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Are paleoclimate estimates biased by foliar physiognomic responses to increased atmospheric CO_2?

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Abstract

Physiognomic analysis of fossil angiosperm leaves has provided an important quantitative database of Tertiary terrestrial paleoclimate. However, atmospheric CO_2 level, a critical control on plant growth, may have been higher in the Tertiary. It is thus crucial to investigate whether elevated CO_2 affects leaf physiognomy. In this study, leaves were collected from white oak (*Quercus alba* L.) seedlings grown in open-top growth chambers at Oak Ridge National Laboratory. The only physiognomic change noted is an increase in length to width ratio with increasing CO_2. In the literature, leaf size has been observed to increase, decrease or remain unchanged for woody C_3 species grown in elevated CO_2. Typically, one sees more variation due to microsite or phenotype than due to CO_2 level. By applying these observed physiognomic trends to two fossil floras, it is argued that estimates of mean annual temperature and growing season precipitation may be biased on the order of 1°C and 20 cm, respectively. These are relatively small effects, as the values are similar to the standard errors of the regression models used to estimate paleoclimate. The lack of data, the variability of response to CO_2 associated with microsite and phenotype, and the question of whether observed short-term trends with elevated CO_2 are sustained make it impossible to propose a correction factor. Adequate sample size and sampling of several sites are the best way to attempt to compensate for CO_2 effects on a given fossil flora until response to CO_2 is better understood.

1. Introduction

Much of our quantitative knowledge of continental paleoclimate is based on fossil floras. Workers have analyzed these assemblages using either the floristic method, which relies on systematics, or the foliar physiognomic method, which is based on leaf morphology and anatomy. Problems arise when the floristic method is applied to pre-Quaternary material, as these ancient plants generally represent extinct species (Chaloner and Creeber, 1990). Thus one identifies a nearest living relative to an extinct form, assuming that the fossil form's ecological range is similar to that of the modern species. The dangers of this assumption are discussed in detail in Wolfe (1971), Wolfe and Schorn (1990), and Chaloner and Creeber (1990).

Foliar physiognomic climate analysis is based on the observation that angiosperm leaves have similar physiognomy, that is morphology and anatomy, in areas of similar climate, regardless of genetic composition (Wolfe, 1979, 1993; Givnish, 1987). This relationship between leaf form and climate may stem from the need to balance carbon gain, water loss, and support structure; this balance will differ for different climates (Taylor, 1975; Givnish, 1984, 1987). Workers have used trends of physiognomy with climate, such as the positive

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linear relationship between entire, that is smooth-margined leaves, and mean annual temperature (MAT) to estimate paleoclimate of fossil floras (i.e. Wolfe, 1971).

Wolfe (1993) expanded on this linear analysis by collecting leaf morphologic data for 29 different morphological character states from woody dicotyledons from 106 sites from the US, Japan, and the Caribbean. By using multivariate statistical techniques, one can obtain estimates of MAT to within $\pm 1.5°C$, as well as estimates of several other climate variables. This method has been used by many workers and has provided an important database of continental paleoclimate (Wolfe, 1992, 1994; Wing and Greenwood, 1993; Povey et al., 1994; Gregory, 1994; Greenwood and Wing, 1995; Gregory and McIntosh, 1996).

The foliar physiognomic method can be applied to fossil floras as long as the environmental conditions of the fossil site are within the range of those of sites in the modern dataset of Wolfe (1993). However, atmospheric CO_2 level, an important control on plant growth, was probably higher in the Tertiary. Using geochemical carbon cycle models, Berner (1991) estimates that early Oligocene levels of CO_2 were up to two times levels today. Cerling (1991) estimates similar values for the Eocene and Miocene by using stable carbon isotopes in paleosols.

The question of whether increased levels of CO_2 in the Tertiary would have affected leaf physiognomy of woody dicotyledons and thus paleoclimate estimates needs to be examined (Idso, 1989). Growth chamber experiments suggest that increased CO_2 generally improves growth efficiency by decreasing respiration (Wullschleger and Norby, 1992; Wullschleger et al., 1992a) and increasing photosynthesis (Norby et al., 1992) unless excessive starch accumulation occurs (DeLucia et al., 1985). These effects, along with the often observed partial stomatal closure in elevated CO_2 (Acock and Allen, 1985; Strain, 1987), increase water-use efficiency. Also, carbon allocation may shift, usually with more carbon partitioned to the roots as opposed to the leaves, though whole-plant carbon storage often does not change (Acock and Allen, 1985; Norby and O'Neill, 1991; Norby et al., 1992). In summary, CO_2 generally increases the amount of carbon and water available to a plant in a given climate, though the response to CO_2 enrichment varies with species and resource availability (Bazzaz, 1990). Thus, leaf physiognomy, which varies with climate, could potentially be altered.

This paper is a preliminary look at whether atmospheric CO_2 levels indeed affect leaf physiognomy. Mainly woody C_3 species will be considered, as these are the plants used in the leaf physiognomy method of paleoclimate analysis. Data from the literature, as well as samples collected from white oak seedlings grown in open-top growth chambers, will be discussed. It is shown that, although response varies with microsite conditions and phenology, one can generalize that microsite or phenology have more importance than CO_2 treatment on variation in leaf physiognomy.

2. Previous work

Workers studying the effect of CO_2 on plant growth are generally more concerned with physiological responses and overall measures of plant growth such as total leaf area of the canopy than leaf physiognomy. However, some workers have reported the effects of elevated CO_2 on individual leaf size. These studies document interspecific differences in response, as well as intraspecific differences due to varying environmental conditions. For example, Radoglou and Jarvis (1990) found that CO_2 treatment had no effect on final leaf size for four poplar clones (*Populus* sp.) while there were significant differences in leaf size between the clones (Table 1). The lack of effect on poplar leaf size is corroborated by an unpublished study on two hybrid poplar clones cited by Taylor et al. (1994), which suggested that leaf expansion increased significantly in young leaves under high CO_2 but that final leaf area for both treatments was similar. Tolley and Strain (1984) found a similar lack of effect of increased CO_2 for sweetgum (*Liquidambar styraciflua* L.); they note that increase in total leaf area of sweetgum was due to increase in leaf number and not due to increase of individual leaf size.

Environmental factors are also important.

Table 1
Trends in leaf size for woody angiosperm species grown in different CO_2 treatments

Genera	+150 ppm	+300 ppm	CO_2 ANOVA	Other ANOVA
Yellow-poplar fertilized[a]	↓	↓	$p=0.182$	$p=0.0$ (fertilizer)
Yellow-poplar unfertilized[a]	↓	↓	$p=0.182$	$p=0.0$
Yellow-poplar[b]	–	–	N.S.	
Poplar clones:[c] Columbia River	–	–	N.S.	significant (genus)
Beupre	–	–	N.S.	significant
Robusta	–	–	N.S.	significant
Raspalje	–	–	N.S.	significant
Sweet chestnut[d]		↓	significant	
Sweet chestnut growth chamber[e]		↑	significant	
Sweet chestnut outside		–	N.S.	
Citrus hybrids:[f] Citrange		↑	?	
Citromelo		↑	?	
Sour orange (winter)[g]		↑	?	? (temperature)
Sour orange (summer)		↑	?	?
Sweetgum[h]		–	N.S.	

Arrow indicates direction of change of mean individual leaf size for medium and high CO_2 treatments (specific values given below) as compared to mean leaf size for ambient CO_2. Dash indicates no change. N.S. = trend not significant. Significant = trend significant at 95% confidence level. Other ANOVA = other variable which affected leaf size.

[a]Norby and O'Neill (1991). Mean leaf size calculated from all leaves from 5 *Liriodendron tulipifera* L. seedlings harvested during week 24 from open-top growth chambers. CO_2 levels = 371, 493, and 787 μmol mol^{-1} (ambient, medium, high).

[b]Wullschleger et al. (1992). Mean leaf size calculated from 12 terminal branch position leaves from each treatment from *Liriodendron tulipifera* L. measured after 24 days on 3 year old seedlings grown in open-top growth chambers. CO_2 levels = 349 ± 28, 500 ± 43, and 653 ± 45 μmol mol^{-1}.

[c]Values estimated from Fig. 1, Radoglou and Jarvis (1990). Mean calculated from three, 92 day old seedlings each of 4 *Populus* clones grown in open-top growth chambers. CO_2 levels = 350 ± 30 and 700 ± 30 μmol mol^{-1}.

[d]Mousseau and Enoch (1989). Mean calculated from 24, 2-year old *Castanea sativa* Mill. seedlings grown in open-top growth chambers for approximately 180 days and 12, 2-year old seedlings which underwent the same treatment the succeeding year. CO_2 levels = 350 ± 20 and 700 ± 20 μmol mol^{-1}.

[e]From Mousseau and Saugier (1992). Leaf area of 2 year old *Castanea sativa* Mill. seedling grown in growth chamber or an open top chamber (outside) with either ambient CO_2 or 2 times ambient for a full growing season.

[f]Values estimated from Fig. 3, Koch et al. (1986). Mean leaf size calculated by: (total leaf area for all but the most recent growth flush/21 leaves) from 70 seedlings from two types of citrus hybrid grown for 22 weeks under controlled CO_2 levels of 330 and 660 μmol mol^{-1}.

[g]Values estimated from Fig. 3, Idso et al. (1993). Measured 68 leaves every two months for two years from each of 8, 3-year-old *Citrus aurantium* L. trees grown in open top chambers with ambient or +300 μmol mol^{-1} CO_2.

[h]From Tolley and Strain (1984). Measurements from one year old *Liquidambar styraciflua* seedlings grown in walk-in growth chambers CO_2 levels = 350 ± 10, 675 ± 20, or 1000 ± 40 μmol mol^{-1}

Norby and O'Neill (1991) studying yellow-poplar seedlings (*Liriodendron tulipifera* L.) found that average leaf area declined somewhat with increasing CO_2 at the 24 week harvest (Table 1), though this trend is significant only at a fairly low confidence level of 82%. However, average leaf size of fertilized trees was significantly higher than average leaf size of unfertilized trees at the 99% confidence level. Mousseau and Saugier (1992) found that sweet chestnut seedlings (*Castanea sativa* Mill.) in a growth chamber showed significantly increased average leaf size with elevated CO_2, while the same species in open-air chambers showed significantly smaller leaf size. Mousseau and Enoch (1989) in a separate study found no significant trends in leaf size with CO_2 for sweet chestnut.

Idso et al. (1993) find that temperature plays an important role in determining plant response to CO_2. In a study on sour orange (*Citrus aurantium* L.), they found that leaf size increased in the

higher CO_2 treatment, and that this increase was larger in the summer months than in the winter months (Table 1). It was not reported whether these effects were statistically significant, but the consistency of the observed trends over 4 2/3 years suggests that they are (S.B. Idso, pers. comm., 1995). Koch et al. (1986) found that average mature leaf size increased in elevated CO_2 for two citrus hybrids (*Poncirus trifolata* × *Citrus* sp.) (Table 1), but again, it was not reported whether this increase was statistically significant. The average leaf size for this study was calculated by taking the total leaf area and dividing by the total number of leaves. Leadley and Reynolds (1989) argue that this method of calculating average leaf size is problematic, because the result is biased by abscised leaves and the area and number of lateral leaves.

The importance of temperature and soil nutrients to plant response have also been documented by studies on herbaceous C_3 plants. Ackerly et al. (1992) find that response to CO_2 varies with temperature for *Abutilon* and *Amaranthus*, with an increase in leaf size with CO_2 at mean temperatures of 25°C and a decrease at 34°C while a study on kidney bean (*Phaseolus vulgaris* L.) shows response varies with soil nutrients (Radoglou et al., 1992).

In summary, leaf size trends with increased levels of CO_2 for woody C_3 species are mixed. Of the ten studied genera, five show no effects; three show an increase, though the method of calculating leaf size was problematic for two of the genera and it is not known if the increases were statistically significant; one shows a decrease at a low probability level or no change; and one shows a significant decrease, increase, or no change depending on environmental conditions. In this last case, the experiment which showed an increase in leaf size is problematic, because of all the experiments, the closed growth chamber environment is the furthest removed from natural conditions. In experiments where differences due to microsite of phenotype were tested, trends with CO_2, if any, were less significant than differences due to these factors.

While trends are mixed for leaf size, many workers report an increase in leaf thickness in C_3 species and leaf weight per area with increased CO_2 (Thomas and Harvey, 1983; Tolley and Strain, 1984; Acock and Allen, 1985; Mousseau and Enoch, 1989; Norby and O'Neill, 1989, 1991; Vu et al., 1989; Radoglou and Jarvis, 1990). Stomatal density does not appear to differ significantly (Thomas and Harvey, 1983; Mousseau and Enoch, 1989). Gaudillère and Mousseau (1989) find an increase in stomatal density with elevated CO_2 on poplar, but measurements were only for 4 leaves grown in elevated CO_2 for 30 days.

3. Methodology of leaf sampling, CO_2 sensitivity study

In order to examine the effect of CO_2 on all physiognomic character states in the modern database of Wolfe (1993), leaves from white oak (*Quercus alba* L.) from the Global Change Field Research Faculty, Oak Ridge National Laboratory were analyzed. This growth chamber facility was developed and is maintained by workers in the Environmental Sciences Division of Oak Ridge. White oak seedlings were planted in April 1990 and were grown in open-top growth chambers which allowed for exposure to ambient light, temperature, precipitation, and soil conditions at three CO_2 levels: ambient (349 ± 28 μmol mol^{-1}), +150 ppm (500 ± 43 μmol mol^{-1}), and +300 ppm (653 ± 45 μmol mol^{-1}). The average temperature for June to September is 22.8°C. The seedlings were grown in 9 chambers, 3 for each treatment, with 5 seedlings per chamber. Further details of the experiment are given in Wullschleger et al. (1992b).

Leaf samples were obtained using 3 strategies: (1) grab samples of litter. Leaf litter from 1991 had been collected at three times: October 28th, November 7th, and December 18th, and had been stored in plastic bags. Nine grab samples of approximately 35 leaves per chamber were taken, with 3 samples from each collection date representing each of the CO_2 treatments. This sample was collected to examine leaf size, however, the leaves were often curled and brittle, and thus physiognomy was not scored.

(2) Canopy sample. On-the-tree foliage from fall 1993 was sampled by randomly picking 25 leaves total from the five trees in each chamber.

In this way fresh leaves more suitable for physiognomic scoring were collected. The probable effect of size on the chance of a leaf being picked makes these samples unsuitable for size analysis.

3) Flush sampling. The top 5 leaves of the 1st growth flush of 1993 were collected from one tree in each chamber. This sample was collected in order to examine physiognomy without introducing variance due to location on the tree.

Leaf area was measured on all samples using an area meter (LI-3100 Area meter, Li-Cor Inc.). The litter presented some problem, as in some cases the leaves had dried and curled. These leaves were taken apart, so each piece could be flattened and measured separately. The random and flush samples were scored for the 29 physiognomic character states in Wolfe's (1993) dataset.

These 29 character states fall into 12 categories: Lobed, No teeth, Teeth regularly spaced, Teeth closely spaced, Teeth round, Teeth compound, Leaf size, Apex emarginate, Apex, Base, Length to width ratio, and Shape. Definitions of character states in each category are given in Table 2. In categories such as Lobed in which there are only two possible states, that is Lobed or Unlobed, a sample receives a score of 1.0 if the character is found in all leaves in the sample, 0.5 if some leaves display the character state, and some do not, and 0 if none of the leaves display the characteristic. In physiognomic categories with more than two states, for example the size category, the full range of variation is scored. For example, if a sample has leaves that only fall into one size class, that class receives a score of 1, if leaves fall into two classes, each receives a score of 0.5, if leaves fall into three classes, each receives a score of 0.33 etc. The character states and method of physiognomic scoring are described in more detail in Wolfe (1993).

In addition, two quantitative measures, length to width ratio and percent dissection were calculated for each leaf from the flush and random samples where: %dissection $= D/(D+A)$ with $A =$ the measured area, and $D =$ the difference between the measured area and the area of the oval defined by connecting the tips of the teeth or lobes to form a smooth margin.

4. Results of CO_2 sensitivity study

Physiognomic scores are given in Table 2. Summed scores for the different treatments and chambers within each treatment are identical except for differences in size classes and length to width ratios represented. In the random sample, one ambient and one +150 ppm chamber had smaller leaf size character states represented than the other chambers, while the +150 ppm flush sample had smaller leaf size character states present. Two of the three ambient chambers in the random sample had lower length to width ratio character states present than the other chambers, and the +150 ppm flush sample had lower length to width ratio character states represented.

The average values for leaf size, length to width ratio, and percent dissection are given in Table 3, with significance analyzed by two way ANOVA. In every case, differences between chambers within a treatment are significant. There is a trend of decreasing size for both the litter and the flush samples, however this trend is not statistically significant at the 67% confidence level. No trends are seen between dissection and CO_2 treatment. Length to width ratio showed an increase with increasing CO_2 at the 91% confidence level for the random sample, while the flush sample showed no trend with CO_2 treatment.

5. Discussion of results

The results for white oak suggest that microsite or phenology have more importance than CO_2 treatment on variation in leaf physiognomy for normal growing season temperatures, because in every case, the differences between chambers within a treatment are significant. On the other hand, only one sample showed a significant trend with CO_2 treatment, and the significance level was lower than the widely used 95% confidence level.

Whether the differences in the physiognomic scores in Table 2 arose by chance or because of CO_2 treatment can be evaluated by considering the statistical significance of the differences in quantitative measurements in Table 3. The variation in physiognomic score for leaf size character

Table 2
Physiognomic character state scores for the random and flush samples

Leaf physiognomic	1	6	8	2	4	9	3	5	7	Flush		
character state	Ambient			+150 ppm			+300 ppm			Amb	+150	+300
Lobed	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
No teeth												
Teeth regular	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Teeth close	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Teeth round	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Teeth acute	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Teeth compound	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Leptophyllous 1												
Leptophyllous 2												
Microphyllous 1												
Microphyllous 2	0.25	0.25		0.25	0.33	0.25	0.25	0.25	0.25			
Microphyllous 3	0.25	0.25	0.5	0.25	0.33	0.25	0.25	0.25	0.25	0.33		
Mesophyllous 1	0.25	0.25	0.5	0.25	0.33	0.25	0.25	0.25	0.25	0.5	0.33	0.5
Mesophyllous 2	0.25	0.25		0.25		0.25	0.25	0.25	0.25	0.5	0.33	0.5
Apex emarginate	1	1	1	1	1	1	1	1	1	1	1	1
Apex round	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Apex acute	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Apex attenuate												
Base cordate												
Base round	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Base acute	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
L:W <1:1												
L:W 1–:1	1	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1	0.5
L:W 2–:1			0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5		0.5
L:W 3–:1												
L:W >4:1												
Shape obovate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Shape elliptical	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Shape ovate												

Definitions of character states after Wolfe (1993), categories in capitals: LOBED = leaf pinnately or palmately lobed; NO TEETH = entire-margined; TEETH REGULAR = no more than 33% variation in distance between teeth; TEETH CLOSE = basal flank of teeth $<3\times$ the length of the apical flank; TEETH ROUND = teeth convex or appressed; Teeth acute = teeth form sharp point; TEETH COMPOUND = teeth with smaller teeth; SIZE: Leptophyllous1 = area of leaf 0–25 mm^2; Leptophyllous2 = area of leaf 25–80 mm^2; Microphyllous1 = area of leaf 80–400 mm^2; Microphyllous2 = area of leaf 400–1400 mm^2; Microphyllous3 = 1400–3600 mm^2; Mesophyllous1 = 3600–9000 mm^2; Mesophyllous2 = 9000 + mm^2; APEX EMARGINATE = notched apex; APEX: Apex round = apex convexly curved; Apex acute = apex straight sided and forms point; Apex attenuate = drip tip; BASE: Base cordate = basal portions extend below juncture with petiole; Base round = basal portion convex; Base acute = basal portion with straight or convex margin; LENGTH TO WIDTH RATIO: L:W <1:1, 1–2:1, 2–3:1, 3–4:1 or >4:1; SHAPE: Shape obovate = widest in upper 1/3 of lamina; Shape elliptic = widest in middle 1/3; Shape ovate = widest in basal 1/3. See Wolfe (1993) for more detail and illustrations. Scoring method described in text.

Table 3
Mean values for various physiognomic parameters measured for white oak from different CO_2 treatments

CO_2 treatment[a]	Leaf size litter	Leaf size flush	Dissection[b] random	Dissection flush	L/W ratio random	L/W ratio flush
Ambient avg.	**31.6±20.3**	**81.0±23.2**	**0.15±0.05**	**0.18±0.05**	**1.6±0.2**	**1.7±0.2**
1[a]	39.3±29.7	89.0±22.0	0.17±0.06	0.11±0.01	1.62±0.16	1.71±0.28
6	28.1±13.5	96.1±15.3	0.15±0.04	0.22±0.02	1.59±0.15	1.68±0.08
8	28.0±12.7	57.8±11.1	0.15±0.04	0.20±0.02	1.62±0.23	1.57±0.20
+150 ppm avg.	**30.9±17.6**	**71.8±30.2**	**0.16±0.04**	**0.19±0.05**	**1.8±0.3**	**1.6±0.1**
2	37.3±17.4	80.7±7.1	0.15±0.03	0.20±0.02	1.63±0.20	1.63±0.22
4	30.3±20.7	52.1±10.7	0.18±0.05	0.23±0.04	1.86±0.32	1.49±0.08
9	25.4±12.2	82.5±50.0	0.14±0.04	0.13±0.03	1.82±0.24	1.62±0.07
+300 ppm avg.	**27.1±16.1**	**75.0±23.3**	**0.14±0.04**	**0.17±0.05**	**1.7±0.2**	**1.7±0.2**
3	24.5±11.3	101.1±10.0	0.15±0.04	0.17±0.02	1.68±0.27	1.64±0.06
5	23.2±13.8	51.1±606	0.14±0.04	0.22±0.03	1.68±0.23	1.61±0.20
7	33.3±19.7	72.7±13.5	0.13±0.04	0.13±0.05	1.66±0.19	1.93±0.15
Statistical significance						
CO_2 treat: p	N.S.[c]	N.S.	N.S.	N.S.	0.09	N.S.
F	N.S.	N.S.	N.S.	N.S.	3.6	N.S.
Chamber: p	0.0	0.0	0.01	0.0	0.02	0.04
F	4.1	5.5	2.7	16.7	2.5	2.5

[a]Numbers indicate chamber number. Values reported are means of measurements on 35, 25, and 5 leaves, respectively for the litter, random and flush samples. Values in bold are the average of values from 3 chambers with the same CO_2 treatment.
[b]Variables log transformed for ANOVA.
[c]N.S. = not significant at the 67% confidence level.

state is more due to variation between chambers than CO_2 treatment, because the trends with CO_2 and leaf size are not significant, even at the very low confidence level of 67%. On the other hand, the increase in length to width ratio was significant at the 91% significance level. Because the average length to width ratio of 1.6–1.7 for the random and flush categories was near the boundary of two length to width physiognomic categories (i.e. 1–2:1 and 2–3:1), the trend of increasing length to width ratio was reflected in the physiognomic scores.

Given the observation that increased CO_2 increases water use efficiency, and thus increases the water available to a plant in a given climate, one would expect leaves in elevated CO_2 to be thinner, larger, and wider based on genecological studies of leaf physiognomy. Workers have found that populations of a given species in more xeric, that is drier, environments have thicker, smaller, and narrower leaves than populations from environments with more rainfall, humidity or soil nutrients (Lewis, 1972; Givnish, 1987). Note however, that very narrow leaves, or stenophyllous leaves, are typically found in stream-side environments (Richards, 1952). However, the studies above indicate that trends for size are mixed, that thickness increases, and that at least for white oak, leaves are narrower for increased CO_2. Thus physiognomic response to CO_2 appears to be more complicated that just a response to increased water availability.

6. Effects of CO_2 on paleoclimate estimates

There are several factors one must consider in evaluating how these physiognomic trends affect estimates of paleoclimate using the above multiple regression models. As discussed previously, there are large between-species differences in responses to CO_2 plus within-species differences in responses due to environmental conditions. Thus, it is very

difficult to predict the effect of elevated CO_2 on fossil vegetation.

Another factor to consider in evaluating the possible effects of CO_2 on leaf physiognomy is that it is not clear how long observed trends are sustained as seedlings mature, or as individuals adjust to higher CO_2 levels. For example, in living plants, stomata close partially in response to higher CO_2 and stomatal density appears not to change. However, Van Der Burgh et al. (1993) and Woodward (1987) looked at herbarium specimens collected over the past 150 years and found that stomatal density decreases as CO_2 concentration increases. Beerling and Chaloner (1993) obtain similar results from 1327 BC, pre-332 BC, 1818 AD and 1978 AD *Olea europaea* L. leaves.

Comparison of measures of stem growth for seedlings and mature plants is complicated by different species lists for the two datasets. In one of the longest growth chamber experiments, Idso and Kimball (1993) found increased stem diameter in sour orange trees growing under increased CO_2. Shorter length experiments have shown an increase in stem diameter to occur in yellow-poplar (O'Neill et al., 1987) sweet gum under high irradiance (Koch et al., 1986), american beech (*Fagus grandifolia* Ehrh.) and black cherry (*Prunus serotina* Ehrh.) (Bazzaz et al., 1990). Other experiments show no significant increase of stem diameter for sweetgum under low irradiance, loblolly pine (*Pinus taeda* L.) (Tolley and Strain, 1984), white pine (*Pinus strobus* L.), eastern hemlock (*Tsuga canadensis* (L.) Carr), sugar maple (*Acer saccharum* Marsh.), red maple (*Acer rubrum* L.), and paper birch (*Betula papyrifera* Marsh) (Bazzaz et al., 1990).

In contrast, D'Arrigo and Jacoby (1993) examined 300 year long ring-width series for white spruce (*Picea glauca* (Moen.) V.) at three North American tree-line sites and found no increased growth due to increasing CO_2. Graumlich (1991) found a similar lack of response to CO_2 in foxtail pine (*Pinus balfouriana*), lodgepole pine (*Pinus murrayana*) and western juniper (*Juniperus occidentalis*). Unfortunately, it is not documented how these species respond as seedlings, so it is difficult to tell whether the lack of response is due to phenology or a decrease in the response to CO_2 over time. Graybill and Idso (1993) found that in strip-bark forms of bristlecone pine (*Pinus aristata*, *P. longaeva*), in which almost all carbon is allocated to the cambium, ring width increased with increasing CO_2. However, this result is not applicable to most forest trees.

7. Methodology

With these caveats in mind, the effect of the observed physiognomic trends on paleoclimate estimates using the foliar physiognomic method was tested on two early Oligocene floras from the western US, the Pitch-Pinnacle flora from Colorado and the Goshen flora from Oregon. This analysis is not meant to be definitive, but rather to give an idea of the magnitude of possible effects on paleoclimate estimates. The mean annual temperature and other climate variables for the two Oligocene floras were estimated using multiple regression models (Table 4) developed from the database of Wolfe (1993). These models are further discussed in Gregory and McIntosh (1996), with similar versions developed on an earlier physiognomic database discussed in Gregory (1994).

For the leaf size data discussed above, three out of the eleven genera had a significant change in leaf size with increasing CO_2, assuming that the trends for sour orange are significant. Trends are mixed, with sour orange showing an increase, yellow poplar showing a decrease or lack of change, and sweet chestnut showing an increase, decrease, or lack of change of leaf size depending on the environmental conditions. None of the studies in the literature measured leaf length to width ratio, which was observed to increase with increasing CO_2 in white oak.

In order to examine the possible effect of CO_2 on physiognomic scores, several assumptions must be made. First of all, we will assume that the above ratio, 3 out of 11 species significantly affected by elevated CO_2, is representative of angiosperm response in general. Given the mixed response of leaf size, it is reasonable to assume that response of leaf length to width ratio, observed to increase in white oak, will be similarly mixed, and that a similar number of genera will be

Table 4
Multiple regression models for various climate factors

Climate variables	Character states										Diagnostics			
											Const.	r^2(%)	s.e.	F
MAT	NoT	**Le2**	**LW<1**	BAct	AEmg									
	16.656	−9.200	−5.594	5.137	4.879						1.768	92.0	1.5	169.6
CMM	**Me1**	NoT	TLob	AEmg										
	25.155	16.266	−14.541	11.717							−12.450	90.4	2.2	172.5
WMM	TRnd	**Me2**	NoT	TCmp	TRg	**Le2**	**Mi2**							
	−27.063	−26.011	−24.050	−20.686	−16.780	−16.478	−9.413				90.660	80.0	2.5	61.1
MART	**Me1**	TCmp	ARnd	**Me2**	**LW<1**	**Mi1**	**Mi2**	TAct	**LW2–3**	NoT				
	−26.997	−23.175	−17.689	−17.047	15.770	−15.524	−15.476	13.498	−12.306	−11.73	072.608	71.1	3.8	26.8
GSP	Me2	**LW<1**	AAtn	SElp	BRnd	TAct								
	141.368	−136.34	130.616	93.936	−79.774	−52.386					48.050	80.4	16	68.6
SEAS	**LW<1**	BRnd	NoT	SOb	**Me1**	AAtn								
	1.196	1.011	−0.986	0.796	0.722	−0.616					−0.143	75.5	0.19	55.0

Climate variables: MAT = mean annual temperature (°C), CMM = cold month mean temperature (°C), WMM = warm months mean temperature (°C), GSP = growing season precipitation (cm), SEAS = seasonality, where SEAS = (mean annual precipitation − GSP)/mean annual precipitation.
Character states: AAtn = Apex attenuate; AEmg = Apex emarginate; ARnd = Apex round; BAct = Base acute; BRnd = Base round; Le2 = Leptophyllous 2; LW < 1 = Length to width ratio < 1:1; LW2–3 = Length to width ratio 2–3:1; Me1 = Mesophyllous 1; Me2 = Mesophyllous 2; Mi1 = Microphyllous 1; Mi2 = Microphyllous 2; NoT = No teeth (entire); SElp = Shape elliptic; SOb = Shape obovate; TAct = Teeth acute; TCmp = Teeth compound; TLob = Teeth lobed; TRg = Teeth regularly spaced; TRn = Teeth round. Character states in **bold** lettering are those that could be affected by increased CO_2 (see section "effect of trends with elevated CO_2 on paleoclimate estimates.").
Numbers below character state = x_n in multiple regression equation of the form: Climate variable = $x_1(q_1) + x_2(q_2) + \ldots x_n(q_n)$ + Constant where q = arcsin $(((p+0.5)/100)^{1/2})$ where p = raw character state score as described by Wolfe (1993). More detailed explanation in Gregory and McIntosh (in prep.) or Gregory (1994).
Diagnostics: Const. = constant in above equation, s.e. = standard error, F = F ratio.
Note that the MAT and CMM models should only be used to estimate climate for floras with CMM > −2°C and that model for GSP should only be used for floras with GSP < 222 cm. See Gregory and McIntosh (1996) for additional models.

affected. We will then assume that half of the affected species have sizes or length to width ratios near the boundary of physiognomic categories, and that these species will thus have a physiognomic score that will be affected by the trends with CO_2. Thus the ratio used is 3 species affected for a given character per 22 species.

The effects were modeled in two ways. In one method, the species with physiognomic scores to be changed are chosen to maximize or minimize the effects on the paleoclimate estimate, in order to get an idea of the maximum possible bias. In the other method, species are chosen randomly, in order to get an idea of average bias.

For the Pitch-Pinnacle flora, with 18 forms, one form had a larger size class added and one had a smaller added, and for one it was randomly chosen whether the size would increase, decrease or remain the same based on the ratio discussed above. The same strategy was used for length to width ratio. For the Goshen flora, which has 48 forms and is subtropical, it was assumed that the higher mean annual temperature (MAT) would favor an increase in leaf size. Therefore, four forms were increased one size class and two were decreased one size class. As the effect of higher temperatures on the length to width ratio is not known, two forms had a higher length to width ratio class added, two had a smaller added, and for two it was randomly chosen whether the length to width ratio would increase, decrease, or remain the same. The resulting modified physiognomic scores are then plugged into the regression models (Table 4). Results are given in Fig. 1.

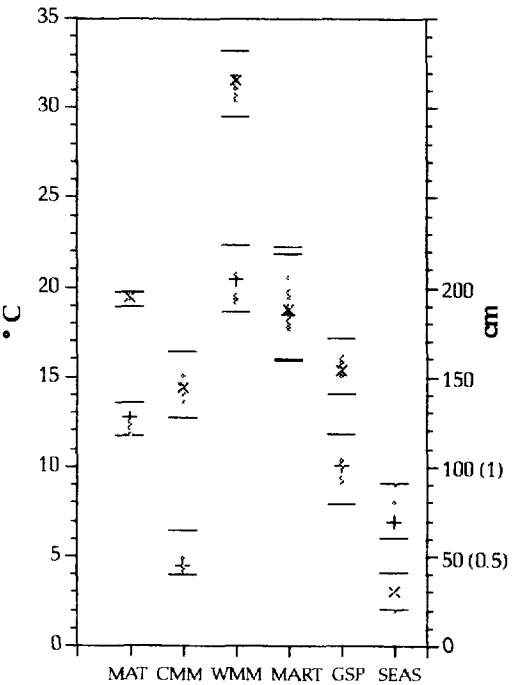

Fig. 1. Plot of modelled effects of CO_2 on the earliest Oligocene Pitch Pinnacle and Goshen floras. + and x mark the paleoclimate estimate for the Pitch Pinnacle and Goshen floras, respectively, as derived from physiognomic scores input into multiple regression models (Table 4). Gray square = paleoclimate estimated from modified physiognomic scores, affected forms chosen randomly. Bar = maximum and minimum paleoclimate estimated by choosing forms to be modified that maximized effects. Definition of climate variable abbreviations given in Table 4. 0–35°C scale for MAT, CMM, WMM, and MART. 0–200 scale for GSP. 0–1 scale for SEAS.

8. Results and discussion of simulation

The results indicate that the effects of increased CO_2 on paleoclimate estimates are generally equal or less than one standard error for the regression models. For example, maximum error for estimates of MAT is on the order of 1°C, compared to a standard error of 1.5°C (Table 4). Note that the maximum possible effect on MAT is less for the warmer Goshen flora, because warmer/wetter floras tend to have larger leaf sizes. Thus the small Leptophyllous 2 leaf size class, which is an important variable in the regression (Table 4) is unlikely to be affected by any changes due to increased CO_2. The maximum error estimated for growing season precipitation (GSP) is on the order of 20 cm, which is slightly more than the standard error of 16 cm. The other climate variables all have estimated maximum CO_2 effects which are equal to or less than the standard error calculated for the regression models.

This analysis is certainly not definitive, given the profound uncertainties in predicting response to CO_2, but it gives a preliminary idea of the magnitude of the possible effect on paleoclimate estimates. This bias appears to be small enough that physiognomic analysis of paleoclimate is still

useful for time periods with double or triple ambient CO_2. Additional sources of error for the physiognomic method are discussed in Gregory (1994) and Gregory and McIntosh (1996).

Because of the large amount of variability associated with microsite and phenology, it is possible that physiognomic scores would not reflect the influence of CO_2 if enough leaves from enough individuals were collected. For example, if physiognomic scores for the random white oak sample were combined for each CO_2 treatment, physiognomic scores would be identical (Table 2); the sample size in this case is 105 leaves from a total of 15 trees per treatment. Thus in fossil localities, one would want to collect from either a large stratigraphic thickness, or from quarries separated by at least the distance of assumed canopy height in order to sample more than one individual of a species (Burnham et al., 1992). However, it would be difficult to obtain large samples of leaves from rare species even with this strategy (Burnham et al., 1992). Thus, in fossil collections, forms with small numbers of leaves or collections from one quarry site from a limited stratigraphic thickness may have physiognomic scores that reflect the effects of elevated CO_2.

9. Summary

In summary, increased atmospheric CO_2 levels cause a decrease or increase in leaf size and increase in length to width ratio for some woody C_3 species growing under certain environmental conditions. These trends can bias physiognomic-based climate estimates on the order of one standard error for the regression model used to generate the estimate. However, not enough is known about either actual CO_2 levels in the past or the effect of phenotype, environmental factors, and length of exposure time on response to increased CO_2 to propose a correction factor for fossil sites. As of now, adequate sample size and sampling of several localities is the best way to attempt to compensate for CO_2 effects for a given fossil flora.

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