



Lomax, Barry H. and Fraser, Wesley T. and Smith, Andrew (2015) Palaeoproxies: botanical monitors and recorders of atmospheric change. *Palaeontology*, 58 (5). pp. 759-768. ISSN 1475-4983

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# FRONTIERS IN PALAEOONTOLOGY

3 PALAEOOPROXIES: BOTANICAL MONITORS AND  
RECORDERS OF ATMOSPHERIC CHANGE

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## **Abstract**

21

The integration of plant and Earth sciences offers the opportunity to develop and test palaeobotanical monitors and recorders (palaeoproxies) of past atmospheric change that are understood from a mechanistic perspective, with the underpinning responses being identified and understood at the genetic level. This review highlights how this approach has been used to deliver two distinct palaeoproxies. The first is based on the negative relationship between stomata (breathing pores found on the leaf surface) and atmospheric CO<sub>2</sub> concentration; the second is based on tracking chemical changes seen in the composition of pollen and spores to reconstruct changes in the flux of UV-B radiation on the Earth's surface and from this infer changes in stratospheric processes linked to the eruption and emplacement of Large Igneous Provinces. Here we highlight the potential of integrating a new rapid, inexpensive chemical analysis technique with existing, robust palynological methods, opening the door to a deeper understanding of past environments via the palaeo-biological record. A look to the future suggests a combined solar radiation-CO<sub>2</sub> concentration approach could be readily applied across the geological record.

36

## **Key words**

39 atmospheric change, Fourier transform infrared (FTIR) spectroscopy, palaeoproxies, stomata, sporopollenin, CO<sub>2</sub> and UV-B.

42 The fifth International Panel on Climate Change (IPCC) assessment AR5 (IPCC 2013), states  
“Warming of the climate system is unequivocal” and that “It is extremely likely that human  
influence has been the dominant cause of the observed warming since the mid-20th century”.  
45 Being able to place anthropogenic warming into context and establish how the Earth system  
responds to climate perturbation is becoming an ever more important area of study; one to  
which the paleontological community is in a unique position to contribute to, providing  
48 impact that is both relevant to policy maker and wider society.

Throughout the majority of the Phanerozoic the Earth’s climate state has been that of a  
greenhouse world, with equitable temperatures, a low tropic to pole heat gradient and little  
51 polar ice (Frakes et al, 1992). The unlocking of this record will enhance our understanding of  
and capacity to predict future climate change scenarios and importantly help to reduce  
uncertainty around these predictions. To achieve this it is necessary to develop well  
54 constrained and robustly tested paleo-proxies (Lomax et al. 2012a). Plants are innately  
coupled to the atmosphere requiring both sunlight and CO<sub>2</sub> to drive photosynthesis and  
carbon assimilation. When combined with their resilience and persistence (discussed below),  
57 the study of plant responses to climate change in concert with the analysis of fossil plants  
offer the opportunity to monitor past atmospheric conditions and infer palaeoclimate change.  
Here we highlight how this approach has led to the development of mechanistic  
60 palaeoproxies tested on palaeobotanically relevant extant species showing that plant fossils  
can be used as both monitors and geochemical recorders of atmospheric changes.

The evolutionary pressures on plants and animal are intrinsically different; effectively  
63 plants dance to an “evolutionarily different beat” (Traverse 1988). This was recognised in  
the late 1950’s with Gothan and Remy (1957) proposing the division of the Phanerozoic into  
three eras (Palaeophytic, Mesophytic and Cenophytic) based on major plant transitions that  
66 are clearly distinct from faunal extinctions (Traverse 1988). Viewing the stratigraphic column

through the lens of plant evolution fundamentally alters the timing of these major transitions and demonstrates the innate resilience of plants to abiotic forcing. The Palaeophytic/ Mesophytic transition is defined as a switch in dominance from Rhyniophytic (Eotrachyophytic) (Gray, 1993; Cleal et al. 2014) to cycadophytes, ginkgophytes and conifers, and occurs in the Early upper Permian. The Mesophytic/ Cenophytic transition is identified by the occurrence of Angiosperms in the Early Cretaceous. Consequently Traverse (1988) suggested that a land plant division of the Phanerozoic is based on originations and/ or changes to the dominance of ecological groups rather than extinctions. This fundamental mismatch has recently been highlighted by Cascales-Miñana and Cleal (2013), who in their family level investigation showed just – two major extinction events – in the plant fossil record; as opposed to the big five recognised in the marine realm (Raup and Sepkoski 1982). Furthermore others have argued that only one of these, the end Permian, was common to both databases (Cascales-Miñana and Cleal 2013). Plant responses to mass extinction typically take the form of ecological reorganisation, floral turnover and species level extinction e.g. Wolfe and Upchurch (1986) reviewed in McElwain and Punyasena (2007) and/or migrations (e.g Wing and Harrington 2001). These data indicate that plants have inherent resilience; achieved via a combination of factors related to reproduction (sexual and asexual) and dormancy, reviewed in Traverse (1988).

As sessile organisms, plants must be able to adapt to their local environment and retain a certain amount of capacity for acclimation. Often these responses can take the form of physiological adjustment(s) and/or changes in biochemistry. This gives plants further flexibility in the face of environmental pressure and offers a suite of mechanisms to safeguard against extinction. This in built resilience when coupled with our increased understanding of extant plant adaptation to a changing climate, often understood at the genetic level which is enabling the development of well constrained palaeoproxies which are understood in a

mechanistic manner. In this review we discuss how this bottom up mechanistic approach is  
93 leading to the enhanced understanding of two key environmental parameters of the  
palaeoatmosphere: atmospheric CO<sub>2</sub> and UV-B flux

## 96 PALAEOATMOSPHERE

The study of past atmospheric composition has gained traction since the 1980's, via a  
combination of geochemical modelling approaches e.g. the BLAG model of Berner et al.  
99 (1983) and the development of palaeoproxies both to test and validate model outputs, and  
further our understanding of the atmosphere e.g. (McElwain and Chaloner 1995). This  
integrated, interdisciplinary approach has been at the focus of research in this area since  
102 initiation. For example a joint meeting of the Paleontological Association and Linnaean  
Society held at the Geological Society produced a thematic set of papers on Biota and  
Palaeoatmospheres (Chaloner and Cocks 1989). Given the concurrent discovery of the  
105 stratospheric 'ozone hole' above Antarctica (Farman et al. 1985) and the dawning realization  
of the consequences of fossil fuel combustion, a major aim of aforementioned meeting  
(Chaloner and Cocks 1989) and subsequent research has been to place anthropogenic  
108 perturbations of the stratosphere and atmosphere into geological context. Hudson (1989)  
considered atmospheric change over a wide range of time scales and discussed the use of  
fossils as monitors and geochemical recorders of atmospheric changes. Since initial studies  
111 these complementary fields have developed in concert with model refinement (e.g. Bergman  
et al. 2004, Berner 2006) and palaeoproxy development (e.g. Fletcher et al 2008; Franks et al.  
2014). The importance of developing an understanding on climate change over geological  
114 time has been recognised by the wider scientific community with palaeoclimate work being  
included in assessment report four and five of the IPCC (IPCC 2007, 2013).

117 PALAEOBOTANICAL MONITORS: CO<sub>2</sub>

Stomata are small pores on the leaf surface which regulate gas exchange between the plant and the atmosphere. In his ground breaking paper Woodward (1987) demonstrated a significant negative relationship between that stomatal density (SD, the number of stomata per unit area) and stomatal index (SI, the number of stomata as a ratio of the number of stomata to epidermal cells expressed as a percentage) and atmospheric CO<sub>2</sub>. Through the analysis of herbarium samples spanning 200 years this paper provided the first direct evidence for human acceleration (via the combustion of fossil fuels) of the carbon cycle, based on evidence that is was driving plant adaptation /acclimation. Since this publication, *p*CO<sub>2</sub> has continued its rapid rise and today the mean annual CO<sub>2</sub> concentration is close to exceeding 400ppm. The paper linked observations from herbaria with experimental work to demonstrate causation as well as correlation; Woodward (1987) indicates that the negative relationship between CO<sub>2</sub> and SD/ SI is a function of plants adaptation to maximise carbon gain whilst minimising water loss, providing a mechanistic basis for the relationship (Fig. 1). The genes responsible for the stomatal CO<sub>2</sub> responses have since been identified (Grey et al. 2000) and the systemic nature of the signal has been established (Lake et al. 2001), demonstrating a causal link between CO<sub>2</sub> and stomata, which in turn provides underpinning confidence in the ability to use the distribution of stomata on fossil leaf surfaces to reconstruct palaeoatmospheric CO<sub>2</sub> concentration.

Both SD and SI are known to vary in response to a number of abiotic stimuli (see for example Tichá 1982). Because SD is linked to leaf expansion, it is inherently more variable than SI which is a measure of stomatal initiation. As a result of this much work has tended to focus on SI. However, Lake and Woodward (2008) reason that a SI can change when either

SD or epidermal cell density (ED) remain constant and either ED or SD change, also because  
141 guard cell length (GCL) is relatively stable (Lomax et al. 2009, 2014) and ED is highly  
dynamic SI does not reflect stomatal initiation which is lost as leaf development continues  
(Lake and Woodward 2008). Furthermore ED detail is often obscured as a function of fossil  
144 preservation making it difficult to calculate SI. This issue was highlighted in the recent study  
of Smith et al (2010) working on Ginkgo cuticle who observed that the anticlinal wall is often  
obscured by papillae again making the calculation of SI problematic. Therefore, although  
147 palaeo-CO<sub>2</sub> reconstructions based on SI may have theoretical advantages practical  
considerations combined with our new understanding of SI (Lake and Woodward 2008) may  
negate this advantage and estimates based on SD could be more robust (Smith et al 2010).

150 Changes in SD and SI have been used both in a semi-quantitative and quantitative way to  
predict changes in atmospheric CO<sub>2</sub> (Lomax et al 2012a). Semi-quantitative reconstructions  
are based on comparing fossil data with that of their nearest living relative (NLR) and/ or  
153 their nearest living ecological equivalent (NLE) (McElwain and Chaloner 1995, Chaloner and  
McElwain 1997, McElwain et al 1999) and CO<sub>2</sub> estimates are calculated based on the ratio of  
stomata in NLR and/ or NLE to their fossil counterpart. Quantitative use of SD and SI to  
156 estimate palaeo-CO<sub>2</sub> is based on the development of species specific (NLR/ NLE) training  
sets which describe the mathematical relationships between SD and SI to known changes in  
atmospheric CO<sub>2</sub>. This relationship is then used to predict palaeo-CO<sub>2</sub> from counts SD and  
159 SI made from fossil cuticle. palaeo-CO<sub>2</sub> fossil cuticle. These studies are increasingly linking  
experimental work on plants grown in high and low CO<sub>2</sub> growth chambers to information  
recovered from plants held in herbaria. This combined approach captures stomatal responses  
162 to a wide range of atmospheric CO<sub>2</sub> concentrations from sub-ambient through to elevated  
greenhouse conditions representative of Phanerozoic. For example calibration curves using  
this approach have been constructed for *Ginkgo biloba* and *Metasequoia glyptostroboides*



165 with an upper experimental CO<sub>2</sub> concentration of 790ppm to constrain Paleogene CO<sub>2</sub>  
(Royer et al 2001a) and using *Stenochlaena palustris* grown at 2300ppm, Beerling et al  
(2002) estimated changes in CO<sub>2</sub> across the Cretaceous/ Paleogene boundary. Stomatal  
168 analysis of the Cretaceous Paleogene indicates super greenhouse conditions in the earliest  
Paleogene, with CO<sub>2</sub> concentrations in excess of 2000ppm (Beerling et al., 2002). These  
findings support the lower range of modelled CO<sub>2</sub> perturbations as a function of the  
171 degassing of limestone from the Chicxulub limestone platform (O'Keefe and Ahrens 1989)  
but these stomatal estimates are lower than palaeo-CO<sub>2</sub> estimates derived from palaeosols  
(Nordt, Atchley and Dworkin 2002, Huang et al., 2013). The apparent discrepancy between  
174 stomatal and paleosol reconstructions could be related to stratigraphic position above the  
Cretaceous Paleogene boundary with stomata data been recovered from within the fern spike,  
which has an estimated duration of a few hundred to a few thousand years (Vajda and  
177 McLoughlin 2007).

The major rate limiting step in developing CO<sub>2</sub> estimates based on stomatal analysis is  
the recognition that the response appears to be species specific, with plants showing an  
180 increase, a decrease or no change in stomatal numbers in response to elevated CO<sub>2</sub> (Royer  
2001b). Furthermore, the pattern of response seems to be independent of phylogeny with  
closely related species behaving in differently (reviewed in Jordan 2011). These data  
183 suggests that quantitative CO<sub>2</sub> reconstructions based on stomatal analysis of NLR could be  
hindered by the need to work within characterised and constrained phylogenetic groups.  
Consequently the power of the technique to explore variations in CO<sub>2</sub> over geological time  
186 may become more limited (an increase in uncertainty around the predicted CO<sub>2</sub>) when it is  
applied further back in geological time when the relationship between NLRs and the fossil  
target species becomes obscured. This maybe further compromised if fossil values of SD  
189 and/or SI are outside of the range of training set. This results in statistical uncertainty around

predicted palaeo-CO<sub>2</sub> concentration resulting in large error bars around the predicted value. However, how much of the stomatal variation identified by Royer (2001) and Jordan (2011) represents actual biological variation in response to changing atmospheric CO<sub>2</sub> concentration has been questioned recently (Lake and Woodward 2008). Experimental data indicates that changes in humidity can suppress stomatal responses to an increase in atmospheric CO<sub>2</sub> concentration. Data from *Arabidopsis thaliana* shows that in response to a doubling of CO<sub>2</sub> SD decreased by 13% in low relative humidity (RH 45%) conditions, whereas when RH was increased to 65% SD declined by 32% (Lake and Woodward 2008). Therefore the lack of RH control in experimental settings might contribute to some variations recorded in response to changes in CO<sub>2</sub>; it may also explain why there is variation in SD with changes in altitude (Lake and Woodward 2008). These findings suggest that if hydrology can be controlled for, SD may effectively monitor changes in atmospheric CO<sub>2</sub>. Given the longstanding acknowledgment of wetland bias in the plant fossil record (e.g. Spicer 1981, Channing and Edwards 2013) from a palaeobotanical standpoint this may indeed be achievable.

Franks et al (2014) have developed an alternative approach to address the need for phylogenetically well constrained training sets via the development of a mechanistic model. The model was developed by linking the universal gas exchange equation (Farquhar, Caemmerer and Berry 1980) to anatomical and geochemical information that can be readily recovered from the plant fossil record (Franks et al. 2014). Applying this model (at a low resolution) over the Phanerozoic suggests that large scale, first order fluctuations in the atmospheric carbon cycle may not have occurred. Specifically their model suggests that atmospheric CO<sub>2</sub> has remained relatively stable and below 1000ppm (Franks et al. 2014) since the evolution and radiation of forests in the Middle Devonian (Stein et al. 2012). These data and modelled solutions for CO<sub>2</sub> concentrations are low when compared to other CO<sub>2</sub> proxy records (Eckart et al. 1999) but compare favourably to recent modelled solutions of

the long-term carbon cycle (Berner et al. 2006) and the proxy record generated from the  
216 carbon isotope analysis of fossil liverworts (Fletcher et al. 20008 ). If correct these, data  
suggest that the Earth system, specifically temperature, is much more sensitive to changes in  
the carbon cycle than previously thought (Franks et al 2014). However, what is required to  
219 fully evaluate this controversial finding is full testing of the model boundary conditions and  
the ecophysiological responses that underpin the model over a wider range of atmospheric  
CO<sub>2</sub> concentrations using a broader phylogenetic spectrum of plants, with the with analysis  
222 being conducted at a finer temporal resolution. However these preliminary findings  
demonstrate the importance of developing proxies to understanding past climatic conditions  
which can be robustly tested. Furthermore, they demonstrate how palaeoclimate work can be  
225 used to understand how the Earth system may respond to future, anthropogenic climate  
forcing.

## 228 PALAEOBOTANICAL GEOCHEMICAL RECORDERS: UV-B

The sporomorph (pollen and spore) fossil record is one of the most complete records  
available to palaeontologist both in terms of temporal and spatial range. This richness is a  
231 function of the amount produced (for example Traverse 2008 “guestimates” that an average  
hectare of woodland in eastern North America would produce at least 3000 litres of  
sporomorphs) and their recalcitrant chemistry. The biopolymer sporopollenin is a major  
234 component of the outer walls (exine) of terrestrial plant-derived pollen and spores (Brooks  
and Shaw 1968, 1978; Hemsley et al. 1996) and it is this biopolymer which is responsible for  
their high preservation potential of sporomorphs. Recent work (e.g. Lomax et al 2008, Fraser  
237 et al. 2011, 2012) has focused on using Fourier transform infrared (FTIR) spectroscopy to  
further the understanding of the structure of sporopollenin via the detection and quantification

of functional groups. The topic of spectroscopy and its application to fossils was the subject  
240 of a recent *Frontiers in Palaeontology* review (Marshall and Marshall 2015). Sporopollenin is  
constructed of two groups of chemicals, phenolic derivatives (*para*-coumaric and ferulic  
acids) and straight chain aliphatic moieties in the range C8-C22. The aromatic ring common  
243 to the phenolic compounds within sporopollenin absorbs and dissipates the energy associated  
with ultraviolet radiation (Rozema et al. 2001; Rozema et al. 2002), while the straight chain  
aliphatic moieties do not absorb UV-B radiation (Fraser et al. 2011). Recent work further  
246 suggests that the chemical composition of sporopollenin is a function of the local  
environment with plants responding to an increase or decrease in ambient UV-B by up or  
down regulating the phenolic component of sporopollenin (Figure 2; Fraser et al. 2011).

249       Geochemical analysis demonstrates that sporopollenin was present in essentially the  
same chemical form in sporomorphs dating to the Early-Middle Pennsylvanian (~310 Ma)  
(Fraser et al. 2012), and that it is very similar to Silurian cryptospores with recognised  
252 embryophytic affinity (Steemans et al. 2010). Furthermore sporopollenin chemistry is  
structurally similar to the zygote wall of the closely related charophycean alga *Coleochaete*  
(Delwiche et al. 1989). Fraser et al. (2012) indicate that these findings suggest that the  
255 chemical structure of sporopollenin has remained highly conserved since the embryophytes  
invaded land in the Middle Ordovician (Rubinstein et al. 2010). Biomolecular work has  
identified the protein (UVR8) responsible for the perception and subsequent upstream  
258 regulation of plant responses to UV-B radiation (Rizzini et al. 2011) and the mechanism  
behind this response has been identified at the genetic level in *Arabidopsis thaliana* with  
orthologous genes being reported in algae and mosses, strongly suggesting evolutionary  
261 conservatism in UV-B perception (Christie et al. 2012). Most recently, evolutionary  
development work has presented evidence that the genetic basis of sporopollenin production,

and subsequent generation of a spore wall, was recruited early on in land plants and has been  
264 highly conserved since (Wallace et al. 2015).

When seeking to establish if biogeochemical information extracted from fossil spores  
and pollen grains can be used in either a quantitative or semi- quantitative manner it is  
267 important to consider the fate of sporomorphs as they undergo diagenesis and the transitions  
from the biosphere to the geosphere. Analysis of geological material from sites recognised as  
having being subjected to low grade diagenetic alteration indicated that the primary  
270 biogeochemical signature can be recovered (Steemans et al. 2010; Fraser et al. 2012).  
Experimental analysis using lycopodium spores further supports these findings (Fraser et al.  
2014) in these experiments fresh lycopods spores were heated in a hydrous bomb over a  
273 range of temperatures (0, 100, 150, 200, 250, 300, and 350 °C) to simulate varying intensities  
of diagenetic alteration from immature to thermally mature. Results specifically  
demonstrated that the phenolic content of sporopollenin remains unaffected at lower grades  
276 of diagenesis and are stable up to an experimental temperature of 200-250°C. Similarly,  
oxidative processes, whether geologically driven, or preparative in the laboratory, are proving  
to have minimal impact upon sporopollenin chemistry demonstrating a relatively broad  
279 window of opportunity in terms of viability of samples for successful analysis (Jardine et al.  
2015).

Taken collectively these findings show that sporopollenin chemistry and the perception  
282 of UV-B radiation within plants is from an evolutionary perspective highly conserved. It is  
possible to recover primary biogeochemical information from the fossil record, and that  
within extant plants the functional chemistry of sporopollenin is influenced by the local  
285 environment; plants respond to an increase in the ambient UV-B flux by producing more  
phenolic compounds (Figure 2; Lomax et al. 2008; Fraser et al. 2011). These findings  
indicate that quantification of the phenolic component within sporopollenin may provide the

288 ability to track changes in ambient UV-B through geological time, effectively using the  
sporomorph record as a geochemical recorder of stratospheric change.

The development of a new proxy to track UV-B flux, and from this infer changes in  
291 stratospheric process over geological time, allows for the testing and development of new  
investigative pathways. For example, a growing body of work has linked the end Permian  
mass extinction event to a global collapse of the stratospheric ozone layer as a consequence  
294 of the eruption and emplacement of the Siberian Traps Large Igneous Province (Beerling et  
al. 2007; Black et al. 2014). The occurrence of abnormal lycopsid spores (Visscher et al.  
2004) and gymnosperm pollen (Foster and Affoni 2005) has been interpreted as evidence for  
297 UV-B driven mutagenesis, with recent experimental evidence confirming pollen mutation in  
*A. thaliana* in response to elevated UV-B flux (Murphy and Mitchell 2013). Therefore  
chemical analysis of the end Permian sporomorph record may provide an opportunity to test  
300 this hypothesis and determine stratospheric and atmospheric perturbations through this likely  
tumultuous period in Earth history.

Looking beyond direct stratospheric processes, in the modern world the UV-B flux is  
303 positively correlated to altitude, with the magnitude of the UV-B flux being controlled by  
latitude (Lomax et al. 2012b). This altitudinal relationship is governed by the physical  
properties of the atmosphere, surface albedo, and pathlength of incoming solar radiation.  
306 Geochemical analysis of extant sporomorphs has demonstrated a statistically significant  
relationship between altitude and the phenolic component of sporopollenin (Lomax et al.  
2012b). This pattern is also repeated in other plant tissues (Rozema et al. 1997) and the  
309 relationship is found to hold across phylogenetically disparate groups (Sullivan et al. 1992).  
These data indicate that the chemical record of pollen and spore assemblages may have the  
potential to be exploited as a novel palaeoaltimetry proxy (Lomax et al. 2012b) and record  
312 elevation history.

## CONCLUSIONS AND FUTURE DIRECTIONS:

315 The research reviewed in this paper demonstrates that the plant fossil record has the  
capacity to be used in a mechanistically understood framework to record and monitor  
changes in environmental conditions over geological time. These data could therefore allow  
318 for historical and current anthropogenic climate change to be placed within a broader  
geological context as originally proposed by Chaloner and Cocks (1989), demonstrating the  
broader impact and utility of paleontologically based research.

321 Specifically the development of a mechanistic model to estimate palaeo-CO<sub>2</sub>  
independent of the need for phylogenetically constrained training sets would enable CO<sub>2</sub>  
reconstructions of the lower Palaeozoic atmosphere, allowing for the development of  
324 Phanerozoic CO<sub>2</sub> curves. These could then be used to test/ validate long term carbon cycle  
models. This mechanistic approach may lead an increase in certainty of the predicted  
atmospheric CO<sub>2</sub> concentration and importantly minimise the uncertainty underpinning these  
327 prediction.

The chemistry of sporopollenin is regulated by UV-B exposure and that these signals can  
be recovered from the fossil record. Prior to this work differences in the chemical signature  
330 of sporopollenin (and fossil macromolecules in general) were generally attributed to either  
evolution or diagenetic alteration, or a combination of both. The realisation that the  
environment alters the primary configuration of plant macromolecules and that this  
333 information can be recovered from the fossil record has the potential to transform how we  
see/use these data. The future for chemical palynological investigations should look to  
address global challenges that are underpinned by changes in energy input to the Earth  
336 system from the Sun, such as: global hydrology on geological timescales (increased

atmospheric moisture is likely to impair transmittance of ultraviolet radiation); verification of orbital cyclicity within the sedimentary record, and from this chemical palynology could potentially act as a tuning device for correlation work. Looking outside of palaeoclimate reconstruction the nature of sporopollenin itself spanning geological/evolutionary time - the exact nature of sporopollenin remains enigmatic, but recent had made some headway; if such work continues a much deeper understanding of the role of sporopollenin in plants survival and adaptation since invading the terrestrial realm is a realistic possibility.

A key advantage of the using FTIR to interrogate sporopollenin is low cost and relative ease of analysis, this provides the opportunity to integrate such a technique within the course of "standard" palynological investigations, thus developing a greater understanding of the ancient ecosystems being studied. A key aspect that provides the greatest potential for a chemical palynology proxy for solar radiation is the long-term preservation that is possible; the pervasive nature of sporomorphs throughout the sedimentary record suggests a globally abundant information resource is waiting to be analysed. Combining biogeochemical analysis of the sporomorph record to track changes in incoming total solar irradiance with stomatal estimates of CO<sub>2</sub> could provide key information on long term climate forcing mechanisms.

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360 *Acknowledgements*

We thank Andrew Smith for the invitation to contribute to this review series in  
Palaeontology, the NERC (NER/A/S/2002/00865, NE/K005294/1) the Leverhulme trust  
363 (ECF/2006/0492) and the Royal Society (RG120535) for funding that initiated and facilitated  
our research into palaeo-UV-B flux and two reviewers for their helpful suggestions. We  
dedicate this paper to Bill Chaloner and Ian Woodward for their incisive and pioneering work  
366 that has done much to develop these fields of enquiry.

369 **REFERENCES**

BEERLING, D.J., LOMAX, B. H., ROYER, D.L., UPCHURCH, G. R. Jr., and KUMP, L. R.  
372 2002. An atmospheric  $p\text{CO}_2$  reconstruction across the Cretaceous-Tertiary boundary  
from leaf megafossils. *Proceedings of the National Academy of Science USA*, **99**,  
7836–7840,

375

BEERLING, D.J., HARFOOT, M., LOMAX, B., and PYLE, J.A. 2007. The stability of the  
stratospheric ozone layer during the end-Permian eruption of the Siberian Traps.  
378 *Philosophical Transactions of the Royal Society A* **365**, 1843–1866.

BERGMAN, N.M., LENTON, T.M. and WATSON, A.J. 2004. COPSE: a new model of  
381 biogeochemical cycling over Phanerozoic time. *American Journal of Science* 304, 397–  
437.

384 BERNER, R. A., LASAGA, A.C. and GARRELS, R.M. 1983. The carbonate-silicate  
geochemical cycle and its effect on atmospheric carbon-dioxide over the past 100 million  
years. *American Journal of Science* **283**, 641–683.

387

BERNER, R. A. 2006. GEOCARBSULF: A combined model for Phanerozoic atmospheric  
 $\text{O}_2$  and  $\text{CO}_2$ . *Geochimica et Cosmochimica Acta* **70**, 5653–5664.

390

BLACK, B.A., LAMARQUE, J., SHIELDS, C.A., ELKINS-TANTON, L.T., and KIEHL JT.

2014. Acid rain and ozone depletion from pulsed Siberian Traps magmatism. *Geology*.

393 42, 67–70.

BROOKS, J. and SHAW, G. 1968. Chemical structure of the exine of pollen walls and a new

396 function for carotenoids in nature. *Nature*, **219**, 532–533.

BROOKS, J. and SHAW, G. 1978. Sporopollenin: A review of its chemistry,

399 palaeochemistry and geochemistry. *Grana* **17**, 91–97.

CASCALES-MIÑANA, B. and CLEAL C.J. 2013. The plant fossil record reflects just two

402 great extinction events. *Terra Nova*, **26**, 195–200.

CHALONER, W.G. and COCKS, L.R.M. 1989. Biota and Palaeoatmospheres. *Journal of*

405 *the Geological Society of London*, **146**, 145–146.

CHALONER, W.G. and McELWAIN, J, 1997. The fossil plant record and global climatic

408 change. *Review of Palaeobotany and Palynology* **95**, 73–82.

- CHANNING, A. and EDWARDS, D. 2013. Wetland megabias: ecological and  
411 ecophysiological filtering dominates the fossil record of hot spring floras.  
*Palaeontology* **56**, 523–556.
- 414 CHRISTIE, J.M., ARVAI, A.S., BAXTER, K.J., HEILMANN, M., PRATT, A.J., O'HARA,  
A., KELLY, S.M., HOTHORN, M. SMITH, B.O. HITOMI, K. JENKINS, G.I. and  
GETZOFF, E.D. 2012. Plant UVR8 photoreceptor senses UV-B by tryptophan-mediated  
417 disruption of cross-dimer salt bridges. *Science*, **335**, 1492–1496.
- CLEAL, C.J. and CASCALES-MIÑANA, B. 2014. Composition and dynamics of the great  
420 Phanerozoic Evolutionary Floras. *Lethaia* **47**, 469-484.
- DELWICHE, C.F., GRAHAM, L.E. and THOMSON N. 1989. Lignin-like compounds and  
423 sporopollenin in *Coleochaete*, an algal model for land plant ancestry. *Science* **245**, 399–  
401.
- 426 EKART, D.D., CERLING, T.E., MONTANEZ, I.P. and TABOR, N.J. 1999. A 400 million  
year carbon isotope record of pedogenic carbonate: Implications for paleoatmospheric  
Carbon Dioxide. *American Journal of Science* **299**, 805–827.
- 429
- FARMAN, J.C., GARDINER, B.G. and SHANKLIN, J.D. 1985. Large losses of total ozone  
in Antarctica reveal seasonal CLO<sub>x</sub>/NO<sub>x</sub> interaction. *Nature* **315**, 207–210.

432

FARQUHAR, G. D., VON CAEMMERER, S., and Berry, J. A. 1980. A biochemical model of photosynthetic CO<sub>2</sub> assimilation in leaves of C<sub>3</sub> plants, *Planta* **149**, 78–90.

435

FLETCHER, B.J., BRENTNALL, S.J. ANDERSON C.W., BERNER R.A. and BEERLING, D. J. 2008. Atmospheric carbon dioxide linked with Mesozoic and early Cenozoic climate change. *Nature Geoscience* **1**, 43–48.

438

FRAKES, L.A., FRANCIS, J.E., and SYKTUS, J.I. 1992. Climate models of the Phanerozoic: The history of the Earth's Climate over the past 600 Million Years. Camb. Univ. Press.

441

444 FRANKS, P.J., ROYER, D.L., BEERLING, D.J., VAN DE WATER, P.K., CANTRILL, D.J., BARBOUR M.M. and BERRY, J. A. 2014. New constraints on atmospheric CO<sub>2</sub> concentration for the Phanerozoic. *Geophysical Research Letters*, **41**, 4685–4694, doi:10.1002/2014GL060457.

447

FRASER, W.T., SEPHTON, M.A., WATSON, J.S., SELF, S., LOMAX, B.H., JAMES, D.I. WELLMAN, C.H., CALLAGHAN, T.V. and BEERLING, D.J. 2011. UV-B absorbing pigments in spores: biochemical responses to shade in a high-latitude birch forest and implications for sporopollenin-based proxies of past environmental change. *Polar Research* **30**, art. 8312. DOI: 10.3402/polar.v30i0.8312

453

- FRASER, W.T., SCOTT, A.C., FORBES, A.E.S. GLASSPOOL, I.J., PLOTNICK, R.E.,  
456 KENIG, F. and LOMAX, B.H. 2012. Evolutionary stasis of sporopollenin biochemistry  
revealed by unaltered Pennsylvanian spores. *New Phytologist* **196**, 397–401.
- 459 FRASER, W.T., WATSON, J.S., SEPHTON, M.A., LOMAX, B.H., HARRINGTON, G.,  
GOSLING, W.D. & SELF, S. 2014. Changes in spore chemistry and appearance with  
increasing maturity. *Review of Palaeobotany and Palynology* **201**, 41–46.
- 462
- FOSTER C.B. and AFONIN S.A. 2005. Abnormal pollen grains: An outcome of  
deteriorating atmospheric conditions around the Permian-Triassic boundary. *Geological*  
465 *Society of London Journal*, **162** 653–659
- GOTHAN, W. and REMY W. 1957. *Steinkohlenpflazen* Essen. Verlag. Glückauf.
- 468
- GRAY, J.E., HOLROYD, G.H., VAN DER LEE, F.M., BAHRAMI, A.R., SIJMONS P.C.,  
WOODWARD F.I., SCHUCH, W. and HETHERINGTON A.M. 2000. The HIC  
471 signalling pathway links CO<sub>2</sub> perception to stomatal development. *Nature*, **408**, 713–  
716.

- 474 GRAY, J. 1993. Major Paleozoic land plant evolutionary bio-events. *Palaeogeography, Palaeoclimatology, Palaeoecology* **104**, 153-169.
- 477 HEMSLEY, A.R., SCOTT, A.C., BARRIE, P.J. and CHALONER, W.G. 1996. Studies of fossil and modern spore wall biomacromolecules using  $^{13}\text{C}$  solid state NMR. *Annals of Botany* **78**, 83–94.
- 480
- HUANG, C., RETALLACK, G.J., WANG, C. and HUANG, Q. 2013. Paleatmospheric pCO<sub>2</sub> fluctuations across the Cretaceous–Tertiary boundary recorded from paleosol carbonates in NE China. *Palaeogeography, Palaeoclimatology, Palaeoecology* **385**, 95–105.
- 483
- 486 HUDSON, J.D. 1989. Palaeoatmospheres in the Phanerozoic. *Journal of the Geological Society of London* **146**, 155–160.
- 489
- IPCC, 2007: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 996 pp.
- 492
- 495

IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working  
Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate  
498 Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A.  
Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press,  
Cambridge, United Kingdom and New York, NY, USA, 1535 pp.

501

JARDINE, P.E., FRASER, W.T., LOMAX, B.H. and GOSLING, W.D. 2015. The impact  
of oxidation on spore and pollen chemistry. *Journal of Micropalaeontology*. doi:  
504 10.1144/jmpaleo2014-022

JORDAN, G. 2011. A critical framework for the assessment of biological proxies: predicting  
507 past climate and levels of atmospheric CO<sub>2</sub> from fossil leaves. *New Phytologist*, **192**,  
29–44.

510 LAKE, J.A., QUICK, W.P., BEERLING D.J. and WOODWARD, F.I. 2001. Plant  
development: Signals from mature to new leaves. *Nature*, **411**, 154.

513 LAKE, J.A. and WOODWARD, F.I. 2008. Response of stomatal numbers to CO<sub>2</sub> and  
humidity: control by transpiration rate and abscisic acid. *New Phytologist*. **179**, 397–404.

516 LOMAX, B.H, FRASER, W.T., SEPHTON, M.A., CALLAGHAN, T.V., SELF, S.,  
HARFOOT, M., PYLE, J.A., WELLMAN, C.H. and BEERLING D.J. 2008. Plant



- spore walls as a record of long-term changes in ultraviolet-B radiation. *Nature*  
519 *Geoscience* 1, 592–596.
- LOMAX, B.H., WOODWARD, F.I., LEITCH, I.J., KNIGHT, C.A. and LAKE, J.A. 2009.  
522 Genome size as a predictor of guard cell length in *Arabidopsis thaliana* is independent of  
environmental conditions. *New Phytologist*, **181**, 311–314.
- 525 LOMAX, B.H., KNIGHT, C.A. and LAKE, J.A. 2012a. An experimental evaluation of the  
use of C3  $\delta^{13}\text{C}$  plant tissue as a proxy for the palaeoatmospheric  $\delta^{13}\text{CO}_2$  signature of air.  
*Geochemistry Geophysics and Geosystems*, **13**, Q0AI03, doi:10.1029/2012GC004174.
- 528
- LOMAX, B.H., FRASER, W.T., HARRINGTON, G., BLACKMORE, S., SEPHTON, M.A.,  
and HARRIS, N.B.W. 2012b. A novel palaeoaltimetry proxy based on spore and  
531 pollen wall chemistry. *Earth and Planetary Science Letters*, **353–354**, 22–28.
- LOMAX, B.H., HILTON, J., BATEMAN, R.M., UPCHURCH, G.R. Jr., LAKE, J.A.,  
534 LEITCH, I.J., CROMWELL, A. and KNIGHT, C.A. 2014. Reconstructing relative  
genome size of vascular plants through geological time. *New Phytologist*, **201**, 636–644.
- 537 MARSHALL, A.O. and MARSHALL, CP. 2015. Vibrational spectroscopy of fossils.  
*Palaeontology*, **58**, 201–211.

- 540 McELWAIN, J.C. and CHALONER, W.G. 1995. Stomatal density and index of fossil plants  
track atmospheric carbon dioxide in the Palaeozoic. *Annals of Botany*, **76**, 389–395.
- 543 McELWAIN, J.C., BEERLING, D.J. and WOODWARD, F.I. 1999. Fossil plants and global  
warming at the Triassic–Jurassic boundary. *Science* **285**, 1386–1390.
- 546 McELWAIN, J.C. and PUNYASENA, S.W. 2007. Mass extinction events and the plant  
fossil record. *Trends in Ecology and Evolution* **22**, 548–557.
- 549 MURPHY, B.R. and MITCHEL, F.J.G. 2013. An association between past levels of ozone  
column depletion and abnormal pollen morphology in the model angiosperm *Arabidopsis*  
*thaliana* L. *Review of Palaeobotany and Palynology* **194**, 12–20.
- 552
- NORDT, L., ATCHLEY, S. and DWORKIN S.I. 2002. Paleosol barometer indicates extreme  
fluctuations in atmospheric CO<sub>2</sub> across the Cretaceous-Tertiary boundary. *Geology* 30, 7  
555 03–706.
- O'KEEFE, J.D. and AHRENS T.J.1989. Impact production of CO<sub>2</sub> by the  
558 Cretaceous/Tertiary extinction bolide and the resultant heating of the Earth. *Nature* **3**  
**38**, 247–249.

- 561 RAUP, D.M and SEPKOSKI, J.J. Jr. 1982. Mass Extinctions in the Marine Fossil Record.  
*Science*. **215**, 1501–1503.
- 564 RIZZINI, L., FAVORY, J.J., CLOIX, C., FAGGIONATO, D., O'HARA, A., KAISERLI, E.  
BAUMEISTER, R., SCHAFER E., NAGY, F. JENKINS, G.I. and ULM, R. 2011.  
Perception of UV-B by the *Arabidopsis* UVR8 Protein. *Science*, **332**, 103–106.
- 567
- ROYER, D.L., WING, S.L., BEERLING, D.J., JOLLEY D.W., KOCH, P. L. HICKEY, L.J.,  
and BERNER, R.A. 2001a. Paleobotanical evidence for near present-day levels of  
570 atmospheric CO<sub>2</sub> during part of the Tertiary. *Science*, **292**, 2310–2313.
- ROYER, D.L. 2001b. Stomatal density and stomatal index as indicators of paleoatmospheric  
573 CO<sub>2</sub> concentration. *Review of Palaeobotany and Palynology*, **114**, 1–28.
- ROZEMA, J., CHARDONNENS, A., TOSSERAMS, M., HAFKENSCHIED, R. and  
576 BRUIJNZEEL, S. 1997. Leaf thickness and UV-B absorbing pigments of plants in  
relation to an elevational gradient along the Blue Mountains, Jamaica. *Plant Ecology*  
**128**, 151–159.
- 579

ROZEMA, J., NOORDIJK, A.J., BROEKMAN, R.A., van BEEM, A., MEIJKAMP, B.M.,  
de BAKKER, N.V.J., van de STAAL, J.W.M., STROETENGA, M., BOHNCKE, S.J.P.,  
582 KONERT, M., KARS, S., PEAT, H., SMITH, R.I.L. and CONVEY, P. 2001.  
(Poly)phenolic compounds in pollen and spores of Antarctic plants as indicators of solar  
UV-B: A new proxy for the reconstruction of past solar UV-B? *Plant Ecology* **154**, 11–  
585 26.

ROZEMA, J., van GEEL, B., BJORN, L. O., LEAN, J. and MADRONICH, S. 2002.  
588 Paleoclimate: Toward solving the UV puzzle. *Science* **296**, 1621–1622.

RUBINSTEIN, C.V., GERRIENNE, P., de la PUENTE, G.S., ASTINI, R.A. and  
591 STEEMANS, P. 2010. Early Middle Ordovician evidence for land plants in Argentina  
(eastern Gondwana). *New Phytologist*, **188**, 365–369.

594 SMITH, R.A., GREENWOOD, D.R. and BASINGER, J.F. 2010. Estimating  
paleoatmospheric  $p\text{CO}_2$  during the Early Eocene Climatic Optimum from of Ginkgo,  
Okanagan Highlands, British Columbia, Canada. *Palaeogeography, Palaeoclimatology,*  
597 *Palaeoecology* **293**, 120–131.

SPICER, R.A. 1981. The sorting and deposition of allochthonous plant material in a modern  
600 environment at Silwood Lake, Silwood Park, Berkshire, England. *US Geological*  
*Survey Professional Papers*, **1143**, 1–77.

603 STEEMANS, P., LEPOT, K., MARSHALL, C.P., Le HÉRISSÉ, A. and JAVAUX, M.J.  
2010. FTIR characterisation of the chemical composition of Silurian miospores  
(cryptospores and trilete spores) from Gotland, Sweden. *Review of Palaeobotany and*  
606 *Palynology* **162**, 577–590.

STEIN, W.E., BERRY, C. M., HERNICK, L VanAller and MANNOLINI, F. 2012.  
609 Surprisingly complex community discovered in the mid-Devonian fossil forest at Gilboa.  
*Nature*. **483**, 78–81

612 SULLIVAN, J.H., TERAMURA, A.H., and ZISKA, L.H., 1992. Variation in UV-B  
sensitivity in plants from a 3,000m elevational gradient in Hawaii. *American Journal of*  
*Botany*. **79**, 37–743.

615

TICHÁ, I. 1982. Photosynthetic characteristics during ontogenesis of leaves. 7. Stomata  
density and sizes. *Photosynthetica*, **16**, 375–471

618

TRAVERSE, A. 1988. Plant evolution dances to a different beat. Plant and animal  
evolutionary mechanisms compared. *Historical Biology*, **1**, 277–301.

621

TRAVERSE, A. 2008. Paleopalynology. Second edition. Springer Dordrecht Netherlands.  
813pp

624

VAJDA, V. and McLOUGHLIN, S. 2007. Extinction and recovery patterns of the vegetation  
across the Cretaceous–Palaeogene boundary – a tool for unravelling the causes of the  
627 end-Permian mass-extinction. *Review of Palaeobotany and Palynology* **144**, 99–112.

630

VISSCHER, H., LOOY, C.V., COLLINSON, M.E., BRINKHUIS, H., CITTERT, J.,  
end-Permian ecological crisis. *Proceedings of the National Academy of Science USA*,  
**101**, 12952–12956.

633

WALLACE, S., CHATER, C.C., KAMISUGI, Y., CUMING, A.C., WELLMAN, C.H.,  
BEERLING, D.J. and FLEMING, A.J. 2015. Conservation of *Male Sterility 2* function  
636 during spore and pollen wall development supports an evolutionarily early recruitment of  
a core component in the sporopollenin biosynthetic pathway. *New Phytologist*, **205**, 390-  
401.

639

WATSON, J.S., FRASER, W.T. and SEPHTON, M.A. 2012. Formation of a polyalkyl  
macromolecule from the hydrolysable component within sporopollenin during  
642 heating/pyrolysis experiments with Lycopodium spores. *Journal of Analytical and  
Applied Pyrolysis* **95**, 138–144.

645 WATSON, J.S., SEPHTON, M.A., SEPHTON, S.V., SELF, S., FRASER, W.T., LOMAX,  
BH. GILMOUR, I., WELLMAN, C.H. and BEERLING, D.J. 2007. Rapid  
determination of spore chemistry using thermochemolysis gas chromatography-  
648 mass spectrometry and micro-Fourier transform infrared spectroscopy. *Photochemical  
and Photobiological Sciences*, **6**, 689–694.

651 WING, S.L. and HARRINGTON, G.J. 2001. Floral response to rapid warming at the  
Paleocene/Eocene boundary and implications for concurrent faunal change.  
*Paleobiology*, **27**, 539–563.

654

WOLFE, J.A. and UPCHURCH G.R. Jr. 1986. Vegetation, climatic and floral changes at the  
Cretaceous-Tertiary boundary. *Nature*, **324**, 148–152.

657

WOODWARD, F. I. 1987. Stomatal numbers are sensitive to increases in CO<sub>2</sub> from pre-  
industrial levels. *Nature*, **327**, 617–618.

660

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666

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675

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681 **Figure Legends**

684 **FIG. 1.** Schematic representation of changes in stomatal density and index as a function of declining atmospheric CO<sub>2</sub> over geological time. A, CO<sub>2</sub>. B, Water availability is indicated



by the solid blue line, dotted blue line indicates changes in plant water use efficiency (*WUE*) as a function of changing stomatal numbers in response to atmospheric CO<sub>2</sub>. A shift to the right relative to the solid blue line indicates a reduction in *WUE* whereas a shift to the left indicates an increase in *WUE*. C, schematic representation of a 1mm<sup>-2</sup> of leaf cuticle SD, stomatal density and SI stomatal index.

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**FIG. 2.** Schematic representation demonstrating the mechanistic process driving chemical changes within sporopollenin composition. Specific regions (ultraviolet-B) within the electromagnetic spectrum of incoming solar radiation (insolation) trigger genetically controlled chemical responses in plants. UV-B radiation stimulation leads to the up-regulation of two specific phenolic-based components of sporopollenin, which are then incorporated into the exine of pollen/spores, providing a passive protective mechanism against damage to internal structures, proteins and DNA by UV-B radiation. Once released from the plant, pollen/spores retain this phenolic-based chemical signature, which can be later recovered if these pollen/spores are conserved within the geological record. The image of the Sun is courtesy NASA/JPL-Caltech.

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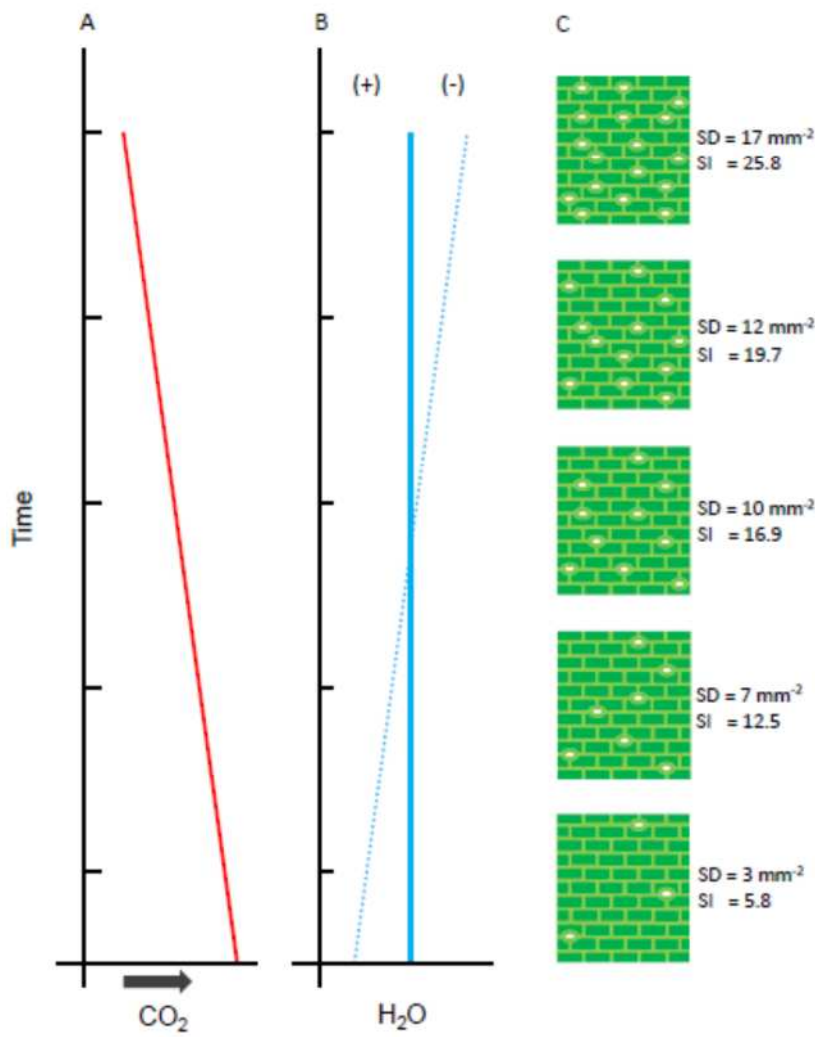


Fig.1.  
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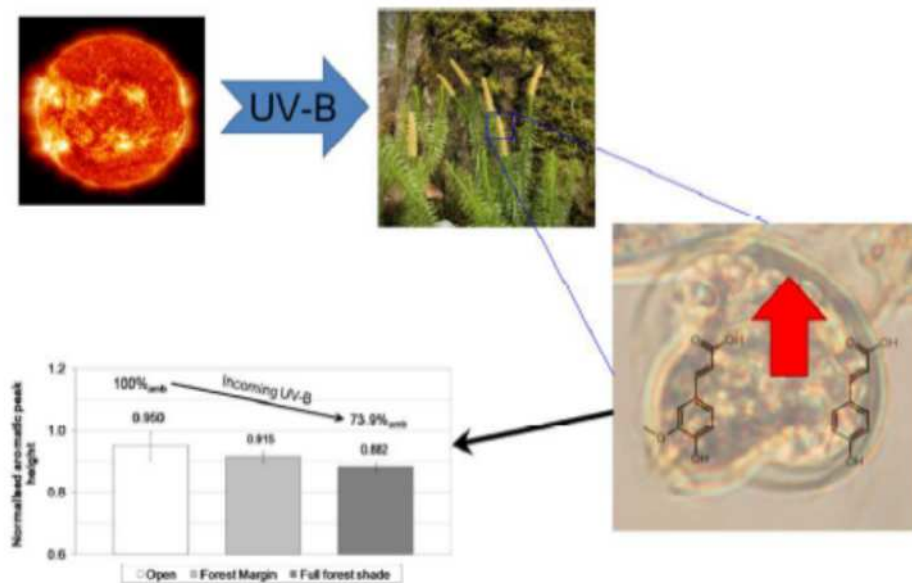


Fig. 2.

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