Soy Free-Air Concentration Enrichment technology (SoyFACE)
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Goals: We propose (1) to examine the physiological and molecular bases of pathology to the soybean crop resulting from rising tropospheric ozone levels, and how this will interact with diseases of the crop; (2) to determine the extent to which germplasm and engineered plants with altered oxidative signaling to increase tolerance of ozone show altered field disease susceptibility; and, (3) to assess the degree of interaction between simultaneous plant disease and changes in tropospheric CO2 and ozone. These will be critical first steps in establishing biotechnological approaches to increasing tolerance/resistance to changing atmospheric conditions.

Procedures: Although rising tropospheric ozone has long been recognized as a factor causing a pathological loss in crop yields, all prior experiments have been conducted within chamber environments (Long *et al.*, 2004, Morgan *et al.*, 2003). As with disease, the pathology of plants treated with ozone is likely to differ greatly between greenhouse and open field environments. Soybean will be grown under fully open air conditions controlling ozone treatment with Free-Air Concentration Enrichment technology (www.soyface.uiuc.edu; Morgan *et al.*, 2004). From germination to harvest, replicate plots (70 ft. diameter) will be treated with ambient+25% ozone, elevated ozone plus CO_2 elevated to 550 ppb, plus four control plots. Half of each plot will include a single major commercial soybean variety, the other half divided into 20 sub-plots each containing a variety selected or engineered for varied ozone or disease tolerance. One sub-plot will include a range of *Arabidopsis* lines (Miyazaki *et al.*, 2004). Disease incidence and severity will be monitored throughout development via observation, *in situ* physiological assessment, and genomic and proteomic analysis (Miyazaki *et al.*, 2004, Morgan *et al.*, 2004). Yield will be measured at harvest for three growing seasons.

Timelines: Treatments will be repeated annually for three years. *Year 1:* Physiological analysis of pathology of elevated ozone, disease incidence, and germplasm assessment. Assessment of *Arabidopsis* lines, including transcriptome analysis. *Year 2.* Use of soybean (and *Arabidopsis*) lines selected for low and high tolerance to atmospheric changes from year 1. Assessment of impact of ozone on controlled introduction of pathogens in small soil isolated sub-plots, potentially SDS. Assessment of soybean transcriptome (expressed genes) of control and treated plants. *Year 3.* Further selection of lines for low and high tolerance, including engineered lines.

Impact of the Soy Free-Air Concentration Enrichment technology (SoyFACE): The U.S. along with other countries has seen a substantial rise in tropospheric ozone concentrations. Through a series of economic and atmospheric chemistry models, the UN Intergovernmental Panel on Climate Change project that substantial increase will continue through this century; 20% by 2050 and 45% by 2100 (Prather, 2001, Prather *et al.*, 2003). Soybean is one of the most sensitive crops to ozone, showing pathological symptoms and yield loss at a peak daytime summer concentration of 40 ppb (Ashmore, 2002; Morgan *et al.* 2003). Today, peak daytime summer concentrations average 60 ppb in Illinois and are expected to rise to 75 ppb by 2050, at a rate of about 0.3 ppb per year (Prather, 2001, Prather *et al.*, 2003). Chamber experiments suggest that soybean will loose 1% of yield for every 1ppb increase above 40 ppb. This predicts that for every 3 years yield will decrease by 1%, all other factors being equal (Ashmore, 2002). This potentially impacts competitiveness of the US crop, since the S. American growing areas are not expected to be exposed to damaging ozone levels (Prather, 2001). Therefore, ozone represents a specific threat to the US soybean crop. However, these effects have not been established under open-air field conditions. This proposal will be a unique test of the predictions made in controlled environments. In addition, rising ozone is known to alter susceptibility to fungal diseases – in particular rusts. For example, (Karnosky *et al.*, 2002) have reported a 3- to 5-fold increase in aspen leaf rust, caused by *Melampsora medusae*, under elevated O_3 over several growing seasons. This disease was particularly enhanced under O_3 for O_3-sensitive aspen clones. If the same applies to soybean rust, this disease could have a much greater impact under the higher ozone conditions of the Midwest than it has had in S. America. Ozone and disease may have a complex interaction. Programmed cell death (PCD) is an important defense against spread of infection but is also induced by ozone and is one of the mechanisms by which ozone lowers yield. Therefore, germplasm which displays tolerance of ozone, may have decreased capacity for PCD and for disease resistance. Documenting these interactions under field conditions will be critical for guiding appropriate biotechnological approaches to improvement.

Dr. Long's research team has surveyed all prior studies of the impacts of rising ozone and CO_2 on soybean production, physiology and pathology using meta-analytical statistics (Ainsworth *et al.*, 2002, Morgan *et al.*, 2003). These studies have indicated substantial increases in yield and photosynthesis under elevated CO_2, substantial decreases under elevated O_3, and increased pest and disease vulnerability with both changes. The SoyFACE facility is now providing us with the opportunity to determine whether these protected environment data differ substantially from the open field approach (Long et al., 2004). The open-air experiments are critical for disease since chambers prevent any natural dispersal of spores (Long et al., 2004). Our preliminary observations are that incidents of both diseases and pest damage to leaves can be increased up to 50% by the elevated CO_2 and also O_3. Water use is decreased substantially by both treatments, leaf nitrogen is increased by O_3 and soluble carbohydrates by CO_2, while cuticle and stomatal damage may also result from O_3. All are factors that may lead to increased infection. Responses of physiology are found to be different under open-air treatment. Elevated CO_2 causes smaller increases in photosynthesis than predicted by chamber studies, larger increases in leaf soluble carbohydrates and a significant decrease in the major leaf protein Rubisco (Rogers et al. 2004; Bernacchi et al. 2005). Elevated ozone has less of an impact on photosynthesis and production than observed in chambers prior to flowering, but pathological symptoms develop at R5 and beyond depressing photosynthesis and resulting in significantly lighter seeds at maturation (Morgan et al. 2004). Some significant differences within the germplasm have been identified but will need to be further tested.