University of Florida
Energy Intensive Crop Development

PIs: Gary Peter, Matias Kirst, Don Rockwood
Students: Alejandro Riveros-Walker, (Ph.D.), Jianxing Zhang, (Ph.D.), Patricio Munoz (Ph.D.)

Description: To build a commercially viable, industrial scale system to produce transportation fuels and electricity from biomass requires both efficient conversion technologies and environmentally sustainable, cost effective supplies of biomass. In the US, Florida ranks first in its annual growth of plant biomass, because of its large cultivable land area and its subtropical climate, even though substantial land areas that can be planted are not currently in agricultural or forest production. The development of high yielding production systems for dedicated energy crops is considered essential for a sustainable, biomass to energy industry to be established, because the long-term availability of sufficient amounts of reasonably priced biomass is one of the most important factors in the site selection for new biofuel and bioenergy facilities. Dedicated energy crops are ones that 1) have high yields with minimum energy inputs in terms of agronomic practices, water and nutrient applications, 2) can be harvested, transported and processed efficiently into fuel or power, and 3) can be grown sustainably for generations without adverse environmental affects, or significantly impacting the food supply. We will evaluate likely energy crop species, *Eucalyptus* and southern pine to provide important yield and best management practices for growing these species for bioenergy conversion. We will also provide important chemical composition information that will impact the conversion efficiency of this biomass to ethanol, and identify and characterize important genes that regulate wood chemical composition.

Budget: $240,000
Universities: UF
External Collaborators: N/A

Progress Summary

Research Objectives for Current Reporting Period: 1) To develop rapid methods for determining wood and grass (in collaboration with the team from Agronomy) cell wall chemical composition, and 2) To establish field plantings of Eucalyptus for testing agronomic practices acquiring yield information.

Progress Made toward Objectives During Reporting Period: <u>Objective 1:</u> Genetic analysis of wood chemical composition is limited by the cost and throughput of direct analytical methods. Indirect methods such as Fourier transform near infrared (FT-NIR) offer an alternative for rapid, low cost method. In FT-NIR, calibration models and their predictions are typically developed and validated from small sample sets. These models are subsequently used to estimate wood chemical composition from larger sets of new samples. However, no direct comparison of direct and indirect estimates of wood chemical composition and the genetic parameter estimates have been reported for the same population. Here we compared for a single poplar family genetic parameter estimates obtained for wood chemical composition with data from pyrolysis molecular beam mass spectrometry (pyMBMS) and FT-NIR. Over 1500 young greenhouse grown wood samples were analyzed for chemical composition with pyMBMS. Randomly selected sample sets of 150, 250, 500 and 750 were used to build a Fourier transform near infrared (FT-NIR) calibration and validate a model based on partial least squares for lignin, G-lignin, S-lignin, S/G ratio and sugars (C5 and C6). The coefficient of determination (R^2) for the calibrations increased slightly, up to 500 samples. For 500 samples, the coefficient of determination (R^2) for the calibrations ranged from 0.56 to 0.87, and the prediction model R^2 ranged from 0.37 to 0.81. Stronger calibration and prediction statistics were obtained with lignin compared with carbohydrates, with the best prediction being R^2 = 0.81 for total lignin. For carbohydrates, the strongest prediction statistics ($R^2 = 0.70$) were obtained for the m/z 144 ion which comes from cellulose. Comparison of genetic parameters showed that all heritabilities were lower for FT-NIR predictions but genetic correlations were similar for two-thirds of the traits, and 10 more quantitative trait loci (QTL) were mapped with pyMBMS. This analysis shows that larger sample sizes make better FT-NIR predictions for the chemical composition of unknown samples.

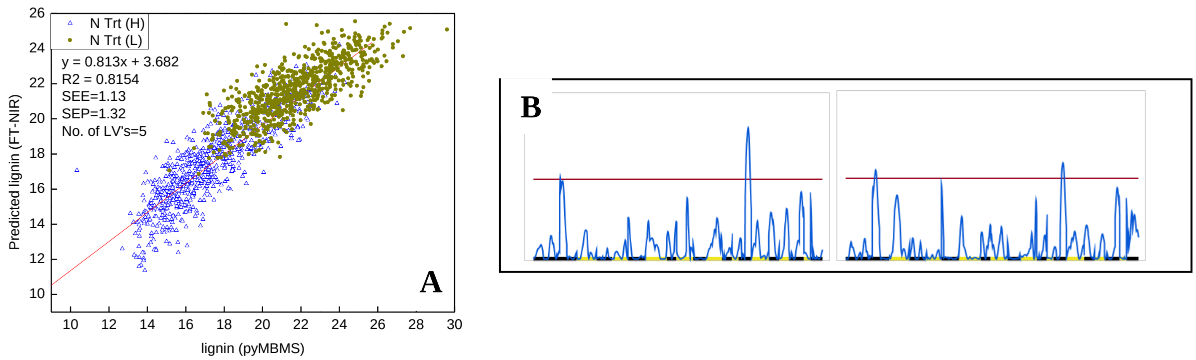

Figure 1: A) Comparison of total lignin content of 1500 poplar wood samples using FT-NIR spectra and py-MBMS. B) Comparison of QTL for lignin from py-MBMS (left) and FT-NIR (right).

<u>Objective 2:</u> In 2009, field plantings were established in central Florida with half-sib seedlings and the four locally adapted UF-IFAS *Eucalyptus grandis* cultivars and in north Florida with half-sib seedlings and clones of *Eucalyptus amplifolia* cultivar. The north Florida planting is about 16 acres and was put in by Buckeye Cellulose. The central Florida planting was about 3 acres and was put in by Mosaic Corp. Height and diameter measurements and biomass samples were taken this fall and will provide the data for growth and yield estimates.

Funds leveraged/new partnerships created:
A new $6.3 million project funded by ARPA-E, "Commercial Production of Terpene Biofuels in Pine" was obtained. This project includes new partnerships with the University of California Berkeley and DOE's Joint BioEnergy Institute, as well as with National Renewable Energy Lab and ArborGen, LLC.

A new alliance is being created to establish a collaborative breeding program for Eucalyptus in Florida and the southeastern US. This program leverages past breeding efforts and growth studies done with FESC. We have obtained substantial interest from the following companies in joining the alliance: ArborGen, Buckeye Cellulose, Foley Timber and Land, Florida Crystals, DeForsa, Evans Property, International Forest Company, International Paper, International Wood Fuels, Lykes Ranch, MeadWestvaco, Rayonier, Rotation Renewables, Rucks Nursery and Weyerhaeuser. We expect this alliance to begin in the summer of 2012. The breeding effort will focus on developing fast growing, disease resistant and cold hardy Eucalyptus hybrids for central and north Florida.

2011 Annual Report

Biomass to Energy:
Dedicated energy crops are ones that 1) have high yields with minimum energy inputs in terms of agronomic practices, water and nutrient applications, 2) can be harvested, transported and processed efficiently into fuel or power, and 3) can be grown sustainably for generations without adverse environmental affects, or significantly impacting the food supply.

For woody crops a broad number of conversion routes to bioenergy are possible and include, wood pellets, co-firing of chips and coal, and biomass combustion for heat, steam and power production, and production of biofuels, such as ethanol or longer chain hydrocarbons, via a number of different conversion technologies. In Florida, wood pellets are produced commercially from southern pine wood, and the city of Gainesville is building a biomass/wood power plant. There is substantial interest in producing biofuels from wood because of it is readily available and can be supplied steadily to a conversion facility. Three main aspects are needed for commercial production of bioenergy: 1) adequate supply of biomass with reasonable cost to site a facility; 2) efficient and cost effective conversion technology that can scale, and 3) markets for use of the bioenergy.

We are evaluating likely energy crop species, *Eucalyptus* and southern pine to provide important yield and best management practices for growing these species for bioenergy conversion. We will also provide important chemical composition information that will impact the conversion efficiency of this biomass to ethanol, and identify and characterize important genes that regulate wood chemical composition.

Objective 1

To conduct fundamental research to identify genes controlling biomass chemical composition and productivity that can be used ultimately to tailor crops for improved conversion efficiencies to biofuels and electricity.

Deliverables:

(1) Validate the candidate genes and pathways implicated in the regulation of biomass productivity and wood chemical composition useful for improvement of *Populus* and pine for biofuel and bioenergy conversion.

- We discovered a gene, cpg13, which regulates partitioning of carbon between lignin and carbohydrates and affects growth rate. We have created up and down regulated versions of this gene in transgenic poplars and are evaluating the effects on lignin to carbohydrate ratio, growth and metabolite levels.

(2) Application of discovered genetic markers to improve pine breeding populations, targeting higher biomass productivity and superior properties for conversion to biofuels.

- Genomic selection is increasingly considered vital to accelerate genetic improvement. However, it is unknown how accurate genomic selection prediction models remain when used across environments and ages. This knowledge is critical for breeders to apply this strategy in genetic improvement.

- We evaluated the utility of genomic selection in a *Pinus taeda* L. population of ~800 individuals clonally replicated and grown on four sites, and genotyped for 4,825 SNP markers. Prediction models were estimated for diameter and height at multiple ages using genomic random regression BLUP.

- Accuracies of prediction models ranged from 0.65–0.75 for diameter, and 0.63–0.74 for height. The selection efficiency per unit time was estimated as 53–112% higher using genomic selection compared to phenotypic selection, assuming a reduction of 50% in the breeding cycle. Accuracies remained high across environments as long as they were used within the same breeding zone. However, models generated at early ages did not perform well to predict phenotypes at age 6.

- These results demonstrate the feasibility and remarkable gain that can be achieved by incorporating genomic selection in breeding programs, as long as models are used at the relevant selection age and within the breeding zone in which they were estimated.

(3) Training of graduate students on the use of the most advanced genomics tools and approaches for analysis of complex traits.

- We are training 3 students in genomics and quantitative genetic analyses of complex traits.

Objective 2

To conduct applied research with Eucalyptus, to provide important agronomic practice, yield, and chemical composition information for Florida growers, producers and policy makers.

Deliverables:

(1). Regionally tested *Eucalyptus* species and cultivar yields and initial best management practices for biomass accumulation in south, central and north Florida.

- In 2009, field plantings were established in central Florida with half-sib seedlings and the four locally adapted UF-IFAS *Eucalyptus grandis* cultivars and in north Florida with half-sib seedlings and clones of *Eucalyptus amplifolia* cultivar. The north Florida planting is about 16 acres and was put in by Buckeye Cellulose. The central Florida planting was about 3 acres and was put in by Mosaic Corp. Height and diameter measurements and biomass samples were taken this fall and will provide the data for growth and yield estimates.

(2). Chemical composition of candidate biomass species and impact of genetic and silvicultural treatments.

- Genetic improvement of forest trees, such as Eucalyptus, *Populus*, and Pine species has increased growth, and improved form and disease resistance, leading to significant gains in field productivity. However, forest tree breeders have not directly selected for wood chemical composition, despite the substantial economic interest and knowledge about genes involved in synthesis of cellulose, hemicelluloses and lignin. With new interest in biomass chemical composition from the developing biofuels industry, there is a need for robust low cost and rapid methods to use in breeding. These methods need to support both traditional breeding practices and recent advances that utilize genetic markers such as genomic selection.

- A variety of methods for measuring the chemical composition of lignocellulosic biomass have been applied to understand genetic control and environmental effects. Although classic wet chemical methods have been widely used, their application for genetic analyses is limited by their high cost and low throughput. Composition data from classical chemical methods were used to calculate genetic parameters for wood chemical composition in loblolly pine and eucalyptus, and map quantitative trait loci (QTL) in loblolly pine. Pyrolysis mass spectrometry methods are more rapid and have been used to characterize lignocellulosic components in herbaceous and woody biomass. In particular, pyrolysis molecular beam mass spectrometry (pyMBMS) has been used to analyze large genetic trials to understand the genetic architecture, map QTL and identify genes that affect cellulose, hemicelluloses and lignin content in pine and poplar. Near infrared (NIR) spectroscopy is an indirect method that has been used for qualitative and quantitative chemical analyses in many fields and industrial applications, including, agricultural products, food, and forestry.

- The goals of this research are to calibrate a FT-NIR model based on the chemical components determined from pyMBMS, test the calibration model's performance with a separate prediction set, and utilize the FT-NIR predictions to estimate the genetic parameters and detect QTLs. Compare the genetic parameters from FT-NIR with those obtained with pyMBMS.

- Here we compared for a single poplar family genetic parameter estimates obtained for wood chemical composition with data from pyrolysis molecular beam mass spectrometry (pyMBMS) and FT-NIR. Over 1500 young greenhouse grown wood samples were analyzed for chemical composition with pyMBMS[2]. We randomly selected 500 samples to build a Fourier transform near infrared (FT-NIR) calibration and validate a model based on partial least square for lignin, G-lignin, S-lignin, S/G ratio and sugars (C5 and C6). A FT-NIR spectrometer, equipped with an X-Y stage auto-sampler was used to improve the scanning efficiency. The sample set was randomly divided into calibration (500) and prediction (1005) sets. The coefficient of determination (R^2) for the calibrations ranged from 0.56 to 0.87, and the prediction model R^2 ranged from 0.37 to 0.81. Stronger calibration and prediction statistics were obtained with lignin compared with carbohydrates. For lignin the best prediction (R^2 = 0.81) was obtained. For carbohydrates, the strongest prediction statistics (R^2 = 0.70) were obtained for the m/z 144 ion which comes from cellulose.

  Genetic analysis of pyMBMS data and FT-NIR predictions was compared to evaluate the utility of the indirect FT-NIR method relative to the direct pyMBMS method for parameter estimates. QTL analysis was used to compare the results between pyMBMS and FT-NIR

(3). Training of graduate students in production physiology, chemical composition, and management of Eucalyptus short rotation woody crops in Florida.

- We have trained one student in chemical composition analysis

Publications:

Resende, M.F.R., Jr., Munoz, P., Acosta, J.J., Peter, G.F., Davis, J.M., Grattapaglia, D., Resende, M.D.V., Kirst, M. 2011. Accelerating the domestication of trees using genomic selection: accuracy of prediction models across ages and environments. *New Phytologist* In press

Zhang, J., Novaes, E., Kirst, M., Peter, G.F. Comparison of pyrolysis mass spectrometry and near infrared spectroscopy for genetic analysis of lignocelluloses composition in Populus Biomass Bioenergy submitted