# Final Progress Report

## Environmental Influences on Wood Chemistry and Density of *Populus* and Loblolly Pine

**Prepared for:**
**U.S. Department of Energy**
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OAK RIDGE NATIONAL LABORATORY
MANAGED BY UT-BATTELLE FOR THE DEPARTMENT OF ENERGY

# FINAL PROGRESS REPORT

**Project Title:** Environmental Influences on Wood Chemistry and Density of *Populus* and Loblolly Pine

**Covering Period:** April 1, 2002 through January 31, 2004

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**Recipient:** Oak Ridge National Laboratory
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**Project Objective:** The objectives of the study are to: 1) determine the degree to which physical and chemical wood properties vary in association with environmental and silvicultural practices in *Populus* and loblolly pine and 2) develop and verify species-specific empirical models in an effort to create a framework for understanding environmental influences on wood quality.

**Background:** This project began in August 2002 and is a renewal effort for a previous Agenda 2020 project entitled: “Development and validation of marker-aided selection methods for wood property traits in loblolly pine and hybrid poplar.” Because environmental factors confound our estimates of genetic parameters, complicate gene discovery, and impact product quality this project was initiated in an effort to quantify these effects on wood properties.

# Acknowledgments and Disclaimer


## Acknowledgments

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## 1. Progress

### 1.1 Loblolly Pine Field Data Collection

The pine portion of this study is located in a mature loblolly pine forest at the Duke Forest Free Air Carbon Exchange (FACE) site in Orange County, North Carolina. The FACE prototype ring was set up in 1994 and $CO_2$ enrichment of 200 ppm above ambient is achieved from April to October. In 1998 the prototype and reference rings were divided and half of each was fertilized to meet optimal nutrition of foliage. This covers four treatments: 1) non-fertilized ambient, 2) fertilized ambient, 3) non-fertilized elevated $CO_2$ 4) fertilized elevated $CO_2$.

We continued to monitor 10 trees (2 sweetgum and 8 loblolly pine) in each $CO_2$ treatment -- 2 sweetgum and 2 loblolly pines for diameter increment continued, along with the hourly and daily records for the climatic variables. For loblolly pine, the first complete data sets began in April 2002. These data were used in a preliminary analysis to predict incremental diameter growth. The resulting multiple regression produced an equation -- DBH = 260173 + 2094*(Soil Temp) – 4.14*(Soil Moisture), that predicted diameter at breast height with an $R^2 = 0.81$.

The summer of 2002 was one of the driest on record at the Duke Forest. Following normal development of diameter growth in the spring, growth rate reduces greatly over time until early July (Fig. 1-3). From July until near the end of August, soil moisture was so low that most trees actually decreased in diameter during this time. There were a few small rain events during this period where minor growth and diurnal diameter variation was observed. In September, rain was plentiful and rapid diameter growth was reinitiated but almost all trees ceased growth by early October. Although overall growth was the lowest measured in these plots in nine years, enhancement of growth was observed due to both fertilization and elevated atmospheric $CO_2$. The severe drought conditions experienced this past summer will likely provide a strong contrast to growth in the next two years if precipitation increases closer or even above the long-term average for the site.

All data from this year have been collected up to 1/30/04. At the beginning of September the data loggers we use for recording the data ran out of memory. All data was downloaded and William Gensler was able to recover about 2 weeks of data that could not be accessed. This data is currently being error checked. Data transformation is completed through 12/31/03. During August all bands were inspected for reduction in clearance due to diameter growth. In all, 70% of the bands were moved back away from the stem. One tree in the control $CO_2$ plot died and was replaced with new tree. Data transformation is completed through 08/15/03.

### 1.2 *Populus* Density Assessments

The *Populus* portion of this study was conduct in a retrospective manner using an existing experiment established by Union Camp Corp., now owned by International Paper Co. Several data sets for various silvicultural and climatic variables, including mean, maximum and minimum daily temperature, mean, maximum and minimum daily vapor pressure deficit, mean, maximum

and minimum daily solar radiation, mean, maximum and minimum daily wind speed, hourly precipitation, mean, maximum and minimum daily air and soil temperature, daily fertigation schedules, daily irrigation schedules, weekly soil moisture, monthly leaf area index, and incremental weekly diameter growth for 1998, 1999, and 2000 were available for analysis (Fig. 4-7). In addition, four fertilizer and irrigation treatments plus a control were applied to 8 clones of eastern cottonwood. From these treatment clones combinations, 364 *Populus* increment cores were collected and have been being processed in preparation for contiguous 100 um density scans and similar incremental cell wall chemistry estimates that will ultimately be used in conjunction with the *Populus* climatic and silvicultural data sets.

The MicroCAT results suggest there is considerable variation across clones, treatments and years in overall wood density (Fig. 8-9). The control treatments tend to display greater fluctuation in density values. Although radial increment may have occurred early in the study, final cell wall density is likely not to be determined until the cell wall assembly is complete. Thus, we are attempting to associate wood density with wood chemistry across entire growing seasons for each clone in each year. The NIR apparatus was used to measure syringyl/guaiacyl (S/G) ratio as a function of radial position of a test hybrid poplar core. A NIR patent to latent structure (PLS) model to determine S/G ratio was constructed from 25 ground poplar wood samples. The range of S/G ratios in the standard samples ranged from 1.1 to 2.5 as determined by thioacidolysis. Figure 6 shows the S/G ratios determined from NIR spectra collected at 2.5 mm increments along a hybrid poplar core. The smaller dips in S/G ratio, most noticeable between 150 and 200 mm correspond to areas along the cores where annual latewood pores predominate.

Preliminary MicroCAT scanner results suggested that three *Populus* clones (72 total cores), representing high, medium and low densities, warranted further analysis. These 72 increment cores were vacuum desiccation was used to dry the cores before scanning. Several test scans were run to obtain the proper configuration of the scanner for the resolution of growth ring structure and to find the quickest method of positioning cores for high-throughput. More tests of reconstruction and imaging software were conducted in an effort to produce the highest quality images. Based on the results of these tests, we are now actively scanning cores and reconstructing the images for later analysis. All 72 increment cores have been rescanned on the latest version of the MicroCAT scanner with high-speed reconstruction capability. High-speed reconstruction allowed for the reconstruction of three-dimensional images with a higher resolution than was available with the older MicroCAT scanner. Using ImageJ imaging software, the three dimensional images were re-sliced to obtain an internal image one pixel wide and the length of each core and free from any surface artifacts. Currently, WinDendro software, a semiautomatic image analysis system specifically designed for tree ring and wood density measurement, is being adapted to acquire absolute wood density and ring width from our images (Fig. 8).

Work has also been progressing on determination of absolute density based on wood samples of known specific gravity. MicroCAT scans of 17 species of known density were acquired for software calibration. The species were scanned under the same conditions and scanner parameters as the increment cores. The scanner's x-ray voltage was set at 60 kVp, the anode

current was 500 μA. The source and detector's total rotation was 360 degrees accomplished in 600 steps. The resulting scanner data was reconstructed into 512 x 512 x 768 pixel dimension images and analyzed with ImageJ software. The species ranged in density from Balsa at 0.104 g/cc to Hickory at 0.939 g/cc (Fig. 8).

### 1.3 *Populus* Wood Chemistry Assessments

Due to the retrospective nature of the study most silvicultural and environmental data sets have large segments of missing data. For example, all climatic data from Day of Experiment (DOE) 165 to 370 are missing due to a bad circuit board in the recorder. There were significant treatment effects with irrigation yielding greater biomass accumulation (data not shown). The irrigation treatments also affected soil temperature over the course of the 1999-growing season, causing soil temperatures to be lower in the irrigated treatments. Clones tended to respond similarly to treatments with the exception of clone R. All clones showed an ever-increasing growth advantage under irrigation with no differences between the high and low treatments. Clone R was the only clone to display a difference between the low irrigation and high irrigation treatments. The bi-weekly dendrobands indicate that radial growth in cottonwood starts before leaf out and is approximately 90% completed by the end of May. As a result, much of the precision in associating growth with wood formation is lost in bi-weekly measures. Moreover, the season-long radial increment is affected by clone, treatment and year. Adjusted R-square values for radial increment and the environmental data set indicate that early season increment in 1999, a wet year, was uniformly affected by solar radiation (Table 1). Over the course of the entire 1999-growing season, potential evapotranspiration was a better predictor of incremental growth for the control treatment and soil temperature was the best predictor for the irrigated treatments (Table 1). In 2000, a dry year, over the course of the entire growing season, potential evapotranspiration was the best predictor over all treatments.

All 364 *Populus* increment cores have been collected and prepared for MicroCAT x-ray computed tomography scan. The scanner data has been used to reconstruct 3 dimensional images of the cores. These images will subsequently be analyzed to determine wood density. Vacuum desiccation was used to dry the cores before scanning. In addition, we have been analyzing samples to improve the predictive Projection to Latent Structure (PLS) models that will be used for this project. An acid hydrolysis lignin determination was performed on a set of poplar samples that were exhaustively ethanol extracted prior to the analysis. Each sample was subjected to 72% sulfuric acid treatment at 30 $^{o}$C for one hour before being autoclaved at 121 $^{o}$C for an additional hour at an acid concentration of 4%. Acid soluble lignin was determined by UV spectrometer at 205 nm. Acid insoluble lignin was determined gravimetrically. All samples were run in duplicate and reported on a dry whole wood weight basis. A PLS model was constructed (r=0.92, RMSEP=0.67) that can be used to estimate lignin in the poplar cores (Table 2). Work has also been progressing to develop the experimental setup and techniques for measuring cell wall chemistry as a function of core position. The experimental setup consists of a linear stage that is accurately moved using a computer controlled stepper motor, NIR spectrometer, and a holder for the NIR probe and light source. The probe holder is designed to be able to move up and down to compensate for surface roughness and to insure the NIR probe remains a fixed distance from the core surface.

Work has been progressing to improve the experimental setup and techniques for measuring cell wall chemistry as a function of core position. During the year, several improvements have been made to the experimental apparatus to improve the spatial resolution and reproducibility. The spatial resolution was improved by integrating a light microscope with spectrometer optics. Integrating the light microscope resulted in two additional benefits. The first benefit was that it became easier to illuminate the area of the sample that was being scanned because the optics were further from the sample and shadowing of the illumination light beam was eliminated. The second benefit was that it became easier to focus the NIR fiber optics on a specific area of the sample.

The experimental reproducibility was improved by modifying the software program controlling the linear stage movement. The improvements consisted of rewriting the programming code to move the linear stage back to a preset location after the scanning was completed. These improvements will allow for multiple scans (replicates) of a single core allowing for the experiment to better determine if changes in the NIR spectra are due to changes in cell wall chemistry or experimental artifacts.

We have been analyzing samples to improve the predictive PLS models that will be used for this project. An acid hydrolysis lignin determination was performed on a set of poplar samples that were exhaustively ethanol extracted prior to the analysis. Each sample was subjected to 72% sulfuric acid treatment at 30$^{o}$C for one hour before being autoclaved at 121$^{o}$C for an additional hour at an acid concentration of 4%. Acid soluble lignin was determined by UV spectrometer at 205nm. Acid insoluble lignin was determined gravimetrically. All samples were run in duplicate and reported on a dry whole wood weight basis. A PLS model was constructed (r=0.92, RMSEP=0.67) that can be used to estimate lignin in the poplar cores.

Alpha cellulose determinations were performed to obtain cellulose values the samples. Some samples were ethanol extracted and some were not; extraction did not seem to have an effect on the results. The wood was first degraded to holocellulose by reaction with chlorine dioxide at 70$^{o}$C. The alpha cellulose was then isolated from the holocellulose by extraction with a 17.5% sodium hydroxide solution. The final alpha cellulose number was determined gravimetrically with moisture and ash removed. All numbers are reported on a whole wood basis. We attempted to construct (without success) a PLS model for alpha cellulose. Work is continuing to determine the cause of the lack of fit between the predicted and measured cellulose values.

We have begun to collect spectra on the poplar cores and to develop protocols for assessing the changes in wood chemistry along the cores related to growth conditions. These experiments are being performed to begin to identify methods to relate wood chemistry determined by traditional analytical chemistry methods with NIR spectra collected on solid wood cores. Our initial experiments have been directed at determining whether regressions models developed on ground wood samples can be applied to solid samples. These experiments involve determining lignin and syringyl/guaiacyl (S/G) ratios along a core using NIR spectroscopy and then verifying the results using analytical pyrolysis (Fig. 10).

These experiments were carried out on samples excised from a wood disk taken from a 7-year-old poplar tree. Positions along the core were selected to ensure that spectra were collected from both springwood and summerwood. PyMBMS spectra were acquired on small samples that were carefully cut from a neighboring matched wedge cut from the same disk. A principal component analysis (PCA) of the mass spectra collected from samples taken along the wedge indicated that the lignin content was higher earlier in the tree growth and began to level off after year 3. A comparison of the pyMBMS spectra for springwood and summerwood selected from the same year did not detect a consistent change in wood chemistry. However, this experiment is being repeated using a larger diameter disk wood selected from a different tree collected from the same area in which it is easier to identify spring and summer wood. NIR spectra are also being collected for the areas selected for pyMBMS analysis.

**2.0 Figures**

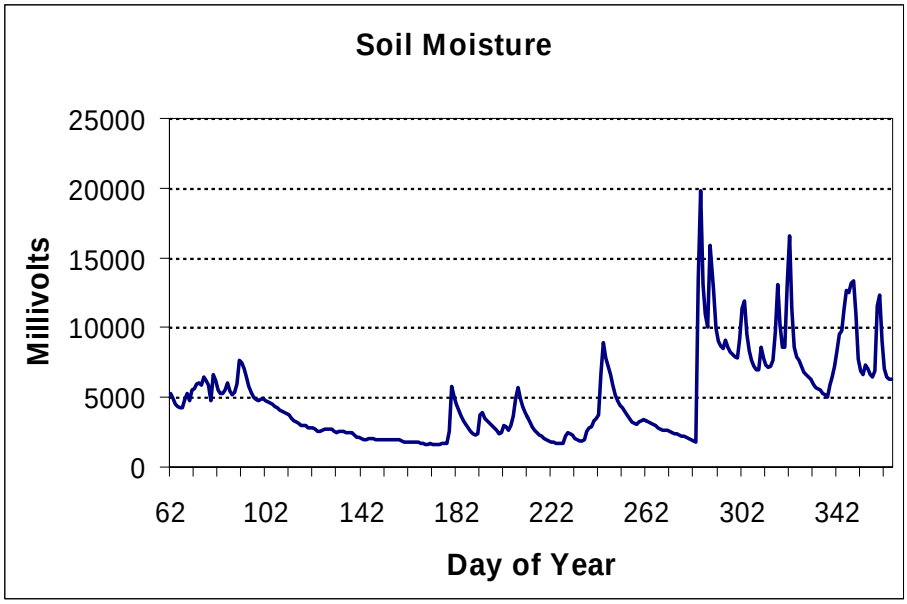


**Figure 1.** Daily variation in soil moisture recorded every 15 minutes in a loblolly pine FACE experiment located on Duke Forest, Raleigh, NC.

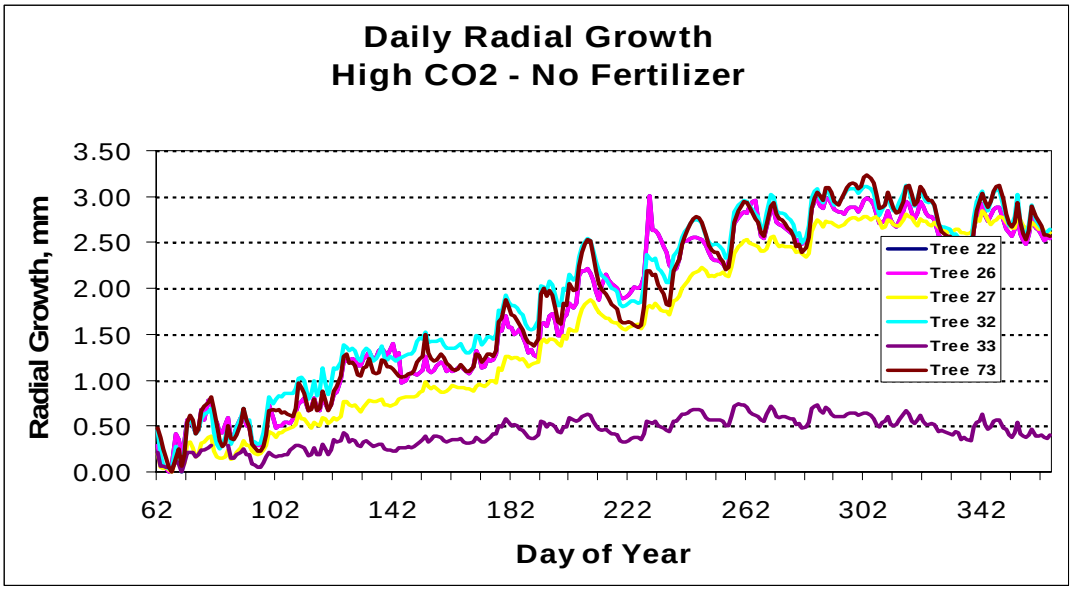


**Figure 2.** Radial growth for selected trees recorded every 15 minutes in a loblolly pine FACE experiment located on Duke Forest, Raleigh, NC.

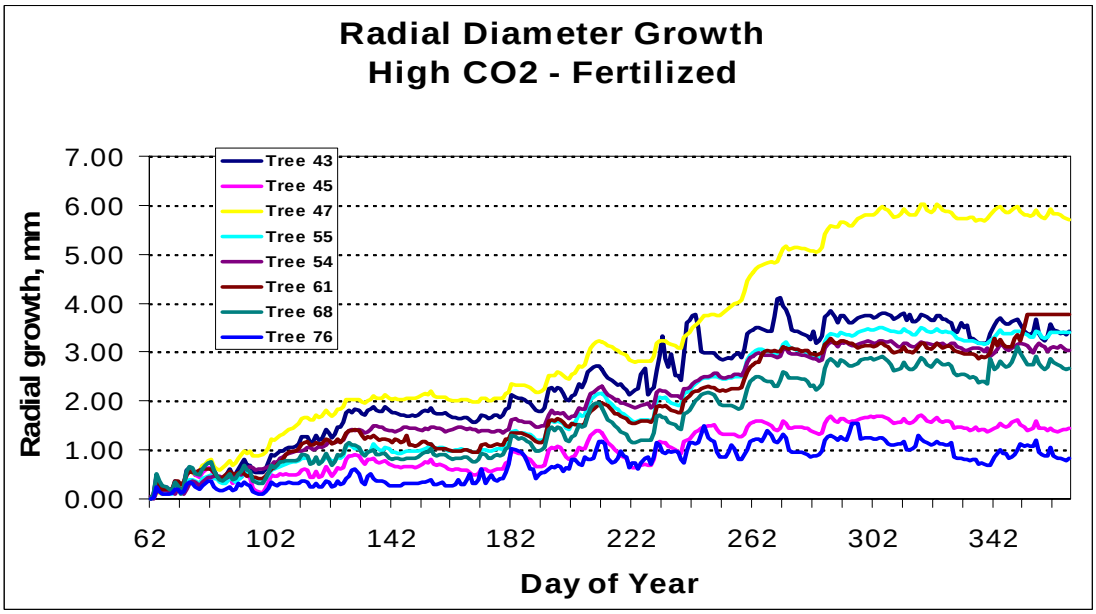


Figure 3. Radial growth for selected trees recorded every 15 minutes in a loblolly pine FACE experiment located on Duke Forest, Raleigh, NC.

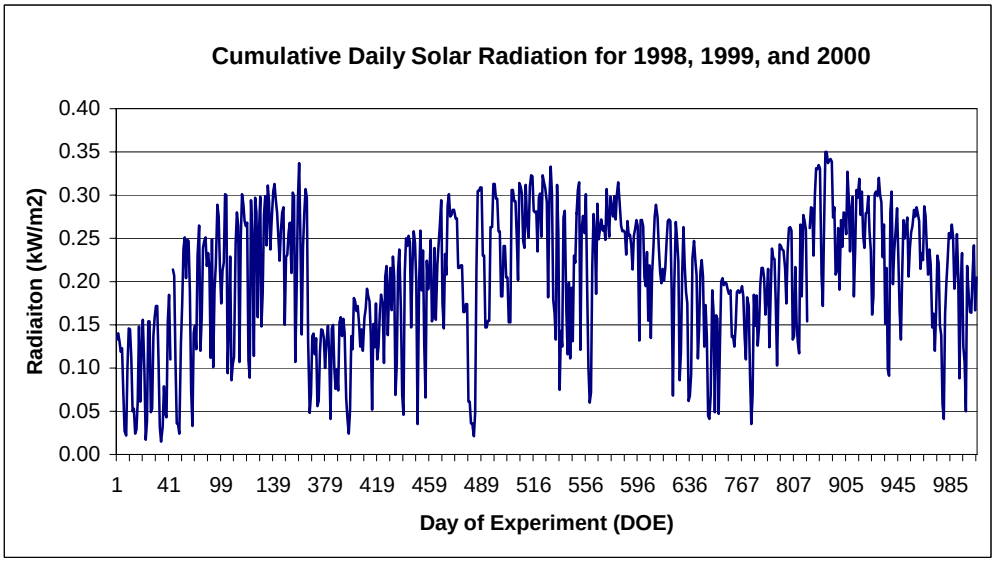


**Figure 4.** Changes in cumulative daily solar radiation for years 1998, 1999 and 2000 as part of a fertigation study conducted at Sumter, SC.

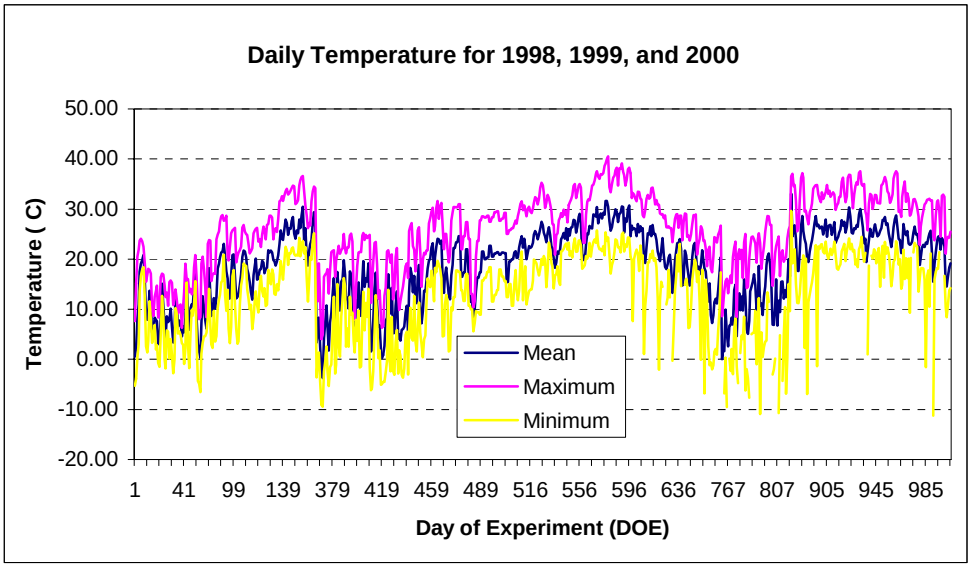


**Figure 5.** Changes in daily air temperature for years 1998, 1999 and 2000 as part of a fertigation study conducted at Sumter, SC.

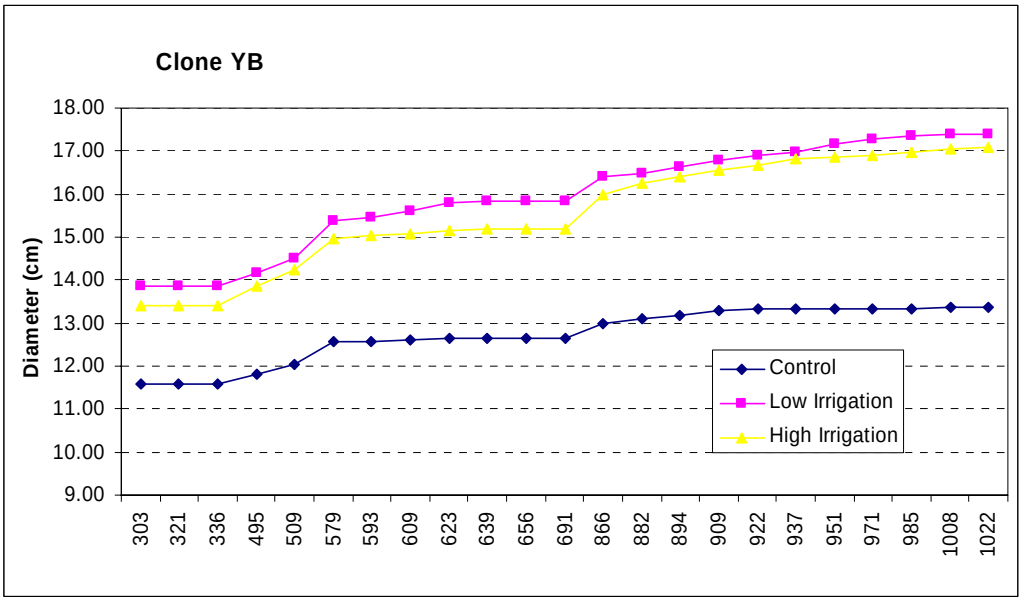


**Figure 6.** Diameter growth for cottonwood clone YB grown in Sumter, SC under no, low and high irrigation levels from 1998 through 2000.

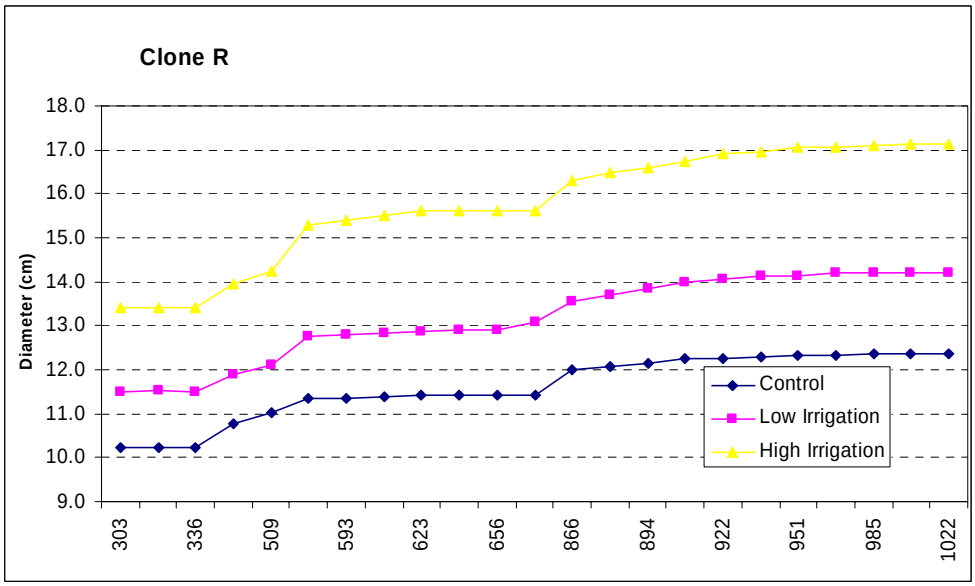


**Figure 7.** Diameter growth for cottonwood clone R grown in Sumter, SC under no, low and high irrigation levels from 1998 through 2000.

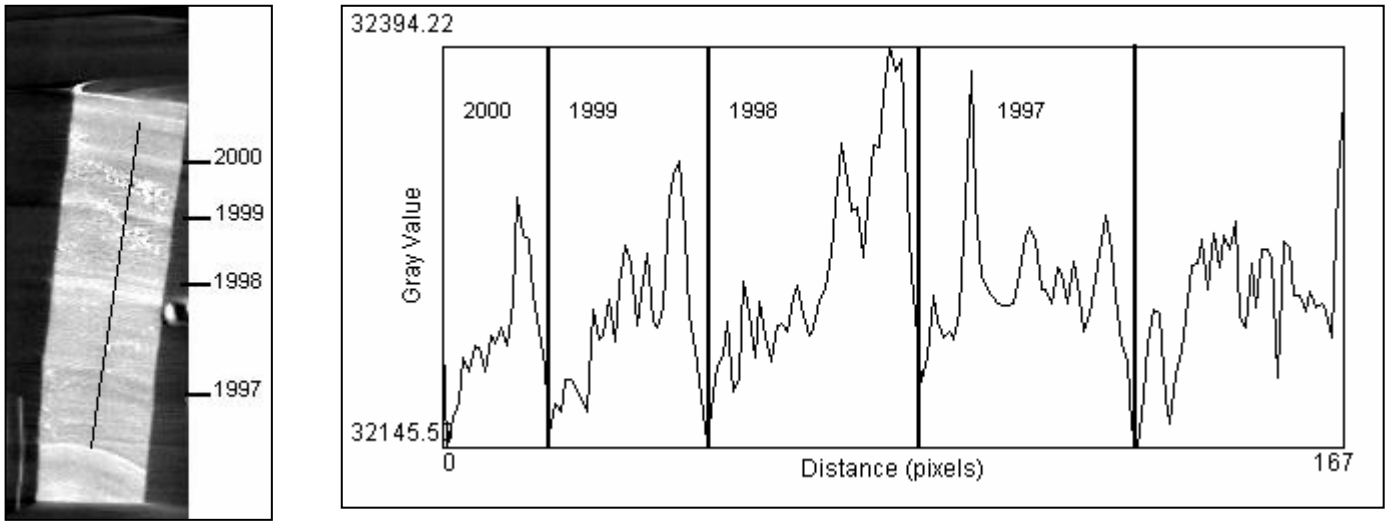


**Figure 8.** Intraannual variation in wood density for cottonwood clone RB grown in Sumter, SC under low irrigation levels from 1996 through 2000.

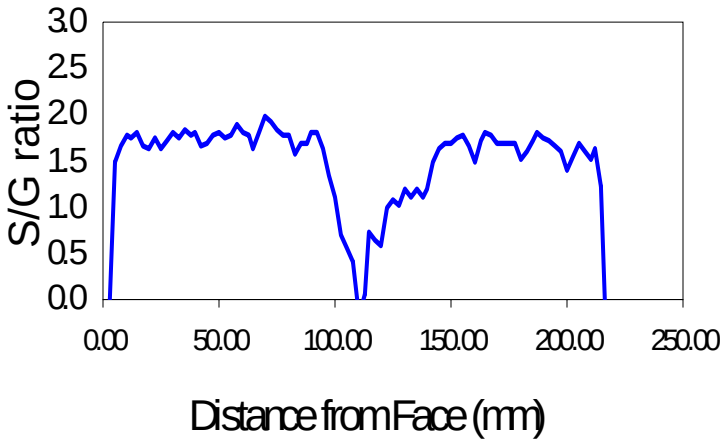


**Figure 9.** Intraannual variation in syringyl to guaiacyl lignin for cottonwood clone RB grown in Sumter, SC under low irrigation levels from 1996 through 2000.

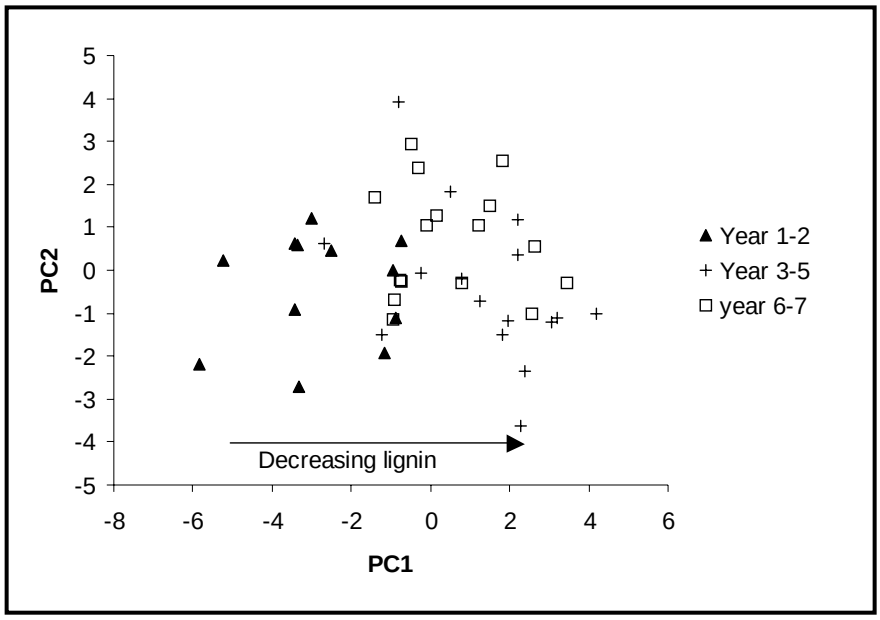


**Figure 10.** Scatter plot of principal component 1 vs. principal component 2 of pyMBMS data obtained from wood sections taken as a function of growth.

### 3.0 Tables

**Table 1.** Adjusted $R^2$ values for individual climatic and/or silvicultural data and weekly diameter measures from fertigated eastern cottonwood clonal trial.

| Early 1999 | Solar Radiation | Potential Evapotranspiration | Soil Temperature | Pecepitation | Irrigaiton Levels |
|---|---|---|---|---|---|
| R Control | 0.95 | 0.92 | 0.88 | 0.85 | na |
| R High Irrigation | 0.99 | 0.99 | 0.99 | na | 0.99 |
| YB Control | 0.97 | 0.96 | 0.94 | 0.92 | na |
| YB High Irrigation | 0.98 | 0.97 | 0.96 | na | 0.96 |
| W Control | 0.96 | 0.95 | 0.92 | 0.89 | na |
| W High Irrigation | 0.98 | 0.97 | 0.97 | na | 0.96 |
| Full 1999 | | | | | |
| R Control | 0.85 | 0.86 | 0.85 | 0.51 | na |
| R High Irrigation | 0.93 | 0.94 | 0.97 | na | 0.84 |
| YB Control | 0.88 | 0.89 | 0.89 | 0.55 | na |
| YB High Irrigation | 0.91 | 0.92 | 0.96 | na | 0.80 |
| W Control | 0.88 | 0.88 | 0.88 | 0.54 | na |
| W High Irrigation | 0.86 | 0.89 | 0.93 | na | 0.74 |
| Full 2000 | | | | | |
| R Control | 0.91 | 0.91 | 0.88 | 0.84 | na |
| R High Irrigation | 0.90 | 0.91 | 0.86 | na | 0.85 |
| YB Control | 0.72 | 0.73 | 0.66 | 0.61 | na |
| YB High Irrigation | 0.94 | 0.94 | 0.91 | na | 0.91 |
| W Control | 0.75 | 0.75 | 0.68 | 0.62 | na |
| W High Irrigation | 0.81 | 0.81 | 0.75 | na | 0.75 |

**Table 2.** Lignin and cellulose values for a set of *Populus* standards used to calibrate the NIR prediction models.

| Sample ID | Lignin | Holocellulose | Alpha cellulose |
|---|---|---|---|
| 998-069 | 21.6% | 87% | 53% |
| 998-069 | 21.6% | 83% | 55% |
| 93968 | 21.6% | 83% | 58% |
| 331-1780 | 22.4% | 82% | 50% |
| 998-070 | 23.8% | 82% | 52% |
| 80-3xtd | 24.0% | 89% | 51% |
| 73-1xtd | 24.1% | 88% | 51% |
| N2 xtd | 24.2% | 88% | 47% |
| 80-1xtd | 24.3% | 89% | 47% |
| 998-071 | 24.4% | 80% | 52% |
| 352xtd | 25.8% | 82% | 56% |
| 148xtd | 26.7% | 83% | 58% |
| 328xtd | 27.1% | 79% | 54% |
| 147 | 27.5% | 84% | 55% |
| 147xtd | 27.5% | 85% | 55% |
| 212xtd | 27.6% | 83% | 53% |

"xtd" indicates ethanol extraction before cellulose determination

**4.0 Publications/Presentations:**

Tuskan, G.A. 2002. Annual summary/presentation “Environmental influences on wood chemistry and density of *Populus* and loblolly pine” to the AF&PA Review committee in Savannah, GA, June 14, 2002.

Davis, M., G.A. Tuskan, M.M. Payne and R. Meilan. 2006. Assessment of *Populus* wood chemistry following the introduction of a Bt toxin gene. Tree Physiology 26:557–564.