Title: A Rapid Semi-Automated Phenotyping System to Capture the Highly Useful Diameter at Breast Height In *Populus trichocarpa*

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Project Goals: The Center for Bioenergy Innovation (CBI) vision is *to accelerate domestication of bioenergy-relevant, non-model plants, and microbes to enable high-impact innovations at multiple points in the bioenergy supply chain.* CBI addresses strategic barriers to the current bioeconomy in the areas of 1) high-yielding, robust feedstocks, 2) lower capital and processing costs via consolidated bioprocessing (CBP) to specialty biofuels, and 3) methods to create valuable byproducts from the lignin. CBI will identify and utilize key plant genes for growth, composition and sustainability phenotypes as a means of achieving lower feedstock costs, focusing on poplar and switchgrass. We will convert these feedstocks to specialty biofuels (C4 alcohols, C6 esters and hydrocarbons) using CBP at high rates, titers and yield in combination with cotreatment, pretreatment or catalytic upgrading. CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

Abstract text: The goal of this project is to reduce the complexity and difficulties associated with capturing accurate precise phenotypes (traits) and encoding the resulting data into easily useable formats for bioinformatic analysis. Here we discuss the implementation of a novel scientific instrument that logs a sample identity via a radio frequency tag on the specimen and records image information. This novel device and more like it will reduce human error and rapidly increase phenotyping efforts.

Diameter at breast height (DBH) is an important phenotype in tree breeding, often correlating with many other phenotypic traits such as height and biomass which are important for biofuel production. In the post genomic revolution, phenotyping has become a major bottleneck in scientific research. Manual measurements are prone to human error as is recording or transcribing to electronic records. Therefore, we have developed a novel hand-held semi-automated DBH camera that captures sample IDs through encoded RFID tags and rapid highly calibrated time-stamped measurements through image processing.

We assessed how this instrument compared to manual phenotyping in a common garden in East Tennessee. Field studies often have many hundreds to thousands of samples, for example, some CBI field sites have up to 4,500 trees. As such, scalable, accurate phenotyping is very important. Speed trials of two people with a manual tape measure and manual clipboard measured 14 trees in 20 minutes, where one person with the DBH Camera captured 60 tree DBH measurements in the same amount of time, or 180 trees in 60 minutes. This 4.3x increase in efficiency means two

people can phenotype 4,500 trees in 12.5 hours (≈25 person hours) with minimal human error, versus 107.14 hours using two people or 214.28 person hours. Thus, the new device requires only 23.34% of the original labor effort, not including the arduous manual typing of sample names and measurements. In contrast, the DBH camera data is automatically captured and produces formats common for analysis software making it near instantly accessible. Thus, such handheld automated phenotyping devices enable the same number of people to capture a wider array of phenotypes with more accuracy and precision in smaller time-windows. This reduces the associated costs for fieldwork campaigns meaning more traits can be used for crop improvement methods.

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