Testing Unifying Theories of Ozone Response in C_4 Bioenergy Grasses

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Project Goals: The objectives of this study are to: i) quantify genotypic and species variation in O_3 sensitivity among C_4 bioenergy crop species, and ii) identify the factors underlying interspecific variability in sensitivity to O_3.

Abstract: Tropospheric ozone (O_3) is a damaging and widespread air pollutant that is detrimental to human and ecosystem health worldwide[1,2]. Current background O_3 concentration is estimated to decrease the yields of maize (*Zea mays*) up to 10% in the United States and reduce global crop yield (maize, soybean, rice and wheat combined) by 227 Tg annually, which portends a significant threat to future global food and energy security[3,4]. However, it is unclear how other bioenergy feedstocks, including switchgrass (*Panicum virgatum*)[5], sorghum (*Sorghum bicolor*)[6], and miscanthus (*Miscanthus ×giganteus*), respond to O_3 stress, or whether these species share a similar O_3 sensitivity as maize.

Tropospheric O_3 negatively impacts plant growth and development at various biological processes from molecular to whole plant scales[1,2]. Although plant response to O_3 is complex, it is well-recognized that some plant species are more sensitive to O_3 than others. There are several mechanisms that potentially explain genotypic and/or species variability in O_3 sensitivity in C_3 species. For example, species-specific variation sensitivity to O_3 in C_3 tree species depends upon stomatal flux of ozone per unit leaf area, so both stomatal conductance and leaf mass per unit area (LMA) are important traits determining sensitivity[7,8]. However, there is currently a lack of unifying mechanistic explanation for variation among C_4 plants in response to O_3. Considering that leaf Kranz anatomy enables the concentration of CO_2 around Rubisco in the bundle sheath cells, limited photorespiration, and lower stomatal conductance, C_4 species may respond differently to O_3 than C_3 species.

In this study, we examined the photosynthetic response of 22 genotypes of four C_4 bioenergy species (switchgrass, sorghum, maize and miscanthus) to elevated O_3 using the unique capabilities of Free Air Concentration Enrichment (FACE) technology, which provides elevated concentrations of O_3 (100 nL L^{-1}) in open-air plots at the field scale. Because different species display different visual symptoms of O_3 damage, we proposed the reductions of photosynthetic traits (the maximum carboxylation capacity of phosphoenolpyruvate (V_{pmax}) and the maximum CO_2-saturated photosynthetic capacity (V_{max})) to O_3 as a proxy to estimate O_3 sensitivity. The

studied species displayed strong variability in V_{pmax} and V_{max} within each species. Across all species, V_{pmax} and V_{max} varied 3.8- and 2.1-fold, respectively. Elevated O_3 concentration did not alter V_{pmax} in any genotypes of switchgrass and miscanthus and reduced V_{max} in maize lines, indicating variation among C_4 species in O_3 sensitivity. O_3-induced reduction in V_{pmax} was positively associated with LMA, but negatively correlated with stomatal conductance on either an area (g_{sa}) or a mass (g_{sm}) basis. However, O_3-induced reduction in V_{max} was not correlated with LMA, but scaled negatively with g_{sa} and g_{sm}. Structural equation models provided further evidence that both V_{pmax} and V_{max} were directly related to stomatal conductance rather than to LMA. We demonstrate that there is significant variation in O_3 sensitivity among C_4 species, with maize and sorghum more sensitive to O_3 than switchgrass and miscanthus. We also demonstrate genotypes with higher stomatal conductance were more sensitive to O_3 compared to genotypes with lower stomatal conductance, and interspecific variation in O_3 sensitivity is determined by direct effects of stomatal conductance and indirect effects of LMA. Such a side-by-side field comparison study has not been conducted so far, and to our knowledge, this is the first study to provide a test of unifying theories explaining variation in O_3 sensitivity in C_4 bioenergy grasses. This information could aid in optimal placement of diverse C_4 bioenergy feedstocks across a polluted landscape.

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