, environmental integrity, and ecosystem services.

Physiological Plasticity Among Differentially Adapted Genotypes of Widespread Warm-Season Grasses Under Altered Precipitation.

Because plants are not mobile organisms they must respond to changes in their environment via adjustments to their growth, form or function. This responsiveness or 'plasticity' can determine whether individual plants survive or persist, and could govern the adaptive capacity of species as a whole. However, populations or individuals within species frequently vary in plasticity for complex and often unknown reasons. Likewise, individuals of the same species often vary in genome size, yet associations between genome size and plasticity are unclear. To address these uncertainties we grew eight individuals or 'genotypes' of switchgrass originating from different habitats under varying precipitation regimes at two sites, and hypothesized that genotypic variation in physiological plasticity would be associated with their source climate. We also hypothesized that genotype's differing in genome size would vary in physiological plasticity. Our results indicated that genotypes with larger genomes were generally less plastic but more tolerant of low precipitation while genotypes with smaller genomes were more plastic but less tolerant of low precipitation. After accounting for genome size differences, genotypes showed little variation in plasticity and no association between plasticity and source climate. Our results highlight how genome size variation within species might result in differential responses to environmental change.

Integrating Transcriptional, Metabolic, and Physiological Responses to Drought Stress and Recovery in Switchgrass.

Switchgrass is an important grass species native to North America. It is also an important bioenergy crop. However, drought may limit the productivity of switchgrass for bioenergy use. In this study we conducted a detailed investigation of how switchgrass responds to drought. We documented how different genes turn off and on depending on how much water is available to switchgrass plants, and we determined how different genes are associated with plant growth and water use. These results provide a foundation for understanding switchgrass response to drought, and will help guide the development of drought tolerant switchgrass grass varieties. These results improve our understanding of plant responses to drought and will help improve switchgrass bioenergy production.

Perennial Biomass Grasses and the Mason-Dixon Line: Comparative Productivity Across Latitudes in the Southern Great Plains.

Understanding latitudinal adaptation of switchgrass and giant miscanthus in the southern Great Plains is key to maximizing productivity by matching each grass variety to its ideal production environment. Objectives of this study were: i) to quantify latitudinal variation in production of representative upland switchgrass ecotypes (Blackwell, Cave-In-Rock; CIR, Shawnee), lowland switchgrass ecotypes (Alamo, Kanlow), and miscanthus in the southern half of the U.S. Great Plains, and ii) to investigate the environmental factors affecting yield variation. Leaf area and yield were measured on plots at ten locations in MO, AR, OK, and TX. More cold winter days led to decreased subsequent Alamo switchgrass yields and increased subsequent upland switchgrass yields. More hot growing season days led to decreased Kanlow and miscanthus yields. Increased drought intensity also contributed to decreased miscanthus yields. Alamo switchgrass had the greatest radiation use efficiency (RUE) and water use efficiency (WUE). Best RUE values for other varieties ranged from 67 to 80% of Alamo's RUE value and 67% to 87% of Alamo's WUE. These results provide inputs to process based models to realistically simulate these important perennial grasses and to assess the environmental impacts of production on water use and nutrient demands. In addition, it will be useful for landowners and companies choosing the best grasses for biofuel production.

Genotypic Variation in Traits Linked to Climate and Aboveground Productivity in Widespread Warm-Season Grasses: Evidence for a Functional Trait Syndrome.
Natural variation in growth and function among plants of a given species is important for understanding how and why plants become adapted to their local environment. In this study, we examined how switchgrass, a native tallgrass prairie species, collected from sites ranging from Nebraska to south Texas, differed in growth and function. We found that switchgrass from cooler sites started growing later in the season, flowered early, used water less efficiently, and were smaller than switchgrass from warmer sites. These results have implications for natural and managed grasslands under predicted changes in climate.

Soil and Variety Effects on the Energy and Carbon Balances of Switchgrass-Derived Ethanol.
We examined the effects of soil and switchgrass variety on how sustainable and environmentally friendly switchgrass-based ethanol production can be. Using the Agricultural Land Management Alternatives with Numerical Assessment Criteria (ALMANAC) computer simulation model, switchgrass production was simulated for several soils and varieties. The yields were fed to the Integrated Biomass Supply Analysis and Logistics (IBSAL) model to compute energy use and carbon emissions in the biomass supply chain, which then were used to compute how much energy is produced and how soil carbon is affected. Values increased for lighter soils and for more southerly and lowland varieties, although these differences decreased in the direction of lower to higher latitudes. The values increased with increasing precipitation. Differences among varieties were smaller in a dry year than in a wet year. The study demonstrated that the sustainability and eco-friendliness of switchgrass-based ethanol production could be increased with alternative soil and switchgrass variety options.

Corn Residue Removal Effects on Soybean Yield and Nitrogen Dynamics in the Upper Mississippi River Basin.
When using corn leaves and stems as biofuel, there can be impacts on subsequent crops in the rotation, in particular soybean. We used the APEX computer simulation model to assess the impacts of four rates of corn residue removals; 0%, 40%, 60%, and 80%, on subsequent soybean yields across highly erodible (HEL) and non HEL land types; three broad soil textural classes; clayey, loamy, and sandy, and the four soil hydrologic groups. We looked at N dynamics for 3703 farm fields within the Upper Mississippi River Basin. Overall, residue removal reduced soybean N fixation by 6%, and yields by 8%. Of the other factors, soil texture and hydrologic group differences impacted yields more than did land type. Soil N storage with no residue removal increased by a maximum of 229 kg per ha, while the 40%, 60% and 80% residue removal rates reduced soil N storage relative to the 0% residue removal rate by approximately 221, 280 and 346 kg per ha, respectively. Nitrogen losses during the soybean-years are relatively small, and on average 56% lower than losses in the corn-years. Corn residue removal-induced reduction in N fixation and soil N storage should impact the size of the soybean N credit generally used in N fertilizer recommendations for corn/soybean rotations in the USA Corn Belt. This study underscores the need for holistic approaches to sustainably manage corn/soybean

biofuel feedstocks production systems to meet the food and fiber needs of the nation, while also providing the much needed feedstocks for the emerging bioenergy industry.

Comparing Productivity via Radiation Use Efficiency for Two Grass Giants of the Biofuel Field.
Two highly productive perennial grasses commonly considered for biofuel are switchgrass and Miscanthus. Their comparative productivity is of critical importance to the biofuel industry. The radiation use efficiency (RUE), when derived in an environment with non-limiting soil water and soil nutrients, provides one measure of relative productivity. The objective of this study was to compare Miscanthus with high productivity switchgrass cultivars, using established methods of measurements to allow calculation of RUE of each at two diverse sites. Measurements of fraction intercepted light and dry matter were taken on plots at Elsberry, Missouri (Miscanthus and three cultivars of switchgrass) and at Gustine, Texas (with Miscanthus and Alamo switchgrass, irrigated with dairy wastewater and a non-irrigated control). Miscanthus overall mean RUE (4.10) was nearly identical to Alamo switchgrass overall mean RUE (4.17). At Elsberry, the more northern lowland switchgrass cultivar, Kanlow, also showed nearly identical mean RUE (3.70) as Miscanthus (3.71). The northern upland cultivar, Cave-in-Rock, at Elsberry had a mean RUE (3.17) that was only 85 percent of the mean for Miscanthus at that site. Stress (water and nutrients) had a greater effect on Miscanthus RUE than on switchgrass RUE at Gustine. These results provide realistic values for simulating these important biofuel grasses in diverse environmental conditions.

Productivity of Well-Watered Switchgrass Does Not Increase with CO_2 Enrichment.
Rising atmospheric CO_2 has been shown to increase aboveground net primary productivity (ANPP) in water-limited perennial grasslands, in part by reducing transpiration, thereby increasing photosynthetic water use efficiency and reducing depletion of soil moisture. However little is known about CO_2 effects on monocultures of perennial warm-season grasses such as switchgrass, a native grass of tallgrass prairie and a potential bioenergy crop. We hypothesized that increased CO_2 would not increase switchgrass ANPP, because photosynthetic rates of this warm-season grass would not increase and because reduced transpiration from reduced stomatal conductance at elevated CO_2 would provide little additional benefit in increased soil moisture. The findings confirmed this hypothesis, and also revealed evidence for meristem limitation of productivity at elevated CO_2 and for increased physiological coupling to CO_2 concentration on the plants established.

Sediment Measurement and Transport Modeling: Impact of Riparian and Filter Strip Buffers.
Watershed models such as the Soil and Water Assessment Tool (SWAT) are widely used to simulate watershed hydrologic processes and the effect of conservation practices such as those used by private landowners participating in selected USDA conservation programs. Lack of data for parameterization and evaluation remains a weakness to modeling globally. Research was conducted in southwestern Oklahoma within the Cobb Creek sub-watershed to develop more cost-effective methods to collect parameterization and validation data for modeling in watersheds with sparse data. The specific objectives were to use: 1) rapid geomorphic assessment (RGA) data to parameterize SWAT stream channel variables, 2) long-term average annual reservoir sedimentation rates obtained from a bathymetric survey at the Crowder Lake

using the acoustic profiling system (APS) to validate sediment delivery rates simulated by the calibrated SWAT model, and 3) use the validated SWAT model to simulate impacts of riparian forest and bermudagrass filter strip buffers on sediment yield (from overland erosion only) and concentration (includes contributions from overland and channel erosion for the contributing sub-basins). Additionally, the calibrated and validated SWAT model was used to simulate impacts of riparian forest buffer and bermudagrass filter strip buffer on sediment yield and concentration in the sub-watershed. The average annual sediment delivery rate to Crowder Lake simulated by the calibrated SWAT model was 1.9 mt per ha per yr, which is in the same order of magnitude as the long-term average annual reservoir sedimentation rates obtained using the APS between 1.2 and 2.6 mt per ha per yr depending on sediment bulk densities between 450 and 950 kg m^{-3}. Application of buffer strip across cropped fields resulted in a 72% reduction of sediment delivery to the stream, while the riparian buffer and the combined riparian buffer and buffer strip reduced the suspended sediment concentration at the sub-watershed outlet by 67% and 70%, respectively. Effective riparian practices have potential to increase reservoir life. These results indicate that the RGA and APS techniques are potential cost-effective methods to obtain data to parameterize and evaluate watershed models in ungauged watersheds globally. In addition, the RGA data alongside a calibrated and validated SWAT model is a potential method that can be used to evaluate the impact of the riparian forest buffer on water quantity and quality.

Potential Carbon and Nitrogen Mineralization in Soils from a Perennial Forage Production System Amended with Class B Biosolids.

Nutrient management is the most critical issue for land application of biosolids, and effective management practices must be used to ensure the long-term agronomic and economic benefits from land-based biosolids recycling. Soils are an integral component of these beneficial reuse systems, and changes in soil microbial activities could change how soils store carbon (C) and provide nutrients such as nitrogen (N) for agricultural production. In this study, we found that surface applications of Class B municipal biosolids to a coastal bermudagrass hay production system resulted in increased soil C and N concentrations. Readily available forms of soil C as water soluble organic C (WSOC) derived from biosolids were higher in more-recently applied soils, regardless of application rate. Biosolids additions stimulated microbial activity, but increases in soil C due to amendments were greater than potential soil C losses from microbial respiration (i.e. losses through CO_2). In conclusion, surface applications of Class B municipal biosolids at the lowest rate could provide C storage while plant growth derived from the nutrients in the biosolids would limit environmental impacts of biosolids-derived N, as well as other compounds (i.e. phosphorus, trace metals). Long-term applications at the lowest rates, however, may still result in excess soil N levels and must be monitored.

Variability in Light-Use Efficiency for Gross Primary Productivity on Great Plains Grasslands.

The amount of carbon (C) that plants capture via the process of photosynthesis (gross primary productivity; GPP) determines plant productivity and affects the amount of C that is stored in terrestrial ecosystems. GPP often is estimated at regional and global scales by multiplying the amount of light absorbed by the plant canopy by a value of light use efficiency, C uptake per unit of absorbed light. Light use efficiency usually is assumed either to be constant or to change predictably as temperature and other environmental conditions change. An implicit assumption of this approach is that environmental effects on light use efficiency do not differ from year-to-year or among ecosystems. We used measurements of C uptake and release from 3 native grasslands in the central Great Plains of the USA to determine whether environmental effects on light use efficiency varied among years and grasslands. Light use efficiency was measured as the slope of the linear relationship between GPP and the amount of light that plants absorbed. Light use efficiency declined as the amount of absorbed light increased and varied among grasslands and among years on each of the grasslands. Light use efficiency was greater during years when the mean air temperature was high than low. Our results indicate that we must account for site-to-site variation in plant and environmental variables in order to accurately predict GPP.

Soil Microbial Activity under Different Grass Species: Underground Impacts of Biofuel Cropping.

We measured the amounts of carbon that native grasses contribute to soil as compared to conventional farming. The study was primarily focused on the effect of different grass species and conventional corn on soil microbes. The study found that soil microbes are much more active under native grass species than conventional farming practices. Increased soil microbial activity is a healthy sign in terms of increased soil quality. This research has broad implications on our ability to manage climate change by using native grass species when and where appropriate. In addition it provides baseline data for conversion of cropping systems to native grass biofuel production for Blackland soils in central Texas.

Funded Bioenergy Grants: As a result of the funding status of our CRIS at Temple, we have sought additional resources from a number of granting agencies to further leverage ARS research.

2010-2014. Department of Navy, Office of Naval Research – Resource Assessment Framework for Dependable Feedstock Supply to Produce Advanced Biofuels in Hawaii. PI's J. R. Kiniry and J. G. Arnold. Award to J. R. Kiniry and J. G. Arnold: $2,796,273.

Selected Publications

1. Aspinwall, M.J., D.B. Lowry, S.H. Taylor, T.E. Juenger, C.V. Hawkes, M.V. Johnson, J.R. Kiniry, and P.A. Fay. 2013. Genotypic variation in traits linked to climate and aboveground productivity in a widespread C_4 grass: Evidence for a functional trait syndrome. New Phytologist 199:966-980.

2. Behrman, K.D., J.R. Kiniry, M. Winchell, T.E. Juenger, and T.H. Keitt. 2013. Spatial forecasting of switchgrass productivity under current and future climate change scenarios. Ecological Applications 23(1):73-85.

3. de Mazancourt, C., F. Isbell, A. Larocque, F. Berendse, E. De Luca, J.B. Grace, B. Haegeman, H.W. Polley, C. Roscher, B. Schmid, D. Tilman, J. Van Ruijven, A. Weigelt, B.J. Wilsey, and M. Loreau. 2013. Predicting ecosystem stability from community composition and biodiversity. Ecology Letters 16:617-625.

4. Fay, P.A., H.W. Polley, V.L. Jin, and M.J. Aspinwall. 2012. Productivity of well-watered *Panicum virgatum* does not increase with CO_2 enrichment. Journal of Plant Ecology. 5(4):366-375.

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6. Jin, V.L., M. Johnson, R.L. Haney, and J.G. Arnold. 2011. Potential carbon and nitrogen mineralization in soils from a perennial forage production system amended with Class B biosolids. Agriculture, Ecosystems and Environment. 141:461-465. Available: DOI: 10.1016/j.agee.2011.03.016.

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8. Kiniry, J.R., M. Johnson, S.B. Bruckerhoff, J.U. Kaiser, R.L. Cordsiemon, and R.D. Harmel. 2012. Clash of the Titans: Comparing productivity via radiation use efficiency for two grass giants of the biofuel field. BioEnergy Research 5(1):41-48.

9. Lowry, D.B., K.D. Behrman, P. Grabowski, G.P. Morris, J.R. Kiniry, and T.E. Juenger. 2014. Adaptations between ecotypes and along environmental gradients in *Panicum virgatum*. The American Naturalist 183:682-692.

10. Meki, M.N., J.L. Snider, J.R. Kiniry, R.L. Raper, and A.C. Rocateli. 2013. Energy sorghum biomass harvest thresholds and tillage effects on soil organic carbon and bulk density. Industrial Crops and Products 43:172-182.

11. Moriasi, D.N., J.L. Steiner, and J.G. Arnold. 2011. Sediment measurement and transport modeling: Impact of riparian and filter strip buffers. Journal of Environmental Quality 40:807-814.

12. Osorio, J. J. Jeong, K. Bieger, J.G. Arnold. 2014. Influence of potential evapotranspiration on the water balance of sugarcane fields in Maui, Hawaii. Accepted for publication in Journal of Water Management and Protection.

13. Polley, H.W., B.L. Phillips, A.B. Frank, J.A. Bradford, P.L. Sims, J.A. Morgan, and J.R. Kiniry. 2011. Variability in light-use efficiency for gross primary productivity on Great Plains grasslands. Ecosystems 14:15-27.

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Other Publications

1. Youkhana, A., S. Crow, M. Meki, J. Kiniry, R. Ogoshi, and M. Nakahata. Belowground biomass and C Dynamics in Sugarcane, Ratooning Energycane cultivated as biofuel production in Hawaii. (Abstract). Proc. ASA-CSSA-SSSA Meetings. ASA-CSSA-SSSA Meetings. October, 2013. Tampa, FL.

2. Meki, M.N., J.R. Kiniry, K.D. Behrman, M.N. Pawlowski, and S.E. Crow. The Role of Simulation Models in Monitoring Soil Organic Carbon Storage and Greenhouse Gas Mitigation Potential in Bioenergy Cropping Systems. In Claudia do Rosario Vaz Morgado and Victor Paulo Pecanha Esteve (Eds), CO_2 Sequestration and Valorization. InTech Europe - Open science | Open minds.

3. Meki, M.N., and J.R. Kiniry. 2013. A Dynamic Tool - Resource assessment framework for dependable feedstock supply to produce advanced biofuels in Hawaii. International Innovation: The Global Forecast, October 2013. Research Media, UK, p118-120, ISSN 2051-8544.

4. Meki, M.N., J.R. Kiniry, A.H. Youkhana, M.H. Nakahata, and J.J. Steiner. 2013. Key crop parameters for ALMANAC modeling of high biomass bioenergy sorghum growth and productivity. Oral Presentation at ASA-CSSA-SSSA Meetings. October, 2013. Tampa, FL.

5. Ganjegunte, G., M. Meki, and J. Kiniry. 2013. Field Evaluation of Bioenergy Crops Performance On Saline Soils Under Arid Conditions. (Abstract). Proc. ASA-CSSA-SSSA Meetings. ASA-CSSA-SSSA Meetings. October, 2013. Tampa, FL.

6. Meki, M.N., J.L. Snider, J.R. Kiniry, R.L. Raper, and A.C. Rocateli. 2012. Energy sorghum biomass harvest thresholds and tillage effects on soil organic carbon and bulk density. Industrial Crops and Products 43, 172-182.

7. Meki, M.N., J.R. Kiniry, A.H. Youkhana, M.H. Nakahata, S.E. Crow, R.M. Ogoshi, and J.J. Steiner. 2012. Parameterization of the ALMANAC model to evaluate novel high biomass crops on Maui Island, Hawaii. (Abstract). Proc. ASA-CSSA-SSSA Meetings. October, 2012. Cincinnati, OH.

8. Meki, M.N., M. Nakahata, J. Arnold, J. Kiniry, and J. Steiner. 2011. Optimizing Sugar Cane Water Use Efficiency with the Aid of the ALMANAC Model. (Abstract). Proc. ASA-CSSA-SSSA Meetings. October, 16-19 2011. San, Antonio, TX.

9. Osorio, J., J. Jeong, J. Arnold, R. Tirado-Corbala, and R. Anderson. 2013. Comparison of soil properties and weather datasets to assess the influence of evapotranspiration estimates on the water balance of sugarcane cropping system in the Hawaiian island of Maui. Oral presentation at the AWRA Annual Meeting. November 7, 2013, Portland, Oregon.

10. Jeong, J., J. Osorio, J.G. Arnold, R. Srinivasan (2013) “Integration of SWAT into a real-time web-based DS tool for sugarcane irrigation management” 2013 International SWAT Conference and Workshop, July 17-19, 2013, Toulouse, France

11. Osorio, J., J. Jeong, J.G. Arnold (2013) “Influence of evapotranspiration estimates on the water balance of sugarcane cropping system in the Hawaiian island of Maui” 2013 International SWAT Conference and Workshop, July 17-19, 2013, Toulouse, France

12. Jeong, J., J.G. Arnold, C. A. Jones, R. Srinivasan, M. Nakahata (2012) “Development of irrigation decision support tool for sugarcane fields using real-time data and SWAT model” ISSCT Agronomy and Agricultural Engineering Workshop, Sep. 9-14, 2012, Townsville, Australia

13. Jeong, J., J.G. Arnold, C. A. Jones, R. Srinivasan, M. Nakahata (2012) “A SWAT-based decision support tool for Hawaii Sugarcane Plantation” 2012 ASABE Annual International Meeting, July 29 - August 1, 2012, Dallas, Texas

14. Jeong, J., J.G. Arnold, C. A. Jones, R. Srinivasan, M. Nakahata (2012) “Application of the SWAT model to support feedstock production of biofuels in Hawaii” 21st Century Watershed Technology Conference and Workshop, May 27-June 1, 2012, Bari, Italy

<u>Tifton, GA</u>: Crop Protection and Management Research Unit (NP301, NP 304); Crop Genetics and Breeding Research Unit (NP 215); and Southeast Watershed Research Unit (NP211, NP212)

The accomplishments listed below summarize the Tifton, GA research for incorporating bioenergy crops in cropping systems of the Southeastern U.S. Since 2003, we have been evaluating the improvement and production of bioenergy crops as well as the potential environmental impacts that may result from the conversion of cropland acreage to feedstock production. We have conducted research on perennial grass biomass (switchgrass, napiergrass, miscanthus, energy cane) as well as the incorporation of annual biomass feed stocks (biomass sorghum, sweet sorghum, corn), and summer winter crops (energy beet, rye, wheat) into existing crop rotation systems. We have also begun new experiments evaluating the potential for perennial grasses (energy cane and napiergrass) to function as a harvestable component of riparian conservation buffer systems.

Determination of Potential Perennial Grass Feedstock Yields Under No Inputs

Warm-season perennial grasses are a promising source of biomass for energy production in the Southeast U.S., and low-input production is desirable. With only residual fertility in the soil and no irrigation, this test compared biomass yields of eight grasses under low-input production: L 79-1002 energycane (*Saccharum* hyb.), Merkeron and N51 napiergrass (*Pennisetum purpureum* Schum.), three clones of giant reed (*Arundo donax* L.), and two switchgrass (*Panicum virgatum* L.) lines. For the first two years napiergrass maintained dry matter (DM) yields over 25 Mg DM $ha^{-1}yr^{-1}$, and energycane yielded over 20 Mg DM $ha^{-1}yr^{-1}$ for three years. Switchgrass yields were lower (8.6 Mg DM $ha^{-1}yr^{-1}$ average of four years), but the biomass contained less moisture at harvest than the other, larger-stemmed grasses. Switchgrass biomass also had the lowest concentrations of N, K, and ash. Average yields of giant reeds were also low (6.4 Mg DM $ha^{-1}yr^{-1}$), while ash and N concentrations were relatively high compared to switchgrass and energycane. In four years of production, energycane and napiergrass removed between 269–386 kg N ha^{-1} and 830–1159 kg K ha^{-1}, while the other grasses removed significantly less of these nutrients. Giant reed removed 126 kg N ha^{-1} and 193 kg K ha^{-1}, and switchgrass removed 83 kg N ha^{-1} and 140 kg K ha^{-1}. The results of this study provide important information to potential growers and the bioenergy industry on limitations and potential of production of biomass from perennial grasses with minimal inputs in the Southeast. (Knoll et al., 2012b)

Organic and Inorganic Fertilizer Needs of Grasses for the Southeast

Napiergrass has shown promise as a biomass feedstock for the Southeast. However, specific production practices need to be determined to guide growers. ARS Researchers at Tifton, GA grew napiergrass (also called elephantgrass) in a study with either poultry litter or inorganic fertilizer (Nitrogen, Phosphorus, Potassium = 100, 40 and 80 kg ha^{-1}) over four years on a non-irrigated Tifton loamy sand soil. For the first two years dry matter yields did not differ among treatments, but yields declined in all treatments, especially in unfertilized check plots in years three and four. In general, nitrogen removal exceeded the amount applied, suggesting that higher fertilizer application rates are necessary. Total soil carbon increased by over 3000 kg ha^{-1} among the three treatments indicating that use of napiergrass as a biomass feedstock would be either carbon neutral or carbon-positive relative to petroleum. (Knoll et al., 2013)

Optimum Time and Method for Propagating Perennial Grass Feedstocks in the Southeast
Research was conducted to evaluate planting methods and cutting lengths for napiergrass, and to determine the optimal planting date for napiergrass and energycane in the Coastal Plain of Georgia, USA. The results of a greenhouse study with Merkeron napiergrass show that either horizontal buried planting or vertical planting with one node exposed can be used to establish cuttings. Cuttings taken from the lower portion of the parent stem were superior to younger material from the upper portion. The rooting hormone indole-3-butyric acid (IBA) did not have significant effects on propagation success. Nine napiergrass genotypes were compared for response to cutting length in the field (one, two, five, or ten nodes/cutting, and ten nodes/plot). For most genotypes, single-node cuttings tended to produce fewer tillers per plot and less total biomass, measured the following spring. Generally, five and ten-node cuttings resulted in better stands, but the more vigorous genotypes were not affected by cutting size. Seven genotypes of napiergrass and two of energycane were compared for response to planting date. Five bi-weekly plantings were made beginning 17-Sept, 2009, and six plantings beginning 2-Sept, 2010. In both years, spring regrowth was greatest in the early plantings, but the effect was greater in 2010. Energycanes showed little effect of planting date in 2009. In the Coastal Plain planting no later than mid-September is recommended for both species. (Knoll and Anderson, 2012)

Evaluation of Energycane Genotypes for Sugar and Ethanol Yields over Time of Harvest
Energycane (Saccharum hyb.) is a perennial bioenergy crop derived from sugarcane, but with higher fiber, greater biomass yields, and better cold tolerance than typical sugarcane. ARS researchers grew two commercial sugarcanes, two high-sugar (Type I) energycanes, and five high-fiber (Type II) energycanes at Tifton, GA. Beginning in October, 2008 (plant-cane crop year), five monthly samples were taken to assess the effects of delaying harvest on biomass composition and quality for ethanol production. The monthly harvests were repeated in the winter of 2010-11 (second-ratoon crop year). Delaying harvest into the winter months resulted in minimal reductions in biomass moisture and N mass fractions, while K mass fraction decreased significantly. Free sugar mass fraction also decreased, thus causing the biomass to have an apparent increase in relative mass fractions of ash and neutral detergent fiber (NDF). The reduction in free sugars was more pronounced in the colder harvest season (2010-11). The composition of biomass fiber (cellulose, hemicellulose, and lignin) was generally stable over time. A bench-top partial saccharification and co-fermentation (PSCF) protocol employing xylose-fermenting Escherichia coli was used to assess ethanol yields from the sequentially harvested biomass. Ethanol yield from sugarcanes and Type I energycanes was more variable over time, due to degradation of free sugars. Thus, early harvest is recommended to avoid loss of fermentable sugars. Type II energycanes can be harvested later during the winter months with little change in conversion properties. Information from this study will help direct the bioenergy industry and growers on types and methods of high-yielding biomass grasses for the Southeast. (Knoll et al., 2012a)

Evaluation of Forage Bermudagrass as Feedstock for Biofuels
Bermudagrass is an attractive candidate as a feedstock for biofuel production because over 4 million hectares of bermudagrass are already grown for forage in Southern United States. Forage grasses have been improved through breeding to obtain high biomass yields and better forage digestibility. This improved biodegradability is conferred through lower lignin content and altered cell wall composition, which may have a direct impact on their quality for use as a

feedstock in biofuel production. Because both rumen digestion and biochemical conversion to ethanol depend upon enzymatic conversion of the fibers into fermentable sugars, it is probable that grasses bred for increased forage quality would be amenable for ethanol production. The objective of this research was to determine relationships between ethanol production evaluated by simultaneous saccharification and fermentation (SSF), ruminal *in vitro* dry matter digestibility (IVDMD), *in vitro* ruminal gas production after 24 and 96 h (NNG24, NNG96), and biomass composition for 50 genetically diverse bermudagrass accessions. Ethanol production was moderately correlated with IVDMD (r = 0.55) but highly correlated with NNG24 (r = 0.93). Regression models indicate that NDF and pentose sugar concentrations had highly significant effects on ethanol production. Variation among entries for IVDMD was affected by variability of NDF, pentose sugar concentrations, and biomass nitrogen content. Variation in lignin content had minor impacts on ethanol production and IVDMD. (Anderson et al., 2010)

Genetic Relationships Among Napiergrass Accessions for the Biofuel Industry
Napiergrass is a perennial grass used for forage and has considerable potential as a biofuel feedstock primarily because of its high biomass yield. ARS researchers at Tifton, GA used amplified fragment length polymorphisms (AFLPs) to assess the genetic variation and genetic relatedness among 89 accessions from the Tifton nursery. Using 218 polymorphic markers, the 89 accessions were clustered into 5 groups using a dendrogram (or genetic tree). These five groups include three groups collected from Kenya, a group from Puerto Rico, and accessions derived from the cultivar Merkeron. This research was the first molecular characterization of the Tifton nursery (a nursery based on over 40 years worth of collections and breeding) and displays the relationships among accessions. Furthermore this work provides potential parents for napiergrass and pearl millet breeding improvement that is currently underway in collaboration with university personnel at Florida and with the bioenergy industry. Data from this manuscript will be used to identify genes involved in height and nitrogen use efficiency in napiergrass. (Harris et al., 2010).

Use of Winter Cover Crops as Banker Plants for Beneficial Insects on Summer Row Crops
The use of novel crops for bio-fuel production requires evaluating the potential for sound ecological and economical implementation in a particular region. We examined the pest and generalist beneficial insect species associated with various winter cover crops (including narrowleaf lupin, white vetch, Austrian winter pea, crimson clover, faba bean, and rye) as sources of colonists in two subsequent summer crops, sorghum and cotton. Sorghum is a potential cellulosic bio-fuel crop and cotton is commonly grown in the region and could be a viable low-input rotation for biofuel sorghum. Insects were sampled weekly over three years in winter cover plots beginning in early spring and in the later planted crop plots beginning at the 15 cm height stage of the crops and continuing for 3-6 weeks. Of the predators, coccinellids dominated and were consistently abundant in vetch, faba and lupin, as was the pea aphid, *Acyrthosiphon pisum* and the aphid parasitoid, *Lysiphlebus testaceipes. Orius* spp. dominated in lupin. Of the pest species, thrips spp. were highest in lupin and pea, and stink bugs were highest in clover. No differences in chinch bugs were found among the covers. There was a ‘relay’ of these species into all of the summer crop plots from living winter crops. Boll damage from stink bugs was highest in the cotton following lupin, pea and fallow with fertilizer; there was no damage from chinch bugs in sorghum. Faba beans had declining stands over the three years, suggesting that this species would not be a reliable winter crop in this system. Vetch and lupin may be the best candidates as banker plants because of their ability to consistently sustain pea

aphids and coccinellids, the former which is a non-pest of sorghum and cotton. (Olson et al., 2012)

Hydrologic Impacts of Projected Land-Use Changes to Accommodate Bio-energy Production. USDA goals for meeting renewable fuels standards by 2022 indicate that nearly 50% of the advanced biofuels to be produced in the U.S. are expected to come from the Southeastern U.S. Meeting these goals will require conversion of existing cropland to high-yielding biomass crops. Changes in water resources, both quantity and quality, are anticipated with these changes. Computer simulations indicated conversion of existing production land into grass biomass crops will result in: 1) decreased evapotranspiration; 2) increased stream flow; 3) decreased sediment loading; and 4) seasonal shifts in stream flow. Results also indicate an increase in forested area for biomass production will result in: 1) increased evapotranspiration; 2) decreased stream flow; 3) decreased sediment loading; and 4) seasonal shifts in stream flow. These results give an indication of the hydrologic changes which would accompany large scale changes in land-use associated with biomass production.

Estimation of Biofuels Feedstock Production Potentials from Non-Forested Riparian Zones and Grassed Waterways. USDA's Regional Roadmap to Meeting the Biofuels Goals of the Renewable Fuels Standard by 2022 targets the southeastern U.S. for delivery of 49.8% of the feedstock contributions needed to meet the advanced biofuels goal of 79.5 billion liters per year. Using corporate estimates that 14,160 ha must be dedicated to feedstock production within 40 km of a 136 million liter per year biofuel conversion facility converting perennial grass feedstocks via cellulosic ethanol production, we used field trial data to estimate that from 6% to 38% of the needed acreage could be gained from riparian buffers and grassed waterways. The remaining acreage, if taken from agricultural land in the 40 km radius would be from 3% to 18% of current agricultural lands. The analysis suggests a potential to produce> 1.98 billion liters of ethanol per year (at 270 liters per Mg dry matter and 33 Mg ha-1 yr-1 dry matter) from riparian zones alone around 11 case study cities in the coastal plain of South Georgia. Another 814 million liters per year could come from nonprime agricultural lands (at 22 Mg ha-1 yr-1 dry matter).

Established new plantings of *Miscanthus giganteus* in a long term research watershed. Cropland in a small watershed that has been the subject of long term research has been converted to *Miscanthus* by the cooperator Lewis Taylor Farms/New Energy Farms. A new gauging station for hydrology and water quality measurements has been established. The new watershed site samples an area that is about 19 ha with 10.7 ha of *Miscanthus* planted and 8.3 ha of forest. Hydrology and water quality measurements will be compared to long-term measurements at a downstream site to determine the effects of *Miscanthus* on watershed processes.

Evaluation of Regional Yields of Energycane in the Southeast
Energycanes are hybrids of a cold-tolerant sugarcane relative (*S. spontaneum*) with domestic varieties. They are typically low in sugar, but high in fiber and yield. As part of a regional team across the United States, five energycane hybrids developed at the USDA/ARS Sugarcane Research Unit at Houma, LA were evaluated for yield and agronomic characteristics. Replicated field trials of five genotypes (Ho 02-144 & 147; Ho 06-9001 & 9002; and Ho 72-114) were conducted across five states in the Southeast to evaluate the potential production and

sustainability of energycane as a bioenergy feedstock resource. Test locations were: Tifton & Athens, GA; Starkville & Raymond, MS; St. Gabriel, LA: and Beamont & College Station, TX. Data collected by location years included: date of emergence, monthly height and °Brix (a measure of sugar content), and end of season biomass yield. After two full years' growth, data indicated greatest plant height was observed during mid to late Sept. at all locations. Termination of growth corresponded to a decrease in soil temperature below 30°C. °Brix varied with location and genotype, but maximum °Brix was observed in mid Oct. A genotype by location interaction was also observed for yield. Generally genotypes that did the best at the southern most locations yielded the worst at the northern most location. Yields in the first full year of production ranged from 3.89 T/A (Ho02-144 @ Raymond, MS) to 25.44 (Ho 06-9001 @ Beaumont, TX). Record cold weather did impact yield, but no genotype was lost. Lines Ho 06-9001 and Ho 06-9002 continued to increase in yield at Tifton through the third year and these lines look particularly cold tolerant and adapted for northern locations.

Ethanol and Co-products from Pretreatment with Pressurized Hot Water of Napiergrass and Bermudagrass

Various warm-season grasses have been proposed as good biomass sources for conversion to ethanol or petroleum replacement fuels. Due to the fact that only cellulose and hemi-cellulose can be converted with biochemical conversion and the presence of lignin within cell walls of these plants, pretreatment of the grasses is required to maximize ethanol yield during fermentation. In collaboration with the University of Georgia USDA/ARS scientists at Tifton, GA, T85 bermudagrass and Merkeron napiergrass (*Pennisetum purpureum* Schumach.) samples were either left untreated or were pretreated with pressurized batch hot water (PBHW) for 2 minutes at 230°C at 5% w/v whole grass solids loading. Following a 24 h enzymatic digestion, untreated and PBHW pretreated grasses were evaluated for ethanol production and co-product generation including potential fermentation inhibitors. Fermentations of PBHW-pretreated grasses with *Escherichia coli* LY01 produced twice the ethanol of their untreated counterparts. PBHW-pretreated Merkeron napiergrass produced 224.5 mg/g grass ethanol (73% maximum theoretical yield) and PBHW pretreated T85 bermudagrass reached 213.0 mg/g grass (70% maximum theoretical ethanol yield). PBHW pretreatment produced potential fermentation inhibitors such as acetic acid, formic, cinnamic acids, and aldehydes. Despite some of these inhibitors remaining with the solids after PBHW pretreatment, there was greater reduction of cellulose and hemicellulose to sugars during the enzymatic digestion of the grasses prior to fermentation when compared to the untreated grasses. This resulted in increased ethanol yields during fermentation with bacteria as the catalyst. (Brandon et al., 2011)

Winter-grown Energy Beet Incorporates into Traditional Summer Crop Rotations

Energy beets (*Beta vulgaris*), which are sugar beets grown for non-food sources, are a potential winter cash crop for growers in the southeastern U.S. that are planted in the autumn and harvested in the spring, complementing current summer crop rotations. The end-product from energy beets will be industrial sugars that can be processed into ethanol/butanol, biodegradable plastics, or some other non-food product. Unlike other potential energy crops, beets have established varieties, agronomic practices, pesticides, and equipment. The challenge will be to adopt those practices developed in other regions of the U.S. to a winter-based system in the southeastern U.S. Studies were conducted by USDA-ARS scientists in collaboration with University of Georgia researchers to evaluate the yield potential of energy beets when harvested at different times during the spring and summer. Studies were planted 6 October 2011 and 1 November 2012, with seven harvest times, initiated approximately 187 days after planting and continuing every three weeks through 318 days after planting. Data collection at harvest included: root biomass, foliar biomass, and an estimate of sugar content; roots were liquefied and total solids of the beet juice measured. Yields were lowest at the first harvest in 2012 (53 to 65 Mg/ha) and 2013 (46 to 64 Mg/ha) and nearly doubled within 6 to 9 weeks, as yields were maximized in mid-June at 250 days after planting in 2012 (103 to 129 Mg/ha) and 230 days after planting in 2013 (90 to 129 Mg/ha). Plant pathogens (Sclerotium root rot and Cercospora leaf spot) were low at early harvests, increasing in intensity as air temperatures and humidity increased. Beet yield declined in July and August (in response to increased disease severity), with final yields ranging from 70 to 110 Mg/ha in 2012 and 28 to 61 Mg/ha in 2013. Winter beets harvested in the spring and early summer had yields that were at least equivalent to average yields in the Midwest U.S., and consistent with the average yields in the Imperial Valley of California 89 Mg/ha, where peak yields approach 142 Mg/ha. Theoretical ethanol yields ranged from 4,760 to 6,730 l/ha (509 to 720 gal/a) at the first harvest and 9,315 to 13,350 l/ha (995 to 1,427 gal/a) in mid-June. There is high potential for successful beet production in the coastal plain of the southeastern U.S., providing an agronomic cash crop during the typical winter fallow period with minimal disruption to traditional summer cash crops.

Bioenergy Grants and Partnerships: Tifton scientists have sought additional resources from a number of granting agencies to further leverage ARS research. We have also entered into cooperative agreements with private companies to access sufficient acreage to evaluate watershed-level responses to upland conversions from annual crops to perennial biofuels feed stocks.

Scientists from the Southeastern Biomass Research Center cooperated with scientists from Ft. Valley State University and other ARS units to submit a successful proposal to the NIFA-AFRI-Sustainable Bioenergy Program "Carbon Sequestration and Nitrogen Cycling for Green House Gas Mitigation by Southeastern US Annual and Perennial Energy Crops"

Scientists from SEBRC have developed a Collaborative Research and Development Agreement with BP Biofuels 2010-2013 for Breeding and selection of napiergrass (*Pennisetum purpureum*) for conversion to biofuels.

Established a Specific Cooperative Agreement with a local farm, Lewis Taylor Farms/New Energy Farms to do cooperative research on Miscanthus and other bioenergy crops. Work condicted under this agreement led to establishment of Miscanthus small watershed study.

Selected Publications:

1. Anderson, W.F., Dien, B.S., Jung, H.G., Vogel, K., and Weimer, P.J. 2010. Effects of forage quality and cell wall constituents of bermudagrass on biochemical conversion to ethanol. Bioenerg. Res. 3:225-237.

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13. Lowrance, R. and Davis, A. 2014. Environmental sustainability of cellulosic energy cropping systems. *In*: Cellulosic Energy Cropping Systems D. L. Karlen (ed), John Wiley and Sons. 15p. doi: 10.1002/9781118676332

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16. Richard, E.P., and Anderson, W.F. 2014. Sugarcane, energy cane and napier grass *In*: Cellulosic Energy Cropping Systems D.L. Karlen (ed.), John Wiley and Sons, pp 91-108.

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1. Knoll, J.E., Anderson, W.F., Strickland, T.M. and Hubbard, R.K. 2010. Field performance of potential biomass feedstocks under no inputs in South Georgia. Proceedings of 32nd Symposium on biotechnology for Fuels and Chemicals, Clearwater, FL April 19-22, 2010.

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7. Anderson, W.F., Knoll, J., Strickland, T.M., and Hubbard, R.K. 2010. Sustainability of Perennial Grass Yields as Bioenergy Feedstock for the Southeast. ASA-CSSA-SSSA International Meetings, Long Beach, CA, Oct 31-Nov 3, CDRom.

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10. Knoll, J.E., Anderson, W.F., Strickland, T.M., and Hubbard, R.K. 2011. Production of napiergrass (*Pennisetum purpureum* Schum) for bioenergy under organic versus inorganic fertilization in the Southeast USA. ASA-CSSA-SSSA International Meetings, San Antonio, TX, Oct 16-20, CDRom.

11. Anderson, W.F., Lamb, J., Wright, D., Tubbs, S., Novak, J. 2012. New modes of use and opportunities for research in forage plants. Proceedings of the 49th Brazilian Society of Animal Science (SBZ), July 23 – 26, 2012, Brasilia, Brazil

12. Knoll, J.E., Lee, D.R., Anderson, W.F., and Johnson, J. 2012. Cellulosic ethanol production from warm-season perennial grasses. ASA-CSSA-SSSA International Meetings, Cincinnati, OH. Oct 21-24, CDRom.

13. Lowrance, R., Singh, B.P., Anderson, W.F., Singh, H.P. and Sainju, U.M. 2012. Sustainability aspects of perennial grass feedstock production in the Southeastern Coastal Plain. ASA-CSSA-SSSA International Meetings, Cincinnati, OH. Oct 21-24, CDRom.

14. Davis, R.F., and Anderson, W.F. 2012. Identification of widely varying levels of resistance to *Meloidogyne incognita* in sweet sorghum. J. of Nem. proceedings (Abst.)

15. Baldwin, B., Anderson, W., Blumenthal, J., Brummer, E.C., Gravois, K., Hale, A.L., Parish, J.R., and Wilson, L.T. 2012. Regional testing of energycane (*Saccharum* spp.) genotypes as a potential bioenergy crop. Proc. of the 2012 Nat. Conf., *Science for Biomass Feedstock Production and Utilization, Oct 2-5, 2012, New Orleans, L.*

16. Dien, B.S., Anderson, W.F., O'Bryan, P.J., and Cotta, M.A. 2014. Effect of agronomics on production and conversion quality of Napiergrass. (Abst.) Proceeding of the 36th Symposium on Biotechnology for Fuels and Chemicals (April 28-May 1, 2014, Clearwater, FL).

<u>Florence, SC</u>: Coastal Plain Soil, Water and Plant Research Center (CPSWPRC) (NP212)

<u>Accomplishments:</u> The accomplishments listed below summarize the CPSWPRC research efforts for assessing the impact of bioenergy crop harvesting on soil sustainability and the utilization of byproducts from agricultural bioenergy production to improve soil health in the Southeastern USA Coastal Plain region. As part of the Sun Grant Initiative, between 2008 to 2012, scientists at the location evaluated the selective removal of different amounts of corn stover biomass for bioenergy production, soil sustainability, and stover yields. Crop and soil data from this location for 2008 through 2012 has been submitted to the USDA REAPnet and to the DOE KDF database through either direct data entry or electronic transfer. Additionally, thought an interagency agreement (IA) with the EPA, we have been designing biochars with specific characteristics as amendments to improve targeted soil deficiencies. Below are the accomplishments from our ARS Research Unit.

Determining Sustainable Corn Stover Mass Harvesting Amounts for Bioenergy Production from Sandy Coastal Plain Soils.
Among the bioenergy crops proposed for cultivation in the Southeastern USA, corn (Zea maize) is the preferred crop because it has the highest above-ground biomass production. However, considering that the sandy soils of the Coastal Plain region have meager organic carbon (OC) contents, low abilities to retain nutrients and moisture, any removal of corn stover may further stress their soil health qualities. Over a 5-year period, different amounts (0, 50 and 100%) of corn stover were harvested from plots across a soil landscape catena. During the study period, crop samples from the grain and corn stover, as well as topsoil samples to 15 cm deep were collected. Long-term data showed that corn grain yields and stover quality were influenced more by rainfall patterns than stover harvest removal. However, removal of stover did cause reductions in topsoil P and K concentrations along with shifts in the bacterial to fungal ratio. This means that farmers will need to replace lost nutrients using fertilizers and that soil microbial health characteristics are impacted. Results from this project were uploaded into the USDA's REAP and the DOEs KDF data bases for the general public and university modelers to assess the impact of corn stover removal under different climatic and soil conditions. Additionally, scientists from the location authored and coauthored five journal publications in Bioenergy Research.

Interagency Agreement (IA) Between the USDA-ARS and EPA Established to Design Biochars to Resolve Specific Deficiencies in Sandy Coastal Plain Soils.
In 2010, scientists at the CPSWPRC submitted a Regional Applied Research Effort (RARE) proposal to the EPA Region 4 office (Atlanta). The proposal was selected for funding, thereafter an IA was constructed. The IA had goals to conduct collaborative research to improve fertility and water storage in agricultural soils of the Coastal Plain region. Biochars from locally available crop residue and animal manure feedstocks and their blends were initially pelletized. The pellets were then pyrolyzed at specific temperatures (350 to 700°C). Initial chemical and physical characterization showed that the biochar pellets were not similar in properties, in fact, the biochars possessed unique properties such as high aromatic character, alkaline pH levels, abundant plant nutrients and pore spaces (Novak et al., 2013). Capitalizing on the dissimilarity in properties, biochars were then selected to target improvement in specific soil deficiencies. In

turn, laboratory and greenhouse experiments showed that a certain biochars made from shell nuts specifically improved soil fertility levels (Novak et al., 2009), biochar with high aromaticity were more suitable for long-term soil OC sequestration (Sigua et al., 2014), biochar made from switchgrass increased plant available soil water (Novak et al., 2012) and pelletized biochars made from a 80:20 blend of chicken litter/pine chips rebalanced soil P concentrations (Novak et al., 2014). These findings are a huge advancement because more effective use of biochars can be obtained by having producers, customers, and fertility experts use guidelines to match the right biochar to the right soil. The EPA was pleased with the results and the high degree of cooperative research between the two federal agencies. In 2014, administrators from the EPA encouraged submission of another RARE proposal from ARS scientists to design biochars to capture P or reduce soluble P concentrations along waste streams of USA dairies operations.

Development of Global Guidelines for Biochar Usage as a Soil Amendment.
Biochar has gained global attention as a soil amendment to rebuild soil health by improving organic carbon (OC) contents, increasing nutrient retention, and decreasing greenhouse production, however, universal guidelines for its use as a soil amendment and climate mitigation tool are lacking. Current biochar usage policies have so far been based on fragmented management platforms relevant to specific countries, soil types, and climatic patterns. Due to the immense complexity in soils and biochar types, our ability to predict these beneficial effects in different soils and land uses across the globe is currently limited. Therefore, ARS scientists at the Florence, Kimberly and St. Paul location teamed up with scientists in Germany and Spain to construct the Designchar4Food (D4F) research proposal. In October 2013, the proposal was submitted to a competitive grant review process through the FACCE-JPI organization. In November 2013, we were informed that the D4F proposal ranked 3 out of 23 submitted proposal, so it was selected for funding. We are currently finishing paperwork with the USDA-NIFA organization (source of funds) to have the grant award made and establish operational accounts. After funds are secured, we will have a kickoff meeting during the 2014 ASA meeting. The benefit of creating a worldwide scientific biochar exchange network through the D4F project, will allow the development of suitable biochar management strategies at regional levels to be evaluated and integrated into a coherent global policy platform that sustains agricultural productivity and food security.

Funded Bioenergy and Biochar Grants:
To supplement our NP212 CRIS project, we have obtained additional funding from the following sources:

1. 2008-2013, USDOE Sun Grant Initiative through South Dakota State University to the USDA-ARS entitled "Regional Corn Stover Removal Impact Study. Award to J.M. Novak: $220,000.
2. 2010-2016, USEPA-RARE Grants Program for Region 4. Competitive research grant entitled "Using biochar to improve the fertility of Coastal Plain Soils". Award: $200,000. Award to J.M. Novak: $160,000.
3. 2013-2016, Joint Programming Initiative on Agricultural, Food Security, and Climate Change (FACCE-JPI), Competitive grant for Multi-partner Call on Agricultural Greenhouse Gas Research with funding support from the USDA-National Institute of Food and Agriculture. Award:$110,000. Award to J.M. Novak $100,000.

Selected Publications: The following publications involve authorship from scientists at the Florence locations (shown as bold):

1. **Cantrell, K**., **Novak, J.M**., Frederick, J., Karlen, D., and **Watts, D.W**. Influence of corn residue harvest on grain, stover, and energy yields. Bioenergy Research. Doi 10.19007/s12155-014-9433-9. 2014.

2. Johnson, J.M.F., **Novak, J.M**., Varvel, G.E., Stott, D.E., Osborne, S.L., Karlen, D.L., Lamb, J.A., Baker, J.M., and Adler, P.R. (2014). Crop residue mass needed to maintain soil organic carbon levels: Can it be determined? Bioenergy Research, Doi 10.1007/s12155-073-9402-8.

3. Karlen, D.L., Birrell, S.J., Johnson, J.M.F., Osborne, S.L., Schumacher, T.E., Varvel, G.E., Ferguson, R.B., **Novak, J.M**., Frederick, J.R., Baker, J.M., Lamb, J.A., Adler, P.R., Roth, G.W., and Nofziger, E.D. (2014). Multi-location corn stover harvest effects on crop yields and nutrient removal. Bioenergy Research, Doi.10.1077/s12155-014-9419-7.

4. Lehmann, R.M., **Ducey, T.F**., Jin, V.L., Acosta-Martinez, V., Ahlschwede, C.M., Jeske, E.S., Drijber, R.A., **Cantrell, K.B**., Frederick, J.R., Fink, D.M., Osborne, S., **Novak, J.M**., Johnson, J.M.F., and Varvel, G.E. (2014). Soil microbial community responses to corn stover harvesting under rain-fed, no-till conditions at multiple US locations. Bioenergy Research. Doi. 10.1007/s12155-014-9417-9.

5. Mourtzinis, S., **Cantrell, K.B**., Arriaga, F.J., Balkcom, K.S., **Novak, J.M**., Frederick, J.R., and Karlen, D.L. (2014). Distribution of structural carbohydrates in corn plants across the Southeastern USA. Bioenergy Res. Doi. 10.1007/S12155-014-9429-5.

6. **Novak, J.M., Busscher**, W.J., Laird, D.L., Ahmedna, M., **Watts, D.W**., & Niandou, M.A.S. (2009). Impact of biochar amendment on fertility of a southeastern coastal plain soil. Soil Science, 174:105-112.

7. **Novak, J.M., Busscher**, W.J., Ippolito, J.A, Lima, I.M., Gaskins J., Das, K.C., Steiner, C., Ahmedna, M., Rehrah, D., Amonette, J., Bae, S., Schomberg, H. and **Watts, D.W**. (2012). Biochars impact on soil moisture storage in an Ultisol and Aridisol. Soil Science, 177:310-320.

8. **Novak, J.M., Cantrell, K.B**., & **Watts, D.W**. (2013). Compositional and thermal evaluation of lignocellulosic and poultry litter chars via high and low temperature pyrolysis. Bioenergy Research, 6:114-130.

9. **Sigua, G.G**., **Novak, J.M**., **Watts, D.W., Cantrell, K.B., Shumaker, P.D., Szogi, A.A.,** and Johnson, M.G. (2014). Carbon mineralization in two Ultisols amended with different sources and forms of pyrolyzed biochar. Chemosphere, 103:313-321.

10. **Novak, J.M**., **Cantrell, K.B., Watts, D.W.,** and Johnson, M.G. (2014). Designing relevant biochars as soil amendments using lignocellulosic and manure-based feedstocks. Journal of Soil and Sediments, 14:330-343.

Auburn, AL: National Soil Dynamics Laboratory (NSDL) (NP 216)

Accomplishments: The accomplishments listed below summarize bioenergy research conducted at the NSDL, specifically in the conservation systems unit. Our primary focus has been to examine crop productions systems that include annual crops (corn and forage sorghums that include photo-period sensitive and sweet sorghums) designed to maximize biomass production with conservation tillage systems that include winter cover crops. In some cases, we have examined the possibility of also harvesting winter cover crop biomass as a potential feedstock, while determining the impact on soil productivity. Our accomplishments are summarized below.

Carbohydrate Distribution in Corn Plants Across Southeastern USA.
Corn stover, the above-ground stalk, leaves, tassels, and cobs remaining after grain harvest have been identified as an important potential feedstock for second generation biofuel production. Therefore, it is important to know more about the anticipated ash, lignin and structural carbohydrate (cellulose and hemicelluloses) composition of these materials and how they are affected from year to year by management practices such as growing a cover crop and different rates of stover harvest. We found significant variations in the distribution of all three plant components. But neither the use of a rye cover crop nor the stover harvest rates affected carbohydrate concentrations within the various plant fractions or across soil types. Total precipitation and average air temperature during the growing season were strongly correlated with stover composition indicating the effect of weather conditions on biofuel production. When compared to the above-ear fractions, bottom plant partitions contained greater lignin and cellulose concentrations. Cellulose and hemicellulose concentrations were consistently greater in cobs, tops and above-ear fractions at every location. This study suggests that the cob, top and above-ear plant portions have the most desirable characteristics for bioethanol production via fermentation and will be useful to developing those industries in the southeastern U.S.

A Simplified Method for Carbohydrate Analysis of Corn Stover.
Constituent determination of biomass for theoretical ethanol yield (TEY) estimation requires the removal of non-structural carbohydrates, prior to analysis, to prevent interference with the analytical procedure. The U.S. Department of Energy-National Renewable Energy Laboratory (NREL) recommends a two-stage process for analysis of cellulosic biomass composition. However, the first stage of this process is time consuming and costly. An older procedure, which was developed for quantifying cell wall constituents for ruminant nutrition research, uses a neutral detergent fiber (NDF) to quantify the insoluble components of biomass by quickly extracting the soluble fraction. The older procedure should be quicker and cheaper, but the reliability of this method for predicting TEY has not been tested. Average TEY per unit area between methods varied only by 1.9% suggesting that the older procedure could be used as an alternative to the current two-stage process used to estimate TEY. This can be of value when time and cost are a constraint. Identifying an alternative method that is faster and just as accurate as the current method will be more cost effective for research and industry personnel estimating future ethanol yields.

Corn Grain and Stover Yield Prediction.

Corn grain and stover yield estimation early in the growing season is an appealing idea. An accurate estimation of the yield of the final product could benefit farmers, as well as corn related industries in order to balance supply and demand logistics to keep various processing plants operating efficiently. The goal was to develop prediction models that could estimate corn grain and stover yield at harvest using simple physiological measurements early in the growing season. The regression was significant at early vegetative growth stages with the amount of explainable variability maximized at R1 stage. For the grain yield model, maximum R^2 was 0.7967 and for the stover model maximum R^2 reached 0.8612. Results from this study suggest that total precipitation from planting until R1 growth stage, and simple physiological measurements can be used to predict corn grain and stover yield. This type of information could be used by the manager of any biofuel facility requiring corn. This manager could estimate grain or stover yields across acreages within a certain radius of their plant to ensure transportation costs are minimized, while maintaining an adequate supply of feedstock.

Biomass Sorghum Production Under Different Irrigation/Tillage Systems.

Successful production of large biomass crops in the southeastern US will be required to produce large amounts of biofuels. Ideally, these production methods will minimize the need for excessive tillage or irrigation. Annual crops, such as biomass sorghum, could fit within existing annual crop production systems without excessively disrupting current markets. A photoperiod-sensitive sorghum variety can maximize biomass production over the entire growing season, but if a shorter season was desired, forage sorghum could be used and harvested in a much quicker time frame. Conservation systems that minimize tillage can be productive and promote more biomass production during drier growing seasons, while irrigation can also enhance biomass production during adverse growing conditions. These results should enable producers and biorefineries to make decisions about production practices, as well as, potential yields of biomass and biofuels using these crops.

Harvest of Winter Cover Crops for Bioenergy.

There is potential in the southeast US to harvest winter cover crops from cotton fields for biofuels or animal feed use, but this practice could threaten agricultural productivity of systems that rely on the beneficial effects of cover crops. In general, cotton productivity and growth parameters were enhanced when cover crop residue was retained in the field. These effects were more pronounced when hot, dry growing conditions persisted during the summer months. However, it should be noted that including a cover crop, despite being harvested for bioenergy production, was more beneficial than not using a cover crop in the first place. These findings support evidence for cover crop adoption across the Southeast. However, growers that choose to harvest their cover crop residue as an additional source of income reduce the benefits associated with cover crops. This residue could serve as a bioenergy feedstock when traditional supplies would be limited, despite storage. These findings reflect short-term changes and require verification across a longer time frame to ensure sustainability for modelers and policymakers.

Selected Publications:

1. Ducamp, F., F.J. Arriaga, K.S. Balkcom, S.A. Prior, E. van Santen, and C.C. Mitchell. 2012. Cover crop biomass harvest influences cotton nitrogen utilization and productivity. Int. J. Agron. 2012:12.

2. Mourtzinis, S., F.J. Arriaga, K.S. Balkcom, and B.V. Ortiz. 2013. Corn grain and stover yield prediction at R1 growth stage. Agron. J. 105:1045-1050.

3. Mourtzinis, S., F.J. Arriaga, D. Bransby, and K.S. Balkcom. 2014. A simplified method for monomeric carbohydrate analysis of corn stover biomass. GCB Bioenergy 6:300-304.

4. Mourtzinis, S., K.B. Cantrell, F.J. Arriaga, K.S. Balkcom, J.M. Novak, J.R. Frederick, and D.L. Karlen. 2014. Distribution of structural carbohydrates in corn plants across the southeastern USA. Bioenerg. Res. 7:551-558.

5. Rocateli, A.C., R.L. Raper, K.S. Balkcom, F.J. Arriaga, and D.I. Bransby. 2012. Biomass sorghum production and components under different irrigation/tillage systems for the southeastern U.S. Ind. Crops Prod. 36:589-598.

Other Publications:

1. Arriaga, F.J., K.S. Balkcom, and L.M. Duzy. 2011. Cover crop biomass harvest for bioenergy: Implications for crop productivity. Sustainable Feedstocks for Advanced Biofuels. Available at: http://www.swcs.org/documents/filelibrary/roadmap/SFAB_Program_and_Abstract_Book__FIN_8607CF9B02A16.pdf (verified 1 May, 2014).

2. Arriaga, F.J., K.S. Balkcom, L.M. Duzy, and V. Acosta-Martinez. 2010. Cotton yield and soil properties are affected by the harvest of a winter cover crop for bioenergy production. In Annual Meetings Abstracts [CD-ROM]. ASA, CSSA, SSSA, Madison, WI.

3. Arriaga, Francisco J., Fernando Ducamp, Kipling S. Balkcom, and Charles C. Mitchell. 2009. Short-term impact of winter cover crop biomass removal on soil physical properties. In Annual Meetings Abstracts [CD-ROM]. ASA, CSSA, SSSA, Madison, WI.

4. Arriaga, Francisco J., Randy L. Raper, and Kipling S. Balkcom. 2009. Biomass production for bioenergy: A Southeastern perspective. ASABE Annual International Meeting, Reno, Nevada, June 21 – 24, 2009.

5. Balkcom, Kipling S. and Francisco J. Arriaga. 2011. Inorganic and organic nitrogen sources for optimal rye cover crop biomass production. Sustainable Feedstocks for Advanced Biofuels Workshop. Available at: http://www.swcs.org/documents/filelibrary/roadmap/SFAB_Program_and_Abstract_Book__FIN_8607CF9B02A16.pdf (verified 1 May, 2014).

6. Balkcom, Kipling S., Francisco J. Arriaga, Charles C. Mitchell, Dennis P. Delaney, and Jason S. Bergtold. 2009. Nitrogen applications to increase cover crop biomass and benefits in the Southeast. In Annual Meetings Abstracts [CD-ROM]. ASA, CSSA, SSSA, Madison, WI.

7. Balkcom, Kipling S., Francisco J. Arriaga, and Leah M. Duzy. 2012. High biomass sorghum production across tillage systems and nitrogen rates. In Annual Meetings Abstracts [CD-ROM]. ASA, CSSA, SSSA, Madison, WI

8. Balkcom, K.S., F.J. Arriaga, and L.M. Duzy. 2013. Nitrogen rates for biomass sorghum production across tillage systems. In K.V. Iversen (ed.) Proc. 33rd Southern Conservation Agricultural Systems Conf., Norman, OK. February 19-20, 2013. Available at: http://www.ag.auburn.edu/auxiliary/nsdl/scasc/.

9. Duzy, Leah M., Francisco J. Arriaga, and Kipling S. Balkcom. 2010. Rye cover crop as a source of biomass feedstock: An economic perspective. In D.M. Endale and K.V. Iversen (eds.) Proc. 32nd So. Conserv. Agric. Syst. Conf., Jackson, TN. July 20–22, 2010. Available at http://www.ag.auburn.edu/auxiliary/nsdl/scasc.

10. Mourtzinis, S., F.J. Arriaga, K.S. Balkcom, D. Bransby, and B. Ortiz. 2010. Corn stover for bioenergy: Effect of N fertilization, winter cover crop and stover harvest on vertical biomass distribution and composition. In Annual Meetings Abstracts [CD-ROM]. ASA, CSSA, SSSA, Madison, WI.

11. Mourtzinis, Spyridon, Francisco Arriaga, Kipling Balkcom, Stephen Prior, and David Bransby. 2012. Soil carbon and nitrogen dynamics after corn stover harvest. In Annual Meetings Abstracts [CD-ROM]. ASA, CSSA, SSSA, Madison, WI.

12. Mourtzinis, Spyridon, Keri B. Cantrell, Francisco J. Arriaga, Kipling S. Balkcom, Jeffrey M. Novak, James R. Frederick, and Douglas L. Karlen. 2013. Corn stover biofuel potential and nutrient removal across the southeastern US. In Annual Meetings Abstracts [CD-ROM]. ASA, CSSA, SSSA, Madison, WI.

13. Rocateli, Alexandre, Randy Raper, Francisco Arriaga, Kipling Balkcom, and David Bransby. 2010. Producing sorghum biomass under different irrigation tillage systems for cellulosic bioenergy production in southeastern U.S. In Annual Meetings Abstracts [CD-ROM]. ASA, CSSA, SSSA, Madison, WI.

14. Rocateli, Alexandre, Randy Raper, Kipling Balkcom, Francisco Arriaga, and David Bransby. 2010. Effect of sorghum biofuel production systems on soil characteristics in southeastern U.S. In Annual Meetings Abstracts [CD-ROM]. ASA, CSSA, SSSA, Madison, WI.

15. Rocateli, Alexadre C., Randy L. Raper, Francisco, J. Arriaga, Kipling S. Balkcom, and David Bransby. 2009. A new spin on an old crop for bioenergy: Sorghum. In Annual Meetings Abstracts [CD-ROM]. ASA, CSSA, SSSA, Madison, WI.

16. Rocatelli A., R.L. Raper, Francisco J. Arriaga, Kip S. Balkcom, and David Bransby. 2009. Effect of conservation systems and irrigation on potential bioenergy crops. In M.S. Endale (ed.) Proc. 31th So. Conserv. Agric. Syst. Conf., Painter, Virginia, July 20-23, 2009. Available at: http://www.ag.auburn.edu/auxiliary/nsdl/scasc/

17. Rocateli, A.C., R.L. Raper, F.J. Arriaga, K.S. Balkcom, and D. Bransby. 2010. A new spin on an old crop for bioenergy: Sorghum. In Southern Branch American Society of Agronomy Annual Meetings Abstracts [CD-ROM]. ASA, Madison, WI.

18. Rocateli, A.C., R.L. Raper, F.J. Arriaga, K.S. Balkcom, D.I. Bransby, and B.V. Ortiz. 2011. Producing sorghum cellulosic feedstock for advanced biofuels production and its impact

on soil physical properties [abstract]. Sustainable Feedstocks for Advanced Biofuels. Available at: http://www.swcs.org/documents/filelibrary/roadmap/SFAB_Program_and_Abstract_Book__FIN_8607CF9B02A16.pdf (verified 2 May, 2014).

<u>Houma, LA</u>: Sugarcane Research Unit (NP 211, 212, 213, 301)

Notice of Release of a High Fiber Sugarcane Variety Ho 02-113

The United States Department of Agriculture's Agricultural Research Service, Sugarcane Research Unit (SRU); the Louisiana Agricultural Experiment Station of the LSU Agricultural Center; and the American Sugar Cane League of the U.S.A, Inc., released a new high-fiber variety, Ho 02-113 to meet the needs of a cellulosic processing biorefinery. Ho 02-113 is derived from a cross made in 2001 between SES-234 (female Saccharum spontaneum parent) and LCP 85-384 (male parent). Testing has indicated that the variety is vigorous with high stalk population and good ratooning ability and because of its fiber content would be a better candidate variety for the production of bioenergy than commercial sugar cane varieties. The variety was evaluated in replicated tests at the Diamond W Ranch near Welsh, LA and the SRU's Spanish Trail Farm near Schriever, LA in 2005. Plant-cane, first-, second-, and third-ratoon crops were harvested and compared to L 79-1002 for each location in 2006 through 2009, respectively. Yield components estimated included gross cane (tons cane/acre), Brix (%), Fiber (%), and the total solids per acre. The tons of solids per acre were broken down into soluble solids (Brix), insoluble solids (fiber), and total solids (Brix + Fiber). When data from the two locations were combined, Ho 02-113 performed as well as L 79-1002 (a=0.05) for all calculated yield parameters, but when the data were analyzed by location, Ho 02-113 was significantly higher yielding than L79-1002. Throughout the five years of testing, no signs of brown rust, smut, leaf scald, or mosaic were observed in this variety under natural field conditions. Field studies do not indicate that Ho 02-113 is susceptible to the sugarcane borer. The variety Ho 02-113 offers growers an alternative to other commercial energy cane varieties, such as L79-1002, as a dedicated feedstock for the production of biofuels.

Breeding Sugarcane for Cold Climates

In recent studies, we have used a growth chamber to expose diverse wild varieties of sugarcane to freezing temperatures. Two wild sugarcane varieties showed significantly higher survival of above ground buds than the commercial varieties (L 97-128 and Ho 95-988), and four were identified with more emerged shoots following a six-day freeze of the below-ground stubble. A test planted in Booneville, Arkansas was allowed to overwinter in freezing conditions. Thirty varieties have been identified that were able to withstand an Arkansas winter and are being returned to Louisiana for evaluation in the sugarcane industry and for use as parental cold-tolerant material. In an additional study, varieties were selected in 2002 that remained green following a freeze at the SRU's research farm. These varieties have been planted in a regional test spanning 8 southern states, some in areas where low temperatures range from 0-10 degrees C. In northerly regions, this cold tolerant material appears to be surviving well, and has produced ratoon crops, indicating a high survival rate of underground buds in frozen soil. Results indicate that these varieties also have significantly less juice degradation than currently available commercial varieties. Identified cold-tolerant individuals will be used in future breeding efforts to enhance cold tolerance in sugarcane. Efforts for breeding cold tolerant sugarcane varieties are expected to contribute to future commercial "Ho" varieties able to withstand freezes to extend both the growing and harvest seasons.

Sugar and Energy Cane Date of Planting Effects on Cane, Sucrose, and Fiber Yields

Production practices may change depending if sugar cane is grown primarily for sucrose (sugar cane) or as a biofuels feedstock (energy cane). Research was conducted to determine if planting date affects yields of both sugar and energy canes. Three sugar cane varieties and one energy cane variety were compared at the planting dates of August 1, September 1, and October 1. Averaged across both sugar and energy cane varieties in plant-cane, the August planting date produced more cane, sucrose, and dry biomass than the September and October plantings. The September planting had higher cane and sucrose yields than the October planting, but there were no differences between the dry biomass yields for these planting dates. Growers should attempt to plant both sugar and energy cane in August to maximize yields. However, if plantings are delayed into September for both sugar and energy cane, it is best to plant sugar cane first, instead of energy cane, during this time period because sucrose yield continued to decline with an October planting while dry biomass yields were consistent with September and October plantings.

Energycane Harvest Date Effects on Biomass, Sugar, and Fiber Yields

Reliable and high yielding biomass feedstocks are a prerequisite to a viable bioenergy industry. Energycane, a sugarcane variety grown solely as a biomass feedstock, is an excellent candidate for the Southeastern U.S. The most recent commercially released energycane variety is ‘Ho 02-113.’ Our objective was to determine the yield and biomass characteristics of Ho 02-113 harvested across an 8 month time span from August through March 2012-2013. Stalks of Ho 02-113 were hand cut each month from two tests near Houma, LA, and analyzed for biomass, Brix (total soluble solids in juice, w/w), theoretically recoverable sucrose (TRS), and fiber content. Yield was estimated using a stalk population of 252,000 /ha. Biomass peaked at 155 Mg/ha in December 2012, and was lowest in August (118 Mg/ha). The average across the 8 month harvest period was 132 Mg/ha. Brix was lowest in August (70 g/kg) and increased each month until it reached a high of about 110 g/kg in November that lasted through March. TRS also was lowest in August (22 g sucrose /kg cane), indicative of immature stalks. TRS increased each month and peaked in February at 49 g/kg. However, TRS for Ho 02-113 was low when compared to the 2012 Louisiana commercial TRS average of 114 g/kg. Fiber content peaked in December-January at 360 g/kg which equaled about 56 Mg dry biomass /ha. Calorimeter tests revealed the energy content of the fiber to be 16.7 Gj/Mg. Energycane yield, Brix, TRS, and fiber content varied throughout the harvest season. The biomass processing strategy will determine the optimal harvest time for energycane; early fall (lower biomass, lower Brix values), late fall (high biomass, higher Brix), winter (highest biomass, highest fiber content), or early spring (high biomass, higher Brix).

Sweet Sorghum Production on Fallow Sugarcane Fields in Louisiana

Sweet sorghum has been grown as a minor crop for syrup production for generations. Its potential as a biofuel feedstock, both through sugar and fiber production, has created interest in utilizing sweet sorghum as a crop that could be grown during the fallow year in the sugarcane cropping cycle in south Louisiana. Sweet sorghum could be planted on the raised beds used for growing sugarcane in Louisiana. It can be harvested with sugarcane equipment without delays for allowing the sorghum seed to mature. The obstacles to growing sweet sorghum in fallowed sugarcane fields are first, weed control would need to be adequate so not to impact future cane crops, second, little is known about the impact of sweet sorghum on the establishment of subsequent sugarcane crops, and third, managements practices, such as crop fertility and seeding rate need to be explored in order to maximize production of both sugars and fiber. Experiments have been and are being conducted to evaluate weed control options for sweet sorghum, to evaluate sugarcane establishment following planting, and to evaluate nitrogen fertilizer rates and sweet sorghum populations. Controlling weeds during sweet sorghum establishment is the most critical time period; afterwards sweet sorghum becomes competitive with developing weeds. Increasing nitrogen rates increases biomass production, but too high of fertility can lower Brix. Higher plant populations may also increase total biomass and Brix production.

Selected Publications:

1. Hale, A.L. 2010. Notice of release of a high fiber sugarcane variety Ho 02-113. Sugar Bulletin. 88(10):28-29.

2. Viator, R.P., White Jr., P.M., Richard Jr., E.P. 2011. Sustainable production of energycane for bio-energy in the Southeastern U.S. American Chemical Society Book Series, Sustainability of Sugarcane for Sugar and Bioenergy. 1058:147-161.

3. Hale, A.L. 2011. Breeding sugarcane for cold climates. Sugar Journal. January 2011:16-21.

4. Viator, R.P., Richard Jr, E.P. 2012. Sugar and energy cane date of planting effects on cane, sucrose, and fiber yields. Biomass and Bioenergy. 40:82-85.

5. Fageria, N.K., Moreira, L.A., Hale, A.L., Viator, R.P. 2013. Sugarcane and energycane. In: Singh, B. (ed.) Biofuel Crops: Production, Physiology and Genetics. CABI, Walingford UK. p. 151 - 171.

Other Publications:

1. Richard Jr, E.P. 2010. USDA-ARS Efforts in Expanding the Region for Growing Sugar Cane and Complimentary Sugar Crops for Bioenergy. Sugar Journal. 72(10):10-11.

2. Gravois, K., Grisham, M.P., Viator, R.P. 2013. Exploiting sugarcane for energy. Sugar Journal. 75(8):8-12.

3. White Jr, P.M., Viator, R.P., Richard, E.P., Grisham, M.P. 2012. Green-cane harvest of sugarcane effects on biomass and energy yields and nutrient removal. In: Proceedings of Sun Grant National Conference: Science for Biomass Feedstock Production and Utilization, October 3 - 5, 2012. New Orleans, LA. Available: http://sungrant.tennessee.edu/NatConference/

4. Grisham, M.P., Hale, A.L., Johnson, R.M. 2012. Disease concerns in energycane. Proceedings of Sun Grant National Conference: Science for Biomass Feedstock Production and Utilization, October 3 - 5, 2012. New Orleans, LA. Available: http://sungrant.tennessee.edu/NatConference/

5. Dalley, C.D. 2013. Sweet sorghum production on fallow sugarcane fields in Louisiana. Proceeding 245th American Chemical Society National Meeting and Exposition, April 7 - 11, 2013, New Orleans, LA.

New Orleans, LA: Southern Research Center: Commodity Utilization Research (NP 213, 306)

Accomplishments: The accomplishments of the Southern Research Center toward bioenergy feedstocks are centered around the properties and use of bio-char as well as the evaluation of sugar crops for conversion to bio-based fuels and chemicals. These aspects are described in detail in the accomplishments listed below.

Physicochemical and Adsorptive Properties of Fast-Pyrolysis Bio-Chars and Their Steam Activated Counterparts

Fast pyrolysis is rapid heating in the absence of oxygen resulting in decomposition of organic material. When applied to biomass, it produces bio-oil, bio-char and gas. The Agricultural Research Service (ARS) of the USDA has studied fluidized-bed fast pyrolysis of several biomass sources including perennial energy crops and plant and animal wastes for bio-energy production. Owing to the short residence time in the fluidized-bed pyrolyzer, required for maximizing bio-oil production, the bio-char structure tend to be underdeveloped thereby impacting its full potential application as a value-added co-product. In this study, we investigate ex-situ steam activation as one upgrading technology for adding value to the said fast-pyrolysis bio-chars. The as-produced bio-chars of several substrates from the fluidized-bed pyrolyzer and their respective steam-activated counterparts were characterized for their surface areas and adsorption of a suite of metal ions (copper, cadmium, nickel and zinc). Surface areas increased with activation from around zero up to 837 m2/g with concomitant pore development evidenced by scanning electron microscopy. Affinity to Cu^{2+} was highest with adsorption efficiencies for 1mM solutions ranging from 60 to 85%. Metal ion adsorption was feedstock dependent and increased with activation possibly due to improved access to highly reactive adsorption sites associated with the inorganic material present in the feedstock. Because of their higher yields and metal ion uptake, broiler litter and alfalfa stems appear to be the feedstock of choice when considering fast pyrolysis bio-char for metal ion uptake. However, if the development of large surface areas is required, guayule bagasse and soybean straw could be the preferred feedstock. Ultimately the fine balance relies on the ability to produce a good quality bio-oil as fuel and a usable bio-char and/or activated char from low value agriculture waste materials.

Contaminant Immobilization and Nutrient Release by Biochar Soil Amendment: Roles of Natural Organic Matter

Contamination of soil interstitial waters by labile heavy metals such as CuII, CdII, and NiII is of worldwide concern. Carbonaceous materials such as char and activated carbon have received considerable attention in recent years as soil amendment for both sequestering heavy metal contaminants and releasing essential nutrients like sulfur. Information is currently lacking on how aging impacts the integrity of biochars as soil amendment for both agricultural and environmental remediation purposes. Major contributors to biochar aging in soils are: sorption of environmental constituents, especially natural organic matter (NOM), and oxidation. To investigate the impact of NOM and organic fractions of chars, we employed broiler litter-derived chars and steam activated carbons that underwent varying degrees of carbonization, in the presence and absence of NOM having known carboxyl contents. For aging by oxidation, we employed phosphoric acid activated carbons that underwent varying degrees of oxidation during activation. Our results suggest that the organic fractions of biochars, and NOM having high carboxyl contents can mobilize CuII retained by alkaline soil. Base treatment of broiler litter-derived char formed at low pyrolysis temperature (350^{o}C) improved the immobilization of all heavy metals investigated, and the extent of immobilization was similar to, or slightly greater than pecan shell-derived phosphoric acid activated carbons. Portions of total sulfur were released in soluble form in soil amended with broiler litter-derived carbons, but not pecan shell-derived phosphoric acid activated carbons.

Immobilization of Heavy Metal Ions (CuII, CdII, NiII, and PbII) by Broiler Litter-Derived Biochars in Water and Soil
Chars, a form of environmental black carbon resulting from incomplete burning of biomass, can immobilize organic contaminants by both surface adsorption and partitioning mechanisms. The predominance of each sorption mechanism depends upon the proportion of organic to carbonized fractions comprising the sorbent. Information is currently lacking in the effectiveness of char amendment for heavy metal immobilization in contaminated (e.g., urban and arms range) soils where several metal contaminants coexist. The present study employed sorbents of a common biomass origin (broiler litter manure) that underwent varying degrees of carbonization (chars formed by pyrolysis at 350 and 700 degrees C and steam-activated analogues) for heavy metal (CdII, CuII, NiII, PbII) immobilization in water and soil. ATR-FTIR, 1H NMR, and Boehm titration results suggested that higher pyrolysis temperature and activation lead to the disappearance (e.g., aliphatic –CH2 and –CH3) and the formation (e.g., C-O) of certain surface functional groups, portions of which are leachable. Both in water and soil, pH increase by the addition of basic char enhanced the immobilization of heavy metals. Heavy metal immobilization resulted in nonstoichiometric release of protons, i.e., several orders of magnitude greater total metal concentration immobilized than the protons released. Our results suggest that with higher carbonized fractions and loading of chars, heavy metal immobilization by cation exchange becomes increasingly outweighed by other controlling factors such as the coordination by pie electrons (C=C) of carbon and precipitation.

Post-Harvest Changes in Sweet Sorghum I: Brix and Sugars
Sweet sorghum is a crop that stores a lot of sugar in the stalk. That sugar can be used to make ethanol for fuel and other products. We grew three varieties of sweet sorghum in Louisiana, and harvested it at three times: 90 days after planting (DAP), 115 DAP, and 140 DAP. We then removed the leaves and seed head, and divided the harvested stalks into four treatments: stalks left whole, stalks cut into 20-cm or 40-cm pieces called billets, or chopped into small pieces. The sorghum was then stored for up to 4 days before removing the juice. We measured juice Brix, which measures the total amount of dissolved solids in the juice, and the sugars glucose, fructose and sucrose. We showed that the sugars decreased rapidly after harvest in the chopped sorghum. We observed significant differences after only one day of storage. In the other treatments, sugar did not decrease rapidly. We also showed that juice Brix was not a good way to monitor sugar deterioration in sweet sorghum, because it didn’t always decrease with the sugar. We concluded that harvesting methods that cut either whole stalks or billets would be acceptable for the production of ethanol from sweet sorghum.

Stereoregularity of Poly (Lactic Acid) and Their Model Compounds as Studied by NMR and Quantum Chemical Calculations

Poly(lactic acid) (PLA) is a commercial polymer derived from agricultural resources, such as sugarcane, corn starch, and tapioca products. It is biodegradable and finds uses in diverse areas, ranging from biomedical (sutures, stents, dialysis media) to bioplastics (agricultural mulch films, compost bags, and food packaging). The properties of PLA depend on its stereoregularity. Thus, poly-L-lactide has a melting temperature of about 175°C, but a blend of poly-L-lactide and poly-D-lactide has a melting point that is 40-50° higher. The stereoregularity of PLA is usually achieved with NMR spectroscopy; whereas the NMR spectra of PLA have been mostly assigned, the origin of the different peaks due to stereochemical sequences ("tacticity splitting") is not previously known. It is important to gain a better understanding of the structure/chemical shift correlation in order to maximize the information available from the NMR data. In this work monomer and dimer model compounds were synthesized and their 1H and 13C chemical shifts observed experimentally and also calculated via a quantum chemical method. The good agreement between the observed and the calculated results suggest that the origin of tacticity splitting in PLA dimer is due to both time-averaged conformations and the chemical shifts of the conformations. An attempt has also been made to rationalize the tacticity splitting of PLA polymer at the diad level on the basis of these chemical shift calculations.

Feasibility of Removing Furfurals from Sugar Solutions Using Activated Biochars Made from Agricultural Residues

Lignocellulosic feedstocks are often prepared for ethanol fermentation by treatment with a dilute mineral acid catalyst that hydrolyzes the hemicellulose and possibly cellulose into soluble carbohydrates. The acid catalyzed reaction scheme is sequential whereby released monosaccharides are further degraded to furans and other chemicals that are inhibitory to the subsequent fermentation step. This work summarizes the use of agricultural residues (e.g., plant waste) as starting materials for making activated biochars to adsorb these degradation products. Results show that both furfural and hydroxymethylfurfural (HMF) are adsorbed by phosphoric acid-activated and steam-activated biochars prepared from residues collected from cotton and linen production. Best results were obtained with steam activated biochars. The activated biochars adsorbed about 14% (by weight) of the furfurals at an equilibrium concentration of 0.5 g/L, and by adding 2.5% of char to a sugar solution, with either furfural or HMF (at 1 g/L), 99% of the furans were removed.

Development of a Sweet Sorghum Juice Clarification Method in the Manufacture of Industrial Feedstocks for Value-Added Products

In recent years, there has been a dramatic increase in interest of sweet sorghum (Sorghum bicolor L. Moench) for small, medium, and large-scale manufacture of renewable, biobased fuels and chemicals. New fermentation organisms hold tremendous potential for the production of biobased fuels, chemicals, and materials from industrial sugar feedstocks, in particular syrups. Clarification of sweet sorghum juice will be critical to the production of stable, intermediate syrup feedstocks for efficient transport, storage, and year-round supply. Juices extracted from physiologically mature sweet sorghum hybrids as well as immature cultivar Topper 76-6 (Topper), were clarified using heat, heat-milk of lime, and heat-milk of lime-polyanionic flocculant at various temperatures and target limed pHs, and compared to the clarification of sugarcane (Saccharum spp. hybrids) juice. There was no significant loss of fermentable sugars (sucrose+glucose+fructose) across clarification for any of the clarification methods examined. Preheating the sweet sorghum juice from 85 to 100 °C not only produced clarified juices of low turbidity, but also with excellent turbidity control. For the cultivars studied, a minimum limed juice pH of ~6.3 was optimum for the clarification of sweet sorghum juice preheated to ~85 °C with 5 ppm polyanionic flocculant addition with respect to clarified juice turbidity, protein, calcium, starch, and to a lesser extent phosphate levels. Clarified juice non-sugar values are also discussed along with possible effects on later processing into value-added products. There was a strong effect of cultivar on juice quality, clarification performance, and clarified juice quality, which warrants further research.

Post-Harvest Changes in Sweet Sorghum II: pH, Acidity, Protein, Starch, and Mannitol
This experiment was conducted to evaluate the effect of four harvesting methods on juice quality and storability in sweet sorghum. Three cultivars (Dale, Theis, and M81-E) were harvested at 90, 115, and 140 days after planting. Stalks were stripped of leaves and topped at the peduncle, then divided into four treatments (whole-stalk, 20-cm or 40-cm billets, or chopped). The sorghum was stored outside at ambient temperature, and juice was extracted from samples removed at 0, 1, 2, and 4 days after harvest. Changes in juice Brix and sugars were reported in an earlier paper (Lingle, S., Tew, T., Rukavina, H., Boykin, D. 2012. Post-harvest changes in sweet sorghum I: Brix and sugars. BioEnergy Research 5:158-167). In this paper we report changes in juice pH, titratable acidity (TA), protein, starch, and mannitol concentration. Juice pH dropped rapidly after harvest in chopped sorghum, but changed little during 4 days of storage in whole stalks or billets. Similarly, TA increased with storage time in chopped samples, but was unchanged in whole stalks and billets. Protein concentration was highly variable, and no pattern with treatment or storage time could be discerned. In whole stalks and billets, starch content slowly decreased during storage, while in chopped samples starch appeared to increase. This was most likely a result of an increase in dextran synthesized by microorganisms in those samples, which was also detected by the enzymatic starch assay. The concentration of mannitol, produced by Leuconostoc mesenteroides bacteria, increased with storage time in chopped samples, but not in whole stalks or billets. Within a harvest date, pH was highly correlated with total sugar but not Brix, while TA and mannitol were highly negatively correlated with total sugar but not Brix. Results confirm that whole stalks and billets were little changed over 4 days of storage, while chopped sorghum was badly deteriorated by 1 day after harvest. Changes in pH, TA or mannitol could be used to monitor deterioration in sweet sorghum after harvest.

Measurement and Analysis of the Mannitol Partition Coefficient in Sucrose Crystallization Under Simulated Industrial Conditions
Mannitol is a major deterioration product of Leuconstoc mesenteroides bacterial deterioration of both sugarcane and sugar beet. The effect of crystallization conditions on the mannitol partition coefficient (Keff) between impure sucrose syrup and crystal has been investigated in a batch laboratory crystallizer and a batch pilot plant-scale vacuum pan. Laboratory crystallization was operated at 65.5 °C (150 °F), 60.0 °C (140 °F), and 51.7 °C (125 °F) with a 78.0 Brix (% refractometric dissolved solids) pure sucrose syrup containing 0, 0.1, 0.2, 1.0, 2.0, 3.0, and 10% (at 65.5 °C only) mannitol on a Brix basis. Produced mother liquor and crystals were separated by centrifugation and their mannitol contents measured by ion chromatography with integrated pulsed amperometric detection (IC-IPAD). The extent of mannitol partitioning into the crystals depended strongly on the mannitol concentration in the feed syrup and, to a lesser extent, the

crystallization temperature. At 65.5 and 60.0 °C, the Keff varied from ~0.4 to 3.0% with 0.2 to 3.0% mannitol in the feed syrup, respectively. The mannitol Keff was lower than that reported for dextran (~9-10% Keff), another product of Leuconstoc deterioration, under similar sucrose crystal growth conditions. At 10% mannitol concentration in the syrup at 65.5 °C, co-crystallization of mannitol with sucrose occurred and the crystal growth rate was greatly impeded. In both laboratory and pilot plant crystallizations (95.7% purity; 78.0 Brix; 65.5 °C), mannitol tended to cause conglomerates to form, which became progressively worse with increased mannitol syrup concentration. At the 3% mannitol concentration, crystallization at both the laboratory and pilot plant scales was more difficult. Mannitol incorporation into the sucrose crystal results mostly from liquid syrup inclusions but adsorption onto the crystal surface may play a minor role at lower mannitol concentrations.

Homo- and Heterofermentative Lactobacilli Differently Affect Sugarcane-Based Fuel Ethanol Fermentation

The antagonism between by yeast and lactobacilli is largely dependent on the initial population of each organism. While homo-fermentative lactobacillus present higher inhibitory effect upon yeast when in equal cell number, in industrial fuel ethanol conditions where high yeast cell densities prevail hetero-fermentative lactobacillus are more deleterious, since they succeed in competing with yeast for sugars during fermentation. Both bacteria metabolic types caused reduced ethanol yield during yeast fermentation, but this effect was more pronounced with the hetero-fermentative strain in industrial fuel ethanol conditions. The greater glycerol formation by yeast as well as higher concentrations of bacterial metabolites produced (lactic and acetic acids, plus mannitol) and higher bacterial growth, all led to a greater sugar consumption and decreased ethanol yield in industrial yeast fermentation contaminated with the hetero-fermentative lactobacillus strain. Studies are in progress on the prevalence of homo- and hetero-fermentative lactobacilli in Brazilian fuel ethanol plants and on the differential effect of these two types of bacteria on glycerol production by yeast.

Pilot Plant Clarification of Sweet Sorghum Juice and Evaporation of Raw and Clarified Juices

One of the fundamental processing areas identified by industry for the commercial, large-scale manufacture of liquid biofuels and bioproducts from sweet sorghum (Sorghum bicolor L Moench) is the clarification of juice to make it suitable for concentration into syrup for long-term storage, year-round supply, efficient transport, and acceptable fermentation yields. Pilot plant studies were conducted to evaluate the clarification of juices (80 °C; target limed pH of 6.3; 5 ppm polyanionic flocculant) from a sweet sorghum hybrid and cultivar M81E on three sample dates across a 3-month (Sept to Nov) processing season in 2011. Turbidity removal across pilot plant clarification was 95-98% after only 30-50 min retention time (Rt). The higher Rt at the pilot than laboratory scale caused a slight loss of total fermentable sugars (sucrose + glucose + fructose) to acid degradation, thus a slightly higher target limed pH of ~6.5 is recommended to preserve sugars during clarification and downstream thermal evaporation. Under non-optimized fermentation conditions (S. cerevisiae yeast 10% w/w; 35 °C; 14 h; 18 Brix), higher and more precise bioethanol yields with less foam formation occurred under sterile than non-sterile conditions for both raw and clarified syrups. Ethanol yields ranged from 7.1 to 8.2% (56.0 to 64.7 g/L) and 5.8 to 8.4% (45.8 to 66.3 g/L) and sterile and non-sterile conditions, respectively. Moreover, under sterile conditions, there were no significant differences at the 5% probability level for ethanol yields between the raw and clarified syrups, indicating clarification did not impede fermentation. Overall, clarification of the juices reduced the loss of fermentable sugars during the evaporation stage, and allowed for better storage.

Simultaneous Detoxification, Saccharification, and Ethanol Fermentation of Weak-Acid Hydrolyzates

Lignocellulosic feedstocks can be prepared for ethanol fermentation by pre-treatment with a dilute mineral acid catalyst that hydrolyzes the hemicellulose and opens up the plant cell wall fibers for subsequent enzymatic saccharification. The acid catalyzed reaction scheme is sequential whereby released monosaccharides are further degraded to furans and other chemicals that are inhibitory to the next fermentation step. This work evaluated the use of agricultural residue (flax shive) as starting material for making activated biochar to adsorb these degradation products. Results show that both furfural and hydroxymethylfurfural (HMF) are adsorbed by steam-activated biochar prepared from flax shive. Decontamination of the hydrolyzate significantly improved the fermentation behavior by Saccharomyces cerevisiae yeast, including significantly reducing the lag phase of the fermentation, when the amount of biochar added to the fermentation broth was 2.5% (w/v). No negative effects were noted from addition of activated char to the process.

Investigation of the Stabilization and Preservation of Sweet Sorghum Juices

Sweet sorghum juice is extremely vulnerable to microbial spoilage during storage because of its high water activity and rich sugar medium, and this represents a major technical challenge. The effects of clarification (80 ºC; limed to pH 6.5; 5 ppm polyanionic flocculant) and UV-C irradiation were investigated as stabilization and preservation treatments for juices stored at ambient temperature (~25 ºC). Juices were extracted by roller press from various sweet sorghum cultivars grown in humid and dry environments in Louisiana and Tennessee, respectively. Raw juices contained up to 10^9 total bacteria cfu/mL. Initial results indicated that pilot plant clarified juice was considerably more stable than raw or UV-C irradiated (15 W lamp aquaculture system) juice, irrespective of cultivar. Further experiments were undertaken to elucidate if heating (80 ºC; 30 min) or impurity precipitation or both of these components of the clarification process were responsible for improved juice stability. Clarification or heating both achieved 3- to 4-log reductions of lactic acid bacteria in juices to negligible levels (<150 cfu/mL), and also significantly ($P<0.05$) reduced total bacterial counts. Juice heating gave similar results as the whole clarification process up to ~24 h storage, but became slightly worse between 24 - 28 h. Overall, clarified or heated (80 ºC; 30 min) juice stored at 25 ˚C can be stored for at least 48 h before unacceptable spoilage occurs. Fingerprint ion chromatography with integrated pulsed amperometric detection (IC-IPAD) oligosaccharide profiles can be used to monitor sweet sorghum juice spoilage >100 cfu/mL lactic acid bacteria.

Storage Technologies for Raw and Clarified Syrups from Sweet Sorghum (*Sorghum bicolor* L. Moench)

Attention is currently focused on developing sustainable supply chains of sugar feedstocks for new, flexible biorefineries. Fundamental processing areas identified by industry for the large-scale manufacture of biofuels and bioproducts from sweet sorghum (*Sorghum bicolor* L. Moench) are stabilization and concentration of juice into syrup for long-term storage, year-round supply, efficient transport, and acceptable fermentation yields. Pilot plant studies were conducted to evaluate the storage (up to 160 days at ~25 ˚C) of raw and clarified syrups from sweet sorghum hybrid and cultivars. Clarified syrups were manufactured after clarification of juice (80 °C; 5 ppm polyanionic flocculant) at various target limed pHs (6.1 to 6.8) and then vacuum evaporation. All 70 Brix raw syrups were susceptible to microbial deterioration on the surface during storage, and raw syrups were more susceptible than clarified syrups. Deterioration was mainly fungal with little or no bacterial deterioration because of low water activity and high sugar content. Clarification of the juices reduced the loss of fermentable sugars during the evaporation stage and, generally allowed for better storage of syrup up to 80 days. There was a dramatic effect of the target clarification pH on the storage of clarified 70 Brix syrups with more acidic pHs reducing fungal deterioration; the pH of the stored syrups may need to be lowered to < pH 6.1 for long-term storage. Further studies are now warranted on the post-evaporation adjustment of the pH of raw and clarified syrups on storage. Inexpensive soy bean oil and candellila wax showed promise as surface sealants to preserve syrups for at least 80 days of storage at ~25 ˚C, and warrant further investigation.

Selected Publications:

1. Uchimiya, M., Wartelle, L.H., Lima, I.M., Klasson, K.T. 2010. Sorption of deisopropylatrazine on broiler litter biochars. Journal of Agricultural and Food Chemistry. 58(23):12350-12356.

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4. Uchimiya, M., Lima, I.M., Klasson, K.T., Wartelle, L.H. 2010. Contaminant Immobilization and Nutrient Release by Biochar Soil Amendment: Roles of Natural Organic Matter. Chemosphere. 80 (8):935-940.

5. Cheng, H.N., Gross, R.A. 2010. Green polymer chemistry: biocatalysis and biomaterials. In: Cheng, H.N., Gross, R.A., editors. Green Polymer Chemistry: Biocatalysis and Biomaterials. Washington, DC: ACS Symposium Series, Vol. 1043. pp 1-14.

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15. Eggleston, G., Wu Tiu Yen, J., Alexander, C., Gober, J. 2012. Measurement and analysis of the mannitol partition coefficient in sucrose crystallization under simulated industrial conditions. Carbohydrate Research. 355:69-78.

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20. Klasson, K.T., Dien, B.S., Hector, R.E. 2013. Simultaneous detoxification, saccharification, and ethanol fermentation of weak-acid hydrolyzates. Industrial Crops and Products. 49:292-298.

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1. Uchimiya, S.M., Lima, I.M., Klasson, K.T., Wartelle, L.H. 2010. Contaminant immobilization and nutrient release by carbonized biomass in water and soils (abstract). 2010 U.S. Biochar Initiative Conference, June 27-30, 2010, Iowa State University, Ames, IA.

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