

How will future elevated levels of CO₂ and O₃ affect antioxidant status in soybean?

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Introduction

By 2050, ecosystems in the U.S. will experience 50% higher concentrations of atmospheric carbon dioxide ([CO₂]) and 20% higher concentrations of tropospheric ozone ([O₃]) than current levels. **Elevated [CO₂] stimulates photosynthesis and growth, while elevated [O₃] impairs photosynthesis and causes accelerated leaf senescence.**

Changes in the oxidative status of leaf tissue are associated with these physiological responses. **Elevated [CO₂] is expected to decrease ROS production in the chloroplast by increasing photochemical quenching of light energy. Ozone enters the leaf through the stomata and generates ROS in mesophyll cells. Elevated [O₃] will increase flux into the leaf. Both elevated [CO₂] and [O₃] decrease stomatal conductance, and it is unknown how the combination of gases will affect antioxidant status.**

In 2005, total leaf antioxidant capacity increased over the growing season. Also, the combination of elevated [CO₂] and elevated [O₃] increased antioxidant capacity late in the growing season. It is unknown which specific antioxidants drive changes in overall antioxidant status.

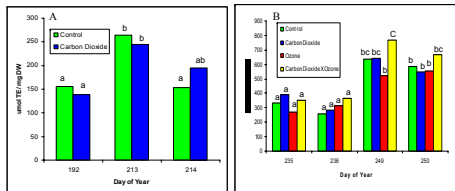


Figure 1. Antioxidant capacity as measured early during reproductive growth (A) and late during reproductive growth (B). Values are least squared means + SE. The statistically significant differences among treatments and sampling periods are indicated by a different letter ($P < 0.1$).

Objectives

How do different components of the antioxidant system in field grown soybean respond to:

- (1) Growth at elevated [CO₂] and [O₃] levels predicted for 2050?
- (2) Changes in leaf age?
- (3) Seasonal variation in weather conditions?

Methods



Field Site Description
Glycine max is grown at the SoyFACE experimental facility at University of Illinois at Urbana-Champaign. Plants are grown in one of four treatments: ambient CO₂ and O₃, elevated CO₂ (550 ppm), elevated O₃ (20% over current ambient), or a combination of both elevated CO₂ and O₃. A cohort of leaves was tagged on day of year (DOY) 171 and tissue was collected every 7-10 days until the onset of leaf senescence. Leaf disks were sampled at midday and immediately frozen in liquid N and stored at -80°C until analysis. Data is presented as the least squared mean +/- SE (n=4 experimental plots).

Antioxidant Assays
Ascorbate was quantified according to Okamura *et al.* (1980). Total phenolic concentration was quantified by a modified Folin-Ciocalteu method based on Singleton and Rossi (1965). Total antioxidant capacity was measured utilizing the Oxygen Radical Absorbance Assay adapted for the microplate (Huang *et al.* 2002).

Results

➤ Total antioxidant capacity increases with leaf age at all [CO₂] and [O₃].

➤ The elevated [CO₂] and elevated [O₃] combination treatment increased total antioxidant capacity in mature leaves.

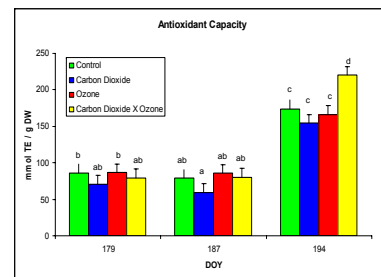


Figure 2. Antioxidant capacity as measured across leaf development during vegetative growth in 2006. Values are least squared means + SE. The statistically significant differences among treatments and sampling periods are indicated by a different letter ($P < 0.1$).

Results

➤ Total ascorbate concentration varies across leaf age and among treatments, while the ratio of DHAsA : AsA increases significantly in older leaves.

➤ Total phenolic concentration is variable across leaf age and among treatments.

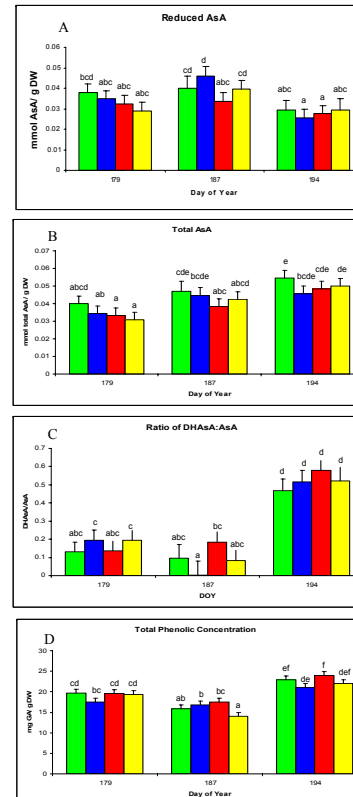


Figure 3. Reduced Ascorbate (A), Total Ascorbate (B), Ratio of Dehydroascorbate : Ascorbate (C), and Total Phenolic Concentration (D) as measured across leaf development during vegetative growth. Values are least squared means + SE. The statistically significant differences among treatments and sampling periods are indicated by a different letter ($P < 0.1$).

Discussion

➤ By measuring total antioxidant capacity along with several aspects of the antioxidant system, we are closer to understanding how field grown plants respond to oxidative stress when exposed to conditions predicted for 2050.

➤ Total soybean leaf antioxidant capacity significantly increased in older leaves.

➤ As expected, elevated CO₂ decreased antioxidant capacity, although not significantly, at all leaf ages measured.

➤ Despite our working hypothesis that O₃ would increase oxidative stress, a 20% increase in O₃ did not consistently alter total leaf antioxidant capacity or any parameter of the antioxidant system that we have measured.

➤ The combination of elevated CO₂ and O₃ significantly increased the total leaf antioxidant capacity in the oldest leaves measured, although this increase is not reflected in any of the components of the antioxidant system measured.

➤ Because the total antioxidant capacity of soybeans grown in elevated CO₂ decreased, there is the potential for greater damage due to acute episodes of high tropospheric O₃ during times when the total oxidative stress response is not primed.

➤ Previous work done at SoyFACE has shown that O₃ damage is cumulative and most significant during reproductive growth, therefore it will be important to also follow a cohort of leaves that develop through reproductive growth this season.

➤ The antioxidant system consists of many metabolites and enzymes. We have only measured some of the key metabolites in the antioxidant system. In order to obtain a more complete picture, it will be important to extend this research into the other contributing parameters such as the different enzymes involved in maintaining cellular redox status.

➤ In order to link this antioxidant system work to the observed physiological effects of elevated CO₂ and elevated O₃, we will combine these results with the other physiological and molecular results from SoyFACE such as: photosynthesis measurements, yield and biomass data, metabolite profiling, and gene transcript analysis.

Acknowledgments

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