

Stomatal density and stomatal index as indicators of paleoatmospheric CO₂ concentration

D.L. Royer*

Yale University Department of Geology and Geophysics, P.O. Box 208109, New Haven, CT 06520-8109, USA

Received 9 February 2000; received in revised form 24 August 2000; accepted for publication 26 September 2000

Abstract

A growing number of studies use the plant species-specific inverse relationship between atmospheric CO₂ concentration and stomatal density (SD) or stomatal index (SI) as a proxy for paleo-CO₂ levels. A total of 285 previously published SD and 145 SI responses to variable CO₂ concentrations from a pool of 176 C₃ plant species are analyzed here to test the reliability of this method. The percentage of responses inversely responding to CO₂ rises from 40 and 36% (for SD and SI, respectively) in experimental studies to 88 and 94% (for SD and SI, respectively) in fossil studies. The inconsistent experimental responses verify previous concerns involving this method, however the high percentage of fossil responses showing an inverse relationship clearly validates the method when applied over time scales of similar length. Furthermore, for all groups of observations, a positive relationship between CO₂ and SD/SI is found in only $\leq 12\%$ of cases. Thus, CO₂ appears to inversely affect stomatal initiation, although the mechanism may involve genetic adaptation and therefore is often not clearly expressed under short CO₂ exposure times.

Experimental responses of SD and SI based on open-top chambers (OTCs) inversely relate to CO₂ less often than greenhouse-based responses ($P < 0.01$ for both SD and SI), and should be avoided when experimental responses are required for CO₂ reconstructions. In the combined data set, hypostomatous species follow the inverse relationship more often than amphistomatous species (56 vs. 44% for SD; 69 vs. 32% for SI; $P < 0.03$ for both comparisons). Both the SD and SI of fossil responses are equally likely to inversely relate to CO₂ when exposed to elevated versus subambient CO₂ concentrations (relative to today). This result casts doubt on previous claims that stomata cannot respond to CO₂ concentrations above present-day levels. Although the proportion of SD and SI responses inversely relating to CO₂ are similar, SD is more strongly affected by various environmental stresses, and thus SI-based CO₂ reconstructions are probably more accurate. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: carbon dioxide; stomatal frequency; paleoatmosphere; paleoclimatology; leaf anatomy; cuticles

1. Introduction

The increase in atmospheric CO₂ concentration since industrialization (Friedli et al., 1986; Keeling et al., 1995) and the predicted continued increase into the near future (Houghton et al., 1995) forces

the need to understand how the biosphere operates under elevated (relative to pre-industrial) CO₂ levels. The geologic record affords a wealth of such information. Fundamental to the use of the geologic record, however, is a reliable estimate of CO₂ concentration throughout the intervals of interest. The results of a computer-based model for the Phanerozoic (Berner, 1994; see Fig. 1), based on rates of Ca–Mg silicate weathering and burial as carbonates, weathering and

* Fax: +1-203-432-3134.

E-mail address: dana.royer@yale.edu (D.L. Royer).

burial of organic carbon, CO₂ degassing, vascular land plant evolution, and solar radiation, have gained considerable use (e.g. Retallack, 1997; Kump et al., 1999). Proxy data are still crucial, however, for both testing and refining this model. Currently used proxies include $\delta^{13}\text{C}$ from pedogenic carbonates (Cerling, 1991, 1992; Mora et al., 1991, 1996; Ekart et al., 1999), $\delta^{13}\text{C}$ from trace carbonates contained within goethite (Yapp and Poths, 1992, 1996), $\delta^{13}\text{C}$ from phytoplankton (Freeman and Hayes, 1992; Pagani et al., 1999a,b), and $\delta^{11}\text{B}$ from planktonic foraminifera (Pearson and Palmer, 1999). To a first approximation, these proxies largely support the model of Berner (1994) (Fig. 1). A discrepancy exists during the late Carboniferous and early Permian between the pedogenic carbonate-derived data of Ekart et al. (1999) and the model of Berner (1994). However, this discrepancy disappears if the $\delta^{13}\text{C}$ values for marine carbonates of Popp et al. (1986) are used during this time interval instead of those of Veizer et al. (1999) in calculating CO₂ from the data of Ekart et al. (1999) (Berner, R.A., unpublished data; see Fig. 1b).

Another emerging proxy relies on the plant species-specific inverse relationship between atmospheric CO₂ concentration and stomatal density and/or stomatal index. Concerns have been raised regarding this method's reliability (Körner, 1988; Poole et al., 1996), and it is the purpose of this paper to address these concerns via an extensive analysis of the literature. Analysis includes stomatal responses from fossil observations as well as short-term (experimental, natural CO₂ springs, altitudinal transects, and herbaria) observations, as responses from the latter category are often used to generate standard curves for estimating CO₂ from fossil observations (van der Burgh et al., 1993; Beerling et al., 1995; Kürschner, 1996; Kürschner et al., 1996; Rundgren and Beerling,

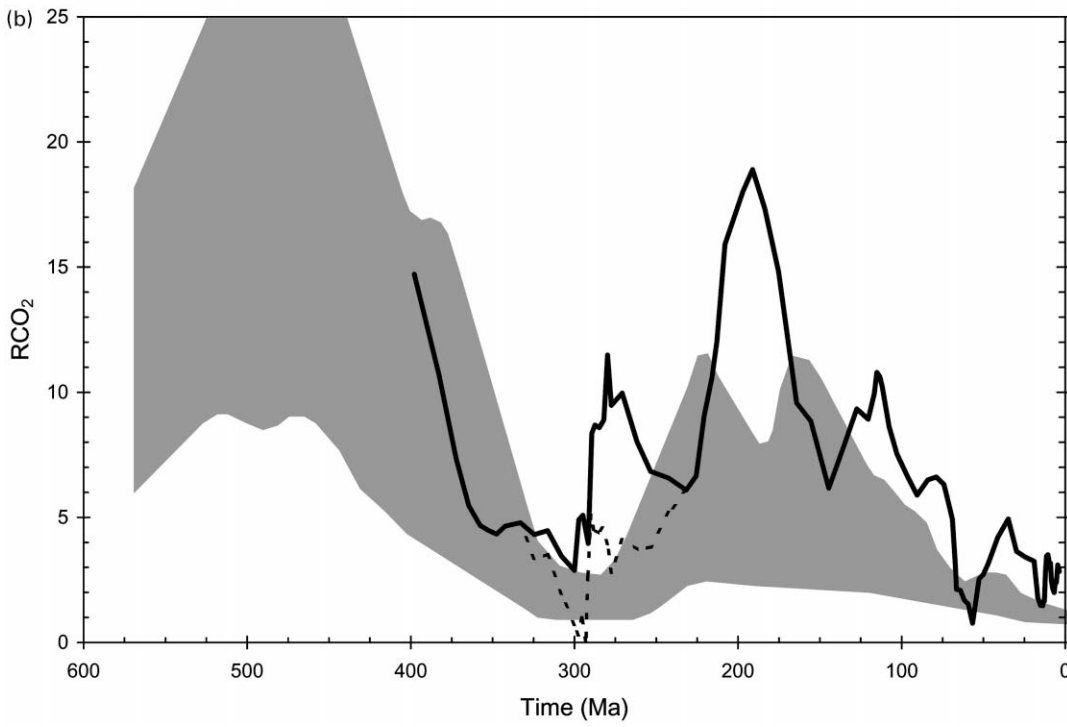
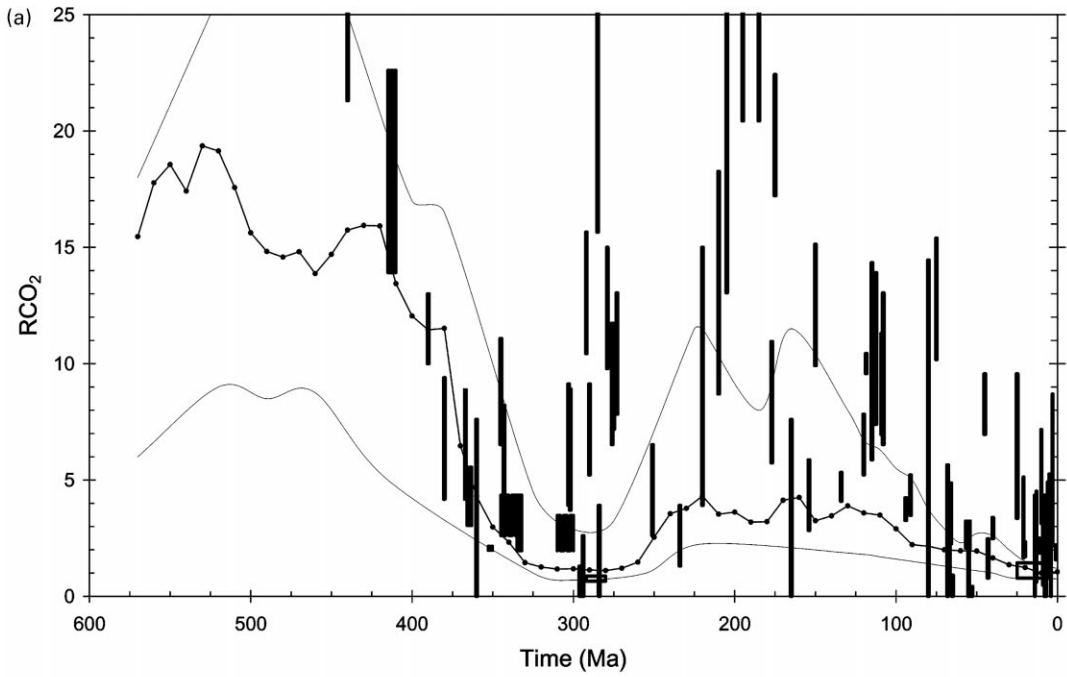
1999; Wagner et al., 1999). Specifically, the utility of stomatal indices will be examined, an approach not analyzed in previous reviews (Beerling and Chaloner, 1992, 1994; Woodward and Kelly, 1995).

2. Mechanism controlling stomatal density

Stomata are pores on leaf surfaces through which plants exchange CO₂, water vapor, and other constituents with the atmosphere. They form early in leaf development, and typically mature by the time the leaf reaches 10–60% of its final leaf size (Tichá, 1982). Thus, the timing for the mechanism(s) of stomatal initiation lies early in leaf ontogeny (Gay and Hurd, 1975; Schoch et al., 1980). Currently, no mechanism or combination of mechanisms adequately explains the expression of stomatal initiation, although genetic work may provide insights in the near future (e.g. Berger and Altmann, 2000). Proposed mechanisms include irradiance (Gay and Hurd, 1975; Schoch et al., 1980), humidity (Salisbury, 1927), and $p\text{CO}_2$ (Woodward, 1986; Beerling and Chaloner, 1992; Woodward and Kelly, 1995; Beerling and Woodward, 1996).

A common theory for why CO₂ should (partially) control stomatal initiation is as follows (e.g. Woodward, 1987). Water vapor and CO₂ constitute the two main fluxes across the leaf epidermis. It is generally advantageous for plants to conserve water loss while maximizing CO₂ uptake, two typically antithetical processes. As CO₂ rises for a given water budget, for example, a plant can 'afford' to reduce its stomatal conductance without suffering a reduction in carbon assimilation rates. Two main pathways driving this response are smaller stomatal pores (Bettarini et al., 1998) and a reduction in stomatal numbers

Fig. 1. Atmospheric CO₂ versus time for the Phanerozoic. RCO_2 = ratio of mass of paleo-CO₂ to time-averaged pre-industrial value (230 ppmV, the mean CO₂ over at least the last 400 k.y. (Petit et al., 1999)). The centerline joining filled circles (10 m.y. time steps) represents the best estimate from the model of Berner (1994, 1998). The two straddling lines represent error estimates based on sensitivity analyses. Boxes in (a) represent 91 non-stomatal-based proxy estimates of varying RCO_2 resolution (data from Suchocky et al., 1988; Platt, 1989; Cerling, 1991, 1992; Freeman and Hayes, 1992; Koch et al., 1992; Muecher et al., 1993; Sinha and Stott, 1994; Andrews et al., 1995; Ghosh et al., 1995; Mora et al., 1996; Yapp and Poths, 1996; Ekart et al., 1999; Elick et al., 1999; Lee, 1999; Lee and Hisada, 1999; Pagani et al., 1999a, 1999b; Pearson and Palmer, 1999). The heavy line in (b) is a five-point running average of the mean RCO_2 of every box in (a). This approach smoothes short-term CO₂ fluctuations and is more directly comparable with the model of Berner (1994, 1998). The dashed line in (b) is a five-point running average incorporating a recalculation of Ekart et al. (1999) data during the late Carboniferous and early Permian using the marine carbonate $\delta^{13}\text{C}$ data of Popp et al. (1986) (see text for details).



(Woodward, 1987). Conversely, a drop in CO₂ requires an increase in stomatal conductance to maintain assimilation rates, but at the cost of increased water loss.

2.1. Stomatal index

Stomatal density (SD) is a function of both the number of stomata plus the size of the epidermal cells. Thus, SD is affected both by the initiation of stomata and the expansion of epidermal cells. This expansion is a function of many variables (e.g. light, temperature, water status, position of leaf on crown, and intra-leaf position), and can overprint the signal reflective of stomatal initiation. As it turns out, CO₂ plays a stronger role in stomatal initiation than in epidermal cell expansion (this is discussed in detail below). Salisbury (1927) introduced the concept of stomatal index (SI), which normalizes for the effects of this expansion (i.e. density of epidermal cells). It is defined as:

$$\text{SI}(\%) = \frac{\text{stomatal density}}{\text{stomatal density} + \text{epidermal cell density}} \times 100$$

where stomata consist of the stomatal pore and two flanking guard cells.

2.2. C₄ plants

The fundamental photosynthetic differences between C₃ and C₄ plants have consequences for stomatal-based CO₂ reconstructions. Carbon in C₃ plants is fixed within the spongy and palisade mesophyll where CO₂ concentrations (c_i) are approximately 70% of the atmospheric value. As atmospheric CO₂ fluctuates, so too does c_i to maintain this ~0.7 ratio (Polley et al., 1993; Ehleringer and Cerling, 1995; Beerling, 1996; Bettarini et al., 1997). Thus the stomatal pore area is sensitive to changing atmospheric CO₂ levels. C₄ plants, in contrast, fix carbon within their bundle sheath cells. The endodermis enclosing these bundle sheath cells is highly impervious to CO₂, and consequently CO₂ concentrations within these cells can reach 1000–2000 ppmV (Lambers et al., 1998). One would therefore anticipate, based on the proposed mechanism between CO₂

and stomatal initiation discussed above, that even moderate changes in atmospheric CO₂ have little influence on stomatal pore area and, by extension, SD and SI (Raven and Ramsden, 1988). Of the nine responses derived from C₄ plants documented here, only one inversely responds to CO₂ (see Appendix A1). This marked insensitivity in C₄ plants lends indirect support for the proposed mechanism. Because of the above physiological reasons, none of the analyses considered here include responses from C₄ plants.

3. Stomatal density and stomatal index as CO₂ indicators

A database consisting of 285 SD responses and 145 SI responses to variable CO₂ concentrations was compiled to elucidate salient patterns (Appendices A1–3). 176 species are represented. This database is an expansion of previous reviews (Beerling and Chaloner, 1994; Woodward and Kelly, 1995) and includes, for the first time, stomatal indices.

Each response was first placed in one of three categories: experimental, subfossil, and fossil. Experimental responses stem from experimentally controlled CO₂ environments, typically in greenhouses, which last from 14 days to five years in length. For studies that measured SD and/or SI at several different times and/or CO₂ levels, typically only the response corresponding to the longest exposure time and highest CO₂ level was used. Most subfossil responses stem from dated herbarium specimens (from the last 240 years), where corresponding CO₂ concentrations are known from ice core data (Neftel et al., 1985; Friedli et al., 1986). Data from altitudinal transects and natural CO₂ springs are also placed in the subfossil category, as this category represents the closest match in terms of CO₂ exposure time. Finally, fossil responses consist of well-dated fossil material. Methods for obtaining reference CO₂ concentrations for the fossil responses are discussed below.

Each response was assigned as either increasing ($P < 0.05$), decreasing ($P < 0.05$), or remaining the same ($P > 0.05$) relative to controls. Where P -values were not reported, a test for overlapping standard deviations was used, which typically yields a conservative estimate for statistical significance (relative to the $\alpha = 0.05$ level).

Table 1
Statistical summary of stomatal responses to changing CO₂ concentrations

	Experimental				Subfossil				Fossil				Combined			
	SD ^a		SI ^b		SD		SI		SD		SI		SD		SI	
	% ^c	(n)	%	(n)	%	(n)	%	(n)	%	(n)	%	(n)	%	(n)	%	(n)
Total	40	(127)	36	(74)	50	(133)	34	(35)	88	(25)	94	(36)	49	(285)	50	(145)
Elevated CO ₂ ^d	39	(109)	29	(65)	–	–	–	–	100	(13)	96	(24)	45	(232)	41	(116)
Subambient CO ₂	50	(18)	89	(9)	–	–	–	–	89	(9)	89	(9)	75	(40)	88	(25)
Opposite response ^e	9	(127)	4	(74)	11	(133)	9	(35)	12	(25)	3	(36)	11	(285)	5	(145)
Hypostomatous ^f	59	(27)	65	(17)	50	(80)	38	(24)	–	–	–	–	56	(121)	69	(70)
Amphistomatous ^g	36	(90)	27	(55)	49	(49)	25	(8)	–	–	–	–	44	(149)	32	(71)
Abaxial	40	(45)	24	(29)	41	(22)	–	–	–	–	–	–	41	(68)	21	(33)
Adaxial	29	(42)	31	(26)	55	(22)	–	–	–	–	–	–	38	(65)	33	(30)
Experiments using OTCs ^h	13	(31)	13	(24)	–	–	–	–	–	–	–	–	–	–	–	–
Experiments using greenhouses	48	(95)	48	(50)	–	–	–	–	–	–	–	–	–	–	–	–
Herbarium studies only	– ^j	–	–	–	57	(93)	89	(9)	–	–	–	–	–	–	–	–
Repeated species ⁱ	–	–	–	–	–	–	–	–	–	–	–	–	57	(28)	55	(11)

^a Stomatal density.

^b Stomatal index.

^c Percentage of responses inversely correlating with CO₂.

^d CO₂ concentrations are higher than controls.

^e Percentage of responses positively correlating with CO₂.

^f Leaves with stomata only on abaxial (lower) side.

^g Leaves with stomata on both surfaces.

^h OTC = open-top chamber; typically cone-shaped with an open top.

ⁱ For species with multiple responses with ≥ 1 inversely correlating with CO₂, percentage that consistently inversely correlate.

^j Not applicable or sample size too small for meaningful comparison.

3.1. Experimental responses

127 SD and 74 SI responses from a pool of 68 species are represented here. For SD, 40% of the experimental responses inversely respond (at the $\alpha = 0.05$ level) to CO₂; the proportion for stomatal indices is similar (36%) (Table 1).

Plants exposed to subambient CO₂ are more likely to inversely respond than plants exposed to elevated CO₂ for both SD (50 vs. 39%; $P = 0.36$) and SI (89 vs. 29%; $P < 0.001$). These results support previous claims that plants more strongly express the CO₂–SD/SI inverse relationship when exposed to subambient versus elevated CO₂ concentrations (Woodward, 1987; Woodward and Bazzaz, 1988; Beerling and Chaloner, 1993a; Kürschner et al., 1997). A common explanation for this CO₂ ‘ceiling’ phenomenon is that plants today have not experienced elevated CO₂ levels (350 + ppmV) for at least the entire Quaternary and possibly longer (Pagani et al.,

1999a; Pearson and Palmer, 1999). Thus, for short time scales where only plant plasticity is tested, plants respond more favorably to CO₂ conditions which they most recently experienced, namely subambient concentrations (Woodward, 1988; Beerling and Chaloner, 1993a). The implication for stomatal-based CO₂ reconstructions is that experimental evidence based on elevated CO₂ treatments may not reflect the reliability of the method. Over 85% of the experimental responses analyzed here stem from elevated CO₂ treatments. Another related concern raised with experimental results is that CO₂ is shifted in one step in contrast to the smoother, longer-term trend in nature (Beerling and Chaloner, 1992; Kürschner et al., 1997).

An alternative explanation for the CO₂ ceiling is that while CO₂ is limiting for photosynthesis at CO₂ concentrations below present-day levels, it is not limiting at elevated levels. Therefore, for example, if CO₂ decreases in a subambient CO₂ regime

(where CO₂ is limiting for photosynthesis), a mechanism exists to increase stomatal pore area and, by extension, CO₂ uptake. The same may not be true at elevated CO₂ concentrations if CO₂ is not limiting for photosynthesis under such conditions (Wagner et al., 1996; Kürschner et al., 1998). Empirical data do not strongly support this alternative hypothesis. While assimilation rates generally decrease at subambient CO₂ levels (Polley et al., 1992; Robinson, 1994), they also typically increase in response to CO₂ concentrations of at least 700 ppmV (Long et al., 1996; Curtis and Wang, 1998). CO₂ therefore usually continues to limit photosynthesis in most plants above present-day CO₂ levels, even if the effects of this excess CO₂ are partially mediated by a reduction in photorespiration and enhancement in RuBP regeneration (the primary substrate used to fix CO₂ in C₃ plants), and so only affect photosynthesis indirectly. Therefore, there is no reason to expect a CO₂ ceiling coincident with current CO₂ levels. It is likely, however, that the rate of change in assimilation rates is reduced at elevated CO₂ concentrations (Farquhar et al., 1980), which could reduce the sensitivity of SD and SI responses under such conditions.

Experimental manipulations are usually conducted in either enclosed greenhouses or open-top chambers (OTCs). Most OTCs have less control over humidity and temperature. Significant ‘chamber effects’ have been detected for stomatal parameters (Knapp et al., 1994; Apple et al., 2000), and results generated here support such claims. Plants in OTCs inversely respond to CO₂ in far fewer cases than greenhouse grown plants for both SD (13 vs. 48%; $P < 0.001$) and SI (13 vs. 48%; $P < 0.01$). Thus, it appears OTCs introduce confounding factors and should be avoided in SD/SI work.

Although the proportion of experimental responses inversely responding to CO₂ may appear low (40 and 36% for SD and SI, respectively), in part from the factors discussed above, it is important to note that the percentage of responses showing a positive relationship ($P < 0.05$) is very low (9 and 4% for SD and SI, respectively). Thus, the vast majority of plants either respond inversely to experimental exposure to CO₂ or do not respond at all.

3.2. Subfossil responses

133 SD and 35 SI responses from a pool of 95 species are represented here. For SD, 50% of the subfossil responses inversely relate (at the $\alpha = 0.05$ level) to CO₂. Thus, subfossil responses, which are based on longer exposure times, more often inversely relate to CO₂ than do experimental responses (50% vs. 40%; $P = 0.11$). For SI, only 34% of the responses show a significant inverse relationship, however the sample size is disproportionately small ($n = 35$) (Table 1).

As outlined above, three types of studies comprise the subfossil responses: altitudinal transects, natural CO₂ springs, and herbaria. If only herbarium responses are analyzed ($n = 93$ and $n = 9$ for SD and SI, respectively), the proportion showing an inverse response to CO₂ improves to 57 and 89%, respectively. Responses from altitudinal transects and natural CO₂ springs may therefore be of less value for paleo-CO₂ reconstructions. This dichotomy in response fidelity may be an expression of the CO₂ ceiling phenomenon discussed above. As CO₂ levels rose to current levels over the last 240 + years, the majority of plants (57 and 89% for SD and SI, respectively) responded with significant decreases in SD and/or SI. At higher CO₂ levels, however, as expressed near natural CO₂ springs, a smaller proportion of plants responded with lower SD (30%; $n = 30$) and/or SI (16%; $n = 25$). If, on the other hand, current CO₂ concentrations do *not* represent a true genetic ceiling for plants, than these data show that the majority of plants cannot adapt to CO₂ levels above today’s within the special residence time near natural CO₂ springs (10²–10³ years?).

In accordance with the experimental responses, a very small proportion of the subfossil observations positively respond to CO₂ (11 and 9% for SD and SI, respectively). Most plants either inversely respond to CO₂ or do not respond at all. If CO₂ exerts any influence on stomatal initiation, it must be of an inverse behavior.

3.3. Fossil responses

25 SD and 36 SI responses from a pool of 28 species are represented here. For SD, 88% of the observations show an inverse relationship (at the $\alpha =$

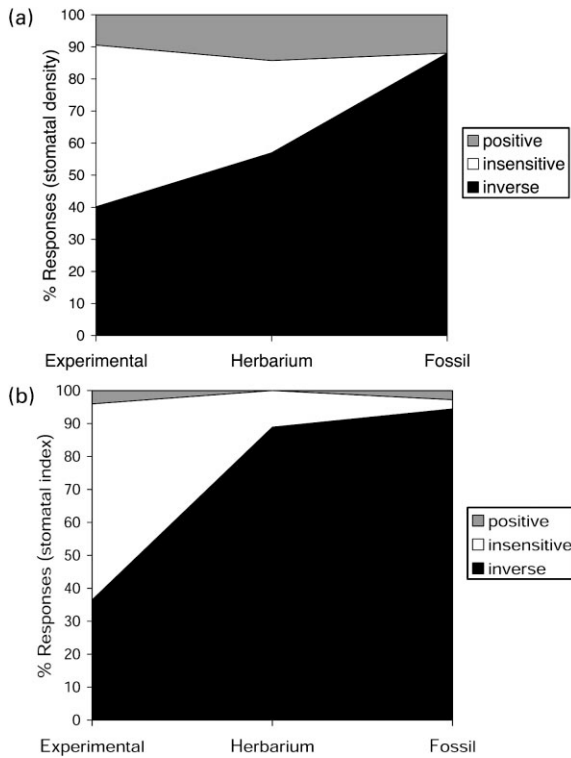


Fig. 2. The percentage of responses for (a) SD and (b) SI that inversely relate to CO₂ ('inverse'), show no significant change to CO₂ ('insensitive'), or respond positively to CO₂ ('positive') in each of three categories. Note that only herbarium responses compose the subfossil category.

0.05 level) to CO₂; for SI, the proportion is 94% (Table 1). Only 12 and 3% of the observations positively respond to CO₂ for SD and SI, respectively. Thus, the robustness of the SD/SI method improves with increased CO₂ exposure time (Fig. 2), supporting earlier hypotheses (Beerling and Chaloner, 1992, 1993a).

Qualitatively, the transition between dominance of stomatal response by plasticity within a given gene pool and genetic adaptation appears to occur for most plants between 10² and 10³ years (i.e. intermediate between CO₂ exposure times typical for subfossil and fossil responses). This conclusion hinges on the assumption that CO₂ exerts a consistent genetic pressure on stomatal initiation, and given sufficient exposure time will overprint the smaller scale plastic responses (including changes in individual stomatal pore size). The fact that the increase in responses

showing an inverse relationship to CO₂ as a function of exposure time comes at the expense of insensitive responses (Fig. 2) supports this assumption. 10² to 10³ years is slightly longer than previous estimates (Beerling and Chaloner, 1993a), and should give rise to some caution in using experimental and sub-fossil responses in paleo-CO₂ reconstructions (i.e. comparing responses due mainly to plasticity versus genetic adaptation).

The fossil data cast doubt on the notion that stomata cannot respond to CO₂ concentrations above present-day levels. The proportion of fossil responses showing an inverse relationship based on subambient CO₂ exposure are nearly equal to those fossil observations based on elevated CO₂ exposure for both SD (89 and 100%, respectively) and SI (89 and 96%, respectively), although sample sizes are fairly small (Table 1). Some groups of plants respond to CO₂ levels of at least 2700 ppmV (McElwain and Chaloner, 1995; Appendix C). This result does not discount, however, that stomatal parameters may be less *sensitive* at elevated than at subambient (relative to today) CO₂ levels. The CO₂ ceiling observed in experimental responses therefore appear to stem from the short-term inability of plants to respond to elevated CO₂, not a long-term genetic limit. Interestingly, Woodward (1988) noted that plants with short generation times (e.g. annuals) are often capable of decreasing their stomatal densities when experimentally exposed to elevated CO₂ levels (for ≥1 year), probably because of their quicker genetic adaptation rates (Woodward, 1988). This suggests that the exposure time required to mitigate the CO₂ ceiling may not be much beyond typical experimental exposure times, and in fact may not exist at all for some plants.

Caution is urged with regard to several features concerning the fossil responses. First, in several studies stomatal comparisons between fossil and modern plants were made with two separate but ecologically equivalent sets of species (McElwain and Chaloner, 1995, 1996; McElwain, 1998; McElwain et al., 1999). In addition to the long-term influence of CO₂ on SD and SI for a given species, it has also been shown, for example, that high CO₂ selects for groups of plants with lower mean stomatal densities/indices (Beerling, 1996; Beerling and Woodward, 1997) (Fig. 3). Thus, it is not particularly surprising that stomatal densities and indices from times of high

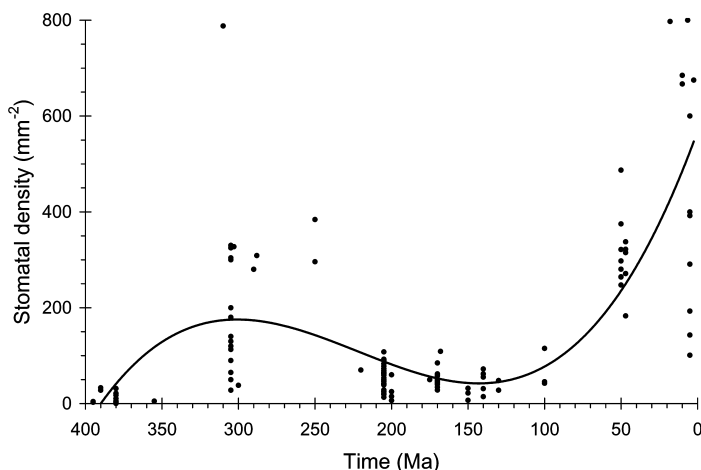


Fig. 3. SD versus time for the Phanerozoic. Redrawn from Beerling and Woodward (1997), with additional data plotted from McElwain and Chaloner (1996), Edwards et al. (1998), McElwain (1998), Cleal et al. (1999) and McElwain et al. (1999). Regression is a third order polynomial ($r^2 = 0.57$; $n = 132$). Compare trend with Fig. 1.

CO₂ are lower than for ecologically equivalent modern species. Ideally, these two effects should be kept separate.

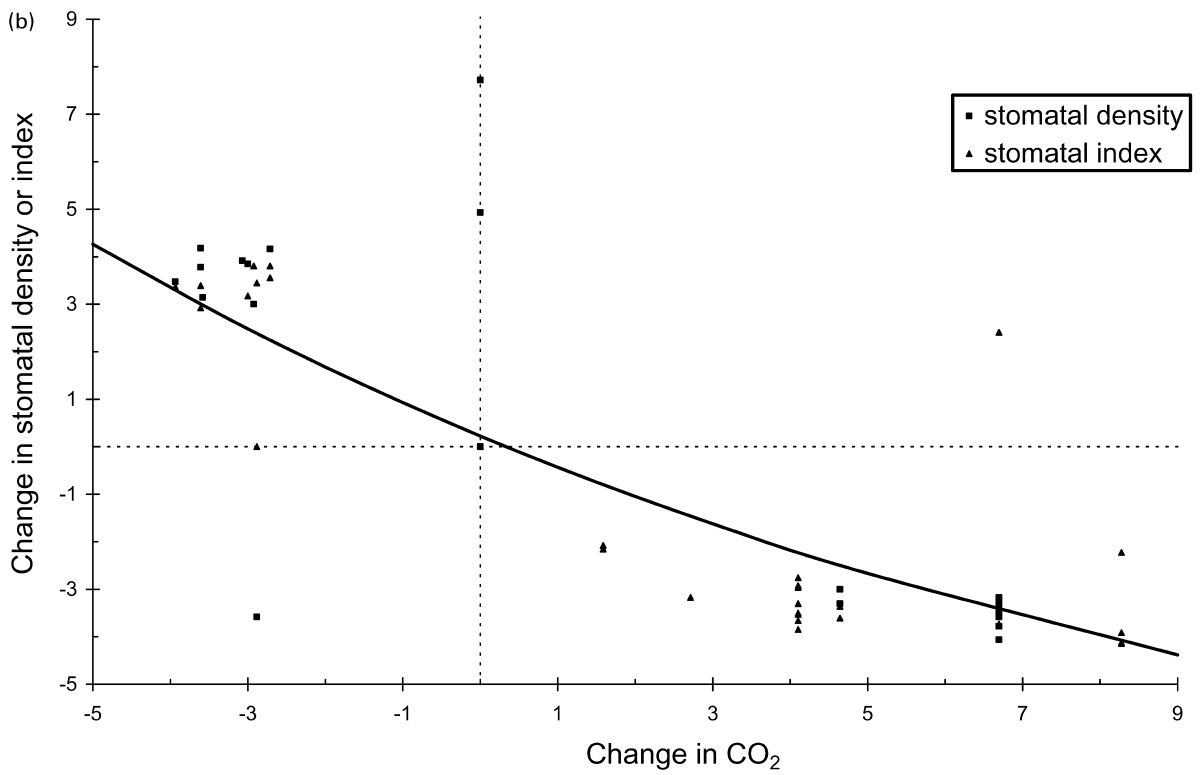
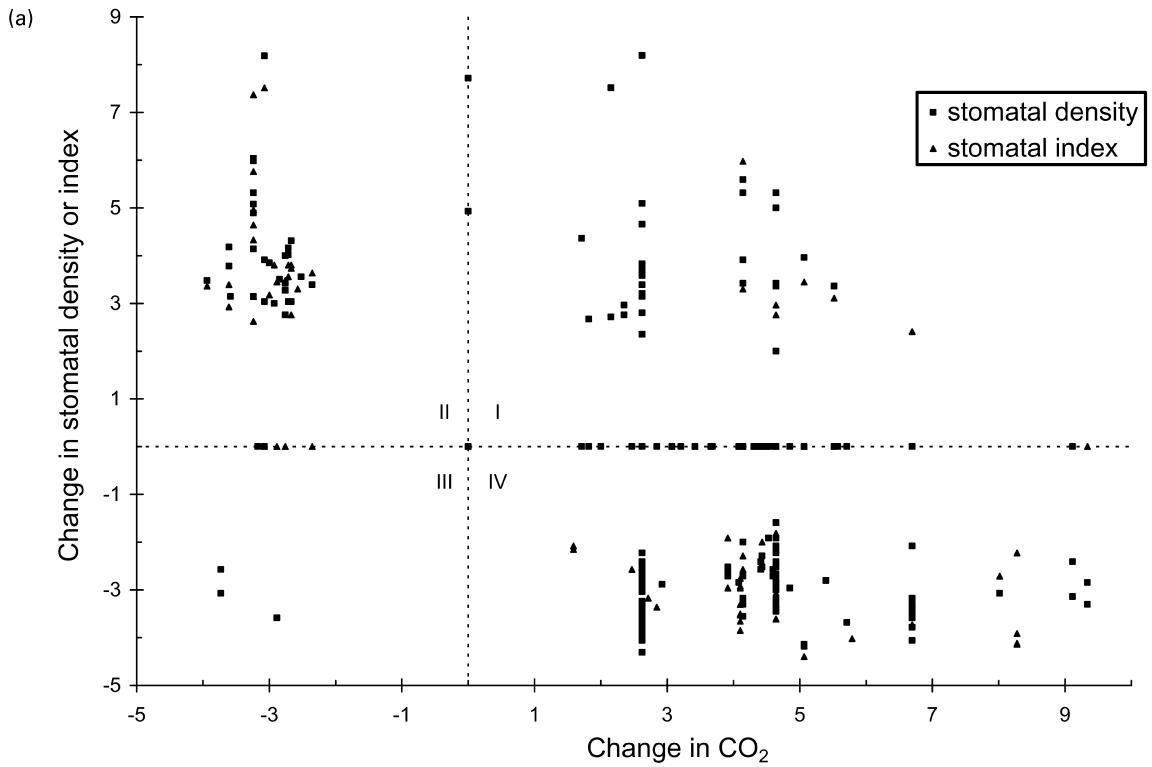
Second, estimates of CO₂ for the fossil responses are invariably not as accurate as those estimates for experimental and subfossil responses. Ice core derived data are used for the last 150 k.y., and the model of Berner (1994) or other proxy data are most often used for pre-ice core responses. In particular, estimates from Berner's curve are highly approximate due to its sizable error envelope and coarse 10 m.y. time resolution (see Fig. 1); brief but large CO₂ excursions discernable with the various proxy methods are probably too temporally constrained to influence Berner's model (Montañez et al., 1999). In cases where experimental and subfossil responses are used to generate a standard curve upon which CO₂ concentrations are directly calculated from fossil responses, ice core data (Beerling et al., 1995; Wagner et al., 1999; Rundgren and Beerling, 1999) or the presence of temperature excursions (van der Burgh et al., 1993; Kürschner, 1996; Kürschner et al., 1996) are used to corroborate the stomatal-based estimates.

3.4. Combined data set

Based on the combination of the above three categories, both SD and SI inversely correlate with CO₂ ca. 50% of the time ($n = 285$ and 145 , respectively) (Table 1). Very rarely do the responses positively correlate with CO₂ (11 and 5% for SD and SI, respectively). For species that have been analyzed repeatedly by different researchers, those that inversely respond to CO₂ tend always to respond in such a way (57% ($n = 28$) and 55% ($n = 11$) for SD and SI, respectively). Woodward and Kelly (1995) reported a similar behavior, where 76% of their sensitive species consistently responded.

Thus, although response times differ (see above and Fig. 2), CO₂ is highly negatively correlated with stomatal initiation. A scatterplot of all data shows an overall inverse relationship between SD/SI and CO₂ (Fig. 4a). Although the overall regression is not robust ($r^2 = 0.26$; $n = 420$), this principally stems from equivocal experimental and natural CO₂ spring data. The fossil data, when regressed independently, yield an r^2 of 0.68 ($n = 59$) (Fig. 4b). Given the species-specific and

Fig. 4. (a) Scatterplot of all data ($r^2 = 0.26$; $n = 420$) showing the cube root transform of percentage change in SD and SI in response to percentage change in atmospheric CO₂ concentration. Responses in quadrants II and IV inversely relate to CO₂ while responses in quadrants I and III positively relate. (b) Similar scatterplot for fossil data only. Regression equation of untransformed data: $y = 112.43\exp(-0.0026x) - 100$. ($r^2 = 0.68$; $n = 59$).



probable multi-mechanistic nature of the relationship, this regression is surprisingly robust.

Curiously, based solely on the combined results, it appears SD is equally reliable as SI as a CO₂ indicator (Table 1). The implications are tempting, as epidermal cells are often difficult to resolve in fossil material (Beerling et al., 1991; McElwain and Chaloner, 1996). This issue is discussed in the section below.

Most vascular land plants have stomata on either both surfaces (amphistomatous) or only the abaxial (lower) surface (hypostomatous). Woodward and Kelly (1995) reported no strong differences in responses between the two leaf types, although in experimental responses amphistomatous species appeared more likely to inversely relate to CO₂. Results here indicate hypostomatous species more often inversely respond to CO₂ for both SD (56 vs. 44%; $P < 0.03$) and SI (69 vs. 32%; $P < 0.001$). For amphistomatous species, neither the abaxial nor adaxial (upper) surface yield statistically different responses (Table 1).

4. Potential confounding factors

CO₂ is likely not the sole factor determining stomatal density and stomatal index. As discussed above, SD is sensitive to both stomatal initiation and epidermal cell expansion, while SI is sensitive only to stomatal initiation. The influence of natural variability, water stress, irradiance, temperature and other factors on stomatal parameters are briefly discussed below. More thorough reviews are given by Salisbury (1927), Tichá (1982) and Woodward and Kelly (1995).

4.1. Natural variability

In general, stomatal density increases from leaf base to tip (Salisbury, 1927; Sharma and Dunn, 1968, 1969; Tichá, 1982; Smith et al., 1989; Ferris et al., 1996; Zacchini et al., 1997; Stancato et al., 1999). SD also often increases from leaf midrib to

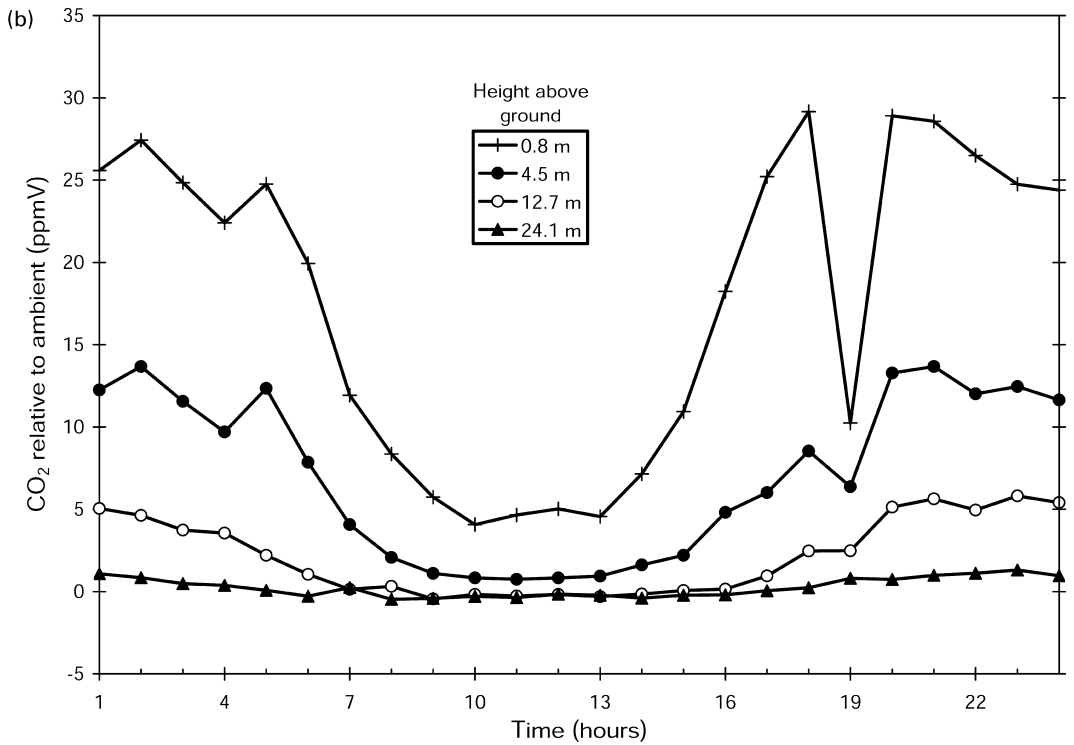
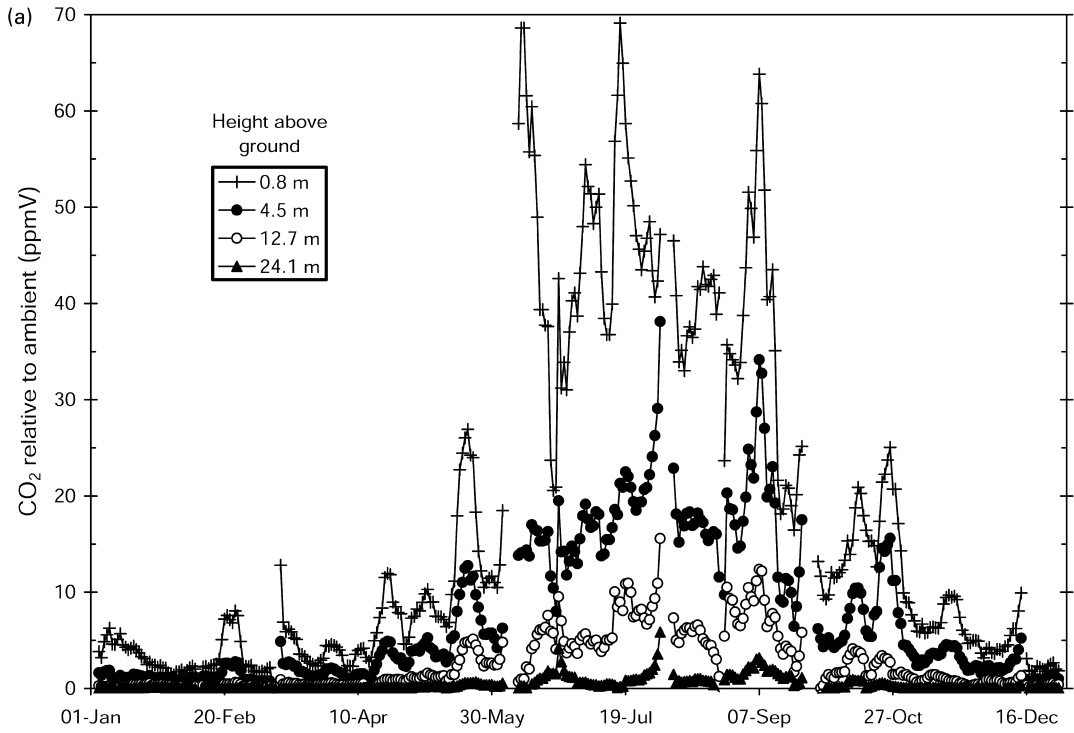
margin (Salisbury, 1927; Sharma and Dunn, 1968; Smith et al., 1989), although sometimes the differences are not significant (Sharma and Dunn, 1969; Tichá 1982). In contrast, very little intra-leaf variation in SI is present (Salisbury, 1927; Rowson, 1946; Sharma and Dunn, 1968, 1969; Rahim and Fordham, 1991), although Poole et al. (1996) found significant intra-leaf variation in *Alnus glutinosa*. For amphistomatous species, the distribution of stomata are generally more uniform on the abaxial surface (Rowson, 1946; Sharma and Dunn, 1968, 1969), and so for all species typically the mid-lamina of the abaxial surface yields the least variation.

Stomatal density also increases from the basal to distal regions of the plant (Salisbury, 1927; Gay and Hurd, 1975; Tichá, 1982; Oberbauer and Strain, 1986; Zacchini et al., 1997), primarily as a consequence of decreased water potential. Decreased water potential stimulates xerophytic traits, which include smaller epidermal cells, which in turn promote closer packing of stomata, and thus increased SD. Little effect is reported for SI (Rowson, 1946), although evergreen species may exhibit a significant gradient (Kürschner, W.M., personal communication, 2000). Conflated with this trend are the differences between sun and shade leaves. Again, SD is consistently higher in sun leaves while SI values remain conservative (Salisbury, 1927; Poole et al., 1996; Kürschner, 1997; Wagner, 1998) with the exception of the study of Poole et al. (1996), who found a small 7% decline in SI for shade versus sun leaves. For fossil studies, since sun leaves in allochthonous assemblages are preferentially preserved (Spicer, 1981), this issue is often not a concern even for SD-based work. For example, Kürschner (1997) observed that 90% of his Miocene *Quercus pseudocastanea* leaves were sun morphotypes.

4.2. Water stress

In general, water stress correlates with increased SD (Salisbury, 1927; Sharma and Dunn, 1968, 1969; Tichá, 1982; Abrams, 1994; Estiarte et al., 1994;

Fig. 5. (a) CO₂ relative to ambient concentrations for four heights within a tree canopy in 1996. Canopy height is ca. 24 m. Ordinate represents seven day running average of daily averages of hourly measurements at each height ($n = 5311$ for each height). Measurements at 29.0 m height taken as ambient value (mean for time interval at this height = 370 ppmV). (b) Diurnal trend of CO₂ relative to ambient concentrations (data from 9 April–13 July 1996). Ordinate represents mean for each hour at each height ($n = 1388$ for each height). Standard errors approximate size of symbols. Raw data used with permission of S. Wofsy.



Clifford et al., 1995; Heckenberger et al., 1998; Pääkkönen et al., 1998). Some studies, however, report no response (Estiarte et al., 1994; Dixon et al., 1995; Pritchard et al., 1998; Centritto et al., 1999). No studies report a decrease. SI consistently appears insensitive to water stress (Salisbury, 1927; Sharma and Dunn, 1968, 1969; Estiarte et al., 1994; Clifford et al., 1995).

Salisbury (1927) proposed humidity as a mechanism for controlling stomatal initiation, and thus SI. Increased humidity slightly increased SI ($P > 0.05$) for *Scilla nutans*, however, Tichá (1982) concluded that humidity may actually reduce stomatal index. Sharma and Dunn (1968, 1969) found no effect. Thus, the current data are equivocal.

4.3. Irradiance

Not surprisingly, light intensity usually positively correlates with SD (Sharma and Dunn, 1968, 1969; Gay and Hurd, 1975; Tichá, 1982; Oberbauer and Strain, 1986; Solárová and Pospíšilová, 1988; Stewart and Hoddinott, 1993; Ashton and Berlyn, 1994; Furukawa, 1997; Zacchini et al., 1997). This response is driven (partially) by enhanced water stress. Light intensity may also positively affect SI (Sharma and Dunn, 1968, 1969; Furukawa, 1997), although some report no response (Salisbury, 1927; Sharma and Dunn, 1968, 1969) and Rahim and Fordham (1991) observed a small decrease with increasing irradiance. In the case of Sharma and Dunn (1968, 1969), they speculated that the low irradiance levels required to depress SI could not sustain plants in a competitive environment.

In experimental manipulations, photoperiod strongly affects both SD and SI (Schoch et al., 1980; Zacchini et al., 1997). Schoch et al. (1980) observed that even one day of low irradiance levels during the critical period of stomatal initiation could affect SD and SI. Given that SI is typically conservative in deciduous species within a given crown, it is possible the effects of photoperiod on SI observed in experiments are minimal in nature.

4.4. Temperature

Temperature appears positively correlated with SD (Ferris et al., 1996; Reddy et al., 1998; Wagner, 1998; but see Apple et al., 2000), a likely consequence of

enhanced water stress. Temperature may also affect SI (Ferris et al., 1996; Wagner, 1998), suggesting an influence on stomatal initiation. Reddy et al. (1998), however, found no response. The influence of temperature on stomatal initiation may be inconsequential, though, as most plants partially normalize for fluctuating temperatures by adjusting the timing of leaf development, and so the temperature at which stomata form remains fairly constant (Wagner, 1998).

4.5. Canopy CO₂ gradient

If CO₂ concentrations within canopies deviate significantly from ambient concentrations, CO₂ estimates based on stomatal parameters could be skewed. Empirical evidence, however, does not suggest such large deviations. Hourly measurements of CO₂ at eight different heights (0.3, 0.8, 4.5, 7.5, 12.7, 18.3, 24.1 and 29.0 m above the ground surface) have been recorded for several consecutive years from an atmospheric tower in the Harvard Forest (data available at <http://www-as.harvard.edu/chemistry/hf/profile/profile.html>). This forest, in north central Massachusetts, USA, consists of mixed hardwoods and conifers. As shown in Fig. 5a, above 4.5 m, where the bulk of leaves from mature trees form, canopy CO₂ levels are virtually indistinguishable from ambient levels. Furthermore, all deviations diminish during the middle of the day (Fig. 5b), a period when cell respiration and division is highest. Thus, CO₂ gradients within canopies are likely not strong enough to influence stomatal initiation rates.

4.6. Paleotaxonomy

Paleobotanical species identification via morphological comparison with modern representatives is often tenuous, particularly with pre-Neogene material. There are methods, however, to bolster confidence in such morphologically based species identification. These include comparing the sedimentological and ecological contexts with the proposed extant representative. For example, if a strictly swamp margin fossil species is morphologically identical to a modern representative that is also restricted to swamp margins, then one can be more confident that the two are identical species. Independent of species identification, however, it is also possible that a single species may develop, for example, different stomatal

behaviors through time. This, in turn, could affect paleo-CO₂ reconstructions. One way to circumvent this problem is through the study of the species' closest extant sister group (e.g. de Queiroz and Gauthier, 1990). If the stomata in both extant species show a similar response to CO₂, then it can be assumed that this stomatal behavior in both species is conservative in time back to at least when the species branched.

4.7. Other potential confounding factors

Through the comparison of 100 species, neither growth form (woodiness vs. non-woodiness; trees vs. shrubs), habitat (cool vs. warm), nor taxonomic relatedness strongly correlated with SD (Woodward and Kelly, 1995). Habitat has also been found not to affect SI (Rowson, 1946). Analysis of the data set presented here shows that for genera represented by >1 species, only 19% ($n = 16$) and 14% ($n = 7$) of these genera respond in a consistent fashion to CO₂ (i.e. positive, negative, or insensitive) for SD and SI, respectively. These results provide further support for the taxonomic independence of stomatal responses to CO₂.

An increase in ploidy level is associated with lower SD (Rowson, 1946; Mishra, 1997). No clear trend is found in SI (based on two studies), with Rowson (1946) reporting a decrease and Mishra (1997) no change. Given the widespread variability of ploidy levels in the fossil record (Masterson, 1994), this may have important consequences for stomatal-based CO₂ reconstructions.

Elevated levels of ozone increase SD in *Betula pendula* (Frey et al., 1996; Pääkkönen et al., 1998), *Fraxinus excelsior* (Wiltshire et al., 1996) and *Olea europaea* (Minnocci et al., 1999). Effects on SI were not reported.

Although largely untested, atmospheric oxygen may influence SD and SI. Elevated O₂/CO₂ ratios increase photorespiration in C₃ plants, suppressing CO₂ assimilation rates. One pathway to mediate this decline is increasing SD and/or SI. Experimental work on *Hedera helix* and *Betula pubescens* show slightly higher stomatal indices in a 35% versus 21% O₂ atmosphere (Beerling et al., 1998b). If correct, this factor may be particularly important during the Carboniferous and early Permian when O₂ concentrations are

modeled to exceed 30% (Berner and Canfield, 1989; Berner et al., 2000).

5. Summary

Based on the data presented here, nearly all species appear responsive on the time scales inherent in most fossil CO₂ reconstructions (>10² years) (Fig. 2; Table 1). Only 40–50% of species are responsive over the time scales of experimental and subfossil studies (~10⁻²–10² years), and so those conducting studies requiring such responses must take care in choosing sensitive species (Appendices A and B). Another potential weakness in utilizing experimental and subfossil responses is that they are more reflective of plasticity within given gene pools, and may display different behaviors than their respective fossil responses (which are more reflective of genetic adaptation).

SD and SI are both equally likely to inversely relate to CO₂. SD, however, is sensitive to factors affecting cell expansion such as water stress, temperature, and irradiation. SI, in contrast, is sensitive only to factors affecting cell initiation, of which CO₂ appears to be one factor. Thus, even if SD and SI show similar responses for a given species (e.g. both positive or negative), SI should yield more accurate CO₂ estimates.

5.1. Applications of method

Although experimental work has been carried out for many years, Woodward (1987) was the first to document the inverse CO₂–SD/SI relationship over longer time scales (200 years). Beerling et al. (1991, 1993) extended the applicability of the method to 140 k.y. with *Salix herbacea*, where stomatal densities showed a general inverse relationship with ice core reconstructed CO₂ concentrations. This method has also proven successful with 9.2 ka *Salix cinerea* (McElwain et al., 1995), 13 ka *Betula nana* (Beerling, 1993), and 28 ka *Pinus flexilis* (van de Water et al., 1994).

While the above studies validate the relationship over time scales useful for fossil studies, they do not generate independent estimates of paleo-CO₂. For this, fossil responses must be fitted to a standard curve based on experimental, subfossil, and fossil

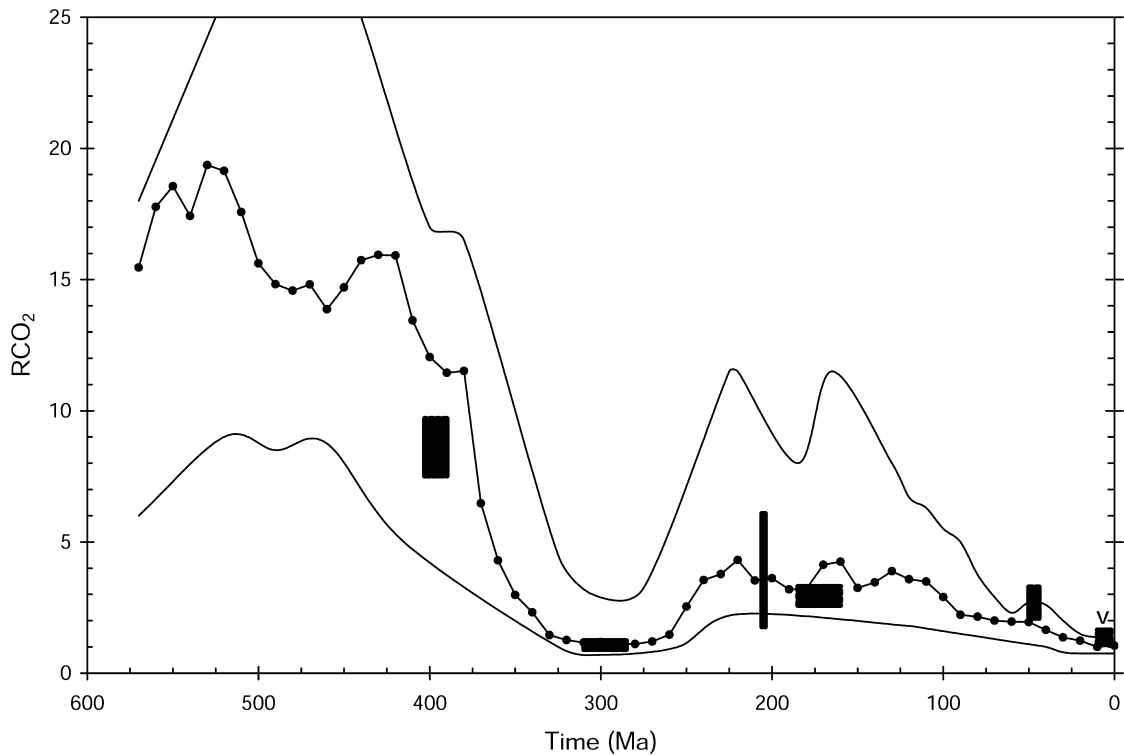


Fig. 6. Estimates of CO_2 for the Devonian, Carboniferous–Permian, Triassic, Jurassic, and Eocene (unmarked boxes) calculated from the stomatal ratio technique of McElwain and Chaloner (1995) superimposed over the CO_2 curve and corresponding error envelope of Berner (1994, 1998). Stomatal ratio scale calibrated to RCO_2 scale using a 1:1 correspondence; this is the ‘Recent standard’ of McElwain (1998). RCO_2 and stomatal ratio defined in Fig. 1 and text, respectively. Data from McElwain (1998) and McElwain et al. (1999). Estimates of CO_2 for the Miocene (“v”) calculated from a herbaria-based training set. Data from van der Burgh et al. (1993) and Kürschner et al. (1996).

responses (from the last 400 k.y., for which ice core data exist; e.g. Petit et al., 1999) of the *same* species. This approach has been successful in the Holocene with *Salix herbacea* (Beerling and Chaloner, 1993a; Beerling et al., 1995; Rundgren and Beerling, 1999) and *Betula pubescens* and *B. pendula* (Wagner et al., 1999), and in the Miocene with *Quercus petraea* and *Betula subpubescens* (van der Burgh et al., 1993; Kürschner, 1996; Kürschner et al., 1996). While this approach produces the most accurate CO_2 reconstructions, it is limited by its requirement to compare identical species (or highly similar species within a genus; Wagner et al., 1999). There are, however, single species spanning most or all of the late Cretaceous and Tertiary (e.g. *Ginkgo adiantoides/biloba*, several members of Taxodiaceae), and so CO_2 estimates for this interval are possible.

One clear advantage of the stomatal method over

other proxies and models is its high temporal resolution. The temporal resolution of late Quaternary fossil material often exceeds that of ice cores (Beerling et al., 1995; Wagner et al., 1999), and similar high resolution data have been used to document CO_2 excursions across the Allerød/Younger Dryas (Beerling et al., 1995) and Triassic/Jurassic (McElwain et al., 1999) boundaries. Another advantage over other proxies and models is its higher level of precision (compare Fig. 1a with Fig. 6).

Estimating pre-Cretaceous CO_2 levels proves more difficult. McElwain and Chaloner (1995) developed a technique comparing responses of fossil species to those of their Nearest Living Equivalents (NLEs). NLEs are defined ecologically, not taxonomically, and represent the ecologically closest living analog to the fossil species. SI ratios of the fossils:NLEs were calculated, and in order to estimate CO_2 the

Carboniferous:NLE stomatal ratio was normalized to the Phanerozoic CO₂ curve of Berner (1994), with the remainder of the ratios scaled accordingly in a linear fashion. Given that this method assumes a linear response and is not a true independent CO₂ indicator, reconstructed CO₂ concentrations from the Devonian, Carboniferous, Permian, and Jurassic all matched Berner's values remarkably well (McElwain and Chaloner, 1995, 1996). Later (McElwain, 1998), in order to reduce the method's dependence on Berner (1994), data (including new material from the Eocene) were plotted assuming a 1:1 correspondence between stomatal ratios and RCO₂ (RCO₂ = ratio of mass of paleo-CO₂ to pre-industrial value; see Fig. 1). Using this more independent technique, all but the Devonian material agreed well with the estimates of Berner (1994). Recent changes in Berner's model, however, have pushed back the sharp drop in Paleozoic CO₂ ~40 m.y. (Berner, 1998), resulting in closer agreement between the two methods for the Devonian (Fig. 6).

There is growing interest in quantifying Tertiary CO₂ concentrations (Cerling et al., 1997; Pagani et

al., 1999a, 1999b; Pearson and Palmer, 1999), primarily fueled by the question of whether CO₂ and temperature are coupled during this interval. Estimates from stomatal indices have the potential to help resolve this question. As for pre-Cretaceous estimates, the less quantitative stomatal ratio method of McElwain and Chaloner (1995) remains the best option.

Acknowledgements

I thank W.M. Kürschner, W.G. Chaloner, D.J. Beerling, L.J. Hickey, J. Carroll, and J.C. McElwain for constructive comments on the manuscript. I thank R.A. Berner for constructive comments and help in drafting Figs. 1 and 6. I also thank L.M. Huggins for providing an age for his Miocene material. I am grateful to S. Wofsy, B. Munger, M. Goulden, and C. Barford for providing canopy CO₂ data from Harvard Forest. This work was funded by DOE grant DE-FGO₂-95ER14522, a NSF Graduate Research Fellowship, and a Yale University Fellowship.

Appendix A1

Experimental stomatal responses

Experiment length (days)	CO ₂ levels (relative to controls ^a)	Species used	Side of leaf	SD response	SI response	Source
?	↑ 300%	<i>Phaseolus vulgaris</i>	abaxial adaxial	* ↓ 9% ↔	–	O'Leary and Knecht, 1981 ^b
14	↑ 2067%	<i>Marsilea vestita</i>	abaxial adaxial	* ↓ 91% ↔	–	Bristow and Looi, 1968 ^b
	↑ ~10 ⁵ %	<i>Marsilea vestita</i>	abaxial adaxial	* ↓ 99% ↔	–	
15	↑ 100%	<i>Populus euroamericana</i>	–	↑ 38%	↔	Gaudillère and Mousseau, 1989 ^b
20	↑ 80%	<i>Phaseolus vulgaris</i>	abaxial adaxial	↔ ↔	↔ ↔	Ranasinghe and Taylor, 1996 ^b
21	↑ 86%	<i>Tradescantia (fluminensis?)</i>	abaxial	↔	↔	Boetsch et al., 1996 ^b
21	↓ 29%	<i>Vaccinium myrtillus</i>	abaxial adaxial	↔ ↔	↔ ↔	Woodward, 1986 ^b
	↑ 29%	<i>Vaccinium myrtillus</i>	abaxial adaxial	* ↑ 548% ↔ ↔	* ↑ 424% ↔ ↔	
21	↓ 34%	<i>Acer pseudoplatanus</i> <i>Geum urbanum</i>	abaxial abaxial adaxial	* ↑ 220% * ↑ 31% * ↑ 214%	* ↑ 122% * ↑ 18% * ↑ 191%	Woodward and Bazzaz, 1988 ^b
		<i>Quercus robur</i> <i>Rhamnus catharticus</i> <i>Rumex crispus</i>	abaxial abaxial abaxial adaxial	* ↑ 131% * ↑ 117% * ↑ 71% * ↑ 150%	* ↑ 81% * ↑ 100% * ↑ 31% * ↑ 400%	

Appendix A1 (continued)

Experiment length (days)	CO ₂ levels (relative to controls ^a)	Species used	Side of leaf	SD response	SI response	Source
	↑ 100%	<i>Amaranthus retroflexus</i>	abaxial	* ↓ 35%	* ↓ 23%	
			adaxial	* ↓ 38%	* ↓ 26%	
		<i>Ambrosia artemisiifolia</i>	abaxial	* ↓ 11%	↔	
			adaxial	* ↓ 24%	* ↓ 25%	
		<i>Setaria faberii</i>	abaxial	↔	* ↓ 21%	
			adaxial	* ↓ 22%	* ↓ 21%	
2199–35	↑ 94%	<i>Lolium perenne</i>	adaxial	↔	–	Ryle and Stanley, 1992 ^b
26	↑ 86%	<i>Lycopersicum esculentum</i>	abaxial	* ↓ 17%	↔	Madsen, 1973 ^b
			adaxial	* ↓ 14%	↔	
	↑ 814%	<i>Lycopersicum esculentum</i>	abaxial	* ↓ 23%	↔	
			adaxial	* ↓ 36%	↔	
~28	↑ 33%	<i>Lolium temulentum</i>	adaxial	↔	–	Gay and Hauck, 1994 ^b
28	↑ 100%	<i>Phaseolus vulgaris</i>	abaxial	↔	↔	Radoglou and Jarvis, 1992 ^c
			adaxial	↔	↔	
~40	↑ 91%	<i>Raphanus raphanistrum</i>	abaxial	↔	↔	Case et al., 1998 ^b
45	↑ 71%	<i>Anthyllis vulneraria</i>	abaxial	* ↓ 32%	* ↓ 17%	Ferris and Taylor, 1994 ^b
			adaxial	↔	↔	
		<i>Lotus corniculatus</i>	abaxial	↑ 60%	↔	
			adaxial	↑ 40%	↔	
		<i>Plantago media</i>	abaxial	* ↓ 20%	↔	
			adaxial	* ↓ 36%	* ↓ 12%	
		<i>Sanguisorba minor</i>	abaxial	↑ 175%	↑ 36%	
			adaxial	↑ 150%	↑ 213%	
45	↑ 100%	<i>Vicia faba</i>	abaxial	↔	↔	Radoglou and Jarvis, 1993 ^c
			adaxial	↔	↔	
45	↑ 168%	<i>Glycine max</i>	abaxial	↑ 38%	↔	Thomas and Harvey, 1983 ^c
			adaxial	↔	↔	
		<i>Liquidambar styraciflua</i>	abaxial	↔	↑ 30%	
		<i>Zea mays</i> (C ₄)	abaxial	↔	↔	
			adaxial	↔	↔	
~50	↑ 68%	<i>Anthyllis vulneraria</i>	abaxial	* ↓ 23%	↔	Bryant et al., 1998 ^c
		<i>Sanguisorba minor</i>	abaxial	↔	↔	
		<i>Bromopsis erecta</i>	abaxial	↔	↔	
50	↓ ~32%	<i>Avena sativa</i>	abaxial	↔	–	Malone et al., 1993 ^c
			adaxial	↔	–	
		<i>Prosopis glandulosa</i>	abaxial	↔	–	
			adaxial	↔	–	
		<i>Schizachyrium scoparium</i> (C ₄)	abaxial	↔	–	
		<i>Triticum aestivum</i>	abaxial	↔	–	
			adaxial	↔	–	
54	↑ 93%	<i>Boehmeria cylindrica</i>	–	↔	–	Woodward and Beerling, 1997 ^b
56	↑ 100%	<i>Coleus blumei</i>	abaxial	* ↓ 9%	* ↓ 4%	Beerling and Woodward, 1995 ^b
		<i>Tropaeolum majus</i>	abaxial	* ↓ 4%	* ↓ 10%	
59	↑ 186%	<i>Pelargonium hortorum</i>	abaxial	↔	–	Kelly et al., 1991 ^b
			adaxial	* ↓ 50%	–	
60	↓ 29%	<i>Salix herbacea</i>	combined	* ↑ 28%	–	Beerling et al., 1995 ^b
	↑ 100%		combined	* ↓ 41%	–	
60	↑ 93%	<i>Ochroma lagopus</i>	abaxial	↔	–	Oberbauer et al., 1985 ^b
			adaxial	↔	–	
63	↑ 157%	<i>Panicum tricanthum</i>	abaxial	* ↓ 22%	–	Tipping and Murray, 1999 ^b
		<i>Panicum antidotale</i> (C ₄)	abaxial	↑ 28%	–	

Appendix A1 (continued)

Experiment length (days)	CO ₂ levels (relative to controls ^a)	Species used	Side of leaf	SD response	SI response	Source
< 66	↑ 100%	<i>Gossypium hirsutum</i>	abaxial adaxial	↔ ↔	↔ ↔	Reddy et al., 1998 ^b
72	↑ 89%	<i>Lolium perenne</i>	abaxial	↑	* ↓	Ferris et al., 1996 ^c
80	↑ 100%	<i>Betula pendula</i>	abaxial	↔	↔	Wagner, 1998 ^b
90	↑ 100%	<i>Quercus ilex</i>	abaxial	* ↓ 27%	–	Paoletti et al., 1997 ^b
90–120	↑ 100%	<i>Andropogon gerardii</i> (C ₄)	abaxial adaxial	* ↓ 28% ↑ 75%	– –	Knapp et al., 1994 ^c
		<i>Salvia pitcheri</i>	abaxial adaxial	↑ 40% ↑ 125%	– –	
92	↑ 100%	<i>Populus trichocarpa</i>	abaxial adaxial	↔ ↔	↔ ↔	Radoglou and Jarvis, 1990 ^b
93	52%	<i>Oryza sativa</i>	abaxial adaxial	↓ 29% ↓ 17%	– –	Rowland-Bamford et al., 1990 ^b
	↑ 173%	<i>Oryza sativa</i>	abaxial adaxial	↔ ↔	– –	
105	↑ 186%	<i>Pelargonium hortorum</i>	abaxial adaxial	↔ ↔	– –	Kelly et al., 1991 ^b
114	↑ 87%	<i>Arachis hypogaea</i>	abaxial adaxial	* ↓ 12% * ↓ 16%	↔ * ↓ 8%	Clifford et al., 1995 ^b
120	↑ 757%	<i>Rhizophora mangle</i> <i>Laguncularia racemosa</i> <i>Musa apiculata</i>	abaxial abaxial abaxial adaxial	* ↓ 14% * ↓ 31% ↔ ↔	– – – –	Beerling, 1994 ^b
120	↑ 100%	<i>Populus trichocarpa</i> <i>Populus deltoides</i>	abaxial adaxial adaxial	* ↓ 19% ↔ * ↓ 27% * ↓ 33%	* ↓ 31% ↔ * ↓ 36% ↔	Ceulemans et al., 1995 ^c
120	↑ 100%	<i>Quercus petraea</i>	abaxial	* ↓ 25%	* ↓ 14%	Kürschner et al., 1998 ^b
123	↑ 93%	<i>Pentaclethra macroloba</i>	abaxial adaxial	* ↓ 7% ↔	– –	Oberbauer et al., 1985 ^b
125	↑ 49%	<i>Triticum aestivum</i>	abaxial adaxial	↔ ↔	↔ ↔	Estiarte et al., 1994 ^c
135	↑ 100%	<i>Prunus avium</i>	abaxial	↔	–	Centritto et al., 1999 ^c
150	↑ 100%	<i>Chlorophytum picturatum</i> <i>Hedera helix</i> <i>Hypoestes variegata</i>	abaxial abaxial abaxial	* ↓ 7% * ↓ 10% * ↓ 9%	* ↓ 23% * ↓ 29% * ↓ 6%	Beerling and Woodward, 1995 ^b
217	↑ 100%	<i>Maranthus corymbosa</i>	abaxial	* ↓ 14%	–	Eamus et al., 1993 ^b
~240	↑ 98%	<i>Picea sitchensis</i>	abaxial	↔	–	Barton and Jarvis, 1999 ^b
270	↑ 114%	<i>Pinus banksiana</i>	–	↔	–	Stewart and Hoddinott, 1993 ^b
300	↑ 97%	<i>Eucalyptus tetrodonta</i>	abaxial	* ↓ 20%	–	Berryman et al., 1994 ^{b,c}
~365	↑ 71%	<i>Rumex obtusifolius</i>	abaxial adaxial	* ↓ 8% ↔	– –	Pearson et al., 1995 ^b
~400	↑ 100%	<i>Rhizophora mangle</i>	abaxial	* ↓ 16%	↔	Farnsworth et al., 1996 ^b
~425	↑ 71%	<i>Bromus erectus</i> <i>Plantago media</i>	abaxial adaxial abaxial adaxial	↔ ↔ ↔ ↔	↔ ↔ ↔ ↔	Lauber and Körner, 1997 ^c
		<i>Sanguisorba minor</i>	abaxial	↔	↔	
570	↑ 100%	<i>Prunus avium</i> <i>Quercus robur</i>	abaxial abaxial	↔ ↑ ~150%	– –	Atkinson et al., 1997 ^b
600	↑ 97%	<i>Pinus palustris</i>	–	↔	–	Pritchard et al., 1998 ^c

Appendix A1 (continued)

Experiment length (days)	CO ₂ levels (relative to controls ^a)	Species used	Side of leaf	SD response	SI response	Source
~730	↑ 100%	<i>Picea abies</i>	–	↔	–	Dixon et al., 1995 ^c
		<i>Quercus rubra</i>	abaxial	↑ 8%	–	
730	↑ 60%	<i>Tussilago farfara</i>	abaxial	–	* ↓ 26%	Beerling and Woodward, 1997 ^b
750	↑ 97%	<i>Mangifera indica</i>	abaxial	* ↓ 17%	–	Goodfellow et al., 1997 ^b
~840	↑ 99%	<i>Scirpus olneyi</i>	–	↔	–	Drake, 1992 ^c
3 years	↑ 60%	<i>Pinus sylvestris</i>	abaxial	* ↓ 16%	–	Beerling, 1997 ^b
			adaxial	* ↓ 18%	–	
3 years	↑ 60%	<i>Ginkgo biloba</i>	abaxial	* ↓ 20%	* ↓ 7%	Beerling et al., 1998a ^b
1155	↑ 50%	<i>Pseudotsuga menziesii</i>	abaxial	↔	–	Apple et al., 2000 ^b
~5 years	↑ ~82%	<i>Citrus aurantium</i>	abaxial	↔	↔	Estiarte et al., 1994 ^c
Meta-analysis		43 species (60% showed SD reductions)		* ↓ (9.0 ± 3.3% s.e.)	–	Woodward and Kelly, 1995

* response inversely relates ($P < 0.05$) to CO₂ concentration.

↔ no significant change ($P > 0.05$).

– not reported.

^a Typically between 340 and 360 ppmV.

^b Plants grown in enclosed greenhouses or chambers.

^c Plants grown in open-top chambers (OTCs).

Appendix A2

Subfossil stomatal responses

Age of material (years)	CO ₂ levels (relative to controls ^a)	Species used	Side of leaf	SD response	SI response	Source
#	↓ 5%	<i>Salix herbacea</i>	abaxial	↔	–	Beerling et al., 1992
			adaxial	* ↑ 83%	–	
#	↓ 13%	<i>Eucalyptus pauciflora</i>	combined	* ↑ 26%	–	Körner and Cochrane, 1985
#	↓ 6%	<i>Griselinia littoralis</i>	combined	↔	–	Körner et al., 1986
	↓ 13%	<i>Nothofagus menziesii</i>	abaxial	* ↑ 21%	–	
	↓ 8%	<i>Ranunculus grahamii</i>	combined	↔	–	
#	↓ 10%	<i>Vaccinium myrtillus</i>	abaxial	↓ 20%	–	Woodward, 1986
			adaxial	* ↑ 425%	–	
#	↓ 6%	<i>Nardus stricta</i>	abaxial	↔	–	Woodward and Bazzaz, 1988
			adaxial	* ↑ 19%	–	
@	↑ 194%	<i>Tussilago farfara</i>	abaxial	–	* ↓ 65%	Beerling and Woodward, 1997
@	↑ 100%	<i>Scirpus lacustris</i>	–	* ↓ 19%	–	Bettarini et al., 1997
@	↑ 100%	<i>Allium sphaerocephalon</i>	abaxial	↔	–	Bettarini et al., 1998
		<i>Buxus sempervirens</i>	abaxial	↔	↔	
		<i>Convolvulus arvensis</i>	abaxial	↔	↑ 26%	
		<i>Convolvulus cantabrica</i>	abaxial	↔	↔	
		<i>Conyza canadensis</i>	abaxial	* ↓ 26%	↑ 21%	
		<i>Fraxinus ornus</i>	abaxial	* ↓ 35%	↔	
		<i>Geranium molle</i>	abaxial	↔	↔	

Appendix A2 (continued)

Age of material (years)	CO ₂ levels (relative to controls ^b)	Species used	Side of leaf	SD response	SI response	Source
		<i>Globularia punctata</i>	abaxial	↔	↔	
			adaxial	↔	↔	
		<i>Hypericum perforatum</i>	abaxial	↔	↔	
		<i>Plantago lanceolata</i>	abaxial	↔	↔	
			adaxial	↔	↔	
		<i>Potentilla reptans</i>	abaxial	↔	↔	
		<i>Pulicaria sicula</i>	abaxial	↔	↔	
		<i>Ruscus aculeatus</i>	abaxial	↔	–	
		<i>Scabiosa columbaria</i>	abaxial	↔	↔	
		<i>Silene vulgaris</i>	abaxial	↔	↔	
		<i>Stachys recta</i>	abaxial	* ↓ 11%	↔	
		<i>Trifolium pratense</i>	abaxial	↔	↔	
@	↑ ~130%	<i>Bauhinia multinervia</i>	abaxial	↑ 62%	↑ 41%	Fernández et al., 1998
			adaxial	* ↓ 71%	* ↓ 73%	
		<i>Spathiphyllum cannifolium</i>	abaxial	↔	↔	
			adaxial	* ↓ 72%	* ↓ 85%	
@	↑ 40%	<i>Quercus pubescens</i>	abaxial	↔	↔	Miglietta and Rasci, 1993
@	↑ 515%	<i>Arbutus unedo</i>	abaxial	* ↓ 29%	* ↓ 20%	Jones et al., 1995
@	↑ 114%	<i>Quercus ilex</i>	abaxial	* ↓ 26%	–	Paoletti et al., 1998
@	↑ 50%	<i>Boehmeria cylindrica</i>	abaxial	↔	–	Woodward and Beerling, 1997
@	↑ ~71%	<i>Phragmites australis</i>	abaxial	↔	–	van Gardingen et al., 1997
			adaxial	* ↓ 45%	–	
37	↑ 15% ^b	<i>Metasequoia glyptostroboides</i>	abaxial	↔	* ↓ 17%	D.L. Royer, unpublished data
43	↑ 15% ^b	<i>Betula pendula</i>	abaxial	* ↓ 30%	* ↓ 32%	Wagner et al., 1996
70	↑ 18% ^c	<i>Acer campestre</i>	abaxial	↔	–	Beerling and Kelly, 1997
		<i>Acer pseudoplatanus</i>	abaxial	↔	–	
		<i>Alliaria petiolata</i>	abaxial	↑ 22%	–	
		<i>Allium ursinum</i>	abaxial	↔	–	
		<i>Alnus glutinosa</i>	abaxial	↑ 132%	–	
		<i>Anemone nemorosa</i>	abaxial	↔	–	
		<i>Arum maculatum</i>	abaxial	* ↓ 61%	–	
			adaxial	* ↓ 80%	–	
		<i>Betula pendula</i>	abaxial	* ↓ 39%	–	
		<i>Betula pendula</i>	abaxial	* ↓ 43%	–	
		<i>Betula pubescens</i>	abaxial	* ↓ 56%	–	
		<i>Carpinus betulus</i>	abaxial	↑ 13%	–	
		<i>Castanea sativa</i>	abaxial	* ↓ 24%	–	
		<i>Chamaenerion angustifolium</i>	abaxial	↔	–	
		<i>Circaea lutetiana</i>	abaxial	* ↓ 25%	–	
		<i>Cirsium palustre</i>	abaxial	* ↓ 22%	–	
		<i>Cornus sanguinea</i>	abaxial	* ↓ 16%	–	
		<i>Corylus avellana</i>	abaxial	* ↓ 50%	–	
		<i>Crataegus monogyna</i>	abaxial	* ↓ 36%	–	
		<i>Dipsacus fullonum</i>	abaxial	↑ 54%	–	
			adaxial	↑ 550%	–	
		<i>Epilobium montanum</i>	abaxial	* ↓ 28%	–	
			adaxial	↔	–	
		<i>Fagus sylvatica</i>	abaxial	↑ 33%	–	
		<i>Fagus sylvatica</i>	abaxial	↔	–	
		<i>Fraxinus excelsior</i>	abaxial	↑ 39%	–	
		<i>Geranium dissectum</i>	abaxial	↔	–	
		<i>Geranium robertianum</i>	abaxial	* ↓ 58%	–	
			adaxial	↑	–	
		<i>Geum rubanum</i>	abaxial	* ↓ 21%	–	
			adaxial	↔	–	
		<i>Glechoma hederacea</i>	abaxial	* ↓ 23%	–	
		<i>Hedera helix</i>	abaxial	↑ 101%	–	

Appendix A2 (continued)

Age of material (years)	CO ₂ levels (relative to controls ^a)	Species used	Side of leaf	SD response	SI response	Source
		<i>Heracleum sphondylium</i>	abaxial	* ↓ 14%	–	
			adaxial	↔	–	
		<i>Hyacinthoides non-scripta</i>	abaxial	↑ 56%	–	
			adaxial	↔	–	
		<i>Hypericum hirsutum</i>	abaxial	* ↓ 11%	–	
		<i>Hypericum perforatum</i>	abaxial	* ↓ 56%	–	
		<i>Ilex aquifolium</i>	abaxial	↑ 31%	–	
		<i>Lamium galeobdolon</i>	abaxial	↔	–	
		<i>Lathyrus pratensis</i>	abaxial	↔	–	
			adaxial	* ↓ 38%	–	
		<i>Ligustrum vulgare</i>	abaxial	* ↓ 67%	–	
		<i>Lonicera periclymenum</i>	abaxial	* ↓ 27%	–	
		<i>Luzula sylvatica</i>	abaxial	* ↓ 44%	–	
		<i>Lysimachia nummularia</i>	abaxial	* ↓ 56%	–	
			adaxial	* ↓ 67%	–	
		<i>Mercurialis perennis</i>	abaxial	* ↓ 17%	–	
		<i>Oxalis acetosella</i>	abaxial	↔	–	
		<i>Populus nigra</i>	abaxial	↑ 46%	–	
		<i>Primula vulgaris</i>	abaxial	* ↓ 14%	–	
		<i>Prunella vulgaris</i>	abaxial	* ↓ 47%	–	
			adaxial	* ↓ 55%	–	
		<i>Prunus avium</i>	abaxial	* ↓ 20%	–	
		<i>Pteridium aquilinum</i>	abaxial	↔	–	
		<i>Quercus petraea</i>	abaxial	* ↓ 14%	–	
		<i>Quercus robur</i>	abaxial	↔	–	
		<i>Ranunculus ficaria</i>	abaxial	* ↓ 21%	–	
			adaxial	↔	–	
		<i>Rosa canina</i>	abaxial	* ↓ 28%	–	
		<i>Sambucus nigra</i> (sun)	abaxial	↔	–	
		<i>sambucus nigra</i> (shade)	abaxial	↔	–	
		<i>Scrophularia nodosa</i>	abaxial	* ↓ 18%	–	
		<i>Silene dioica</i>	abaxial	↑ 49%	–	
			adaxial	* ↓	–	
		<i>Sorbus aucuparia</i>	abaxial	↔	–	
		<i>Stellaria holostea</i>	abaxial	* ↓ 28%	–	
		<i>Taxus baccata</i>	abaxial	↔	–	
		<i>Tilia cordata</i>	abaxial	* ↓ 34%	–	
		<i>Ulmus glabra</i>	abaxial	↔	–	
		<i>Vaccinium myrtillus</i>	abaxial	↔	–	
			adaxial	↔	–	
		<i>Vicia cracca</i>	abaxial	* ↓ 57%	–	
			adaxial	* ↓ 20%	–	
		<i>Vicia sepium</i>	abaxial	* ↓ 43%	–	
		<i>Viola odorata</i>	abaxial	↔	–	
91	↑ 20% ^c	<i>Betula nana</i>	abaxial	* ↓ 29%	–	Beerling, 1993
98	↑ 24% ^c	<i>Salix herbacea</i>	combined	–	* ↓ 21%	Rundgren and Beerling, 1999
110	↑ 25% ^c	<i>Betula pubescens</i>	abaxial	* ↓ 45%	* ↓ 35%	Kürschner, 1996
118	↑ 24% ^c	<i>Quercus petraea</i>	abaxial	–	* ↓ 34%	van der Burgh et al., 1993
126	↑ 14% ^c	<i>Salix herbacea</i>	combined	* ↓ 22%	–	Beerling et al., 1993
~127	↑ 24% ^c	<i>Salix cinerea</i>	abaxial	* ↓ 22%	* ↓ 17%	McElwain et al., 1995
144	↑ 23% ^c	<i>Salsola kali</i> (C ₄)	abaxial	–	↔	Raven and Ramsden, 1988
144	↑ 27% ^c	<i>Ginkgo biloba</i>	abaxial	↔	* ↓ 44%	D.L. Royer, unpublished data
150	↑ 14% ^c	<i>Salix herbacea</i>	combined	* ↓ 26%	–	Beerling et al., 1995
151	↑ 27% ^c	<i>Quercus robur</i>	abaxial	* ↓ 23%	–	Beerling and Chaloner, 1993b
173	↑ 25% ^c	<i>Olea europaea</i>	abaxial	* ↓ 24%	–	Beerling and Chaloner, 1993c
181	↑ 26% ^c	<i>Fagus sylvatica</i>	abaxial	* ↓ 43%	–	Paoletti and Gellini, 1993
		<i>Quercus ilex</i>	abaxial	* ↓ 28%	–	

Appendix A2 (continued)

Age of material (years)	CO ₂ levels (relative to controls ^a)	Species used	Side of leaf	SD response	SI response	Source
190	↑ 27% ^c	<i>Quercus petraea</i>	abaxial	* ↓ 40%	* ↓ 31%	Kürschner et al., 1996
200	↑ 24% ^c	<i>Acer pseudoplatanus</i> , <i>Carpinus betulus</i> , <i>Fagus sylvatica</i> , <i>Populus nigra</i> , <i>Quercus petraea</i> , <i>Q. robur</i> , <i>Rhamnus catharticus</i> , <i>Tilia cordata</i>	abaxial	* ↓ 40% (mean)	–	Woodward, 1987
240	↑ 25% ^c	<i>Alnus glutinosa</i> , <i>Amaranthus caudatus</i> , <i>Betula pendula</i> , <i>Buxus sempervirens</i> , <i>Ceratonia siliqua</i> , <i>Cynodon dactylon</i> , <i>Gentiana alpina</i> , <i>Helleborus foetidus</i> , <i>Juniperus communis</i> , <i>Papaver alpinum</i> , <i>Pinus pinea</i> , <i>P. uncinata</i> , <i>Pistacia lentiscus</i> , <i>Rhododendron ferrugineum</i>	combined	* ↓ 17% (mean)	↔ (mean)	Peñuelas and Matamala, 1990
3318	↑ 22% ^d	<i>Olea europaea</i>	abaxial	* ↓ 33%	–	Beerling and Chaloner, 1993c

* response inversely relates ($P < 0.05$) to CO₂ concentration.

↔ no significant change ($P > 0.05$).

– not reported.

data from an altitudinal study; thus, the ‘age’ is however long the population has existed at the sampled altitudes.

@ data from a natural CO₂ spring area; thus, the ‘age’ is however long the population has existed at the location, assuming constant CO₂ emissions.

^a Typically between 340 and 360 ppmV; for herbarium studies, control corresponds with oldest material.

^b From direct measurements from Mauna Loa Observatory, Hawaii and South Pole (Keeling et al., 1995).

^c From Siple Station ice core (Neftel et al., 1985; Friedli et al., 1986).

^d From Taylor Dome ice core (Indermühle et al., 1999).

Appendix A3

Fossil stomatal responses

Age of material (years)	CO ₂ levels (relative to controls ^a)	Species used	Side of leaf	SD response	SI response	Source
9000	↓ 25% ^{b,c}	<i>Salix herbacea</i>	combined	–	* ↑ 55%	Rundgren and Beerling, 1999
9190	↓ 27% ^b	<i>Salix cinerea</i>	abaxial	* ↑ 57%	* ↑ 32%	McElwain et al., 1995
9800	↑ 20% ^{d,j}	<i>Betula pubescens</i> , <i>B. pendula</i>	abaxial	–	* ↓ 32% (mean)	Wagner et al., 1999
10750 (Allerød/Y. Dryas)	↓ 25% ^{c,k}	<i>Salix herbacea</i>	combined	* ↑ 27%	–	Beerling et al., 1995
11500	↓ 24% ^b	<i>Salix herbacea</i>	combined	↓ 46%	↔	Beerling et al., 1992
13000	↓ 29% ^b	<i>Betula nana</i>	abaxial	* ↑ 60%	–	Beerling, 1993
16500	↓ 47% ^b	<i>Salix herbacea</i>	combined	* ↑ 54%	* ↑ 25%	Beerling et al., 1993
28000	↓ 46% ^b	<i>Pinus flexilis</i>	–	* ↑ 31%	–	van de Water et al., 1994
140,000	↓ 47% ^b	<i>Salix herbacea</i>	combined	* ↑ 73%	* ↑ 39%	Beerling et al., 1993
2.5 m.y.	↑ 4% ^e	<i>Quercus petraea</i>	abaxial	–	* ↓ 10%	van der Burgh et al., 1993; Kürschner et al., 1996

Appendix A3 (continued)

Age of material (years)	CO ₂ levels (relative to controls ^a)	Species used	Side of leaf	SD response	SI response	Source
6.5 m.y.	↓ 20% ^c	<i>Quercus petraea</i>	abaxial	–	* ↑ 55%	van der Burgh et al., 1993; Kürschner et al., 1996
6.5 m.y.	↓ 24% ^{e,l}	<i>Fagus attenuata</i>	abaxial	–	* ↑ 41%	Kürschner, 1996
10 m.y.	↓ 20% ^d	<i>Betula subpubescens</i>	abaxial	* ↑ 72%	* ↑ 45%	
10 m.y.	↑ 4% ^c	<i>Quercus petraea</i>	abaxial	–	* ↓ 9%	van der Burgh et al., 1993; Kürschner et al., 1996
10 m.y.	↔ ^d	<i>Betula subpubescens</i>	abaxial	* ↔	* ↔	Kürschner, 1996
15.5 m.y.	↑ ^f	<i>Chamaecyparis linguaefolia</i> , <i>Cunninghamia chaneyi</i> , <i>Metasequoia occidentalis</i> , <i>Pinus harneyana</i> , <i>Pinus</i> sp., <i>Taxodium dubium</i>	combined	* ↓ (mean)	–	Huggins, 1985
44–50 m.y. (M. Eocene)	↑ 43% ^g	<i>Lindera cinnamomifolia</i> , <i>Lindera</i> sp. ⁿ	abaxial	* ↓ 36% (mean)	* ↓ 47% (mean)	McElwain, 1998
		<i>Litsea bournensis</i> , <i>L. edwardsii</i> , <i>L. hirsuta</i> ⁿ	abaxial	* ↓ 27% (mean)	* ↓ 38% (mean)	
160–185 m.y. (M. Jurassic)	↑ 149% ^g	<i>Brachyphyllum crucis</i> ⁿ	abaxial	* ↓ 54%	* ↓ 39%	McElwain and Chaloner, 1996
		<i>B. mamillare</i> ⁿ	abaxial	* ↓ 39%	* ↓ 52%	McElwain, 1998
		<i>Ginkgo huttonii</i> ⁿ	abaxial	* ↓ 32%	–	
160–185 m.y. (M. Jurassic)	↑ 149% ^g	<i>Baeira furcata</i> ⁿ	abaxial	* ↓ 44%	–	McElwain, 1998
		<i>Ctenis exilis</i> , <i>C. kaneharai</i> , <i>C. sulcicaulis</i> ⁿ	adaxial abaxial	* ↓ 67% * ↓ 46% (mean)	– ↑ 14% (mean)	
		<i>Pagiophyllum kurrrii</i> , <i>P. maculosum</i> , <i>P. ordinatum</i> ⁿ	abaxial	* ↓ 36% (mean)	* ↓ 39% (mean)	McElwain et al., 1999
~205 m.y. (Latest Triassic)	↑ 69% ^h	<i>Baeira boeggildiana</i> ⁿ	abaxial	–	* ↓ 44%	
		<i>B. minuta</i> ⁿ	abaxial	–	* ↓ 49%	McElwain et al., 1999
		<i>B. paucipartiata</i> ⁿ	abaxial	–	* ↓ 25%	
		<i>Baeira</i> sp. ⁿ	abaxial	–	* ↓ 36%	McElwain et al., 1999
		<i>Ctenis minuta</i> , <i>C. nilssonii</i> ⁿ	abaxial	–	* ↓ 43% (mean)	
		<i>C. nilssonii</i> ⁿ	abaxial	–	* ↓ 21%	McElwain et al., 1999
		<i>Ginkgo acosmica</i> ⁿ	abaxial	–	* ↓ 26%	
		<i>G. obovatus</i> ⁿ	abaxial	–	* ↓ 57%	McElwain et al., 1999
~205 m.y. (Earliest Jurassic)	↑ 567% ^h	<i>Baeira longifolia</i> ⁿ	abaxial	–	* ↓ 60%	
		<i>B. spectabilis</i> ⁿ	abaxial	–	* ↓ 71%	McElwain et al., 1999
		<i>Nilssonina polymorpha</i> ⁿ	abaxial	–	* ↓ 70%	
		<i>Stenopteris dinosaurensis</i> ⁿ	abaxial	–	* ↓ 11%	McElwain and Chaloner, 1995
285–290 m.y. (E. Permian)	↔ ^g	<i>Lebachia frondosa</i> ⁿ	abaxial	↑ 120%	* ↔	
290–303 m.y. (L. Penn.)	↑ ^l	<i>Neuropteris ovata</i>	abaxial	* ↓ 40%	* ↓ 27%	Cleal et al., 1999
310 m.y. (L. Penn.)	↔ ^g	<i>Swillingtonia denticulata</i> ⁿ	abaxial	↑ 460%	* ↔	McElwain and Chaloner, 1995
388–373 m.y. (M. Devonian)	↓ 61% ^{g,m}	<i>Drepanophycus spinaeformis</i>	–	* ↑ 42%	* ↑ 38%	Edwards et al., 1998

Appendix A3 (continued)

Age of material (years)	CO ₂ levels (relative to controls ^a)	Species used	Side of leaf	SD response	SI response	Source
390–403 m.y. (E. Devonian)	↑ 657% ^g	<i>Aglaophyton major</i> ⁿ	combined	* ↓ 99%	* ↓ 84%	McElwain and Chaloner, 1995
		<i>Sawdonia ornata</i> ⁿ	combined	* ↓ 98%	* ↓ 78%	

* response inversely relates ($P < 0.05$) to CO₂ concentration.

↔ no significant change ($P > 0.05$).

– not reported.

^a Typically between 340 and 360 ppmV.

^b From Vostok (Barnola et al., 1987) and Taylor Dome (Indermühle et al., 1999) ice cores.

^c From stomatal response of recent *Salix herbacea*, where CO₂ concentrations are known; values match ice core data (refer table footnote 9).

^d From stomatal responses of recent *Betula pubescens* and *Betula pendula*, where CO₂ concentrations are known.

^e From stomatal response of recent *Quercus petraea*, where CO₂ concentrations are known; values correlate with temperature curve.

^f From Freeman and Hayes, 1992; Cerling et al., 1997 (c.f. Pagani et al., 1999a).

^g From ‘best estimate’ of Berner (1994, 1998).

^h From stomatal ratios (McElwain and Chaloner, 1995, 1996; McElwain, 1998).

^j The control group is prior to the CO₂ spike (260 ppmV CO₂ (refer table footnote 11)).

^k The control group is the late Allerød material, prior to CO₂ drop (273 ppmV CO₂ (refer table footnote 10)).

^l The control group is the 10 Ma material (370 ppmV CO₂ (refer table footnote 12)).

^m The control group is the 388 Ma material (2600 ppmV CO₂ (refer table footnote 14)).

ⁿ Stomatal responses compared with corresponding Nearest Living Equivalents (NLEs); method described in text.

References

- Abrams, M.D., 1994. Genotypic and phenotypic variation as stress adaptations in temperate tree species: a review of several case studies. *Tree Physiol.* 14, 833–842.
- Andrews, J.E., Tandon, S.K., Dennis, P.F., 1995. Concentrations of carbon dioxide in the Late Cretaceous atmosphere. *J. Geol. Soc. London* 152, 1–3.
- Apple, M.E., Olszyk, D.M., Ormrod, D.P., Lewis, J., Southworth, D., Tingey, D.T., 2000. Morphology and stomatal function of Douglas fir needles exposed to climate change: elevated CO₂ and temperature. *Int. J. Plant Sci.* 161, 127–132.
- Ashton, P.M.S., Berlyn, G.P., 1994. A comparison of leaf physiology and anatomy of *Quercus* (section *Erythrobalanus* — Fagaceae) species in different light environments. *Am. J. Bot.* 81, 589–597.
- Atkinson, C.J., Taylor, J.M., Wilkins, D., Besford, R.T., 1997. Effects of elevated CO₂ on chloroplast components, gas exchange and growth of oak and cherry. *Tree Physiol.* 17, 319–325.
- Barnola, J.M., Raynaud, D., Korotkevich, Y.S., Lorius, C., 1987. Vostok ice core provides 160,000-year record of atmospheric CO₂. *Nature* 329, 408–414.
- Beerling, D.J., 1993. Changes in the stomatal density of *Betula nana* leaves in response to increases in atmospheric carbon dioxide concentration since the late-glacial. *Spec. Pap. Palaeontol.* 49, 181–187.
- Beerling, D.J., 1994. Palaeo-ecophysiological studies on Cretaceous and Tertiary fossil floras. In: Boulter, M.C., Fisher, H.C. (Eds.), *Cenozoic Plants and Climates of the Arctic*. Springer, Berlin, pp. 23–33.
- Beerling, D.J., 1996. Palaeo-ecophysiological perspectives on plant responses to global change. *Trends Ecol. Evol.* 11, 20–23.
- Beerling, D.J., 1997. Carbon isotope discrimination and stomatal responses of mature *Pinus sylvestris* L. trees exposed in situ for three years to elevated CO₂ and temperature. *Acta Ecol.* 18, 697–712.
- Beerling, D.J., Birks, H.H., Woodward, F.I., 1995. Rapid late-glacial atmospheric CO₂ changes reconstructed from the stomatal density record of fossil leaves. *J. Quat. Sci.* 10, 379–384.
- Beerling, D.J., Chaloner, W.G., 1992. Stomatal density as an indicator of atmospheric CO₂ concentration. *Holocene* 2, 71–78.
- Beerling, D.J., Chaloner, W.G., 1993a. Evolutionary responses of stomatal density to global CO₂ change. *Biol. J. Linnean Soc.* 48, 343–353.
- Beerling, D.J., Chaloner, W.G., 1993b. The impact of atmospheric CO₂ and temperature change on stomatal density: observations from *Quercus robur* lammas leaves. *Ann. Bot.* 71, 231–235.
- Beerling, D.J., Chaloner, W.G., 1993c. Stomatal density responses of Egyptian *Olea europaea* L. leaves to CO₂ change since 1327 BC. *Ann. Bot.* 71, 431–435.
- Beerling, D.J., Chaloner, W.G., 1994. Atmospheric CO₂ changes since the last glacial maximum: evidence from the stomatal density record of fossil leaves. *Rev. Palaeobot. Palynol.* 81, 11–17.
- Beerling, D.J., Chaloner, W.G., Huntley, B., Pearson, J.A., Tooley,

- M.J., 1991. Tracking stomatal densities through a glacial cycle: their significance for predicting the response of plants to changing atmospheric CO₂ concentrations. *Global Ecol. Biogeogr. Lett.* 1, 136–142.
- Beerling, D.J., Chaloner, W.G., Huntley, B., Pearson, J.A., Tooley, M.J., 1993. Stomatal density responds to the glacial cycle of environmental change. *Proc. R. Soc. London B251*, 133–138.
- Beerling, D.J., Chaloner, W.G., Huntley, B., Pearson, J.A., Tooley, M.J., Woodward, F.I., 1992. Variations in the stomatal density of *Salix herbacea* L. under the changing atmospheric CO₂ concentrations of late- and post-glacial time. *Philos. Trans. R. Soc. London B336*, 215–224.
- Beerling, D.J., Kelly, C.K., 1997. Stomatal density responses of temperate woodland plants over the past seven decades of CO₂ increase: a comparison of Salisbury (1927) with contemporary data. *Am. J. Bot.* 84, 1572–1583.
- Beerling, D.J., McElwain, J.C., Osborne, C.P., 1998a. Stomatal responses of the living fossil *Ginkgo biloba* L. to changes in atmospheric CO₂ concentrations. *J. Exp. Bot.* 49, 1603–1607.
- Beerling, D.J., Woodward, F.I., 1995. Stomatal responses of variegated leaves to CO₂ enrichment. *Ann. Bot.* 75, 507–511.
- Beerling, D.J., Woodward, F.I., 1996. Palaeo-ecophysiological perspectives on plant responses to global change. *Trends Ecol. Evol.* 11, 20–23.
- Beerling, D.J., Woodward, F.I., 1997. Changes in land plant function over the Phanerozoic: reconstructions based on the fossil record. *Bot. J. Linn. Soc.* 124, 137–153.
- Beerling, D.J., Woodward, F.I., Lomas, M.R., Wills, M.A., Quick, W.P., Valdes, P.J., 1998b. The influence of Carboniferous palaeoatmospheres on plant function: an experimental and modelling assessment. *Philos. Trans. R. Soc. London B353*, 131–140.
- Berger, D., Altmann, T., 2000. A subtilisin-like serine protease involved in the regulation of stomatal density and distribution in *Arabidopsis thaliana*. *Genes Dev.* 14, 1119–1131.
- Berner, R.A., 1994. Geocarb II: a revised model of atmospheric CO₂ over Phanerozoic time. *Am. J. Sci.* 294, 56–91.
- Berner, R.A., 1998. Sensitivity of Phanerozoic atmospheric CO₂ to paleogeographically induced changes in land temperature and runoff. In: Crowley, T.J., Burke, K.C. (Eds.), *Tectonic Boundary Conditions for Climate Reconstructions*. Oxford University Press, New York, pp. 251–260.
- Berner, R.A., Canfield, D., 1989. A new model for atmospheric oxygen over Phanerozoic time. *Am. J. Sci.* 289, 333–361.
- Berner, R.A., Petsch, S.T., Lake, J.A., Beerling, D.J., Popp, B.N., Lane, R.S., Laws, E.A., Westley, M.B., Cassar, N., Woodward, F.I., Quick, W.P., 2000. Isotope fractionation and atmospheric oxygen: implications for Phanerozoic O₂ evolution. *Science* 287, 1630–1633.
- Berryman, C.A., Eamus, D., Duff, G.A., 1994. Stomatal responses to a range of variables in tree species grown with CO₂ enrichment. *J. Exp. Bot.* 45, 539–546.
- Bettarini, I., Miglietta, F., Raschi, A., 1997. Studying morphophysiological responses of *Scirpus lacustris* from naturally CO₂-enriched environments. In: Raschi, A., Miglietta, F., Tognetti, R., van Gardingen, P.R. (Eds.), *Plant Responses to Elevated CO₂*. Cambridge University Press, Cambridge, pp. 134–147.
- Bettarini, I., Vaccari, F.P., Miglietta, F., 1998. Elevated CO₂ concentrations and stomatal density: observations from 17 plant species growing in a CO₂ spring in central Italy. *Global Change Biol.* 4, 17–22.
- Boetsch, J., Chin, J., Ling, M., Croxdale, J., 1996. Elevated carbon dioxide affects the patterning of subsidiary cells in *Tradescantia* stomatal complexes. *J. Exp. Bot.* 47, 925–931.
- Bristow, J.M., Looi, A.-S., 1968. Effects of carbon dioxide on the growth and morphogenesis of *Marsilea*. *Am. J. Bot.* 55, 884–889.
- Bryant, J., Taylor, G., Frehner, M., 1998. Photosynthetic acclimation to elevated CO₂ 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 Environ.* 21, 159–168.
- van der Burgh, J., Visscher, H., Dilcher, D.L., Kürschner, W.M., 1993. Paleoatmospheric signatures in Neogene fossil leaves. *Science* 260, 1788–1790.
- Case, A.L., Curtis, P.S., Snow, A.A., 1998. Heritable variation in stomatal responses to elevated CO₂ in wild radish, *Raphanus raphanistrum* (Brassicaceae). *Am. J. Bot.* 85, 253–258.
- Centritto, M., Maonani, F., Lee, H.S.J., Jarvis, P.G., 1999. Interactive effects of elevated [CO₂] and drought on cherry (*Prunus avium*) seedlings. II. Photosynthetic capacity and water relations. *New Phytol.* 141, 141–153.
- Cerling, T.E., 1991. Carbon dioxide in the atmosphere: evidence from Cenozoic and Mesozoic paleosols. *Am. J. Sci.* 291, 377–400.
- Cerling, T.E., 1992. Use of carbon isotopes in paleosols as an indicator of the P(CO₂) of the paleoatmosphere. *Global Biogeochem. Cycles* 6, 307–314.
- Cerling, T.E., Harris, J.M., MacFadden, B.J., Leakey, M.G., Quade, J., Eisenmann, V., Ehleringer, J.R., 1997. Global vegetation change through the Miocene/Pliocene boundary. *Nature* 389, 153–158.
- Ceulemans, R., van Praet, L., Jiang, X.N., 1995. Effects of CO₂ enrichment, leaf position and clone on stomatal index and epidermal cell density in poplar (*Populus*). *New Phytol.* 131, 99–107.
- Cleal, C.J., James, R.M., Zedrow, E.L., 1999. Variation in stomatal density in the Late Carboniferous gymnosperm frond *Neuropteris ovata*. *Palaios* 14, 180–185.
- Clifford, S.C., Black, C.R., Roberts, J.A., Stronach, I.M., Singleton-Jones, P.R., Mohamed, A.D., Azam-Ali, S.N., 1995. The effect of elevated atmospheric CO₂ and drought on stomatal frequency in groundnut (*Arachis hypogaea* (L.)). *J. Exp. Bot.* 46, 847–852.
- Curtis, P.S., Wang, X., 1998. A meta-analysis of elevated CO₂ effects on woody plant mass, form, and physiology. *Oecologia* 113, 299–313.
- Dixon, M., Le Thiec, D., Garrec, J.-P., 1995. The growth and gas exchange response of soil-planted Norway spruce [*Picea abies* (L.) Karst.] and red oak (*Quercus rubra* L.) exposed to elevated CO₂ and to naturally occurring drought. *New Phytol.* 129, 265–273.
- Drake, B.G., 1992. A field study of the effects of elevated CO₂ on

- ecosystem processes in a Chesapeake Bay Wetland. *Aust. J. Bot.* 40, 579–595.
- Eamus, D., Berryman, C.A., Duff, G.A., 1993. Assimilation, stomatal conductance, specific leaf area and chlorophyll responses to elevated CO₂ of *Maranthus corymbosa*, a tropical monsoon rain forest species. *Aust. J. Plant Physiol.* 20, 741–755.
- Edwards, D., Kerp, H., Hass, H., 1998. Stomata in early land plants: an anatomical and ecophysiological approach. *J. Exp. Bot.* 49, 255–278.
- Ehleringer, J.R., Cerling, T.E., 1995. Atmospheric CO₂ and the ratio of intercellular to ambient CO₂ concentrations in plants. *Plant Physiol.* 15, 105–111.
- Ekart, D.D., Cerling, T.E., Montañez, I.P., Tabor, N.J., 1999. A 400 million year carbon isotope record of pedogenic carbonate: implications for paleoatmospheric carbon dioxide. *Am. J. Sci.* 299, 805–827.
- Elick, J.M., Mora, C.I., Driese, S.G., 1999. Elevated atmospheric CO₂ levels and expansion of early vascular land plants: stable isotope evidence from the Battery Point Fm. (Early to Middle Devonian), Gaspé Bay, Canada. *GSA Abstracts with Programs*, vol. 31, p. A-159.
- Estiarte, M., Peñuelas, J., Kimball, B.A., Idso, S.B., LaMorte, R.L., Pinter, P.J., Wall, G.W., Garcia, R.L., 1994. Elevated CO₂ effects on stomatal density of wheat and sour orange trees. *J. Exp. Bot.* 45, 1665–1668.
- Farnsworth, E.J., Ellison, A.M., Gong, W.K., 1996. Elevated CO₂ alters anatomy, physiology, growth, and reproduction of red mangrove (*Rhizophora mangle* L.). *Oecologia* 108, 599–609.
- Farquhar, G.D., von Caemmerer, S., Berry, J.A., 1980. A biochemical model of photosynthetic CO₂ assimilation in leaves of C3 species. *Planta* 149, 78–90.
- Fernández, M.D., Pieters, A., Donoso, C., Tezara, W., Azkue, M., Herrera, C., Rengifo, E., Herrera, A., 1998. Effects of a natural source of very high CO₂ concentration on the leaf gas exchange, xylem water potential and stomatal characteristics of plants of *Spatiphyllum cannifolium* and *Bauhinia multinervia*. *New Phytol.* 138, 689–697.
- Ferris, R., Nijs, I., Behaeghe, T., Impens, I., 1996. Elevated CO₂ and temperature have different effects on leaf anatomy of perennial ryegrass in spring and summer. *Ann. Bot.* 78, 489–497.
- Ferris, R., Taylor, G., 1994. Stomatal characteristics of four native herbs following exposure to elevated CO₂. *Ann. Bot.* 73, 447–453.
- Freeman, K.H., Hayes, J.M., 1992. Fractionation of carbon isotopes by phytoplankton and estimates of ancient CO₂ levels. *Global Biogeochem. Cycles* 6, 185–198.
- Frey, B., Scheidegger, C., Günthardt-Goerg, M.S., Matyssek, R., 1996. The effects of ozone and nutrient supply on stomatal response in birch (*Betula pendula*) leaves as determined by digital image-analysis and X-ray microanalysis. *New Phytol.* 132, 135–143.
- Friedli, H., Lötscher, H., Oeschger, H., Siegenthaler, U., Stauffer, B., 1986. Ice core record of the ¹³C/¹²C ratio of atmospheric CO₂ in the past two centuries. *Nature* 324, 237–238.
- Furukawa, A., 1997. Stomatal frequency of *Quercus myrsinaefolia* grown under different irradiances. *Photosynthetica* 34, 195–199.
- van Gardingen, P.R., Grace, J., Jeffrey, C.E., Byari, S.H., Miglietta, F., Raschi, A., Bettarini, I., 1997. Long-term effects of enhanced CO₂-concentrations on leaf gas exchange: research opportunities using CO₂ springs. In: Raschi, A., Miglietta, F., Tognetti, R., van Gardingen, P.R. (Eds.), *Plant Responses to Elevated CO₂*. Cambridge University Press, Cambridge, pp. 69–86.
- Gaudillère, J.P., Mousseau, M., 1989. Short term effect of CO₂ on leaf development and gas exchange of young poplars (*Populus euramericana* cv. I 214). *Oecol. Plant.* 10, 95–105.
- Gay, A.P., Hauck, B., 1994. Acclimation of *Lolium temulentum* to enhanced carbon dioxide concentration. *J. Exp. Bot.* 45, 1133–1141.
- Gay, A.P., Hurd, R.G., 1975. The influence of light on stomatal density in the tomato. *New Phytol.* 75, 37–46.
- Ghosh, P., Bhattacharya, S.K., Jani, R.A., 1995. Palaeoclimate and palaeovegetation in central India during the Upper Cretaceous based on stable isotope composition of the palaeosol carbonates. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 114, 285–296.
- Goodfellow, J., Eamus, D., Duff, G., 1997. Diurnal and seasonal changes in the impact on CO₂ enrichment on assimilation, stomatal conductance and growth in a long-term study of *Mangifera indica* in the wet-dry tropics of Australia. *Tree Physiol.* 17, 291–299.
- Heckenberger, U., Roggatz, U., Schurr, U., 1998. Effect of drought stress on the cytological status in *Ricinus communis*. *J. Exp. Bot.* 49, 181–189.
- Houghton, J.T., Meira Filho, L.G., Callander, B.A., Harris, N., Kattenberg, A., Maskell, K., 1995. *Climate Change. The IPCC Scientific Assessment*. Cambridge University Press, Cambridge.
- Huggins, L.M., 1985. Cuticular analyses and preliminary comparisons of some Miocene conifers from Clarkia, Idaho. In: Smiley, C.J. (Ed.), *Late Cenozoic history of the Pacific Northwest*. American Association for the Advancement of Science, San Francisco, pp. 113–138.
- Indermühle, A., Stocker, T.F., Joos, F., Fischer, H., Smith, H.J., Wahlen, M., Deck, B., Mastroianni, D., Tschumi, J., Blunier, T., Meyer, R., Stauffer, B., 1999. Holocene carbon-cycle dynamics based on CO₂ trapped in ice at Taylor Dome, Antarctica. *Nature* 398, 121–126.
- Jones, M.B., Brown, J.C., Raschi, A., Miglietta, F., 1995. The effects on *Arbutus unedo* L. of long-term exposure to elevated CO₂. *Global Change Biol.* 1, 295–302.
- Keeling, C.D., Whorf, T.P., Wahlen, M., van der Plicht, J., 1995. Interannual extremes in the rate of atmospheric carbon dioxide since 1980. *Nature* 375, 666–670.
- Kelly, D.W., Hicklenton, P.R., Reekie, E.G., 1991. Photosynthetic response of geranium to elevated CO₂ as affected by leaf age and time of CO₂ exposure. *Can. J. Bot.* 69, 2482–2488.
- Knapp, A.K., Cocks, M., Hamerlynck, E.P., Owensby, C.E., 1994. Effect of elevated CO₂ on stomatal density and distribution in a C4 grass and a C4 forb under field conditions. *Ann. Bot.* 74, 595–599.
- Koch, P.L., Zachos, J.C., Gingerich, P.D., 1992. Correlation between isotope records in marine and continental carbon reservoirs near the Palaeocene/Eocene boundary. *Nature* 358, 319–322.

- Körner, Ch., 1988. Does global increase of CO₂ alter stomatal density? *Flora* 181, 253–257.
- Körner, Ch., Bannister, P., Mark, A.F., 1986. Altitudinal variation in stomatal conductance, nitrogen content and leaf anatomy in different plant life forms in New Zealand. *Oecologia* 69, 577–588.
- Körner, Ch., Cochrane, P.M., 1985. Stomatal responses and water relations of *Eucalyptus pauciflora* in summer along an elevational gradient. *Oecologia* 66, 443–455.
- Kump, L.R., Arthur, M.A., Patzkowsky, M.E., Gibbs, M.T., Pinkus, D.S., Sheehan, P.M., 1999. A weathering hypothesis for glaciation at high atmospheric pCO₂ during the Late Ordovician. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* 152, 173–187.
- Kürschner, W.M., 1996. Leaf stomata as biosensors of paleoatmospheric CO₂ levels. *LPP Contrib. Series* 5, 1–153.
- Kürschner, W.M., 1997. The anatomical diversity of recent and fossil leaves of the durmast oak (*Quercus petraea* Lieblein/*Q. pseudocastanea* Goepfert) — implications for their use as biosensors of palaeoatmospheric CO₂ levels. *Rev. Palaeobot. Palynol.* 96, 1–30.
- Kürschner, W.M., Stulen, I., Wagner, F., Kuiper, P.J.C., 1998. Comparison of palaeobotanical observations with experimental data on the leaf anatomy of durmast oak [*Quercus petraea* (Fagaceae)] in response to environmental change. *Ann. Bot.* 81, 657–664.
- Kürschner, W.M., van der Burgh, J., Visscher, H., Dilcher, D.L., 1996. Oak leaves as biosensors of late Neogene and early Pleistocene paleoatmospheric CO₂ concentrations. *Mar. Micropaleontol.* 27, 299–312.
- Kürschner, W.M., Wagner, F., Visscher, E.H., Visscher, H., 1997. Predicting the response of leaf stomatal frequency to a future CO₂-enriched atmosphere: constraints from historical observations. *Geol. Rundsch.* 86, 512–517.
- Lambers, H., Chapin, F.S., Pons, T.L., 1998. *Plant Physiological Ecology*. Springer, New York.
- Lauber, W., Körner, Ch., 1997. In situ stomatal responses to long-term CO₂ enrichment in calcareous grassland plants. *Acta Oecol.* 18, 221–229.
- Lee, Y.I., 1999. Stable isotopic composition of calcic paleosols of the Early Cretaceous Hasandong Formation, southeastern Korea. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 150, 123–133.
- Lee, Y.I., Hisada, K., 1999. Stable isotopic composition of pedogenic carbonates of the Early Cretaceous Shimonoseki Subgroup, western Honshu, Japan. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 153, 127–138.
- Long, S.P., Osborne, C.P., Humphries, S.W., 1996. Photosynthesis, rising atmospheric carbon dioxide concentration and climate change. In: Breymeyer, A.I., Hall, D.O., Melillo, J.M., Ågren, G.I. (Eds.), *Global Change: Effects on Coniferous Forests and Grasslands*. Wiley, New York, pp. 121–159.
- Madsen, E., 1973. Effect of CO₂-concentration on the morphological, histological and cytological changes in tomato plants. *Acta Agric. Scand.* 23, 241–246.
- Malone, S.R., Mayeux, H.S., Johnson, H.B., Polley, H.W., 1993. Stomatal density and aperture length in four plant species grown across a subambient CO₂ gradient. *Am. J. Bot.* 80, 1413–1418.
- Masterson, J., 1994. Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. *Science* 264, 421–424.
- McElwain, J.C., 1998. Do fossil plants signal palaeoatmospheric CO₂ concentration in the geological past? *Philos. Trans. R. Soc. London B* 353, 83–96.
- McElwain, J.C., Beerling, D.J., Woodward, F.I., 1999. Fossil plants and global warming at the Triassic–Jurassic boundary. *Science* 285, 1386–1390.
- McElwain, J.C., Chaloner, W.G., 1995. Stomatal density and index of fossil plants track atmospheric carbon dioxide in the Palaeozoic. *Ann. Bot.* 76, 389–395.
- McElwain, J.C., Chaloner, W.G., 1996. The fossil cuticle as a skeletal record of environmental change. *Palaios* 11, 376–388.
- McElwain, J., Mitchell, F.J.G., Jones, M.B., 1995. Relationship of stomatal density and index of *Salix cinerea* to atmospheric carbon dioxide concentrations in the Holocene. *Holocene* 5, 216–219.
- Miglietta, F., Raschi, A., 1993. Studying the effect of elevated CO₂ in the open in a naturally enriched environment in central Italy. *Vegetatio* 104/105, 391–400.
- Minnocci, A., Panicucci, A., Sebastiani, L., Lorenzini, G., Vitaliano, C., 1999. Physiological and morphological responses of olive plants to ozone exposure during a growing season. *Tree Physiol.* 19, 391–397.
- Mishra, M.K., 1997. Stomatal characteristics at different ploidy levels in *Coffea* L. *Ann. Bot.* 80, 689–692.
- Montañez, I.P., Tabor, N.J., Ekart, D., Collister, J.W., 1999. Evolution of Permian atmospheric pCO₂ as derived from latest Carboniferous through Permian paleosol carbonates, Midland and Paradox Basins, U.S.A. and northern Italian Alps. *GSA Abstracts with Programs*, vol. 31, p. A-326.
- Mora, C.I., Driese, S.G., Colarusso, L.A., 1996. Middle and Late Paleozoic atmospheric CO₂ levels from soil carbonate and organic matter. *Science* 271, 1105–1107.
- Mora, C.I., Driese, S.G., Seager, P.G., 1991. Carbon dioxide in the Paleozoic atmosphere: evidence from carbon-isotope compositions of pedogenic carbonate. *Geology* 19, 1017–1020.
- Muchez, P., Peeters, C., Keppens, E., Viaene, W.A., 1993. Stable isotopic composition of paleosols in the Lower Visian of eastern Belgium: evidence of evaporation and soil-gas CO₂. *Chem. Geol.* 106, 389–396.
- Neftel, A., Moor, E., Oeschger, H., Stauffer, B., 1985. Evidence from polar ice cores for the increase in atmospheric CO₂ in the past two centuries. *Nature* 315, 45–47.
- Oberbauer, S.F., Strain, B.R., 1986. Effects of canopy position and irradiance on the leaf physiology and morphology of *Pentaclethra macroloba* (Mimosaceae). *Am. J. Bot.* 73, 409–416.
- Oberbauer, S.F., Strain, B.R., Fetcher, N., 1985. Effect of CO₂-enrichment on seedling physiology and growth of two tropical tree species. *Physiol. Plant.* 65, 352–356.
- O’Leary, J.W., Knecht, G.N., 1981. Elevated CO₂ concentration increases stomata numbers in *Phaseolus vulgaris* leaves. *Bot. Gaz.* 142, 438–441.
- Pääkkönen, E., Vahala, J., Pohjola, M., Holopainen, T., Kärenlampi, L., 1998. Physiological, stomatal and ultrastructural ozone responses in birch (*Betula pendula* Roth.) are modified by water stress. *Plant, Cell Environ.* 21, 671–684.

- Pagani, M., Arthur, M.A., Freeman, K.H., 1999a. Miocene evolution of atmospheric carbon dioxide. *Paleoceanography* 14, 273–292.
- Pagani, M., Freeman, K.H., Arthur, M.A., 1999b. Late Miocene atmospheric CO₂ concentrations and the expansion of C4 grasses. *Science* 285, 876–879.
- Paoletti, E., Gellini, R., 1993. Stomatal density variation in beech and holm oak leaves collected over the last 200 years. *Acta Oecol.* 14, 173–178.
- Paoletti, E., Miglietta, F., Raschi, A., Manes, F., Grossoni, P., 1997. Stomatal numbers in holm oak (*Quercus ilex* L.) leaves grown in naturally and artificially CO₂-enriched environments. In: Raschi, A., Miglietta, F., Tognetti, R., van Gardingen, P.R. (Eds.), *Plant Responses to Elevated CO₂*. Cambridge University Press, Cambridge, pp. 197–208.
- Paoletti, E., Nourrisson, G., Garrec, J.P., Raschi, A., 1998. Modifications of the leaf surface structures of *Quercus ilex* L. in open, naturally CO₂-enriched environments. *Plant, Cell Environ.* 21, 1071–1075.
- Pearson, M., Davies, W.J., Mansfield, T.A., 1995. Asymmetric responses of adaxial and abaxial stomata to elevated CO₂: impacts on the control of gas exchange by leaves. *Plant, Cell Environ.* 18, 837–843.
- Pearson, P.N., Palmer, M.R., 1999. Middle Eocene seawater pH and atmospheric carbon dioxide concentrations. *Science* 284, 1824–1826.
- Peñuelas, J., Matamala, R., 1990. Changes in N and S leaf content, stomatal density and specific leaf area in 14 plant species during the last three centuries. *J. Exp. Bot.* 41, 1119–1124.
- Petit, J.R., Jouzel, J., Raynaud, D., Barkov, N.I., Barnola, J.-M., Basile, I., Bender, M., Chappellaz, J., Davis, M., Delaygue, G., Delmotte, M., Kotlyakov, V.M., Legrand, M., Lipenkov, V.Y., Lorius, C., Pépin, L., Ritz, C., Saltzman, E., Stievenard, M., 1999. Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. *Nature* 399, 429–436.
- Platt, N.H., 1989. Lacustrine carbonates and pedogenesis: sedimentology and origin of palustrine deposits from the Early Cretaceous Rupelo Formation, W. Cameros Basin, N. Spain. *Sedimentology* 36, 665–684.
- Polley, H.W., Johnson, H.B., Marino, B.D., Mayeux, H.S., 1993. Increase in C3 plant water-use efficiency and biomass over Glacial to present CO₂ concentrations. *Nature* 361, 61–64.
- Polley, H.W., Johnson, H.B., Mayeux, H.S., 1992. Growth and gas exchange of oats (*Avena sativa*) and wild mustard (*Brassica kaber*) at subambient CO₂ concentrations. *Int. J. Plant Sci.* 153, 453–461.
- Poole, I., Weyers, J.D.B., Lawson, T., Raven, J.A., 1996. Variations in stomatal density and index: implications for palaeoclimatic reconstructions. *Plant, Cell Environ.* 19, 705–712.
- Popp, B.N., Anderson, T.F., Sandberg, P.A., 1986. Brachiopods as indicators of original isotopic composition in some Paleozoic limestones. *Bull. Geol. Soc. Am.* 97, 1262–1269.
- Pritchard, S.G., Mosjidis, C., Peterson, C.M., Runion, G.B., Rogers, H.H., 1998. Anatomical and morphological alterations in long-leaf pine needles resulting from growth in elevated CO₂: interactions with soil resource availability. *Int. J. Plant Sci.* 159, 1002–1009.
- de Queiroz, K., Gauthier, J., 1990. Phylogeny as a central principle in taxonomy: phylogenetic definitions of taxon names. *Syst. Zool.* 39, 307–322.
- Radoglou, K.M., Jarvis, P.G., 1990. Effects of CO₂ enrichment on four poplar clones. II. Leaf structure properties. *Ann. Bot.* 65, 627–632.
- Radoglou, K.M., Jarvis, P.G., 1992. The effects of CO₂ enrichment and nutrient supply on growth, morphology and anatomy of *Phaseolus vulgaris* L. seedlings. *Ann. Bot.* 70, 245–256.
- Radoglou, K.M., Jarvis, P.G., 1993. Effects of atmospheric CO₂ enrichment on early growth of *Vicia faba*, a plant with large cotyledons. *Plant, Cell Environ.* 16, 93–98.
- Rahim, M.A., Fordham, R., 1991. Effect of shade on leaf and cell size and number of epidermal cells in garlic (*Allium sativum*). *Ann. Bot.* 67, 167–171.
- Ranasinghe, S., Taylor, G., 1996. Mechanism for increased leaf growth in elevated CO₂. *J. Exp. Bot.* 47, 349–358.
- Raven, J.A., Ramsden, H.J., 1988. Similarity of stomatal index in the C4 plant *Salsola kali* L. in material collected in 1843 and 1987: relevance to changes in atmospheric CO₂ content. *Trans. Bot. Soc. Edinb.* 45, 223–233.
- Reddy, K.R., Robana, R.R., Hodges, H.F., Liu, X.J., McKinion, J.M., 1998. Interactions of CO₂ enrichment and temperature on cotton growth and leaf characteristics. *Environ. Exp. Bot.* 39, 117–129.
- Retallack, G.J., 1997. Early forest soils and their role in Devonian global change. *Science* 276, 583–585.
- Robinson, J.M., 1994. Speculations on carbon dioxide starvation. Late Tertiary evolution of stomatal regulation and floristic modernization. *Plant, Cell Environ.* 17, 345–354.
- Rowland-Bamford, A.J., Nordenbrock, C., Baker, J.T., Bowes, G., Allen, L.H., 1990. Changes in stomatal density in rice grown under various CO₂ regimes with natural solar radiation. *Environ. Exp. Bot.* 30, 175–180.
- Rowson, J.M., 1946. The significance of stomatal index as a differential character. Part III. Studies in the genera *Atropa*, *Datura*, *Digitalis*, *Phytolacca* and in polyploid leaves. *Quart. J. Pharm. Pharmaceut.* 19, 136–143.
- Rundgren, M., Beerling, D., 1999. A Holocene CO₂ record from the stomatal index of sub-fossil *Salix herbacea* L. leaves from northern Sweden. *Holocene* 9, 509–513.
- Ryle, G.J.A., Stanley, J., 1992. Effect of elevated CO₂ on stomatal size and distribution in perennial ryegrass. *Ann. Bot.* 69, 563–565.
- Salisbury, E.J., 1927. On the causes and ecological significance of stomatal frequency, with special reference to the woodland flora. *Philos. Trans. R. Soc. London B* 216, 1–65.
- Schoch, P.G., Zinsou, C., Sibi, M., 1980. Dependence of the stomatal index on environmental factors during stomatal differentiation in leaves of *Vigna sinensis* L. I. Effect of light intensity. *J. Exp. Bot.* 31, 1211–1216.
- Sharma, G.K., Dunn, D.B., 1968. Effect of environment on the cuticular features in *Kalanchoe fedtschenkoi*. *Bull. Torrey Bot. Club* 95, 464–473.
- Sharma, G.K., Dunn, D.B., 1969. Environmental modifications of leaf surface traits in *Datura stramonium*. *Can. J. Bot.* 47, 1211–1216.
- Sinha, A., Stott, L.D., 1994. New atmospheric pCO₂ estimates from

- paleosols during the late Paleocene /early Eocene global warming interval. *Global Planet. Change* 9, 297–307.
- Smith, S., Weyers, J.D.B., Berry, W.G., 1989. Variation in stomatal characteristics over the lower surface of *Commelina communis* leaves. *Plant, Cell Environ.* 12, 653–659.
- Solárová, J., Pospíšilová, J., 1988. Stomatal frequencies in the adaxial and abaxial epidermes of primary bean leaves affected by growing irradiances. *Acta Univ. Carolinae — Biol.* 31, 101–105.
- Spicer, R.A., 1981. The sorting and deposition of allochthonous plant material in a modern environment at Sillwood Lake, Sillwood Park, Berkshire, England. *US Geol. Surv. Prof. Pap.* 1143, 1–77.
- Stancato, G.C., Mazzoni-Viveiros, S.C., Luchi, A.E., 1999. Stomatal characteristics in different habitat forms of Brazilian species of *Epidendrum* (Orchidaceae). *Nord. J. Bot.* 19, 271–275.
- Stewart, J.D., Hoddinott, J., 1993. Photosynthetic acclimation to elevated atmospheric carbon dioxide and UV irradiation in *Pinus banksiana*. *Physiol. Plant.* 88, 493–500.
- Sucecky, R.K., Hubert, J.F., Birney de Wit, C.C., 1988. Isotopic imprint of climate and hydrogeochemistry on terrestrial strata of the Triassic–Jurassic Hartford and Fundy Rift Basins. *J. Sediment. Petrol.* 58, 801–811.
- Thomas, J.F., Harvey, C.N., 1983. Leaf anatomy of four species grown under continuous CO₂ enrichment. *Bot. Gaz.* 144, 303–309.
- Tichá, I., 1982. Photosynthetic characteristics during ontogenesis of leaves. 7. Stomata density and sizes. *Photosynthetica* 16, 375–471.
- Tipping, C., Murray, D.R., 1999. Effects of elevated atmospheric CO₂ concentration on leaf anatomy and morphology in *Panicum* species representing different photosynthetic modes. *Int. J. Plant Sci.* 160, 1063–1073.
- Veizer, J., Ala, D., Azmy, K., Bruckschen, P., Buhl, D., Bruhn, F., Carden, G.A.F., Diener, A., Ebneith, S., Godderis, Y., Jasper, T., Korte, C., Pawellek, F., Podlaha, O.G., Strauss, H., 1999. ⁸⁷Sr/⁸⁶Sr, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ evolution of Phanerozoic seawater. *Chem. Geol.* 161, 59–88.
- Wagner, F., 1998. The influence of environment on the stomatal frequency in *Betula*. *LPP Contrib. Series* 9, 1–102.
- Wagner, F., Below, R., De Klerk, P., Dilcher, D.L., Joosten, H., Kürschner, W.M., Visscher, H., 1996. A natural experiment on plant acclimation: lifetime stomatal frequency response of an individual tree to annual atmospheric CO₂ increase. *Proc. Natl Acad. Sci. USA* 93, 11705–11708.
- Wagner, F., Bohncke, S.J.P., Dilcher, D.L., Kürschner, W.M., van Geel, B., Visscher, H., 1999. Century-scale shifts in early Holocene atmospheric CO₂ concentration. *Science* 284, 1971–1973.
- van de Water, P.K., Leavitt, S.W., Betancourt, J.L., 1994. Trends in stomatal density and ¹³C/¹²C ratios of *Pinus flexilis* needles during last glacial–interglacial cycle. *Science* 264, 239–243.
- Wiltshire, J.J.J., Wright, C.J., Colls, J.J., Craigon, J., Unsworth, M.H., 1996. Some foliar characteristics of ash trees (*Fraxinus excelsior*) exposed to ozone episodes. *New Phytol.* 134, 623–630.
- Woodward, F.I., 1986. Ecophysiological studies on the shrub *Vaccinium myrtillus* L. taken from a wide altitudinal range. *Oecologia* 70, 580–586.
- Woodward, F.I., 1987. Stomatal numbers are sensitive to increases in CO₂ from pre-industrial levels. *Nature* 327, 617–618.
- Woodward, F.I., 1988. The responses of stomata to changes in atmospheric levels of CO₂. *Plants Today* 1, 132–135.
- Woodward, F.I., Bazzaz, F.A., 1988. The responses of stomatal density to CO₂ partial pressure. *J. Exp. Bot.* 39, 1771–1781.
- Woodward, F.I., Beerling, D.J., 1997. Plant–CO₂ responses in the long term: plants from CO₂ springs in Florida and tombs in Egypt. In: Raschi, A., Miglietta, F., Tognetti, R., van Gardingen, P.R. (Eds.). *Plant Responses to Elevated CO₂*. Cambridge University Press, Cambridge, pp. 103–113.
- Woodward, F.I., Kelly, C.K., 1995. The influence of CO₂ concentration on stomatal density. *New Phytol.* 131, 311–327.
- Yapp, C.J., Poets, H., 1992. Ancient atmospheric CO₂ pressures inferred from natural goethites. *Nature* 355, 342–344.
- Yapp, C.J., Poets, H., 1996. Carbon isotopes in continental weathering environments and variations in ancient atmospheric CO₂ pressure. *Earth Planet. Sci. Lett.* 137, 71–82.
- Zacchini, M., Morini, S., Vitagliano, C., 1997. Effect of photoperiod on some stomatal characteristics of in vitro cultured fruit tree shoots. *Plant, Cell, Tissue Organ Culture* 49, 195–200.