**Abstract**

DECHERD, SARA MAURINE. Primary Productivity and Forage Quality of *Ginkgo biloba* in Response to Elevated Carbon Dioxide and Oxygen – An Experimental Approach to Mid-Mesozoic Paleoecology. (Under the direction of Reese Barrick and Barry Goldfarb.)

Atmospheric composition was unique during the Late Jurassic and Early Cretaceous Periods (~180-90 mya) due to concurrent elevations of $CO_2$ and $O_2$. Experimental methodology, an under-utilized technique in paleoecology, is used to address the physiological responses of Ginkgo biloba seedlings to these conditions. Experimental results have implications to Mid-Mesozoic paleoecology. Plants were exposed to these atmospheric treatments in hyperbaric chambers:

Control: 1 atm pressure, 370 ppm $CO_2$, and 20.9% $O_2$

CO2: 1.25 atm pressure, 2000 ppm $CO_2$, and 20.9% $O_2$

CO2&O2: 1.25 atm pressure, 2000 ppm $CO_2$, and 30% $O_2$

Gas exchange parameters were measured after 24-Hour and 35-Day exposure to evaluate photosynthetic rate and primary productivity. G. biloba photosynthesis was stimulated by $CO_2$, but experienced photosynthetic down-regulation after 35 days. Elevated $O_2$ did not decrease photosynthetic rate.

The concentrations of protein, lignin, sugar, and starch, and the C:N ratio and non-structural to structural carbohydrate ratio of experimental G. biloba leaves were measured to assess foliage quality. Nutritive content was reduced while digestibility was increased in response to elevated $CO_2$ and $O_2$.

Observed changes in G. biloba suggest mid-Mesozoic primary productivity could have been increased 200-300% over control levels; plant growth rate and fecundity may have increased. Changes in foliar quality could have stimulated herbivore and detritivore biomass and affected foraging strategies during the mid-Mesozoic.

Stomatal density and index values of experimental leaves were compared with gas exchange data and stomatal values from mid-Mesozoic Ginkgo fossils to determine if stomatal frequency is a proxy for photosynthesis in fossil and shed leaves. Stomatal frequency correlated with conductance but not photosynthesis. Experimental and fossil values did not compare favorably. Stomatal density and index are inappropriate proxies for photosynthetic rate.

This study used experimental techniques to examine G. biloba physiological responses to atmospheric conditions that existed during the distant past. The use of experimental methods for paleoecological investigation is a powerful technique.

# PRIMARY PRODUCTIVITY AND FORAGE QUALITY OF *GINKGO BILOBA* IN RESPONSE TO ELEVATED CARBON DIOXIDE AND OXYGEN – AN EXPERIMENTAL APPROACH TO MID-MESOZOIC PALEOECOLOGY

by

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## BIOGRAPHY

Sara Decherd was born in Dillingham, Alaska, but grew up amongst the wetlands and Douglas Fir trees of the Willamette Valley in northwest Oregon. This backdrop instilled a love of the outdoors and an understanding of the inherent interconnectedness of all living things. She has tried to bring this love and understanding into her professional life.

Sara studied geology at Carleton College in Northfield, MN and graduated Cum Laude with a B.S. in June of 2001. During her stay in Minnesota, she learned the art of Ultimate Frisbee, the meaning of a true friend, and found a passion for reconstructing paleoecological history. Sara gained hands-on geologic mapping and interpretive experience through fieldwork in southern Minnesota and in the Apennine Mountains of Italy during an Off-Campus Studies Program to that country. She learned paleontologic field techniques as a student fieldworker on the Royal Tyrrell Museum of Paleontology's (RTMP) Field Experience program during the summers of 1999 and 2000 at Dinosaur Provincial Park (DPP) in Alberta, Canada. As a part of her work with RTMP, Sara designed an interpretive and interactive exhibit for the DPP Field Station focusing on recent discoveries by Dr. Michael Ryan and Dr. Don Brinkman, both of RTMP.

Sara moved to Raleigh, North Carolina, in the fall of 2001 to begin her graduate work in paleontology and paleoecology at North Carolina State University (NCSU) with advisors Dr. Reese Barrick and Dr. Barry Goldfarb. The present work has allowed her to return to her early loves of ecology and plants, but still incorporate deep history and geologic inference.

During her tenure in North Carolina, Sara presented her research at Society of Vertebrate Paleontology annual meetings and at Geological Society of America regional and national meetings. Her talks and posters were very well received and her current research has generated publicity in such notable popular science publications as New Scientist, Science News, and National Geographic. Her research has also attracted the attention of NHK (Japan Public Broadcasting) and Sara's work and ideas will soon be incorporated into a documentary on the causes and consequences of large dinosaur body size.

While her research endeavors have attracted national and international interest, Sara has also earned acclaim as a geology laboratory instructor in the Marine, Earth, and Atmospheric Sciences Department at NCSU. She helped to develop two hands-on field- and inquiry-based introductory science courses, Honors Introductory Geology, and Water Resource Management, both of which were well-regarded by her students. In fact, on the cusp of her graduation, Sara recognizes that nearly half of the undergraduate geology majors in her department took at least one of their introductory classes with her, and some still stop by for a quick explanation. Sara received a University Outstanding Teaching Assistant Award in 2003 for her skill in laboratory instruction. She also participated in the Certificate of Accomplishment in Teaching program to develop her teaching skills further during the 2005/2006 school year.

Sara looks forward to continuing her paleoecological studies in the future in whatever forms these may take, but hopes especially to continue to incorporate the elements of travel, teaching, and research into her long-term career goals.

## ACKNOWLEDGEMENTS

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The majority of this work was funded through a GSA student research grant (2004), but the mechanics of my research were made possible by a large group of professionals and students. The staff of the Center for Hyperbaric Medicine and Environmental Physiology, Dr. Claude Piantadosi, Owen Doar, Barry Castle, Albert Boso, and Ben Comfort, bent over backwards to support the fulfillment of this research for little more than thanks, a few jokes, and leftover trees. Part of this dissertation rightfully belongs to Owen for his help and hard work, and I hope that we can continue to work together in the future. Carmen Lanz-Garcia, Ross Whetton, Hou-Minh Chang, James Mickle, Anthony Lebude, Jennifer Wittmeyer, and Jon Karr provided assistance in the laboratory and greenhouse, and taught me all I know about biochemistry. Alan Coulson, Bill Straight, and Jeff Bartlett deserve special thanks for their friendship, collegial support, and for being my paleo-gurus; I'm glad to have met each of them, and look forward to seeing them at the next professional meeting.

Finally, on a more personal note, this process has not been easy, and I would not have made it without the support of my loved ones. I am eternally grateful to my parents, Martha and Glenn Decherd, my brother, Ari, and my grandmother and grad school mentor, Bobe (Roberta L. Weil, PhD). My best friend, Sarah C, my long-lost cousin, Laura, and especially my boyfriend, Solomon, deserve even more thanks because they're not actually related to me. ☺ Thank you to all of you for always being there to listen, support, debate, and inspire me to meet the continuing challenges presented by my pursuit of this degree. I am stronger for sticking through it, and hope that in time I can repay the favor to each of you.

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24-Hour

Experiment……...Gas exchange experiment involving a 24-hour acclimation period

35-Day

Experiment……...Gas exchange experiment incorporating a 5-week acclimation period

A……..…… Net photosynthetic rate

ANOVA……... Analysis of Variance

$C_3$ plants……... Plants that use the $C_3$ photosynthetic pathway

$C_4$ plants……... Plants that use the $C_4$ photosynthetic pathway

$C_i$…….. Carbon dioxide concentration within the pore spaces inside a leaf

CO2…….. Elevated carbon dioxide treatment: 1.25 atm pressure, 2000ppm carbon dioxide and 20.9% oxygen

CO2&O2…….. Elevated carbon dioxide and oxygen treatment: 1.25 atm pressure, 2000ppm carbon dioxide and 30% oxygen

Control……. Control treatment: I atm pressure, 370ppm carbon dioxide, and 20.9% oxygen

CSP…….. Carbon Saturation Point. It is the photosynthetic rate at which increases in carbon dioxide no longer causes increases in photosynthetic rate)

GLM…….General Linear Models procedure (SAS Statistical Software)

LSP……...Light Saturation Point. It is the photosynthetic rate at which increases in light level no longer cause increases in photosynthetic rate

PAR……..Photosynthetically Active Radiation

PE ……Photosynthetic Efficiency. It is the slope of the linear portion of the curve comparing photosynthetic rate with carbon dioxide concentration.

ppm…….. Parts Per Million; a unit of concentration used for gasses

Non-adjusted..…..Chlorophyll data as measured

Rubisco…… Ribulose 1,5 Bisphosphate Carboxylase/Oxygenase, the initial enzyme involved in photosynthesis. It can react with both carbon dioxide and oxygen molecules

QE …….. Quantum Efficiency. It is the slope of the linear portion of the curve comparing photosynthetic rate with light level

TNC……. Total Non-structural Carbohydrates (concentration of sugar and starch)

TNC-free……..Chlorophyll data as corrected for possible dilution from TNC

**CHAPTER 3**: *The nutritive content and digestibility of Ginkgo biloba seedlings exposed to elevated atmospheric carbon dioxide and oxygen: Implications for Mesozoic food webs and decomposition rates*

35-Day Experiment……...Gas exchange experiment incorporating a 5-week acclimation period

ANOVA……... ANalysis Of VAriance

ADL……. Acid-Detergent Lignin

BSA……. Bovine Serum Albumine

%C……... Percent Carbon

C:N ratio……...The ratio between Carbon and Nitrogen

CO2…… Elevated carbon dioxide treatment: 1.25 atm pressure, 2000ppm carbon dioxide and 20.9% oxygen

CO2&O2…… Elevated carbon dioxide and oxygen treatment: 1.25 atm pressure, 2000ppm carbon dioxide and 30% oxygen

Control…… Control treatment: I atm pressure, 370ppm carbon dioxide, and 20.9% oxygen

DDI…….. Distilled Deionized (water)

EA……... Elemental Analyzer

GLM…….General Linear Models procedure (SAS Statistical Software)

%N……... Percent Nitrogen

NaOH……... Sodium hydroxide

Non-adjusted..…... Data as measured

ppm…….. Parts Per Million; a unit of concentration used for gasses

Rubisco…… Ribulose 1,5 Bisphosphate Carboxylase/Oxygenase, the initial enzyme involved in photosynthesis. It can react with both carbon dioxide and oxygen molecules

RuBP…… Ribulose Bisphosphate, the sugar substrate for Rubisco during photosynthesis

TNC……. Total Non-structural Carbohydrates (concentration of sugar and starch)

TNC-free……..Data as corrected for possible dilution from TNC

TNC:TSC……..... The ratio between Total Non-structural Carbohydrates and Total Structural Carbohydrates

TSC…….. Total Structural Carbohydrates

**CHAPTER 4:** *Potential effects of elevated atmospheric carbon dioxide and oxygen on mid-Mesozoic terrestrial ecosystems: Inferences based on changes in Ginkgo biloba foliage quality and quantity*

35-Day

Experiment……...Gas exchange experiment incorporating a 5-week acclimation period

$C_3$ plants……...Plants that use the $C_3$ photosynthetic pathway

$C_4$ plants……...Plants that use the $C_4$ photosynthetic pathway

CAM plants..…... Plants that use the Crassulacean Acid Metabolism (CAM) photosynthetic pathway

C:N ratio……...The ratio between Carbon and Nitrogen in leaf tissue

CO2…… Elevated carbon dioxide treatment: 1.25 atm pressure, 2000ppm carbon dioxide and 20.9% oxygen

CO2&O2…… Elevated carbon dioxide and oxygen treatment: 1.25 atm pressure, 2000ppm carbon dioxide and 30% oxygen

Control…… Control treatment: I atm pressure, 370ppm carbon dioxide, and 20.9% oxygen

Detritivore……An animal that consumes plant material that has been shed from a plant or other organism. The consumption of this material helps to break it down through decomposition and recycle the nutrients in the detritus back to the soil system

Herbivore……. An animal that primarily consumes plant material to meet its nutritional needs

ppm…….. Parts Per Million; a unit of concentration used for gasses

TNC:TSC……..... The ratio between Total Non-structural Carbohydrates and Total Structural Carbohydrates

**CHAPTER 5**: *Applying experimental physiological results to the fossil record: Can the stomatal density and index of Ginkgo biloba provide a meaningful link between experimentally produced leaves and Ginkgo fossils?*

35-Day

Experiment……...Gas exchange experiment incorporating a 5-week acclimation period

ANOVA……... Analysis of Variance

CO2…… Elevated carbon dioxide treatment: 1.25 atm pressure, 2000ppm carbon dioxide and 20.9% oxygen

CO2&O2…… Elevated carbon dioxide and oxygen treatment: 1.25 atm pressure, 2000ppm carbon dioxide and 30% oxygen

Control…… Control treatment: I atm pressure, 370ppm carbon dioxide, and 20.9% oxygen

ED…… Epidermal Density (# cells per $mm^2$ leaf surface)

Experimental

Dataset……..SD and SI datasets produced through measurement of SD and SI on Ginkgo leaves that were produced during exposure to elevated carbon dioxide and oxygen atmospheric treatments during experimentation.

GLM…….General Linear Models procedure (SAS Statistical Software)

mya……..… Millions of Years Ago

NLE……..…Nearest Living Equivalent is the title given to an extant plant that most closely resembles an extinct fossil plant both ecologically and taxonomically. Used in the measurement of stomatal ratio.

ppm…….. Parts Per Million; a unit of concentration used for gasses

$R^2$……..…... A statistic that is the percentage of variation in a dataset that is explained by the explanatory variable (in this case photosynthetic or conductance rates)

RTMP…….. Royal Tyrrell Museum of Paleontology, Drumheller, Alberta, Canada

SD…… Stomatal Density (# stomata per $mm^2$ leaf surface)

SI…….. Stomatal Index. SI = SD / ED + SD

SR…… Stomatal Ratio. This is the ratio of SI of fossil leaf to the SI of a nearest living relative modern leaf

**CHAPTER 6:** *Future Directions*

FACE……... Free-Air Carbon dioxide Enrichment; A technique for studying plant phsysiological responses to elevated carbon dioxide

ppm…….. Parts Per Million; a unit of concentration used for gasses

# CHAPTER 1:

## Introduction

## Introduction

Paleoecology describes the interactions among extinct plants and animals and their environments. While modern ecologists examine these relationships in the present day using directly measurable dynamic parameters of living organisms such as photosynthesis and metabolic rate (e.g. Nobel 1980, Williams et al. 1993), paleoecologists must rely on fragmentary fossil and geologic evidence for ecological inference that does not allow the same level of statistical certainty (Chin 1997, Johnson 1999, Sander et al. 2004, Tiffney 1997).

Environments during the Late Jurassic and Early Cretaceous Periods (~180-90 mya (mid-Mesozoic)) were different from those of the present day. The continents were arranged differently (Scotese 2002) and global temperatures were elevated (Retallack 2002). Along with these physical differences, world fauna was dominated by dinosaurs (e.g. Carpenter et al. 2002, Dal Sasso 2003), and angiosperms were a minor, local component of world flora (Friis et al. 2003, Sun et al. 1998). Additionally, atmospheric carbon dioxide and oxygen levels were concurrently elevated during these Periods relative to the modern atmospheric composition (Fig. 1) (Berner et al. 2003, Royer et al. 2001), which is of specific interest to this study. Carbon dioxide concentration fluctuated between 1000 ppm to 2000 ppm, while oxygen concentration hovered around 25-30% of the atmosphere (Berner et al. 2003, Royer et al. 2001). These values are large in comparison with present ambient values of 370 ppm carbon dioxide and 20.9% oxygen.

Atmospheric carbon dioxide and oxygen values for the mid-Mesozoic are supported by several independent lines of evidence (Berner 2004). Carbon dioxide

concentration has been determined through mathematical modeling of the long term carbon cycle (Berner 2004). These models use measured carbon concentrations in reservoirs, such as the atmosphere, mantle, and biosphere, to solve steady state equations representing the fluxes between reservoirs, such as weathering, deposition, and mantle degassing, on million-year intervals. This technique suggests a fluctuating trend of carbon dioxide concentration throughout time (redrawn in Fig. 1). Model predictions agree with independent lines of evidence, including stomatal frequency data (e.g. Royer et al. 2001), stable carbon isotope values from marine phyto-plankton, and boron isotopes (see Berner 2004 for discussion and references).

Oxygen concentration in the atmosphere during the mid-Mesozoic has been estimated using rock-abundance models that track the sedimentation and weathering rates of carbonates and pyrite-rich rocks and provide an estimate of oxygen evolution rates throughout time (Fig. 1) (Berner and Canfield 1989). Additional models use isotope mass balance calculations for carbon and sulfur isotopes in oceanic sediments (Berner 2001). Stable carbon isotope values from plants (Beerling et al. 2002a). Insect size and the frequency of paleo-fires (Robinson 1989) also correlate with the oxygen modeling results. Mesozoic atmospheric carbon dioxide and oxygen values were different enough from modern atmospheric conditions that organisms, communities, and whole ecosystems likely functioned differently during those periods than they do today.

The overall purpose of this dissertation is to investigate how Ginkgo biloba L., a living relative of a mid-Mesozoic floral lineage (Tralau 1968), responds physiologically and morphologically to the atmospheric composition of the mid-Mesozoic. G. biloba

seedlings were exposed to elevated carbon dioxide and oxygen atmospheres within a hyperbaric chamber for 35 days. The photosynthetic rate, foliage quality, and stomatal density parameters of leaves produced during exposure were measured. The results of these experiments were used to infer plant physiological, primary productivity, and food web characteristics for mid-Mesozoic terrestrial ecosystems.

G. biloba was chosen because it is a widely cultivated modern tree from a very ancient lineage. The family Ginkgoaceae, which includes the genera Ginkgo L., Ginkgoites Seward, Baiera F. Braun, and Sphenobaiera Florin (Watson et al. 1999), extends back to the Permian (Tralau 1968). For these studies, extant G. biloba seems appropriate to use as an analog for fossil Ginkgoaceae because the morphological differences between fossil and extant Ginkgo are minimal. There are differences in margin morphology (e.g. Denk and Velitzelos 2002, Hill and Carpenter 1999) and some features of the cuticle and the reproductive structures (Royer et al. 2003, Watson et al. 1999, Zhou and Zheng 2003). The morphological similarity between fossil and extant Ginkgo leaves suggests similarity of function. G. biloba is also appropriate as an experimental subject because Ginkgoaceae achieved their greatest diversity during the elevated atmospheric carbon dioxide and oxygen of the Early Cretaceous Period (Tralau 1968). During this time, Ginkgo was a major component of high latitude (> 40°) forests (Royer et al. 2003, Tralau 1968). Additionally, stomatal frequencies of Ginkgo leaves have been used to infer ancient carbon dioxide levels throughout the mid-Mesozoic (e.g. Retallack 2002, Royer 2000). Ginkgo is therefore an ideal experimental subject for this study.

## Specific Questions Addressed

The photosynthetic rate and primary productivity responses of G. biloba to elevated carbon dioxide and oxygen are presented in Chapter 2. In Chapter 3, the differences in nutritive content and digestibility among Ginkgo leaves in response to different carbon dioxide and oxygen atmospheric treatments are assessed. In Chapter 4, the implications of plant physiological, productivity, and foliage quality responses to elevated gaseous levels in Mesozoic terrestrial ecosystems are discussed. Finally, in Chapter 5, the fitness of stomatal parameters as proxies for photosynthetic rates in shed G. biloba and fossil Ginkgo affinity leaves is assessed.

Photosynthetic Rate and Primary Productivity.- Very little is known about the actual gas exchange responses of fossil plants to elevated atmospheric carbon dioxide and oxygen. Multi-generational responses of fossil plants to elevated carbon dioxide and oxygen have been addressed using theoretical physiology from an evolutionary standpoint (e.g. Ackerly et al. 2000, Gale et al. 2001, Tolbert et al. 1995), but data on instantaneous photosynthetic rate responses of living relatives of fossil plants are rare. Generalized photosynthetic rates for the mid-Mesozoic have been measured by coupling a mathematical model for $C_3$ photosynthesis (Farquhar et al. 1980) with the long-term carbon cycle model (Beerling 1994, Berner 2004). However, existing models have not yet accounted for dynamic long-term photosynthetic rate responses such as photosynthetic down-regulation because they focus on potential rather than actual photosynthetic rates (Beerling 1994).

The photosynthetic rate and primary productivity responses of extant plants to both carbon dioxide and oxygen have been explored by plant physiologists (e.g. Leegood et al. 1995, Saralabai et al. 1997). However, these studies have only examined extant plants that were not a part of the Mesozoic flora, e.g., wheat (Adam et al. 2000), grasses (Shaw et al. 2002), and ponderosa pine (Tissue et al. 1999). Additionally, these studies have generally not examined photosynthetic rate responses to carbon dioxide levels greater than 700 ppm (e.g. Norby et al. 1999), and have not examined the photosynthetic rate response to elevated levels of both carbon dioxide and oxygen (e.g. Badger 1985).

Chapter 2 presents a study that examined how photosynthetic rate and primary productivity of mid-Mesozoic plants might have been affected by mid-Mesozoic atmospheric conditions. Ginkgo biloba seedlings were exposed to one of three gaseous composition treatments (elevated carbon dioxide, elevated oxygen, or elevated levels of both gasses) for either five weeks or 24 hours, and photosynthetic rate and other gas exchange parameters were measured. G.biloba photosynthetic rate was strongly stimulated over both durations by carbon dioxide (rates were 200-300% higher with 2000 ppm carbon dioxide than with 370 ppm). Oxygen did not have a negative effect on photosynthetic rate, as no significant rate differences were observed between 20.9% oxygen and 30% when carbon dioxide was at 2000 ppm. These photosynthetic rate stimulations suggest G, biloba primary productivity increases in response to elevated carbon dioxide and oxygen atmospheres.

Nutritive Content and Digestibility of Foliage.- Stimulation of photosynthetic rate may have resulted in different relative amounts of primary metabolites within ancient leaf

tissue (Billings et al. 2003, Kause et al. 1999). Extant herbivores and detritivores are strongly affected by changes in foliar tissue quality (e.g. Geluso and Hayes 1999, Sariyildiz and Anderson 2003): food quality can affect body size (Demment and Van Soest 1985), metabolism (Williams et al. 1993), reproductive success (Davis and Graham 1991), and biomass (Coe et al. 1976). Therefore, the primary metabolite composition responses of G. biloba leaves to mid-Mesozoic-like atmospheres are important for an understanding of herbivorous diets during that time. However, few papers have examined the primary metabolite responses of leaves to highly elevated levels of carbon dioxide and oxygen (Cotrufo et al. 1998, Fritschi et al. 1999, Tingey et al. 2003).

In Chapter 3, the primary chemical constituents of G. biloba leaves were measured after seedling exposure to elevated carbon dioxide and oxygen treatments. Nutritive content and digestibility of the leaves, two relative measures of leaf quality, were assessed using these primary metabolite data. Protein concentration was not reduced by elevated carbon dioxide and oxygen, but carbon to nitrogen ratio was elevated due to a strong stimulation of starch content. Hence, nutritive content was not reduced by elevated carbon dioxide and oxygen. Digestibility was increased, however, due to the increase in easily digestible carbohydrates including starch.

Implications for Mesozoic Terrestrial Ecosystems.- Chapter 4 summarizes the results of the gas exchange and leaf quality experiments and discusses the implications of these results for mid-Mesozoic terrestrial ecosystems. Under the assumptions that G. biloba is an appropriate proxy plant for most mid-Mesozoic flora on the basis of the common $C_3$ photosynthetic pathway, and that the experimental results can be

extrapolated to the entire lifespan of the plant, the experimental G. biloba results can be tentatively applied to mid-Mesozoic terrestrial ecology.

This chapter draws on literature about the size, metabolism, and behavioral responses of present-day herbivores from diverse taxonomic groups in response to different quality diets (Brand et al. 2003, Clemens and Maloiy 1982, Lundberg and Palo 1993, Schluter 1984) and on decomposition rates and the microbial responses to elevated carbon dioxide and oxygen in the modern world (Arnone III and Bohlen 1998, Bollmann and Conrad 1998, Jin et al. 2002, Rouhier et al. 1994). This information is incorporated with information about the temperature and continental landmasses of the mid-Mesozoic (e.g. Retallack 2004, Scotese 2002), floral and faunal assemblages of that time (e.g. Ziegler et al. 1993), and hypothesized ancient herbivore metabolic rates (Farlow 1976, Seymour and Lillywhite 2000, Weaver 1983). The stimulated productivity observed under elevated carbon dioxide and oxygen and changes to the quality of the foliage have the potential to have affected many aspects of mid-Mesozoic ecology including herbivore size and metabolism, and decomposition rates.

Stomatal Frequency Responses.- The stomatal frequency of leaves (number of gas exchange pores on the surface of the leaf per area) responds to both environmental and physiological variations. For this reason, stomatal frequency parameters (stomatal density, index, and ratio) have been used to examine gas exchange and stress responses of modern and ancient plants (e.g. Beerling 1997, Farquhar and Sharkey 1982, Jarvis et al. 1999) and the carbon dioxide composition of the atmosphere over the past 300 million years (e.g. Beerling et al. 2002b, Retallack 2001), despite their high spatial density

variation within individual leaves (Greenwood et al. 2003, Poole et al. 2000). Stomatal frequency data therefore may help with inference of photosynthetic and conductance rate data from Ginkgo leaves and well-preserved Ginkgoaceae fossil leaves.

In Chapter 5, the stomatal density and index response of G. biloba leaves to elevated carbon dioxide and oxygen are assessed. These values are then compared with photosynthetic and conductance rate data from Chapter 2 in order to assess the relationship between gas exchange and stomatal frequency. Finally, the stomatal density and index data from G. biloba are compared with values for previously unexamined Late Cretaceous Ginkgoaceae leaves and published stomatal data for Ginkgoaceae. Stomatal index and density did not respond to either elevated carbon dioxide or oxygen, despite the well-documented inverse relationship between carbon dioxide and stomatal frequency (Royer 2001, Woodward and Bazzaz 1988). G. biloba stomatal frequency values correlated with conductance rate, but not photosynthetic rate, suggesting that inference of photosynthetic rates from shed leaves may be inappropriate. Additionally, stomatal frequency values from G. biloba leaves produced under elevated carbon dioxide and oxygen were not comparable with Ginkgo lineage fossils from the mid-Mesozoic. These comparisons demonstrate that stomatal density and index are inappropriate proxies for gas exchange parameters in extant and fossil Ginkgo leaves.

## Conclusions

The current study investigates the photosynthetic and foliage quality responses of modern Ginkgo biloba seedlings to atmospheric compositions that were most likely

found during the mid-Mesozoic. The impacts of atmospheric gas compositions on photosynthesis and foliage quality have implications for Mesozoic ecology, and demonstrate that experimental methods can be applied to paleoecological inquiry.

## References Cited


Ackerly, D., S. Dudley, S. Sultan, J. Schmitt, J. Coleman, C. Linder, D. Sandquist, M. Geber, A. Evans, T. Dawson, and M. Lechowicz. 2000. The evolution of plant ecophysiological traits: Recent advances and future directions. BioScience 50(11):979-995.

Adam, N., G. Wall, B. Kimball, P. Pinter, R. LaMorte, D. Hunsaker, F. Adamsen, T. Thompson, A. Matthias, S. Leavitt, and A. Webber. 2000. Acclimation response of spring wheat in a free-air $CO_2$ enrichment (FACE) atmosphere with variable soil nitrogen regimes. I. Leaf position and phenology determine acclimation response. Photosynthesis Research 66:65-77.

Arnone III, J., and P. Bohlen. 1998. Stimulated $N_2O$ flux from intact grassland monoliths after two growing seasons under elevated atmospheric $CO_2$. Oecologia 116:331-335.

Badger, M. 1985. Photosynthetic oxygen exchange. Annual Review of Plant Physiology 36:27-53.

Beerling, D. 1994. Modelling palaeophotosynthesis: Late Cretaceous to present. Philosophical Transactions of the Royal Society, London, B 346:421-432.

Beerling, D. 1997. Carbon isotope discrimination and stomatal responses of mature Pinus sylvestris L. trees exposed in situ for three years to elevated $CO_2$ and temperature. Acta Oecologia 18(6):697-712.

Beerling, D., J. Lake, R. Berner, L. Hickey, D. Taylor, and D. Royer. 2002a. Carbon isotope evidence implying high $O_2/CO_2$ ratios in the Permo-Carboniferous atmosphere. Geochimica et Cosmochimica Acta 66(21):3757-3767.

Beerling, D., B. Lomax, D. Royer, G. Upchurch, and L. Kump. 2002b. An atmospheric $pCO_2$ reconstruction across the Cretaceous-Tertiary boundary from leaf megafossils. PNAS 99(12):7836-7840.

Berner, R. 2004. The Phanerozoic Carbon Cycle: $CO_2$ and $O_2$. Oxford University Press, Oxford.

Berner, R., D. Beerling, R. Dudley, J. Robinson, and R. Wildman. 2003. Phanerozoic Atmospheric Oxygen. Annual Review of Earth and Planetary Sciences 31:105-34.

Berner, R., and D. Canfield. 1989. A new model for atmospheric oxygen over Phanerozoic time. American Journal of Science 289:333-361.

Berner, R. A. 2001. Modeling atmospheric $O_2$ over Phanerozoic time. Geochimica et Cosmochimica Acta 65(5):685-694.

Billings, S., S. Zitzer, H. Weatherly, S. Schaeffer, T. Charlet, J. Arnone, and R. Evans. 2003. Effects of elevated carbon dioxide on green leaf tissue and leaf litter quality in an intact Mojave Desert ecosystem. Global Change Biology 9:729-735.

Bollmann, A., and R. Conrad. 1998. Influence of $O_2$ availability on NO and $N_2O$ release by nitrification and denitification in soils. Global Change Biology 4:387-396.

Brand, Z., T. Brand, and C. Brown. 2003. The effect of dietary energy and protein levels on body condition and production of breeding male ostriches. South African Journal of Animal Science 32(4):231-239.

Carpenter, K., T. DiCroce, D. Gilpin, B. Kinneer, F. Sanders, V. Tidwell, and A. Shaw. 2002. Origins of the Early and 'Middle' Cretaceous dinosaurs of North America: Implications for plate tectonics. Pp. 289-308. Proceedings of the International Symposium on New Concepts in Global Tectonics. Otero Junior College, La Junta, CO.

Chin, K. 1997. What did dinosaurs eat? Coprolites and other direct evidence of dinosaur diets. Pp. 371-382. *In* J. Farlow, and M. Brett-Surman, eds. The Complete Dinosaur. Indiana University Press, Bloomington, IN.

Clemens, E. T., and G. M. O. Maloiy. 1982. The digestive physiology of three East African herbivores: the elephant, rhinoceros and hippopotamus. Journal of Zoology, London 198:141-156.

Coe, M., D. Cumming, and J. Phillipson. 1976. Biomass and production of large African herbivores in relation to rainfall and primary production. Oecologia 22:341-354.

Cotrufo, M., P. Ineson, and A. Scott. 1998. Elevated $CO_2$ reduces the nitrogen concentration of plant tissues. Global Change Biology 4(1):1365-2486.

Dal Sasso, C. 2003. Dinosaurs of Italy. Comptes Rendus Palevol 2:45-66.

Davis, W., and D. Graham. 1991. The influence of food on reproductive strategies in a monogamous kingfisher (Chloroceryle amazona). The Auk 108:780-789.

Demment, M. W., and P. J. Van Soest. 1985. A nutritional explanation for body-size patterns of ruminant and nonruminant herbivores. The American Naturalist 125(5):641-672.

Denk, T., and D. Velitzelos. 2002. First evidence of epidermal structures of Ginkgo from the Mediterranean Tertiary. Review of Palaeobotany and Palynology 120:1-15.

Farlow, J. O. 1976. A consideration of the trophic dynamics of a Late Cretaceous large-dinosaur community (Oldman Formation). Ecology 57:841-857.

Farquhar, G., and T. Sharkey. 1982. Stomatal conductance and photosynthesis. Annual Review of Plant Physiology 33:317-345.

Farquhar, G., S. von Caemmerer, and J. Berry. 1980. A biochemical model of photosynthetic $CO_2$ assimilation in leaves of $C_3$ species. Planta 149:78-90.

Friis, E. M., J. Doyle, P. K. Endress, and Q. Leng. 2003. Archaefructus - angiosperm precursor of specialized early angiosperm? Trends in Plant Science 8(8):369-373.

Fritschi, F., K. Boote, L. Sollenberger, and L. J. Allen. 1999. Carbon dioxide and temperature effects on forage establishment: tissue composition and nutritive value. Global Change Biology 5:743-753.

Gale, J., S. Rachmilevitch, J. Reuveni, and M. Volokita. 2001. The high oxygen atmosphere toward the end-Cretaceous; a possible contributing factor to the K/T boundary extinctions and to the emergence of $C_4$ species. Journal of Experimental Botany 52(357):801-809.

Geluso, K., and J. Hayes. 1999. Effects of dietary quality on basal metabolic rate and internal morphology of European Starlings (Sturnus vulgaris). Physiological and Biochemical Zoology 72(2):189-197.

Greenwood, D., M. Scarr, and D. Christophel. 2003. Leaf stomatal frequency in the Australian tropical rainforest tree Neolitsea dealbata (Lauraceae) as a proxy measure of atmospheric $pCO_2$. Palaeogeography, Palaeoclimatology, Palaeoecology 196:375-393.

Hill, R., and R. Carpenter. 1999. Ginkgo leaves from Paleogene sediments in Tasmania. Australian Journal of Botany 47:717-724.

Jarvis, A., T. Mansfield, and W. Davies. 1999. Stomatal behavior, photosynthesis and transpiration under rising $CO_2$. Plant, Cell and Environment 22:639-648.

Jin, Z., B. Chung, K. Iiyama, and S. Watanabe. 2002. Changes in chemical components of leaf litter of Ginkgo biloba during mulching. Journal of Arboriculture 28(4):171-177.

Johnson, K. 1999. The megaflora of the Hell Creek Formation, southwestern North Dakota: biostratigraphy and paleoecology of the End-Cretaceous terrestrial vegetation. GSA - Abstracts with Programs:A-72.

Kause, A., V. Ossipov, E. Haukioja, K. Lempa, S. Hanhimaki, and S. Ossipova. 1999. Multiplicity of biochemical factors determining quality of growing birch leaves. Oecologia 120:102-112.

Leegood, R., P. Lea, M. Adcock, and R. Hausler. 1995. The regulation and control of photorespiration. Journal of Experimental Botany 46(Special Issue):1397-1414.

Lundberg, P., and R. T. Palo. 1993. Resource use, plant defenses, and optimal digestion in ruminants. Oikos 68:224-228.

Nobel, P. 1980. Water vapor conductance and $CO_2$ uptake for leaves of a $C_4$ desert grass, Hilaria rigida. Ecology 61(2):252-258.

Norby, R., S. Wullschleger, C. Gunderson, D. Johnson, and R. Ceulemans. 1999. Tree responses to rising $CO_2$ in field experiments: implications for the future forest. Plant, Cell and Environment 22:683-714.

Poole, I., T. Lawson, D. Weyers, and J. Raven. 2000. Effect of elevated $CO_2$ on the stomatal distribution and leaf physiology of Alnus glutinosa. New Phytologist 145:511-521.

Retallack, G. 2001. A 300-Million year record of atmospheric carbon dioxide from fossil plant cuticles. Nature 411:287-290.

Retallack, G. 2002. Carbon dioxide and climate over the past 300 Myr. Philosophical Transactions of the Royal Society of London A 360:659-673.

Retallack, G. 2004. Soils and global change in the carbon cycle over geologic time. Pp. 581-605. *In* H. Holland, and K. Turekian, eds. Treatise on Geochemistry. Elsevier, Amsterdam.

Robinson, J. 1989. Phanerozoic $O_2$ variation, fire, and terrestrial ecology. Palaeogeography, Palaeoclimatology, Palaeoecology 75(3):223-240.

Rouhier, H., G. Billés, A. El Kohen, M. Mousseau, and P. Bottner. 1994. Effect of elevated $CO_2$ on carbon and nitrogen distribution within a tree (Castanea sativa Mill.) - soil system. Plant and Soil 162:281-292.

Royer, D. 2000. Estimating latest Cretaceous and Early Tertiary atmospheric $pCO_2$ from stomatal indices. GSA - Abstracts with Programs:A-196.

Royer, D. 2001. Stomatal density and stomatal index as indicators of paleoatmospheric $CO_2$ concentration. Review of Palaeobotany and Palynology 114:1-28.

Royer, D., R. Berner, and D. Beerling. 2001. Phanerozoic atmospheric $CO_2$ change: evaluating geochemical and paleobiological approaches. Earth-Science Reviews 54:349-392.

Royer, D., L. Hickey, and S. Wing. 2003. Ecological conservatism in the 'living fossil' Ginkgo. Paleobiology 29(1):84-104.

Sander, P., N. Klein, E. Buffetaut, G. Cuny, V. Suteethorn, and J. Le Loeuff. 2004. Adaptive radiation in sauropod dinosaurs: bone histology indicates rapid evolution of giant body size through acceleration. Organisms Diversity & Evolution 4(3):165-173.

Saralabai, V., M. Vivekanandan, and R. Babu. 1997. Plant responses to high $CO_2$ concentration in the atmosphere. Photosynthetica 33(1):7-37.

Sariyildiz, T., and J. Anderson. 2003. Interactions between litter quality, decomposition and soil fertility: a laboratory study. Soil Biology and Biochemistry 35:391-399.

Schluter, D. 1984. Body size, prey size and herbivory in the Galapagos lava lizard, Tropidurus. Oikos 43:291-300.

Scotese, C. 2002. Paleomap Project. www.scotese.com.

Seymour, R., and H. Lillywhite. 2000. Hearts, neck posture and metabolic intensity of sauropod dinosaurs. Proceedings of the Royal Society of London B 267:1883-1887.

Shaw, M., E. Zavaleta, N. Chiarello, E. Cleland, H. Mooney, and C. Field. 2002. Grassland responses to global environmental changes suppressed by elevated $CO_2$. Science 298:1987-1990.

Sun, G., D. Dilcher, S. Zheng, and Z. Zhou. 1998. In search of the first flower: a Jurassic angiosperm, Archaefructus, from Northeast China. Science 282:1692-1695.

Tiffney, B. H. 1997. Land plants as food and habitat in the Age of Dinosaurs. Pp. 352-370. *In* J. Farlow, and M. Brett-Surman, eds. The Complete Dinosaur. Indiana University Press, Bloomington, IN.

Tingey, D., R. McKane, D. Olszyk, M. Johnson, P. Rygiewicz, and E. Lee. 2003. Elevated $CO_2$ and temperature alter nitrogen allocation in Douglas-fir. Global Change Biology 9:1038-1050.

Tissue, D., K. Griffin, and J. Ball. 1999. Photosynthetic adjustment in field-grown ponderosa pine trees after six years of exposure to elevated $CO_2$. Tree Physiology 19:221-228.

Tolbert, N., C. Benker, and E. Beck. 1995. The oxygen and carbon dioxide compensation points of $C_3$ plants: possible role in regulating atmospheric oxygen. Proceedings of the National Academy of Sciences 92:11230-11233.

Tralau, H. 1968. Evolutionary trends in the genus Ginkgo. Lethaia 1:63-101.

Watson, J., S. Lydon, and N. Harrison. 1999. Consideration of the genus Ginkgoites Seward and a redescription of two species from the Lower Cretaceous of Germany. Cretaceous Research 20:719-734.

Weaver, J. C. 1983. The improbable endotherm: the energetics of the sauropod dinosaur Brachiosaurus. Paleobiology 9(2):173-182.

Williams, J., W. Siegfried, S. Milton, N. Adams, W. Dean, M. Du Plessis, and S. Jackson. 1993. Field metabolism, water requirements, and foraging behavior of wild ostriches in the Namib. Ecology 74(2):390-404.

Woodward, F., and F. Bazzaz. 1988. The response of stomatal density to $CO_2$ partial pressure. Journal of Experimental Botany 39(209):1771-1781.

Zhou, Z., and S. Zheng. 2003. Palaeobiology: the missing link in Ginkgo evolution. Nature 423:821-822.

Ziegler, A., J. Parrish, Y. Jiping, E. Gyllenhaal, D. Rowley, J. Parrish, N. Shangyou, A. Bekker, and M. Hulver. 1993. Early Mesozoic phytogeography and climate. Philosophical Transactions of the Royal Society of London B 341:297-305.

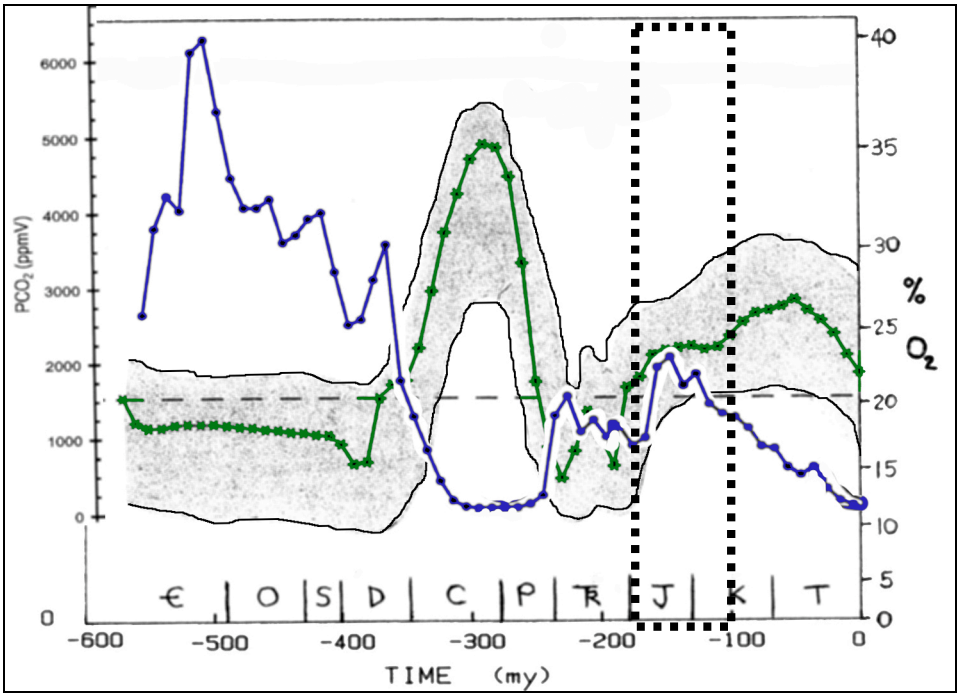


Figure 1: Carbon dioxide (blue circles) and oxygen (green stars) concentration variation over Phanerozoic time. The carbon dioxide curve is redrawn from Royer et al (2001), while the oxygen curve is redrawn from Berner and Canfield (1989). Shading surrounding the oxygen curve indicates the margin of error in the model measurements generated through sensitivity analysis. The box (dotted line) delineates the time of interest, the Late Jurassic and Early Cretaceous Periods, when both carbon dioxide and oxygen were elevated relative to present-day ambient conditions.

# CHAPTER 2

## Photosynthetic rate responses of Ginkgo biloba seedlings to elevated atmospheric carbon dioxide and oxygen: an experimental approach to studying the paleoecology of the Mesozoic Era

Abstract.-

Biogeochemical cycling models provide evidence that atmospheric carbon dioxide and oxygen were concurrently enriched during the Late Jurassic and Early Cretaceous Periods, but the possible effects of this enriched atmosphere on terrestrial ecosystems are still poorly understood. Ginkgo biloba L. seedlings were exposed to atmospheric treatments containing combinations of ambient carbon dioxide (370 ppm), ambient oxygen (210,000 ppm), elevated carbon dioxide (2000 ppm), and elevated oxygen (300,000 ppm) in order to elucidate the gas exchange and physiological responses of Ginkgo to these atmospheric conditions. Results differed according to the duration of exposure. Twenty-four-hour exposure to enriched carbon dioxide levels resulted in increased photosynthetic rates. Photosynthetic rate was not depressed in response to elevated oxygen regardless of whether it was accompanied by either ambient or elevated carbon dioxide. 35-day exposure to elevated carbon dioxide alone and with elevated oxygen resulted in a significant photosynthetic rate increase relative to ambient carbon dioxide. However, these increases were lower than those observed in response to 24-hour exposure, due to apparent photosynthetic down-regulation. There is some evidence that the treatment with elevated oxygen may not have experienced down-regulation to the same extent as the treatment with elevated carbon dioxide alone. Reduced down-regulation caused by elevated oxygen in the atmosphere has implications for the primary productivity of the mid-Mesozoic.

## Introduction

Over the past 600 million years the carbon dioxide and oxygen concentrations of the atmosphere have varied greatly (Berner 2001, 2003, Rothman 2002, Royer et al. 2001). During the Mesozoic Era, carbon dioxide levels may have been enriched up to 4000 ppm (10 times present levels) (Royer et al. 2001) and oxygen may have comprised up to 30% of the atmosphere, relative to present levels of 20.9% (Berner 2003). Enrichments of oxygen to these levels would have been accompanied by an increase in atmospheric pressure to 1.25 atm (Berner et al. 2003). Generally, reduced levels of atmospheric oxygen accompany high levels of atmospheric carbon dioxide, although the relationship is not precisely an inverse relationship. During the Late Jurassic and Early Cretaceous Periods both gasses were concurrently enriched (Berner 2001, 2004, Royer et al. 2001). This enrichment can be expected to have affected the physiology of individual plants and may have resulted in changes to primary productivity.

Research on modern plants has identified complex physiological responses to enriched atmospheric carbon dioxide and oxygen. The response of $C_3$ plants to both carbon dioxide and oxygen gasses is mediated through the enzyme Ribulose 1,5 Bisphosphate Carboxylase/Oxygenase (Rubisco) as both gasses are substrates for this enzyme. Carboxylation results in photosynthesis – the formation of sugars and the net gain of carbon by the plant. Oxygenation results in photorespiration – the oxidation of sugars and net loss of carbon from the plant (Berner and Canfield 1989, Tolbert et al. 1995).

Two to 10 times ambient carbon dioxide levels have a mixed effect on photosynthesis (Bazzaz and Fajer 1992, Saralabai et al. 1997). Elevated carbon dioxide causes a strong and transient stimulation of photosynthetic rate by saturating the carboxylation function of Rubisco and reducing photorespiration by occupying binding sites that could be taken by oxygen molecules (Webber et al. 1994, Woodrow 1994). However, when the elevated carbon dioxide level is maintained over time, the photosynthetic apparatus undergoes photosynthetic down-regulation, a process by which photosynthetic rate is reduced to values that are lower than the initial stimulation, but are still elevated relative to rates at ambient carbon dioxide (Saralabai et al. 1997). Even the down-regulated long-term increases in photosynthetic rate result in a relatively increased growth rate, more rapid ontogenetic development, and increased biomass production over ambient carbon dioxide levels (Hamerlynck et al. 2000, Hunt et al. 1991). Photosynthetic responses to elevated carbon dioxide have not been tested with concurrent elevations of oxygen concentration.

Oxygen gas affects net photosynthetic rate and carbon dioxide uptake through two mechanisms. First, oxygen competitively inhibits the binding of carbon dioxide to reaction sites on Rubisco by binding to these sites, which causes the breakdown of carbon skeletons within the plant through photorespiration – respiration in the light that does not generate chemical energy in the form of ATP (Beerling and Berner 2000, Leegood et al. 1995, Takeba and Kozaki 1998). Therefore, carbon cycling, photosynthesis, growth, and reproduction are retarded in the presence of excess oxygen gas. Second, dark respiration,

the metabolic break-down of plant compounds and subsequent production of ATP, is increased by elevated oxygen (Poskuta and Frankiewicz-Jozko 1975, Tolbert et al. 1995), which may affect carbon balance and resource partitioning in photosynthesizing leaves. However, most studies of photorespiration and dark respiration (see Takeba and Kozaki 1998 for references) have examined the effect of elevated oxygen at sub-ambient carbon dioxide levels. The effect of oxygen combined with high levels of carbon dioxide on productivity has yet to be examined.

Ginkgo biloba is an ideal experimental subject for a study seeking to examine physiological parameters in the fossil record. It is a cultivated, extant, broad-leaved, deciduous, gymnosperm tree using the $C_3$ photosynthetic pathway (Royer et al. 2003). It is also a "living fossil:" The Ginkgoaceae (Ginkgo L., Ginkgoites Seward, Baiera F. Braun, and Sphenobaiera Florin (Watson et al. 1999)) was speciose throughout the Jurassic and Cretaceous (Tralau 1968). Paleoenvironmental reconstructions place the Ginkgo lineage as an abundant component of ancient higher-latitude and polar forests (Royer et al. 2003). Today, however, there is only one species, G. biloba. It is morphologically identical to G. adiantoides, which first appeared during the Early Cretaceous (Royer et al. 2003). G. biloba is the focus of much paleoecological research (e.g. Beerling et al. 1998, Kürschner 2001, Retallack 2001, Royer 2000) because of the morphological similarity between G. biloba and Ginkgoaceae (Watson et al. 1999, Zhou and Zheng 2003).

The objective of this study is to address the gas exchange responses of G. biloba, the living relative of a Mesozoic lineage, to a strong concurrent increase in atmospheric carbon dioxide and oxygen through an experimental approach.

## Methods

Facilities.- 35-Day and 24-Hour gas exchange experiments were performed in hyperbaric chambers at the Center for Hyperbaric Medicine and Environmental Physiology at the Duke University Medical Center (Durham, NC). These chambers were used as growth chambers because of their utility in creating and sustaining altered atmospheric conditions, including elevated atmospheric pressure and specific gas concentrations.

Plant Material.- Ginkgo biloba seedlings were obtained from Musser Forests, Inc. (Indiana, PA). They were purchased as dormant 1-2 year old bare-root seedlings and stored in a 4°C walk-in cooler until potting.

24-Hour Experiment.- Seedlings were potted in three-gallon pots using a 4:1 hand-mixed soil:sand potting mixture with 1 tablespoon fertilizer per gallon substrate, five to six weeks before experiment initiation. Once potted, seedlings were placed on an outdoor pad in order to encourage leaf production. Average outdoor temperatures ranged from 9-15°C and 12-13 hours of daylight in March and April to 24-27°C and 14 hours of daylight in June and July. After seedlings had produced leaves, pots were transferred to a

600 cu. ft. chamber at the Hyperbaric Center to initiate the 24-hour (24-Hour) experiments.

During experimentation, seedlings were exposed to one of five different atmospheric acclimation treatments based on gaseous concentrations that biogeochemical cycling models suggest for the Late Jurassic and Early Cretaceous Periods (Berner et al. 2003, Royer et al. 2001). Treatment atmospheres were the following:

1. Present atmospheric composition: 1 atm pressure, 370 ppm $CO_2$, and 20.9% $O_2$, (Control)
2. Pressure increased to 1.25 atm, gas mix same as Control (Pressure)
3. Pressure increased to 1.25 atm, $CO_2$ increased to 2000 ppm, $O_2$ maintained at Control level (CO2)
4. Pressure increased to 1.25 atm, $O_2$ increased to 30%, $CO_2$ maintained at Control level (O2)
5. Pressure increased to 1.25 atm, $CO_2$ increased to 2000 ppm and $O_2$ increased to 30%. (CO2&O2)

Plants in the 24-Hour Experiment were watered before 24-hour acclimation began, but were not watered during acclimation. Seedling canopy temperature inside the chamber was maintained at 27°C through the use of an air-conditioned stream of air that blew directly through the canopy. Humidity was held at 50%. Light levels were constant, but ranged spatially from 300-500 $\mu M \bullet m^{-2} \bullet sec^{-1}$ Photosynthetically

Active Radiation (PAR). Day-length was 13 hours, maintained through a manual light switch.

The experimental design was a randomized complete block design. Repetition over time was the statistical blocking factor and three repetitions (blocks) of each experiment were performed. Treatments were applied sequentially to a random selection of 6 seedlings. Treatment order within replications was randomized.

35-Day Experiment.- Seedlings were potted in one-gallon pots using the same soil mixture as in the 24-Hour Experiment three to seven days before experiment initiation. Environmental conditions prior to experimentation were the same as for the seedlings before the 24-Hour Experiment. To initiate experimentation, plants were transported to a 390.5 cu. ft. chamber at the hyperbaric center for a 35 day acclimation period (35-Day Experiment). During acclimation seedlings emerged from quiescence and produced leaves. After the acclimation period, they were exposed to one of three atmospheric treatments:

1. Present atmospheric composition: 1 atm pressure, 370 ppm $CO_2$, and 20.9% $O_2$ (Control)
2. Pressure increased to 1.25 atm, $CO_2$ increased to 2000 ppm, $O_2$ maintained at Control level (CO2)
3. Pressure increased to 1.25 atm, $CO_2$ increased to 2000 ppm and $O_2$ increased to 30%. (CO2&O2)

During acclimation, seedlings were watered using a remote watering system every day until water dripped from the pots. Light level was reduced in the 35-Day Experiment relative to the 24-Hour Experiment in order to reduce the risk of light and heat damage in the smaller chamber. Light levels were constant but ranged spatially within the chamber from 200-350 $\mu M \bullet m^{-2} \bullet sec^{-1}$ PAR. Day-length, temperature and humidity were maintained as in the 24-Hour Experiment. Experimental design was also the same as in the 24-Hour Experiment.

Carbon dioxide concentrations varied during the 35-Day Experiment due to presumed high levels of dark respiration. Carbon dioxide levels were highest in the mornings. They varied from 370-500 ppm, with brief excursions up to 700 ppm, in the Control treatment, and from 1800-2600 ppm in the CO2 and CO2&O2 treatments. Oxygen varied less and did not follow a pattern. Oxygen ranged from 20.5% to 21.7% in the Control and CO2 treatments, and from 29.9% to 30.2% in the CO2&O2 treatment. In all treatments the extreme values observed were brief events (less than 12 hours in duration).

## Measurement and Analysis

Gas Exchange.- Following the acclimation period in both experiments, instantaneous net photosynthetic rate, conductance rate, and internal carbon dioxide concentration ($C_i$) of Ginkgo seedlings were measured using a Li-Cor 6400 Portable Photosynthesis System (LiCor Systems, Lincoln, NE). This open-flow system uses an

infrared gas analyzer to measure carbon dioxide and water vapor concentrations in a stream of air before and after it passes over a photosynthesizing leaf. Net photosynthesis, conductance and $C_i$ are calculated by the system using measured data and the gas exchange equations derived by von Caemmerer and Farquhar (1981).

Photosynthetic rate data were collected along a carbon dioxide concentration gradient created by the $CO_2$ mixer of the system (370-2000 ppm). Data were also collected along a PAR gradient (0-2000 $\mu M \bullet m^{-2} \bullet sec^{-1}$). The $CO_2$ curves were used to calculate photosynthetic efficiency (PE) and the carbon dioxide saturation points (CSP) for each treatment. The PAR curves were used to calculate the quantum efficiency (QE) and light saturation points (LSP). QE and PE are calculated as the slope of the linear portion of their respective curve, while CSP and LSP are the photosynthetic rate value at 95% of the maximum $CO_2$ or PAR value. These parameters describe the responsiveness of photosynthetic rate to different environmental conditions. For each curve, one leaf from each seedling was measured repeatedly; the leaf used was always the largest leaf closest to the base of the highest long shoot of each plant. Each measurement took three to five minutes and approximately 15 minutes passed between measurements of the same leaf.

While carbon dioxide concentrations varied during measurement of the $CO_2$ concentration gradient, they were maintained at acclimation level during gas exchange measurement at various PAR levels. Oxygen levels were not varied during measurement; all gas exchange measurements are at the acclimation oxygen concentration. Treatment

effects on photosynthetic and conductance rates were assessed using the acclimation conditions for each treatment.

Chlorophyll a and b.- All leaves per seedling were collected after gas exchange measurements in the 35-Day Experiment only and transported to the laboratory on ice. Brown tissue was removed, leaves were flash-frozen at -80° C, and then lyophilized using a Labconco FreeZone 18 Liter Freeze Dry System (Labconco, Kansas City, MO) for 48 hours. Freeze-dried leaves were ground to a fine powder with a mortar and pestle and stored at -80°C until chlorophyll was measured. Powder from all leaves produced during a single treatment / block combination was pooled for the chlorophyll assay.

10 mg leaf powder was mixed with 2ml of 80% acetone, and extracted for 30 minutes. After centrifugation, aliquots of the supernatant were taken for spectrophotometric measurement at 663 nm (chlorophyll a) and 645 nm (chlorophyll b). Both wavelengths were measured on the same aliquot.

Chlorophyll a (mg/g) = 1.07 (abs 663 nm) - .094 (abs 645 nm)

Chlorophyll b (mg/g) = 1.77 (abs 645 nm) – 0.28 (abs 663 nm)

Total Chlorophyll = chl a + chl b

Total chlorophyll concentration is reported both as measured (non-adjusted dataset) and as adjusted for transient starch and sugar (total non-structural carbohydrate (TNC)) concentrations in the leaf (TNC-free dataset). The concentration of TNC expressed as %TNC per gram (Chapter 3, Table 1) was subtracted from an idealized gram of leaf material (100%). Total chlorophyll data (mg/g) were then expressed in

terms of this TNC-free gram (less than 100%). Further details and rationale can be found in Chapter 3.

Leaf Production (biomass).- Biomass production was estimated by determining the weight of the total amount of lyophilized and ground leaf material produced by all seedlings in each treatment / block combination. The total weight of the powder is reported on a per-seedling basis by dividing the powder weight by the number of seedlings producing leaves in each treatment (max. 6 seedlings, min. 2 seedlings).

Other Chemical Constituents.- Concentrations of sugar, starch, and protein were also measured from the Long Duration Experiment. These data are reported in Chapter 3, Tables 1 and 2.

Statistical Analysis.- All data sets, including both 35-Day and 24-Hour photosynthetic rate and conductance rate data, were analyzed using SAS Statistical Software (Cary, NC). Proc GLM (ANOVA) was used to determine which experimental factors had a significant effect on the measured parameters. Additionally, Fisher's least significant difference t-tests were performed (means statement) to determine significant differences among treatments in each dataset. Significance was determined at $p \leq 0.1$, as there were only three independent observations made for each treatment. Due to the exploratory nature of this study, it was decided that a p-value of $\leq 0.05$ would represent too high a probability of Type II error.

## Results

24-Hour Experiment.- Net photosynthetic rate had a strong and statistically significant response to elevated carbon dioxide in the 24-Hour Experiment (Table 1). The photosynthetic rates of both the CO2 treatment and the CO2&O2 treatment were significantly elevated over Control values. However, photosynthetic rate did not respond to elevated oxygen: the O2 treatment was not significantly different from the Control treatment and the presence of elevated oxygen in the CO2&O2 treatment did not result in a significant difference from the CO2 treatment. Similarly, elevated atmospheric pressure had no significant effect on Ginkgo photosynthetic rate. Conductance rates, PE, and CSP did not differ significantly among treatments in the 24-Hour Experiment.

35-Day Experiment.- Net photosynthetic rate in the 35-Day Experiment increased significantly in response to elevated carbon dioxide (Table 2). The CO2 and CO2&O2 treatments resulted in significantly higher photosynthetic rate than the Control treatment. Oxygen in the 35-Day Experiment appeared to have a stimulatory effect on photosynthetic rate; the CO2&O2 treatment had a higher mean value than the CO2 treatment, although this comparison was not significant ($p = 0.15$).

Conductance rates in the 35-Day Experiment did not differ significantly among treatments and had a similar range of values to those observed in the 24-Hour Experiment. The PE of seedlings exposed to the 35-Day Experiment did not vary significantly among treatments. However, CSP did vary among treatments in this

experiment. Seedlings in the CO2 treatment had a significantly lower CSP than did seedlings in the Control or CO2&O2 treatments.

When photosynthetic rate parameters were measured along a PAR concentration gradient, LSP did not differ significantly among treatments. However, QE was highest in the CO2 treatment and lowest in the Control treatment, while the CO2&O2 treatment had an intermediate value.

There was no apparent substantial effect of chamber size or differences in experimental light level on the Control photosynthetic rate of the plants between the 24-Hour and 35-Day Experiments as the values obtained for the Control treatments were very similar in both experiments (the 35-Day rate was 103% of the 24-Hour rate) (Fig. 1). This suggested that photosynthetic rate differences between the 24-Hour and 35-Day Experiments found in other treatments were due to differences in the length of acclimation to atmospheric treatments rather than to slight differences in experimental conditions.

The photosynthetic rate of control plants was similar across experimental treatments. In contrast, photosynthetic rate for plants in the CO2 treatment of the 35-Day Experiment was 58% of the rate observed in the 24-Hour Experiment. The photosynthetic rate of the 35-Day CO2&O2 treatment was 81% of the 24-Hour rate, reflecting an apparent smaller rate reduction in this treatment between the two experiments. These reductions in the photosynthetic rates of the 35-Day Experiment were also most pronounced in the CO2 treatment when plotted as a function of $C_i$ (Fig. 2).

Chlorophyll and Leaf Biomass.- Chlorophyll concentration (chlorophyll a + chlorophyll b) did not vary among the treatments in either the non-adjusted or TNC-free datasets (Table 4). However, there did appear to be a trend of reduced chlorophyll in the CO2 treatment as compared with the Control treatment in the non-adjusted data ($p = 0.11$). Leaf biomass did not vary significantly among treatments (Table 4).

## Discussion

Effects of Elevated Carbon Dioxide.- Results from the both the 24-Hour and 35-Day Experiments demonstrate that carbon dioxide alone and in combination with elevated oxygen stimulates photosynthetic rate. These results indicate that Ginkgo trees growing in atmospheres with highly enriched levels of carbon dioxide (up to five times ambient) will experience stimulated photosynthetic rates that could persist over the long term. This inference is also supported by the elevated QE in the CO2 and CO2&O2 treatments of the 35-Day Experiment. Of the environmental factors examined in this study (carbon dioxide, oxygen, and atmospheric pressure), carbon dioxide had the greatest effect on photosynthetic rate.

Although Ginkgo leaf biomass was not significantly affected by carbon dioxide and oxygen in the CO2 and CO2&O2 treatments, the observed increases in net photosynthesis may translate into increases in primary productivity in plants exposed to elevated carbon dioxide and oxygen. For this increase to occur, constant rates of litter fall and herbivory must be assumed (Ricklefs 2001). Changes in tree growth parameters

are not observed in most studies until after multiple growing seasons have passed (e.g. Centritto et al. 1999, Gielen and Ceulemans 2001). Additionally, leaf biomass of deciduous plants is often determined by the amount of carbohydrate reserves stored by the plant the previous fall (Cheng and Fuchigami 2002), the values measured in this study could have been influenced by conditions during the previous season rather than by current experimental conditions. The significantly elevated carbon to nitrogen ratio of the CO2 and CO2&O2 treatments (Chapter 3, Table 2) suggests that root biomass might have increased as a mechanism for gathering additional nitrogen over a longer acclimation period (Gielen and Ceulemans 2001, Pearson and Brooks 1995, Sage 1996). However, root biomass was not measured in this study. Additionally, a 35-day acclimation period is not long enough to adequately assess differences in root biomass and resource allocation (Centritto et al. 1999, Idso 1999). Additional experiments looking at longer growth periods and assessing overall plant and root biomass changes are necessary to demonstrate greater biomass productivity in response to elevated photosynthetic rate.

In plants exposed to twice ambient carbon dioxide levels over longer exposure (weeks to years), photosynthetic rates undergo a process called photosynthetic down-regulation that occurs when an initial highly stimulated photosynthetic rate causes sugars to accumulate within the leaf (Saralabai et al. 1997). The presence of photosynthetic down-regulation in Ginkgo seedlings in the current study in response to five times ambient carbon dioxide is indicated by the differences between the $A/C_i$ curve slopes from the 24-Hour and 35-Day Experiments in the CO2 and CO2&O2 treatments relative

to the difference in the Control treatment and by the significantly reduced CSP in the CO2 treatment of the 35-Day Experiment.

Soluble sugars within the leaves slow photosynthetic rates by reducing the rate at which photosynthetic genes are transcribed (Van Oosten and Besford 1994). This complex feedback inhibition mechanism is accepted as the primary cause of photosynthetic down-regulation, although other causes have been proposed (e.g. Allen et al. 1988, Bryant et al. 1998). Van Oosten and Besford (1994) demonstrated that a build up of soluble sugars in the leaf (specifically hexose, glucose, and sucrose, measured as soluble sugars in this study) initiates a chain of reactions culminating in the suppression of gene transcription of Rubisco and other key photosynthetic enzymes and pigments (Furbank and Taylor 1995, Moore et al. 1999, Stitt 1991, Van Oosten and Besford 1995, Winters et al. 1995). Over time, this reduction in transcription rates results in lower concentrations of photosynthetic enzymes and pigments, which consequently lowers the photosynthetic capacity of the leaf. Gas exchange rates can also be reduced through reductions in conductance due to stomatal closure, but we did not observe any differences in conductance either among treatments or between experiments in the current study (Tables 1 and 2).

Carbon to nitrogen ratio (Chapter 3, Table 2) of Ginkgo seedlings was increased in response to elevated carbon dioxide in the current study. This change is consistent with a response to carbon dioxide that includes the down-regulation of genes coding for various compounds of the photosynthetic apparatus, including chlorophyll and protein.

However, neither chlorophyll (Table 4) nor protein (Chapter 3, table 2) was reduced in response to carbon dioxide, and soluble sugar concentration (Chapter 3, Table 1) did not differ by treatment. Given these results, we cannot confirm that decreases in gene-transcription rates were responsible for the observed photosynthetic down-regulation.

Starch concentration in Ginkgo leaves reached 60% of the leaf on a dry weight basis in response to elevated carbon dioxide (Chapter 3, Table 1), a value considerably higher than the 14% found in Control leaves. The Control treatment value is comparable with starch values measured on broadleaved trees (e.g. Rey and Jarvis 1998) and the deciduous conifers Metasequoia (Dawn Redwood), and Taxodium (Cypress) (Osborne and Beerling 2003). Other studies have found similarly large starch concentration enrichments in response to lower levels of carbon dioxide than used in this study. For example, Sholtis et al. (2004) found starch levels were 27% higher in plants grown at ~650 ppm that in control plants. With continued carbon dioxide exposure, the amount of starch enrichment varies seasonally, but stays enriched from year to year (Osborne and Beerling 2003, Rey and Jarvis 1998, Schapendonk et al. 2000). While previous research has not documented direct involvement of starch in feedback inhibition, it seems reasonable that such large increases in starch concentration could have resulted in transient stimulations of soluble sugar concentration, since starch is converted to sugars for export from the leaf. These fluctuating spikes in sugar concentration (e.g. Moing et al. 1994) may have resulted in feedback inhibition of photosynthetic rate.

Research on trees exposed to roughly doubled levels of carbon dioxide has shown that the stimulation of photosynthetic rate in response to carbon dioxide can lead to an early stimulation of ontogenetic development, which only occurs in the absence of photosynthetic down-regulation (Arp 1991, Centritto et al. 1999, Rogers and Ellsworth 2002). This means that saplings transplanted into high carbon dioxide environments mature at an accelerated rate until down-regulation occurs, and then proceed to age at the normal rate experienced by that species, even though the carbon dioxide level remains enriched. Accelerated growth may or may not be associated with the ontogenetic stimulation, and the effects of this accelerated development on reproductive success has not been addressed thus far in the literature. Accelerated ontogeny might result in the appearance of mature characteristics, such as reproductive organs and dwarf shoots (in some gymnosperms), earlier in the life span of the plant and may translate into an overall shorter life expectancy. The effects of the extremely elevated carbon dioxide levels examined in this study on ontogenetic rate could not be assessed due to the short duration of the acclimation periods, but it is reasonable to predict a similar pattern if acclimation periods had been extended. The magnitude of the ontogenetic stimulation experienced may be greater for plants exposed to the atmospheric compositions examined in this study because a much higher carbon dioxide concentration was employed here than in other studies.

Effects of Elevated Carbon Dioxide and Oxygen.- Contrary to predictions of heightened rates of photorespiration (e.g. Leegood et al. 1995, Takeba and Kozaki 1998),

elevated oxygen did not reduce Ginkgo net photosynthetic rates when combined with either ambient or elevated carbon dioxide in the current study. In comparing treatments that had oxygen (the O2 treatment of the 24-Hour Experiment and the CO2&O2 treatment of the 35-Day Experiment) with treatments that did not have oxygen (the Control treatment of the 24-Hour Experiment and the CO2 treatment of the 24-Hour and 35-Day Experiments), no significant differences were observed.

This observed lack of an oxygen effect on Ginkgo photosynthetic rate is surprising, given existing knowledge about the detrimental effects of oxygen on photosynthetic rate (Gale et al. 2001, Leegood et al. 1995, Tolbert et al. 1995). However, most of the studies that have examined photorespiration rates were performed in the absence of or at sub-ambient levels of carbon dioxide, in order to specifically examine the effects of oxygen (e.g. Leegood et al. 1995, Ogren 1975, Sharkey 1988). In response to low carbon dioxide levels and high oxygen concentrations, plants generally experience high levels of photorespiration, reducing photosynthetic rates to dangerous levels (Sharkey 1988, Tolbert et al. 1995). Some authors contend that this physiological stress in response to elevated oxygen may have contributed to the evolution of the $C_4$ photosynthetic pathway (Cerling et al. 1995), and the ecophysiological regulation of atmospheric oxygen concentration (Beerling and Berner 2000, Berry et al. 1994, Tolbert 1994). Recent authors have recognized a beneficial effect of photorespiration, in that it can dissipate free oxygen radicals generated by excessive exposure to light, reducing photoinhibition and oxidative stress in leaves (Ogren 1994, Takeba and Kozaki 1998),

but this effect only occurs in light-stressed plants ( ≥ 2000 PAR), so it is not applicable to this study. Despite a beneficial affect under certain circumstances, oxygen has been shown to have a detrimental affect on photosynthetic rate at sub-ambient carbon dioxide levels.

The dominance of photosynthesis over photorespiration in Ginkgo seedlings observed in the 24-Hour Experiment may have been due to the sensitivity of the $C_3$ photosynthetic apparatus to the relative increases in carbon dioxide and oxygen concentrations examined. While carbon dioxide in the CO2 treatment was only equivalent to 0.2% of the atmosphere, this concentration is 500% greater than the Control level of 0.037%. Oxygen concentration was increased from 20.9% to 30%, an increase of only about 50%. In absolute value, oxygen concentration was much greater than carbon dioxide concentration, but Michaelis-Menton enzyme activity constants for Rubisco, which were calculated using equations by Faruquhar et al. (1980), demonstrate that Rubisco is much more sensitive to changes in carbon dioxide concentration than to changes in oxygen concentration. Rubisco catalyzes 460 $\mu$mol carbon dioxide per $m^2 \bullet sec^{-1}$, but only 330 $\mu$mol oxygen per $m^2 \bullet sec^{-1}$. The heightened sensitivity of Rubisco to carbon dioxide rather than to oxygen most likely translated into the lack of Ginkgo photosynthetic rate response to elevated oxygen in this study because the increase in carbon dioxide level relative to the increase in oxygen maintained a high $CO_2/O_2$ ratio for Rubisco (e.g. Berner 2004, Igamberdiev et al. 2004). These responses suggest that

oxygen does not have an effect on Ginkgo photosynthetic rate at ambient or elevated carbon dioxide levels.

Increased atmospheric oxygen with elevated carbon dioxide may help maintain a stimulated photosynthetic rate over the long-term by increasing the rate of dark respiration, which would in turn increase the rate of sugar export from leaves, alleviating the build up of carbohydrates and potentially slowing photosynthetic down-regulation. Dark respiration rate is positively affected by oxygen concentration and is not decreased by carbon dioxide enrichment (Davey et al. 2004). Dark respiration may also have been enhanced in the current study by the optimal growing conditions during experimentation or the immature nature of the seedlings in this study, both of which might have caused dark respiration rates above expected values. In any case, elevated oxygen in this study did not detrimentally affect photosynthetic rate at either ambient or elevated levels of carbon dioxide.

## Conclusions

As expected, carbon dioxide concentrations at 500% of present-day ambient levels stimulated photosynthetic rate after both 24-Hour and 35-Day acclimation periods. However, at 30% atmospheric oxygen, photosynthetic rate was not reduced at either ambient or elevated levels of carbon dioxide irrespective of acclimation period. This result was unanticipated. While the experiments undertaken here examined only a short period in the usual lifespan of Ginkgo, the responses observed point to the possibility of

significant effects on primary productivity and terrestrial paleoecology of Mesozoic ecosystems assuming that Mesozoic Ginkgoaceae responded in the same way as modern G. biloba and the physiologic responses observed here persist over the lifespan of the plant.

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## References Cited

Allen, L., J. Vu, R. Valle, K. Boote, and P. Jones. 1988. Nonstructural carbohydrates and nitrogen of soybean grown under carbon dioxide enrichment. Crop Science 28:84-94.

Arp, W. 1991. Effects of source-sink relations on photosynthetic acclimation to elevated $CO_2$. Plant, Cell and Environment 14:869-875.

Bazzaz, F., and E. Fajer. 1992. Plant life in a $CO_2$-rich world. Scientific American:68-74.

Beerling, D., and R. Berner. 2000. Impact of a Permo-Carboniferous high $O_2$ event on the terrestrial carbon cycle. PNAS 97(23):12428-12432.

Beerling, D., J. McElwain, and C. Osborne. 1998. Stomatal responses of the 'living fossil' Ginkgo biloba L. to changes in atmospheric $CO_2$ concentration. Journal of Experimental Botany 49(326):1603-1607.

Berner, R. 2001. Modeling atmospheric $O_2$ over Phanerozoic time. Geochimica et Cosmochimica Acta 65(5):685-694.

Berner, R. 2003. The long-term carbon cycle, fossil fuels and atmospheric composition. Nature 426:323-326.

Berner, R. 2004. The Phanerozoic Carbon Cycle: $CO_2$ and $O_2$. Oxford University Press, Oxford.

Berner, R., D. Beerling, R. Dudley, J. Robinson, and R. Wildman. 2003. Phanerozoic Atmospheric Oxygen. Annual Review of Earth and Planetary Sciences 31:105-34.

Berner, R., and D. Canfield. 1989. A new model for atmospheric oxygen over Phanerozoic time. American Journal of Science 289:333-361.

Berry, J., G. Collatz, R. Guy, and M. Fogel. 1994. The compensation point: can a physiological concept be applied to global cycles of carbon and oxygen. Pp. 234-248. *In* N. Tolbert, and J. Preiss, eds. Regulation of Atmospheric $CO_2$ and $O_2$ by Photosynthetic Carbon Metabolism. Oxford University Press, Oxford.

Bryant, J., G. Taylor, and M. Frehner. 1998. Photosynthetic acclimation to elevated $CO_2$ is modified by source:sink balance in three component species of chalk grassland swards grown in a free air carbon dioxide enrichment (FACE) experiment. Plant, Cell and Environment 21(2):159-168.

Centritto, M., H. Lee, and P. Jarvis. 1999. Increased growth in elevated [$CO_2$]: an early, short-term response? Global Change Biology 5:623-633.

Cerling, T., J. Ehleringer, and J. Harris. 1995. Carbon dioxide starvation, the development of $C_4$ ecosystems, and mammalian evolution. Philosophical Transactions of the Royal Society, London, B 353:159-171.

Cheng, L., and L. Fuchigami. 2002. Growth of young apple trees in relation to reserve nitrogen and carbohydrates. Tree Physiology 22:1297-1303.

Davey, P., S. Hunt, G. Hymus, E. DeLucia, B. Drake, D. Karnosky, and S. Long. 2004. Respiratory oxygen uptake in not decreased by an instantaneous elevation of [$CO_2$], but is increased with long-term growth in the field at elevated [$CO_2$]. Plant Physiology 134:520-527.

Farquhar, G., S. von Caemmerer, and J. Berry. 1980. A biochemical model of photosynthetic $CO_2$ assimilation in leaves of $C_3$ species. Planta 149:78-90.

Furbank, R., and W. Taylor. 1995. Regulation of photosynthesis in $C_3$ and $C_4$ plants: a molecular approach. The Plant Cell 7:797-807.

Gale, J., S. Rachmilevitch, J. Reuveni, and M. Volokita. 2001. The high oxygen atmosphere toward the end-Cretaceous; a possible contributing factor to the K/T boundary extinctions and to the emergence of $C_4$ species. Journal of Experimental Botany 52(357):801-809.

Gielen, B., and R. Ceulemans. 2001. The likely impact of rising atmospheric $CO_2$ on natural and managed Populus: a literature review. Environmental Pollution 115:335-358.

Hamerlynck, E., T. Huxman, R. Nowak, S. Redar, M. Loik, D. Jordan, S. Zitzer, J. Coleman, J. Seemann, and S. Smith. 2000. Photosynthetic responses of Larrea tridentata to a step-increase in atmospheric $CO_2$ at the Nevada Desert FACE Facility. Journal of Arid Environments 44:425-436.

Hunt, R., D. Hand, M. Hannah, and A. Neal. 1991. Response to $CO_2$ enrichment in 27 herbaceous species. Functional Ecology 5:410-421.

Idso, S. 1999. The long-term response of trees to atmospheric $CO_2$ enrichment. Global Change Biology 5:493-495.

Igamberdiev, A., T. Mikkelson, P. Ambus, H. Bauwe, P. Lea, and P. Gardestrom. 2004. Photorespiration contributes to stomatal regulation and carbon isotope fractionation: a study with barley, potato, and Arabidopsis plants deficient in glycine decarboxylase. Photosynthesis Research 81:139-152.

Kürschner, W. M. 2001. Leaf sensor for $CO_2$ in deep time. Nature 411:247-248.

Leegood, R., P. Lea, M. Adcock, and R. Hausler. 1995. The regulation and control of photorespiration. Journal of Experimental Botany 46(Special Issue):1397-1414.

Moing, A., A. Escobar-Gutierrez, and J.-P. Gaudillere. 1994. Modeling carbon export out of mature peach leaves. Plant Physiology 106:591-600.

Moore, B., S.-H. Cheng, D. Sims, and J. Seemann. 1999. The biochemical and molecular basis for photosynthetic acclimation to elevated atmospheric $CO_2$. Plant, Cell and Environment 22:567-582.

Ogren, W. 1975. Control of photorespiration in soybean and maize. Pp. 45-52. *In* R. Marcelle, ed. Environmental and Biological Control of Photosynthesis. Dr. W. Junk Publishers, The Hague.

Ogren, W. 1994. Energy utilization by photorespiration. Pp. 115-125. *In* N. Tolbert, and J. Preiss, eds. Regulation of Atmospheric $CO_2$ and $O_2$ by Photosynthetic Carbon Metabolism. Oxford University Press, Oxford.

Osborne, C., and D. Beerling. 2003. The penalty of a long, hot summer. Photosynthetic acclimation to high $CO_2$ and continuous light in "living fossil" conifers. Plant Physiology 133:803-812.

Pearson, M., and G. Brooks. 1995. The influence of elevated $CO_2$ on growth and age-related changes in leaf gas exchange. Journal of Experimental Botany 46(292):1651-1659.

Poskuta, J., and A. Frankiewicz-Jozko. 1975. Enhanced dark $CO_2$ fixation by maize leaves in relation to previous illumination and oxygen concentration. Pp. 89-105. *In* R. Marcelle, ed. Environmental and Biological Control of Photosynthesis. Dr. W. Junk, Publishers, The Hague.

Retallack, G. 2001. A 300-Million year record of atmospheric carbon dioxide from fossil plant cuticles. Nature 411:287-290.

Rey, A., and P. Jarvis. 1998. Long-term photosynthetic acclimation to increased atmospheric $CO_2$ concentration in young birch (Betula pendula) trees. Tree Physiology 18:441-450.

Ricklefs, R. 2001. The Economy of Nature. W.H. Freeman and Company, New York.

Rogers, A., and D. Ellsworth. 2002. Photosynthetic acclimation of Pinus taeda (loblolly pine) to long-term growth in elevated $pCO_2$ (FACE). Plant, Cell and Environment 25:851-858.

Rothman, D. 2002. Atmospheric carbon dioxide levels for the last 500 million years. PNAS:4167-4171.

Royer, D. 2000. Estimating latest Cretaceous and Early Tertiary atmospheric $pCO_2$ from stomatal indices. GSA - Abstracts with Programs:A-196.

Royer, D., R. Berner, and D. Beerling. 2001. Phanerozoic atmospheric $CO_2$ change: evaluating geochemical and paleobiological approaches. Earth-Science Reviews 54:349-392.

Royer, D., L. Hickey, and S. Wing. 2003. Ecological conservatism in the 'living fossil' Ginkgo. Paleobiology 29(1):84-104.

Sage, R. 1996. Atmospheric modification and vegetation responses to environmental stress. Global Change Biology 2:79-83.

Saralabai, V., M. Vivekanandan, and R. Babu. 1997. Plant responses to high $CO_2$ concentration in the atmosphere. Photosynthetica 33(1):7-37.

Schapendonk, H., M. van Oijen, P. Dijkstra, C. Pot, W. Jordi, and G. Stoopen. 2000. Effects of elevated $CO_2$ concentration on photosynthetic acclimation and productivity of two potato cultivars grown in open-top chambers. Australian Journal of Plant Physiology 27:1119-1130.

Sharkey, T. 1988. Estimating the rate of photorespiration in leaves. Physiologia Plantarum 73:147-152.

Sholtis, J., C. Gunderson, R. Norby, and D. Tissue. 2004. Persistent stimulation of photosynthesis by elevated $CO_2$ in a sweetgum (Liquidambar styraciflua) forest stand. New Phytologist 162:343-354.

Stitt, M. 1991. Rising $CO_2$ levels and their potential significance for carbon flow in photosynthetic cells. Plant, Cell and Environment 14:741-762.

Takeba, G., and A. Kozaki. 1998. Photorespiration is an essential mechanism for the protection of $C_3$ plants from photooxidation. Pp. 15-36. *In* K. Satoh, and N. Murata, eds. Stress Responses of Photosynthetic Organisms. Elsevier Science.

Tolbert, N. 1994. Role of photosynthesis and photorespiration in regulating atmospheric $CO_2$ and $O_2$. Pp. 8-33. *In* N. Tolbert, and J. Preiss, eds. Regulation of Atmospheric $CO_2$ and $O_2$ by Photosynthetic Carbon Metabolism. Oxford University Press, Oxford.

Tolbert, N., C. Benker, and E. Beck. 1995. The oxygen and carbon dioxide compensation points of $C_3$ plants: possible role in regulating atmospheric oxygen. Proceedings of the National Academy of Sciences 92:11230-11233.

Tralau, H. 1968. Evolutionary trends in the genus Ginkgo. Lethaia 1:63-101.

Van Oosten, J., and R. Besford. 1994. Sugar feeding mimics effect of acclimation to high $CO_2$- rapid down regulation of RuBisCo small subunit transcripts but not of the large subunit transcripts. Journal of Plant Physiology 143:306-312.

Van Oosten, J., and R. Besford. 1995. Some relationships between the gas exchange, biochemistry, and molecular biology of photosynthesis during leaf development of tomato plants after transfer to different carbon dioxide concentrations. Plant, Cell and Environment 18:1253-1266.

von Caemmerer, S., and G. Farquhar. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153:376-387.

Watson, J., S. Lydon, and N. Harrison. 1999. Consideration of the genus Ginkgoites Seward and a redescription of two species from the Lower Cretaceous of Germany. Cretaceous Research 20:719-734.

Webber, A., G.-Y. Nie, and S. Long. 1994. Acclimation of photosynthetic proteins to rising atmospheric $CO_2$. Photosynthesis Research 39:413-425.

Winters, A., J. Gallagher, C. Pollock, and J. Farrar. 1995. Isolation of a gene expressed during sucrose accumulation in leaves of Lolium temulentum L. Journal of Experimental Botany 46(Special Issue):1345-1350.

Woodrow, I. 1994. Optimal acclimation of the $C_3$ photosynthetic system under enhanced $CO_2$. Photosynthesis Research 39:401-412.

Zhou, Z., and S. Zheng. 2003. Palaeobiology: the missing link in Ginkgo evolution. Nature 423:821-822.

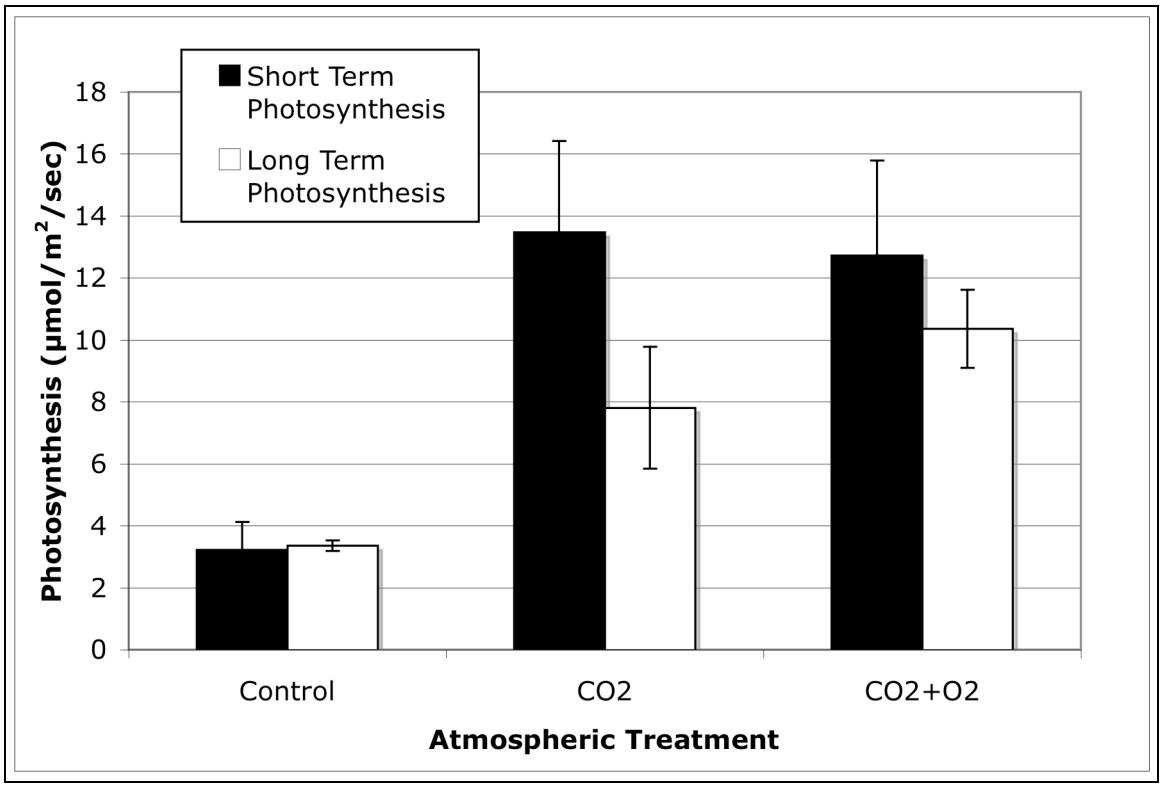
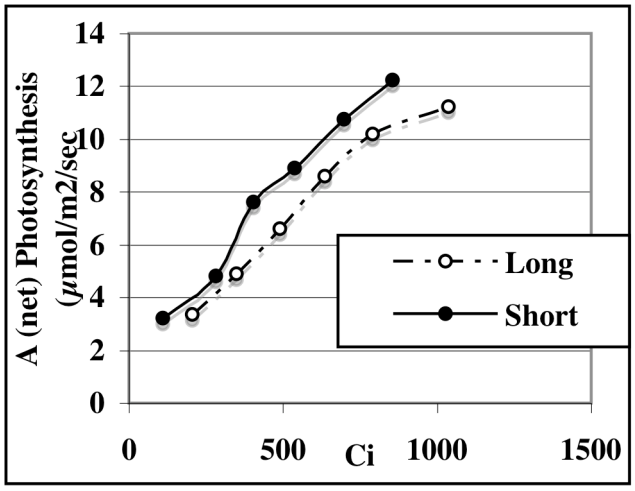



Figure 1: Comparison of net photosynthetic rate after 24-hour acclimation to net photosynthetic rate after five-week acclimation during the 35-Day Experiment. Data represent means of three repetitions of each experiment. Error bars are one standard deviation wide.

Figure 2: $A/C_i$ curves generated during experimentation for the Control treatment (A), CO2 treatment (B), and CO2&O2 treatment (C) from both the 24-Hour (filled symbols, solid line) and 35-Day (empty symbols, dashed line) Experiments. Curves were generated by measuring photosynthetic rate along a carbon dioxide concentration gradient using the Li-Cor 6400, and used to calculate PE (photosynthetic efficiency, the slope of the linear portion of the curve) and CSP (carbon dioxide saturation point, 95% of maximal photosynthetic rate value). Data points are mean values from three repetitions of each experiment.

A.


A (net) Photosynthesis (μmol/m2/sec
14
12
10
8
6
4
2
0
0
500
Ci
1000
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Long
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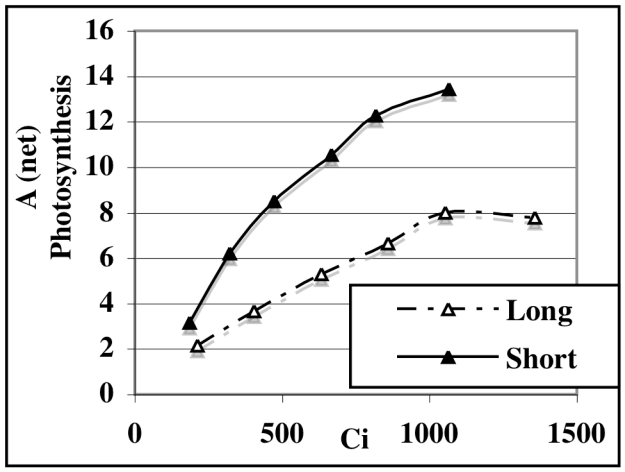
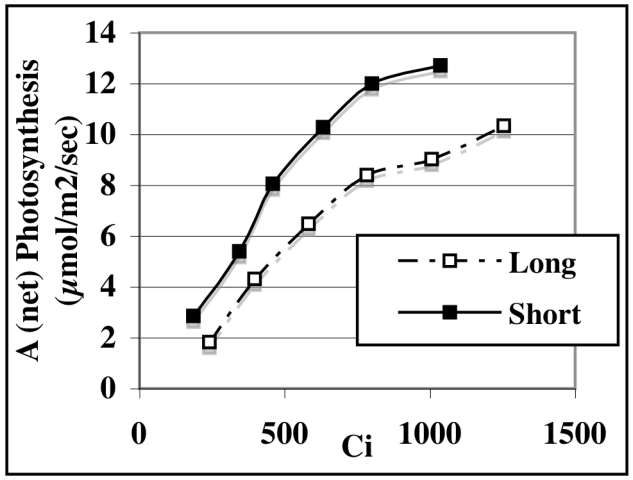
B.


A (net) Photosynthesis
16
14
12
10
8
6
4
2
0
0
500
Ci
1000
1500
Long
Short

C.


A (net) Photosynthesis (μmol/m2/sec)
14
12
10
8
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4
2
0
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1000
1500
Long
Short

Figure 2

Table 1: Treatment means (standard errors) for net photosynthesis, conductance, photosynthetic efficiency (PE), and carbon dioxide saturation point (CSP) of Ginkgo leaves exposed to various gaseous conditions for a 24-hour acclimation period.

| | **Control** | **Pressure** | **CO2** | **O2** | **CO2+O2** |
|---|---|---|---|---|---|
| **Photosynthesis** ($\mu$mol/m$^2$/sec$^{-1}$) | 3.22 **B**[1] (0.89) | 3.92 **B** (0.47) | 13.46 **A** (2.95) | 3.70 **B** (0.91) | 12.72 **A** (3.07) |
| **Conductance** ($\mu$mol/m$^2$/sec$^{-1}$) | 0.023 **A** (0.004) | 0.029 **A** (0.006) | 0.027 **A** (0.008) | 0.036 **A** (0.008) | 0.025 **A** (0.001) |
| **PE** | 0.007 **A** (0.001) | -[2] | 0.007 **A** (0.001) | - | 0.007 **A** (0.002) |
| **CSP** ($\mu$mol/m$^2$/sec$^{-1}$) | 1881.0 **A** (20.0) | - | 1821.0 **A** (80.0) | - | 1774.3 **A** (28.2) |

1. Values in the same row with the same letters are not significantly different from each other, based on a Fisher's Least Significant Difference t-test at $p \leq 0.1$.

2. Data not collected for these treatments (-)

Table 2: Treatment means (standard errors) for net photosynthesis, conductance, photosynthetic efficiency (PE), and carbon dioxide saturation point (CSP) of Ginkgo leaves exposed to various gaseous conditions for a 35-Day acclimation period.

| | **Control** | **CO2** | **CO2+O2** |
|---|---|---|---|
| **Photosynthesis** ($\mu$mol/m$^2$/sec$^{-1}$) | 3.35 **B**[1] (0.18) | 7.80 **A** (1.97) | 10.35 **A** (1.26) |
| **Conductance** ($\mu$mol/m$^2$/sec$^{-1}$) | 0.028 **A** (0.002) | 0.027 **A** (0.010) | 0.025 **A** (0.005) |
| **PE** | 0.0054 **A** (0.0017) | 0.0046 **A** (0.0015) | 0.0058 **A** (0.0014) |
| **CSP** ($\mu$mol/m$^2$/sec$^{-1}$) | 1861.0 **A** (80.0) | 1674.3 **B** (323.9) | 1801.0 **A** (70.7) |

1. Values in the same row with the same letters are not significantly different from each other, based on a Fisher's Least Significant Difference t-test at $p \leq 0.1$.

Table 3: Means (standard errors) for quantum efficiency (QE) and light saturation point (LSP) data of Ginkgo leaves exposed to various gaseous conditions for the 35-Day acclimation period, and measured repeatedly along a Photosynthetically Active Radiation (PAR) gradient.

| | **Control** | **CO2** | **CO2&O2** |
|---|---|---|---|
| **QE** | 0.0069 **A**[1] (0.0037) | 0.0264 **B** (0.0021) | 0.0229 **C** (0.0035) |
| **LSP** ($\mu$mol/m$^2$/sec$^{-1}$) | 1121.0 **A** (876.8) | 23.5 **A** (10.6) | 1201.0 **A** (622.3) |

1. Values in the same row with the same letters are not significantly different from each other, based on a Fisher's Least Significant Difference t-test at $p \leq 0.1$.

Table 4: Means (standard errors) of chlorophyll and leaf biomass from Ginkgo exposed to various gaseous conditions for 35 days.

| | **Control** | **CO2** | **CO2&O2** |
|---|---|---|---|
| Non-adjusted **Chlorophyll** ($\mu$g/mg) | 1.91 **A**[1] (0.59) | 0.84 **A** (0.21) | 1.11 **A** (0.53) |
| TNC-free | 2.15 **A** (0.87) | 3.07 **A** (2.49) | 5.52 **A** (3.86) |
| **Leaf Biomass** (g) | 1.49 **A** (0.94) | 1.90 **A** (0.79) | 1.80 **A** (1.40) |

1. Values in the same row with the same letters are not significantly different from each other, based on a Fisher's Least Significant Difference t-test at $p \leq 0.1$.

# CHAPTER 3:

## The nutritive content and digestibility of Ginkgo biloba seedlings exposed to elevated atmospheric carbon dioxide and oxygen: Implications for Mesozoic paleoecology

Abstract.-

Biogeochemical cycling models provide evidence that atmospheric carbon dioxide and oxygen may have been concurrently enriched during the Late Jurassic and Early Cretaceous. Primary productivity increases with enrichments of these gasses, but the effects of this enrichment on the quality of plants as forage material and on litter decomposition is not known. Ginkgo biloba seedlings were grown in three carbon dioxide and oxygen concentration treatments (ambient levels of both, 370 ppm and 209,000 ppm respectively, 2000 ppm carbon dioxide with ambient oxygen, and 2000 ppm carbon dioxide with 300,000 ppm oxygen) and relative nutritive content and digestibility of leaves were assessed using the ratio of carbon to nitrogen (C:N), protein concentration, lignin concentration, and the ratio of total non-structural carbohydrates to total structural carbohydrates (TNC:TSC). Elevated carbon dioxide alone and with elevated oxygen resulted in an increased C:N ratio of Ginkgo leaves but protein concentration did not change. TNC:TSC ratio was increased in response to carbon dioxide and oxygen treatments. Lignin concentration did not vary significantly among treatments. These results have implications to secondary productivity, herbivore physiology, decomposition rates, and nutrient cycling in mid-Mesozoic terrestrial ecosystems.

## Introduction

Plants are the primary producers in terrestrial ecosystems, providing forage material for all herbivores in the food web and creating the majority of the biomass in the ecosystem. Alterations to photosynthetic rates and other plant physiological processes have the potential to affect secondary productivity (East 1984, Olff et al. 2002); foraging behavior (Demment and Van Soest 1985), life history (Brand et al. 2003), and evolutionary patterns (Espinoza et al. 2004) of herbivores; and litter decomposition rates (Sariyildiz and Anderson 2003) through changes in the composition of primary metabolites within plant tissue. Previous studies have focused on the effects of twice ambient levels of carbon dioxide on plant physiology (e.g. Osborne and Beerling 2002, Royer et al. 2002, Staudt et al. 2001), and on herbivorous feeding rates (Dury et al. 1998, Fritschi et al. 1999, Williams et al. 1998), but few studies have examined the responses of plant quality to carbon dioxide increases above twice ambient levels, or to concurrent enrichments of oxygen. This study examines the effects of highly elevated atmospheric carbon dioxide and oxygen concentrations on primary plant biochemical constituent concentrations within leaves in order to establish a basis for inferring the effects of these atmospheric enrichments on herbivores and detritivores.

During the Mesozoic Era (245-65 million years ago) atmospheric composition was substantially enriched in both carbon dioxide and oxygen relative to modern levels, based on mathematical mass balance, elemental cycling, and rock abundance models (Berner 1998, 2004, Berner et al. 2003, Royer et al. 2001) and on various other proxies including stomatal frequency (e.g. Retallack 2001) and fire stratigraphy (Robinson 1989,

Wildman et al. 2004). The absolute levels of atmospheric carbon dioxide and oxygen were not constant throughout this span, however. The Late Jurassic and Early Cretaceous Periods (~180 mya to ~125 mya) saw the extremes of the enrichment, with carbon dioxide and oxygen levels possibly reaching up to 2000 ppm and 30% respectively, as compared with modern levels of 370 ppm and 20.9% (Berner et al. 2003, Royer et al. 2001).

Enriched levels of atmospheric carbon dioxide and oxygen affect net photosynthetic rate and primary productivity (Hamerlynck et al. 2000, Leegood et al. 1995, Saralabai et al. 1997, Takeba and Kozaki 1998), and may also affect the primary metabolite composition of the leaves. Elevated carbon dioxide stimulates photosynthetic rate over the long term (Adam et al. 2000, Marek et al. 1995, Rogers and Ellsworth 2002) and may result in complex changes to plant life history and productivity (Beerling et al. 1999, Rogers and Ellsworth 2002). Oxygen, however, can deter plant productivity through increased photorespiration in the absence of carbon dioxide (Badger 1985, Sharkey 1988), but it did not reduce net photosynthetic rate in Ginkgo biloba, when oxygen was combined with ambient or elevated levels of carbon dioxide (Chapter 2). In fact, under concurrent enrichments of both gasses, the net photosynthetic rate of G. biloba was stimulated up to 300% over present-day ambient conditions (Chapter 2). Therefore, elevated carbon dioxide and oxygen together may have a stimulatory affect on net photosynthetic rate, despite the reciprocal affects of photosynthesis and photorespiration. This stimulation could affect the chemical constituent composition of leaves.

The concentration of nitrogen (N)-bearing compounds is often reduced in leaves

exposed to elevated carbon dioxide due to photosynthetic rate stimulation and subsequent accumulation of carbohydrates (carbon (C) compounds) within the leaves. The increase in carbohydrates reduces the proportion of N-compounds in the leaf tissue on a dry-weight basis (Cotrufo et al. 1998, Krapp et al. 1991). N-compounds are also removed through photosynthetic down-regulation (Van Oosten and Besford 1994, Yelle et al. 1989b) – a process which occurs after continued exposure to elevated carbon dioxide, and results in a reduction of the transcription rate of genes that code for photosynthesis-specific N-compounds. In contrast, elevated levels of oxygen increase N-compounds within the leaf through the promotion of photorespiration (Leegood et al. 1995, Noctor et al. 2002, Novitskaya et al. 2002). Studies have shown that increases in photorespiration result in an increase in both the % and absolute amount of amino acids and other N-compounds within the leaf (Novitskaya et al. 2002) that may be partially due to an interconnection between the biochemical photorespiratory pathway and N metabolism within leaves (Leegood et al. 1995, Novitskaya et al. 2002). Photorespiration can also increase the proportional amount of N-compounds in leaf tissue by reducing the assimilation rate of C-compounds through reductions in net photosynthetic rate (Bertrand et al. 2003, Haupt-Herting and Fock 2002).

The relationship between atmospheric composition and forage digestibility has been sparsely addressed in the literature. Elevated photosynthetic rate, which is stimulated by elevated carbon dioxide, results in an accumulation of non-structural carbohydrates (TNC), which are highly digestible (Allen et al. 1988, Chapin et al. 1986, Madsen 1968). However, potential changes in structural carbohydrate (TSC)

concentrations are less well understood. Kilpeläinen et al (2005, 2003) found that lignin concentration responded to changes in temperature, but not to carbon dioxide concentration. They did not examine the effects of oxygen concentration. Gibeaut et al (2001) found no changes in cell wall composition in response to elevated carbon dioxide. No studies to date have examined how elevated oxygen affects cell wall composition either when alone or in combination with carbon dioxide. Additional work is needed to elucidate how cell wall composition is affected by elevated carbon dioxide and oxygen.

In order to assess the forage quality of leaves grown under extremely elevated levels of carbon dioxide and oxygen, Ginkgo biloba seedlings were exposed to hypothesized mid-Mesozoic carbon dioxide and oxygen levels. G. biloba seedlings were used as experimental subjects because of this genus' extensive fossil record (Tralau 1968) and morphological similarity to fossil specimens (Zhou and Zheng 2003).

## Methods

Experimentation and Plant Material.- Experiments were performed as in the 35-Day Experiment of Chapter 2. Seedlings used in the 35-Day Experiment in Chapter 2 were sampled for various leaf parameters to assess nutritive content and digestibility in the current study.

## Measurement and Analysis

Leaf Preparation.- After the 35-day acclimation period in the 35-Day Experiment (see Chapter 2 for a full description of experimental methods), leaves were collected and transported on ice to the laboratory. Brown tissue was removed and leaves were flash-frozen at -80° C for five to ten minutes and lyophilized using a Labconco FreeZone 18 Liter Freeze Dry System (Labconco, Kansas City, MO) for 48 hours. Freeze-dried leaves were ground to a fine powder with a mortar and pestle and stored at -80°C until chemical analyses were performed. Powder from all leaves produced during a single treatment/block combination was pooled for all chemical analyses.

C:N Ratio.- 4 mg of leaf powder per sample were packed in a silver boat and analyzed for %C and %N using an elemental analyzer (EA). Blocks 1 and 2 were analyzed at the Duke Environmental Stable Isotope Laboratory (Duke University, Durham, NC) using a Carlo Erba EA, and Block 3 was analyzed at the Idaho Stable Isotope Laboratory (University of Idaho, Moscow) with an NC 2500 EA. The average %C and %N values of two samples from each treatment/block combination were assessed. C:N ratio was calculated by dividing %C by %N for each sample, and then the two samples per treatment/block combination were averaged to get a mean value for each treatment/block combination. N=3 %C, %N, and C:N values per treatment were averaged to calculate the reported means.

Protein.- Total protein concentration was estimated using the Bradford Protein Assay (Bradford 1976), as modified for plant material by Jones et al (1989), except that

Bovine Serum Albumine (BSA) was used as the protein standard instead of RuBP to reduce analysis expenditure. Briefly, 10 mg of leaf powder was extracted for 30 minutes using 5 mL of a 1 N solution of NaOH. After the extraction, 10 µL of supernatant was diluted to 100 µL with DDI water and then mixed with 5 ml of 1:4 diluted Bradford Dye Reagent and 3 mg/mL soluble polyvinylpyrollidone (PVP). Absorbance was measured at 595 nm and protein concentration was determined through comparison to a BSA standard concentration curve.

Sugar.- Sugar concentration was determined using a modified Phenol-Sulfuric Acid assay (Chow and Landhausser 2004, Sturgeon 1990). Soluble sugars were extracted three times from 50 mg leaf powder at 95°C using 5 mL of 80% ethanol. The resulting extract was diluted 1:1 with 80% ethanol.

To control for substances in the ethanol extract that might have reacted with the phenols, causing spectrophotometric analysis error, parallel assays were performed on two separate 0.5 mL aliquots of each sample. Aliquot 1 was mixed with 1 ml 2% phenol solution, while aliquot 2 was mixed with 1 mL DDI water. 2.5 mL concentrated sulfuric acid was added to each aliquot, samples were diluted to 8 mL with water, and absorbance was measured at 490 nm. Sugar concentration was determined by comparison with a (1:1:1) glucose: fructose: galactose standard curve, and was calculated using the following formula:

$$\text{Sugar } (\mu g/mg) = [\text{abs (aliquot 1)} - \text{abs (aliquot 2)}] / [\text{abs coefficient (aliquot 1)} - \text{abs coefficient (aliquot 2)}]$$

Absorbance coefficients used in the current study were the following:

abs coefficient (aliquot 1) = 0.3489

abs coefficient (aliquot 2) = 0.0488

Starch.- Starch analysis was performed using Enzyme Method 2 from Rose et al. (1991), as modified by Chow and Landhausser (2004). 12 mg leaf powder was extracted as described for sugar. The insoluble pellet was incubated with 2 mL 0.1 N NaOH at 50°C for 30 minutes to solubilize the starch. 2.5 mL 0.1 N acetic acid (pH 5.1) was added as a buffer, and 0.5 mL of enzyme solution (1000 U Alpha-amylase, 5 U amylo-glucosidase) was added to cleave the glycosidic bonds over a 20-hour incubation period at 50°C. Hydrolized starch samples were then centrifuged for 10 minutes and 200 µL aliquots were taken for colorimetric analysis. 2 mL of glucose oxidase/peroxidase/o-dianisidine solution was added and a brown color developed for 45 minutes in the dark. Samples were moved to a 22°C water bath and 0.5 mL 75% sulfuric acid was added in order to stop color development and stabilize the color. Absorbance was read at 525 nm, and concentration was determined by comparison with a glucose standard curve.

TNC:TSC.- Total Non-Structural Carbohydrates (TNC) was measured by summing the concentrations of sugars and starch. Total Structural Carbohydrates (TSC) was measured by finding the difference between total carbon in the sample (calculated from mass spectrometer %C output) and the TNC.

Lignin Concentration.- The Soxhlet refluxing procedure was used to purify leaf powder for lignin measurement. Methanol-soluble compounds (extractives) were removed from leaf powder by reflux with methanol at room temperature for 8-12 hours.

After the extraction, the remaining leaf material was dried under vacuum at 55°C until reaching constant weight. Powder was stored in a desiccator until analysis.

Lignin % of the cell wall was determined using the Klason lignin procedure (Dence 1992). Aliquots of 100 mg (W1) extracted powder were solubilized in 1.5 mL 72% sulfuric acid, stirred with a glass rod at 10 minute intervals for two hours, and diluted to 50 mL volume with DDI water. The leaf-acid solution was sealed in a glass bottle, placed in a pressure cooker with 500 mL water, and cooked for one hour. After overnight cooling, the cooked leaf-acid solution was filtered under suction through a pre-weighed medium sintered glass filter (W2). The volume of filtrate (containing acid soluble lignin) was adjusted to 100 mL volume and diluted 1:10 with DDI water, and measured by absorbance at 280 nm. The filter (residue is the acid insoluble lignin) was washed three times with hot DDI water to remove any residual acid and dried for 2 hours at 105°C. After drying, the filter was weighed (W3). Lignin fraction concentrations were calculated as follows:

Acid Soluble Lignin % (of cell wall) = $(abs_{205nm} * 10) / 110$

Acid Insoluble Lignin % (of cell wall) = $(W3 – W2) / W1$

Total Lignin % = Acid Soluble Lignin % + Acid Insoluble Lignin %

Only the Acid Insoluble Lignin fraction was reported in this study, as the measured absorbance was abnormally high for all aliquots (Chang 2006). These very high values were most likely due to phenolic substances in the acid solution that absorbed at 205 nm but were not lignin (Chang 2006, Dence 1992).

Acid-Detergent Lignin (ADL), a common procedure for lignin measurement, and

the Klason lignin procedure both measure lignin concentration directly, but consistently return different estimates due to the variable structure of lignin (Fukushima and Hatfield 2004), making comparison of lignin concentrations between studies that use different procedures difficult. Klason lignin values range from only a few percent to a 100% increase in concentration relative to ADL values. Using bomb calorimetry, Jung et al (1999) have shown that these higher estimates are not an overestimation of concentration, but are instead more accurate estimates. It is inadvisable to directly compare Klason lignin results with ADL results, as studies using the ADL procedure will generally report less lignin than those using the Klason procedure.

Potential Dilution Effect of High TNC.-TNC levels are variable within leaves, due to fluctuating assimilation (photosynthesis) and export rates (Krapp and Stitt 1995, Mauney et al. 1979, Yelle et al. 1989a). Therefore, TNC can vary in response to carbon dioxide concentration, individual leaf differences, leaf age, and on daily and seasonal cycles. The experimental design controlled for carbon dioxide concentration, leaf age, time of day, and season, but individual variation in leaf TNC concentration was not controlled. Hence, the concentrations of protein and %N may have been affected by dilution from variable levels of TNC in leaves. In order to correct for this potential source of error, protein and %N concentrations were reported as measured (non-adjusted dataset) and as corrected for TNC (TNC-free dataset). Corrections were calculated by subtracting the concentration of TNC from the total amount of leaf powder prior to calculating concentrations of protein and N.

Statistical Analysis.- C:N ratio, protein concentration, TNC:TSC ratio, and lignin concentration data (including both non-adjusted and TNC-free datasets) were analyzed using SAS Statistical Software version 6.12 (Cary, NC). For all data sets, proc GLM (ANOVA) was used to determine which experimental factors had a significant effect on the measured variables. Additionally, Fisher's Least Significant Difference t-tests were performed (means statement) to determine significance among treatments in each dataset. Significance was determined at $p \leq 0.1$. Datasets were small; $n = 3$ for each treatment for the C:N ratio, protein, sugar, and starch datasets, while $n = 2$ for the lignin dataset due to limited leaf material in the third rep.

## Results

Nutritive Content.- Table 1 gives data for protein, %N, %C, and C:N ratio. Protein concentration did not differ significantly among treatments when reported on either a non-adjusted or TNC-free basis, although the variability of the mean was much greater on a TNC-free basis, most likely due to large differences in TNC among treatment/block combinations. A weak trend of depression in the non-adjusted protein data in the CO2 treatment relative to the other two experimental treatments superficially resembled a similar (and significant) trend in the non-adjusted %N data, but was not detected as significant in this dataset ($p = 0.23$). TNC-free %N data were not significantly different among treatments, but were highly variable. %C data did not differ due to treatment. However, C:N ratio was significantly higher in those treatments with elevated

carbon dioxide, as compared with the Control.

Digestibility.- Sugar, starch, TNC:TSC ratio, and lignin concentration are reported in Table 2. Sugar concentration did not vary significantly among treatments, and comprised less than 1% of total leaf material. Starch concentration had a strong and significant positive response to elevated carbon dioxide, composing approximately 60% of the dry matter of the leaf in the CO2 and CO2&O2 treatments. TNC:TSC ratio was significantly increased with exposure to the CO2 and CO2&O2 treatments relative to the response to the Control treatment. Lignin concentration did not vary significantly by treatment.

## Discussion

Alterations to the composition of primary metabolites within leaves can have a strong affect on herbivores of all sizes and types, affecting growth, development, longevity and fecundity (e.g. Brand et al. 2002, 2003, Schoenian 2003). These affects are reflected in two plant tissue parameters: the nutritive content (Apori et al. 2000, Awoyinka et al. 1995, Fritschi et al. 1999, Salawu et al. 2002) – estimated by the protein concentration and proportion of N-compounds (C:N ratio) in the leaf – and the digestibility (Dada et al. 2002, Fritschi et al. 1999, Zhiliang et al. 1996) – estimated through lignin concentration and the relative amount of digestible carbohydrates (TNC:TSC ratio) in the leaf. For generalist herbivores, such as ungulates and horses, the toughness, or digestibility, of the forage is the most important test of the potential

nutritional benefits of that food source (Demment and Van Soest 1985, Lundberg and Palo 1993), since the nutrients within indigestible food items cannot be utilized. For herbivores that can afford to be more selective during foraging, such as insects, birds, and smaller mammals, the nutritive content of the tissue selected will have a greater affect on diet (Cipollini et al. 2002, Geluso and Hayes 1999, Kinnear et al. 1979, Scheirs et al. 2003). Despite these differences between herbivores, forage quality is generally elevated by increases in both nutritive content and digestibility.

Nutritive Content.- Modern herbivores of diverse taxonomy have dietary requirements for adequate nutrition that include different relative amounts of both N-based compounds like proteins and pigments, and C-based compounds like sugars and starch (Aganga et al. 2000, Campbell 1996, Herrel et al. 2004, Schoenian 2003). Some plant compounds, including certain amino acids, vitamins, and fatty acids, which are predominantly N-based (Campbell 1996, Goodwin and Mercer 1983), are essential to the herbivorous diet, however, because they must be ingested prefabricated; animals cannot synthesize them from their inorganic parts (Campbell 1996, Cilliers et al. 1999, Schoenian 2003). Since these essential nutrients are relatively rare within plants on a dry-weight basis (Brand et al. 2002, Salawu et al. 2002), plants with a lower C:N ratio have a higher nutritive content than those with a higher C:N ratio. Therefore, C:N ratio is used in this paper to estimate nutritive content.

Nutritive content results for Ginkgo leaves were reported both as TNC-free datasets and as non-adjusted datasets (Table 1). TNC-free data reflected changes in relative abundance of protein and %N only, while non-adjusted data included both a TNC

component and a protein or %N component. Both datasets were reported in order to distinguish the varying affects of TNC and protein or %N on nutritive content.

The TNC-free datasets did not respond to either elevated carbon dioxide or elevated oxygen, indicating that neither protein nor %N in Ginkgo leaves varied among treatments. However, non-adjusted %N was significantly reduced and C:N ratio was significantly increased in the CO2 and CO2&O2 treatments relative to the Control, while protein concentration was slightly, but non-significantly, reduced in the CO2 treatment. These minimal variations in Ginkgo nutritive content were most likely due to increases in TNC instead of decreases in protein or %N, because only the non-adjusted datasets, which included a TNC component, responded to treatment. Additionally, the content of starch, a significant component of TNC, was increased by elevated carbon dioxide (Table 2). Overall, these results demonstrate that Ginkgo leaf nutritive content was not strongly affected by elevated carbon dioxide, despite the significantly reduced C:N ratio of leaves produced in the CO2 and CO2&O2 treatments.

The increase in Ginkgo leaf TNC in response to elevated carbon dioxide was most likely due to extreme increases in starch concentration (Table 2). 60% starch is an unexpectedly high concentration for Ginkgo because many modern gymnosperms have less than 5% starch (e.g. Rogers and Ellsworth 2002). Ginkgo has broad leaves rather than needles like conifers. The Control treatment value (~14%) is comparable with starch values of many broadleaved trees (e.g. Rey and Jarvis 1998), suggesting that leaf morphology influences starch concentration more than taxonomy. The large increase in

starch concentration (46%) observed in this study is also not unreasonable because other studies using lower carbon dioxide enrichments had similar results. Sholtis et al. (2004), for instance, report a persistent 27% increase in starch in Liquidambar styraciflua (Sweetgum, a dicotyledonous tree) in response to only ~650 ppm carbon dioxide.

Other authors have observed reductions in nutritive content in response to elevated carbon dioxide (e.g. Fritschi et al. 1999, Williams et al. 1998). In addition to an abundant accumulation of certain carbon compounds that make up TNC, such as starch (Saralabai et al. 1997), the decrease seen in other studies may be partially due to a plant physiological process known as photosynthetic down-regulation (Nie et al. 1995, Rey and Jarvis 1998, Rogers and Ellsworth 2002, Webber et al. 1994). Accumulation of excess carbohydrates in leaves, in response to prolonged exposure to elevated atmospheric carbon dioxide, reduces initial photosynthetic rate. Most studies have found that the reduction in photosynthetic rate during down-regulation is accompanied by a decrease in foliar protein and nitrogen, which has been attributed to a reduced rate of protein synthesis in the leaf (Van Oosten et al. 1994). Some studies suggest that this reduction may be due to dilution of foliar protein and nitrogen by TNC (e.g. Krapp et al. 1991, Nie et al. 1995), as was found in this study; but in others, the reduction was demonstrated more conclusively (e.g. Sheen 1990, Sholtis et al. 2004, Yelle et al. 1989b). Photosynthetic down-regulation was observed in the net photosynthetic rate responses of Ginkgo seedlings exposed to the CO2 and CO2&O2 treatments in the current study (Chapter 2), but protein and %N concentrations were not reduced by this process.

The non-adjusted protein and %N data of Ginkgo leaves may demonstrate a response to oxygen concentration through photorespiration stimulation. Increased rates of photorespiration can reduce TNC within leaves (Bertrand et al. 2003, Haupt-Herting and Fock 2002) and stimulate protein synthesis (Novitskaya et al. 2002). A non-significant trend supporting this suggestion is apparent in the mean values of several datasets, including protein concentration, C:N ratio, and %N, in which the Control and CO2 treatments had the extreme values, and the CO2&O2 treatment had the intermediate value. If additional experimentation demonstrates that these trends accurately describe real plant responses, then elevated oxygen could counter potential reductions in Ginkgo leaf nutritive content in response to elevated carbon dioxide through photorespiration. Additional work is needed to more fully elucidate how the nutritive quality of Ginkgo is affected by elevated atmospheric oxygen in combination with elevated carbon dioxide.

Digestibility**.-** The effects of leaf tissue digestibility on herbivore behavior and life history are complex, and are partially dependent on the digestive physiology of the herbivore (e.g. Demment and Van Soest 1985, Wikelski et al. 1993). Different herbivores have different digestive efficiencies and are therefore variably able to digest forages (Lundberg and Palo 1993). For an herbivore with a low digestive efficiency, highly indigestible plant tissues will not yield much sustenance, and so the herbivore either increases its consumption to compensate (Demment and Van Soest 1985, Owen-Smith and Novellie 1982) or adopts a more selective foraging habit, as do some non-ruminant ungulates (Demment and Van Soest 1985, Kinnear et al. 1979), birds (Geluso

and Hayes 1999) and insects (Caswell et al. 1973). For herbivores with high digestive efficiencies, which include reptiles (Wikelski et al. 1993) and ruminants (Lundberg and Palo 1993), more types of forage can be digested, allowing these herbivores to be less selective in their foraging strategy.

In Ginkgo leaves, the TNC:TSC ratio was significantly increased in both treatments with elevated carbon dioxide. This ratio compares the digestible component of cells – TNC – with the indigestible component – TSC. The observed increase indicates an increase in Ginkgo leaf digestibility in response to elevated carbon dioxide.

Lignin concentration did not vary by treatment, however. Lignin is a complex polymer of xylem cell walls and cannot easily be broken down enyzmatically irrespective of digestive efficiency (Goodwin and Mercer 1983). Unless lignin is physically broken through oral or gut processing, it remains a significant barrier to herbivores (Demment and Van Soest 1985). Nevertheless, the observation that elevated carbon dioxide alone and together with oxygen resulted in an increased TNC:TSC ratio strongly suggests an increase in overall digestibility in plants grown under these conditions.

Increases in TNC:TSC ratio were due to the extremely high starch concentrations in response to elevated carbon dioxide that made up the TNC component (Table 2). The apparent paradox of an increased starch concentration without a concurrent %C response was most likely due to a change in resource partitioning within the leaf (McBee and Miller 1990, Staudt et al. 2001). Partitioning is a plant physiological process by which different sink organs determine the end form of assimilated carbohydrates (Huber et al. 1984, Wardlaw 1990). These end forms can include non-structural carbohydrates as well

as structural carbohydrates that are incorporated into cell walls (McBee and Miller 1990, Sturm and Tang 1999). Under ambient conditions, a greater proportion of the assimilated carbon could have been used for carbon compounds not measured in this study, such as cellulose and hemicellulose, than was used for those compounds under elevated carbon dioxide. The significant increase in starch content observed in this study may reflect a persistent change in partitioning (Osborne and Beerling 2003, Rey and Jarvis 1998, Schapendonk et al. 2000). Concurrent decreases are expected in other carbon compounds, such as the structural carbohydrates cellulose and hemicellulose, and would further increase the digestibility of the leaves in response to elevated carbon dioxide. Additional work is needed in this area to more fully assess the carbon partitioning response of plants to the conditions examined in this study.

## Conclusions

This study examined the nutritive content (C:N ratio and protein concentration) and digestibility (TNC:TSC ratio and lignin concentration) responses of Ginkgo biloba, a modern tree from an ancient lineage, to atmospheric compositions hypothesized to have existed during the mid-Mesozoic Era. 35-Day growth chamber experiments testing elevated levels of carbon dioxide and oxygen resulted in changes to Ginkgo foliage chemistry. Ginkgo leaf nutritive content did not change appreciably, but digestibility was increased in Ginkgo leaves under these conditions. These alterations to forage quality have the potential to add to our understanding of mid-Mesozoic ecosystem functioning

through changes in herbivore diet, foraging behavior, and physiology and to decomposition and nutrient cycling rates.

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## References Cited

Adam, N., G. Wall, B. Kimball, P. Pinter, R. LaMorte, D. Hunsaker, F. Adamsen, T. Thompson, A. Matthias, S. Leavitt, and A. Webber. 2000. Acclimation response of spring wheat in a free-air $CO_2$ enrichment (FACE) atmosphere with variable soil nitrogen regimes. I. Leaf position and phenology determine acclimation response. Photosynthesis Research 66:65-77.

Aganga, A., T. Adogia-Bessa, U. Omphile, and K. Tshireletso. 2000. Significance of browses in the nutrition of Tswana goats. Archivos de Zootecnia 49(188):469-480.

Allen, L., J. Vu, R. Valle, K. Boote, and P. Jones. 1988. Nonstructural carbohydrates and nitrogen of soybean grown under carbon dioxide enrichment. Crop Science 28:84-94.

Apori, S., R. Long, F. Castro, and E. Ørskov. 2000. Chemical composition and nutritive value of leaves and stems of tropical weed Chromolaena odorata. Grass and Forage Science 55:77-81.

Awoyinka, A., V. Abegunde, and S. Adewush. 1995. Nutrient content of young cassava leaves and assessment of their acceptance as a green vegetable in Nigeria. Plant

Foods for Human Nutrition 47:21-28.
Badger, M. 1985. Photosynthetic oxygen exchange. Annual Review of Plant Physiology 36:27-53.
Beerling, D., F. Woodward, and P. Valdes. 1999. Global terrestrial productivity in the mid-Cretaceous (100 ma): model simulations and data. Geological Society of America Special Paper 332:385-390.
Berner, R. 1998. The carbon cycle and $CO_2$ over Phanerozoic time: the role of land plants. Philosophical Transactions of the Royal Society of London B 353:75-82.
Berner, R. 2004. The Phanerozoic Carbon Cycle: $CO_2$ and $O_2$. Oxford University Press, Oxford.
Berner, R., D. Beerling, R. Dudley, J. Robinson, and R. Wildman. 2003. Phanerozoic Atmospheric Oxygen. Annual Review of Earth and Planetary Sciences 31:105-34.
Bertrand, A., Y. Castonguay, P. Nadeau, S. Laberge, R. Michaud, G. Bélanger, and P. Rochette. 2003. Oxygen deficiency affects carbohydrate reserves in overwintering forage crops. Journal of Experimental Botany 54(388):1721-1730.
Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72:248-254.
Brand, Z., T. Brand, and C. Brown. 2002. The effect of dietary energy and protein levels during a breeding season of ostriches (Struthio camelus domesticus) on production the following season. South African Journal of Animal Science 32(4):226-230.
Brand, Z., T. Brand, and C. Brown. 2003. The effect of dietary energy and protein levels on body condition and production of breeding male ostriches. South African Journal of Animal Science 32(4):231-239.
Campbell, N. 1996. Biology. The Benjamin/Cummings Publishing Company, Inc., Menlo Park, California.
Caswell, H., F. Reed, S. Stephenson, and P. Werner. 1973. Photosynthetic pathways and selective herbivory: a hypothesis. The American Naturalist 107(956):465-480.
Chang, H.-M. 2006. Lignin determination with the Klason Lignin procedure. P. Discussion of Ginkgo foliage Klason Lignin laboratory results in relation to Klason Lignin results from woods.
Chapin, F., J. McKendrick, and D. Johnson. 1986. Seasonal changes in carbon fractions in Alaskan Tundra plants of differing growth form: implications for herbivory. Journal of Ecology 74:707-731.
Chow, P., and S. Landhausser. 2004. A method for routine measurements of total sugar and starch content in woody plant tissues. Tree Physiology 24:1129-1136.
Cilliers, S., J. Sales, J. Hayes, A. Chwalibog, and J. Du Preez. 1999. Comparison of metabolisable energy values of different foodstuffs determined in ostriches and poultry. British Poultry Science 40:491-494.
Cipollini, M., E. Paulk, and D. Cipollini. 2002. Effect of nitrogen and water treatment on leaf chemistry in horsenettle (Solanum carolinense), and relationship to herbivory

by flea beetles (Epitrix spp.) and tobacco horworm (Manduca sexta). Journal of Chemical Ecology 28(12):2377-2398.

Cotrufo, M., P. Ineson, and A. Scott. 1998. Elevated $CO_2$ reduces the nitrogen concentration of plant tissues. Global Change Biology 4(1):1365-2486.

Dada, S., A. Akinsoyinu, J. Smith, and K. Dashiell. 2002. The effect of leaf-pruning on nutrient intake and in vivo digestibility of soybean stovers by sheep. Journal of Sustainable Agriculture 19(4):5-14.

Demment, M. W., and P. J. Van Soest. 1985. A nutritional explanation for body-size patterns of ruminant and nonruminant herbivores. The American Naturalist 125(5):641-672.

Dence, C. W. 1992. Methods in lignin chemistry. Springer-verlag, New York.

Dury, S., J. Good, C. Perrins, A. Buse, and T. Kaye. 1998. The effects of increasing $CO_2$ and temperature on oak leaf palatability and the implications for herbivorous insects. Global Change Biology 4(1).

East, R. 1984. Rainfall, soil nutrient status and biomass of large African savanna mammals. African Journal of Ecology 22:245-270.

Espinoza, R., J. Wiens, and C. Tracy. 2004. Recurrent evolution of herbivory in small, cold-climate lizards: Breaking the ecophysiological rules of reptilian herbivory. PNAS 101(48):16819-16824.

Fritschi, F., K. Boote, L. Sollenberger, and L. J. Allen. 1999. Carbon dioxide and temperature effects on forage establishment: tissue composition and nutritive value. Global Change Biology 5:743-753.

Fukushima, R., and R. Hatfield. 2004. Comparison of the Acetyl Bromide spectrophotometric method with other analytical lignin methods for determining lignin concentration in forage samples. Journal of Agricultural and Food Chemistry 52:3713-3720.

Geluso, K., and J. Hayes. 1999. Effects of dietary quality on basal metabolic rate and internal morphology of European Starlings (Sturnus vulgaris). Physiological and Biochemical Zoology 72(2):189-197.

Gibeaut, D., G. Cramer, and J. Seemann. 2001. Growth, cell walls, and UDP-Glc dehydrogenase activity of Arabidopsis thaliana grown in elevated carbon dioxide. Journal of Plant Physiology 158(5):569-576.

Goodwin, T., and E. Mercer. 1983. Introduction to Plant Biochemistry. Pergamon Press, Ltd, New York.

Hamerlynck, E., T. Huxman, R. Nowak, S. Redar, M. Loik, D. Jordan, S. Zitzer, J. Coleman, J. Seemann, and S. Smith. 2000. Photosynthetic responses of Larrea tridentata to a step-increase in atmospheric $CO_2$ at the Nevada Desert FACE Facility. Journal of Arid Environments 44:425-436.

Haupt-Herting, S., and H. Fock. 2002. Oxygen exchange in relation to carbon assimilation in water-stressed leaves during photosynthesis. Annals of Botany 89:851-859.

Herrel, A., B. Vanhooydonck, R. Joachim, and D. Irschick. 2004. Frugivory in

polychrotid lizards: effects of body size. Oecologia 140:160-168.
Huber, S., H. Rogers, and D. Israel. 1984. Effects of $CO_2$ enrichment on photosynthesis and photosynthate partitioning in soybean (Glycine max) leaves. Physiologia Plantarum 62:95-101.
Jones, C. G., J. D. Hare, and S. J. Compton. 1989. Measuring plant protein with the Bradford Assay. Journal of Chemical Ecology 15(3):979-992.
Jung, H., V. Varel, P. Weimer, and J. Ralph. 1999. Accuracy of Klason Lignin and Acid Detergent Lignin Methods as assessed by bomb calorimetry. Journal of Agricultural and Food Chemistry 47:2005-2008.
Kilpelainen, A., H. Peltola, A. Ryyppo, and S. Kellomaki. 2005. Scots pine responses to elevated temperature and carbon dioxide concentration: growth and wood properties. Tree Physiology 25:75-83.
Kilpelainen, A., H. Peltola, A. Ryyppo, K. Sauvala, K. Laitinen, and S. Kellomaki. 2003. Wood properties of Scots pines (Pinus sylvestris) grown at elevated temperature and carbon dioxide concentration. Tree Physiology 23:889-897.
Kinnear, J., A. Cockson, P. Christensen, and A. R. Main. 1979. The nutritional biology of the ruminants and ruminant-like mammals -- a new approach. Comparative Biochemistry and Physiology 64A:357-365.
Krapp, A., W. Quick, and M. Stitt. 1991. Ribulose-1,5-bisphosphate carboxylase-oxygenase, other Calvin-cycle enzymes, and chlorophyll decrease when glucose is supplied to mature spinach leaves via the transpiration stream. Planta 186:58-69.
Krapp, A., and M. Stitt. 1995. An evaluation of direct and indirect mechanisms for the "sink-regulation" of photosynthesis in spinach: Changes in gas exchange, carbohydrates, metabolites, enzyme activities and steady-state transcript levels after cold-girdling source leaves. Planta 195:313-323.
Leegood, R., P. Lea, M. Adcock, and R. Hausler. 1995. The regulation and control of photorespiration. Journal of Experimental Botany 46(Special Issue):1397-1414.
Lundberg, P., and R. T. Palo. 1993. Resource use, plant defenses, and optimal digestion in ruminants. Oikos 68:224-228.
Madsen, E. 1968. Effect of $CO_2$-concentration on the accumulation of starch and sugar in tomato leaves. Physiologia Plantarum 21:168-175.
Marek, M., J. Kalina, and M. Matouskova. 1995. Response of photosynthetic carbon assimilation of Norway spruce exposed to long-term elevation of $CO_2$ concentration. Photosynthetica 31(2):209-220.
Mauney, J., G. Guinn, K. Fry, and J. Hesketh. 1979. Correlation of photosynthetic carbon dioxide uptake and carbohydrate accumulation in cotton, soybean, sunflower and sorghum. Photosynthetica 13(3):260-266.
McBee, G., and F. Miller. 1990. Carbohydrate and lignin partitioning in sorghum stems and blades. Agronomy Journal 82:687-690.
Nie, G., S. Long, R. Garcia, B. Kimball, R. LaMorte, P. Pinter, G. Wall, and A. Webber. 1995. Effects of free-air $CO_2$ enrichment on the development of the

photosynthetic apparatus in wheat, as indicated by changes in leaf proteins. Plant, Cell and Environment 18:855-864.

Noctor, G., S. Veljovic-Jovanovic, S. Driscoll, L. Novitskaya, and C. Foyer. 2002. Drought and oxidative load in the leaves of $C_3$ plants: a predominant role for photorespiration. Annals of Botany 89:841-850.

Novitskaya, L., S. Trevanion, S. Driscoll, C. Foyer, and G. Noctor. 2002. How does photorespiration modulate leaf amino acid contents? A dual approach through modelling and metabolite analysis. Plant, Cell and Environment 25:821-835.

Olff, H., M. Ritchie, and H. Prins. 2002. Global environmental controls of diversity in large herbivores. Nature 415:901-904.

Osborne, C., and D. Beerling. 2002. Sensitivity of tree growth to a high $CO_2$ environment: consequences for interpreting the characteristics of fossil woods from ancient greenhouse worlds. Palaeogeography, Palaeoclimatology, Palaeoecology 182:15-29.

Osborne, C., and D. Beerling. 2003. The penalty of a long, hot summer. Photosynthetic acclimation to high $CO_2$ and continuous light in "living fossil" conifers. Plant Physiology 133:803-812.

Owen-Smith, N., and P. Novellie. 1982. What should a clever ungulate eat? The American Naturalist 119(2):151-178.

Retallack, G. 2001. A 300-Million year record of atmospheric carbon dioxide from fossil plant cuticles. Nature 411:287-290.

Rey, A., and P. Jarvis. 1998. Long-term photosynthetic acclimation to increased atmospheric $CO_2$ concentration in young birch (Betula pendula) trees. Tree Physiology 18:441-450.

Robinson, J. 1989. Phanerozoic $O_2$ variation, fire, and terrestrial ecology. Palaeogeography, Palaeoclimatology, Palaeoecology 75(3):223-240.

Rogers, A., and D. Ellsworth. 2002. Photosynthetic acclimation of Pinus taeda (loblolly pine) to long-term growth in elevated $pCO_2$ (FACE). Plant, Cell and Environment 25:851-858.

Royer, D., R. Berner, and D. Beerling. 2001. Phanerozoic atmospheric $CO_2$ change: evaluating geochemical and paleobiological approaches. Earth-Science Reviews 54:349-392.

Royer, D., C. Osborne, and D. Beerling. 2002. High $CO_2$ increases the freezing sensitivity of plants: implications for paleoclimatic reconstructions from fossil floras. Geology 30(11):963-966.

Salawu, M., A. Adesogan, M. Fraser, R. Frychan, and R. Jones. 2002. Assessment of the nutritive value of whole crop peas and intercropped pea-wheat bi-crop forages harvested at different maturity stages for ruminants. Animal Feed Science and Technology 96:43-53.

Saralabai, V., M. Vivekanandan, and R. Babu. 1997. Plant responses to high $CO_2$ concentration in the atmosphere. Photosynthetica 33(1):7-37.

Sariyildiz, T., and J. Anderson. 2003. Interactions between litter quality, decomposition

and soil fertility: a laboratory study. Soil Biology and Biochemistry 35:391-399.
Schapendonk, H., M. van Oijen, P. Dijkstra, C. Pot, W. Jordi, and G. Stoopen. 2000. Effects of elevated $CO_2$ concentration on photosynthetic acclimation and productivity of two potato cultivars grown in open-top chambers. Australian Journal of Plant Physiology 27:1119-1130.
Scheirs, J., L. Bruyn, and R. Verhagen. 2003. Host nutritive quality and host plant choice in two grass miners: primary roles for primary compounds. Journal of Chemical Ecology 29(6):1373-1389.
Schoenian, S. 2003. An introduction to feeding small ruminants. Maryland Small Ruminant Page. www.sheepandgoat.com.
Sharkey, T. 1988. Estimating the rate of photorespiration in leaves. Physiologia Plantarum 73:147-152.
Sheen, J. 1990. Metabolic repression of transcription in higher plants. The Plant Cell 2:1027-1038.
Sholtis, J., C. Gunderson, R. Norby, and D. Tissue. 2004. Persistent stimulation of photosynthesis by elevated $CO_2$ in a sweetgum (Liquidambar styraciflua) forest stand. New Phytologist 162:343-354.
Staudt, M., R. Joffre, S. Rambal, and J. Kesselmeier. 2001. Effect of elevated $CO_2$ on monoterpene emission of young Quercus ilex trees and its relation to structural and ecophysiological parameters. Tree Physiology 21:437-445.
Sturgeon, R. 1990. Monosaccharides. Pp. 1-37. Methods in Plant Biochemistry. Academic Press Limited.
Sturm, A., and G.-Q. Tang. 1999. The sucrose-cleaving enzymes of plants are crucial for development, growth and carbon partitioning. Trends in Plant Science 4(10):401-407.
Takeba, G., and A. Kozaki. 1998. Photorespiration is an essential mechanism for the protection of $C_3$ plants from photooxidation. Pp. 15-36. *In* K. Satoh, and N. Murata, eds. Stress Responses of Photosynthetic Organisms. Elsevier Science.
Tralau, H. 1968. Evolutionary trends in the genus Ginkgo. Lethaia 1:63-101.
Van Oosten, J., and R. Besford. 1994. Sugar feeding mimics effect of acclimation to high $CO_2$- rapid down regulation of RuBisCo small subunit transcripts but not of the large subunit transcripts. Journal of Plant Physiology 143:306-312.
Van Oosten, J., D. Wilkins, and R. Besford. 1994. Regulation of the expression of photosynthetic nuclear genes by $CO_2$ is mimicked by regulation by carbohydrates: a mechanism for the acclimation of photosynthesis to high $CO_2$? Plant, Cell and Environment 17:913-923.
Wardlaw, I. 1990. Tansley Review No. 27: The control of carbon partitioning in plants. New Phytologist 116:341-381.
Webber, A., G.-Y. Nie, and S. Long. 1994. Acclimation of photosynthetic proteins to rising atmospheric $CO_2$. Photosynthesis Research 39:413-425.
Wikelski, M., B. Gall, and F. Trillmich. 1993. Ontogenetic changes in food intake and digestion rate of the herbivorous marine iguana (Amblyrhynchus cristatus, Bell).

Oecologia 94:373-379.
Wildman, R., L. Hickey, M. Dickinson, R. Berner, J. Robinson, M. Dietrich, R. Essenhigh, and C. Wildman. 2004. Burning of forest materials under late Paleozoic high atmospheric oxygen levels. Geology 32(5):457-460.
Williams, R., D. Lincoln, and R. Norby. 1998. Leaf age effects of elevated $CO_2$-grown white oak leaves on spring-feeding lepidopterans. Global Change Biology 4:235-246.
Yelle, S., R. Beeson, M. Trudel, and A. Gosselin. 1989a. Acclimation of two tomato species to high atmospheric $CO_2$. I Sugar and starch concentration. Plant Physiology 90:1465-1472.
Yelle, S., R. Beeson, M. Trudel, and A. Gosselin. 1989b. Acclimation of two tomato species to high atmospheric $CO_2$. II Ribulose-1,5-bisphosphate carboxylase/oxygenase and phophoenolpyruvate carboxylase. Plant Physiology 90:1473-1477.
Zhiliang, T., C. Huiping, and X. Tingxian. 1996. Comparative study on fibre characteristics of rye and wheat straws. AJAS 9(1):51-56.
Zhou, Z., and S. Zheng. 2003. Palaeobiology: the missing link in Ginkgo evolution. Nature 423:821-822.

Table 1: Means (standard errors) of %C, %N, protein concentration, and C:N ratio of Ginkgo biloba leaves in response to 35-day exposure to three atmospheric compositions.

| | **Control** | **CO2** | **CO2&O2** |
|---|---|---|---|
| **%C** | 51.04 **A**[1] (1.79) | 49.64 **A** (0.97) | 49.65 **A** (0.47) |
| **%N** Non-adjusted | 2.39 **A** (0.20) | 1.29 **B** (0.32) | 1.45 **B** (0.38) |
| **%N** TNC-free | 2.61 **A** (0.34) | 4.56 **A** (3.76) | 6.92 **A** (4.56) |
| **Protein** ($\mu$g/mg) Non-adjusted | 128.80 **A** (28.75) | 90.76 **A** (11.08) | 108.06 **A** (12.25) |
| **Protein** ($\mu$g/mg) TNC-free | 140.99 **A** (38.24) | 288.10 **A** (150.50) | 570.77 **A** (466.78) |
| **C:N ratio**[2] | 21.39 **A** (1.19) | 40.34 **B** (10.47) | 35.90 **B** (9.18) |

1. Values in the same row with the same letters are not significantly different from each other, based on a Fisher's Least Significant Difference t-test at $p \leq 0.1$.

2. C:N ratio mean values cannot be calculated from the %C and %N values reported due to the different averaging paths used. See text for further explanation.

Table 2: Means (standard errors) of sugar, starch, Klason lignin and Total Non-Structural Carbohydrate to Total Structural Carbohydrate (TNC:TSC) ratio of Ginkgo biloba leaves in response to 35-day exposure to three atmospheric compositions.

| | **Control** | **CO2** | **CO2&O2** |
|---|---|---|---|
| **Sugar** ($\mu$g/mg) | 1.62 **A**[1] (0.52) | 1.69 **A** (0.34) | 1.93 **A** (0.37) |
| **Starch** ($\mu$g/mg) | 148.30 **A** (85.63) | 604.15 **B** (242.26) | 682.94 **B** (217.30) |
| **Klason Lignin** (% cell wall)[2] | 21.42 **A** (7.68) | 16.66 **A** (1.37) | 20.46 **A** (8.87) |
| **TNC:TSC** | 0.002 **A** (0.002) | 0.012 **B** (0.004) | 0.014 **B** (0.004) |

1. Values in the same row with the same letters are not significantly different from each other, based on a Fisher's Least Significant Difference t-test at $p \leq 0.1$.

2. Klason lignin does not include the soluble component due to the presence of substances that interfered with the absorption measurement.

# CHAPTER 4

## Potential effects of elevated atmospheric carbon dioxide and oxygen on mid-Mesozoic terrestrial ecosystems: Inferences based on changes in Ginkgo biloba foliage quality and quantity

Abstract.-

The potential effects of extremely elevated atmospheric carbon dioxide and oxygen levels on mid-Mesozoic (Late Jurassic through Early Cretaceous) terrestrial ecosystems are discussed. This discussion is based on the results of recent gas exchange and leaf quality experiments on Ginkgo biloba seedlings and on a review of the relevant plant physiology, dinosaur herbivory, dinosaur metabolism, and microbial decomposition literature. Experimental work demonstrated that atmospheric carbon dioxide and oxygen stimulate the net photosynthetic rate and primary productivity of G. biloba and alter the nutritive content and digestibility of its foliage. Under the assumption that these changes are maintained throughout the lifespan of G. biloba and that other Mesozoic plants had similar physiological responses, the experimental results suggest that mid-Mesozoic plants may have been more productive than modern plants, and would have had higher foliar carbon to nitrogen (C:N) ratios and more starch in the leaves, decreasing the nutritive content and increasing the digestibility of foliage at that time. Among the host of possible consequences of such changes for mid-Mesozoic terrestrial ecosystems are a shortening of plant maturation rates, decreased plant life span, and increased amounts of leaf litter and changes in its decomposition rate. Changes in the quantity and nutritive quality of mid-Mesozoic plants may also help to explain the large body sizes of mid-Mesozoic herbivores. Changes in plant quantity and nutritive quality in response to atmospheric composition variation can have a prominent affect on terrestrial ecosystems.

## Introduction

During the Late Jurassic and Early Cretaceous Periods (~180 – 125 mya (mid-Mesozoic)), the carbon dioxide and oxygen partial pressures of the atmosphere were most likely elevated relative to present-day ambient conditions, based on mathematical mass balance, elemental cycling, and rock abundance models (Berner 1998, 2004, Berner et al. 2003, Royer et al. 2001). Carbon dioxide at that time may have reached 2000 ppm in the atmosphere, a 540% increase over present-day levels (370 ppm) (Royer et al. 2001), while oxygen may have reached up to 30% of the atmosphere, a 43% increase over present-day levels (20.9%) (Berner et al. 2003). Also during the mid-Mesozoic, the continents were arranged into a super continent that was just beginning to break up (Scotese 2002) and global temperatures were most likely elevated (Retallack 2002), resulting in large continental arid regions and some global sea level rise due to the melting of the polar ice caps (Scotese 2002).

Populating the terrestrial ecosystems during this time was a fauna in which the primary herbivores were dinosaurs (e.g. Carpenter et al. 2002, Dal Sasso 2003). These herbivores were large (Appenzeller 1994), are hypothesized to have been gregarious (Day et al. 2002), and may have had faster metabolisms than those of modern reptiles (Seymour and Lillywhite 2000). Long bone histology supports incredibly fast growth rates for even the largest dinosaurs (Horner et al. 2000, Sander et al. 2004), including the sauropods Janenschia, Brachiosaurus, Dicraeosaurus, and Barosaurus (Sander 2000). Additionally, fossil evidence such as sauropod trackways (Day et al. 2002, Lockley et al. 2002a, Lockley et al. 2002b), herd mass death assemblages (Varricchio and Horner

1993), and nesting sites (Chiappe and Coria 2004, Horner 1999, Horner et al. 2000, Salgado et al. 2005) suggest that herbivorous dinosaurs may have participated in energy-expensive activities such as migration and active parenting.

Herbivorous dinosaurs must have eaten large quantities of food in order to support their bulk, fast growth rates, and energy-dependent lifestyles (Day et al. 2002, Sander et al. 2004). The flora during the mid-Mesozoic included a range of plants such as conifers, ferns, seed ferns, cycads and related plants, and ginkgos (Ash 1999, Basinger 1997, Miller and LaPasha 1984, Tidwell 1990, Wolfe and Upchurch 1987, Ziegler et al. 1993). Most plants that lived during the mid-Mesozoic are resistant to herbivory today (Freeland and Janzen 1974, Tiffney 1997a, 1997b), due to the presence of defense chemicals, thick cuticles, and spines (Bond et al. 2004, Freeland and Janzen 1974, Rafferty et al. 2005). However, coprolite (Chin 1995, Sharma et al. 2005) and phytolith (Krauss 2001) evidence demonstrates that these plants were ingested by herbivorous dinosaurs of the Late Jurassic and Early Cretaceous.

Recent experimental studies on Ginkgo biloba seedlings (Chapter 2) have demonstrated a 200% increase in G. biloba photosynthetic rate in response to 2000 ppm carbon dioxide (CO2 treatment), and increases up to 300% in response to 2000 ppm carbon dioxide and 30% oxygen (CO2&O2 treatment), conditions hypothesized for the mid-Mesozoic. These strong stimulations of photosynthetic rate suggested a proportional increase in primary productivity over the long term in G. biloba seedlings continually exposed to these conditions (Ricklefs 2001). Additionally, the quality of G. biloba foliage was altered similarly in response to both the CO2 and CO2&O2 treatments

(Chapter 3); no differences in leaf quality were observed between these treatments. In both the CO2 and CO2&O2 treatments, elevated carbon dioxide stimulated the production of starch within the Ginkgo leaves, which reduced the C:N ratio, lowering G. biloba leaf nutritive content. Elevated levels of starch in the leaves also increased the Total Non-Structural Carbohydrate (sugars and starches) to Total Structural Carbohydrate (cell wall carbohydrates) Ratio (TNC:TSC ratio) of those leaves. Hence, the apparent digestibility of G. biloba foliage was increased in response to elevated carbon dioxide. Similar changes in plant quality during the mid-Mesozoic could have influenced the foraging and feeding behaviors of mid-Mesozoic herbivores, and may have affected leaf litter decomposition rates due to the abundance of easily degraded compounds in these leaves.

The purpose of this paper is to explore the various potential implications of elevated carbon dioxide and oxygen atmospheres for mid-Mesozoic terrestrial ecosystems, using the G. biloba experimental results as a starting point. To achieve this goal, the literature on how productivity and quality changes in foliage affect herbivory, herbivores, and decomposition rates in modern ecosystems is reviewed, and this information is applied to the mid-Mesozoic through a generalization of the G. biloba experimental results to all Mid-Mesozoic plants.

In order to be able to generalize the results of the G. biloba physiological experiments to the Mesozoic flora, several assumptions were made. These include (1) G. biloba, the only surviving species of the Ginkgo genus (Royer et al. 2003, Tralau 1968), has identical ecophysiological responses to all of Ginkgoaceae, (2) the gas exchange,

productivity, and carbon partitioning responses of G. biloba to elevated carbon dioxide and oxygen are maintained throughout the lifespan of the plant when gaseous exposure is continued, and (3), Ginkgo photosynthetic rate, carbon partitioning, and productivity responses are conserved throughout the mid-Mesozoic flora. Using these assumptions, the potential implications of stimulated photosynthetic rate and high starch content in Ginkgo leaves are tentatively applied to the mid-Mesozoic world.

## Plant Physiology in the mid-Mesozoic

A highly stimulated photosynthetic rate, such as that observed in G. biloba in response to elevated atmospheric carbon dioxide and oxygen (Chapter 2) may have instigated various plant physiological adjustments in mid-Mesozoic plants. For instance, carbohydrates – the end-products of photosynthesis (Fader and Koller 1983) – are transported throughout each individual plant to various sink organs where they are made into a variety of compounds used for growth, respiration, storage, and reproduction (Huber et al. 1984, Krapp and Stitt 1995, Wardlaw 1990). Genetic and environmental variability generally determine how carbohydrates are partitioned within the plant (Körner 2003). However, with a continued stimulated photosynthetic rate during the mid-Mesozoic, carbohydrate production could have been very high; a higher yield of seeds (Allen et al. 1987), faster growth (Centritto et al. 1999, Sturm and Tang 1999), and greater carbohydrate storage (Fader and Koller 1983) could have resulted. These changes have the potential to have increased the abilities of mid-Mesozoic plants to withstand adverse seasonal climate variations (Cheng and Fuchigami 2002, Kozlowski and Pallardy

2002), including solar winters in high-latitude Mesozoic forests (e.g. Francis 1988) and the dry season in arid climates (Hamerlynck et al. 2000). Carbohydrate partitioning adjustments in response to elevated carbon dioxide may have also affected plant life history and fitness if an increase in carbohydrates affected seed yield or quality (Allen et al. 1987).

Elevated photosynthetic rates also temporarily stimulate ontogenetic development in modern trees (Arp 1991, Centritto et al. 1999, Rogers and Ellsworth 2002). However, this observation may be an artifact of experimental methodology, as the ontogenetic stimulation has generally been observed when trees are initially exposed to elevated carbon dioxide (Arp 1991, Centritto et al. 1999), and may not persist after the tree acclimates to its environment through a homeostatic mechanism called photosynthetic down-regulation (Rogers and Ellsworth 2002). Given the experimentally derived nature of this effect, it is doubtful that mid-Mesozoic plants experienced faster maturation than plants do presently since mid-Mesozoic plants spent their entire lives in an elevated carbon dioxide and oxygen environment and were presumably already acclimated to it. However, if ontogeny remains stimulated past acclimation, and therefore could have occurred in mid-Mesozoic plants, accelerated ontogeny could have had a strong affect on these plants. Stimulated ontogeny could have led to the early appearance of mature characteristics, including reproductive organs and dwarf shoots, and could have translated into a shorter life expectancy for plants growing under elevated carbon dioxide and oxygen during the mid-Mesozoic. These effects could have meant that mid-Mesozoic

plant communities had higher turnover rates and shorter life spans than would the same plants growing under modern atmospheric conditions.

Lastly, the strong photosynthetic rate response of G. biloba to the highly enriched carbon dioxide and oxygen levels tested here may give insights into the fitness of Ginkgo in the Mesozoic world. During the Jurassic and Early Cretaceous Periods, Ginkgo was ubiquitous above 40° latitude and speciose (Czier 1998). As world climate began to change and angiosperms radiated during the Late Cretaceous and Tertiary, this genus was restricted to only G. biloba (Royer et al. 2003, Tralau 1968). This may indicate that G. biloba was one of the luckiest or hardiest of the Ginkgoaceae, given that it survived an additional 65 million years of environmental change. The strong positive photosynthetic rate response of this taxon to experimental conditions may demonstrate that Ginkgo was highly adapted to survive under very different environmental conditions than those in the present-day world. Since present-day atmospheric conditions include much lower levels of both carbon dioxide and oxygen than existed during the mid-Mesozoic, this may in part help to explain the lack of diversity in the modern Ginkgo lineage after such diverse beginnings.

## Effects of Elevated Primary Productivity For Mid-Mesozoic Terrestrial Ecosystems

Experimental ecological work in the modern world has suggested that marginal environments, such as swamps, estuaries, and river systems, are more highly productive than non-marginal and arid environments (Lieth 1973, Likens 1973, Whittaker and

Likens 1973). During the Late Jurassic, arid terrestrial environments were common, but during the Early Cretaceous, continental land masses were drifting apart from each other, producing a greater proportion of marginal environments (Scotese 2002). It is likely that the higher proportion of marginal environments during the Cretaceous meant that overall primary productivity was relatively higher then than during the Late Jurassic (Miller and LaPasha 1984, Tidwell 1990).

Regardless of these changes in environment over time, the elevated photosynthetic rates observed in both the CO2 and CO2&O2 treatments most likely translated into higher rates of primary productivity in all terrestrial environments in both the Late Jurassic and Early Cretaceous Periods (Chapter 2). Primary productivity is essentially an accounting term for plant biomass (Ricklefs 2001). It is determined by subtracting the amount of mass lost from the plant due to herbivory and litter fall from the amount of mass gained through growth due to net photosynthetic rate (Ricklefs 2001, Whittaker and Likens 1973). Therefore, assuming constant rates of herbivory and litter fall, primary productivity is proportional to net photosynthetic rate (Ricklefs 2001). An increase in primary productivity of mid-Mesozoic plants most likely affected both herbivores and detritivores.

Effects on Herbivores.- Elevated primary productivity may have produced a greater amount of forage during the mid-Mesozoic. Both the size and metabolism of an herbivore have a strong effect on its energy and food intake requirements. Therefore, given the increased productivity assumed for that time, mid-Mesozoic terrestrial

ecosystems may have been able to feed even the biggest and most energetically demanding herbivores.

Larger animals, as a general rule, require more food to support themselves than do smaller animals (Burness 2002, Farlow 1987, Weaver 1983). Elephants, the largest terrestrial animals today (5-7 metric tons), eat 170 kg of food per day (Guy 1975). Elephant food requirements scaled by body mass up to the size of a large sauropod, Brachiosaurus (15-78 metric tons), indicate that this dinosaur would have needed to eat a minimum of 250 kg per day, assuming an ectothermic metabolism, but up to 1200 kg of plant material a day assuming an endothermic metabolism (Weaver 1983). Other dinosaurs would also have needed to eat similarly large amounts of food (Farlow 1976, 1987). Primary productivity stimulated up to 200-300% during the mid-Mesozoic in response to elevated carbon dioxide and oxygen atmospheres could have provided more of the plant material required to support these large herbivores.

While size plays a large role in the amount of food consumed by an animal, the metabolism also affects energy requirements (Burness 2002, Farlow 1987, Weaver 1983). Endothermic animals must eat more food per unit of body weight to support their metabolisms than ectothermic animals do. An endothermic Brachiosaurus, for instance, could have required up to 3 times as much food as an ectothermic Brachiosaurus of the same body size (Weaver 1983). Dinosaur metabolic rates have not been determined with certainty, although varying lines of evidence suggest fast growth rates (Erickson et al. 2001, Horner et al. 2000, Padian et al. 2001, Sander et al. 2004) and endothermic-like metabolisms (Barrick et al. 1996, Farlow 1987, Fricke and Rogers 2000, Padian 1997,

Reid 1997). If dinosaurian herbivores had endothermic or endothermic-like metabolisms, elevated primary productivity due to elevated carbon dioxide and oxygen atmospheres could have helped to provide the food they required.

Effects on Detritivores.- Microbial decomposers feed on leaf litter, decomposing it using a variety of biochemical pathways (Nealson 1997). Elevated primary productivity stimulates microbial activity (Billings et al. 2002, Cannell and Hornley 1998, Comins and McMurtrie 1993, Cotrufo et al. 2005) and biomass (De Graaff et al. 2004, Rønn et al. 2003) through additional litter contributions to the soil system (Booker et al. 2005). Additional microbial activity and biomass during the mid-Mesozoic in response to elevated primary productivity may therefore have translated into faster decomposition rates and quicker nutrient turnover within the soil system.

## Consequences of Plants with Lower C:N Ratios and Higher Digestibility For Mid-Mesozoic Terrestrial Ecosystems

From the herbivore's perspective, there are two parameters that determine the potential benefit of food. (1) Nutritive content, and (2) digestibility (Demment and Van Soest 1985, Hanley 1982, Milton et al. 1994, Owen-Smith and Novellie 1982). Nutritive content speaks to the amount of essential nutrients that are contained in the potential food item (Apori et al. 2000, Awoyinka et al. 1995, Fritschi et al. 1999, Salawu et al. 2002) and was estimated in Chapter 3 by the protein concentration and proportion of N-compounds (C:N ratio) in the leaf. Digestibility describes how much of the plant material

can be utilized by the herbivore (Dada et al. 2002, Fritschi et al. 1999, Zhiliang et al. 1996). In Chapter 3, this parameter was estimated through lignin concentration and the relative amount of digestible carbohydrates (TNC:TSC ratio) in the leaf.

In G. biloba leaves produced during 35-day exposure to elevated carbon dioxide and oxygen atmospheric treatments, the amount of essential nutrients in the leaves was not altered (despite an increase in C:N ratio) and digestibility was increased, predominantly due to strong elevations of starch concentration (Chapter 3, Table 2). Therefore, mid-Mesozoic plants most likely had more digestible leaves than do modern plants living under ambient atmospheric conditions.

Herbivore Foraging Behavior and C:N Ratio.- Modern animals are divided broadly into selective and generalist foragers, based on how they select food items while foraging (Gagnon and Chew 2000, Williams et al. 1993). Selective foragers have generally smaller bodies and narrower mouths than generalist foragers. Mice, birds, and deer are a few modern selective herbivores (Dada et al. 2002, Geluso and Hayes 1999, Owen-Smith and Novellie 1982). They are highly particular about which plant parts they select as food (Owen-Smith and Novellie 1982). Selection is based both on avoidance of tough or harmful plant parts, such as wood, bark, spines or leaves with thick cuticle, and acceptance of plant parts that are perceived to have a high nutritive content such as new growing tips, fruits, and seeds (Ginnett and Demment 1995, Hanley 1982, Shipley et al. 1999). Generalist herbivores, in contrast, tend to have large bodies, wide mouths, and adaptations that allow them to take in a great deal of food at once such as a long legs and necks (Clemens and Maloiy 1982, Hanley and Hanley 1982, Kinnear et al. 1979).

Elephants, horses, and large ungulates are modern generalist herbivores (Clemens and Maloiy 1982, Keys et al. 1969, Salawu et al. 2002). They rely either on high digestive efficiencies or high ingestion rates to obtain adequate nutrition and food energy (Demment and Van Soest 1985, Farlow 1987).

Based on general skull morphology and body size, extinct herbivores can be broadly placed within one of these two foraging groups (Zavada and Mentis 1992). Because of their large body size, all dinosaurian herbivores during the mid-Mesozoic most likely used the generalist foraging strategy. Selective herbivores at that time would have included insects, small tetrapods, and mammals. Generally, prosauropods, small ornithopods, and stegosaurs during the Late Jurassic, and ornithopods, early ceratopsians, and iguanodontids during the Early Cretaceous, had more of a selective foraging morphology than did sauropods (Dodson 1990, Norman and Weishampel 1985, Weaver 1983). However, given the large absolute size of all of these dinosaurian herbivores, the slight change in leaf nutritive quality observed in G. biloba in response to mid-Mesozoic-like atmospheric conditions (Chapter 3) would not have had an appreciable affect on their nutritional needs. This is because the nutritive content changes were due to increases in starch concentration rather than to decreases in essential N-bearing compounds, and the dinosaurs would therefore still have ingested the same amount of N-bearing compounds per bite as they would with foliage produced under present-day atmospheric conditions.

Increases in foliar C:N ratio like those observed in G. biloba in response to elevated carbon dioxide and oxygen atmospheres would most likely have had the strongest affect on the highly selective foragers of the mid-Mesozoic, such as insects.

These selective herbivores would have actively searched for plant parts of higher nutritive content (Scheirs et al. 2003, Yang and Joern 1994). But, due to their small bite size and the TNC-dilution of N-compounds in mid-Mesozoic foliage, they would not have been able to get the same amount of N-bearing compounds from this foliage as from foliage produced under present-day atmospheric conditions. A common herbivorous response to lower nutritive content is to eat more; the same amount of essential N-bearing nutrients can be gleaned from a greater amount of lower quality food as from less higher quality food (Campbell 1996, Cipollini et al. 2002, Dury et al. 1998, Scheirs et al. 2003, Yang and Joern 1994). The elevated primary productivity of the mid-Mesozoic (Chapter 2) would have provided a greater amount of plant tissue. This may have helped to relieve the nutritional situation for these selective herbivores.

The increase in foliar C:N ratio and starch concentration observed for G. biloba in response to atmospheric conditions like those during the mid-Mesozoic may help to explain the extremely large body sizes of dinosaurian herbivores at that time. Generalist foragers have larger bodies in part in order to house longer digestive tracts (Burness et al. 2001, Demment and Van Soest 1985, Geluso and Hayes 1999, Schluter 1984, Yang and Joern 1994). These longer digestive tracts allow food to be retained for longer periods, ensuring that the animal can digest a higher proportion of the material (Demment and Van Soest 1985, Keys et al. 1969). Additionally, larger mouths mean larger bite sizes that allow more food to be ingested per bite, and longer legs make moving from food patch to food patch more energetically efficient, ensuring a high ingestion rate (Ginnett and Demment 1995, Owen-Smith and Novellie 1982, Shipley et al. 1999). Therefore, larger

body size may be at least in part a response to the increase in foliar C:N ratio and starch concentration observed for G. biloba in response to the atmospheric conditions of the mid-Mesozoic.

Dinosaur Diet and Digestive Physiology.- Generally, dinosaur diets and digestive efficiency are reconstructed based on observations of teeth and gastroliths, and on coprolites (i.e. fossilized dung) and fossilized stomach contents. The nutritive content and digestibility results from G. biloba gas exchange experiments provide additional perspective to these lines of inquiry.

The structure and arrangement of herbivorous dinosaur teeth is often used to estimate the diet and digestive efficiency of dinosaurs (Farlow 1987, Norman and Weishampel 1985). Herbivores with complex dental batteries such as iguanodonts, hadrosaurines, and advanced ceratopsians most likely had relatively higher digestive efficiencies due to their ability to break food down orally. Dinosaur groups such as sauropods, thyreophorans, and most ornithopods, which had less extensive dental batteries, are not expected to have fully orally processed their food (Farlow 1987). Instead, they would have relied upon long digestive tracts with long residence times in order to ferment and chemically break down their food (Keys et al. 1969, Lundberg and Palo 1993), which may have translated into lower digestive efficiencies. Until recently, sauropods were thought to thoroughly macerate their food using gastroliths in the stomach or crop. However, recent observations suggest that many of the stones previously described as gastroliths may not be and were smoothed through stream

abrasion (Lucas 2000). If sauropods did not have gastroliths to process their food prior to digestion, they may have had extremely low digestive efficiencies.

However, the increased digestibility hypothesized for mid-Mesozoic plants based on experiments on G. biloba seedlings (Chapter 3) suggests that oral and gut processing of mid-Mesozoic food by sauropods may not have been as necessary as it would have been if they had been eating plants grown under present-day atmospheric conditions. Given the massive size of sauropods and their presumably long digestive tracts (Dodson 1990, McIntosh 1990, Sander 2000), sauropods may not have needed to chew their food or macerate it in a crop in order to digest it well enough to remove the essential nutrients, since all animals with long digestive tracts, including ectotherms, have high digestive efficiencies (Schluter 1984, Valido and Nogales 2003). The higher starch content of the mid-Mesozoic plants may have increased the digestibility of the foliage enough that maceration may not have been necessary, and a high gut residence time would have been adequate for sauropod digestion.

Coprolites and stomach contents of herbivorous dinosaurs are direct evidence for diet and digestive efficiency, assuming that the dinosaur that created the coprolite can be determined (Chin 1997a, 1997b). Animals that eat low fiber diets or have high digestive efficiencies tend to expel feces with very few identifiable remains, while there is a predominance of identifiable elements in fecal matter from animals with low digestive efficiencies or that eat high fiber diets. Coprolites with both high (Chin 1995) and low (Sharma et al. 2005) plant material concentration have been attributed to sauropods based on size, which may indicate differences in diet or digestive efficiency within this group.

The gut contents of an ankylosaur have also been described (Molnar and Clifford 2000). They are shown to consist of only 10% fibrous plant remains and seeds, which may indicate that ankylosaurs had high digestive efficiencies or a generally low fiber diet. The increase in digestibility of G. biloba in response to the elevated carbon dioxide and oxygen atmospheres of the mid-Mesozoic suggests that irrespective of diet choice or digestive physiology, mid-Mesozoic plants were more easily digested than modern plants (Chapter 3). Therefore, low-fibrous coprolites and stomach contents such as those observed for sauropods and ankylosaurs could indicate that these dinosaurs had high digestive efficiencies, given the more digestible food produced at that time.

Increases in mid-Mesozoic plant digestibility also would have increased the amount of food available to each dinosaurian herbivore. In highly digestible leaf material, more material per bite can be assimilated by the herbivore, effectively further increasing the amount of primary productivity that can be translated into secondary productivity (Demment and Van Soest 1985, Ginnett and Demment 1995). Given the extremely large size and hypothesized high metabolic rates of most mid-Mesozoic herbivorous dinosaurs, the greater energy content conferred to them from these more highly digestible foods would have helped to support their bulk and metabolism.

Decomposition Rates and Decomposers.- Decomposition rates are also affected by changes in nutritive content and digestibility of leaf material. During leaf senescence, leaf material is transferred to the ground where bacteria slowly digest it. Bacteria generally have very low C:N ratios, which they need to maintain by consuming litter (Rønn et al. 2003). Therefore, increased C:N ratios in ancient leaf litter may have slowed

decomposition over the long-term by reducing the nutrients available for microbial biomass (Williams et al. 2001). However, an accumulating body of research demonstrates that slight changes in C:N ratio and other leaf chemical parameters do not significantly affect the rate of decay under modern atmospheric compositions (Booker et al. 2005, Hall et al. 2006), which may be due to concurrent increases in digestibility, as was observed for G. biloba leaves. However, the difference in C:N ratio between the Control and the CO2 treatments (Chapter 3) is much larger than the differences found in other studies. Booker et al (2005), for instance, only observed an increase in C:N from 40 at 370 ppm carbon dioxide to 42 at 700 ppm, while an increase in G. biloba C:N ratio from 21 at 370 ppm carbon dioxide to 40 at 2000 ppm was observed. Given that the nutritive content difference reported in Chapter 3 is much greater than that observed in other studies, leaf litter produced under elevated carbon dioxide and oxygen atmospheres might have decomposed at a slower rate during the mid-Mesozoic than today.

However, changes in the digestibility of leaf litter can affect decomposition rates as well. Different compounds in leaf litter degrade at different rates. Decomposition follows a general sequence in the break-down of plant material (Goñi and Hedges 1990, Gupta and Pancost 2004, Hedges et al. 1985, Loranger et al. 2002). Leaching removes up to 50% of the organic matter in the form of proteins and soluble carbohydrates first (Benner et al. 1990). Polysaccharides follow, while cuticle and lignin are the most resistant to degradation. However, this sequence varies slightly in different environments (i.e. wet vs. dry, saline vs. fresh water) (e.g. Benner et al. 1990, Goñi and Hedges 1990).

Lignin can be broken down by the action of selected microbes (Loranger et al. 2002), but it is highly resistant to decay by others. The increase in digestibility observed in G. biloba in response to elevated carbon dioxide and oxygen atmospheres was due to an increase in starch, a polysaccharide, which might suggest an increase in litter decomposition rates during the mid-Mesozoic for plants exposed to elevated carbon dioxide and oxygen atmospheres, despite the fact that no differences in lignin were observed for G. biloba. Given that starch both decreased the nutritive value of leaves by increasing the C:N ratio, which potentially slowed decomposition rates, and increased the digestibility of the tissue, which potentially stimulated decomposition rates, the most likely effect of elevated carbon dioxide and oxygen on decomposition rates during the mid-Mesozoic is uncertain.

## Conclusions

The results of experiments examining photosynthetic rate, primary productivity, and the nutritive content and digestibility of Ginkgo biloba foliage in response to 35-day exposure to elevated carbon dioxide and oxygen atmospheric treatments may help to elucidate the ecology of the Late Jurassic and Early Cretaceous Periods worldwide, as atmospheric composition was highly enriched in both carbon dioxide and oxygen during those times. Potential effects of changes to the quantity and quality of mid-Mesozoic foliage include changes to plant physiological function and stimulation of primary productivity that would have supported large, active herbivores and large populations of

microbial decomposers. Additionally, decreases in nutritive content may help to explain the large body sizes of dinosaurian herbivores, while increases in digestibility may help to explain their apparently high digestive efficiencies even in the absence of gut or oral maceration.

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## References Cited

Allen, L., K. Boote, J. Jones, P. Jones, R. Valle, B. Acock, H. Rogers, and R. Dahlman. 1987. Response of vegetation to rising carbon dioxide: photosynthesis, biomass, and seed yield of soybean. Global Biogeochemical Cycles 1(1):1-14.

Apori, S., R. Long, F. Castro, and E. Ørskov. 2000. Chemical composition and nutritive value of leaves and stems of tropical weed Chromolaena odorata. Grass and Forage Science 55:77-81.

Appenzeller, T. 1994. Argentine dinos vie for heavyweight titles. Science 266:1805.

Arp, W. 1991. Effects of source-sink relations on photosynthetic acclimation to elevated $CO_2$. Plant, Cell and Environment 14:869-875.

Ash, S. 1999. An Upper Triassic upland flora from north-central New Mexico, U.S.A. Review of Palaeobotany and Palynology 105:183-199.

Awoyinka, A., V. Abegunde, and S. Adewush. 1995. Nutrient content of young cassava leaves and assessment of their acceptance as a green vegetable in Nigeria. Plant Foods for Human Nutrition 47:21-28.

Barrick, R., W. Showers, and A. Fischer. 1996. Comparison of thermoregulation of four Onithischian dinosaurs and a varanid lizard from the Cretaceous Two Medicine Formation: Evidence from oxygen isotopes. Palaios 11:295-305.

Basinger, J. 1997. Mesozoic Floras. Pp. 422-432. *In* P. Currie, and K. Padian, eds. Encyclopedia of Dinosaurs. Academic Press, New York.

Benner, R., K. Weliky, and J. Hedges. 1990. Early diagenesis of mangrove leaves in a tropical estuary: Molecular-level analyses of neutral sugars and lignin-derived phenols. Geochimica et Cosmochimica Acta 54(7):1991-2001.

Berner, R. 1998. The carbon cycle and $CO_2$ over Phanerozoic time: the role of land plants. Philosophical Transactions of the Royal Society of London B 353:75-82.

Berner, R. 2004. The Phanerozoic Carbon Cycle: $CO_2$ and $O_2$. Oxford University Press, Oxford.

Berner, R., D. Beerling, R. Dudley, J. Robinson, and R. Wildman. 2003. Phanerozoic Atmospheric Oxygen. Annual Review of Earth and Planetary Sciences 31:105-34.

Billings, S., S. Schaeffer, S. Zitzer, T. Charlet, S. Smith, and R. Evans. 2002. Alterations of nitrogen dynamics under elevated carbon dioxide in an intact Mojave Desert ecosystem: evidence from nitrogen-15 natural abundance. Oecologia 131:463-467.

Bond, W., W. Lee, and J. Craine. 2004. Plant structural defences against browsing birds: a legacy of New Zealand's extinct moas. Oikos 104(3):500-508.

Booker, F., S. Prior, A. Torbert, E. Fiscus, W. Pursley, and S. Hu. 2005. Decomposition of soybean grown under elevated concentrations of $CO_2$ and $O_3$. Global Change Biology 11:685-698.

Burness, G. 2002. Elephants, mice and red herrings. Science 296:1245-1247.

Burness, G., J. Diamond, and T. Flannery. 2001. Dinosaurs, dragons, and dwarfs: the evolution of maximal body size. PNAS 98(25):14518-14523.

Campbell, N. 1996. Biology. The Benjamin/Cummings Publishing Company, Inc., Menlo Park, California.

Cannell, M., and H. Hornley. 1998. N-poor ecosystems may respond more to elevated [$CO_2$] than N-rich ones in the long term. A model analysis of grassland. Global Change Biology 4:431-442.

Carpenter, K., T. DiCroce, D. Gilpin, B. Kinneer, F. Sanders, V. Tidwell, and A. Shaw. 2002. Origins of the Early and 'Middle' Cretaceous dinosaurs of North America: Implications for plate tectonics. Pp. 289-308. Proceedings of the International Symposium on New Concepts in Global Tectonics. Otero Junior College, La Junta, CO.

Centritto, M., H. Lee, and P. Jarvis. 1999. Increased growth in elevated [$CO_2$]: an early, short-term response? Global Change Biology 5:623-633.

Cheng, L., and L. Fuchigami. 2002. Growth of young apple trees in relation to reserve nitrogen and carbohydrates. Tree Physiology 22:1297-1303.

Chiappe, L., and R. Coria. 2004. Auca Mahuevo, an extroardinary Late Cretaceous sauropod dinosaur nesting site, Neuquen, Argentina. Ameghiniana 41(4):591-596.

Chin, K. 1995. The paleobiological implications of herbivorous dinosaur coprolite fabrics from the Two Medicine Formation, Montana. Journal of Vertebrate Paleontology 15(3, Supplement):27A.

Chin, K. 1997a. Coprolites. Pp. 147-150. *In* P. Currie, and K. Padian, eds. Encyclopedia of Dinosaurs. Academic Press, New York.

Chin, K. 1997b. What did dinosaurs eat? Coprolites and other direct evidence of dinosaur diets. Pp. 371-382. *In* J. Farlow, and M. Brett-Surman, eds. The Complete Dinosaur. Indiana University Press, Bloomington, IN.

Cipollini, M., E. Paulk, and D. Cipollini. 2002. Effect of nitrogen and water treatment on leaf chemistry in horsenettle (Solanum carolinense), and relationship to herbivory by flea beetles (Epitrix spp.) and tobacco horworm (Manduca sexta). Journal of Chemical Ecology 28(12):2377-2398.

Clemens, E. T., and G. M. O. Maloiy. 1982. The digestive physiology of three East African herbivores: the elephant, rhinoceros and hippopotamus. Journal of Zoology, London 198:141-156.

Comins, H., and R. McMurtrie. 1993. Long-term response of nutrient-limited forests to $CO_2$ enrichment: equillibrium behavior of plant-soil models. Ecological Applications 3(4):666-681.

Cotrufo, M., P. De Angelis, and A. Polle. 2005. Leaf litter production and decomposition in a poplar short-rotation coppice exposed to free air $CO_2$ enrichment (POPFACE). Global Change Biology 11:971-982.

Czier, Z. 1998. Ginkgo foliage from the Jurassic of the Carpathian Basin. Paleontology 41(part 2):349-381.

Dada, S., A. Akinsoyinu, J. Smith, and K. Dashiell. 2002. The effect of leaf-pruning on nutrient intake and in vivo digestibility of soybean stovers by sheep. Journal of Sustainable Agriculture 19(4):5-14.

Dal Sasso, C. 2003. Dinosaurs of Italy. Comptes Rendus Palevol 2:45-66.

Day, J., P. Upchurch, D. B. Norman, A. S. Gale, and H. P. Powell. 2002. Sauropod trackways, evolution, and behavior. Science 296(5573):1659-1659.

De Graaff, M.-A., J. Six, D. Harris, H. Blum, and C. Van Kessel. 2004. Decomposition of soil and plant carbon from pasture systems after 9 years of exposure to elevated $CO_2$: impact on C cycling and modeling. Global Change Biology 10:1922-1935.

Demment, M. W., and P. J. Van Soest. 1985. A nutritional explanation for body-size patterns of ruminant and nonruminant herbivores. The American Naturalist 125(5):641-672.

Dodson, P. 1990. Sauropod Paleoecology. Pp. 402-407. *In* D. Weishampel, P. Dodson, and H. Osmólska, eds. The Dinosauria. The University of California Press, Berkeley, CA.

Dury, S., J. Good, C. Perrins, A. Buse, and T. Kaye. 1998. The effects of increasing $CO_2$ and temperature on oak leaf palatability and the implications for herbivorous insects. Global Change Biology 4(1):55-61.

Erickson, G., K. Curry Rogers, and S. A. Yerby. 2001. Dinosaurian growth patterns and rapid avian growth rates. Nature 412:429-433.

Fader, G., and H. Koller. 1983. Relationships between carbon assimilation, partitioning, and export in leaves of two soybean cultivars. Plant Physiology 73:297-303.

Farlow, J. O. 1976. A consideration of the trophic dynamics of a Late Cretaceous large-dinosaur community (Oldman Formation). Ecology 57:841-857.

Farlow, J. O. 1987. Speculations about the diet and digestive physiology of herbivorous dinosaurs. Paleobiology 13(1):60-72.
Francis, J. 1988. A 50-million-year-old fossil forest from Strathcona Fiord, Ellesmere island, Arctic Canada: Evidence for a warm polar climate. Arctic 41(4):314-318.
Freeland, W. J., and D. H. Janzen. 1974. Strategies in herbivory by mammals: the role of plant secondary compounds. The American Naturalist 108(961):269-289.
Fricke, H., and R. Rogers. 2000. Multiple taxon-multiple locality approach to providing oxygen isotope evidence for warm-blooded theropod dinosaurs. Geology 28(9):799-802.
Fritschi, F., K. Boote, L. Sollenberger, and L. J. Allen. 1999. Carbon dioxide and temperature effects on forage establishment: tissue composition and nutritive value. Global Change Biology 5:743-753.
Gagnon, M., and A. Chew. 2000. Dietary preferences in extant African Bovidae. Journal of Mammalogy 81(2):490-511.
Geluso, K., and J. Hayes. 1999. Effects of dietary quality on basal metabolic rate and internal morphology of European Starlings (Sturnus vulgaris). Physiological and Biochemical Zoology 72(2):189-197.
Ginnett, T., and M. W. Demment. 1995. The functional response of herbivores: analysis and test of a simple mechanistic model. Functional Ecology 9:376-384.
Goñi, M., and J. Hedges. 1990. The diagenetic behavior of cutin acids in buried conifer needles and sediments from a coastal marine environment. Geochimica et Cosmochimica Acta 54(11):3083-3093.
Gupta, N., and R. Pancost. 2004. Biomolecular and physical taphonomy of angiosperm leaf during early decay: implications for fossilization. Palaios 19:428-440.
Guy, P. 1975. The daily food intake of the African elephant, Loxodonta africana Blumenbach, in Rhodesia. Arnoldia 7(26):1-8.
Hall, M., P. Stiling, D. Moon, B. Drake, and M. Hunter. 2006. Elevated $CO_2$ increases the long-term decomposition rate of Quercus myrtifolia leaf litter. Global Change Biology 12:568-577.
Hamerlynck, E., T. Huxman, R. Nowak, S. Redar, M. Loik, D. Jordan, S. Zitzer, J. Coleman, J. Seemann, and S. Smith. 2000. Photosynthetic responses of Larrea tridentata to a step-increase in atmospheric $CO_2$ at the Nevada Desert FACE Facility. Journal of Arid Environments 44:425-436.
Hanley, T. 1982. The nutritional basis for food selection by ungulates. Journal of Range Management 35(2):146-151.
Hanley, T., and K. Hanley. 1982. Food resource partitioning by sympatric ungulates on Great Basin rangeland. Journal of Range Management 35(2):152-158.
Hedges, J., G. Cowie, J. Ertel, R. Barbour, and P. Hatcher. 1985. Degradation of carbohydrates and lignins in buried woods. Geochimica et Cosmochimica Acta 49(3):701-711.
Horner, J. 1999. Egg clutches and embryos of two hadrosaurian dinosaurs. Journal of Vertebrate Paleontology 19(4):607-611.

Horner, J., A. de Ricqlés, and K. Padian. 2000. Long bone histology of the Hadrosaurid dinosaur Maiasaura peeblesorum: growth dynamics and physiology based on an ontogenetic series of skeletal elements. Journal of Vertebrate Paleontology 20(1):115-129.

Huber, S., H. Rogers, and D. Israel. 1984. Effects of $CO_2$ enrichment on photosynthesis and photosynthate partitioning in soybean (Glycine max) leaves. Physiologia Plantarum 62:95-101.

Keys, J. E., P. J. Van Soest, and E. P. Young. 1969. Comparative study of the digestibility of forage cellulose and hemicellulose in ruminants and nonruminants. Journal of Animal Science 29(1):11-15.

Kinnear, J., A. Cockson, P. Christensen, and A. R. Main. 1979. The nutritional biology of the ruminants and ruminant-like mammals -- a new approach. Comparative Biochemistry and Physiology 64A:357-365.

Kozlowski, T., and S. Pallardy. 2002. Acclimation and adaptive responses of woody plants to environmental stresses. The Botanical Review 68(2):270-334.

Krapp, A., and M. Stitt. 1995. An evaluation of direct and indirect mechanisms for the "sink-regulation" of photosynthesis in spinach: Changes in gas exchange, carbohydrates, metabolites, enzyme activities and steady-state transcript levels after cold-girdling source leaves. Planta 195:313-323.

Krauss, D. 2001. An analysis of the feeding habits of herbivorous dinosaurs through the examination of phytoliths trapped on tooth grinding surfaces. Journal of Vertebrate Paleontology 21(3, Supplement):69A.

Körner, C. 2003. Carbon limitation in trees. Journal of Ecology 91:4-17.

Lieth, H. 1973. Primary production: Terrestrial ecosystems. Human Ecology 1(4):303-332.

Likens, G. E. 1973. Primary Production: freshwater ecosystems. Human Ecology 1(4):347-356.

Lockley, M., A. Schulp, C. Meyer, G. Leonardi, and D. Mamani. 2002a. Titanosaurid trackways from the Upper Cretaceous of Bolivia: evidence for large manus, wide-gauge locomotion and gregarious behavior. Cretaceous Research 23(3):383-400.

Lockley, M., J. Wright, D. White, M. Matsukawa, L. Jianjun, F. Lu, and L. Hong. 2002b. The first sauropod trackways from China. Cretaceous Research 23(3):363-381.

Loranger, G., J.-F. Ponge, D. Imbert, and P. Lavelle. 2002. Leaf decomposition in two semi-evergreen tropical forests: influence of litter quality. Biology and Fertility of Soils 35:247-252.

Lucas, S. 2000. The Gastromyths of 'Seismosaurus,' a Late Jurassic dinosaur from New Mexico. Pp. 61-67. Dinosaurs of New Mexico: New Mexico Museum of Natural History and Science Bulletin.

Lundberg, P., and R. T. Palo. 1993. Resource use, plant defenses, and optimal digestion in ruminants. Oikos 68:224-228.

McIntosh, J. 1990. Sauropoda. Pp. 345-401. *In* D. Weishampel, P. Dodson, and H. Osmólska, eds. The Dinosauria. University of California Press, Berkeley, CA.

Miller, C., and C. LaPasha. 1984. Flora of the Early Cretaceous Kootenai Formation in Montana, conifers. Paleontographica Abt. B 193:1-17.
Milton, S., W. Dean, and W. Siegfried. 1994. Food selection by ostrich in southern Africa. Journal of Wildlife Management 58(2):234-248.
Molnar, R., and H. Clifford. 2000. Gut contents of a small ankylosaur. Journal of Vertebrate Paleontology 20(1):194-196.
Nealson, K. 1997. Sediment Bacteria: Who's there, what are they doing, and what's new? Annual Review of Earth and Planetary Sciences 25:403-434.
Norman, D. B., and D. B. Weishampel. 1985. Ornithopod feeding mechanisms: their bearing on the evolution of herbivory. The American Naturalist 126:151-164.
Owen-Smith, N., and P. Novellie. 1982. What should a clever ungulate eat? The American Naturalist 119(2):151-178.
Padian, K. 1997. Physiology. Pp. 552-556. *In* P. Currie, and K. Padian, eds. Encyclopedia of Dinosaurs. Academic Press, New York.
Padian, K., A. de Ricqlés, and J. Horner. 2001. Dinosaur growth rates and bird origins. Nature 412:405-408.
Rafferty, C., B. Lamont, and M. Hanley. 2005. Selective feeding by kangaroos (Macropus fuliginosus) on seedlings of Hakea species: Effects of chemical and physical defenses. Plant Ecology 177(2):201-208.
Reid, R. 1997. Dinosaurian physiology: the case for 'intermediate' dinosaurs. Pp. 449-473. *In* J. Farlow, and M. Brett-Surman, eds. The Complete Dinosaur. Indiana University Press, Bloomington, IN.
Retallack, G. 2002. Carbon dioxide and climate over the past 300 Myr. Philosophical Transactions of the Royal Society of London A 360:659-673.
Ricklefs, R. 2001. The Economy of Nature. W.H. Freeman and Company, New York.
Rogers, A., and D. Ellsworth. 2002. Photosynthetic acclimation of Pinus taeda (loblolly pine) to long-term growth in elevated $pCO_2$ (FACE). Plant, Cell and Environment 25:851-858.
Royer, D., R. Berner, and D. Beerling. 2001. Phanerozoic atmospheric $CO_2$ change: evaluating geochemical and paleobiological approaches. Earth-Science Reviews 54:349-392.
Royer, D., L. Hickey, and S. Wing. 2003. Ecological conservatism in the 'living fossil' Ginkgo. Paleobiology 29(1):84-104.
Rønn, R., F. Ekelund, and S. Christensen. 2003. Effects of elevated atmospheric $CO_2$ on protozoan abundance in soil planted with wheat and on decomposition of wheat roots. Plant and Soil 251:13-21.
Salawu, M., A. Adesogan, M. Fraser, R. Frychan, and R. Jones. 2002. Assessment of the nutritive value of whole crop peas and intercropped pea-wheat bi-crop forages harvested at different maturity stages for ruminants. Animal Feed Science and Technology 96:43-53.
Salgado, L., R. Coria, and L. Chiappe. 2005. Osteology of the sauropod embryos from the Upper Cretaceous of Patagonia. Acta Palaeontologica Polonica 50(1):79-92.

Sander, M. 2000. Long bone histology of the Tendaguru Sauropods: implications for growth and biology. Paleobiology 26(3):466-488.

Sander, P., N. Klein, E. Buffetaut, G. Cuny, V. Suteethorn, and J. Le Loeuff. 2004. Adaptive radiation in sauropod dinosaurs: bone histology indicates rapid evolution of giant body size through acceleration. Organisms Diversity & Evolution 4(3):165-173.

Scheirs, J., L. Bruyn, and R. Verhagen. 2003. Host nutritive quality and host plant choice in two grass miners: primary roles for primary compounds. Journal of Chemical Ecology 29(6):1373-1389.

Schluter, D. 1984. Body size, prey size and herbivory in the Galapagos lava lizard, Tropidurus. Oikos 43:291-300.

Scotese, C. 2002. Paleomap Project. www.scotese.com.

Seymour, R., and H. Lillywhite. 2000. Hearts, neck posture and metabolic intensity of sauropod dinosaurs. Proceedings of the Royal Society of London B 267:1883-1887.

Sharma, N., R. Kar, A. Agarwal, and R. Kar. 2005. Fungi in dinosaurian (Isisaurus) coprolites from the Lameta Formation (Maastrichtian) and its reflection on food habit and environment. Micropaleontology 51(1):73-82.

Shipley, L., A. Illius, K. Danell, N. Hobbs, and D. Spalinger. 1999. Predicting bite size selection of mammalian herbivores: a test of a general model of diet optimization. Oikos 84:55-68.

Sturm, A., and G.-Q. Tang. 1999. The sucrose-cleaving enzymes of plants are crucial for development, growth and carbon partitioning. Trends in Plant Science 4(10):401-407.

Tidwell, W. 1990. Preliminary report on the megafossil flora of the Upper Jurassic Morrison Formation. Hunteria 2(8):1-10.

Tiffney, B. H. 1997a. Land plants as food and habitat in the Age of Dinosaurs. Pp. 352-370. *In* J. Farlow, and M. Brett-Surman, eds. The Complete Dinosaur. Indiana University Press, Bloomington, IN.

Tiffney, B. H. 1997b. Plants and dinosaurs. Pp. 557-559. *In* P. Currie, and K. Padian, eds. Encyclopedia of Dinosaurs. Academic Press, New York.

Tralau, H. 1968. Evolutionary trends in the genus Ginkgo. Lethaia 1:63-101.

Valido, A., and M. Nogales. 2003. Digestive ecology of two omnivorous Canarian lizard species (Gallotia, Lacertidae). Amphibia-Reptilia 24:331-344.

Varricchio, D., and J. Horner. 1993. Hadrosaurid and Lambeosaurid bone beds from the Upper Cretaceous 2 Medicine Formation of Montana - taphonomic and biologic implications. Canadian Journal of Earth Science 30(5):997-1006.

Wardlaw, I. 1990. Tansley Review No. 27: The control of carbon partitioning in plants. New Phytologist 116:341-381.

Weaver, J. C. 1983. The improbable endotherm: the energetics of the sauropod dinosaur Brachiosaurus. Paleobiology 9(2):173-182.

Whittaker, R., and G. E. Likens. 1973. Primary production: the biosphere and man. Human Ecology 1(4):357-369.

Williams, J., W. Siegfried, S. Milton, N. Adams, W. Dean, M. Du Plessis, and S. Jackson. 1993. Field metabolism, water requirements, and foraging behavior of wild ostriches in the Namib. Ecology 74(2):390-404.

Williams, M., C. Rice, and C. Owensby. 2001. Nitrogen competition in a tallgrass prairie ecosystem exposed to elevated carbon dioxide. Soil Science Society of America Journal 65:340-346.

Wolfe, J., and G. Upchurch. 1987. North American nonmarine climates and vegetation during the Late Cretaceous. Palaeogeography, Palaeoclimatology, Palaeoecology 61:33-77.

Yang, Y., and A. Joern. 1994. Gut size changes in relation to variable food quality and body size in grasshoppers. Functional Ecology 8:36-45.

Zavada, M., and M. Mentis. 1992. Plant-animal interaction: the effect of Permian megaherbivores on the Glossopterid flora. The American Midland Naturalist 127(1):1-12.

Zhiliang, T., C. Huiping, and X. Tingxian. 1996. Comparative study on fibre characteristics of rye and wheat straws. AJAS 9(1):51-56.

Ziegler, A., J. Parrish, Y. Jiping, E. Gyllenhaal, D. Rowley, J. Parrish, N. Shangyou, A. Bekker, and M. Hulver. 1993. Early Mesozoic phytogeography and climate. Philosophical Transactions of the Royal Society of London B 341:297-305.

# CHAPTER 5:

## Applying experimental physiological results to the fossil record: Can the stomatal density and index of Ginkgo biloba provide a meaningful link between experimentally produced leaves and Ginkgo fossils?

Abstract.-

The possibility of using stomatal density (SD) and index (SI) values as proxies for photosynthetic and conductance rates was investigated in Ginkgoaceae leaves. SD and SI values in Ginkgo biloba exposed to elevated carbon dioxide and oxygen were measured and compared with photosynthetic and conductance rate data for the same leaves. SD and SI of the experimental leaves were also compared with SD and SI values for fossil Ginkgoaceae, including values from previously unexamined specimens and those reported in the literature. Elevated levels of carbon dioxide and oxygen did not affect SD or SI of G. biloba leaves, which was an unexpected result. Instantaneous net photosynthetic rate did not correlate with either SD or SI ($R^2$ values were 0.12 and 0.16, respectively), but instantaneous net conductance rate had an inverse correlation with both SD and SI ($R^2$ values were 0.43 and 0.44), suggesting that SD and SI cannot be used as proxies for photosynthetic rate, but are appropriate for the assessment of conductance rate. It was unclear how best to compare experimental and fossil SD and SI values. While SD and SI values correlated with conductance rate, SD and SI are not reliable proxies for gas exchange physiology of mid-Mesozoic Ginkgo.

## Introduction

Several lines of evidence suggest that atmospheric carbon dioxide and oxygen were extremely elevated during the Late Jurassic and Early Cretaceous (mid-Mesozoic) (Berner 2004). Mathematical modeling of the long-term carbon cycle (Berner 1998), stable carbon isotopes from paleosols and boron stable isotopic evidence from carbonates (Berner 2004), and stomatal index and stomatal ratio measurements (e.g. McElwain and Chaloner 1995, Retallack 2001, Royer et al. 2001b) suggest that mid-Mesozoic carbon dioxide levels were as high as 540% of modern day levels (2000 ppm vs. 370 ppm). Additionally, rock-abundance models (Berner 2001), isotope mass balance models (Berner et al. 2000), stable carbon isotope values from plants (Beerling et al. 2002a), and qualitative evidence from insect size and the frequency of fire in the stratigraphic record (McElwain and Chaloner 1995, Robinson 1989) suggest that oxygen levels may have reached 30% of the atmosphere during the mid-Mesozoic, i.e. 148% of the present-day level of 20.9%. The higher carbon dioxide and oxygen levels of the mid-Mesozoic may have affected the terrestrial ecosystems of that time through changes in primary productivity and the primary metabolite composition of the leaves.

Experimental studies have examined the photosynthetic rate and leaf quality responses of Ginkgo biloba seedlings to these enriched atmospheric compositions (Chapters 2 and 3). In response to carbon dioxide and oxygen elevated to the hypothesized mid-Mesozoic atmospheric compositions, G. biloba seedlings had photosynthetic rates 200-300% greater than control levels of both gasses (Chapter 2, Table 2). This extremely high photosynthetic rate also produced a high concentration of

foliar starch (Chapter 3, Table 2), which is expected to increase the digestibility of the leaves and diluted the concentration of proteins and nitrogen-based compounds (Chapter 3, Table 1). If mid-Mesozoic Ginkgo also had high photosynthetic rates and starch concentrations, the nutritive content of the foliage would have been reduced, particularly for arthropods (Chapter 4). The responses of G. biloba to experimental conditions suggest that elevated levels of carbon dioxide and oxygen potentially greatly affected mid-Mesozoic Ginkgo gas exchange rates, productivity, and foliage quality, assuming that the physiology of fossil Ginkgoaceae is similar to G. biloba.

The purpose of the current paper is to explore the use of stomatal density (SD = # stomata / $mm^2$ on the leaf epidermis) and stomatal index (SI = SD / ((# of epidermal cells/ $mm^2$) + SD)) measurements as morphological proxies for photosynthetic rate and conductance rate in fossil leaves. Do G. biloba SD and SI values reflect strongly elevated levels of carbon dioxide and oxygen? Can SD and SI values be used to estimate photosynthetic or conductance rate in a G. biloba leaf? If so, can photosynthetic or conductance rates be estimated in shed fossil Ginkgo leaves?

Both SD and SI are inversely proportional to atmospheric carbon dioxide concentration in a wide range of tree and herb species (e.g. Woodward 1987), at least at levels below 370 ppm (Beerling and Royer 2002b, Royer 2001), so changes in SD and SI are expected in response to elevated carbon dioxide. SD and SI also affect photosynthesis, conductance, and water status (Farquhar and Sharkey 1982, Jarvis et al. 1999, Pons and Welschen 2003, Wilson et al. 2000). As characteristics of the cuticle, SD and SI can be measured in well-preserved compression fossils (Kerp 2002, McElwain

and Chaloner 1996). SD and SI have the potential to be proxies for photosynthetic rate in fossil Ginkgo leaves.

However, Ginkgo SD and SI are influenced by many variables. Factors that affect leaf shape and size, such as water status and light level, can affect the spacing between stomata (Lake et al. 2001, Poole et al. 1996, Royer 2001). Hence, SD, which is an area-based parameter, is affected more by changes in leaf area than is SI (Beerling and Royer 2002b). Variation in SD and SI within a single leaf (Poole et al. 2000, Poole et al. 1996), among leaves on the same plant (Chen et al. 2001), and among plants (McElwain 1998, McElwain and Chaloner 1996) is considerable. Variations in SD and SI may be due to growth environment (Sun et al. 2003), genetic differences (Beerling and Royer 2002b, Royer 2001), or to a combination (Morison 1998). Additionally, in trees that have two shoot morphologies, such as G. biloba, leaves on long shoots (the bilobed leaf form from new growing shoots and seedlings) generally have different SD and SI values than leaves on short shoots (fan-shaped leaves from second-year shoots) (Chen et al. 2001). All of these factors can make interpretation of SD and SI data in extant and fossil leaves problematic (McElwain and Chaloner 1996, Poole et al. 1996, Royer 2001).

Despite these complicating factors, stomatal frequency has been used to estimate water-use-efficiency and photosynthetic rate in both extant and fossil plants (Beerling 1994, 1996, Jarvis et al. 1999, Morison 1998, Poole et al. 2000), and SI values from compression fossils have been used to calculate past atmospheric carbon dioxide concentrations (Beerling et al. 2002b, Beerling and Royer 2002b, Chen et al. 2001, Kouwenberg et al. 2005, Malone et al. 1993, Retallack 2001, Royer et al. 2001c,

Woodward and Bazzaz 1988). Most of these analyses have been performed using techniques that minimize the errors present in SD and SI measurement. These include sampling a wide range of fossil specimens to obtain general trends irrespective of genetic and individual variation (McElwain and Chaloner 1995), sampling extensively over the leaf surface to control for within-leaf variation (Poole et al. 2000), and the use of a stomatal ratio (SR), which controls for genetic differences between fossil and extant leaves by comparing fossil leaf SI values with SI values from extant relatives of the fossil specimens (Chen et al. 2001, McElwain 1998).

In the current study, SD and SI values were determined from G. biloba leaves during experimentation (Chapter 2), and the differences among treatments were assessed. These data were compared with photosynthetic and conductance rate data for the same leaves in order to investigate the relationship between gas exchange and stomatal characteristics of G. biloba leaves. SD and SI values of these samples were also compared with previously reported mid-Mesozoic fossil Ginkgo SD and SI values and additional SD and SI values from previously unexamined Ginkgo fossils. These comparisons assessed the feasibility of using SD and SI as proxies for photosynthetic and conductance rate data in fossil Ginkgo specimens.

## Methods

### Plant Material

Extant Plants.- Ginkgo biloba seedlings were obtained as described in Chapter 2 (experimental dataset).

Fossil Specimens.- SD and SI values reported in the literature (Table 1) and collected from three previously unexamined G. adiantoides fossils of Late Cretaceous age (Tables 2 and 4) were compared with the experimental data. The previously unexamined fossils were obtained from the Royal Tyrrell Museum of Paleontology (RTMP). Specimen numbers, ages, and source geologic formations are reported in Table 2. Specimens chosen for measurement were well-preserved with an obvious imprint and cuticle.

## Experimentation

Experimental Set Up.- Experiments were performed as described in Chapter 2 (35-Day Experiment). In these experiments, leaf age and position, water status, light level, and nutrient status were held constant among trees and treatments. There were non-quantified genetic and gender differences, as seedlings were grown from seed instead of grafted (Flynn 2006). Gender in Ginkgo cannot be determined with certainty until the tree reaches sexual maturity at around 30 years of age (Hara 1997); all seedlings were 1-2 years old at time of experimentation.

Gas Exchange Parameters.- Photosynthetic rate and conductance rate data were collected from G. biloba seedlings in response to acclimation (treatment) conditions. Data collection was performed as described in Chapter 2.

Leaf Preparation

Experimental Leaves.- One leaf per experimental tree (=6 leaves per treatment/block combination) was prepared for SD and SI measurement after the conclusion of experimental acclimation. This leaf was the same leaf as had been used for photosynthetic rate measurement (Chapter 2). Rationale for leaf choice is explained in Chapter 2. Impressions of the abaxial surface of leaves were made using clear nail polish. The nail polish peels were placed on slides for microscopic observation. The right half of the abaxial leaf surface was peeled preferentially. Peels stretched from the base of the leaf blade (just above the petiole) to the margin and ranged from 0.5-2.0 cm in width.

Fossil Leaves.- Fossil cuticle was removed with a dissecting needle. The opaque cuticle was bleached using 20% chromium trioxide (Alvin and Boulter 1974). The cuticle pieces were submerged in chromium trioxide solution, which was exchanged as it was used up (black color) for up to 96 hours. Once the bleaching was complete, the now translucent cuticle was thoroughly rinsed with water to remove any remaining chromium trioxide.

For microscope slide mounting, bleached cuticle was transferred through dilution and repeated pipetting from water to alcohol to Hemo-D solvent. Cuticle pieces were then removed from solution with a dissecting needle, placed in a drop of Canada Balsam (Fisher Scientific International Inc., Hampton, NH) on a microscope slide, and covered with a cover slip. Slides were placed on a warming block for six weeks to cure the Canada Balsam. One to four slides were made per specimen, depending on the volume of cuticle recovery.

SD and SI Measurement

Digital photographs of experimental and fossil cuticle were taken with an Olympus DP 70 digital camera through an optical microscope using a 20x objective lens. Field of view for each photograph was 0.29 $mm^2$. SD and SI were measured by marking and counting individual epidermal cells and stomata using ImageJ image analysis software, version 1.32j (available for download at http://rsb.info.nih.gov/ij/; developed by the National Institute of Health, Bethesda, MD).

SD and SI values were calculated using the following equations:

$$SD = \#\ \text{stomata} / \text{area in } mm^2$$

$$SI = SD / ED + SD$$

$$\text{where } ED = \#\ \text{epidermal cells} / \text{area in } mm^2$$

SI values were used to calculate predicted carbon dioxide concentrations using a Ginkgo-specific inverse regression equation used by Royer et al (2001c):

$$CO_2 = [1 - (0.1564 * SI)] / [0.00374 - (0.0005485 * SI)]$$

The regression curve described by this equation is hyperbolic with an asymptote that approaches SI = 7%.

SD and SI of experimental leaves were measured using an even sampling procedure in which six fields per slide (two fields at the base of the leaf, two in the middle, and two near the margin) were counted. Unfortunately fossil cuticle pieces were not separated by leaf location prior to bleaching and slide mounting. Therefore, the several pieces of cuticle on a single fossil slide were grouped by slide to ensure a

relatively even sampling distribution per fossil leaf since all leaves but one produced more than one slide. This sampling strategy is analogous to the one used for the experimental leaves. Three counts were made from each slide, resulting in a total of 12 counts per fossil.

Statistical Analysis

Experimental SD and SI data were analyzed for among treatment differences using using SAS Statistical Software (Cary, NC). Models were fit using proc GLM (ANOVA). Among treatment differences were assessed using Fisher's Least Significant Difference t-tests (means statement), and significance was determined at $p \leq 0.05$. Gas exchange data and SD and SI data were analyzed for correlation using proc GLM to calculate $R^2$ values.

Qualitative comparisons were made between the fossil and experimental datasets. Ginkgo SD and SI values from the literature and previously unexamined fossils were plotted by geologic age, which allowed comparison of SD and SI values based on atmospheric composition change over geologic time (see Fig. 2 a and b for further explanation). G. biloba SD and SI values were plotted according to the acclimation level of carbon dioxide under which the leaves developed. SD and SI values from leaves that developed under 2000 ppm were therefore plotted between 100 and 175 mya, during the Late Jurassic to Early Cretaceous, when atmospheric carbon dioxide levels most likely reached 2000 ppm. Control SD and SI values, that were produced under 370 ppm carbon

dioxide, were plotted at 0 mya, because present-day conditions include 370 ppm atmospheric carbon dioxide.

## Results

Experimental Leaves.- Experimental SD and SI data were highly variable, both within and among treatments, and were not significantly different among treatments (Table 3). Independent measurements of both parameters were highly variable within a single leaf and among different leaves within the same treatment / block combination. After averaging (all 6 measurements per leaf, and then 6 leaves per treatment), the amount of variability in both parameters was reduced. SD treatment means ranged from 68.4 pores•$mm^{-2}$ to 75.08 pores•$mm^{-2}$, while SI treatment means ranged from 9.05% to 9.69%. Predicted carbon dioxide values for the experimental samples all fell between 330 ppm and 340 ppm, despite their exposure to 2000 ppm carbon dioxide in the CO2 and CO2&O2 treatments.

Gas Exchange Data and Relationship with SD and SI.- Photosynthetic rate and conductance rate data are reported in Chapter 2 (Table 2). The relationships between these data and SD and SI experimental data are shown in Fig. 1. Neither photosynthetic nor conductance rates correlated strongly with either SD or SI, although conductance had a better correlation, with $R^2$ values of 0.41 and 0.42 for SD and SI, respectively, as opposed to $R^2$ values of 0.13 and 0.16 for photosynthetic rate. Conductance rate had an inverse relationship with both SD and SI, while photosynthetic rate had an inverse relationship only with SD.

Fossil Leaves.- SD values from the n=3 Late Cretaceous fossils measured were all similar in value, ranging from 53.89 pores•mm$^{-2}$ to 72.25 pores•mm$^{-2}$ (Table 4). SI values ranged from 5.67 to 8.20%. Carbon dioxide concentrations calculated from Late Cretaceous fossil SI values ranged from 180 ppm carbon dioxide to 468 ppm carbon dioxide, with an average value of 311 ppm.

Comparison of SD and SI Between Extant and Fossil Leaves.- Literature values of SD and SI spanned the entirety of the Jurassic and Cretaceous (Fig. 2a-b), and included some SD and SI values from present-day G. biloba. However, the majority of literature reports of Ginkgo were from the Cretaceous/Tertiary boundary (~65mya) and the earliest Tertiary (55mya), while Late Cretaceous (65-75 mya) values were especially rare. Since the 3 fossils measured in the current study are of Late Cretaceous age, they partially filled in the Late Cretaceous gap in the SD and SI record.

SD values from the literature and fossil datasets were widely ranging in value (Fig. 2a) and did not overlap with one another appreciably. SD values from the experimental leaves fell within the range of the values from the other datasets. SI values in the literature extended over a longer span of time (Fig. 2b). Fossil SI measurements correlated well with literature values. SI values from the experimental dataset were higher than literature values for the Late Jurassic and Early Cretaceous periods.

## Discussion

### SD and SI Responses to Elevated Carbon Dioxide and Oxygen

Neither elevated atmospheric carbon dioxide nor elevated atmospheric oxygen affected G. biloba SD and SI values. A well-documented inverse relationship between stomatal frequency and carbon dioxide concentration exists (Beerling and Royer 2002b, Woodward and Bazzaz 1988); it was reasonable to expect a response to the carbon dioxide levels examined here. Additionally, both carbon dioxide and oxygen have a noticeable effect on photosynthetic rate (Chapter 2) (Haupt-Herting and Fock 2002), which partially affects SD and SI (Igamberdiev et al. 2004, Marek et al. 1995). Therefore, the effect of both carbon dioxide and oxygen concentrations on photosynthetic rate should have been reflected in the SD and SI values. However, it seems that SD and SI are less responsive to acclimation gas composition than is photosynthetic rate. There are several possible explanations for the lack of stomatal response to both carbon dioxide and oxygen. These include: 1) the high levels of carbon dioxide used in the experiments, 2) the timing of SD and SI initiation during Ginkgo leaf development, and 3) the possibility that Ginkgo SD and SI responses are genetically, rather than environmentally, controlled.

Explanation 1.- while carbon dioxide is inversely related to both SD and SI, the relationship is non-linear. Beerling and Royer (2002b, fig. 2b) observed that the relationship between carbon dioxide and SI is linear only at sub-ambient carbon dioxide levels (~280-370 ppm). Above ~370 ppm, Ginkgo SI approaches an asymptote around 7%. Elevated carbon dioxide in the current experimental study was over twice as high as

the highest value in Beerling and Royer (2002a), and even the Control carbon dioxide concentration had short excursions above 370 ppm. Therefore, the lack of among-treatment differences in SD and SI in response to carbon dioxide could be explained by the fact that experimental atmospheric carbon dioxide levels were outside of the narrow range at which stomata are sensitive to them.

SD and SI responses to elevated atmospheric oxygen have not been studied previously, so the lack of response to the conditions examined here cannot be described as unexpected. However, the high levels of carbon dioxide used in this study could have masked or hidden responses of SD and SI to elevated oxygen, given that plants respond more strongly to carbon dioxide than they do to oxygen (Chapter 2) and the experiments used a larger increase in carbon dioxide levels (370 to 2000 ppm, an increase of 540%) than in oxygen levels (210,000 to 300,000 ppm, an increase of 148%). However, the fact remains that elevated oxygen did not affect the values of G. biloba SD or SI.

Explanation 2.- The lack of SD or SI response to elevated carbon dioxide and oxygen may have also been the result of leaf developmental physiology. In the model plant Arabidopsis thaliana, stomatal characteristics in developing leaves are determined by the conditions experienced by mature leaves (Lake et al. 2001, Lake et al. 2002). Developmental signals sent from the mature to the developing leaves largely determine the stomatal patterning on the epidermis of the new leaves (Brownlee 2001). In the current study, plants were exposed to experimental conditions during dormancy prior to budbreak when no mature leaves existed to send signals to the developing leaves. If the mechanism for epidermal cell patterning in G. biloba is similar to that in Arabidopsis, G.

biloba SD and SI values may have been determined during budset prior to experimentation. If this was the case, SD and SI for all treatments were actually determined by present-day ambient atmospheric compositions, rather than by experimental atmospheric conditions. Beerling et al (1998) exposed G. biloba seedlings to 560 ppm carbon dioxide continuously for three years and observed a significant decrease in both SD and SI, which may suggest that stomatal spacing is determined in Ginkgo during budset, rather than during budbreak. Further experimentation is required to resolve this issue. Specifically, experiments that compare G. biloba SD and SI responses between leaves that are produced after seedling exposure to elevated carbon dioxide during budset and leaves exposed to ambient carbon dioxide during budset, would resolve this question.

Explanation 3.- It has been hypothesized that leaf SD and SI values are genetically, rather than environmentally, determined (Royer 2001). This hypothesis arose out of the observation that in experimental growth chamber studies, SD and SI values from extant plants have been as likely to increase (Poole et al. 2000) or not change in response to elevated carbon dioxide (Kouwenberg et al. 2003, Körner 1988, Malone et al. 1993) as they are to have the expected decrease (Beerling et al. 1998, Woodward and Bazzaz 1988). However, in studies that examined fossils or herbarium specimens, SD and SI had a consistently inverse relationship with carbon dioxide concentration (Beerling and Kelly 1997, Beerling et al. 2002b, McElwain and Chaloner 1995, Retallack 2001, Royer 2001, Woodward 1987). Therefore, it may be that the SD and SI values of

G. biloba did not respond to 2000 ppm carbon dioxide because the acclimation period was not long enough to affect these changes.

Relationship Between Gas Exchange and Stomatal Frequency

G. biloba SD and SI values correlated with instantaneous conductance rate data measured after acclimation to one of three atmospheric treatments (Chapter 2). Conductance rate is determined by stomatal frequency and the width of the stomatal aperture (Farquhar and Sharkey 1982, Forseth et al. 1984, Nobel 1980), and responds dynamically to environmental and physiological stimuli. Therefore, conductance is partially determined by SD and SI (Drake et al. 1997). This relationship suggests that SD and SI can be used to estimate the water loss potential of a shed extant or fossil leaf (see Beerling et al. 2001, Kenrick 2001, Osborne et al. 2004).

G. biloba SD and SI values and instantaneous net photosynthetic rate responses to one of three atmospheric treatments (Chapter 2) did not correlate, however. This lack of relationship can be explained by the fact that photosynthetic rate can operate somewhat independently of stomatal frequency and aperture width in plants irrespective of photosynthetic pathway and taxonomy (Gibson 1998, Odening et al. 1974, Saralabai et al. 1997, Wilson et al. 2000). SD and SI affect photosynthetic rate over the long term since carbon dioxide must diffuse through the stomata, and low leaf SD or SI can limit photosynthesis by limiting carbon dioxide diffusion into the leaf (Beerling and Woodward 1997, Norby et al. 1999). On a minute-by-minute basis, photosynthetic rate is further limited by stomatal aperture width (i.e. instantaneous conductance rate), which

can respond to environmental and physiological parameters dynamically (Odening et al. 1974, Saralabai et al. 1997). However, while photosynthetic rate may be limited by conductance through SD, SI, and stomatal aperture width, it is to some extent independent of these parameters (Drake et al. 1997). For example, even a reduced stomatal frequency and narrow stomatal aperture may not reduce photosynthetic rate if atmospheric carbon dioxide concentration is elevated, because of the presence of carbon dioxide within the leaf pore spaces (Ogle and Reynolds 2002, Zhang and Marshall 1995). Therefore, the lack of relationship between stomatal frequency and photosynthetic rate, especially in G. biloba leaves exposed to 370 ppm to 2000 ppm carbon dioxide concentrations, is not surprising, since photosynthesis can operate largely independently of SD and SI (Drake et al. 1997, Saralabai et al. 1997). The lack of relationship between photosynthesis and SD and SI demonstrates that SD and SI are not appropriate as proxies for photosynthetic rate in either shed or fossil leaves.

Comparison of SD and SI Among Extant and Fossil Leaves.- SD and SI values from G. biloba exposed to 370 ppm carbon dioxide correlated well with literature values from present-day G. biloba leaves. However, SD and SI values from leaves that had been exposed to 2000 ppm carbon dioxide were different from mid-Mesozoic Ginkgo literature values. This was most likely due the fact that G. biloba SD and SI values did not respond to elevated carbon dioxide during experimentation, while the SD and SI values of ancient Ginkgoaceae responded to changes in carbon dioxide over millions of years of atmospheric change (Beerling 2002, McElwain et al. 1999, Retallack 2001, Royer et al. 2001c, Rundgren and Beerling 1999). The stomatal frequency response in

plants to elevated carbon dioxide is most likely at least in part a genetic response (Royer 2001). SD and SI responses to gaseous composition change have been observed in G. biloba after exposure over multiple growing seasons (Beerling et al. 1998). Therefore, in order to gather data that would allow SD and SI to be used as proxies for photosynthetic rate comparison between extant G. biloba and fossil Ginkgo leaves, longer experiments are needed. Atmospheric acclimation periods would need to last for multiple years, or at least longer than the 35-day acclimation period employed in the current experiment.

## Conclusions

The stomatal frequency (SD and SI) responses of G. biloba leaves produced under exposure to 2000 ppm carbon dioxide and 30% oxygen were examined. Despite the well-known inverse relationship between carbon dioxide and stomatal frequency, neither SD nor SI responded to either elevated carbon dioxide or oxygen. These SD and SI values were compared with photosynthetic and conductance rate data measured on the same leaves during experimental treatment in order to assess relationships between gas exchange and stomatal frequency parameters. A correlation between G. biloba conductance rate and SD or SI values was observed, but no relationship was observed between photosynthetic rate and SD or SI. SD and SI values of experimental G. biloba and Ginkgoaceae leaves were also compared in order to assess the possibility of applying experimental G. biloba gas exchange results to the fossil record. The lack of observed SD and SI response to elevated carbon dioxide and oxygen, the presence of only a weak correlation between photosynthetic and conductance rates and SD and SI values, and the

lack of strong similarity between SD and SI values from fossil and extant specimens demonstrate that for now SD and SI are inappropriate proxies for Ginkgo photosynthetic rate in fossil Ginkgoaceae. In the future, experiments of longer duration may find that SD and SI can be used in this manner.

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## References Cited

Alvin, K. L., and M. C. Boulter. 1974. A controlled method of comparative study for Taxodiaceous leaf cuticles. Botanical Journal of the Linnean Society 69:277-286.

Beerling, D. 1994. Predicting leaf gas exchange and $\delta^{13}C$ responses to the past 30000 years of global environmental change. New Phytologist 128(3):425-433.

Beerling, D. 1996. Ecophysiological responses of woody plants to past $CO_2$ concentrations. Tree Physiology 16:389-396.

Beerling, D. 2002. Low atmospheric $CO_2$ levels during the Permo-Carboniferous glaciation inferred from fossil lycopsids. PNAS 99(20):12567-12571.

Beerling, D., and D. Jolley. 1998. Fossil plants record an atmospheric $^{12}CO_2$ and temperature spike across the Palaeocene-Eocene transition in NW-Europe. Journal of the Geological Society, London 155:591-594.

Beerling, D., and C. Kelly. 1997. Stomatal density responses of temperate woodland plants over the past seven decades of $CO_2$ increase: a comparison of Salisbury (1927) with contemporary data. American Journal of Botany 84(11):1572-1583.

Beerling, D., J. Lake, R. Berner, L. Hickey, D. Taylor, and D. Royer. 2002a. Carbon isotope evidence implying high $O_2/CO_2$ ratios in the Permo-Carboniferous atmosphere. Geochimica et Cosmochimica Acta 66(21):3757-3767.

Beerling, D., B. Lomax, D. Royer, G. Upchurch, and L. Kump. 2002b. An atmospheric $pCO_2$ reconstruction across the Cretaceous-Tertiary boundary from leaf megafossils. PNAS 99(12):7836-7840.

Beerling, D., J. McElwain, and C. Osborne. 1998. Stomatal responses of the 'living fossil' Ginkgo biloba L. to changes in atmospheric $CO_2$ concentration. Journal of Experimental Botany 49(326):1603-1607.

Beerling, D., C. Osborne, and W. Chaloner. 2001. Evolution of leaf-form in land plants linked to atmospheric $CO_2$ decline in the Late Paleozoic era. Nature 410:352-354.

Beerling, D., and D. Royer. 2002a. Fossil Plants as indicators of the Phanerozoic global carbon cycle. Annual Review of Earth and Planetary Sciences 30:527-556.

Beerling, D., and D. Royer. 2002b. Reading a $CO_2$ signal from fossil stomata. New Phytologist 153:387-397.

Beerling, D., and F. Woodward. 1997. Changes in land plant function over the Phanerozoic: reconstructions based on the fossil record. Botanical Journal of the Linnean Society 124:137-153.

Berner, R. 1998. The carbon cycle and $CO_2$ over Phanerozoic time: the role of land plants. Philosophical Transactions of the Royal Society of London B 353:75-82.

Berner, R. 2001. Modeling atmospheric $O_2$ over Phanerozoic time. Geochimica et Cosmochimica Acta 65(5):685-694.

Berner, R. 2004. The Phanerozoic Carbon Cycle: $CO_2$ and $O_2$. Oxford University Press, Oxford.

Berner, R., D. Beerling, R. Dudley, J. Robinson, and R. Wildman. 2003. Phanerozoic Atmospheric Oxygen. Annual Review of Earth and Planetary Sciences 31:105-34.

Berner, R., S. Petsch, J. Lake, D. Beerling, B. Popp, R. Lane, E. Laws, M. Westley, N. Cassar, F. Woodward, and W. Quick. 2000. Isotope fractionation and atmospheric oxygen: Implications for Phanerozoic $O_2$ evolution. Science 287:1630-1633.

Brownlee, C. 2001. The long and the short of stomatal density signals. TRENDS in Plant Science 6(10):441-442.

Chen, L.-Q., C.-S. Li, W. Chaloner, D. Beerling, Q.-G. Sun, M. Collinson, and P. Mitchell. 2001. Assessing the potential for the stomatal characters of extant and fossil Ginkgo leaves to signal atmospheric change. American Journal of Botany 88(7):1309-1315.

Drake, B., M. González-Meler, and S. Long. 1997. More efficient plants: a consequence of rising atmospheric $CO_2$? Annual Review of Plant Physiology and Plant Molecular Biology 48:609-639.

Farquhar, G., and T. Sharkey. 1982. Stomatal conductance and photosynthesis. Annual Review of Plant Physiology 33:317-345.

Flynn, C. 2006. Personal communication: Ginkgo seedling cultivation at Musser Forests, Inc.

Forseth, I., J. Ehleringer, K. Werk, and C. Cook. 1984. Field water relations of Sonoran Desert annuals. Ecology 65(5):1436-1444.

Gibson, A. 1998. Photosynthetic organs of desert plants. BioScience 48(11):911-920.

Hara, N. 1997. Morphology and Anatomy of Vegetative Organs in Ginkgo biloba. *In* T. Hori, ed. Ginkgo biloba, a global treasure: from biology to medicine. Springer, New York.

Haupt-Herting, S., and H. Fock. 2002. Oxygen exchange in relation to carbon assimilation in water-stressed leaves during photosynthesis. Annals of Botany 89:851-859.

Igamberdiev, A., T. Mikkelson, P. Ambus, H. Bauwe, P. Lea, and P. Gardestrom. 2004. Photorespiration contributes to stomatal regulation and carbon isotope fractionation: a study with barley, potato, and Arabidopsis plants deficient in glycine decarboxylase. Photosynthesis Research 81:139-152.

Jarvis, A., T. Mansfield, and W. Davies. 1999. Stomatal behavior, photosynthesis and transpiration under rising $CO_2$. Plant, Cell and Environment 22:639-648.

Kenrick, P. 2001. Turning over a new leaf. Nature 410:309-310.

Kerp, H. 2002. Atmospheric $CO_2$ from fossil plant cuticles. Nature 415:38-39.

Kouwenberg, L., J. McElwain, W. Kurschner, F. Wagner, D. Beerling, F. Mayle, and H. Visscher. 2003. Stomatal frequency adjustment of four conifer species to historical changes in atmospheric $CO_2$. American Journal of Botany 90(4):610-619.

Kouwenberg, L., R. Wagner, W. Kurschner, and H. Visscher. 2005. Atmospheric $CO_2$ fluctuations during the last millenium reconstructed by stomatal frequency analysis of Tsuga heterophylla needles. Geology 33(1):33-36.

Körner, C. 1988. Does global increase in $CO_2$ alter stomatal density? Flora 181:253-257.

Lake, J., W. Quick, D. Beerling, and F. Woodward. 2001. Signals from mature to new leaves. Nature 411:154.

Lake, J., F. Woodward, and W. Quick. 2002. Long-distance $CO_2$ signalling in plants. Journal of Experimental Botany 53(367):183-193.

Malone, S., H. Mayeux, H. Johnson, and H. Polley. 1993. Stomatal density and aperture length in four plant species grown across a subambient $CO_2$ gradient. American Journal of Botany 80(12):1413-1418.

Marek, M., J. Kalina, and M. Matouskova. 1995. Response of photosynthetic carbon assimilation of Norway spruce exposed to long-term elevation of $CO_2$ concentration. Photosynthetica 31(2):209-220.

McElwain, J. 1998. Do fossil plants signal palaeoatmospheric $CO_2$ concentration in the geologic past? Philosophical Transactions of the Royal Society of London B 353:83-96.

McElwain, J., D. Beerling, and F. Woodward. 1999. Fossil plants and global warming at the Triassic-Jurassic boundary. Science 285:1386-1390.

McElwain, J., and W. Chaloner. 1995. Stomatal density and index of fossil plants track atmospheric carbon dioxide in the Palaeozoic. Annals of Botany 76:389-395.

McElwain, J., and W. Chaloner. 1996. The fossil cuticle as a skeletal record of environmental change. Palaios 11:376-388.

Morison, J. 1998. Stomatal response to increased $CO_2$ concentration. Journal of Experimental Botany 49(Special Issue):443-452.

Nobel, P. 1980. Water vapor conductance and $CO_2$ uptake for leaves of a $C_4$ desert grass, Hilaria rigida. Ecology 61(2):252-258.

Norby, R., S. Wullschleger, C. Gunderson, D. Johnson, and R. Ceulemans. 1999. Tree responses to rising $CO_2$ in field experiments: implications for the future forest. Plant, Cell and Environment 22:683-714.

Odening, W., B. Strain, and W. Oechel. 1974. The effect of decreasing water potential on net $CO_2$ exchange of intact desert shrubs. Ecology 55:1086-1095.

Ogle, K., and J. Reynolds. 2002. Desert dogma revisited: coupling of stomatal conductance and photosynthesis in the desert shrub, Larrea tridentata. Plant, Cell and Environment 25:909-921.

Osborne, C., D. Beerling, B. Lomax, and W. Chaloner. 2004. Biophysical constraints on the origin of leaves inferred from the fossil record. PNAS 101(28):10360-10362.

Pons, T., and R. Welschen. 2003. Midday depression of net photosynthesis in the tropical rainforest tree Eperua grandiflora: contributions of stomatal and internal conductances, respiration and Rubisco functioning. Tree Physiology 23:937-947.

Poole, I., T. Lawson, D. Weyers, and J. Raven. 2000. Effect of elevated $CO_2$ on the stomatal distribution and leaf physiology of Alnus glutinosa. New Phytologist 145:511-521.

Poole, I., J. Weyers, T. Lawson, and J. Raven. 1996. Variations in stomatal density and index: implications for palaeoclimatic reconstructions. Plant, Cell and Environment 19:705-712.

Retallack, G. 2001. A 300-Million year record of atmospheric carbon dioxide from fossil plant cuticles. Nature 411:287-290.

Robinson, J. 1989. Phanerozoic $O_2$ variation, fire, and terrestrial ecology. Palaeogeography, Palaeoclimatology, Palaeoecology 75(3):223-240.

Royer, D. 2001. Stomatal density and stomatal index as indicators of paleoatmospheric $CO_2$ concentration. Review of Palaeobotany and Palynology 114:1-28.

Royer, D., R. Berner, and D. Beerling. 2001a. Phanerozoic atmospheric $CO_2$ change: evaluating geochemical and paleobiological approaches. Earth-Science Reviews 54:349-392.

Royer, D., S. Wing, and D. Beerling. 2001b. Estimating latest Cretaceous and Tertiary atmospheric $pCO_2$ from stomatal indices. Eos. Trans. AGU 82(20 Spring Meeting Supplement):Abstract B21B-04.

Royer, D., S. Wing, D. Beerling, D. Jolley, P. Koch, L. Hickey, and R. Berner. 2001c. Paleobotanical evidence for near present-day levels of atmospheric $CO_2$ during part of the Tertiary. Science 292:2310-2313.

Rundgren, M., and D. Beerling. 1999. A Holocene $CO_2$ record from the stomatal index of subfossil Salix herbacea L. leaves from northern Sweden. The Holocene 9(5):509-513.

Saralabai, V., M. Vivekanandan, and R. Babu. 1997. Plant responses to high $CO_2$ concentration in the atmosphere. Photosynthetica 33(1):7-37.

Sun, B., D. Dilcher, D. Beerling, C. Zhang, D. Yan, and E. Kowalski. 2003. Variation in Ginkgo biloba L. leaf characters across a climatic gradient in China. PNAS 100(12):7141-7146.

Wilson, K., D. Baldocchi, and P. Hanson. 2000. Quantifying stomatal and non-stomatal limitations to carbon assimilation resulting from leaf aging and drought in mature deciduous tree species. Tree Physiology 20:787-797.

Woodward, F. 1987. Stomatal numbers are sensitive to increase in $CO_2$ from pre-industrial levels. Nature 327:617-618.

Woodward, F., and F. Bazzaz. 1988. The response of stomatal density to $CO_2$ partial pressure. Journal of Experimental Botany 39(209):1771-1781.

Zhang, J., and J. Marshall. 1995. Variation in carbon isotope discrimination and photosynthetic gas exchange among populations of Pseudotsuga menziesii and Pinus ponderosa in different environments. Functional Ecology 9:402-412.

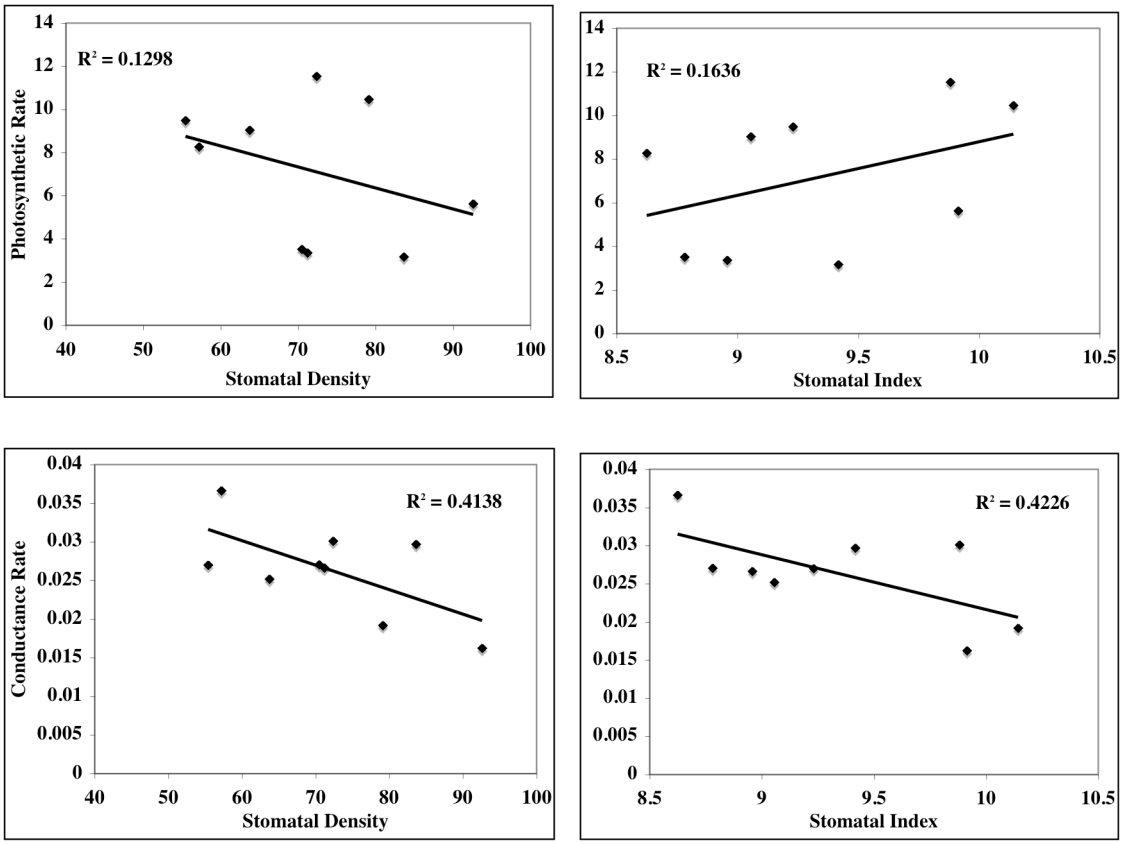
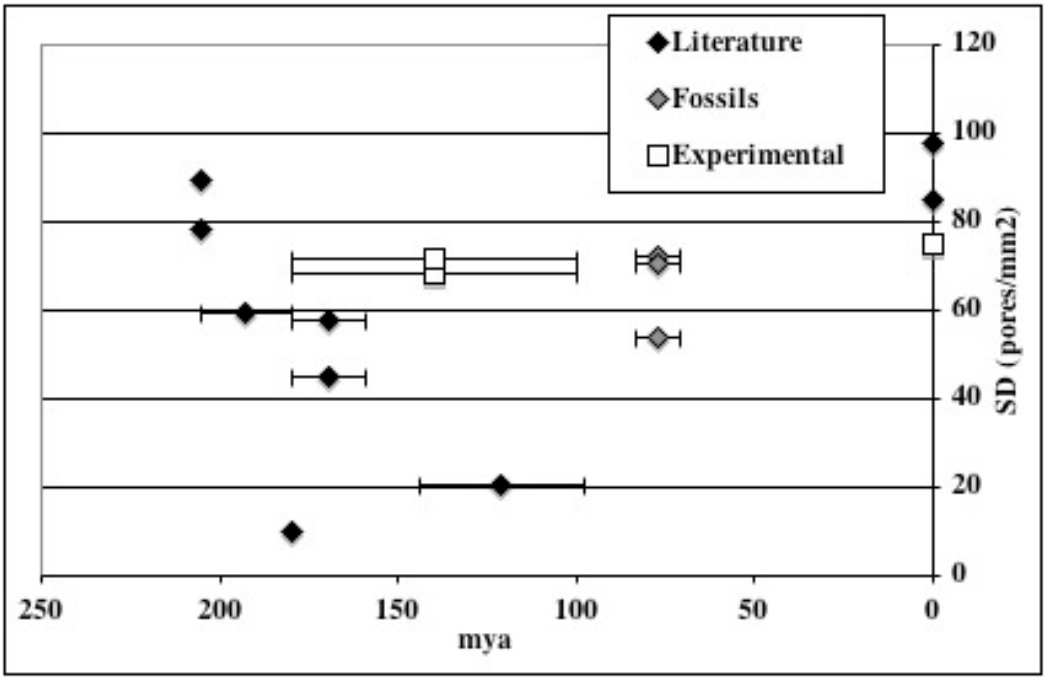



Figure 1: Scatter plots comparing photosynthetic rate and conductance rate with stomatal density and stomatal index. All data were taken from G. biloba seedlings exposed to one of three atmospheric treatments for 35 days (Chapter 2). In all cases, gas exchange and stomatal data plotted as a single point were collected from the same leaves.

Figure 2: Scatter plots comparing SD (a) and SI (b) values from the literature (filled in diamonds) with experimental (hollow squares), and previously unexamined fossil (half-tone diamonds) data. Literature and fossil SD and SI values are plotted by geologic age. SD and SI values produced during experimentation are plotted according to the atmospheric composition under which the leaves were produced. Error bars for experimental SD and SI reflect the range of possible dates for the experimental atmospheric compositions. Hence, SD and SI values that were produced during exposure to 2000 ppm carbon dioxide are plotted as if they had formed during the Late Jurassic to Early Cretaceous. SD and SI values that were observed after exposure to only 370 ppm carbon dioxide are plotted at 0 mya, because current atmospheric composition includes 370 ppm carbon dioxide. Error bars on fossil SD and SI values reflect uncertainty in dates. For carbon dioxide and oxygen concentration curves throughout the mid-Mesozoic, see Chapter 1, Fig. 1 or Berner (2004, 2003) and Royer et al. (2001a).

a.


Literature
Fossils
Experimental
120
100
80
60
40
20
0
SD (pores/mm2)
250
200
150
100
50
0
mya

b.

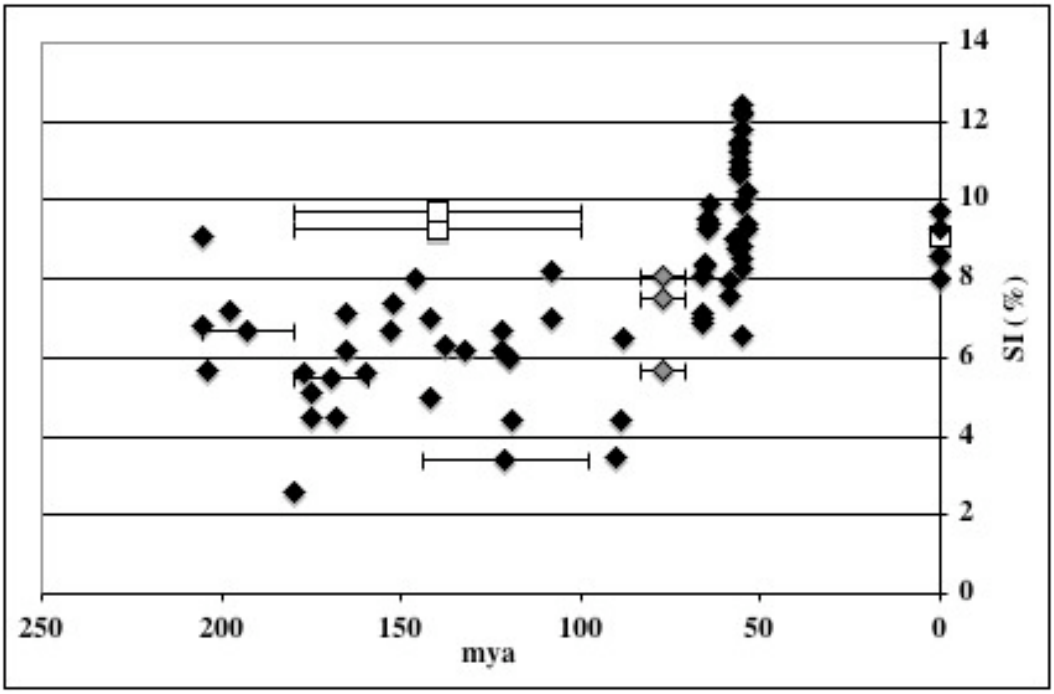

14
12
10
8
6
4
2
0
SI (%)
250
200
150
100
50
0
mya

Figure 2

Table 1: Means of SD and SI values of Ginkgo specimens reported in the literature.

Unless otherwise noted, the number of samples examined was not reported.

| Citation | Age | Species Examined | SD | SI |
|---|---|---|---|---|
| Chen et al. 2001 | Modern | *G. biloba* | 97.7 | 9.3 |
| McElwain and Chaloner 1996 | Modern | *G. biloba* | 84.8 | -[1] |
| Retallack 2001 | Modern | *G. biloba* | - | 8.7[2] |
| Beerling and Royer 2002b | Tertiary (~55mya) | *Ginkgo sp.* | - | 9.9 |
| Royer et al. 2001b | Tertiary (~55mya) | *Ginkgo sp.* | - | 7.76[3] |
| Royer et al. 2001b | Tertiary (~55mya) | *Ginkgo sp.* | - | 10.41[4] |
| Royer et al. 2001b | Tertiary (~55mya) | *Ginkgo sp.* | - | 6.54 |
| Beerling et al. 2002 | Late Cretaceous | *G. adiantoides* | - | 7.7[5] |
| Beerling et al. 2002 | Late Cretaceous | *G. adiantoides* | - | 9.56[6] |
| Beerling et al. 2002 | Late Cretaceous | *G. spitsbergensis* | - | 9.4 |
| Retallack 2001 | Late Cretaceous | *G. pilifera* | - | 4.8[7] |
| Retallack 2001 | Late Cretaceous | *G. adiantoides* | - | 8.1 |
| Retallack 2001 | Late Cretaceous | *G. tzagajanica* | - | 6.9 |
| Chen et al. 2001 | Early Cretaceous | *G. coriacea* | 20.3 | 3.4 |
| Retallack 2001 | Early Cretaceous | *G. manchurica* | - | 5.0 |
| Retallack 2001 | Early Cretaceous | *G. pluripartita* | - | 7.0 |
| Retallack 2001 | Early Cretaceous | *Ginkgoites dissectus* | - | 6.3 |
| Retallack 2001 | Early Cretaceous | *G. coriacea* | - | 6.2[8] |

Table 1 continued…

| Citation | Age | Species Examined | SD | SI |
|---|---|---|---|---|
| Retallack 2001 | Early Cretaceous | *G. polaris* | - | 6.7 |
| Retallack 2001 | Early Cretaceous | *Ginkgoites tigrensis* | - | 6.0 |
| Retallack 2001 | Early Cretaceous | *Ginkgoites ticoensis* | - | 6.0 |
| Retallack 2001 | Early Cretaceous | *Ginkgoites australis* | - | 4.4 |
| Retallack 2001 | Early Cretaceous | *G. paradiantoides* | - | 7.0 |
| Retallack 2001 | Early Cretaceous | *G. delicata* | - | 8.2 |
| Retallack 2001 | Late Jurassic | *G. huttoni* | - | 6.7 |
| Retallack 2001 | Late Jurassic | *G. manchurica* | - | 7.7[8] |
| Chen et al. 2001 | Middle Jurassic | *G. huttoni* | 44.8 | 5.5 |
| Beerling and Jolley 1998 | Middle Jurassic | *G. huttoni* | - | 5.6 |
| Beerling and Royer 2002a | Middle Jurassic | *G. dahlii* | - | 4.5 |
| McElwain and Chaloner 1996 | Middle Jurassic | *G. huttoni* | 58 | - |
| Retallack 2001 | Middle Jurassic | *G. huttoni* | - | 5.3[8] |
| Retallack 2001 | Middle Jurassic | *G. sibirica* | - | 4.5 |
| Retallack 2001 | Middle Jurassic | *G. suluktensis* | - | 7.1 |
| Retallack 2001 | Early Jurassic | *G. taeniata* | - | 7.2 |
| McElwain et al. 1999 | Early Jurassic | *G. obovatus* | 78.2 | 6.8 |
| Retallack 2001 | Middle Jurassic | *G. asiatica* | - | 6.2 |
| Chen et al. 2001 | Early/Middle Jurassic | *G. yimaensis* | 10.1 | 2.6 |

Table 1 continued…

| Citation | Age | Species Examined | SD | SI |
|---|---|---|---|---|
| Chen et al. 2001 | Early Jurassic | *G. obrutschewii* | 59.3 | 6.7 |
| Beerling and Jolley 1998 | Early Jurassic | *Ginkgoites marginata* | - | 5.7 |

1. (-) Indicates no data

2. Mean of four samples

3. Mean of two samples from Alberta, Canada

4. Mean of 18 samples from the Bighorn Basin

5. Mean of four samples from the Hell Creek Fm

6. Mean of three total samples from the Fort Union and Dawson Fm.s

7. Mean of three samples

8. Mean of two samples

Table 2: Specimen numbers, ages, geologic formations, and stomatal (SD and SI) counting distribution from 3 previously unexamined Ginkgo fossils.

| Specimen Number | Age | Formation | # of slides prepared | Total Counts |
|---|---|---|---|---|
| RTMP 97.76.129 | L. Cretaceous | Horseshoe Canyon | 4 | 10 |
| RTMP 97.76.131 1of2 | L. Cretaceous | Horseshoe Canyon | 1 | 3 |
| RTMP 97.76.131 2of2 | L. Cretaceous | Horseshoe Canyon | 2 | 4 |

Table 3: Means (standard errors) of stomatal density and stomatal index values measured on modern Ginkgo leaves after exposure to experimental atmospheric treatments.

| | **Control** | **CO2** | **CO2&O2** |
|---|---|---|---|
| **Stomatal Density** pores•mm$^{-2}$ | 75.08 **A**[1] (7.43) | 68.40 **A** (20.97) | 71.72 **A** (7.70) |
| **Stomatal Index** | 9.05 **A** (0.33) 340 ppm $CO_2$[2] | 9.26 **A** (0.65) 337 ppm $CO_2$ | 9.69 **A** (0.57) 328 ppm $CO_2$ |

1. Values in the same row with the same letters are not significantly different from each other, based on a Fisher's Least Significant Difference t-test at $p \leq 0.05$.

2. Predicted atmospheric carbon dioxide concentration values were calculated from mean SI using an inverse regression equation (Royer et al. 2001c).

Table 4: Sample size (n = # of leaves examined), means, (standard errors), and predicted carbon dioxide concentrations of SD and SI data collected from Late Cretaceous Ginkgo specimens.

| | **Sample size (n)** | **Mean** | **Predicted [$CO_2$]** |
|---|---|---|---|
| **Stomatal Density** pores•$mm^{-2}$ | **3** | 65.49 (10.09) | |
| **Stomatal Index** % | **3** | 7.09 (1.26) | 311 ppm $CO_2$[1] |

1. Predicted atmospheric carbon dioxide value was calculated from mean SI using an inverse regression equation (Royer et al. 2001c).

# CHAPTER 6:

# Future Directions

## Introduction

Given the exploratory nature of this study, questions have been suggested by the results that cannot be resolved yet. The major research inquiries prompted by this research and suggestions for experimental methods that would help to elucidate them are listed below.

## Gas Exchange and Primary Productivity

The gas exchange data presented in Chapter 1 supported an increase in G. biloba photosynthetic rate of 200-300% in response to elevated carbon dioxide and oxygen, which is a previously unobserved phenomenon. However, experiments were only 35 days long, which precluded investigation into the relationships between photosynthetic rate and plant growth, fecundity, and resource partitioning over longer time spans. An understanding of these relationships is vital for an appreciation of the potential range of physiological responses to elevated atmospheric carbon dioxide and oxygen (e.g. Allen et al. 1987, Centritto et al. 1999, Staudt et al. 2001). Future studies examining similar atmospheric conditions that involve longer acclimation periods than 35 days could be used to assess these longer-term physiological responses.

Ginkgo biloba is only one species of a diverse flora that lived during the mid-Mesozoic. There were ferns, Equisetum (horsetails), mosses, lichens, lycopods, early angiosperms, conifers, tree ferns, and cycads (Ash 1999, 2005, 2003, Parker 1975, Pasch 2000, Thorn 2001, Tidwell 1990, Tidwell et al. 1975), and many have morphologically similar extant relatives. In Chapter 4 of the current study, the shared $C_3$ photosynthetic pathway was the basis for generalizing from G. biloba to other Mesozoic plants (Farquhar

et al. 1980). However, the specific photosynthetic rate and primary productivity responses of these different Mesozoic plants was not investigated experimentally. The experimentally observed responses of other extant Mesozoic plants would allow a more detailed description of the plant physiology and productivity responses to elevated carbon dioxide and oxygen during the mid-Mesozoic.

## Plant Quality and Secondary Productivity

G. biloba foliage produced under elevated carbon dioxide and oxygen had a strongly elevated starch concentration in the leaves, which diluted the concentrations of protein and nitrogen (Chapter 3). This starch was predominantly due to photosynthetic rate stimulations prompted by elevated carbon dioxide (Chapter 2). Observations of a non-significant but repeated trend in these datasets in response to elevated oxygen (Chapter 3) suggests a larger role for oxygen in the primary metabolite composition of G. biloba leaves than could be demonstrated in the current study. Future research that expands the small datasets (n=3) of the current study would greatly help to elucidate the primary metabolite response of plants to elevated oxygen levels.

Also in Chapter 2, digestibility was assessed through lignin concentration and the ratio between non-structural and total structural carbohydrates, but the concentrations of individual cell wall carbohydrates, such as cellulose and hemicellulose, were not measured. Only a few authors have examined the effects of elevated carbon dioxide and oxygen on the carbohydrate composition of the cell wall (Kilpelainen et al. 2005, Kilpelainen et al. 2003). However, if cell wall composition is affected by these gasses,

changes in composition could have had noticeable effects on plant physiology (Boyce et al. 2001) and tissue quality (Demment and Van Soest 1985, Smith et al. 1972) during the mid-Mesozoic. Studies that examine the responses of cellulose and hemicellulose to elevated carbon dioxide and oxygen treatments would help to resolve these issues.

Additionally, leaves are not the only plant organs eaten by herbivores. In fact, growing tips, seeds, fruits, roots, wood, and bark are all eaten, often by herbivores that occupy specialized niches in the food web (Geluso and Hayes 1999, Lundberg and Palo 1993, Olofsson et al. 2004, Salawu et al. 2002). Future studies that examine the nutritive value and digestibility of these other plant organs grown under elevated carbon dioxide and oxygen might allow researchers to characterize much of the Mesozoic flora in terms of nutritive quality based on its taxonomy and the type of organ to be consumed. This sort of analysis, to the extent that it is possible given issues of imperfect preservation, would increase our understanding of mid-Mesozoic landscapes and the nutritional pressures faced by herbivores during that time.

**Proxies for Paleoecological Inference**

In Chapter 5, the stomatal density and index values of G. biloba leaves exposed to elevated levels of carbon dioxide and oxygen concentrations were examined, but stomatal density and index in G. biloba did not respond to elevated levels of either gas. Several explanations for this lack of response were suggested, including that the level of carbon dioxide used was outside the range at which stomatal frequency responds to it (e.g. Jarvis et al. 1999, Royer 2001, Woodward and Bazzaz 1988), which may have shielded a

potential oxygen effect on stomatal density and index. In order to rectify this issue, an experiment on G. biloba should be performed in which carbon dioxide levels are set at a specific value within the sub-ambient range at which stomatal density and index are sensitive to carbon dioxide (≤370 ppm), while oxygen level is varied by treatment. If there is an oxygen effect on stomatal frequency, the results from this experiment will elucidate the relationship. Future experiments that examine the effects of oxygen on stomatal frequency would greatly add to our understanding of stomatal physiology.

Another potential reason for the lack of G. biloba stomatal density and index value response to elevated carbon dioxide and oxygen proposed was the lack of certainty about the timing of stomatal initiation. The stomatal frequency of modern G. biloba leaves could have been determined prior to experimental acclimation (Lake et al. 2001), but this hypothesis was not tested in the current research. If the timing of the stomatal response in Ginkgo could be determined with some certainty, it would elucidate G. biloba stomatal physiology and would determine how future stomatal frequency experiments should be performed. A simple experiment that could resolve this question would be to expose a seedling to elevated carbon dioxide and oxygen atmospheres during a single growing season and throughout budset, and then measure the stomatal frequency of the leaves the following spring.

Additionally, stomatal density and index are not the only potential paleoecological proxies for ancient plant physiology. Stable carbon isotope ratios ($\delta^{13}C$) and isotopic discrimination ($\Delta^{13}C$) are also influenced by plant physiology parameters

such as photosynthetic rate and conductance. As such, these values have been used to estimate growth rate, water-use-efficiency (Beerling and Woodward 1995, Ellsworth et al. 1994, Marshall and Zhang 1994, McDowell et al. 2003, Zhang et al. 1994, Zhang and Marshall 1995), and gas exchange rates of plants of diverse taxonomy (Evans et al. 1986, Farquhar et al. 1989). Isotopic parameters have also been used as proxies for paleoclimate reconstruction based on fossil evidence (Beerling 1997, Bettarini et al. 1995, Lockheart et al. 1998, Poole et al. 2000, Poole et al. 1996). Stable carbon isotope ratios from extant and fossil plants may be a fruitful area for future searches for parameters that can be used to determine photosynthetic rate and other physiological parameters from shed G. biloba and fossil Ginkgo affinity leaves.

## Broader Ecological Questions

Experimental studies that examine the response of extant relatives of fossil plants to elevated levels of atmospheric carbon dioxide and oxygen could also be used to address more complex paleoecological questions. If the enclosed growth chamber used in the current study were removed and replaced with Free-Air Enrichment technology in a common garden set-up, questions of inter-species competition, forest structure, decomposition rates, and plant-soil interactions in the mid-Mesozoic and other ancient terrestrial ecosystems could be addressed experimentally. In experiments using Free-Air Enrichment technology, a common garden or existing forest ecosystem is encircled by upright gas-emitting pipes (Gielen and Ceulemans 2001, Hamerlynck et al. 2000, Rogers and Ellsworth 2002). These pipes are as tall as the plants that they essentially fence off,

and emit gas (commonly carbon dioxide (FACE), but could also include oxygen or pollutants (e.g. Liu et al. 2005)) at various levels throughout the canopy. The power of this technology is that the plants are not enclosed in chambers of any kind, precluding complicating factors due to chambers, constricted roots due to small pots, or the exclusive use of seedlings.

Free-Air Enrichment technology also allows studies to be performed on intact and natural ecosystems (e.g. Bryant et al. 1998, e.g. Cotrufo et al. 2005) or common gardens planted in natural soils instead of potting mixtures. In these studies, the entire system is exposed to elevated gaseous levels at the same time. A common garden of living relatives of Mesozoic flora exposed to Free-Air Enrichment would facilitate statistically robust research into decomposition, nutrient cycling, competition among species, and many other dynamic, inter-related processes that could have occurred in Mesozoic ecosystems. Free-Air Enrichment studies in which both carbon dioxide and oxygen are elevated around a Mesozoic common garden would greatly enrich out understanding of past environments, and allow very complex and interesting questions to be examined in a statistically robust manner.

The changing atmospheric composition over Phanerozoic time (Chapter 1, Fig. 1) suggests that experimental studies that are focused on different time ranges, including narrowed in time specifically within the Mesozoic and focused on times before and after this Era, may yield large amounts of ecological, physiological, and evolutionary information. The use of experimental techniques to study mid-Mesozoic paleoecology affords researchers the ability to create empirical data based on responses to

environmental conditions that may not currently exist in the natural world. Gas exchange studies examining the responses of plants to the Permian oxygen spike (Berner et al. 2003), for instance, would be illuminating, and might help to explain the taxonomic changes observed for that time period.

## References Cited


Allen, L., K. Boote, J. Jones, P. Jones, R. Valle, B. Acock, H. Rogers, and R. Dahlman. 1987. Response of vegetation to rising carbon dioxide: photosynthesis, biomass, and seed yield of soybean. Global Biogeochemical Cycles 1(1):1-14.

Ash, S. 1999. An Upper Triassic upland flora from north-central New Mexico, U.S.A. Review of Palaeobotany and Palynology 105:183-199.

Beerling, D. 1997. Interpreting environmental and biological signals from the stable carbon isotope composition of fossilized organic and inorganic carbon. Journal of the Geological Society, London 154:303-309.

Beerling, D., and F. Woodward. 1995. Leaf stable carbon isotope composition records increased water-use efficiency of $C_3$ plants in response to atmospheric $CO_2$ enrichment. Functional Ecology 9:394-401.

Berner, R., D. Beerling, R. Dudley, J. Robinson, and R. Wildman. 2003. Phanerozoic Atmospheric Oxygen. Annual Review of Earth and Planetary Sciences 31:105-34.

Bettarini, J., G. Calderoni, F. Miglietta, A. Raschi, and J. Ehleringer. 1995. Isotopic carbon discrimination and leaf nitrogen content of Erica arborea L. along a $CO_2$ concentration gradient in a $CO_2$ spring in Italy. Tree Physiology 15:327-332.

Boyce, C., A. Knoll, G. Cody, M. Fogel, and R. Hazen. 2001. Evolution of tracheids and lignification in early land plants. Eos. Transactions of the American Geophysical Union 82(20):Abstract B21B-03.

Bryant, J., G. Taylor, and M. Frehner. 1998. Photosynthetic acclimation to elevated $CO_2$ is modified by source:sink balance in three component species of chalk grassland swards grown in a free air carbon dioxide enrichment (FACE) experiment. Plant, Cell and Environment 21(2):159-168.

Centritto, M., H. Lee, and P. Jarvis. 1999. Increased growth in elevated [$CO_2$]: an early, short-term response? Global Change Biology 5:623-633.

Cotrufo, M., P. De Angelis, and A. Polle. 2005. Leaf litter production and decomposition in a poplar short-rotation coppice exposed to free air $CO_2$ enrichment (POPFACE). Global Change Biology 11:971-982.

Demment, M. W., and P. J. Van Soest. 1985. A nutritional explanation for body-size patterns of ruminant and nonruminant herbivores. The American Naturalist 125(5):641-672.

Ellsworth, D., M. Tyree, B. Parker, and M. Skinner. 1994. Photosynthesis and water-use efficiency of sugar maple (Acer saccharum) in relation to pear thrips defoliation. Tree Physiology 14:619-632.

Evans, J., T. Sharkey, J. Berry, and G. Farquhar. 1986. Carbon isotope discrimination measured concurrently with gas exchange to investigate $CO_2$ diffusion in leaves of higher plants. Australian Journal of Plant Physiology 13:281-292.

Farquhar, G., J. Ehleringer, and K. Hubick. 1989. Carbon isotope discrimination and photosynthesis. Annual Review of Plant Physiology 40:503-537.

Farquhar, G., S. von Caemmerer, and J. Berry. 1980. A biochemical model of photosynthetic $CO_2$ assimilation in leaves of $C_3$ species. Planta 149:78-90.

Geluso, K., and J. Hayes. 1999. Effects of dietary quality on basal metabolic rate and internal morphology of European Starlings (Sturnus vulgaris). Physiological and Biochemical Zoology 72(2):189-197.

Gielen, B., and R. Ceulemans. 2001. The likely impact of rising atmospheric $CO_2$ on natural and managed Populus: a literature review. Environmental Pollution 115:335-358.

Hamerlynck, E., T. Huxman, R. Nowak, S. Redar, M. Loik, D. Jordan, S. Zitzer, J. Coleman, J. Seemann, and S. Smith. 2000. Photosynthetic responses of Larrea tridentata to a step-increase in atmospheric $CO_2$ at the Nevada Desert FACE Facility. Journal of Arid Environments 44:425-436.

Jarvis, A., T. Mansfield, and W. Davies. 1999. Stomatal behavior, photosynthesis and transpiration under rising $CO_2$. Plant, Cell and Environment 22:639-648.

Kilpelainen, A., H. Peltola, A. Ryyppo, and S. Kellomaki. 2005. Scots pine responses to elevated temperature and carbon dioxide concentration: growth and wood properties. Tree Physiology 25:75-83.

Kilpelainen, A., H. Peltola, A. Ryyppo, K. Sauvala, K. Laitinen, and S. Kellomaki. 2003. Wood properties of Scots pines (Pinus sylvestris) grown at elevated temperature and carbon dioxide concentration. Tree Physiology 23:889-897.

Lake, J., W. Quick, D. Beerling, and F. Woodward. 2001. Signals from mature to new leaves. Nature 411:154.

Liu, L., J. King, and C. Giardina. 2005. Effects of elevated concentrations of atmospheric $CO_2$ and tropospheric $O_3$ on leaf litter production and chemistry in trembling aspen and paper birch communities. Tree Physiology 25:1511-1522.

Lockheart, M., I. Poole, P. Van Bergen, and R. Evershed. 1998. Leaf carbon isotope compositions and stomatal characters: important considerations for palaeoclimate reconstructions. Organic Geochemistry 29(4):1003-1008.

Lundberg, P., and R. T. Palo. 1993. Resource use, plant defenses, and optimal digestion in ruminants. Oikos 68:224-228.

Marshall, J., and J. Zhang. 1994. Carbon isotope discrimination and water-use efficiency in native plants of the North-Central Rockies. Ecology 75(7):1887-1895.

McDowell, N., J. Brooks, S. Fitzgerald, and B. Bond. 2003. Carbon isotope discrimination and growth response of old Pinus ponderosa trees to stand density reductions. Plant, Cell and Environment 26:631-644.

Olofsson, J., P. Hulme, L. Oksanen, and O. Suominen. 2004. Importance of large and small mammalian herbivores for the plant community structure in the forest tundra ecotone. Oikos 106:324-334.

Parker, L. 1975. The paleoecology of the fluvial coal-forming swamps and associated floodplain environments in the Blackhawk Formation (Upper Cretaceous) of Central Utah. Brigham Young University Geology Studies 22(3):99-116.

Pasch, A. 2000. Plant megafossils associated with dinosaur tackways from the Middle Cretaceous, North Slope, Alaska. GSA - Abstracts with Programs 32(7):450.

Poole, I., T. Lawson, D. Weyers, and J. Raven. 2000. Effect of elevated $CO_2$ on the stomatal distribution and leaf physiology of Alnus glutinosa. New Phytologist 145:511-521.

Poole, I., J. Weyers, T. Lawson, and J. Raven. 1996. Variations in stomatal density and index: implications for palaeoclimatic reconstructions. Plant, Cell and Environment 19:705-712.

Rogers, A., and D. Ellsworth. 2002. Photosynthetic acclimation of Pinus taeda (loblolly pine) to long-term growth in elevated $pCO_2$ (FACE). Plant, Cell and Environment 25:851-858.

Royer, D. 2001. Stomatal density and stomatal index as indicators of paleoatmospheric $CO_2$ concentration. Review of Palaeobotany and Palynology 114:1-28.

Salawu, M., A. Adesogan, M. Fraser, R. Frychan, and R. Jones. 2002. Assessment of the nutritive value of whole crop peas and intercropped pea-wheat bi-crop forages harvested at different maturity stages for ruminants. Animal Feed Science and Technology 96:43-53.

Smith, L., H. K. Goering, and C. Gordon. 1972. Relationships of forage compositions with rates of cell wall digestion and indigestibility of cell walls. Journal of Dairy Science 55(8):1140-1147.

Staudt, M., R. Joffre, S. Rambal, and J. Kesselmeier. 2001. Effect of elevated $CO_2$ on monoterpene emission of young Quercus ilex trees and its relation to structural and ecophysiological parameters. Tree Physiology 21:437-445.

Thorn, V. 2001. Vegetation communities of a high palaeolatitude Middle Jurassic forest in New Zealand. Palaeogeography, Palaeoclimatology, Palaeoecology 168:273-289.

Tidwell, W. 1990. Preliminary report on the megafossil flora of the Upper Jurassic Morrison Formation. Hunteria 2(8):1-10.

Tidwell, W., G. Thayn, and J. Roth. 1975. Cretaceous and Early Tertiary floras of the Intermountain area - A summary. Brigham Young University Geology Studies 22(3):77-98.

Woodward, F., and F. Bazzaz. 1988. The response of stomatal density to $CO_2$ partial pressure. Journal of Experimental Botany 39(209):1771-1781.

Zhang, J., L. Fins, and J. Marshall. 1994. Stable carbon isotope discrimination, photosynthetic gas exchange, and growth differences among Western Larch families. Tree Physiology 14:531-539.

Zhang, J., and J. Marshall. 1995. Variation in carbon isotope discrimination and photosynthetic gas exchange among populations of Pseudotsuga menziesii and Pinus ponderosa in different environments. Functional Ecology 9:402-412.

# CHAPTER 7:

## Summary and Conclusions

## Introduction

During the mid-Mesozoic (Late Jurassic and Early Cretaceous Periods) atmospheric carbon dioxide and oxygen levels may have been elevated up to 2000 ppm and 30%, respectively, relative to present-day ambient levels of 370 ppm and 20.9% (Berner 2004, Berner et al. 2003, Royer et al. 2001). These are large increases of both carbon dioxide and oxygen, and can be expected to have had a strong effect on terrestrial ecosystems during the mid-Mesozoic. In order to examine the photosynthetic rate, primary productivity, and leaf quality responses of Ginkgo biloba, an extant relative of a diverse mid-Mesozoic fossil lineage (Royer et al. 2003, Tralau 1968), to these atmospheric conditions, G. biloba seedlings were exposed to 2000 ppm carbon dioxide and 30% oxygen in a randomized complete block experiment within a hyperbaric chamber (Chapter 2). Instantaneous gas exchange parameters, such as photosynthetic and conductance rates, were measured during exposure (Chapter 2), and foliage quality was assessed after exposure by measuring leaf protein, lignin, non-structural carbohydrates, and C:N ratio (Chapter 3). The stomatal frequency parameters of stomatal density and stomatal index were also measured on the experimental G. biloba leaves (Chapter 5) in order to provide a framework for comparison with Ginkgo affinity fossils of mid-Mesozoic age.

## Experimental Results

Effects of 2000 ppm Carbon Dioxide**.-** Elevated levels of carbon dioxide had a strong effect on G. biloba seedlings. Elevated carbon dioxide resulted in photosynthetic

rate stimulations of 200% in response to carbon dioxide alone, and of 300% in response to elevated levels of both carbon dioxide and oxygen (Chapter 2). Additionally, the stimulated photosynthetic rate of G. biloba caused a strong accumulation of starch molecules within the leaf (Chapter 3). This abundance of foliar starch decreased the C:N ratio of the leaf tissue, reducing its nutritive content by diluting the nitrogen-bearing compounds within the leaves. Elevated levels of starch also increased the digestibility of the leaf tissue by increasing the proportion of non-structural, relative to structural, carbohydrates. Carbon dioxide did not have an effect on either stomatal density or index, however (Chapter 5).

Effects of 30% Oxygen.- Elevated levels of oxygen had a more subtle effect on G. biloba than did elevated levels of carbon dioxide. 30% oxygen did not reduce photosynthetic rate (Chapter 2), as previous studies have suggested it would (e.g. Badger 1985, Gale et al. 2001, Krause 1994). Additionally, oxygen may have actually had a stimulatory effect on photosynthesis, since photosynthetic rate was stimulated 300% in response to elevated oxygen and carbon dioxide together, but the stimulation was only 200% in response to carbon dioxide alone (Chapter 2). Oxygen did not have any significant effect on either nutritive content or digestibility (Chapter 3). Lastly, oxygen did not have an observable effect on either stomatal density or index (Chapter 5).

## Relevance of Experimental Results to the Fossil Record of Mid-Mesozoic Terrestrial Ecosystems

The experimental results were applied to the fossil record of the Late Jurassic and Early Cretaceous Periods using two separate techniques. These were a thought experiment (Chapter 4), and the use of stomatal density and index values as morphological proxies for photosynthetic rate.

Thought Experiment.- In the first technique (Chapter 4), a thought experiment was performed. Certain assumptions were adopted about the potential similarity of responses to elevated carbon dioxide and oxygen between G. biloba and Ginkgo fossils, and between G. biloba and other members of the mid-Mesozoic flora, based primarily on morphological similarities between extant and fossil Ginkgo species (Tralau 1968, Watson et al. 1999) and the shared $C_3$ photosynthetic pathway of mid-Mesozoic plants (Cerling et al. 1995, Furbank and Taylor 1995, Gale et al. 2001), respectively. Using these assumptions, the experimental results were tentatively and generally applied to mid-Mesozoic plants, and the potential implications of these responses to the mid-Mesozoic terrestrial ecosystem were discussed in terms of food webs, plant physiology, primary productivity, and decomposition rates.

Stomatal Density and Index as Proxies for Photosynthetic Rate.- In the second method for application of the responses of G. biloba to elevated carbon dioxide and oxygen to the fossil record, stomatal density and index values were assessed as proxies for photosynthetic rate and other plant physiological parameters in both extant and fossil shed leaves (Chapter 5). Stomatal density and index values from G. biloba leaves were

compared with photosynthetic and conductance rate data from the same leaves (reported in Chapter 2). Photosynthetic rate did not correlate well with stomatal frequency, but conductance rate correlated with both stomatal density and index. Stomatal density and index values were also qualitatively compared between extant G. biloba leaves and fossil Ginkgo affinity leaves in order to assess the value of stomatal characters as plant physiological proxies. However, it was difficult to assess similarity of values between extant and fossil Ginkgo specimens since data in each group was collected in different ways. Hence, while stomatal frequency values have been shown to be responsive to atmospheric carbon dioxide gas (Beerling and Royer 2002, Woodward 1987) and to photosynthetic rate (Igamberdiev et al. 2004), the results of the current study suggest that they are not suitable as proxies for photosynthetic rate in shed Ginkgo leaves (Chapter 5).

## Conclusions

In this dissertation, the experimentally derived physiological responses of G. biloba to elevated carbon dioxide and oxygen atmospheric compositions have been presented and discussed. The use of experiments on extant G. biloba as a means to understand extinct Ginkgo affinity specimens is an under-utilized technique. By examining the effects of atmospheric composition, a parameter that varies only slightly throughout terrestrial ecosystems, in a statistically robust manner, the current study has set up a framework for the examination of how fossil Ginkgo trees worldwide responded to the elevated carbon dioxide and oxygen atmospheres of the Late Jurassic and Early Cretaceous Periods. Like a time machine, these experimental techniques bring us closer

to being able to explore the ancient world with the ease with which we explore our current surroundings.

## References Cited


Badger, M. 1985. Photosynthetic oxygen exchange. Annual Review of Plant Physiology 36:27-53.

Beerling, D., and D. Royer. 2002. Reading a $CO_2$ signal from fossil stomata. New Phytologist 153:387-397.

Berner, R. 2004. The Phanerozoic Carbon Cycle: $CO_2$ and $O_2$. Oxford University Press, Oxford.

Berner, R., D. Beerling, R. Dudley, J. Robinson, and R. Wildman. 2003. Phanerozoic Atmospheric Oxygen. Annual Review of Earth and Planetary Sciences 31:105-34.

Cerling, T., J. Ehleringer, and J. Harris. 1995. Carbon dioxide starvation, the development of $C_4$ ecosystems, and mammalian evolution. Philosophical Transactions of the Royal Society, London, B 353:159-171.

Furbank, R., and W. Taylor. 1995. Regulation of photosynthesis in $C_3$ and $C_4$ plants: a molecular approach. The Plant Cell 7:797-807.

Gale, J., S. Rachmilevitch, J. Reuveni, and M. Volokita. 2001. The high oxygen atmosphere toward the end-Cretaceous; a possible contributing factor to the K/T boundary extinctions and to the emergence of $C_4$ species. Journal of Experimental Botany 52(357):801-809.

Igamberdiev, A., T. Mikkelson, P. Ambus, H. Bauwe, P. Lea, and P. Gardestrom. 2004. Photorespiration contributes to stomatal regulation and carbon isotope fractionation: a study with barley, potato, and Arabidopsis plants deficient in glycine decarboxylase. Photosynthesis Research 81:139-152.

Krause, G. 1994. The role of oxygen in photoinhibition of photosynthesis. Pp. 43-76. *In* C. Foyer, and P. Mullineaux, eds. Causes of Photooxidative stress and Amelioration of Defense Systems in Plants. CRC Press, Ann Arbor, MI.

Royer, D., R. Berner, and D. Beerling. 2001. Phanerozoic atmospheric $CO_2$ change: evaluating geochemical and paleobiological approaches. Earth-Science Reviews 54:349-392.

Royer, D., L. Hickey, and S. Wing. 2003. Ecological conservatism in the 'living fossil' Ginkgo. Paleobiology 29(1):84-104.

Tralau, H. 1968. Evolutionary trends in the genus Ginkgo. Lethaia 1:63-101.

Watson, J., S. Lydon, and N. Harrison. 1999. Consideration of the genus Ginkgoites Seward and a redescription of two species from the Lower Cretaceous of Germany. Cretaceous Research 20:719-734.

Woodward, F. 1987. Stomatal numbers are sensitive to increase in $CO_2$ from pre-industrial levels. Nature 327:617-618.