

Effects of *Claviceps spp./Epicoccum andropogonis* inoculation on switchgrass phenotypic traits

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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: This project is part of the strategic effort to identify, quantify, and evaluate the risk potential of emerging pathogens in switchgrass. This work addresses several under-investigated topics, such as the effects of specific biotic stresses on switchgrass development. The goals of the work presented here are to 1) identify and quantify the causal agent responsible for false smut on switchgrass in Georgia and 2) evaluate the effects of *Claviceps spp./Epicoccum andropogonis* infection on switchgrass phenotypic traits including flowering, seed yield, and biomass production.

Switchgrass is a perennial C4 grass native to North America that has been used for grazing, soil conservation, and as a bioenergy crop. Switchgrass is affected by several diseases that could impact its industrial development. In 2019, black sclerotia with a brain-like appearance were observed on the surface of seeds of accessions from the Center for Bioenergy Innovation (CBI) genome-wide association study (GWAS) panels planted in Watkinsville, GA, Tifton, GA, and Knoxville, TN (Figure 1). The causal agent in all three panels was identified morphologically and molecularly as *Epicoccum andropogonis* independently at the University of Georgia (UGA) and the University of Tennessee



Figure 1: Panicle (A) and close-up of seed (B) infected with *Epicoccum andropogonis*.

(UTK)/Cornell University. *Epicoccum andropogonis* is a mycoparasite responsible for false smut disease and is known to grow saprophytically on the honeydew of *Claviceps* species that cause ergot disease in grasses. To assess the impact of *Claviceps spp./ E. andropogonis* on switchgrass development, cultivar "Alamo" and "Summer" seeds were inoculated with this complex sampled from the Watkinsville, GA field site, and plant development was followed in the greenhouse for 10 months. ANOVA analysis revealed non-significant differences between plants grown from inoculated and non-inoculated seed in plant height at flowering ($p=0.867$ for Alamo, $p=0.0504$ for Summer), the number of tillers ($p=0.583$ for Alamo, $p=0.894$ for Summer), panicle length ($p=0.506$ for Alamo, $p=0.218$ for Summer), and biomass production ($p=0.647$ for Alamo, $p=0.811$ for Summer). However, 10-month old inoculated Alamo and non-inoculated Summer genotypes were significantly taller than non-inoculated Alamo ($p=0.00744$) and inoculated Summer genotypes ($p=1.35e-07$), respectively. In addition, inoculated Alamo genotypes had a lower number of seeds per tiller ($p=0.0135$) and a lower number of spikelets per tiller ($p=0.0031$) than non-inoculated Alamo. No significant difference was observed between inoculated and inoculated Summer genotypes for either trait ($p=0.156$ and $p=0.664$, respectively). Furthermore, chi-square tests showed that inoculated Alamo genotypes had a higher percentage of flowering tillers ($p=0.00057$) than non-inoculated Alamo. Again, no significant difference was observed between inoculated and non-inoculated Summer plants for this trait ($p=0.8256$). Overall, these preliminary results suggest that *Claviceps spp./Epicoccum andropogonis* inoculation more significantly impacts the flowering process and seed production of switchgrass than its biomass production. Additional experiments with a larger number of genotypes need to be carried out to confirm this pattern. Quantifying *Claviceps spp./Epicoccum andropogonis* levels in the plant tissues (panicle and leaf organs) is also needed to better interpret our results.

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