Global Change Biology

Global Change Biology (2010) **16**, 320–330, doi: 10.1111/j.1365-2486.2009.01978.x

Elevated atmospheric carbon dioxide and ozone alter soybean diseases at SoyFACE

DARIN M. EASTBURN*, MELISSA M. DEGENNARO†, EVAN H. DELUCIA‡, ORLA DERMODY§ and ANDREW J. MCELRONE¶

*Department of Crop Sciences, University of Illinois, Urbana-Champaign, Urbana-Champaign, IL 61801, USA, †Department of Biology, Saint Joseph's University, Philadelphia, PA 19131, USA, ‡Department of Plant Biology, University of Illinois, Urbana-Champaign, Urbana-Champaign, IL 61801, USA, §Pioneer Hi-Bred Switzerland S.A., DuPont Agriculture & Nutrition, Via Cantonale/Galleria 3, CH-6928 Manno, Switzerland, and ¶USDA-ARS Crops Pathology and Genetics Research Unit, University of California, Davis, CA 95616, USA

Abstract

Human driven changes in the Earth's atmospheric composition are likely to alter plant disease in the future. We evaluated the effects of elevated carbon dioxide (CO_2) and ozone (O_3) on three economically important soybean diseases (downy mildew, *Septoria* brown spot and sudden death syndrome-SDS) under natural field conditions at the soybean free air concentration enrichment (SoyFACE) facility. Disease incidence and/or severity were quantified from 2005 to 2007 using visual surveys and digital image analysis, and changes were related to microclimatic variability and to structural and chemical changes in soybean host plants. Changes in atmospheric composition altered disease expression, but responses of the three pathosystems varied considerably. Elevated CO_2 alone or in combination with O_3 significantly reduced downy mildew disease severity (measured as area under the disease progress curve-AUDPC) by 39–66% across the 3 years of the study. In contrast, elevated CO_2 alone or in combination with O_3 significantly increased brown spot severity in all 3 years, but the increase was small in magnitude. When brown spot severity was assessed in relation to differences in canopy height induced by the atmospheric treatments, disease severity increased under combined elevated CO_2 and O_3 treatment in only one of the 3 years. The atmospheric treatments had no effect on the incidence of SDS or brown spot throughout the study. Higher precipitation during the 2006 growing season was associated with increased AUDPC severity across all treatments by 2.7 and 1.4 times for downy mildew and brown spot, respectively, compared with drought conditions in 2005. In the 2 years with similar precipitation, the higher daily temperatures in the late spring of 2007 were associated with increased severity of downy mildew and brown spot. Elevated CO_2 and O_3 induced changes in the soybean canopy density and leaf age likely contributed to the disease expression modifications.

Keywords: climate change, elevated CO_2, elevated ozone, fungal pathogens, *Fusarium virguliforme*, *Glycine max*, *Peronospora manshurica*, plant disease, plant–pathogen interactions, *Septoria glycines*, tropospheric composition

Received 8 December 2008 and accepted 27 March 2009

Introduction

Human activities have radically altered the earth's atmospheric composition, and these changes significantly impact plants and their interactions with other organisms (IPCC, 2007). Concentrations of carbon dioxide (CO_2) and tropospheric ozone (O_3) have increased markedly since the inception of the industrial revolution, and will continue to climb well into the 21st century; CO_2 is expected to double the preindustrial levels by 2050 while O_3 is increasing by as much as 2.5% annually (Prather & Ehhalt, 2001; Vingarzan, 2004; IPCC, 2007). Both elevated CO_2 and O_3 alter plant function but in opposite ways. In general, photosyn-

Correspondence: Andrew J. McElrone, tel. + 530 754 9763, fax + 530 752 0382, e-mail: ajmcelrone@ucdavis.edu

thetic capacity, growth and yield are positively affected by elevated CO_2 (Drake *et al*., 1997; Ainsworth *et al*., 2002; Dermody *et al*., 2008) but negatively affected by elevated O_3 (Manning & Tiedemann, 1995; Morgan *et al*., 2003) across a wide range of study species. Despite abundant evidence about the direct effects of atmospheric change on plants, we still need a more thorough understanding of their impact on plant diseases in agricultural ecosystems to properly predict and plan for disease pressure under future climatic conditions (Manning & Tiedemann, 1995; Coakley *et al*., 1999; Runion, 2003; Scarascia-Mugnozza *et al*., 2005; Garrett *et al*., 2006; Chakraborty *et al*., 2008).

Three key components are required for plant disease expression: a susceptible host plant, a prevalent and virulent pathogen, and environmental conditions that favor infection or alter host susceptibility. Interaction of these components can be conceptualized using the disease triangle model to illustrate how shifts in any one of these components can dramatically change the magnitude of disease expression in a given pathosystem (Scholthof, 2007). Changes in environmental conditions are known to exacerbate disease symptoms in plant pathosystems (e.g. McElrone *et al*., 2001) and are implicated in 44% of new disease emergence (Anderson *et al*., 2004). Given the integral role of environmental conditions in disease expression, altered atmospheric composition is expected to modify plant disease expression and pathogen load indirectly through changes in host plants (Manning & Tiedemann, 1995; Chakraborty *et al*., 2000, 2008; Garrett *et al*., 2006). Indeed, Karnosky *et al*. (2002) demonstrated that seasonal exposure to elevated CO_2 and O_3 significantly increases rust disease of aspen trees by altering leaf surface characteristics. Similarly, decreases in a maple leaf spot disease under elevated CO_2 were attributed to changes in stomatal physiology and altered leaf chemistry (McElrone *et al*., 2005). Given the variable responses of pathosystems to altered atmospheric composition studied to date (Manning & Tiedemann, 1995; Chakraborty *et al*., 2000, 2008; Garrett *et al*., 2006; Kobayashi *et al*., 2006), more work is needed to quantify changes in host–pathogen interactions particularly for important crop species under natural conditions.

One crop of particular importance throughout the world is soybean (*Glycine max*). Globally, soybean is the most widely planted dicot crop and has economic significance due to its wide variety of uses ranging from food and health products to printing inks and biodiesel (UN-FAO, 2002; Illinois Soybean Association, 2008). The response of soybean to elevated CO_2 and O_3 has been studied extensively (Ainsworth *et al*., 2002; Morgan *et al*., 2003), but little to no work has evaluated the influence of future atmospheric conditions on soybean diseases. Knowledge of these interactions is especially needed since there are ~ 35 economically important soybean pathogens, and under ambient atmospheric conditions they can cause annual losses of >24 million metric tons worldwide (Wrather *et al*., 1997; Hartman *et al*., 1999).

Until recently, most studies considering the individual and combined effect of elevated CO_2 and tropospheric O_3 have been performed in greenhouses, controlled environment chambers, transparent field enclosures or open top chambers. Data derived from such work may not represent all aspects of natural systems (Long *et al*., 2004) because of the exclusion of contributing abiotic and biotic factors. Implementation of Free Air gas Concentration Enrichment (FACE) systems has allowed researchers to expose study plants to altered atmospheric composition in agricultural and natural ecosystems with minimal impact on microclimate and without limiting the movement of biological organisms (e.g. insects and pathogens) (McLeod & Long, 1999; Scarascia-Mugnozza *et al*., 2005). Soybean Free Air gas Concentration Enrichment (SoyFACE) located at the University of Illinois was the first facility to expose study plants to elevated O_3 under completely open-air conditions within an agricultural field (Morgan *et al*., 2003). Working at SoyFACE allowed us to evaluate the influence of natural variability of meteorological factors (i.e. drought and temperature) in conjunction with the imposed atmospheric treatments. The primary objective of this study was to evaluate the effects of predicted future atmospheric composition (elevated CO_2 and O_3 individually and in combination) on naturally occurring soybean diseases across several growing seasons. Through visual inspection and digital image analysis, we quantified disease incidence and severity and related any changes to physiological, structural, and chemical responses of soybean host plant grown under the varying treatments.

Methods

Experimental field site and species

The SoyFACE facility is located on the University of Illinois campus (40°02′N, 88°14′W; for more site details see http://www.soyface.uiuc.edu) and was designed to examine the effect of elevated tropospheric CO_2 and O_3 on an agroecosystem. In each year of our study, sixteen 20 m diameter octagonal experimental plots were established within a 16 ha soybean field (Hamilton *et al*., 2005), and were divided evenly into four randomized blocks each containing one of the four different atmospheric treatments: ambient, elevated CO_2 (550 μmol m^{-3}), elevated O_3 (1.2 times ambient), and combination of elevated CO_2 and O_3 (550 μmol m^{-3} and 1.2 times

ambient, respectively). Concentrations of CO_2 and O_3 were controlled by an adjustable segmented ring encircling each plot area that released high velocity gas just above the surface of the crop canopy. Wind speed and direction were automatically and continually recorded to adjust the rates and position of gas release to maintain the desired concentrations of atmospheric gases within the plot (Rogers *et al*., 2004). Fumigation was performed during daylight hours from the time of planting and continued until harvest. Fumigation with O_3 was suspended during periods of low wind speeds or leaf wetness from rain or dew due to safety concerns and variability of uptake under these conditions, respectively. All plots were separated by 100 m to minimize cross-plot contamination. In all 3 years, the actual average concentrations of CO_2 in the elevated CO_2 rings ranged from 550 to 552 $\mu mol\,m^{-3}$, while levels in the ambient rings averaged $\sim 400\,\mu mol\,m^{-3}$ from May to August across the 3 years. Mean levels of ambient and elevated O_3 from 10:00 to 18:00 hours were 49.7 and 58.7 $nmol\,m^{-3}$ in 2005, 41.2 and 56.8 $nmol\,m^{-3}$ in 2006, and 46.7 and 82.5 $nmol\,m^{-3}$ in 2007, respectively. The O_3 treatment for 2007 was increased to 1.75 times ambient, because ambient levels in 2005 and 2006 were unusually low and an elevated target concentration significantly above the current ambient range was not being consistently obtained.

In mid-May of each year, soybeans (*Glycine max* L., cv. Pioneer 93B15, Pioneer Hi-Bred, Johnston, IA, USA) were mechanically planted across the 16 ha field at 0.38 m row spacing. Pioneer 93B15 is typical of varieties commonly grown in this region, and is resistant to soybean cyst nematode but susceptible to all three diseases evaluated in this study and described below. The soybean plots were treated with pre-emergence herbicides (Pursuit® Plus and Prowl® H_2O; BASF Corporation, Research Triangle Park, NC, USA) and spot treated with postemergence herbicides Select 2EC® (Valent Corporation, Walnut Creek, CA, USA) and First Rate™ (Dow AgroSciences LLC, Calgary, Alberta, Canada) as needed according to manufacturer suggested rates. Soybeans in this region are not typically treated with foliar insecticides or fungicides, and neither insecticides nor fungicides were applied to soybean plants in the SoyFACE plots during this experiment. The field was not irrigated and plants relied on natural precipitation inputs. Annual variability in precipitation allowed for concurrent evaluation of drought effects in this system. The soybean crop at SoyFACE is rotated annually with corn according to standard agronomic practice in this region. Therefore, the plots in 2005 and 2007 were in the same field location, but the plots in 2006 were in an adjacent location. Standard tillage practices in this region include leaving crop stubble on the soil surface undisturbed in the fall, followed by disking and harrowing in the spring before planting. This leaves some corn debris from the previous season on the soil surface. However, none of the pathogens monitored in this study are able to infect or survive as saprobes on corn tissues. Plots were fertilized with P and K as needed but were not inoculated with Bradyrhizobium, which is ubiquitous. No nitrogen fertilizer was added to the field during the growing season in the year that the rings were planted to soybean. For additional site details see Rogers *et al*. (2004) and Dermody *et al*. (2006).

Assessment of disease incidence and severity

The incidence and/or severity levels of naturally occurring soybean diseases were determined for field-grown plants in each of the four atmospheric treatments beginning approximately 2 weeks after seedling emergence and continuing through the onset of leaf senescence at soybean growth-stage R7. Diseases that occurred consistently and were rated included: brown spot, caused by *Septoria glycines* Hemmi; downy mildew, caused by *Peronospora manshurica* (Naumov) Syd; and sudden death syndrome (SDS), caused by *Fusarium virguliforme* O'Donnell and Aoki.

S. glycines survives in soybean debris and the 1 year rotation to corn reduces, but does not eliminate, sources of inoculum. *P. manshurica* survives as oospores in crop debris and on soybean seed. One year rotations also reduce, but do not eliminate, inoculum sources of this pathogen. Sporangia of this oomycete are disseminated fairly long distances by air currents, so inoculum may originate from a neighboring soybean field. The development of brown spot and downy mildew are fairly ubiquitous on susceptible cultivars in this region. *F. virguliforme* survives in soil in the form of chlamydospores, and other structures, in the absence of soybean plants for many years. Inoculum levels are relatively unaffected by short-term crop rotations. No evaluation of inoculum levels of any of the three pathogens were conducted before beginning this experiment.

Brown spot is primarily a foliar disease, causing irregular, dark brown spots surrounded by chlorotic tissue on both the upper and lower leaf surfaces. Downy mildew causes spots on the upper leaf surface that range from pale green to dark brown depending on leaf age, and the spots are usually surrounded by yellowish green margins. On the lower surface of downy mildew-infected leaves, lesions are covered with tufts of grayish to pale purple sporangiophores. SDS is a soilborne root disease, and visual foliar symptoms first appear on leaves as scattered chlorotic spots,

which may become necrotic, enlarge and coalesce leaving only the midvein and major lateral veins green. Severely affected leaflets detach from the petiole and severe symptoms throughout the canopy can give affected areas a tan to brown cast (Hartman *et al*., 1999). Tissue samples were collected from representative plants showing typical symptoms for all three diseases. These samples were used to verify the causal agents for the disease symptoms through microscopic examination of symptomatic tissues and/or through pathogen isolation on culture media.

Disease incidence levels of brown spot were measured as the number of symptomatic plants per 1 m of row in five randomly selected sections of each plot. The number of symptomatic plants was divided by the total number of plants per 1 m of row to calculate the percent incidence value. Incidence levels of brown spot were recorded until the average percent incidence exceeded 90%. Brown spot disease severity was measured as the maximum height of symptoms in the canopy (Pataky & Lim, 1981) on five randomly selected 1 m sections of row within each plot. Average plant height for each plot was also recorded to allow the percent height of symptoms in the canopy to be calculated. Downy mildew disease severity was measured as the percentage leaf area infected by visual observation of the leaves in the uppermost canopy for five randomly selected 1 m sections of row within each plot. Disease incidence levels of SDS were measured as the number of infected plants, based on the presence of foliar symptoms, per 1 m of row in five randomly selected sections of each plot. Severity levels of brown spot and downy mildew and incidence of SDS were evaluated until leaf senescence or defoliation made accurate rating difficult.

Leaf level assessment of brown spot disease incidence and severity were determined in both 2005 and 2006 by analyzing digital images of leaf samples. Within each of the 16 plots, samples were randomly collected in August of each year from the 3rd to 5th node from 50 to 100 different plants from each treatment ring. Digital images were taken of each individual leaf with a camera (Sony Cyber Shot S85; Sony, New York, NY, USA) placed approximately 50 cm above a light box upon which the leaf sample was placed. Incidence (i.e. % positive or negative infection status and number of lesions) was determined through visual inspection of the leaves. To determine disease severity percentage leaf area infected and individual lesion size) leaf and lesion areas were determined for each leaf using ASSESS: *Image analysis software for plant disease quantification* (American Phytopathological Society, St. Paul, MN, USA). Percentage severity was calculated as lesion area/leaf area $\times 100$. Individual lesion size was determined from total lesion area and the number of lesions.

Soybean stomatal structure and leaf chemistry

Stomatal impressions of both the adaxial and abaxial leaf surfaces were taken to determine stomatal density (SD) and stomatal length (SL). Impressions were made by covering a microscope slide with Polyvinylsiloxane dental impression material ('Extrude' Medium; Kerr Manufacturing CO., Orange, CA, USA) and pressing the leaf surface of a leaf disk ($d = 0.76$ mm) onto the material for approximately 5 min (Maherali *et al*., 2002; McElrone *et al*., 2005). In the lab, the leaf impressions were coated in clear nail polish and this peel was subsequently mounted on a microscope slide for viewing and photographing under a light microscope at $\times 40$ magnification (Olympus BH2 microscope fitted with Olympus DP12 digital camera, Olympus, Center Valley, PA, USA). Stomatal characteristics were quantified using the ASSESS software described above.

Owing to their role in nutritional value and plant defenses, the carbon (C) and nitrogen (N) content of soybean leaf tissue and cuticle wax were analyzed. To determine the C and N concentration, six leaf disks from six different leaves ($d = 0.76$ mm) from each treatment were pooled, and analyzed using an elemental combustion system (ECS; CHNS-O, ECS 4010, Costech Analytical Technologies Inc., Valencia, CA, USA). This was performed by first placing the leaf material in a 70 °C dryer, then grinding all the leaf tissue from each plot together with a bead beater. Approximately 3 mg of ground tissue sample from each plot was weighed in a tin capsule and analyzed in the ECS.

To quantify the amount of wax present in the leaf cuticle, one leaf disk was taken from six separate leaves in each plot. All leaf disks within a treatment were collectively dipped into preweighed 25 mL disposable scintillation vials containing 5 mL of HPLC grade chloroform for 30 s. This step was repeated with a second vial to ensure that all cuticle wax was recovered from the disks. Once the samples were removed from the second vial, the contents of the two vials were combined. The chloroform was then allowed to evaporate under a fume hood for approximately 24 h at which time the vials were reweighed to determine the amount of cuticle wax in grams.

Scanning electron microscopy (SEM) imaging of the leaf surface was also performed to examine the structure of the wax cuticle. Leaf samples were collected from each plot as leaf disks and immediately placed in Trump's fixative (4% formaldehyde, 1% glutaraldehyde, 1.16 g $NaH_2PO4 \cdot H_20$ and 0.27 g NaOH 100 mL^1; pH 7.2–7.4). Samples were rinsed in distilled water, dehydrated in an

ethanol dilution series, and placed in a critical point dryer. Dried samples were affixed to metal stubs using liquid silver. Images were taken from rings 1 to 8 at a $\times 120$, $\times 1200$, and $\times 2600$ magnification.

Statistical analysis

Statistical analysis of field disease rating data was performed using the PROC MIXED program of SAS 9.1 (SAS Institute Inc., Cary, NC, USA), with the atmospheric treatments entered as fixed variables and block entered as a random variable. Data were analyzed using both repeated measures analysis on incidence or severity levels, and using a single area under the disease progress curve (AUDPC) value for each disease in each ring for the season. AUDPC values were calculated using the formula $\text{AUDPC} = \sigma i = 1\text{–}n\ [(\text{DS}_i + \text{DS}_{i-1})/2 \times (X_i - X_{i-1})]$, where n is the number of observations; DS_i the mean disease severity at the ith observation; and X_i the number of days after planting at the ith observation (Tooley & Grau, 1984).

All statistical analyses for the digital image disease assessments and leaf structure and chemistry data were performed using SPSS (SPSS Inc., Chicago, IL, USA). One way ANOVA was used for comparisons of disease incidence, C : N content, and amount of cuticle wax, and a univariate GLM analysis was used for all other measurements. LS Means were used to distinguish between individual treatments when needed.

Results

Precipitation and temperature varied considerably between the 3 years of the study. During the 2005 growing season, the region experienced a severe drought, with a rainfall total of 193 mm for the months of May through August (Fig. 1– upper panels). Rainfall levels in 2006 were slightly above average (i.e. 460 mm), with a total of 510 mm for the May through August period. Drier conditions were experienced again in 2007, with rainfall totals of 226 mm for the May through August period. Mean air temperatures were between 20 and 25 °C for most of the season in all 3 years (Fig. 1– lower panels). Cooler temperatures were seen in May and early June in 2005 and 2006, while early season temperatures in 2007 were notably warmer.

During all growing seasons, weekly field surveys revealed the development of three soybean diseases and their causal agents: downy mildew (*P. manshurica*), SDS (*F. virguliforme*), and brown spot (*S. glycines*). In 2005, symptoms of brown spot were first observed during the week of July 14, while downy mildew symptoms were

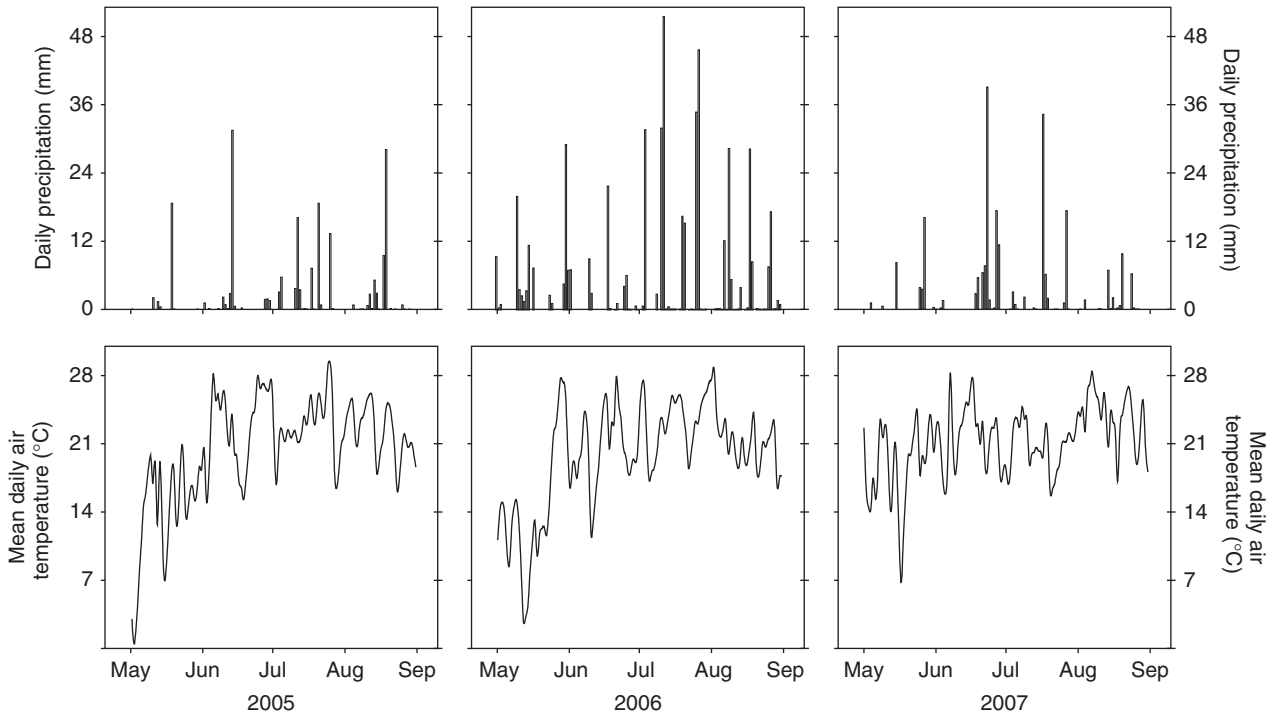

Fig. 1 Microclimatic data recorded at the soybean Free Air gas Concentration Enrichment (SoyFACE) site. Data represent the daily sum precipitation (upper panels) and mean daily temperature (lower panels) from 2005 (left column), 2006 (middle column), and 2007 (right column) growing seasons.

not observed until the week of July 24. Symptoms of SDS appeared much later in the 2005 season and were not observed until the week of August 25. In 2006, symptoms of all three diseases were observed somewhat earlier. Symptoms of brown spot were first observed on June 20, downy mildew symptoms on July 22, and SDS symptoms on August 4. In 2007, brown spot symptoms were first observed on July 6, downy mildew symptoms on August 6, and SDS symptoms on August 15. All three diseases were seen to some extent in the field plots in all years.

Downy mildew symptom severity was consistently reduced under elevated CO_2 and O_3 treatments in all years of the study relative to ambient conditions (Fig. 2; Table 1). Repeated measure analysis of the 2005 data revealed that downy mildew symptom severity was significantly reduced by O_3, CO_2, and combination treatments ($P = 0.0001$) (data not shown). AUDPC analysis showed a similar significant reduction under the combination treatment ($P = 0.04$), while CO_2 ($P = 0.052$) and O_3 ($P = 0.068$) treatments were reduced relative to ambient conditions but at the $P = 0.10$ level (Fig. 2; Table 1). In 2006 and 2007 both analyses of AUDPC values and repeated measures analysis showed significantly lower severity levels ($P = 0.0026$–0.0001) of downy mildew in the plots receiving elevated levels of CO_2 (Fig. 2; Table 1). There were no statistically significant differences in downy mildew severity between the elevated O_3 and ambient treatments, although symptoms were significantly reduced by the combination of CO_2 and O_3 in the second and third years. In a combined analysis of the AUDPC data from all 3 years, the CO_2, O_3 and the interaction term were all significant, with the mean AUDPC value for the ambient treatment significantly higher than those for plants exposed to elevated levels of CO_2, O_3, or both (Table 1). Downy mildew severity was highest in 2006 across all treatments, corresponding to the greater and more frequent rainfall that season (Figs 1 and 2, Table 1). However, the disease was first observed in the plots only slightly earlier in that year compared with 2005 and 2007.

The incidence of SDS and brown spot was evaluated at the SoyFACE site in all 3 years of the study. Symptoms of SDS were first observed between August 4 and 25 in all years. However, the atmospheric treatments had no significant effect on SDS incidence at any time. Similarly, brown spot was abundant throughout the site with incidence averaging $>90\%$ in most plots by mid to late-July, but the atmospheric treatments had no significant effect on disease incidence throughout the study (incidence data not shown for SDS or brown spot). Therefore, all further analyses of brown spot disease detailed below focus on severity ratings.

In all 3 years, brown spot disease severity expressed as actual height of symptoms in the canopy increased slightly but significantly under elevated CO_2 for both the AUDPC and repeated measures analyses (Table 2 and Fig. 3; main CO_2 effect $P < 0.05$; only nonsignificant term was found with repeated measures in 2007). Since plants growing in elevated CO_2 plots were taller, we also analyzed the data controlling for these treatment-induced growth differences (expressed as % canopy

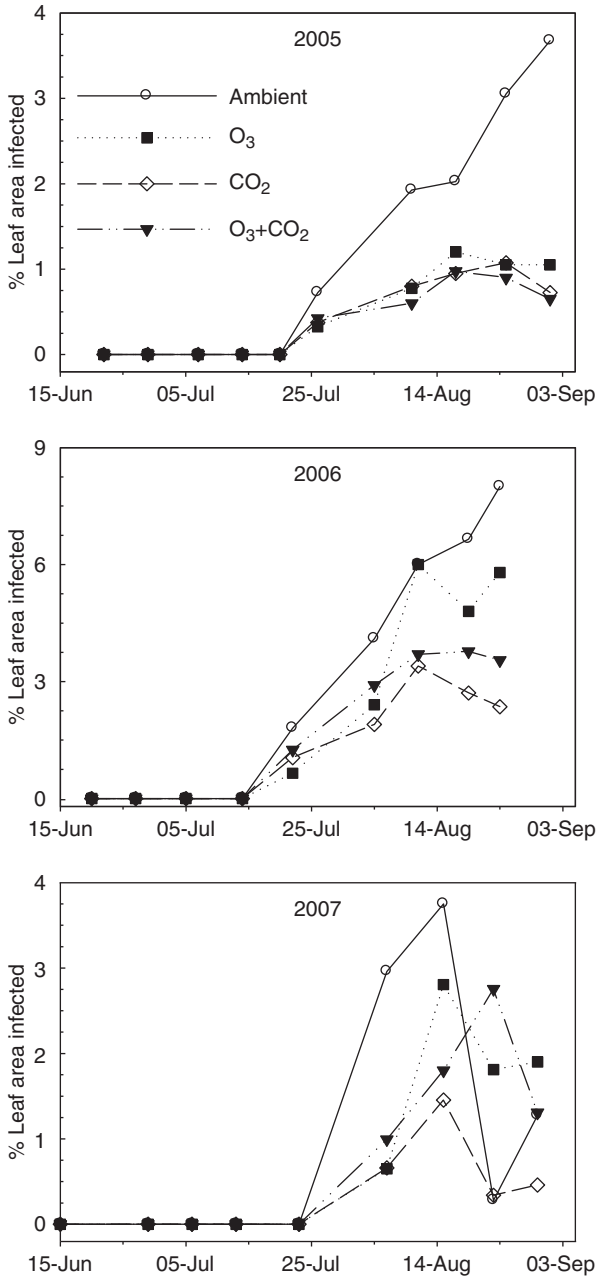

Fig. 2 Disease severity levels of downy mildew of soybean in response to carbon dioxide (CO_2) and ozone (O_3) fumigation treatments over the 2005–2007 growing seasons. Values represent the means of five subsamples per ring and four rings per treatment.

height – Table 2). Analysis of the data expressed as % of canopy height showed a statistically significant CO_2 effect for AUDPC in 2005 (Table 2) and for the repeated measures analysis in 2005 and 2007 (data not shown). The combination of CO_2 and O_3 increased severity for all variables in 2005 (interaction term $P < 0.05$), but had no effect in 2006 and 2007. In both 2006 and 2007, there were no significant differences for any of the fumigation treatments when the AUDPC data were analyzed as % canopy height (Table 2). In general, elevated O_3 alone had no effect on brown spot disease severity throughout the study (only exception is the repeated measures analysis of height in 2007). The lowest levels of brown spot were recorded in 2005, which was the driest year (Fig. 3 and Table 2). Increased brown spot severity in 2007 corresponded with higher daily temperatures in the late spring (Fig. 1, Table 2).

Table 1 Area under the disease progress curves (AUDPC) analysis for downy mildew severity on soybeans growing at the SoyFACE site in Urbana-Champaign, IL, USA

	AUDPC for % leaf area infected with Downy Mildew		
SoyFACE treatment	2005	2006	2007
Ambient	79.7 (28.8)^{a}	168.2 (11.1)^{a}	122.5 (33.4)^{a}
Elevated O_3	32.5 (6.3)^{a,b}	121.6 (11.2)^{a,b}	70.0 (23.4)^{a,b}
Elevated CO_2	30.5 (6.5)^{a,b}	79.0 (20.9)^{b}	39.2 (6.7)^{b}
Elevated $O_3 + CO_2$	27.4 (3.1)^{b}	103.3 (27.9)^{a,b}	45.0 (7.3)^{a,b}

Disease progression was documented throughout the 2005–2007 growing seasons and the entire disease progress curves are reported in Fig. 2. Data represent the mean (SE) of $n = 4$ rings within each treatment. Values within year columns followed by the same letter are not significantly different at $P = 0.05$.
SoyFACE, soybean Free Air gas Concentration Enrichment.

Similar to the field surveys, analysis of the digital images revealed significant differences in brown spot incidence between the 2005 and 2006 growing seasons. Brown spot incidence was significantly lower across all treatments in 2005 when the region experienced an extended drought. The fumigation treatments had no significant effect on disease incidence as measured by the percentage of leaves infected, but elevated CO_2 and O_3 combined significantly reduced the number of lesions per leaf by 51% relative to the ambient conditions in 2005 ($P < 0.05$) (Fig. 4). Ambient and O_3 conditions resulted in a greater numbers of small lesions per leaf, while elevated CO_2 and combination treatments tended to produce fewer lesions of a larger size (Fig. 4). Disease severity expressed as % leaf area showing brown spot symptoms was not significantly affected by atmospheric treatment in either year of the leaf analysis ($P > 0.05$) (Fig. 4). The small magnitude of change in brown spot disease incidence and severity found in the field AUDPC analyses is consistent with the limited significant effects found with the digital image analysis.

Structural and chemical characteristics of soybean leaves showed little to no significant changes across the atmospheric treatments. SD and SL, leaf chemistry (measured by the C : N ratio), and the quantity of cuticular wax were not significantly affected by atmospheric conditions (Table 3; $P > 0.05$). SEM images of the leaf surface revealed a possible alteration in cuticular wax structure. Elevated CO_2 treatments resulted in a smooth structure of the wax, while a plated structure of wax formed with all other treatments (figures not shown).

Discussion

Elevated CO_2 and O_3 directly impact the physiology, growth, and yield of soybeans (see meta-analyses by Ainsworth *et al*., 2002; Morgan *et al*., 2003). Despite the

Table 2 Area under the disease progress curves (AUDPC) analysis for *Septoria* brown spot severity on soybeans growing at the SoyFACE site in Urbana-Champaign, IL, USA

	2005		2006		2007	
SoyFACE treatment	Height (cm)	% Canopy Height	Height (cm)	% Canopy Height	Height (cm)	% Canopy Height
Ambient	1802 (47)^{a,b}	1733 (27)^{a}	2624 (12)^{a}	2985 (44)^{a}	3102 (92)^{a}	3636 (48)^{a}
Elevated O_3	1612 (66)^{a}	1660 (29)^{a}	2616 (49)^{a}	2977 (45)^{a}	3170 (58)^{a}	3676 (102)^{a}
Elevated CO_2	1997 (74)^{b,c}	1768 (31)^{a,b}	2855 (26)^{b}	3018 (43)^{a}	3310 (93)^{a,b}	3560 (124)^{a}
Elevated $O_3 + CO_2$	2113 (93)^{c}	1859 (30)^{b}	2801 (26)^{b}	2924 (46)^{a}	3489 (82)^{b}	3648 (114)^{a}

Disease progression was documented throughout the 2005–2007 growing seasons as height within the canopy and % canopy height to control for differences in plant height imposed by the treatments. The entire disease progress curves are reported in Fig. 3. Data represent the mean (SE) of $n = 4$ rings within each treatment. Values within a measurement column followed by the same letter are not significantly different at $P = 0.05$.
SoyFACE, soybean Free Air gas Concentration Enrichment.

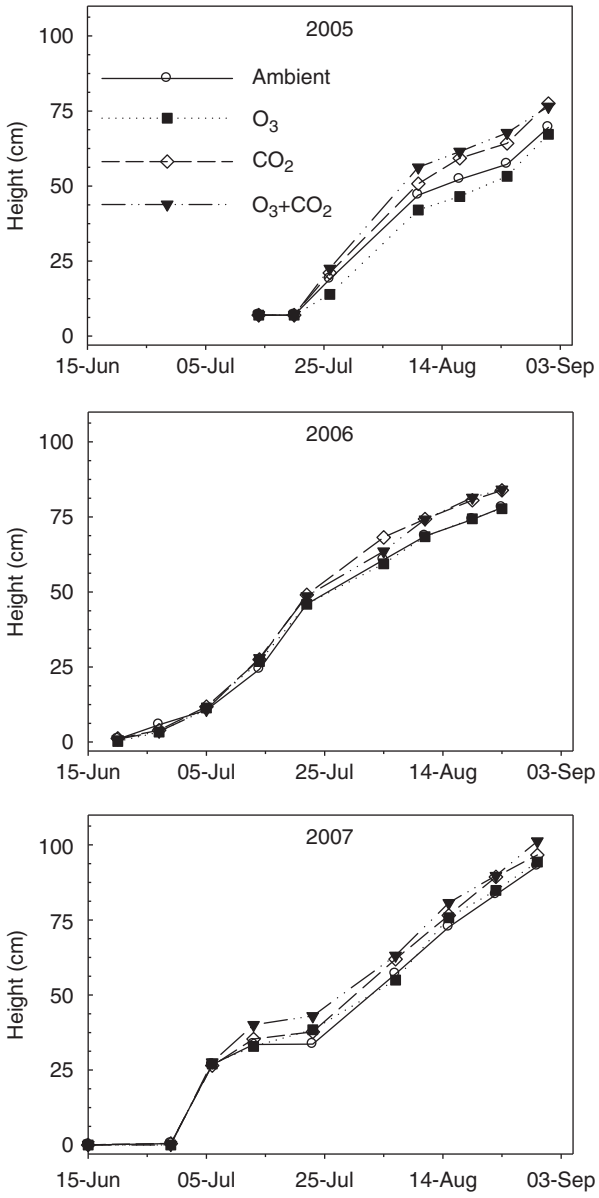

Fig. 3 Mean actual height of brown spot symptoms in the soybean canopy in response to carbon dioxide (CO_2) and ozone (O_3) fumigation treatments evaluated over the 2005–2007 seasons. Values represent means of five subsamples per ring and four rings per treatment.

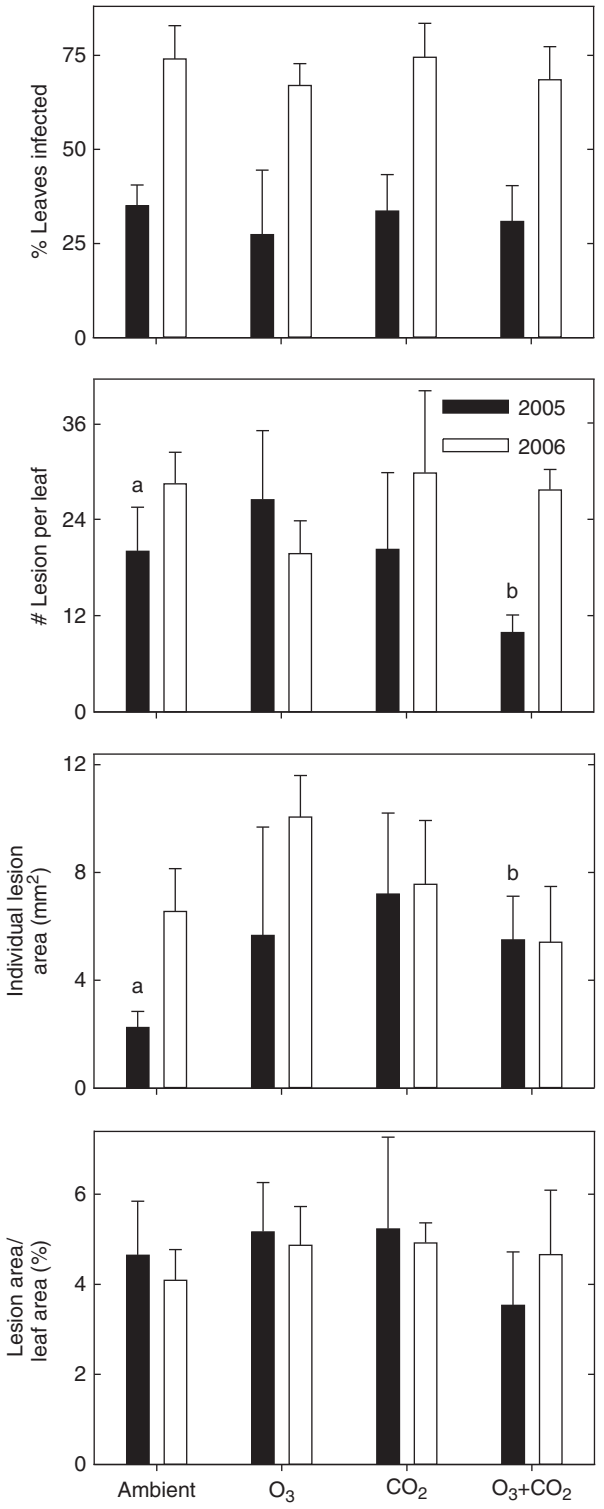

Fig. 4 Brown spot disease incidence and severity estimated from digital images of individual soybean leaves growing at the soybean Free Air gas Concentration Enrichment (SoyFACE) site in the 2005 and 2006 seasons. Values represent the means of 50–100 subsamples per ring and four rings per treatment. Bars within a given growing season labeled with different lowercase letters are significantly different at $P<0.05$.

demonstrable effects on plant function, we still lack a thorough understanding of how soybean diseases will be affected by these future atmospheric conditions. In this study, we showed that predicted changes in CO_2 and O_3 will alter disease expression for important fungal pathogens of soybean. Elevated CO_2 alone or in combination with O_3 consistently decreased downy mildew but increased *Septoria* brown spot albeit to a lower degree. Bilgin *et al*. (2008) also recently demonstrated that altered atmospheric conditions can decrease

Table 3 Structural and chemical characteristics of soybean leaves sampled from plants growing at the SoyFACE site in Urbana-Champaign, IL, USA

SoyFACE treatment	Stomatal density (# mm^{-2})	Stomatal length (μm)	Carbon : nitrogen	Cuticle wax ($mg\,cm^{-2}$)
Ambient	343 (24)[a]	18.3 (0.5)[a]	10.3 (0.1)[a]	7.0 (0.7)[a]
Elevated O_3	355 (16)[a]	17.9 (0.6)[a]	11.3 (0.2)[a]	8.0 (1.4)[a]
Elevated CO_2	383 (24)[a]	17.1 (0.2)[a]	11.5 (0.4)[a]	9.2 (1.6)[a]
Elevated $O_3 + CO_2$	374 (18)[a]	17.6 (0.2)[a]	11.7 (0.8)[a]	8.3 (2.5)[a]

Leaves were sampled in August 2005. Values represent the mean (SE) of five subsamples per ring and $n = 4$ rings within each treatment. Values within a measurement column followed by the same letter are not significantly different at $P = 0.05$.
SoyFACE, soybean Free Air gas Concentration Enrichment.

disease expression of Soybean Mosaic Virus by slowing systemic infection through up-regulation of basal defenses. Conservative annual estimates suggest that worldwide yield losses to all soybean diseases combined are $\sim 11\%$ (Wrather *et al*., 1997), which is equivalent to >24 million metric tons based on current production. Downy mildew, brown spot and SDS are common diseases that consistently contribute to these losses across the global distribution of soybeans (Hartman *et al*., 1999). Given their widespread occurrence and economic significance, our findings suggest that shifts in management will need to occur to account for the changing pressure of these and other (i.e. viral) diseases under future conditions (e.g. Salinari *et al*., 2006).

Previous research has shown that soybean leaf demography and canopy structure are influenced by elevated CO_2 and O_3. Elevated O_3 accelerates soybean leaf senescence (i.e. age more quickly) and decreases canopy density, while elevated CO_2 prolongs leaf area duration and increases canopy density (Dermody *et al*., 2006). Since soybean leaves become more resistant to downy mildew infection with age (Hartman *et al*., 1999), it is not surprising that the O_3 treatment resulted in lower downy mildew severity by reducing the period of susceptibility. In addition, downy mildew and brown spot are both favored by humid conditions, so denser canopies should favor development of these diseases. This trend is apparent for brown spot with the greatest disease levels under elevated CO_2 and lowest in O_3. Pangga *et al*. (2004) similarly found that denser and enlarged canopies of a pasture legume under elevated CO_2 increased capture of anthracnose fungal spores leading to more lesions per plant. However, if canopy structure and leaf age were exclusively responsible for the changes in downy mildew documented here we would expect to see peak disease levels under elevated CO_2. Yet downy mildew severity was highest under ambient conditions, thus the responses were not solely driven by differences in canopy structure and may be related to unmeasured changes in host defensive chemistry.

Studies conducted at FACE facilities allow for realistic disease assessment because plants are exposed to natural pathogens loads and microclimatic conditions (Chakraborty, 2005; Chakraborty *et al*., 2008). Such studies conducted over multiple years allow us to assess the concurrent effects of temperature and precipitation variability. Evidence for global climate warming is now unequivocal, and experts predict that altered precipitation regimes will accompany the increased temperature in many regions (IPCC, 2007). The 3 years examined in this study provided us with varied microclimatic conditions. Increased rainfall in 2006 was linked with greater downy mildew severity (Fig. 2) and *Septoria* brown spot incidence and severity (Fig. 4a) compared with the other 2 years. These results are not surprising given that downy mildew and brown spot are both favored by wet weather and high humidity. These conditions encourage the emergence of sporangiophores of *P. manshurica* from stomata on the lower surface of leaves and promote sporulation and spread of conidia of *S. glycines* by splashing rain (Hartman *et al*., 1999).

In the 2 years with below average rainfall, the higher temperatures in early 2007 were associated with increased downy mildew and brown spot severity relative to 2005. Salinari *et al*. (2006) simulated downy mildew epidemics on grapevines under future climatic conditions by combining a disease model with output from global circulation models (GCMs). The GCMs predicted increases in temperature and a decrease in precipitation for the northwest region of Italy. Their simulations found more severe epidemics and increased downy mildew disease pressure as a direct consequence of more favorable temperatures during late spring to early summer months (Salinari *et al*., 2006). However, their models did not account for the direct impacts of changing atmospheric composition on disease expression like those induced by elevated CO_2 and O_3 in this study. Future management of these diseases will need to consider both the direct impacts of atmospheric change and the resulting alterations in regional climate.

P. manshurica is an obligate parasite which should be very responsive to changes in the structure and function of its host plant. *S. glycines*, by contrast, is a necrotrophic pathogen that can exist as a saprobe (Hartman *et al*., 1999). Physiological changes in the host plant are likely to be more important for obligate pathogens, while microclimate could drive the response for the necrotroph (Manning & Tiedemann, 1995). This may explain why disease severity levels of downy mildew responded to a greater degree to atmospheric changes than did severity levels of brown spot.

Soybean stomata play an important role in the disease cycle for both downy mildew and *Septoria* brown spot. *P. manshurica* uses soybean stomata for germ tube entry into leaves (but can also penetrate between epidermal cells) and emergence of sporangiophores from the lower leaf surface and *S. glycines* uses them exclusively for entry (Hartman *et al*., 1999). Meta-analyses have shown that soybean stomata close by 40% under elevated CO_2 and by 17% under elevated O_3 (Ainsworth *et al*., 2002; Morgan *et al*., 2003). Stomatal closure has recently been linked to reduced *Phyllosticta* leaf spot disease incidence under elevated CO_2 (McElrone *et al*., 2005) and implicated in the incompatibility of soybean leaf infection by *Phytophthora sojae* (McDonald & Cahill, 1999). Given the clear physiological responses of soybean stomata to elevated CO_2 and O_3, it is surprising that disease incidence for downy mildew and brown spot were unaffected by these treatments. However, the lack of significant changes in stomata structure and density under the varying treatments found here may have a stronger influence over disease expression in this system by maintaining the number and size of entry points for these pathogens.

We found that predicted changes in atmospheric composition of CO_2 and O_3 will alter soybean disease expression, but responses of the three pathosystems studied here varied considerably. SDS was unaffected by the atmospheric treatments, while downy mildew severity decreased and brown spot severity increased under elevated CO_2 and/or O_3 conditions. Recent field studies with deciduous trees have found similar divergent responses. For example, aspen rust increased under elevated O_3 alone and in combination with CO_2 (Karnosky *et al*., 2002; Percy *et al*., 2002), while *Phyllosticta* leaf spot of red maple decreased under elevated CO_2 conditions (McElrone *et al*., 2005). These varied results are consistent with those summarized in several reviews, and further illustrate how the specific nature of host-pathogen interactions make it difficult to devise general principles/mechanisms that govern changes across pathosystems (Manning & Tiedemann, 1995; Coakley *et al*., 1999; Chakraborty *et al*., 2000, 2008). To better predict how important agricultural pathosystems will respond to future climatic conditions, additional studies are needed in the natural conditions of FACE facilities and should be combined with other climate change factors (i.e. increased temperatures) (Chakraborty *et al*., 2008).

Acknowledgements

Thanks to J. Lee and J. Foster for help with digital image analysis, K. Snetselaar for help with microscopic analysis of leaves, and Alex Luckew for field disease evaluations. Funding for M. DeGennaro was provided by SJU Sigma Xi grants and a National Science Foundation GK-12 fellowship through the SJU GeoKids program. The SoyFACE experiment was constructed with funds from the Illinois Council on Food and Agricultural Research. This work was also supported in part by The Soybean Disease Biotechnology Center – National Soybean Research Laboratory, The Illinois Soybean Association, USDA Hatch funds, and the Office of Science (BER), Department of Energy Grant no. DE-FG02-04ER63489.

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