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Combined effects of elevated CO_2 and natural climatic variation on leaf spot diseases of redbud and sweetgum trees

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Climatic variation had a greater impact than elevated CO_2 on Cercospora *diseases, especially since leaf photosynthetic efficiency increased under elevated CO_2.*

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ABSTRACT

Atmospheric CO_2 concentrations are predicted to double within the next century and alter climate regimes, yet the extent that these changes will affect plant diseases remains unclear. In this study conducted over five years, we assessed how elevated CO_2 and interannual climatic variability affect *Cercospora* leaf spot diseases of two deciduous trees. Climatic data varied considerably between the five years and altered disease expression. Disease incidence and severity for both species were greater in years with above average rainfall. In years with above average temperatures, disease incidence for *Liquidambar styraciflua* was decreased significantly. When significant changes did occur, disease incidence and severity always increased under elevated CO_2. Chlorophyll fluorescence imaging of leaves revealed that any visible increase in disease severity induced by elevated CO_2 was mitigated by higher photosynthetic efficiency in the remaining undamaged leaf tissue and in a halo surrounding lesions.

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1. Introduction

Anthropogenic emissions are drastically increasing the concentration of atmospheric CO_2 and levels are expected to double sometime in the coming decades (IPCC, 2007). Elevated CO_2 directly alters plant physiology, growth and yield (reviewed in Ainsworth et al., 2002; Drake et al., 1997; Saxe et al., 1998). Elevated CO_2 can also modify plant–pathogen interactions primarily through changes in host plants, but far fewer studies addressed these effects especially under field conditions with natural pathogen loads and ambient abiotic variability (Chakraborty et al., 2000a, 2008; Coakley et al., 1999; Manning and Tiedemann, 1995; Runion, 2003). Given that pathogens persistently reduce plant productivity in forested, agricultural and natural ecosystems worldwide (Pimentel et al., 2000), more work is needed to elucidate how plant disease will respond to the interacting factors of future climatic conditions. Understanding such relationships is vital to making predictions about overall plant and ecosystem health and for managing plants in a range of systems in the future.

Expression of plant disease for any given pathosystem requires a susceptible host, a prevalent/virulent pathogen, and favorable environmental conditions (Scholthof, 2007). Changes in environmental conditions are known to exacerbate plant disease symptoms (e.g. Boyer, 1995; McElrone et al., 2001) and are implicated in 44% of new disease emergence (Anderson et al., 2004). The rapidly increasing atmospheric [CO_2] is not only expected to modify plant–pathogen interactions (Chakraborty et al., 2000a, 2008; Garrett et al., 2006; Manning and Tiedemann, 1995), but also contribute considerably to the predicted changes in the Earth's climate over the coming decades (IPCC, 2001, 2007). According to model predictions, many regions will experience higher temperatures and

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altered precipitation patterns. Because of the importance of abiotic environmental conditions to disease expression, there is a need to evaluate disease responses to atmospheric change concurrently with the effects of altered temperature and precipitation.

In the current study, we assessed how elevated CO_2 affects *Cercospora* leaf spot diseases of *Liquidambar styraciflua* (sweetgum) and *Cercis canadensis* (redbud) saplings. Species of the genus *Cercospora* also affect numerous economically important plant species around the world including grapes, cereals, soybeans, peanuts, orchids, coffee, alfalfa and potatoes (Sinclair et al., 1987). This study was conducted at the Duke Forest Free-Air CO_2 Enrichment (FACE) experiment, and we examined disease incidence and severity for both pathosystems over a five-year period that encompassed a range of natural climatic variability. This longer term dataset allowed us to examine not only the effects of elevated CO_2 but also the interacting effects of interannual changes in temperature and precipitation.

2. Materials and methods

2.1. Field site description

The Duke FACE (Free-Air CO_2 Enrichment) experiment is located in the Blackwood Division of the Duke Forest, Orange County, NC (35°97′N 79°09′W). The site contains a loblolly pine (*Pinus taeda*) plantation established in 1983. Since then, other plant species have been allowed to establish within the plantation. The most abundant understory tree species include sweetgum (*L. styraciflua*), redbud (*C. canadensis*), red maple (*Acer rubrum*), winged elm (*Ulmus alata*), and hickory (*Carya* spp.). Additional site details are available in DeLucia et al. (1999).

Within the Duke FACE site, six 30 m-diameter circular plots (rings) were established in 1996. Each ring is equipped with thirty-two vertical pipes that extend from the forest floor through the canopy and deliver either elevated CO_2 or ambient air. Three experimental rings are fumigated with CO_2 to raise the atmospheric CO_2 concentration 200 μL L^{-1} above ambient (elevated rings, ~586 μL L^{-1} and 577 μL L^{-1} at 0.25 and 1.0 m height, respectively; Knepp et al., 2005). Three additional rings are supplied with ambient air only (~385 μL L^{-1}) and serve as controls to accommodate any effects of air movement on the vegetation (ambient rings). Climate data were collected at the Duke FACE site using relative humidity/air temperature sensors and rain gauges as in Schäfer et al. (2003). The data were provided by R. Oren. The compiled data were used to calculate cumulative precipitation and cumulative degree-days greater than 15 °C in each of the growing seasons.

2.2. Host plants and fungal pathogens

Both *L. styraciflua* (sweetgum) and *C. canadensis* (eastern redbud) are deciduous trees that grow abundantly throughout the eastern US, and are known to tolerate a variety of soils and habitats. Sweetgum is commonly found in bottomlands and can occupy the forest canopy or understory while redbud habitats commonly include the forest understory, riparian zones, open rocky woods and abandoned farmlands (Gleason and Cronquist, 1991; USDA-Natural Resources Conservation Service-Plant Guide: http://plants.usda.gov). Both species are also cultivated and used extensively in landscaped settings.

Cercospora is a large genus of ascomycete fungi, and numerous species cause disease on a variety of host plants. Most diseases caused by *Cercospora* are characterized by chlorotic to necrotic localized lesions, and occur in all temperate habitats with particular abundance in warm, humid regions such as the southeastern US (Sinclair et al., 1987). Visual surveys confirmed that sweetgum and redbud are commonly infected by *Cercospora* leaf spots throughout the Duke FACE site and the surrounding forest. The Plant Disease Clinic at North Carolina State University confirmed our initial surveys using leaf tissue samples tissue for each species, and identified the *Cercospora* species for each host. The *Cercospora* species infecting redbud and sweetgum at the Duke FACE site were identified as *Cercospora liquidambaris* Cooke & Ellis and *Cercospora cercidicola* Ellis (syn. *Passalora cercidicola*), respectively. Both pathogens are co-extensive throughout the hosts' range and occur frequently throughout the eastern and southeastern US (Wolf, 1940). Lesions of both infections typically range from 2 to 10 mm diameter and are angular to nearly round. Lesions caused by *C. liquidambaris* are dark-brown with a purplish black border and a diffuse purplish halo. The pathogen sporulates on both surfaces of the lesions, producing conidia on dark-brown stromata (Sinclair et al., 1987). *C. cercidicola* lesions are rusty-brown to dark-brown with a definite and raised border. These spots become grayish above but remain rusty-brown on the lower surface, and the tissue surrounding the lesions develops a chlorotic halo (Wolf, 1940). Conidophores are produced on both leaf surfaces and emerge through the stomata. The conidia continue to be formed throughout the entire summer whenever moisture conditions are favorable.

2.3. Disease incidence and severity assessment: digital image analysis

In all years of the study, we surveyed disease incidence and severity on even-aged sweetgum and redbud saplings planted in herbivore exclosures within the Duke FACE rings. Eight month old tree saplings were grown from seed and transplanted into eight 1.44 m^2 exclosures inside the canopy of each experimental ring in October 1998. The saplings were grown from locally collected and genetically diverse seeds of each species, were germinated in a greenhouse prior to transplanting, and were planted at 30 cm intervals in a random order (see details in Mohan, 2002).

Throughout the study, *L. styraciflua* and *C. canadensis* saplings were surveyed for disease incidence (% leaves infected) and severity (% leaf area infected and lesion area). A digital camera was used to capture images of leaves while still attached to the plants. Leaves were photographed over a contrasting paper background that contained a fine resolution scale needed for size calibration during image analysis. In each year, fully expanded leaves of both species were randomly selected from numerous saplings in multiple exclosures within each ring (between 174 and 336 leaves were sampled/analyzed for each species in each year). In some years, an unequal number of leaves were sampled in each ring due to differences in sapling survival rate among rings. In the laboratory, incidence was determined through visual inspection of the leaves. To determine disease severity (% leaf area infected, individual lesion size) leaf and lesion areas were measured for each leaf using *ASSESS: Image analysis software for plant disease quantification* (American Phytopathological Society, St. Paul, MN, USA). Percent severity was calculated as (lesion area/leaf area) × 100. Individual lesion sizes within a leaf were determined using the total lesion area and the number of lesions. Disease parameters measured on all leaves within a given ring were averaged into a single replicate ($n = 3$ rings per atmospheric treatment).

2.4. Chlorophyll fluorescence imaging

To determine how photosynthetic capacity surrounding pathogen damage was affected by CO_2 exposure, the spatial pattern of photosystem II operating efficiency (Φ_{PSII}) was quantified on *C. canadensis* leaves still attached to plants with an imaging

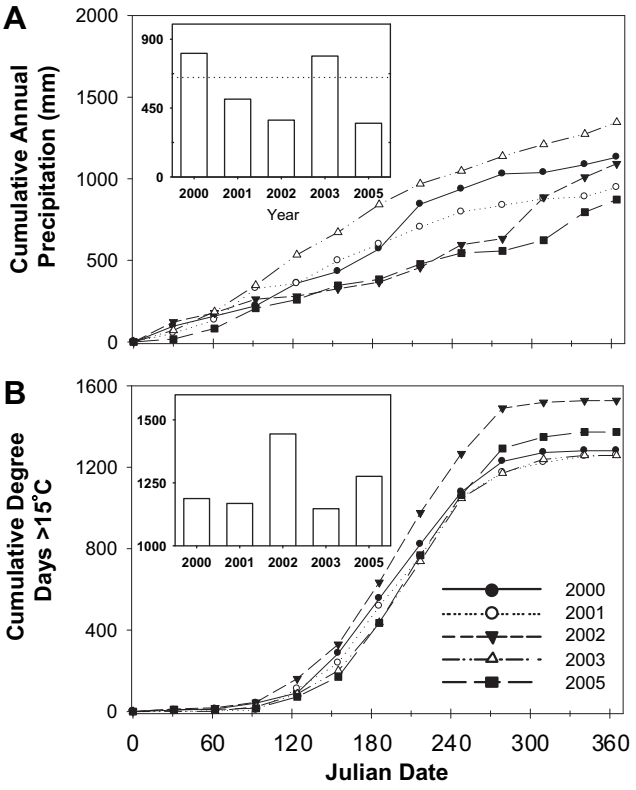

Fig. 1. Weather data collected at the Duke FACE site in Chapel Hill, NC, USA from 2000 to 2003 and 2005. Precipitation (top panel) and cumulative degree-days >15 °C (bottom panel) summed across each growing season is represented by the line and scatter plot in each panel, while the growing season (i.e. when the deciduous tree species are leaved) sum for each parameter is represented by the inset bar graph in each panel. The dashed line on the precipitation inset panel represents the mean growing season precipitation (~650 mm). The cumulative degree-day >15 °C was used as a proxy for representative seasonal temperature.

chlorophyll fluorometer (Walz Imaging PAM, Walz GmbH, Effeltrich, Germany). Both control and infected leaves were measured under steady state, light-adapted conditions (51 μmol m^{-2} s^{-1} PFD; 2.3 × 3.3 cm imaged area). These irradiances were typical for shaded understory conditions (average 121 μmol m^{-2} s^{-1}; median 68 μmol m^{-2} s^{-1}; Singsaas et al., 2000) and optimal for imaging chlorophyll fluorescence using the Walz Imaging PAM at its highest resolution. Each leaf was adapted to the new light environment for 5 min. The minimum fluorescence in the light-adapted state (F') was then recorded with the measuring pulse from the fluorometer (Baker et al., 2001). An image of the maximum fluorescence (F'_m) was recorded following a 1 s saturating pulse (ca. 2500 μmol m^{-2} s^{-1}). Photosystem II efficiency (Φ_{PSII}) was calculated as the quotient $(F'_m - F')/F'_m$ (Baker et al., 2001). At a given incident irradiance and leaf absorptance, Φ_{PSII} is directly proportional to the rate of electron transport through the photosystem II reaction centers and is correlated with the rate of carbon assimilation (Genty et al., 1989; Rolfe and Scholes, 1995). In each atmospheric treatment, measurements were made on 1) uninfected control leaves, 2) undamaged portions of infected leaves, and 3) the regions surrounding visible leaf spots where photosynthesis was suppressed (i.e. halo). At least 5 replicate leaves were used for each of the three measurement categories within a treatment, and numerous lesions were assessed on each infected leaf (see Fig. 5). Additional details about these measurements and the image analysis can be found in Aldea et al. (2006).

2.5. Leaf chemical analysis

In several years of the study, tissue samples were collected with a cork borer (0.6 cm^2) from randomly selected undamaged leaves in each ring. Primary and secondary order veins were avoided during collection of the leaf punches. Approximately 45 leaf punches for each species were dried at 70 °C to constant mass. C and N content per unit dry mass were then measured on ground subsamples using either an Elemental Combustion System (Model 4010-Costech Analytical Technologies, Valencia, CA, USA) or a Carlo Erba Elemental Analyzer.

Total phenolic and tannin content was determined using the Folin–Ciocalteu method (Makkar, 2003) on leaf punches from each ring. Leaf tissue was ground in liquid N_2, extracted five times with 70% acetone, and run in triplicate for each sample. Aliquots (500 μL) were diluted 1:10 with 70% acetone and 475 μL of Folin–Ciocalteu (Sigma, St. Louis, MO, USA) reagent was added. Each solution was stirred, and after 3 min 475 μL of Na_2CO_3 was added to each tube. Tubes were sealed and left for 1 h.

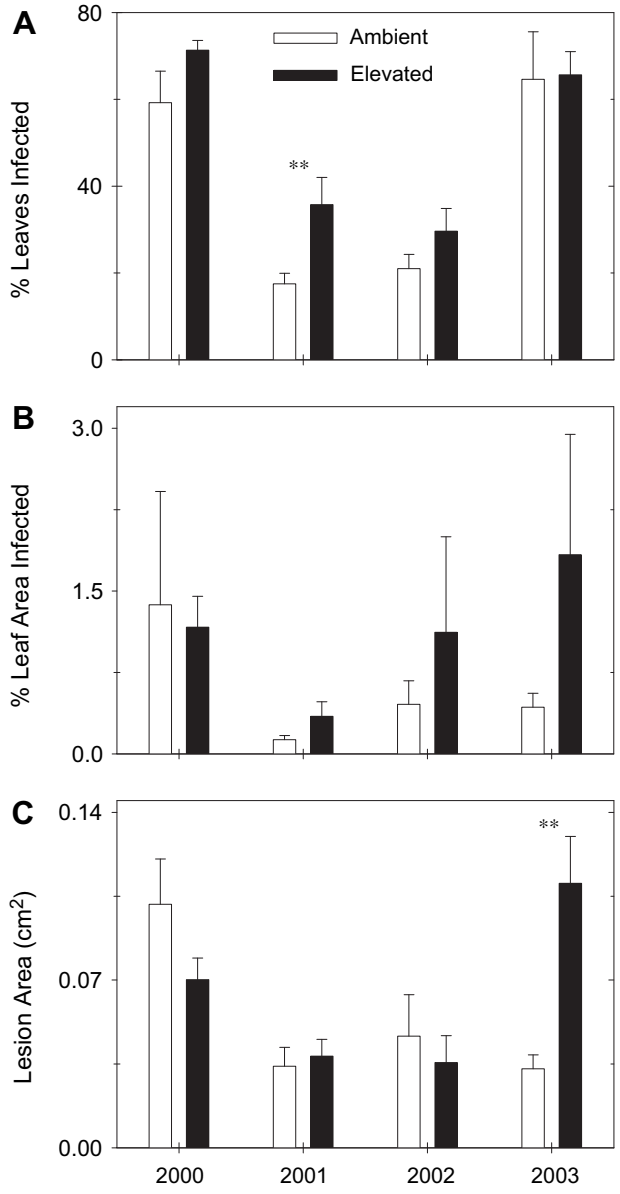

Fig. 2. *Cercospora liquidambaris* leaf spot disease incidence (A) and severity (B and C) measured on understory *L. styraciflua* saplings from 2000 to 2003. The plants were growing in ambient and elevated CO_2 at the Duke FACE site, Durham, NC. Disease incidence was measured as the percentage of leaves infected (A), while measurements of severity included percentage leaf area infected and mean lesion size. Paired CO_2 treatment bars within a year are significantly different at $P < 0.05$ (**); $n = 3$ rings for each treatment bar.

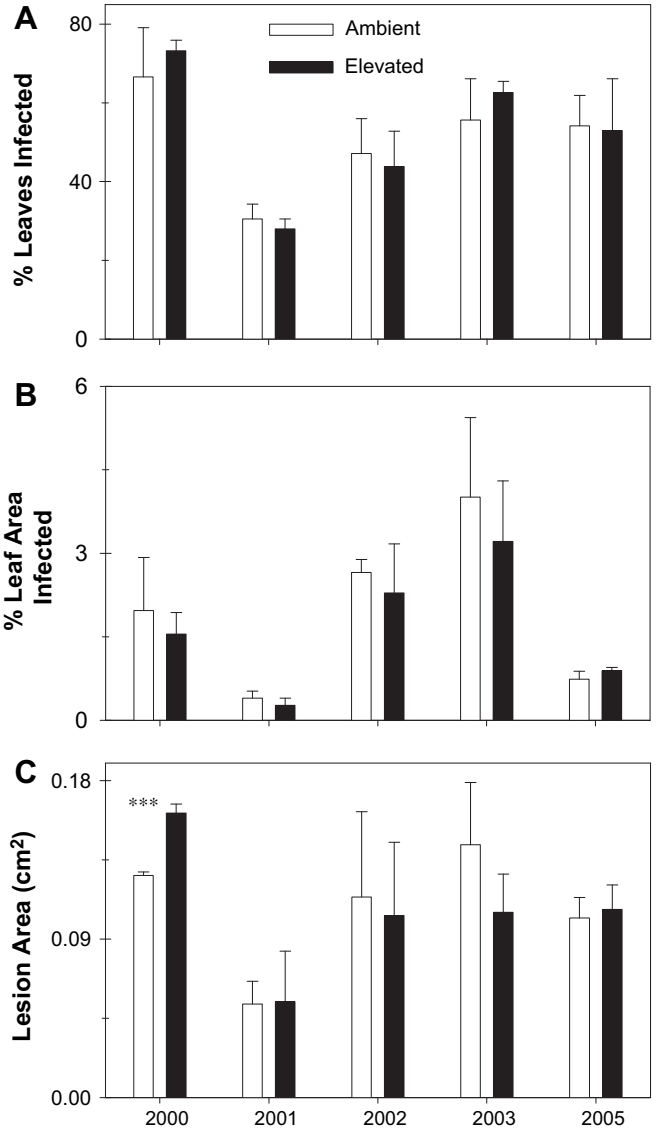

Fig. 3. *Cercospora cercidola* leaf spot disease incidence (A) and severity (B and C) measured on understory *C. canadensis* saplings from 2000 to 2003 and 2005. The plants were growing in ambient and elevated CO_2 at the Duke FACE site, Durham, NC. Disease incidence was measured as the percentage of leaves infected (A), while measurements of severity included percentage leaf area infected and mean lesion size. Paired CO_2 treatment bars within a year are significantly different at $P < 0.001$ (***); $n = 3$ for each treatment bar.

Afterwards, the solutions were centrifuged and absorbance read at 724 nm. Values for multiple leaf samples were averaged within a ring ($n = 3$ per atmospheric treatment). Additional details about these methods can be found in Hamilton et al. (2004).

2.6. Statistical analysis

Treatment effects on disease incidence, severity, C:N, %N, and total phenolics were pooled within rings and evaluated using a Mixed Model Analysis of Variance (ANOVA) with CO_2 treatment as the fixed factor and climatic data (i.e. precipitation and temperature) as random factors. Interactive effects between the atmospheric treatment and the climatic variables were assessed in this model as well. All statistical analyses were completed using SAS 9.2 (SAS Institute, Cary, NC, USA). Analysis details for chlorophyll fluorescence imaging data can be found in Aldea et al. (2006).

3. Results

Climatic data varied considerably throughout the course of the study (Fig. 1). Mean annual precipitation is ~1220 mm at the Duke FACE site and during an average year ~650 mm falls during growing season (Fig. 1A inset). In two of the years of the study (2002 and 2005), cumulative precipitation was well below the annual and growing season averages. By contrast, 2003 exhibited the greatest cumulative and growing season precipitation totals of the 5 years. Seasonal air temperatures also varied considerably throughout the study (Fig. 1B); the two warmest growing season years during the course of the study were 2002 and 2005.

Disease incidence and severity for both pathosystems exhibit clear interannual variability that mirrors changes in climatic parameters. Mean disease incidence ranged from ~17 to 73% across both pathosystems and years of the study (Figs. 2A and 3A). Disease incidence for both species was strongly influenced by precipitation with greater percentage leaves infected in 2000 and 2003, the wettest years (Figs. 2A and 3A; $F = 7.86$, $P = 0.0101$ for red bud; $F = 57.52$, $P < 0.0001$ for sweetgum). This pattern is particularly evident for sweetgum (Fig. 2A). Disease severity, measured by percentage leaf area infected, was lowest for redbud in 2001 and 2005 (Fig. 3B; $F = 11.96$, $P = 0.002$), and lesion area was reduced for both species in 2001 and 2002 when precipitation was below average (Figs. 2C and 3C; $F = 6.06$, $P = 0.022$ for red bud; $F = 7.20$, $P = 0.015$ for sweetgum). Cooler temperatures were found to significantly increase disease incidence for sweetgum (Fig. 2; $F = 5.13$, $P = 0.036$). No significant interactive effects were found for any of the disease parameters for either pathosystems across the study years ($P > 0.23$ for treatment by climate interactions).

When significant differences were found, disease incidence and severity increased under elevated CO_2 (Figs. 2 and 3). *Cercospora* disease incidence was two times greater in sweetgum exposed to elevated CO_2 in 2001 ($P < 0.05$), and similar non-significant trends were exhibited in all years of the study (Fig. 2A). Disease severity (i.e. lesion area) was increased by 235% in sweetgum ($P < 0.05$) and by 28% in redbud ($P = 0.001$) under elevated CO_2 in 2003 and 2000, respectively (Figs. 2C and 3C). The percentage leaf area infected in sweetgum also exhibited a similar non-significant trend of increased severity under elevated CO_2 across the 2001–2003 seasons (Fig. 2B). The high variability seen in all years is typical of the results for the Duke FACE site.

Spatial patterns of the operating efficiency of photosystem II (Φ_{PSII}) revealed varying patterns of damage to the photosynthetic machinery (Fig. 4). Φ_{PSII} of redbud leaves (for all three tissue categories measured) exposed to elevated CO_2 was on average higher than that of leaves in the ambient treatment (Fig. 5; main CO_2 effect $F = 17.86$, $P = 0.0003$). Within an atmospheric treatment, Φ_{PSII} was similar between the tissue from control leaves (i.e. non-infected) and lesion-free portions of infected leaves (Fig. 5; $P > 0.05$). Leaves from both ambient and elevated plots exhibited reduced Φ_{PSII} in a halo that extended away from the visible lesion damage, but the size of the halo was similar between the two treatments. However, Φ_{PSII} of the halo was more depressed under ambient conditions (Fig. 5; $F = 48.2$, $P = 0.0004$).

Leaf chemistry of both tree species was unaltered by the atmospheric treatments across multiple years of the study

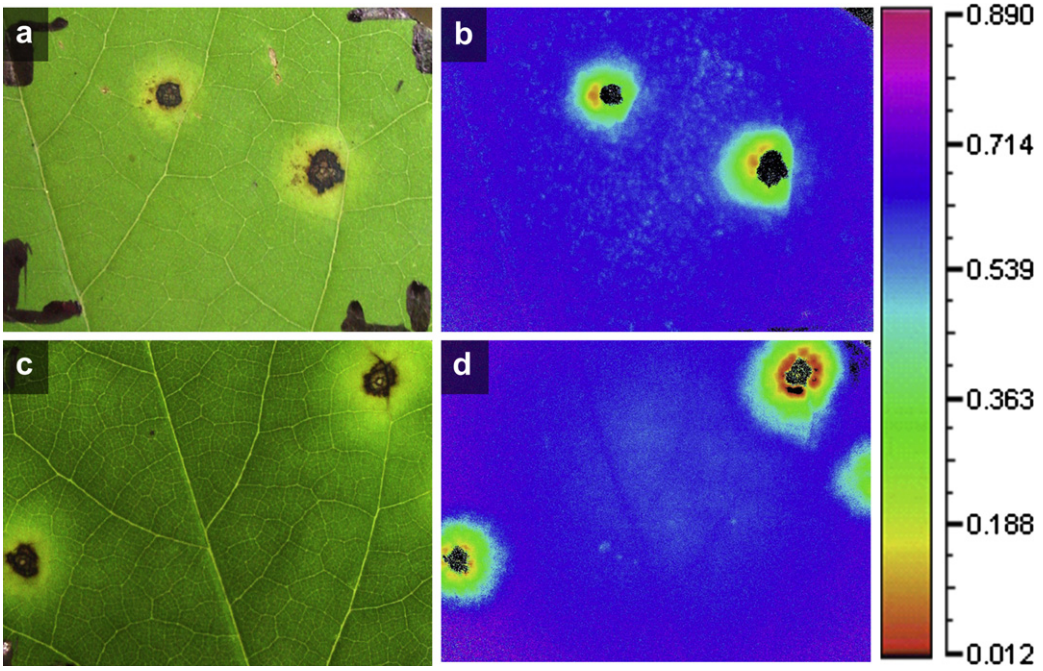

Fig. 4. Representative true-color reflected light images (left column) and their corresponding false-color maps of the spatial patterns of Φ_{PSII} for *Cercis canadensis* leaves. Leaves in these images are infected with *Cercospora cercidicola* leaf spot and growing under ambient (a, b) and elevated CO_2 (c, d). The color scale for the false-color maps is provided to the right of these images. Damaged and halo areas of the infected leaves (see Fig. 5) are represented by dark blue and light blue/aqua, respectively, in these images. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

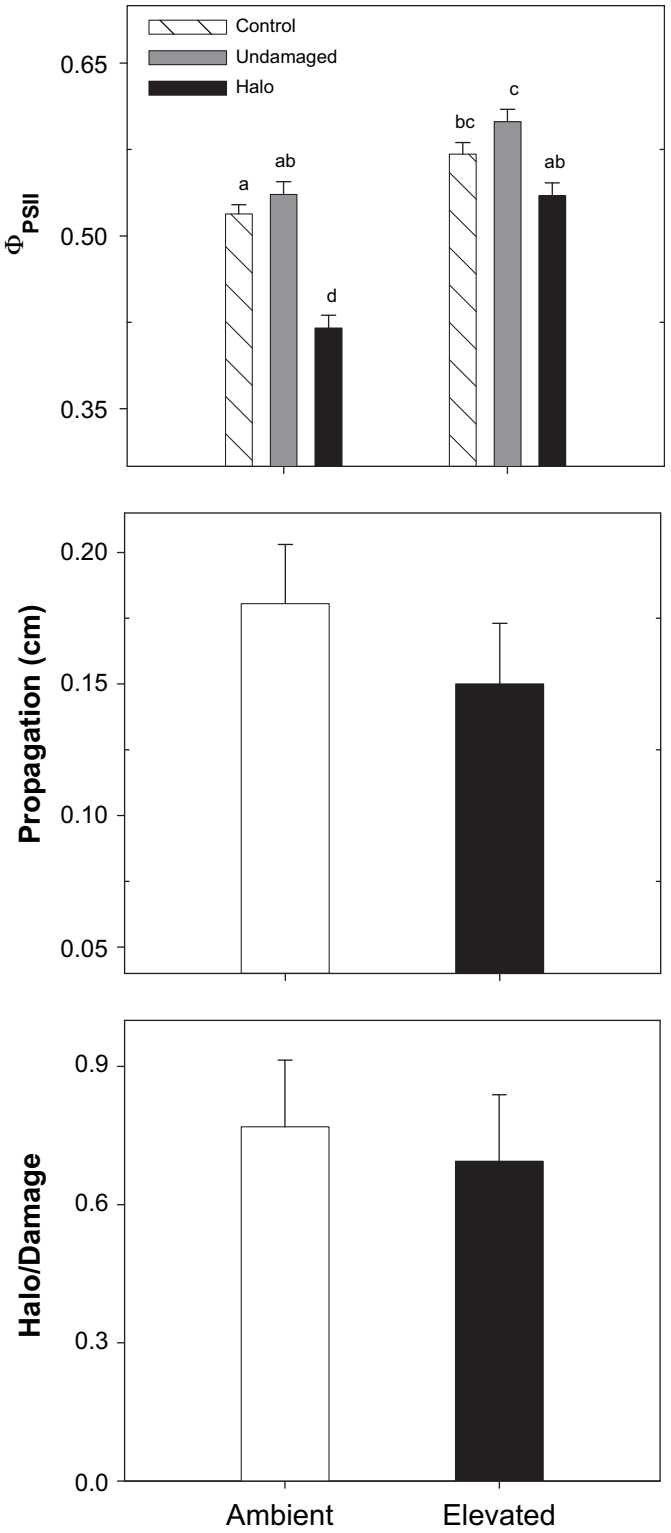

Fig. 5. Image analysis results demonstrating the indirect effects of *Cercospora cercidicola* infection on leaves of *C. canadensis* growing under ambient and elevated CO_2. Φ_{PSII} values (top panel) represent the operating efficiency of Photosystem II. The striped, gray, and black bars represent values of Φ_{PSII} for uninfected control leaves, undamaged portions of infected leaves, and the regions surrounding visible leaf spots where photosynthesis was suppressed (i.e. halo), respectively, for each treatment (A). Propagation (middle panel) is the average linear distance that depressed photosynthesis extended away from the *C. cercidicola* leaf spots. Halo/Damage ratio (bottom panel) represents the total area of depressed Φ_{PSII} divided by the area of visible lesion damage. Bars represent least squared mean ± SE values (25–49 lesions were analyzed per CO_2 treatment), and those with different letters were significantly different at $P \leq 0.05$.

(Table 1). Total N, C:N, and phenolics was not significantly different between ambient and elevated CO_2 both within a given measurement year or when averaged across the study years ($P > 0.05$).

4. Discussion

Recent work with other deciduous forest trees growing in FACE facilities has shown that exposure to altered atmospheric conditions can modify fungal disease expression (Karnosky et al., 2002; McElrone et al., 2005; Percy et al., 2002). In this study, we found that elevated CO_2 increased disease incidence and severity in two *Cercospora* leaf spot pathosystems in some years. When our current results are combined with all previous field studies that evaluated the effects of elevated CO_2 on fungal disease, it is clear that results are varied. Of the 16 pathosystems studied previously, incidence and/or severity increased in five pathosystems (Eastburn et al., 2009; Kobayashi et al., 2006; Mitchell et al., 2003; Thompson and Drake, 1994; current results), decreased in seven (Chakraborty et al., 2000b; Eastburn et al., 2009; Jwa and Walling, 2001; McElrone et al., 2005; Pangga et al., 2004; Thompson et al., 1993; Thompson and Drake, 1994), and remained the same in four (Eastburn et al., 2009; Hibberd et al., 1996; Percy et al., 2002; Tiedemann and Firsching, 2000). These varied results continue to illustrate how the specific nature of host–pathogen interactions makes it difficult to devise general principles that govern changes across fungal pathosystems (Chakraborty et al., 2000a, 2008; Coakley et al., 1999; Manning and Tiedemann, 1995). However, such work is still needed because in the majority of cases (12 out of the 16 compiled above) disease expression will change under an atmosphere with elevated [CO_2].

Chlorophyll fluorescence imaging revealed that any increases in *Cercospora* leaf spot experienced under elevated CO_2 would likely be minimized by enhanced photosynthetic efficiency in the remaining leaf tissue. Aldea et al. (2006) utilized this imaging technique to show that damage caused by biotic agents extends well beyond the visible damage; in some cases the tissue area with depressed Φ_{PSII} surrounding visible damage was equal to that of the lesion area itself (i.e. true damage total is twice that of the visibly damaged area). Our current results demonstrate that Φ_{PSII} was higher in control leaves, in undamaged portions of infected leaves, and in halos surrounding the visible lesion damage for redbud exposed to elevated CO_2. For species like redbud, the negative effects of increased disease under elevated CO_2 will be offset by the positive effects in photosynthetic gains. Future studies will need to consider whether the visible disease damage alone is a true representation of the damage caused by pests.

Evidence for global climate warming is clear and now widely accepted, and models predict that altered precipitation regimes will accompany the increased temperature in many regions (IPCC, 2007). Several recent studies have simulated how altered regional climates will impact future plant disease conditions (see summary by Jeger and Pautasso, 2008). In two of these simulations, disease

Table 1
Leaf chemistry of *Cercis canadensis* and *Liquidambar styraciflua* plants growing under ambient and elevated CO_2 at the Duke FACE site in Durham, North Carolina from 2000 to 2003. Data are presented as means (SE).

Species	Treatment	Total N (% dry wt.)	C:N	Phenolics (g g^{-1} leaf)
Redbud	Ambient	2.58 (0.10)	18.1 (0.6)	0.11 (0.03)
	Elevated	2.50 (0.09)	19.1 (0.2)	0.11 (0.01)
Sweetgum	Ambient	2.11 (0.12)	23.5 (0.2)	0.12 (0.01)
	Elevated	1.98 (0.14)	25.0 (1.4)	0.13 (0.01)

Leaf total N and C:N values are means of each treatment measured in 2000, 2001 and 2003. Phenolics were measured only in 2000 for both species.

models were combined with output from global circulation models that consistently predicted increases in temperature and decreases in precipitation for two different regions of Europe (Desprez-Loustau et al., 2007; Salinari et al., 2006). Increasing temperatures consistently resulted in greater disease for the majority of pathogens analyzed in these simulations, but in many cases it was counterbalanced by a decrease in disease pressure with lower summer precipitation. Because our study was conducted at a FACE facility, we were able to monitor *Cercospora* disease expression in trees exposed to natural climatic variability and pathogen loads. Across the five years, we found that higher rainfall increased *Cercospora* leaf spot disease, results that contradict these recent simulation studies. Changes in disease associated with increased precipitation were to be expected since *Cercospora* diseases are favored by wet weather and high humidity that enhance production and spread of conidia for secondary infections (Sinclair et al., 1987; Wolf, 1940). The Hadley climate model projects significant increases in precipitation for the southeastern US (NAST, 2000). If these projections are correct, disease pressure in these pathosystems could increase significantly by the end of this century.

The recent simulations do not explicitly account for the direct impact of changes in atmospheric gases on disease expression (Desprez-Loustau et al., 2007; Salinari et al., 2006). Eastburn et al. (2009) found that the direct effects of elevated CO_2 and O_3 influence disease expression as much or more so than interannual variability of climatic conditions. Future management and simulations of diseases in agricultural and natural systems will need to consider both the direct impact of atmospheric change and the resulting alterations in regional climates. Long-term studies at FACE facilities provide an ideal setting for such work.

Elevated CO_2 is known to stimulate fungal pathogen growth rates, aggressiveness, and fecundity (Chakraborty et al., 2000b; Coakley et al., 1999; Hibberd et al., 1996; McElrone et al., 2005). For example, an anthracnose pathogen exposed to elevated CO_2 exhibited increases in sporulation per unit of infected leaf area and in aggressiveness towards resistant cultivars after a few infection cycles (Chakraborty et al., 2000b; Chakraborty and Datta, 2003, respectively). The increased disease expression documented here may have resulted from enhanced fungal performance under elevated CO_2. However, if *Cercospora* growth and reproduction were increasing we would expect an increase in pathogen load and disease pressure in the elevated CO_2 plots overtime. Since such a pattern did not occur, the increased disease expression is likely a result of some other change in the host plants.

Changes in disease expression in plants exposed to elevated CO_2 have been attributed to altered canopy structure, leaf demography, and altered host chemistry (McElrone et al., 2005; Pangga et al., 2004). Elevated CO_2 increases leaf area index and promotes canopy closure in many hosts (Dermody et al., 2006), and specifically increases leaf area duration for *L. styraciflua* and *C. canadensis* growing at Duke FACE (Hartz-Rubin and DeLucia, 2001). A larger canopy and increased leaf longevity may have contributed to increased disease incidence by promoting greater spore capture and more favorable humidity conditions around the leaf tissue for a longer period of time.

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