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Stomatal Responses to CO₂: A Comparison of Woody and Herbaceous Species in Arid and Humid Climates

A Collaborative Project between Brookhaven National Laboratory (Dr. David Ellsworth) and Northern Arizona University (Dr. George W. Koch)

George W. Koch,
Associate Professor
Northern Arizona University

Executive Summary

The project involved a study of a fundamental response of terrestrial vegetation to rising atmospheric carbon dioxide (CO₂) concentration, namely, the change in leaf conductance to gas diffusion associated with a change in the aperture of the microscopic pores (stomata) on the surface of leaves. This “stomatal conductance” determines the rate at which water is lost from vegetation during transpiration and the rate at which CO₂ is taken up by leaves during photosynthesis. Many previous studies have documented a decrease in stomatal conductance when leaves are exposed to elevated CO₂ levels similar to those expected toward the latter half of the 21st century (c. 550 ppm vs. c. 360 ppm in 1999). The consequences of a reduction in stomatal conductance are several and important: a reduction in water use by vegetation, assuming the transpiring surface area of leaves is unchanged, 2) a smaller enhancement in photosynthesis than would be realized if stomatal conductance remained unchanged in elevated CO₂, 3) an increase in leaf temperature because evaporative heat loss is reduced when lower stomatal conductance cause a decrease in transpiration.

Previous studies have shown a wide range of stomatal conductance responses to CO₂. It has been unclear whether this range reflects inherent differences in species’ responses or variation due to the methodologies and growing conditions employed in different studies. Our study sought to apply a uniform methodology for assessing stomatal response to elevated CO₂ in studying vegetation from contrasting environments at sites where the same means of exposing plants to elevated CO₂ has been employed. Thus, we used standard methods of leaf gas exchange measurement to determine the short-term and acclimated stomatal conductance responses of vegetation at four different Free-Air CO₂ Enrichment (FACE) study sites distributed across the continental U.S. In addition, we included a comparison of stomatal conductance response to elevated CO₂ of two major woody plant species – piñon pine and one-seeded juniper – that are extensively distributed in the southwestern U.S. and not yet studies in FACE experiments.

Summary of Major Findings.

- We found that the magnitude of the stomatal conductance response to elevated CO₂ was directly related to the stomatal conductance in ambient CO₂; species with higher conductance showed larger reductions in conductance when exposed to increased CO₂.
- Dominant species of woody plants, notably coniferous trees, showed minor changes in stomatal conductance in elevated CO₂, a pattern contrary to that of most herbaceous species and which has been the basis for conductance changes used in models of dynamic interactions between climate, atmospheric composition, and vegetation physiology.
- We demonstrated the value of applying a standard methodology for gas exchange measurements.
- Our findings will inform the scientific and policy communities regarding the potential magnitude of changes in biosphere-atmosphere CO₂ and H₂O exchange in response to a doubling of pre-industrial atmospheric CO₂ concentration.
- The project benefits the public by providing an improved scientific basis for policy decisions related to regulation of greenhouse gas emissions based on an understanding of the likely range of plant response to future atmospheric CO₂ levels.

II. Summary of project activities for entire funding period

Project Objective: The overall objective was to provide a systematic comparison of the response of stomata to rising atmospheric CO₂ concentration in diverse plant species in established free-air CO₂ enrichment (FACE) experiments.

Original hypotheses

Our primary hypothesis at the outset of the study was that plant species with the highest stomatal conductance would show the largest stomatal conductance response to elevated CO₂.

Specific Objectives:

(1) To measure stomatal responses to elevated CO₂ during acclimation to elevated CO₂ for diverse plant species at FACE sites in arid and humid climates.

(2) To investigate the mechanism of these responses in a subset of study species through detailed analysis of relationships among photosynthesis, conductance and intercellular CO₂ for acclimating and non-acclimated leaves.

Research Approach and Methodologies

The investigators visited each of the 4 principle FACE sites (Nevada, Minnesota, Wisconsin, Duke) a total of 3 to 4 times between 1997 and 2000. These studies covered a total of 18 plant species. In addition we studied two additional species, piñon pine (*Pinus edulis*) and one-seeded juniper (*Juniperus monosperma*) in northern Arizona. These conifers, while not the subject of an existing FACE study, extended our survey of short-term response to elevated CO₂ to include an additional major vegetation type (arid montane evergreen). Table 1 lists the species for which stomatal response to CO₂ was examined in a consistent manner using the methodology developed during Year 1.

During each FACE site visit, stomatal conductance and associated gas exchange parameters were monitored for plants in Ambient (blower controls, ca. 360 ppm) and Elevated CO₂ (550 ppm) rings. Based on more extensive diurnal measurements by on-site investigators, our measurements were concentrated in the morning when gas exchange rates are high. To ensure intercomparability of all data, a common measurement protocol was used and the Li-Cor 6400 gas exchange systems were checked regularly to minimize calibration drift.

To determine if **acclimation** of stomatal conductance has occurred, we compared “operational” gas exchange rates (i.e. that measured at the growth CO₂ level and ambient temperature and vapor pressure) in the two CO₂ treatments (Amb & Elev). To examine the **dynamics** of stomatal response, we also applied a hour-long step change to the reciprocal CO₂ level (Amb → Elev or Elev → Amb) after these steady-state measurements. Stomatal dynamics were analyzed with a new computer program developed specifically for this purpose. Importantly, data collected at the end of the one hour reciprocal CO₂ treatment were also used to evaluate the extent to which the short-term response of ambient-grown leaves to increased CO₂ mirrored the longer-term, acclimation response of leaves exposed to elevated CO₂ for a season or more.

Table 1. List of primary study species. Species were chosen to complement ongoing core research at each site and to provide a broad life-form comparison across sites.

Species	Abbreviation	Growth form	Reciprocal Measurements ¹	Site ²
<i>Oenothera primiveris</i>	Oepr	Winter annual forb	X	NDFF
<i>Sorghastrum nutans</i>	Sonu	C ₄ Perennial grass	X	CCF
<i>Baileya multiradiata</i>	Bamu	Perennial C ₃ forb	X	NDFF
<i>Populus tremuloides</i>	Potr	Deciduous tree	X	FACTS-II
<i>Achillea millefolium</i>	Acmi	Perennial C ₃ forb	X	CCF
<i>Solidago rigida</i>	Sori	Perennial C ₃ forb	X	CCF
<i>Liquidambar styraciflua</i>	List	Deciduous tree	X	FACTS-I
<i>Betula papyrifera</i>	Bepa	Deciduous tree	X	FACTS-II
<i>Lycium pallidum</i>	Lypa	Deciduous shrub	X	NDFF
<i>Larrea tridentata</i>	Latr	Evergreen shrub	X	NDFF
<i>Pinus taeda</i>	Pita	Evergreen conifer	X	FACTS-I
<i>Acer rubrum</i>	Acru	Deciduous tree		FACTS-I
<i>Cornus florida</i>	Cofl	Deciduous tree	X	FACTS-I
<i>Pinus edulis</i>	Pied	Evergreen conifer		N. AZ
<i>Juniperus monosperma</i>	Jumo	Evergreen conifer		N. AZ
<i>Agropyron repens</i>	Agre	C ₃ perennial grass		CCF
<i>Taxodium distichum.</i>	Tadi	Evergreen conifer		FACTS-I

¹ 'X' denotes a complete set of reciprocal measurements (ambient measured at elevated and *vice versa*) made for the designated species.

² Sites are abbreviated as follows: NDFF, Nevada Desert FACE site; CCF, Cedar Creek, MN FACE site; FACTS-I, Forest-Atmosphere Carbon Transfer and Storage-I (N.C.); FACTS-II, Forest-Atmosphere Carbon Transfer and Storage-II (WI), N. AZ, northern Arizona montane coniferous woodland, Flagstaff, AZ.

III. Summary of Results

A. Stomatal dynamics: conductance response to short-term change in CO₂ concentration.

- Grasses showed the largest responses to CO₂, with the strongest opening and closing responses among the 10 intensively-studied species in FACE experiments (Fig. 1). Among tree species, conifers (*Pinus taeda*, *Pinus edulis*, *Juniperus monosperma*) showed smaller conductance responses to a short-term increase in CO₂ than did the broadleaved species (*Betula*, *Populus*, *Liquidambar*).

Figure 1. Stomatal responses to CO₂ with an hour-long step pulse either +200 ppm (ambient-grown plants; top panel) or -200 ppm (elevated CO₂-grown plants; bottom panel). With the exception of *Pinus edulis* (Pied) and *Juniperus monosperma* (Jumo), species are dominants measured at the 4 FACE sites, ordered according to their maximum *g_s* in ambient CO₂ (same order retained for elevated CO₂-grown plants). Genus names are abbreviated. Measurement year is specified below name for species measured in 1998 and 1999, otherwise measurements are from 1998.

- Year-to-year differences in the magnitude of stomatal responsiveness to CO₂ were not significant for *Pinus taeda* and *Solidago*. Year-to-year differences were apparent for *Betula* and dramatic for *Larrea*, with these differences attributable to large differences in soil moisture at the time of measurements. For example, associated with the record high soil moisture of July '98 at NDFE, *Larrea* had very high stomatal conductance and showed a much larger closing response in elevated CO₂ (both in the short-term and in the high CO₂ FACE rings) than in 1999, when rainfall was much lower. The same pattern was observed for *Betula*; higher conductance in the wetter year (1998) was associated with a greater stomatal response to elevated CO₂.

- **There was little evidence of photosynthetic acclimation in the study species across all FACE sites at the time of our measurements.** Even so, there was hysteresis in the reciprocal response to CO₂ pulses in most cases, suggesting acclimation of the short-term stomatal response to elevated CO₂. For the species with the strongest stomatal responses to CO₂ (*Sorghastrum*, *Agropyron* and *Liquidambar*), there was a tendency for the stomatal responses to reciprocal conditions to be greater for elevated CO₂-grown plants than for ambient plants (Fig. 1). For some of the species with the highest g_s (*Solidago*, *Betula* and *Populus*), stomatal responses were different in magnitude or sign in elevated CO₂-grown plants than expected based on the measured ambient plants (Fig. 1).

- **The C_i/C_a ratio of leaves is very conservative despite large changes in atmospheric CO₂ concentration.** Despite a 50% change in the CO₂ concentration (± 200 ppm) surrounding leaves in FACE experiments and in the pulse-response experiments, C_i/C_a ratios were maintained within 7% of the starting C_i/C_a. Net differences in C_i/C_a between Ambient and Elevated CO₂ plants measured at their growth CO₂ were even smaller than this. Even so, there were consistent if small patterns in C_i/C_a as a result of the reciprocal experiment (Fig. 2). When stomatal opening occurred in response to a step decrease in C_a, C_i/C_a decreased and vice versa. Species not responsive to the step change in C_a were inconsistent in their changes in C_i/C_a during the pulse experiments.

Figure 2. Changes in the C_i/C_a ratio of leaves from gas exchange measurements in the reciprocal pulse experiments. Non-responder species (change in g_s close to zero; *Larrea*-dry, *Pinus* and *Solidago*) have been removed for clarity. Different symbols indicate different species (mean ± s.e.), with open symbols for ambient-grown plants and closed symbols for elevated CO₂-grown plants.

B. Comparing short-term and long-term responses to elevated CO₂.

Figure 3. The relationship between net CO₂ assimilation and stomatal conductance to CO₂ for leaves of plants exposed to 360 ppm (upper panel) or 560 ppm (lower panel) CO₂. Plants grown at ambient CO₂ are depicted by filled symbols, plants grown at elevated CO₂ are depicted by open symbols.

- Elevated CO₂-grown plants show the same relationship between assimilation (A) and conductance (g) as ambient CO₂ grown plants.** Figure 3 shows the A-g relationship for ambient and elevated CO₂ plants, C3 species only. Each different symbol represents a different species-year combination. The upper graph is for leaves measured at ambient CO₂, with elevated-grown plants having open symbols and ambient-grown plants having filled symbols. The lower graph is for leaves measured at elevated CO₂ with the same symbols as above. There is no statistical difference between ambient- and elevated-grown plants when measured at a common CO₂. This implies that, across a wide range of species, acclimation to elevated CO₂ has not altered the fundamental relationship between stomatal conductance and photosynthesis. This result is consistent with there being no qualitative change in the biochemical mechanism(s) that

regulates the relationship between stomatal aperture and CO₂ assimilation. During the coming year we will examine this relationship in more detail for individual species.

- **Water use efficiency (WUE) is greater for elevated CO₂ grown plants than ambient CO₂ grown plants. WUE is greater at the growth CO₂ level than following a short-term change to the reciprocal CO₂ level.** On average, elevated CO₂-grown plants have a higher intrinsic WUE than ambient CO₂ grown plants, as indicated by the greater A for a given g (Figure 3). Ambient and elevated CO₂ plants each have the greater intrinsic WUE (as indicated by the slope of A/g) than the opposite treatment at their growth CO₂. Overall, the mean stomatal response is 12% (12% closing for ambient pulsed to elevated, 12% opening for elevated pulsed to ambient). Elevated CO₂ grown plants have on average a higher A/g than ambient plants for the given standardized mean g across all treatments. This means that there may be stomatal acclimation, but still not statistically significant. Our estimate of WUE is based on short-term gas exchange measurements.

- **The stomatal conductance of plants acclimated to elevated CO₂ can be estimated reasonably well from the conductance of ambient CO₂ grown plants given a short-term exposure to elevated CO₂ (Figure 4).** Given the high variance inherent in measurements of physiological function in natural conditions, it is remarkable that the short and long-term responses to elevated CO₂ fall close to the 1:1 line. In cases where conductance after acclimation to elevated CO₂ is higher than in ambient-grown plants pulsed to elevated CO₂, it may be the case that a system-level increase in soil moisture is allowing improved plant water relations and higher conductance. Interaction with FACE site scientists monitoring soil moisture and plant water status will allow us to address this possibility.

Figure 4. The relationship between stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$) to CO₂ in plants exposed to a short-term (“pulse”) exposure to 560 ppm and stomatal conductance of the same species acclimated to elevated CO₂. Each symbol represents the mean (± 1 s.d.) conductance of a single species from one of the 4 FACE sites.

IV. Dissemination

Presentations and meeting abstracts.

DeMarco, J., C. W. Edgar and G. W. Koch (2002). Stomatal response to CO₂ in conifers of semi-arid regions. 87th Annual meeting of the Ecological Society of America, Tucson, AZ. Aug 7- 11, 2002.

Ellsworth D.S., G.W. Koch, P.B. Reich and G. Katul. 2000. CO₂ responses in 15 species across four free-air CO₂ enrichment (FACE) experiments: is leaf Ci/Ca maintained? 85th Annual meeting of the Ecological Society of America, Salt Lake City, UT, Aug. 6-10 2000.

Edgar C.W., G.W. Koch and D.S. Ellsworth. 1999. Stomatal responses to elevated carbon dioxide among woody and herbaceous desert perennials. 84th Annual meeting of the Ecological Society of America, Spokane, WA, Aug. 8-12 1999.

G. Koch, C. Edgar, D. S. Ellsworth. Stomatal response to elevated atmospheric carbon dioxide: interspecific variation and implications for plant water use and air pollutant uptake. Electric Utilities Environmental Conference, January 11-13, 1999, Tuscon, AZ.

G. W. Koch, C. W. Edgar, D. S. Ellsworth. Patterns of stomatal response to elevated CO₂: an inter FACE site comparison. TCP/PER/NIGEC Joint Science Team Meeting, June 3 – 4, 1999, Indianapolis, IN.

C. W. Edgar, G. W. Koch, and D. S. Ellsworth. Stomatal response to elevated carbon dioxide among woody and herbaceous species from arid and humid environments. Southwestern Association of Biologists Annual Meeting, September 17-19, 1999, Ghost Ranch, New Mexico.

D. S. Ellsworth. Measurements and modeling of carbon dioxide and water vapor fluxes in a uniform pine forest. TCP/PER/NIGEC Joint Science Team Meeting, June 3 – 4, 1999, Indianapolis, IN.

D. S. Ellsworth. Stomatal responses to CO₂ and limitations to photosynthesis. Plant Group seminar, Essex University, 17 Nov. 1998, Essex University, Colchester, England.

Journal Publications

Katul, G.G., D.S. Ellsworth and C. Lai. 2000. Modeling assimilation and intercellular CO₂ from measured conductance: A synthesis of approaches. *Plant, Cell and Environment* 23:1313-1328.

Ellsworth, D. S., P. B. Reich, G. W. Koch, M. E. Kubiske, S. D. Smith. (2004) A comparison of photosynthesis, carboxylation, and leaf nitrogen responses of 16 species to atmospheric CO₂ across four free-air CO₂ enrichment (FACE) experiments. Submitted to *Global Change Biology*.

Koch, G. W., C. W. Edgar, J. DeMarco, and D. S. Ellsworth. Stomatal response to CO₂ in conifers of semi-arid regions. In preparation for *Oecologia*.