FESC
Florida Energy Systems Consortium

## UNIVERSITY OF FLORIDA

# *Energy Intensive Crop Development*

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**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:** University of Florida

## Progress Report

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

**Progress Made Toward Objectives During Reporting Period:**
**Objective 1**: The chemical composition of biomass is a critical determinant of the yield of energy from biomass. Biomass that has higher carbohydrate levels are expected to increase the yield of biofuel with a bioconversion process. In contrast, biofuel yields from gasification and pyrolysis are expected to be greater from biomass with higher lignin and oleoresin contents. To identify genetic and environmental control of biomass chemical content we are testing the utility of near and mid-infrared spectroscopy together with multivariate statistical modeling for rapid biomass chemical composition analyses. A near and mid-infrared spectrometer was purchased from Perkin-Elmer. The instrument is capable of collecting reflectance near and mid IR spectra from 96-well plates as well as individual sample near infrared (NIR) spectra with an integrating sphere and mid IR spectra with an attenuated reflectance probe. A variety of spectra collection and analytical methods have been tested and the results show that NIR reflectance spectra collected with the 96 well plate reader gave good calibration models using the 2nd derivative of the spectra and partial least squares for grass, pine and poplar carbohydrate and lignin content biomass samples. Calibration models have been built for predicting the lignin, cellulose and hemicellulose chemical composition of tropical grass, loblolly pine and poplar wood biomass samples.

- For grass biomass, calibrations were performed with a total of 116 samples, spanning 4 sites and 4 species. Strong calibration regression coefficients ($r^2$) were obtained with recovered glucose

(0.92), xylose (0.93), fiber-glucose (0.90), total recovered (0.93) and total potential sugar (0.91). The grass work is in collaboration with the FESC funded UF teams led by L. Ingram and L. Sollenberger.

- With 396 poplar samples, the calibration regression models gave strong coefficients for total lignin (0.87), guaiacyl lignin (0.81) and syringyl lignin (0.91) and cellulose (0.80). In contrast, the calibration coefficient was moderate for xylan (0.68). These poplar calibration models gave strong prediction regression coefficients with new wood samples for total lignin (0.875), syringyl-lignin (0.83), guaiacyl lignin (0.69), and cellulose (0.74) content while xylan (0.59) was only moderately well predicted. We are now testing how well these calibration equations predict *Eucalyptus* wood chemical content.
- With 308 pine samples, calibration regression models gave strong coefficients for total lignin (0.93), guaiacyl lignin (0.90), xylan (0.90), and cellulose (0.92). Unlike poplar, the models gave only weak prediction regression coefficients with 192 new wood samples: total lignin (0.63),
- In addition, calibration models have been developed for □-pinene (0.99) and □-pinene (0.99), the two most abundant terpenoids of pine oleoresin. The calibration model was used to predict the pinene levels in a genetic trail and estimates of genetic control were high, similar to literature values for southern pine.

**Objective 2:** Eucalyptus species are the fastest growing woody plants. For south and central Florida, we have developed four locally adapted UF-IFAS *Eucalyptus grandis* cultivars and for central and north Florida a half-sib seedlings of *Eucalyptus amplifolia*. In 2009, five tests were established with 3 *E. grandis* cultivars. Table 1 shows the survival and early height growth. The survival ranged from 82.4-100% across sites for all cultivars. The cultivars planted in central and south Florida had minimal to no freeze damage during 2009-2010 freezes. However, the *E. grandis* cultivars planted in north Florida were killed to the ground, but some have produced new shoots from the base of the stem.

## 2010 Annual Report

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

**Progress Made Toward Objectives During Reporting Period:**

**Objective 1**: The chemical composition of biomass is a critical determinant of the yield of energy from biomass. Biomass that has higher carbohydrate levels are expected to increase the yield of biofuel with a bioconversion process. In contrast, biofuel yields from gasification and pyrolysis are expected to be greater from biomass with higher lignin and oleoresin contents. To identify genetic and environmental control of biomass chemical content we are testing the utility of near and mid-infrared spectroscopy together with multivariate statistical modeling for rapid biomass chemical composition analyses.

A near and mid-infrared spectrometer was purchased from Perkin-Elmer. The instrument is capable of collecting reflectance near and mid IR spectra from 96-well plates as well as individual sample near infrared (NIR) spectra with an integrating sphere and mid IR spectra with an attenuated reflectance probe. A variety of spectra collection and analytical methods have been tested and the results show that NIR reflectance spectra collected with the 96 well plate reader gave good calibration models using the 2nd derivative of the spectra and partial least squares for grass, pine and poplar carbohydrate and lignin content biomass samples. Calibration models have been built for predicting the lignin, cellulose and hemicellulose chemical composition of tropical grass, loblolly pine and poplar wood biomass samples.

**Perennial Grasses:** For grass biomass, calibrations were performed with a total of 116 samples, spanning 4 sites and 4 species. Strong calibration regression coefficients ($r^2$) were obtained with recovered glucose (0.92), xylose (0.93), fiber-glucose (0.90), total recovered (0.93) and total potential sugar (0.91) (Table 1). The work with grass samples was done in collaboration with the FESC funded UF teams led by L. Ingram and L. Sollenberger.

**Table 1.** Calibration statistics using partial least squares with the second derivative of the NIR spectra of 116 grass biomass

| Chemical | # Components | % Variance | SEE | SEP | Mean Value |
|---|---|---|---|---|---|
| Recovered Glucose | 4 | 91.62 | 13.62 | 22.37 | 379.4 |
| Recovered Xylose | 5 | 92.56 | 4.567 | 9.688 | 184.9 |
| Fiber-glucose | 5 | 90.32 | 11.62 | 20.16 | 334.7 |
| Total water soluble sugar | 4 | 93.76 | 13.25 | 20.17 | 98.98 |
| Total recovered sugar | 6 | 92.60 | 17.78 | 46.94 | 664.8 |
| Total fiber sugar | 3 | 67.12 | 36.85 | 54.72 | 606.3 |
| Total potential sugar | 5 | 91.29 | 19.10 | 40.42 | 723.7 |
| Ash | 5 | 92.74 | 2.342 | 6.606 | 51.98 |
| Lignin+others | 5 | 94.47 | 15.04 | 45.04 | 297.7 |

We have scanned an additional 100 grass samples and are waiting for the chemical composition data from L. Sollenberger's group. To conduct prediction tests.

**Poplar Wood:** With 396 poplar samples, the multivariate calibration regression models gave strong coefficients for total lignin (0.87), guaiacyl lignin (0.81) and syringyl lignin (0.91) and cellulose (0.80). In contrast, the calibration coefficient was moderate for xylan (0.68). These poplar calibration models gave strong prediction regression coefficients with 99 new poplar wood samples for total lignin (0.875), syringyl-lignin (0.83), guaiacyl lignin (0.69), and cellulose (0.74) content while xylan (0.59) was only moderately well predicted (Figure 1). We are now testing how well these calibration equations predict *Eucalyptus* wood chemical content.

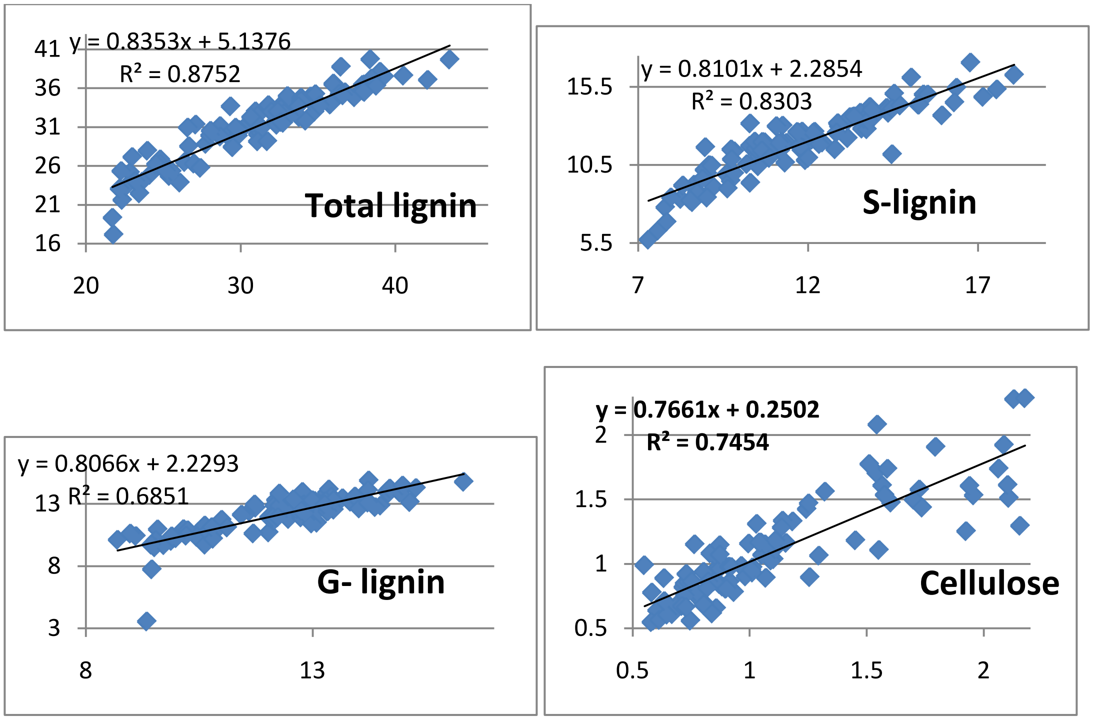


**Figure 1.** Prediction of total lignin, S-lignin, G-lignin and cellulose in 99 samples of poplar wood using calibration models developed with 396 poplar samples.

**Loblolly Pine Wood**: With 79 loblolly pine wood samples, the multivariate calibration regression models gave stronge coefficients for total lignin, G-lignin, C5 and C6 sugars as well as xylan and cellulose (Table 2). The calibrated pine models gave strong prediction regression coefficients with 26 new wood samples for total lignin (0.85) and G-lignin (0.87), but not for the carbohydrates.

**Table 2.** Calibration and prediction statistics for chemical composition of wood from loblolly pine using partial least squares with the second derivative of the NIR spectra of 105 samples

| **Compound** | **# of LV's** | **% Variance** | **SEE** | **SEP** | **Mean value** | **Prediction $R^2$** |
|---|---|---|---|---|---|---|
| C5 sugars | 6 | 94.29 | 0.449 | 1.047 | 24.78 | 0.36 |
| C6 sugars | 6 | 95.43 | 0.721 | 1.875 | 34.7 | 0.34 |
| G-lignin | 3 | 92.09 | 0.978 | 1.271 | 32.3 | 0.87 |
| Total Lignin | 3 | 92.45 | 1.489 | 1.952 | 44.52 | 0.85 |
| xylan | 5 | 90.02 | 0.136 | 0.297 | 2.967 | 0.07 |
| cellulose | 5 | 90.47 | 0.080 | 0.191 | 2.217 | 0.18 |

**Loblolly Pine Oleoresin:** In addition to working with wood samples, we developed calibration models with □-pinene (0.99) and□ -pinene (0.99), the two most abundant terpenoids of pine oleoresin. The calibration model was used to predict the pinene levels in a genetic trail and estimates of genetic control were very high, similar to literature values for southern pine.

**Objective 2:** Eucalyptus species are the fastest growing woody plants. For south and central Florida, we have developed four locally adapted UF-IFAS *Eucalyptus grandis* cultivars and for central and north Florida a half-sib seedlings of *Eucalyptus amplifolia*. In 2009, six tests were established with 3 *E. grandis* cultivars and one with *E. amplifolia*. Table 3 shows the survival and early height growth for 3 cultivars of *E. grandis*. The survival ranged from 82.4-100% across sites for all cultivars. The cultivars planted in central and south Florida had minimal to no freeze damage during 2009-2010 freezes. However, as expected the *E. grandis* cultivars planted in north Florida were killed to the ground, but some have produced new shoots from the base of the stem. The E. amplifolia survived very well in North Florida as expected.

Overall, the cultivars have grown exceptionally well, with uniform crown form (Figure 2 & 3). The tallest tree was in south Florida and measured 6.1m at eight months. Trees in both phosphate mined land sites in central Florida were planted on the same day using the trees from the same batch, but the trees in clay settling area outperformed the trees in reclaimed site. Soil analysis from the two sites indicated that clay settling area had higher nutrients leading to better tree performance.

**Table 3**. Mean height and survival data for *E. grandis* G1, G2 and G3 cultivars growing at six sites planted in 2009.

| **Location** | **Site** | **Soil** | **Age (mo)** | **Response** | **G1** | **G2** | **G3** |
|---|---|---|---|---|---|---|---|
| South | Citrus beds | Poorly drained sand | 9 | Height (m)<br>Survival (%) | 3.3<br>92.3 | 3.3<br>89.4 | 3.5<br>91.3 |
| South | Agricultural land | Sandy muck | 8 | Height (m) up to 6.1 | | | |
| Central | Mined land (Reclaimed site) | Sandy | 7 | Height (m)<br>Survival (%) | 0.5<br>100 | 0.5<br>100 | 0.7<br>100 |
| Central | Mined land (Clay settling) | Clay | 7 | Height (m)<br>Survival (%) | 0.9<br>100 | 1.8<br>100 | 1.3<br>100 |
| Central | Citrus grove windbreak | Well drained sand | 15 | Height (m) | 4.3 | 4.4 | 5.0 |
| North | Former Pine Plantation | Sandy clay | 5 | Height (m)<br>Survival (%) | 0.5<br>100 | 0.6<br>100 | 0.5<br>96 |

**Figure 2.** 9 month old *E. grandis* cultivars growing on a "citrus bed" in south FL.

**Figure 3**. 7 month old *E. grandis* cultivar G2 growing on the clay settling area of phosphate mined land in central FL.

Allocation and biomass data was collected to build allometric relationships with which to predict growth. In addition, a preliminary analysis of growth and yield is being conducted with data collected from a series of older trails with *E. grandis* and *E. amplifolia*. With the available data *E. amplifolia* is yield more than *E. grandis* (Figure 4). The reduced biomass for *E. grandis* is probably due to the poorer quality sites and some cold damage. Measurements of the newly installed field trails will be used together with allometric relationships to predict biomass yields.

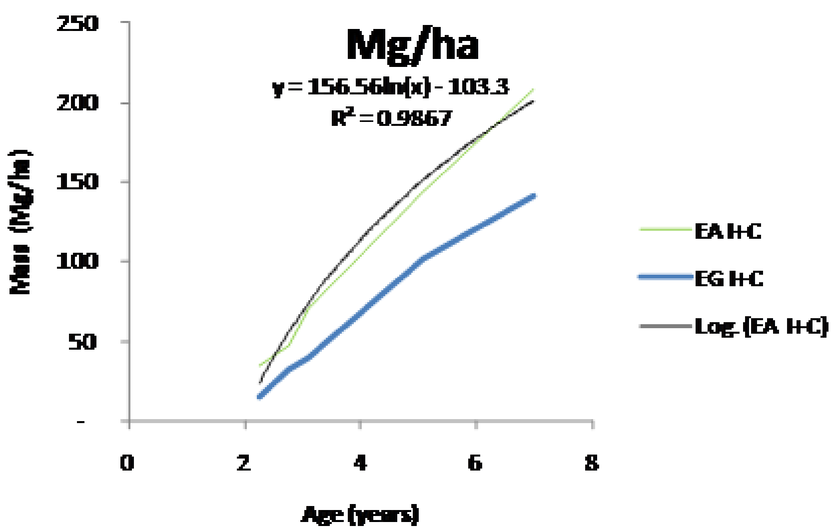


**Figure 4.** Growth and yield analysis of *E. grandis* and *E. amplifolia* from 7 year old trials.