On the relationship between stomatal characters and atmospheric CO_2

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[1] Leaf stomatal characters influence the response of terrestrial evapotranspiration to climate change and are used as proxies for the reconstruction of past atmospheric [CO_2]. We examined the phenotypic response of stomatal index (SI), density (SD) and aperture (AP) to rising atmospheric CO_2 in 15 species after four years exposure to a field CO_2 gradient (200 to 550 μmol mol^{-1} atmospheric [CO_2]) or at three Free Air CO_2 Enrichment (FACE) sites. Along the CO_2 gradient, SI and SD showed no evidence of a decline to increasing [CO_2], while AP decreased slightly. There was no significant change in SI, SD or AP with CO_2 across FACE experiments. Without evolutionary changes, SI and SD may not respond to atmospheric [CO_2] in the field and are unlikely to decrease in a future high CO_2 world. *INDEX TERMS:* 0315 Atmospheric Composition and Structure: Biosphere/atmosphere interactions; 1615 Global Change: Biogeochemical processes (4805); 1851 Hydrology: Plant ecology; 3344 Meteorology and Atmospheric Dynamics: Paleoclimatology. **Citation:** Reid, C. D., H. Maherali, H. B. Johnson, S. D. Smith, S. D. Wullschleger, and R. B. Jackson, On the relationship between stomatal characters and atmospheric CO_2, *Geophys. Res. Lett.*, *30*(19), 1983, doi:10.1029/2003GL017775, 2003.

1. Introduction

[2] Various proxies are used to infer past atmospheric CO_2 concentrations and their consequences for the earth's climate [*Crowley and Berner*, 2001]. Stomatal density (SD; # stomata per unit area) and index (SI; # stomata divided by the sum of stomatal and epidermal cells #) of plants are used because paleontological data [*Woodward*, 1987; *Retallack*, 2001] and growth chamber experiments [*Royer*, 2001] suggest they decline with increasing CO_2 up to 600 μmol CO_2 mol^{-1} [*Beerling and Royer*, 2002]. Stomatal density and the size of the stomatal aperture (AP; length between the junctions of the guard cells at each end of the stoma) partially determine leaf conductance to H_2O and CO_2, a key physiological variable in coupled atmosphere-biosphere models [*Sellers et al.*, 1996; *Kürschner et al.*, 1997; *Collatz et al.*, 2000]. Studies in natural CO_2 vents, which provide a long-term experimental system for adaptation to elevated CO_2, suggest that many species do not alter their SD or SI in response to elevated atmospheric CO_2 [*Jones et al.*, 1995; *Bettarini et al.*, 1998], although some increase AP [*Tognetti et al.*, 2000]. However, there are few direct experimental tests of the CO_2 - SD/SI/AP relationship in the field. Furthermore, to our knowledge, no field experiment has tested the effect of low atmospheric CO_2 concentration on stomatal structure and function, a critical window for paleo-reconstruction. Here, we present data for 15 annuals and perennial herb and woody species suggesting that, in the absence of evolutionary changes, SD and SI do not decrease with increasing CO_2 under field conditions but AP may.

2. Methods

[3] We examined the response of SD, SI, and AP to CO_2 in the field using a unique four-year experiment that maintained a continuous CO_2 gradient from paleo to future atmospheric CO_2 [200 to 550 μmol mol^{-1}; *Gill et al.*, 2002] and three long-term Free Air CO_2 Enrichment (FACE) experiments. We examined woody, herbaceous, and annual species because previous analyses of paleo-correlations between CO_2 concentrations and stomatal characters have relied primarily on woody perennial species but have also used herbs [*Wooller and Agnew*, 2002]. In addition, the annual species *Arabidopsis thaliana* has been instrumental in attempts to elucidate the role of CO_2 signaling on stomatal development [*Gray et al.*, 2001; *Lake et al.*, 2001].

[4] The continuous CO_2 gradient and other environmental variables in the tunnel system have been described previously [*Johnson et al.*, 2000]. Briefly, it consisted of two parallel elongated chambers (1 m tall × 1 m wide × 60 m long) in a Texas grassland, with atmospheric CO_2 maintained from ambient to pre-industrial concentrations (365 to 200 μmol mol^{-1}) along one chamber and from elevated to ambient CO_2 along the other (550 to 350 μmol mol^{-1}). The different CO_2 concentrations along the gradient were maintained by varying the rate and direction of air flow through the chamber. Chamber temperature was controlled to track outside ambient temperatures. Although tunnel daytime temperatures were lower than ambient and differed between the pre-industrial and elevated chambers in the first year of operation [*Johnson et al.*, 2000], temperature control was enhanced in subsequent years so that both chambers were at ambient temperature [H. W. Polley, USDA/ARS, Temple, TX, unpublished data]. In the 4th year of CO_2 exposure, fully expanded sun leaves from each species were sampled in the Spring (*Bromus japonicus*, *Solanum dimidiatum*, *Sorghum halepense*) or Fall (*Convolvulus equitans*, *Paspalum pubiflorum*, *Solidago canadensis*). The dominant *Bothriochloa ischaemum* was sampled during both periods and showed similar results. Only data for

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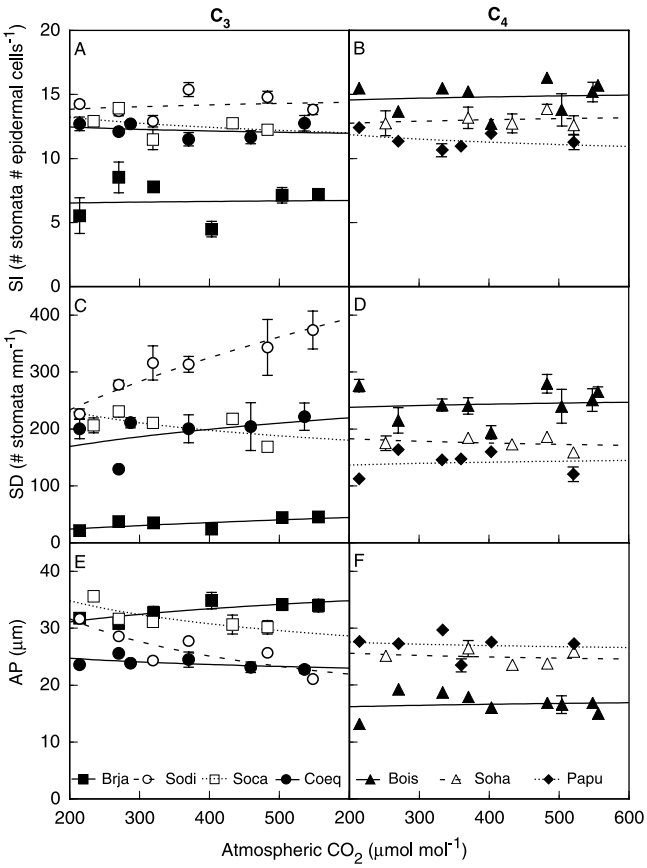

Figure 1. Stomatal characters of dominant species grown at subambient to elevated CO_2 in tunnel chambers for four years. Abaxial stomatal index (SI, the ratio of # stomata to the sum of stomata and epidermal cells #; (A–B), stomatal density (SD, # stomata per unit leaf area; (C–D), and the length of the stomatal aperture (AP, length between junction of the guard cells; E–F) are shown for C_3 grass and forbs (A, C, E): Brja, *Bromus japonicus*; Coeq, *Convolvulus equitans*; Soca, *Solidago canadensis*; Sodi, *Solanum dimidiatum*; and C_4 grasses (B, D, F): Bois, *Bothriochloa ischaemum*; Papu, *Paspalum pubiflorum*; Soha, *Sorghum-halepense*. Data were fitted to a power model in SAS 6.12 (SAS Inst. Cary, NC). (n = 6, mean ± standard error).

species growing along the entire gradient from pre-industrial to elevated CO_2 are presented here.

[5] Free-Air CO_2 Enrichment (FACE) technology is used in a *Pinus taeda* plantation at the Duke Forest, Durham, NC [*DeLucia et al.*, 1999], a desert scrub community at the Nevada Test Site, Las Vegas, NV [*Smith et al.*, 2000], and a *Liquidambar styraciflua* plantation at the Oak Ridge National Environmental Research Park, Oak Ridge, TN [*Norby et al.*, 2001]. All three FACE systems had been operating for at least four growing seasons when the leaves were sampled. CO_2 fumigation began in August 1996 at the Duke Forest, in April 1997 at the Nevada Test Site, and in April 1998 at the Oak Ridge forest. At the Duke Forest, fully expanded leaves were sampled in October 2000 (*L. styraciflua* and *Lonicera japonica*) and May 2001 (*Parthenocissus quinquefolia*, *Polygonatum biflorum*, *P. taeda*). *P. taeda* needles were sampled from the top of the forest canopy using mechanical lifts; the other species were sampled from the forest understory. At the Nevada desert site, plants were sampled in early May 2001. At Oak Ridge, upper canopy leaves of *L. styraciflua* were sampled in May 2001.

[6] At each field site, casts of fully expanded mature leaves were made by pressing leaf sections onto a microscope slide covered with polyvinylsiloxane dental impression material ('*Extrude*' Medium, Kerr Manufacturing Co, Orange, CA, USA; *Williams and Greene* [1988]). For short leaves (<2 cm length), the whole leaf was used. For longer leaves, a 1-cm length starting 2 cm up from the petiole or blade base was used to minimize variability due to position [*Poole et al.*, 1996]. Each impression was analyzed at 100× or 400× on a light microscope interfaced with a solid state TV camera (Model CCD-72-SX; DAGE-MTI Inc., Michigan City, IN, USA) using an image analysis program (NIH Image 1.58; U.S. National Institutes of Health; http://rsb.info.nih.gov/nih-image/). Stomatal and epidermal cell counts were done on 3 to 6 fields-of-view per slide (depending on the variation in the counts) and were averaged for each slide. For the Oak Ridge site, the upper canopy leaves of 3 to 4 trees in each of three replicate ambient and elevated CO_2 rings were harvested. Leaves were placed into plastic envelopes and transported immediately to the laboratory, where leaf surface impressions were taken using clear fingernail polish. The number of stomata within each of three randomly selected fields (0.05 mm^2) per leaf was counted using a light microscope, and stomatal density was averaged per ring.

3. Results and Discussion

[7] Along the CO_2 gradient, SI was not correlated with CO_2 either for any single species ($P > 0.18$ in all species, Figures 1a and 1b) or for the pooled data ($P = 0.87$, Figure 2a). Although SI is preferred to SD, because it is

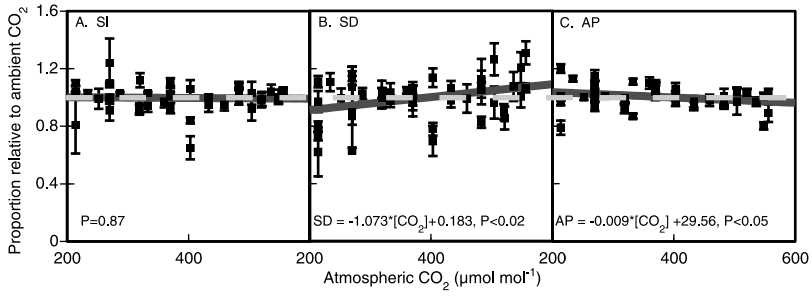

Figure 2. Relative change in stomatal characters of dominant species as a function of growth [CO_2] after four years in tunnel chambers. For all species pooled, each SI, SD, or AP at growth CO_2 is expressed as a proportion of SI, SD or AP at ambient CO_2. (n = 6 for mean ± standard error; dashed line = 1).

Table 1. Abaxial Stomatal Index (SI), Density (SD) and Length of the Stomatal Aperture (AP) of Woody and Herbaceous Species Grown at Current and Elevated Atmospheric CO_2 (FACE) for at Least Four Years

	SI		SD[a]		AP		
Species	Ambient Avg. (#/#)	% Change	Ambient Avg. ($\#mm^{-2}$)	% Change	Ambient Avg. (μm)	% Change	Location
Eriogonum trichopes (forb)[b]	14.7 (0.3)	−0.4	312 (31)	+2.8	18.2 (1.5)	−1.1	Nevada Test Site, NV[d]
Larrea tridentata (shrub)[b]	N/A	N/A	121 (10)	+6.4	19.6 (0.4)	+11.2*	Nevada Test Site, NV
Lepidium lasiocarpum (forb)[b]	13.5 (0.6)	+4.6	246 (16)	+15.6	14.8 (0.5)	−1.3	Nevada Test Site, NV
Liquidambar styraciflua (tree)	N/A	N/A	461 (22)[c]	−3.0	N/A	N/A	Overstory, Oak Ridge TN[e]
Liquidambar styraciflua (tree)	11.4 (0.4)	−8.1	264 (16)	−24.5**	16.1 (0.4)	+3.0	Understory, Durham NC[f]
Lonicera japonica (vine)	11.9 (0.3)	−0.6	237 (10)	+6.8	17.5 (0.3)	+1.5	Understory, Durham NC
Parthenocissus quinquefolia (vine)	8.7 (0.2)	−0.15	121 (5)	+16.6	21.0 (0.5)	+2.2	Understory, Durham NC
Pinus taeda (tree)	N/A	N/A	112 (5)	+1.1	33.4 (1.2)	−1.8	Overstory, Durham NC
Polygonatum biflorum (forb)	16.4 (1.2)	+10.8	49 (8)	+1.5	27.5 (1.3)	+5.2	Understory, Durham NC

The % Change from ambient CO_2 (ca. 360 $\mu mol\ mol^{-1}$) in SI, SD or AP is shown for plants grown at elevated CO_2 (ca. 550−560 $\mu mol\ mol^{-1}$). In each FACE site, leaves of six individuals per species were used for each replicate ambient or elevated CO_2 ring (n = 18, mean ± standard error). CO_2 effect is significant at $P < 0.05$* and at $P < 0.005$** using ANOVA.

[a]Ratio of abaxial to adaxial SD was near 1 for amphistomatous species and was not affected by CO_2.

[b]Amphistomatous leaf.

[c]n = 4.

[d]*Smith et al.* [2000].

[e]*Norby et al.* [2001].

[f]*DeLucia et al.* [1999].

unaffected by changes in epidermal cell expansion with CO_2 or other environmental factors [*Retallack*, 2001; *Royer*, 2001], SD is easier to measure and is often used when epidermal cells cannot be counted accurately. SD showed little evidence of the predicted decline with CO_2. Analyzed for species individually, SD increased significantly with CO_2 (Figure 1c) for *Br. japonicus* ($r^2 = 0.243$, $P < 0.005$) and *S. dimidiatum* ($r^2 = 0.252$, $P < 0.005$), opposite the direction predicted, and decreased only for one species, *S. canadensis* ($r^2 = 0.180$, $P < 0.05$). With all species pooled, SD showed a weak positive correlation with CO_2 ($P = 0.02$, Figure 2B), again opposite the direction predicted. AP showed a weak negative correlation with CO_2 ($P < 0.05$, Figure 2c) suggesting some compensation between stomatal number and size. However, individually, only *S. canadensis* and *S. dimidiatum* showed significant decreases in AP with increasing CO_2 ($r^2 = 0.216$, $P < 0.004$; and $r^2 = 0.542$, $P < 0.0001$, respectively, Figure 1e), while AP for *Br. japonicus* increased significantly ($r^2 = 0.149$, $P < 0.01$, Figure 1e).

[8] Results from the FACE experiments provide no evidence for a decline in SI or AP generally ($P = 0.62$ and $P = 0.33$, respectively) or for any species individually (Table 1). Similarly, the pooled SD data provided no evidence for a decline in SD at high CO_2 ($P = 0.83$, Table 1), also in contrast to current predictions. In fact, in seven of eight species, mean SD tended to be higher at elevated CO_2 (though not significantly), and only *L. styraciflua* when grown in the understory showed decreased SD (Table 1). These data suggest no significant morphological stomatal adjustments from current ambient to elevated CO_2. They also suggest that evolutionary timescales may be required for such responses to projected future CO_2 concentrations, as occurred for a few species in CO_2 vent studies [*Jones et al.*, 1995; *Tognetti et al.*, 2000]. Many trees will experience almost a doubling of atmospheric CO_2 in their current lifetime without the opportunity for such evolutionary changes.

[9] Our long-term experiments suggest that no general association between SD, SI or AP and future atmospheric CO_2 is evident in the field. Available data on SD and SI from short-term CO_2 enrichment studies in open-top field chambers [*Royer*, 2001] and long-term records from near CO_2 springs [*Bettarini et al.*, 1998] are consistent with this conclusion. Individually, species responded from pre-industrial to future CO_2 concentrations by a combination of change in stomatal size and number rather than solely by SD, and many species in all four experiments decrease their stomatal conductance in response to increased CO_2 despite the lack of decrease in SD or SI [e.g., *Maherali et al.*, 2002; *Nowak et al.*, 2001].

[10] Although our experiments do not incorporate long-term evolutionary effects, they reinforce some caveats about using stomatal characters in paleo-reconstruction of atmospheric CO_2, especially when historical factors such as precipitation, temperature, canopy position and leaf age are poorly characterized. Numerous studies have reported the effects of environmental variables other than CO_2 on stomatal development and morphology. For example, light availability and quality [e.g., *Tichá*, 1982; *Schoch et al.*, 1984; *Liu-Gitz et al.*, 2000] affect stomatal development and, hence, a plant can have leaves with different SD depending on leaf position in the canopy [*Ceulemans et al.*, 1995]. Furthermore, signaling of light and CO_2 for stomatal development has been explored and appears similar [*Lake et al.*, 2001]. Likewise, exposure to drought during leaf development reduces SD [*Ciha and Brun*, 1975; *Awada et al.*, 2002], and, in altitudinal gradients where atmospheric CO_2 decreases, SD is determined by precipitation rather than CO_2 concentration [*Körner et al.*, 1986]. Therefore, environmental factors other than CO_2 concentration may be stronger determinants of stomatal characters in natural settings.

[11] Predictions of reduced SD, SI, or AP with rising atmospheric CO_2 are implicit in coupled biosphere-atmosphere models as a mechanism for declining stomatal conductance [*Sellers et al.*, 1996; *Kürschner et al.*, 1997; *Aasamaa et al.*, 2001]. These relationships are also being incorporated into projected forest responses to pollutants

such as ozone [*Evans et al.*, 1996]. Our results indicate that SI, SD, and AP are unlikely to decline in response to future high CO_2, and may not have responded significantly to low CO_2 in the past. Stronger field-based evidence supporting the relationships should emerge before stomatal characters are incorporated into biosphere-atmosphere models.

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