An Integrated Functional Genomics Consortium to Increase Carbon Sequestration in Poplars: Optimizing Aboveground Carbon Gain

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***Executive Summary*:**

This project used gene expression patterns from two forest Free-Air CO_2 Enrichment (FACE) experiments (Aspen FACE in northern Wisconsin and POPFACE in Italy) to examine ways to increase the aboveground carbon sequestration potential of poplars (*Populus*). The aim was to use patterns of global gene expression to identify candidate genes for increased carbon sequestration. Gene expression studies were linked to physiological measurements in order to elucidate bottlenecks in carbon acquisition in trees grown in elevated CO_2 conditions. Delayed senescence allowing additional carbon uptake late in the growing season, was also examined, and expression of target genes was tested in elite *P. deltoides* x *P. trichocarpa* hybrids.

In *Populus euramericana,* gene expression was sensitive to elevated CO_2, but the response depended on the developmental age of the leaves. Most differentially expressed genes were upregulated in elevated CO_2 in young leaves, while most were downregulated in elevated CO_2 in semi-mature leaves. In *P. deltoides* x *P. trichocarpa* hybrids, leaf development and leaf quality traits, including leaf area, leaf shape, epidermal cell area, stomatal number, specific leaf area, and canopy senescence were sensitive to elevated CO_2. Significant increases under elevated CO_2 occurred for both above- and belowground growth in the F-2 generation. Three areas of the genome played a role in determining aboveground growth response to elevated CO_2, with three additional areas of the genome important in determining belowground growth responses to elevated CO_2. In *Populus tremuloides*, CO_2-responsive genes in leaves were found to differ between two aspen clones that showed different growth responses, despite similarity in many physiological parameters (photosynthesis, stomatal conductance, and leaf area index). The CO_2-responsive clone shunted C into pathways associated with active defense/response to stress, carbohydrate/starch biosynthesis and subsequent growth. The CO_2-unresponsive clone partitioned C into pathways associated with passive defense and cell wall thickening. These results indicate that there is significant variation in gene expression patterns between different tree genotypes. Consequently, future efforts to improve productivity or other advantageous traits for carbon sequestration should include an examination of genetic variability in CO_2 responsiveness.

Background, Methods and Activities

This project (POPGENICS) examined ways to increase the aboveground carbon sequestration potential of poplars. The project used gene expression patterns from two forest FACE experiments (Aspen FACE in northern Wisconsin and POPFACE in Italy) in combination with Quantitative Trait Loci (QTL) analysis, using a mapping pedigree of *Populus*. The overall goal was to link patterns of gene expression to candidate genes for increased carbon sequestration, using a combination of transcriptomics and QTL analysis. Ultimately, this information can be linked to specific regions of the recently sequenced poplar genome. In addition, our METLA collaborators examined similar characteristics with birch using samples from our Aspen FACE experiment.

Gene expression and tree productivity. During 2004, the POPFACE site in Italy was subjected to intensive sampling with relevance to the POPGENICS study. Three samples (June, July and

August) were taken during the growing season, whilst four samples were taken during the period of senescence from mid-September through mid-November. On each occasion, samples of leaves that were fully mature (LPI 9) were frozen in liquid nitrogen from all plots (3 ambient and 3 elevated CO_2) and two nitrogen treatments. Both *P.* x *euramericana* and *P. nigra* trees were sampled and RNA was extracted from these leaves. Following this they were used for microarray analysis. These samples were used to test the hypothesis that exposure to elevated CO_2 leads to delayed autumnal senescence and that early signaling genes in this response can be identified using a transcriptomic approach.

During 2004, 2005, and 2006 samples were taken from all three replicates of four treatments at Aspen FACE (control, +CO_2, +O_3 and +CO_2+O_3) at three times (June, July, August) for *Populus tremuloides* and *Betula papyrifera*. A single late autumn (October) sample of *P. tremuloides* was taken for senescence in 2004, and late season samples were taken three times in 2005 and 2006. On each occasion, short-shoot leaves were taken from the mid to upper canopy in full sun. Microarray analysis was conducted, and real time PCR was run to verify microarray analyses. Early results from this work allowed us to compared CO_2-responsive and CO_2-nonresponsive clones (Taylor et al. 2005, Rae et al. 2007).

QTL detection for adaptive traits related to productivity. We subjected an interspecific hybrid poplar pedigree, with 280 F-2 individual genotypes, to either ambient or elevated CO_2, using an open-top chamber facility. This allowed us to map 97 QTL for adaptive traits that may be linked to poplar aboveground productivity, including the phonological trait, 'senescence index'. These QTL showed that both common and differential areas of the genome are responsible for adaptive traits in elevated CO_2. For example, four commonly occurring and two differentially expressed QTL were detected for 'senescence index'. In POPGENICS, we linked these QTL to the physical sequence using a combination of approaches, including the use of microarrays to follow gene expression of extremes in the QTL mapping population.

Physiological measurements. We collected a series of physiological measures throughout the growing season for comparison with gene expression. These included diurnal photosynthesis curves, SPAD readings, and water balance determination. Samples for biochemical and physiological markers for carbon and nitrogen metabolism were taken at both the Aspen FACE and POPFACE sites. Analysis of these samples facilitated attempts to determine if the trees are nitrogen or carbon limited at elevated CO_2 and helped identify metabolic targets that could be manipulated to improve carbon sequestration.

Transformation of elite hybrid poplar genotypes. We obtained unrooted cuttings of 15 of D. Riemenschneider's elite *P. deltoides* x *P. trichocarpa* hybrids backcrossed with *P. deltoides* to increase disease resistance. We rooted these cuttings and have them in various stages of *in vitro* culture in preparation for transformation with gene cassettes to knock out flowering. These genotypes are not easily transformed, and we have continued to optimize transformation regimes throughout the POPGENICS project.

Collaborations and other activities. Our POPGENICS project involved collaboration between scientists from Michigan Technological University, Brookhaven National Laboratory, the

University of Alabama-Huntsville, the University of Southampton (UK), the USFS Northern Research Station, the Canadian Forest Service, the University of Helsinki, METLA (Finland), INRA (France), and Nanjing University (China). Leveraged support, beyond the original DOE support, was obtained from Canadian Forest Service, the University of Helsinki, METLA, INRA, and Nanjing University. We organized a session entitled "Poplars in a Changing World: Understanding Responses to Climate Change" at the Fourth International Poplar Symposium in Nanjing, China on June 5-9, 2006. Several of the talks were directly related to our POPGENICS Consortium

Results

Gene expression and tree productivity.

POPGENICS funding helped Gupta et al. (2005) complete publication of their analysis of 4600 *Populus* expressed sequence tags (ESTs) using trees of trembling aspen (*Populus tremuloides*) clone 216 exposed to elevated CO_2 and/or O_3 at Aspen FACE. A total of 238 genes showed qualitatively similar expression in at least one treatment and were retained for analysis. Under elevated CO_2, a relatively small number of genes was up-regulated, whereas elevated O_3 caused higher expression of many signaling and defense-related genes and lower expression of several photosynthesis and energy-related genes. Senescence-associated genes (SAGs) and genes involved in the flavanoid pathway were also up-regulated under O_3, for both ambient and elevated CO_2. The combined treatment of $+CO_2+O_3$ resulted in the differential expression of genes that were not up-regulated with individual gas treatments.

Taylor et al. (2005) used cDNA microarrays for *Populus euramericana* (clone I-214) that had received 6 years of elevated CO_2 to study long-term changes in genetic expression in response to elevated CO_2. They found gene expression was sensitive to elevated CO_2, but the response depended on the developmental age of the leaves. Less than 50 transcripts differed significantly between elevated CO_2 and control conditions. Most differentially expressed genes were upregulated in elevated CO_2 in young leaves, while most were downregulated in elevated CO_2 in semi-mature leaves. For transcripts related only to the small subunit of Rubisco, upregulation in LPI 3 and downregulation in LPI 6 leaves in elevated CO_2 was confirmed by ANOVA. Similar patterns of gene expression for young leaves were also confirmed independently across year 3 and year 6 microarray data, and using real-time RT-PCR.

During its final years, the POPGENICS team completed comprehensive examinations of leaf transcription profiles, physiological characteristics, and primary metabolites of two *Populus tremuloides* genotypes known to differ in their responses to long-term elevated CO_2 at the Aspen FACE (Cseke et al. 2009). Physiological responses of these clones are similar in photosynthesis, stomatal conductance, and leaf area index under elevated CO_2, yet very different in growth enhancement (0-10% in clone 216; 40-50% in clone 271). While few genes responded to long-term exposure to elevated CO_2, the transcriptional activity of leaf CO_2-responsive genes was distinctly different between the clones, differentially impacting multiple pathways during both early and late growing seasons. Analysis of transcript abundance and carbon/nitrogen biochemistry suggests that the CO_2-responsive clone (271) partitions C into pathways associated

with active defense/response to stress, carbohydrate/starch biosynthesis and subsequent growth. The CO_2-unresponsive clone (216) partitions C into pathways associated with passive defense (e.g. lignin, phenylpropanoid) and cell wall thickening. These results indicate that there is significant variation in expression patterns between different tree genotypes in response to long-term exposure to elevated CO_2. Consequently, future efforts to improve productivity or other advantageous traits for carbon sequestration should include an examination of genetic variability in CO_2 responsiveness.

QTL detection for adaptive traits related to productivity and senescence.

Rae et al. (2006) determined QTL for leaf growth, development, quality and leaf senescence in an F-2 hybrid of *Populus trichocarpa* T. & G and *Populus deltoides* Marsh, following season-long exposure to either current day ambient CO_2 or elevated CO_2 at 600 $\mu l\ l^{-1}$. Leaf growth and development differed between the grandparents such that *P. trichocarpa* showed greater response to elevated CO_2. In the F-2 generation, leaf development and quality traits including leaf area, leaf shape, epidermal cell area, stomatal number, specific leaf area (SLA), and the phenology trait, canopy senescence index, were sensitive to elevated CO_2. This latter finding is consistent with the findings of Taylor et al. (2008) that a significant delay exists in the decline of autumnal canopy leaf area index under elevated CO_2 at both Aspen FACE and POPFACE.

Sixty-nine QTL were mapped for the 19 traits of plants in ambient CO_2, while 60 QTL were mapped for plants in elevated CO_2 (Rae et al. 2006). They found many QTL mapped to common positions in both ambient and elevated CO_2, confirming their importance in determining growth. However, there was also differential genetic control for a number of traits including leaf senescence. Candidate genes were shown to collocate to regions where response QTL mapped. In further work from the same study, Rae et al. (2007) reported significant increases under elevated CO_2 for both above- and below-ground growth in the F-2 generation. Three areas of the genome, on linkage groups I, IX and XII, played a role in determining aboveground growth response to elevated CO_2. Three additional areas of the genome, on linkage groups IV, XVI and XIX, were important in determining belowground growth responses to elevated CO_2.

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