Fossil pollen and spores as a tool for reconstructing ancient solar-ultraviolet irradiance received by plants: an assessment of prospects and challenges using proxy-system modelling

Alistair W.R. Seddon[1,2], Daniela Festi[3,4], T. Matthew Robson[5], Boris Zimmermann[6]

[1]Department of Biological Sciences, University of Bergen, Norway
[2]Bjerknes Centre for Climate Research, University of Bergen, Norway
[3]Department of Botany, University of Innsbruck, Austria
[4]Faculty of Science and Technology, Free University of Bozen-Bolzano, Italy
[5]Organismal and Evolutionary Biology (OEB), Viikki Plant Science Centre (ViPS), Faculty of Biological and Environmental Sciences, University of Helsinki, Finland
[6]Faculty of Science and Technology, Norwegian University of Life Sciences, Norway

*Corresponding author email: alistair.seddon@uib.no

Abstract

Ultraviolet-B radiation (UV-B, 280-315 nm) constitutes less than 1% of the total solar radiation that reaches the Earth's surface but has a disproportional impact on biological and ecological processes from the individual to the ecosystem level. Absorption of UV-B by ozone is also one of the primary heat sources to the stratosphere, so variations in UV-B have important relationships to the Earth's radiation budget. Yet despite its importance for understanding atmospheric and ecological processes, there is limited understanding about the changes in UV-B radiation in the geological past. This is because systematic and satellite measurements of total ozone and surface UV-B only exist since the 1970s, so biological or geochemical proxies from sediment archives are needed to reconstruct UV-B irradiance received at the Earth surface beyond the experimental record. Recent developments have shown that the quantification of UV-B-absorbing compounds in pollen and spores have the potential to provide a continuous record of the solar-ultraviolet radiation received by plants. There is increasing interest in developing this proxy in palaeoclimatic and palaeoecological research. However, differences in interpretation exist between palaeoecologists, who are beginning to apply the proxy under various geological settings, and UV-B ecologists, who question whether a causal dose-response relationship of pollen and spore chemistry to UV-B irradiance has really been established. Here, we use a proxy-system-modelling approach to systematically assess components of the pollen- and spore-based UV-B-irradiance proxy to ask how these differences can be resolved. We identify key unknowns and uncertainties in making inferences about past UV-B irradiance, from the pollen sensor, the sedimentary archive, and through to the laboratory and experimental procedures in order to target priority areas of future work. We argue that an interdisciplinary approach, modifying methods used by plant ecologists studying contemporary responses to solar UV-B radiation specifically to suit the needs of palaeoecological analyses, provides a way forward in developing the most reliable reconstructions for the UV-B irradiance received by plants across a range of timescales.

Keywords

UV-B irradiance; sporomorph chemistry; UV-B absorbing compounds; palaeoecology; sporopollenins.

1. Introduction

1.1 UV-B radiation at the Earth's surface over geological time

Ultraviolet-B radiation (UV-B, 280-315 nm) constitutes less than 1% of the total solar radiation that reaches the Earth's surface[1], but has a disproportional impact on biological and ecological processes from the individual to the ecosystem level. Exposure to high levels of UV-B radiation is known to produce a number of effects on biota, including: DNA damage and mutagenesis, inhibition of photosynthetic processes, reduced membrane function, and lethal cell damage[2-5]. Effects of UV-B at the individual level can scale up to have major ecosystem impacts, both through evolutionary processes[6] and by altering key components of community structure and ecosystem functioning [7,8].

Ozone (O_3) is an effective absorber of UV-B radiation, so the concentration of stratospheric ozone in the Earth's atmosphere plays a key role in determining the amount of UV-B radiation received by plants. Ozone is produced in the stratosphere through a two-stage process involving the photodegradiation of oxygen molecules (O_2) into individual oxygen atoms, each of which are then involved in a binding collision with another oxygen molecule resulting in ozone. Thus, production of ozone is dependent on incident radiation in the upper atmosphere, as well as a supply of atmospheric oxygen as a result of photosynthesis. Indeed, it is thought that the evolution and colonization of land plants was limited by UV-B radiation until enough oxygen had accumulated in the atmosphere to allow sufficient UV-B protection[9]. Since then, variations in stratospheric ozone concentrations, resulting from volcanic events and/ or solar variability, means that the total amount of surface UV-B irradiance has not been constant over Earth's history[10-12]. For example, it has been proposed that large volcanic eruptions across the end-Permian Mass Extinction (~254 million years BP) released ozone-depleting aerosols into the stratosphere, resulting in elevated surface UV-B irradiance for thousands of years[12]. Although there are currently no direct estimates of terrestrial-received radiation for this time period, evidence of unseparated lycopsid-spore tetrads and malformed bisaccate-gymnosperm pollen are present in numerous sedimentary deposits and are thought to be an indication of plant damage to environmental distress under these high UV-B irradiances[13-15].

The amount of UV-B radiation received by biota may also vary as a result of non-ozone-related effects. For example, enhanced UV-B radiation during mountain-building episodes may have been an important driver of present-day phylogenetic and biogeographic patterns. Mountain building would have exposed flora and fauna to higher levels of UV-B irradiance as a result of atmospheric thinning effects, potentially causing changes in diversification rates in affected regions[6,16,17]. Further, because absorption of solar radiation by ozone is one of the

primary heat sources to the stratosphere, UV-B also acts as an important source of information for understanding aspects of past atmospheric and Earth-system processes, including the links between variations in solar or volcanic activity and climate change[8,18-20]. One recent study showed that stratospheric ozone depletion, linked to volcanic eruptions in Antarctica, may have affected atmospheric circulation to such an extent that it triggered abrupt climate warming during the last deglaciation[21]. Variations in solar activity may have been an important driver of changes in regional-scale circulation patterns and associated temperature and precipitation changes in the past [22,23].

However, although systematic instrumental observations of stratospheric ozone over the Antarctic began in 1957, ground-based and satellite measurements of total ozone and surface UV-B only exist since the 1970s[24]. As a result, instrumental records of UV-B are too short to understand the long-term effects of changes in UV-B radiation on biota and most studies investigating the impacts of past variations in UV-B lack independent estimates of incoming solar radiation. UV-B-absorbing pigments, which represent physiological changes in aquatic organisms in lakes, have been proposed as a proxy for local changes in UV-B radiation in palaeolimnological studies[25,26], but factors relating to water depth, transparency, and suspension of UV-B absorbing particles can result in UV-B attenuation in the water column and add complexities to the interpretation of changes in these pigments[27]. Recent developments in using isotopic analysis of ice cores (e.g. sulphur-isotope anomalies and changes in bromine concentrations) are enabling reconstructions of UV-B irradiance at the polar latitudes[21], but these methods are less useful if one aims to reconstruct changes in UV-B irradiance beyond the temporal windows covered by the ice-core record. Thus, there remains no universal and standardised method for reconstructing terrestrial UV-B irradiance beyond the instrumental record. This is severely hindering our ability to infer the extent of past UV-B changes and, by extension, to understand the extent of the impacts that UV-B radiation has had on organisms, populations, communities, and biosphere dynamics over geological timescales.

1.2 The potential of pollen chemistry to yield UV-B reconstructions

Changes in the chemical composition of fossil pollen and spores (hereafter, sporomorphs) could constitute a possible means to reconstruct ancient UV-B irradiance[28-36]. Sporomorph exines (outer walls) are made from sporopollenins, complex biopolymers[37] that are partly composed of phenolic compounds (i.e. phenylpropanoids), such as *para*-coumaric acid and ferulic acid[28,32,33,42]. Plants can produce these compounds after exposure to UV-B radiation through activation of the phenylpropanoid pathway. Because these compounds absorb UV-B radiation, they are thought to provide defence against DNA damage and mutagenesis as well

as quenching reactive oxygen species[4,38-40]. Sporopollenin compounds are highly resistant to corrosion and sporopollenin has been chemically stable over geological time[41]. As result, sporomorphs are readily preserved in lake and bog sediments globally and the analysis of UV-B-absorbing compounds found in pollen and spores may be used to reconstruct UV-B radiation received by plants over thousands, or even millions of years.

Over the past decade, development of this proxy has built on early experimental results to demonstrate that UV-B-absorbing compounds may be found in high concentrations in the pollen of plants that are exposed to high UV-B radiation (Table 1). Initial studies showed that *Vicia faba* pollen accumulated greater amounts of UV-B absorbing pigments in the protective walls of its pollen grains when grown under 10 kJ m^{-2} day^{-1} of biologically-effective UV-B radiation in a greenhouse, as compared to a control group receiving no UV-B radiation[28,42]. Subsequent analyses confirmed that these UV-B absorbing compounds are primarily composed of *para*-coumaric and ferulic acids[33]. Similarly, the phenolic content of *Lycopodium annotinum* and *L. magellanicum* spores, sampled from botanic gardens collected at high-latitude sites in Greenland (67°N) and South Georgia (54°S), was correlated with stratospheric ozone column thickness between 1979 and 1993[31]. In contrast, phenolic compounds in *L. magellanicum* spores from Ecuador, where UV-B irradiance was unchanged during that period, did not increase over time. Likewise, one study demonstrated that the content of UV-B-absorbing compounds was lower in *Lycopodium* spores grown under a shaded forest canopy compared to an unshaded area in northern Sweden[30]. There is also evidence for a positive correlation between the content of UV-B-absorbing compounds in *Pinus*-pollen grains and *Lycopodium* spores and received-UV-B radiation across broad-scale latitudinal[10,29] and elevational[17,35] gradients.

The data emerging from these pollen-chemistry studies are exciting, since they suggest that independent reconstructions of UV-B radiation, a key biological and climatological variable across a range of biomes, are now within reach. Interest in the proxy is growing rapidly and an emerging community of palaeobotanists and palaeoecologists are poised to use it for a suite of applications in the fossil record[10,43-46]. Two published studies have used pollen grains from sediments to reconstruct past changes in incident UV-B radiation beyond the instrumental series that are currently available[10,29].

Yet despite this excitement in the palaeoecological community, a recent UNEP EEAP (United Nations Environmental Program Environmental Effects Assessment Panel) synthesis concluded that "the utility of this proxy for inferring historical changes in stratospheric ozone remains limited"[8], questioning the extent to which the dose-response relationship of the pollen

158 and spore chemistry with incident UV-B radiation has been established. This assessment of
159 the literature suggested that variability in weather patterns, shading from canopies, and
160 complex altitudinal effects might affect incident solar radiation received by the plant, and
161 may make any reconstructions deriving from these methods challenging to interpret.
162 Questions have also been raised as to whether different taxa, which have evolved under very
163 different atmospheric conditions, are able to adapt or acclimate at different rates to changes in
164 any UV radiation they receive during different periods of Earth's history. An important
165 question that follows, therefore, is what steps are now required so that the inconsistencies in
166 perspective, and the conclusions drawn between ecological and palaeoecological studies, can
167 be resolved?
168
169 In this perspective we aim to provide an up-to-date assessment on the potential and current
170 status of a UV-B proxy based on sporopollenin from pollen and spores. By using a proxy-
171 system-modelling framework[47], we identify key unknowns and uncertainties in making
172 inferences about past UV-B irradiance, from the pollen sensor, the sedimentary archive, and
173 through laboratory and experimental procedures in order to target priority areas of future
174 work. Our goal is to highlight the most efficient steps required to achieve the optimum levels
175 of precision and reconstruction skill. An interdisciplinary approach, modifying methods used
176 by plant ecologists who study contemporary responses to solar-UV-B radiation to suit the
177 specific needs of palaeoecological analyses, provides a way forward in developing more
178 reliable reconstructions for UV-B irradiance across a range of timescales.
179
180 **2. A UV-B proxy system model**
181 A proxy-system model describes a set of processes linking the response of a sensor to
182 environmental forcing that is recorded, preserved, and then observed in a sediment archive[47].
183 A complete proxy-system model incorporates understanding of all the components linking an
184 observation made about a change in environmental conditions stimulating a response in a
185 biological proxy sensor (e.g. pollen grains), which is recorded in a proxy archive (e.g. lake
186 sediments), and is then measured by an analyst in the laboratory (e.g. pollen-chemistry
187 measurements using Thermally Assisted Hydrolysis and pyrolysis, combined with Gas
188 Chromatography/Mass Spectrometry, THM-GC-MS) (Figure 1). A proxy-system model can
189 exist in various forms, either as a qualitative description of the components influencing a
190 proxy signal[48], or as a quantitative framework which allows for experimental and proxy-
191 system design[49], data-model validation[50], and error propagation and uncertainty analysis[51].
192 Given that the development of the UV-B proxy remains in its early stages, here we provide a
193 qualitative assessment of a pollen-based UV-B proxy-system model to evaluate uncertainties

and identify future research directions. We address each component of the model individually to highlight knowledge gaps that need to be addressed.

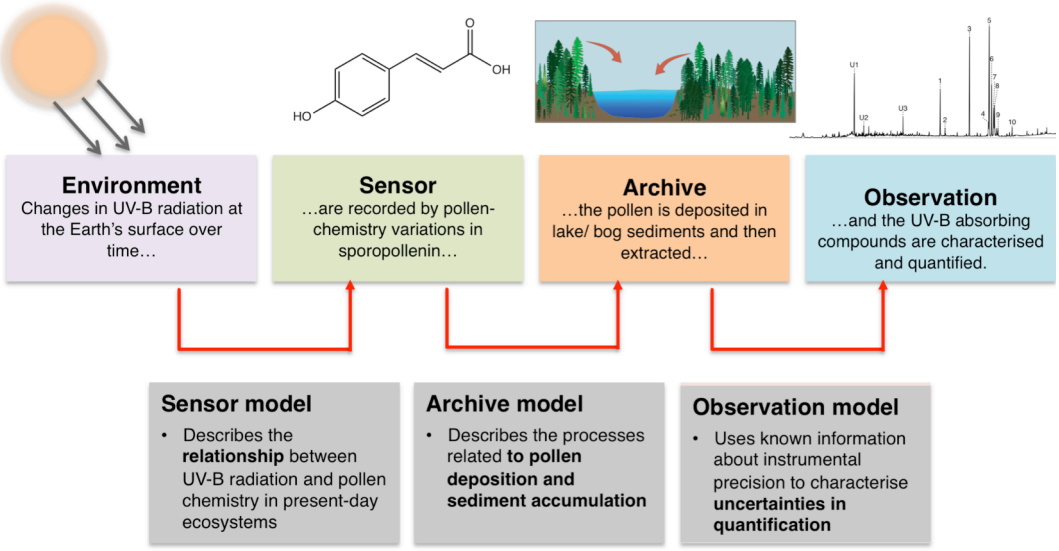


Figure 1: A proxy-system model for reconstructions of UV-B radiation based on sporomorph chemistry. Changes in the environment are recorded by a sensor (in this case, chemical changes in sporopollenin of pollen and spores). This sensor is deposited in an archive such as a lake or bog, from which it is later extracted and analysed to make observations about past changes in the content of UV-B-absorbing compounds within the sporopollenin. Inferences are made about UV-B radiation from these observations. Inferences made between each component (red arrows) are associated with uncertainties, which accumulate through the proxy-system model (Adapted from an original figure by Evans et al. 2013)[47]. We thank Jesse Morris for permission to use the lake/forest cartoon.

3. The sensor model

The key component of any proxy-system model is the sensor, which describes how a biological proxy responds to an environmental driver. So far, the sporomorph-chemistry response to UV-B radiation has been assessed in a range of species across different sections of the plant phylogenetic tree, including: *Vicia faba*[28], three species of *Lycopodium*[10,31], conifers such as *Pinus* spp.[29] and *Cedrus atlantica*[45], and Poaceae[10] (Table 1). Except for one study assessing a time series of UV-B absorbing compounds extracted from herbarium-pollen specimens[34], a common result is that, across different taxa, the content of UV-B-absorbing compounds, such as *para*-coumaric and ferulic acids, tends to be higher in the pollen and spores of plants exposed more UV-B radiation (Table 1, see references therein). Yet while this general positive relationship is a clear strength, providing confidence that the proxy might be broadly applicable; the diverse set of experimental approaches (e.g. greenhouse experiments, latitudinal gradients, calibrations through time) (Table 1) is also a weakness: it is difficult to compare dose-response relationships between these studies because experimental and quantification approaches vary; there are large differences in the way UV-B

212 exposure is measured, both in terms of the wavelength of the incident solar radiation, and the
213 spatial and temporal range of the UV-B forcing using to calibrate the response. The result is
214 that there remains high uncertainty about the dose-response relationship on which any
215 sporomorph-chemical reconstruction is based. To resolve these uncertainties we identify four
216 key challenges for improved understanding of the pollen-UV-B sensor.
217
218 **Table 1** (below) State of the art on the dose-response relationship for spores/pollen and UV-B radiation
219 and TSI (total solar irradiance).

Reference	Taxa	Number of individual/ replicates	Sampling period	Temporal Scale	Type of experiment	UV-B data	Sampling units	Method	Type of data presented	Key findings
Rozema et al. (2001)[28]	*Vicia faba*	6, 3 replications reported in the figure.	6 week flowering period	Annual	Climatized greenhouse	2 treatments: 10.6 kJ m^{-2} day^{-1} UV-B-compared to 0 kJ m^{-2} day^{-1}, PAR supplied was 300 µmol m^{-2} s^{-1}	Individual plants	Sequential extraction of soluble and insoluble fractions/ THM-GC-MS	Original	96% increase in UV-B absorbance (280-320 nm) in acetolysis residue; higher amounts of *para*-coumaric (pCA) and ferulic acid (FA) reported using THM-GC-MS
Rozema et al. (2001)[42]	*Vicia faba*	6, 3 replications reported in the figure.	6 week flowering period	Annual	Climatized greenhouse	3 treatments: PAR; PAR+ UV-A; PAR + UV-A + UV-B. PAR supplied was 300 µmol m^{-2} s^{-1}	Individual plants	Sequential extraction of soluble and insoluble fractions	Original	Difference between the UV-A and UV-B treatment differed significantly ($p \leq 0.05$) from the PAR treatment, but no significant difference between the UV-A and UV-B treatment.
Blokker et al. (2005, 2006)[32,33]	*Vicia faba*	12 plants per treatment	6 week flowering period	Annual	Climatized greenhouse	2 treatments: 12 kJ m^{-2} day UV-B-compared to 0 kJ m^{-2} day=1, PAR supplied was 300 µmol m-2 s^{-1}	Individual plants	THM-GC-MS	Original	Significant differences FA, p=0.004; pCA, p=0.007, and pCA/ FA ratio (p=0.006) between UV-B and non-UV-B treatment
Watson et al. (2007)[35]	*Lycopodium cernuum*	5 individuals	years 1943;1962; 1965; 1976, 1981	Annual	Natural, Altitudinal gradient (650-1981 m a.s.l.)	NA	Herbarium samples, SE Asia 9°S -16°N	FTIR/ THM-GC-MS	Original	Higher abundance of UV-B absorbing compounds in higher elevation samples using FTIR
Lomax et al. (2008)[31]	*Lycopodium annotinum*	15	1906-1993	Decadal/ centennial	Natural	FTIR inferred chemical changes compared to modelled change in UV-B flux from Abisko, Sweden	Herbarium samples, Greenland	FTIR	Original	Correlation between modelled UV-B changes at 300nm at UV-B absorbing compounds.
Lomax et al. (2008)[31]	*Lycopodium magellanicum , L. annotinum*	8 samples per location	Samples represent individual years between 1906-2004	Annual/ decadal	Natural	Inferred from observed ozone thickness values	Herbarium samples; South Georgia, Greenland, Ecuador	FTIR	Original	UV-B absorbing compounds correlated with stratospheric ozone column thickness between 1979 and 1993 (Lomax et al. 2008)
Rozema et al. (2009)[34]	*Alnus glutinosa*	40 samples with 2-4 replicates	Samples represent individual years between 1880-1960	Decadal/ centennial	Natural	Ratio pCA:FA compared against sunspot cycles	Herbarium samples	THM-GC-MS	Original	No correlation observed between sunspot cycle record and UV-B absorbing compound ratio

Willis et al. (2011)[29]	*Pinus sylvestris, P., pinaster, P.canariensis*	18 (3-5 replicate trees per location)	Plants sampled over two growing seasons	Annual/ decadal	Natural	UV-B in satellite-derived surface UV-B dose corrected for cloudiness and ozone 20-year climatological mean	Individual plants, Europe from arboreta, botanic gardens and native populations	THM-GC-MS	Original	Positive relationship between UV-B absorbing compound (para-coumaric acid) and surface UV-B
Fraser et al. (2011)[30]	*Lycopodium annotinum*	30	Spores sampled mid-September 2006	Annual	Ambient shading	Full forest shaded species had 73.6% of ambient (clear sky) UV-B	Individual plants, Sweden	FTIR	Original	UV-B-absorbing compounds content lower in *Lycopodium* spores grown under a shaded forest canopy
Lomax et al. (2012)[17]	*Polygonum/ Lycopodium cernum*	5	See Watson et al. (2007)	Annual/ decadal	Natural	NA	Individual plants; Asia; altitudinal gradient	FTIR	Original/ Watson et al. 2007	Positive relationship between UV-B absorbing compounds and altitude
Jardine et al. (2016)[10]	*Poaceae*	69	NA	Orbital	Natural	Modelled TSI inferred from orbital forcing	Fossil sediment core samples; Ghana	FTIR	Original	Positive relationship between UV-B absorbing compounds and modelled TSI inferred from orbital forcing
Jardine et al. (2016)[10]	*Lycopodium annotinum, L. magellanicum, L. cernuum*	12	See Watson et al. (2007); Lomax et al. (2008)	Annual/ decadal	Natural	Modelled TSI for September	Herbarium samples, field samples	FTIR	Lomax (2008), Watson et al (2007)	Positive relationship between UV-B absorbing compounds and modelled TSI
Bell et al (2018)[44]	*Cedrus atlantica*	95 trees from 16 sampling locations	Pollen sampled from single year.	Annual/decadal	Natural	Average daily mean for June, July and August from Satellite glUV datasets from 2004 and 2013). Erythemally weighted estimate of mean daily UV-B radiation for each month estimated	Individual plants from native populations in Morocco+ botanic gardens and urban parks of Europe and USA	FTIR/ THM-GC-MS	Original	Positive relationship between UV-B absorbing compounds and modelled TSI observed when only samples from native populations (i.e. not-arboretum/ botanic gardens) specimens are not included in the regression model

Jokerud et al. (2017)[43]	*Pinus sylvestris*	10 individuals	4-6 weeks before flowering	Annual	Field (shading cloth covered inflorescences on tree 4-6 weeks before flowering)	UV-B dose not estimated but change compared to clear-sky control from the same tree.	Individual plants, Botanic Garden (10 trees)	THM-GC-MS	Original	Reduction in pCA in samples from shaded inflorescences compared to unshaded inflorescences
Jokerud et al. (2017)[43]	*Pinus sylvestris, P., pinaster, P.cembra, P. mugo*	10 individuals from Geneva botanic gardens	Samples from growing season 2015 and 2016	Annual	Natural	UV-B dose estimated from satellite data for growing season period	Individual plants; Botanic Garden (1-3 tree per species)	THM-GC-MS	Original	Reduced pCA in samples from low UV-B year compared to high UV-B year

i. Is the dose-response relationship consistent across species?

Although the general trend for a positive relationship of UV-B-absorbing compounds and received UV-B radiation has been generally established (Table 1), the ability to distinguish between *within-species* effects and *UV-B effects* remains a key challenge. Two studies using latitudinal gradients are useful examples to demonstrate this point. A training set of *Pinus* spp. was developed to investigate latitudinal differences in *para*-coumaric acid content across a latitudinal gradient in Europe[29]. The majority of samples in this study were from individuals of *Pinus sylvestris* from populations ranging from northern Norway to southern continental Spain. To extend the gradient in UV-B radiation towards lower latitudes (i.e. those populations at locations receiving higher UV-B), populations of *P. sylvestris* were added to with individuals of *P. pinaster* at four locations in Greece, and individuals of *P. canariensis* in the Canary Islands. A significant positive relationship is present between mean annual UV-B irradiance and the content of UV-B absorbing compounds across the entire dataset (Table 2, Figure 2a). This significant positive relationship between *para*-coumaric acid and annual UV-B irradiance is also present when only *Pinus sylvestris* populations are included and the other species are removed. However, the effect size when using this reduced dataset is approximately halved (Table 2, Figure 2a). A similar result was also obtained with a latitudinal gradient using *Lycopodium* spores (Figure 2b)[10]. Here, the strength of the relationship with TSI is reduced by a factor of 5 (p=0.136, n=9) when only using *Lycopodium annotinum,* rather than the full dataset. For other lower latitude populations (i.e. those receiving higher UV-B radiation), the sample size remains too small to make any general conclusions.

One recent study also investigated the difference in *para*-coumaric acid content of ten individuals from five different species of *Pinus* growing in Geneva Botanical Garden between a year when they received high exposure to solar UV-B radiation and a low-UV-B year[43]. Whilst pollen samples from all trees had lower *para*-coumaric acid content during the low-UV-B year compared to the high UV-B year, results also showed that *para*-coumaric acid content was strongly related to pollen size[43]. To account for this covariant, a size correction procedure was used, which involved dividing the total content of UV-B-absorbing compounds in each sample by a scaling factor to correct for the mean pollen surface area. Once pollen surface area was taken into account, the *para*-coumaric acid content was more similar across the different taxa, although species-specific differences in the year-to-year relationship with UV-B irradiance remained[43].

Taken together, these uncertainties have implications when considering interpretations of pollen- and spore-chemistry reconstructions in the sediment record. Although some sporomorph types can be identified to species level using traditional microscopic approaches, there are many that may only be identified to genus, or even family. Thus, whilst a particular sporomorph may be confidently interpreted as representing only one species in some locations (e.g. *Pinus sylvestris* pollen in the Holocene in Norway), in other cases, it may represent a larger number of plant species (e.g. Lateglacial to Holocene sequences of *Pinus* spp. pollen in the Alps, Poaceae pollen). We argue that it remains critical to understand whether the dose-response relationship is consistent across all taxa represented in the pollen record. More work is required to resolve this issue if robust, multi-species calibration datasets are to be developed.

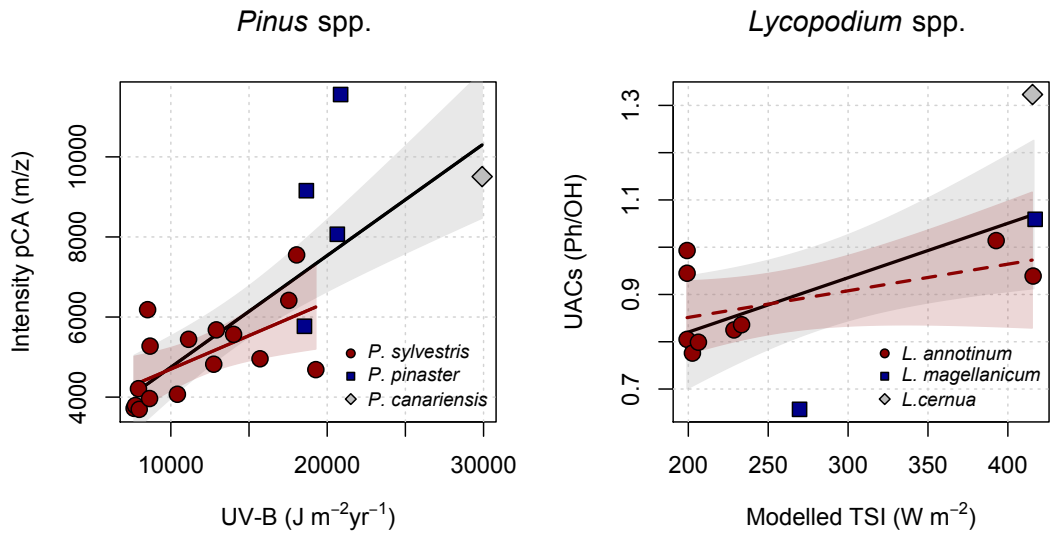


Figure 2: Results from studies of latitudinal gradients of UV-B-absorbing compounds for two proxy systems: (a) *Pinus* spp. [29] and (b) *Lycopodium* spp. [10]. The coloured lines represent species-specific response functions for UV-B-absorbing compounds and annual UV-B radiation or total solar irradiance (TSI). Dashed lines mean the relationship is not significant at p= 0.05. The dark black line is the combined multi-species response function. The y-axes represent quantitative estimation of UV-B absorbing compounds: (a) absolute intensity of the ion 161 m/z, divided by the number of *Pinus* spp. pollen grains, quantified using THM-GC-MS; (b) ratio of the height of the spectral band representing phenylpropanoids at 1510 wavenumbers cm^{-1}, compared to the hyrodxyl vibrational band at 3300 cm^{-1} using Fourier Transform Infrared Spectroscopy (see section 5 in the main text for more information about quantification of UV-B-absorbing compounds). Note the units on the x-axis are different for both studies. (a) Annual UV-B irradiance calculated from satellite derived erythemal daily doses[52] (b) Modelled Total Solar Irradiance[53].

Table 2. Summary statistics of linear regression modelling of latitudinal variations in UV-B absorbing compounds in pollen and spores.

Study	Calibration set	Coefficient estimate	Std Error	Pr	Adj. r^2	% Change in effect size
Willis et al. (2011)	Full dataset	0.279	0.052	0.000036	0.58	NA
	Pinus sylvestris only	0.167	0.058	0.012	0.33	-40.1
Jardine et al. (2016)	Full dataset	0.00115	0.00043	0.023	0.36	NA
	Lycopodium annotinum only	0.00056	0.00033	0.136	0.19	-51.3

ii*) Are results transferable between taxa?*

A second, related issue concerns whether the results of experiments carried out on model species under experimental settings are transferable across broader phylogenetic groups (e.g. between genera/ phyla). This is important if results derived from experiments conducted on a model plant type (e.g. *Vicia faba*[28]) can be directly applied to other pollen sensors. Evidence indicates that the genetic mechanisms used in the perception and subsequent upstream regulation of plant responses identified in *Arabidopsis thaliana*[54], may be similar to those in algae and mosses on account of the presence of orthologous genes[55]. In addition, the genetic basis of sporopollenin production likely developed early in land plant evolution and is highly conserved across taxa[56] and through time[41]. Such results indicate that the genetic mechanisms underlying any UV-B response are likely to have been conserved across the phylogenetic tree, providing hope for the transposition of the method between different species[36,57].

Despite the fact that the photoreceptor-activated signaling pathways are highly conserved, sporopollenin content of pollen from different genera can still contain different relative amounts of UV-B-absorbing compounds, which are namely derivatives of *para*-coumaric and ferulic acids. For example, sporopollenins of northern hemisphere conifers, such as *Pinus* and *Picea*, have extremely high *para*-coumaric/ferulic acid ratios compared to that in *Cedrus*[44,45,58,59] (all within Pinaceae). Thus, although the underlying biomolecular mechanisms involved in UV-B perception may be similar, associated responses related to the composition of UV-B absorbing componds can differ, even within taxa of the same family. This means that it may be necessary to use different indices when quantifying UV-B-absorbing compounds from different plant groups. One study proposed that the ratio of *para*-coumaric acid: ferulic acid would be a useful index for quantification of UV-B absorbing compounds in *Alnus glutinosa* using THM-GC-MS, assuming that *para*-coumaric acid was more sensitive than ferulic acid in its UV-B response[33]. Whilst it is possible that this index would work for *Cedrus* spp., such an index is not useful for *Pinus* spp.[44]. Furthermore, the *relative* response

of the different UV-B-absorbing compounds in different plant taxa remains unknown. From this evidence it is clear that developing species-species specific calibration datasets for pollen-chemistry UV-B reconstructions is a critical goal that has yet to be achieved for many taxa.

Such high variability between taxonomic groups may not be surprising when considering that inter-species variations in the phenolic responses of other plant processes to UV-B radiation are commonly found in ecological studies[60]. For example, an experimental study showed that although UV-B radiation has a negative effect on pollen-tube length for the majority of the taxa they studied (*n*=34), monocotyledons were more sensitive to UV-B exposure than dicotyledons, and trinucleate pollen types more sensitive than binucleate pollen[61]. There is also evidence for differences in UV-B sensitivity according to the flowering period of plants: plant species flowering early in the year are more sensitive than those blooming later in the season, whilst plants that grow under natural conditions can be more sensitive to UV-B radiation than those growing in greenhouses[61]. In addition, experiments on other plant parts indicate that the effect of UV-B radiation on leaf chemistry can differ between species among compounds. For example, only specific phenolic compounds, lutonarin and 3-feruloylquinic acid, accumulated in response to UV-supplementation to two *Hordeum vulgare* (barley) varieties showing differing sensitivities of response [62]. Likewise, leaf flavonoid composition in tree species typically responds specifically to both UV-B and UV-A radiation[63]. Indeed, a common result is that UV-B radiation affects the composition of UV-B absorbing compounds without affecting the total content[64].

iii. What is the critical developmental stage for which pollen is sensitive to UV-B exposure?

Modern ecological evidence indicates that the abundance of phenolics (and other secondary metabolites) in leaves can vary on daily, seasonal and annual timescales[65]. Pollen production in trees from temperate forests can follow a biennial pattern, with the magnitude of the peaks in pollen-production years correlated with temperature or precipitation during the previous growing season[66], but whether the concentration of UV-B-absorbing compounds responds to UV-B exposure over a short developmental period, or integrates a long-term signal spanning a longer time period, remains poorly understood. Experimental studies tend to be short term (e.g. the length of one growing season or shorter), whilst pollen-based UV-B-absorbing compounds have been correlated against climatological means of both annual and seasonal (i.e. covering the developmental period) UV-B irradiance (Table 1). Determining whether the pollen-chemistry signal represents shorter-term seasonal fluctuations in UV-B, or the longer-term changes over multiple years is critical when interpreting any reconstruction of UV-B absorbing compounds from a sediment core.

One recent study provides potential insights into this question[43]. Branches of 10 individuals of *Pinus sylvestris* were covered with shading cloths for 4-weeks before dehiscence (pollen release) and showed that the content of UV-B-absorbing compounds in the pollen was lower than compared to non-exposed branches on the same tree. Although this study did not control for the fact that the shading cloths resulted in a reduction of PAR as well as UV-B (nor temperature and humidity), what these results do show is that the UV-B-absorbing compounds content of pollen and can change rapidly, at least within 4-weeks, in response to changing environmental conditions. In the case of *Pinus* spp, results are in line with current understanding of its reproductive cycle, in which the microspores are coated with the main sporopollenin component ollowing degeneration of the tapetal cells which occurs towards the end of pollen development[67]. Other evidence, which indicates reductions in UV-B-absorbing compounds in five species of *Pinus* spp. in one season with low cumulative UV-B irradiance compared to a season with high cumulative UV-B irradiance[43], also tentatively supports this conclusion. Thus, it appears there is potential for sporomorph chemistry to respond to changes in UV-B radiation within the growing season. Since other studies have also shown that the chemical composition of pollen grains varies in response to drought stress between different years[68], it is possible that sporomorph-chemistry variations may respond to environmental stimuli on seasonal timescales or shorter.

In contrast, a recent study found that, although the content of UV-B-absorbing compounds in *Cedrus atlantica* pollen was positively correlated with seasonal UV-B irradiance in native populations, there was no evidence of a broad-scale latitudinal relationship among trees sampled from botanic gardens across Europe[45]. In fact, they found that the FTIR spectra of pollen from *C. atlantica* growing in botanic gardens closely resembled the FTIR spectra of these native populations growing at their point of origin. Similar relationships are found in studies from other fields beyond aiming to reconstruct UV-B radiation from the chemical contents of fossil pollen. In horticulture, for example, the ratio of different phenolic compounds in the plant leaves have been proposed as a potential tool for fingerprinting different cultivars of a species, although recent findings also acknowledge that the environment has an effect on phenolic content once a cultivar is planted elsewhere[69].

Whether species can demonstrate plastic responses or their phenolic content is representative of longer term, genetic factors has also been studied in the ecological literature in a number of different contexts. For example, plant populations that grow in higher elevations (high UV-B) may differ in their ability to acclimatize to new UV-B environmental conditions compared to low elevations. For example, sensitivity to UV radiation in high- compared with low-

385 elevation populations and species in the Hakkado Mountains, Japan was partly due to
386 differences in DNA damage and repair between populations[70]. Similarly, a few studies have
387 found that some invasive populations of plants have higher concentrations of phenolic
388 compounds compared to native populations, which may result in a competitive advantage in
389 resistance to biotic and abiotic stressors when growing in non-native locations[71-73]. However,
390 these responses are not necessarily universal, since a number of other studies have found no
391 clear differences in leaf flavonoid content between native and non-native species[65,74,75].
392
393 Since tree populations are likely to expand and contract their ranges in response to global-
394 climate shifts on millennial timescales or longer, it is interesting to consider the implications
395 of these findings for the interpretation of chemistry changes in sporomorphs that have been
396 extracted from a lake or sediment core. For example, if long-term genetic effects (i.e.
397 adaptation) are a consistent feature of the chemical response to UV-B in sporopollenin, then
398 in Quaternary sequences from higher latitude sites, the dominant signal of UV-B absorbing
399 compounds inferred from pollen during different interglacial periods may primarily be related
400 to their source populations. Whether this signal is also a function of the time for local
401 adaptation to new conditions is also unknown. Shorter-term fluctuations in the chemical
402 signal of the sporopollenin may be superimposed on this variation as a result of phenotypic
403 plasticity in relation to shorter-term changes for UV-B flux. Given these uncertainties, we
404 propose that determining the relative importance of phenotypic plasticity (i.e. short-term
405 responses) and local adaptation (longer-term inherited changes) is a critical research topic that
406 currently remains unresolved[76,77].
407
408 *iv. What are the effects of other wavelengths on UV-B absorbing compounds?*
409 The motivation behind developing a sporomorph-based proxy for UV-B irradiance was first
410 based on investigating changing concentrations of atmospheric ozone on timescales beyond
411 the experimental record[11,28,34]. Consequently, laboratory and field experiments were designed
412 to investigate how the changing ratio of UV-B to PAR would affect the abundance of UV-B
413 absorbing compounds in pollen[28]. Even in cases where the UV-B effects could not be isolated
414 from other wavelengths of sunlight, UV-B is often still assigned as the main variable causing
415 changes in the response. For example, spores from *Lycopodium annotinum* grown under
416 shaded conditions in a birch-forest understory were shown to have significantly lower
417 abundance of UV-B-absorbing compounds than those exposed to sunlight[30]. Although canopy
418 shading can have major effects on the incident spectra of sunlight[78], it was concluded that it
419 was the response to UV-B radiation that was the most likely explanation for the changes in
420 UV-B absorbing compounds[30]. A similar interpretation has been made when comparing *Pinus*

421 responses under shading cloths, and between low UV-B and higher UV-B years as a result of
422 cloudiness[43].
423
424 As interest in this proxy has grown, palaeoecologists have extended the potential application
425 of this UV-B proxy to understand environmental variability related to other wavelengths of
426 light. Most recently, one study found that UV-B absorbing compounds in Poaceae showed
427 weak but significant relationships with modelled total solar irradiance (TSI) in Ghana ($r2$ =
428 0.11, p = 0.008 when unsmoothed data are correlated against modelled TSI)[10]. Setting aside
429 complications resulting from possible species-specific effects, this calibration through time
430 indicates a shift in the potential use of the pollen-based UV-B proxy towards more direct
431 quantification of total-solar irradiance.
432
433 However, we suggest that there are a number of fundamental knowledge gaps surrounding the
434 sensitivity of the response before these findings can be confirmed. Of major importance is the
435 fact that the relative sensitivity of phenolic compounds to one spectral region (e.g. UV-B
436 radiation) against other regions (e.g UV-A radiation) remains unknown. In other plant
437 processes, action spectra (i.e. the relative strength of response of a biological process
438 produced across a range of different wavelengths) can be highly non-linear across different
439 spectral regions[79,80], and the relative importance of energy from longer wavelengths in the
440 UV-B region can change our estimates of what constitutes a biologically effective UV-B dose
441 for a particular plant response[81]. The action spectrum is presently unknown for UV-B
442 absorbing compounds in pollen, but understanding this represents a major challenge if one
443 aims to develop reliable quantitative reconstructions. Such non-linear dose-response
444 relationships could result in very different sensitivities to solar-radiation exposure under
445 different ambient spectral conditions, with obvious impacts on the interpretation of
446 sporopollenin-chemistry variability inferred from sediment cores.
447
448 Finally, related to this issue is how plants respond to other climatic and non-climatic
449 variables. Although it is accepted that UV-B radiation often stimulates the production of
450 phenolic compounds[64,82-84], there is also widespread evidence that other environmental factors
451 (i.e. temperature, mineral nutrition, water availability, atmospheric CO_2 concentrations,
452 salinity, pathogens) also affect their production and accumulation[69,85-87]. Indeed, UV-B
453 absorbing compounds such as *para*-coumaric acid and ferulic acid represent important
454 building blocks of other compounds related to plant defence and structure (e.g lignins), as
455 well as sporopollenins[88]. Plants can also respond differently when exposed to supplemental
456 UV-B radiation in isolation from the rest of the solar spectrum compared to increases in UV-
457 B radiation as a part of natural sunlight exposure[89]. For example, whilst exposure to UV-B

458 radiation during sunlight hours can induce cyclobutane pyrimidine and pyrimidine (6-4)
459 pyrimidinone dimers, with effects on cell transcription and replication processes in plant
460 epidermal layers[2], subsequent exposure to blue light or UV-A radiation can induce repair
461 mechanisms related to photoreactivation reducing these biological effects[2]. This means that
462 UV-B responses may sometimes have been overestimated when greenhouse or laboratory
463 studies are considered in isolation of other environmental changes[27]. Such effects have yet to
464 be considered in palaeoecological studies based on sporomorphs and more work is required to
465 elucidate the potential for interactive effects of temperature and other variables.
466
467 **4. Archive model**
468 A sediment sample taken from a lake or wetland deposit contains pollen and spores reflecting
469 a biased selection from the regional species pool depending on dispersal, pollen production,
470 plant-population abundance and preservation processes after burial. The archive component
471 of a proxy-system model is then used to take these processes into account by describing the
472 way that pollen grains are transported to the depositional environment, and then preserved or
473 stored until recovery by the analysts for thousands or even millions of years. It is useful to
474 separate the archive model related to the pollen-and spore-UV-B proxy into two key factors,
475 both of which should be considered when interpreting sporomorph-chemistry reconstructions
476 from sediments. Although much of the following analysis is tailored to analysis of Quaternary
477 records, many of the same principles are likely to apply on longer timescales.
478
479 4.1. *Source area and transport*
480 The fundamental principals behind Quaternary palynology were established following the
481 first pollen records presented by Von Post in 1916[90] and 1918[91](see also ref. [92]). Although
482 models of sporomorph deposition and transport have become more sophisticated to enable
483 quantitative reconstructions of vegetation cover around a lake[93,94], the general principals
484 remain the same. Pollen and spore dispersal is primarily a function of pollen size and
485 shape[94,95]. The pollen and spore catchment area of a lake or bog from which they are
486 deposited (known as the pollen-source area for pollen grains) is dependent on basin size and
487 configuration, with large, round lakes integrating pollen from trees from larger source areas.
488 The pollen influx (amount of pollen deposited in a given volume of sediment for a given time
489 period) can vary as a result of population size of the plant in the surrounding basin (larger
490 population size will result in larger pollen influx for a given species); the proximity of the
491 source population to the lake (larger populations, closer to the lake will result in larger pollen
492 influx); the productivity of a plant for a given time period; and the sediment accumulation
493 rates (higher sedimentation rates can mask periods of high pollen production in the
494 environment). In Quaternary sequences sedimentation rates are estimated through modeling

of radiometric ages to account for this[96,97]. Furthermore, pollen productivity also varies greatly among taxa according to their pollination strategy, where wind pollinated taxa produce higher amount of pollen compared to those relying on insect pollination. Thus, distinguishing between small, local populations and pollen representing long-distance dispersal can be challenging. A site which has stable pollen-influx rates might be preferable since it is more likely to reflect stable environmental conditions (see reference[98] for a discussion).

Work is currently ongoing in other areas of palynology to develop sophisticated models to enable quantitative reconstructions of vegetation cover based on these principles[99], in addition to appropriate associated uncertainties[93]. For inferences using pollen, these models generally rely on estimating a pollen-production factor before integrating pollen data from both large and small lakes within the landscape matrix to develop quantitative reconstructions of vegetation cover. Whilst it is unlikely that such models could be applied directly to any sporomorph-chemistry reconstruction at present, what these models can do is provide guidance on how to reduce uncertainty related to source-area effects. For example, based on the understanding of the work into pollen-source area and deposition, it is possible to identify study sites that are more likely to provide reliable results (see reference[98] for a discussion). For an integrated network of sites which allow for reliable reconstructions of UV-B across different geographic regions, sites would ideally have relative stable pollen influx rates for the entire period of investigation, be of the similar basin size and shape to ensure similar pollen-source areas, and contain a target species where the UV-B dose-response relationship is known. Where this is not possible (e.g. for estimating deep-time sedimentary contexts), then the potential source-area effects are more difficult to resolve in any reconstruction.

These general considerations are relevant to any pollen- and spore-based proxy (e.g. land-cover reconstructions from pollen[93,94]; pollen-based-climate reconstructions of temperature and precipitation[100]). However, a number of challenges outlined in section 3 above (the sensor model) have additional specific implications for the archive model related to a sporomorph-based proxy of UV-B. For example, an archive model that only integrates light-demanding taxa, which are directly exposed to solar UV and which are less likely to be influenced by attenuation by shading effects[34], can reduce uncertainties related to shading influences that can result in local variations of UV-B-absorbing compounds. Similarly, the challenges of taxonomic identification down to species level in pollen, combined with uncertainties in our understanding of species-specific dose-response relationships, mean that archives where we can be more confident that only a single species is represented may be more desirable until understanding of species-specific effects is more clear. Sites where large population turnover

or habitat change have occurred may require more complex interpretations, since the populations influencing the sporomorph-chemistry signatures can be influenced by other factors (e.g. colonization of different populations from different source areas, see above). One way to take this into account may be to combine sporomorph-chemistry reconstructions with traditional palynological analyses so that the general information about ecological changes at the site can be realized. For example, Poaceae pollen percentages were included in a regression model to test for relationships between TSI and UV-B absorbing compound abundance in pollen through time[10]. The Poaceae pollen percentages were included to test whether habitat openness was influencing the result. Here, no effect of Poaceae percentage variability on UV-B absorbing compound abundance was found so the so the authors concluded that the main effect they observed was a result of changes TSI related to solar irradiance.

4.2 *Diagenetic effects on sporomorph chemistry*

On longer timescales, stability of sporopollenin under different temperature and pressure regimes may be inferred by apply a colour index to pollen or spores[36,101]. Darker grains tend to indicate more chemical alteration as a result of heat and pressure, too much of which is likely to have an adverse effect on the quantification of UV-B absorbing compounds[101]. It has been suggested that chemistry remains relatively intact in grains up to 250-300ºC, but below this value the chemical structure of sporopollenin remains relatively stable over a wide range of simulated maturation conditions[36,101]. This means that, for more ancient sediments, detailed work on the structure of the embedding rock types or thermal maturation status of the sporomorphs are required for any accompanying pollen chemistry reconstruction so that the diagenetic effects after burial can be accounted for.

For analysis of Quaternary pollen grains, such high temperatures are highly unlikely so these diagenetic effects will be less problematic. However, periods of oxidation (e.g., as a result of low lake levels) can result in corrosion of sporopollenin. Another consideration is whether the content of UV-B absorbing compounds remains stable from the time of pollen release to the point they are analysed in the sediment. Given that a large component of UV-absorbing compounds are stored in the grain wall, either as pigments or as part of the sporopollenin, it is likely that the chemistry of sporopollenin during the pollination is preserved. However, since UV-B-absorbing compounds can absorb UV radiation, exposure to UV-B radiation over time could be expected to affect them as they absorb the UV whilst being ecologically active, and it remains unclear whether this will have a structural effect on the phenolic compounds that would further affect their chemical signatures later.

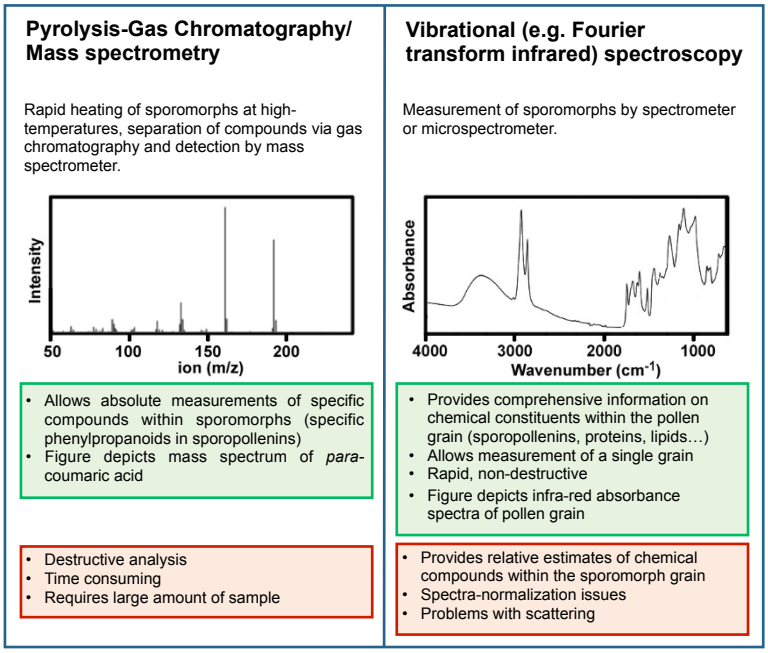
A final consideration is the chemical effects of laboratory treatments used to extract sporomorphs from the sediment prior to chemical analysis. Standard laboratory treatments of fossil sediments typically involves a series of procedures including acid digestion to remove silicates (using hydrofluoric acid), followed by an oxidation step using an acetolysis or warm nitric acid treatment[102] to remove cellulose or other organic debris from the samples. Such procedures are often necessary steps to aid identification and isolation of pollen grains before quantification of UV-B absorbing compounds. Oxidation procedures are also used on modern grains to remove the cellular protoplasm, the cellular intine, and any proteins and lipids, which then helps to emphasize the structures in the exine used in identification[103,104]. Thus, it is necessary to understand how such chemical treatment procedures can affect sporopollenin.

Known physical effects of such oxidation methods include exine darkening, size increases, and corrosion leading to complete destruction[103-107], whilst the absolute total abundance of UV-B absorbing compounds from modern-pollen grains are known to be reduced following these chemical-treatment effects[28,42,108]. Indeed, a clear reduction in UV-B absorbing compounds from cell protoplasm, intine, to sporopollenin has been shown following sequential extraction using methanol, sodium hydroxide and acetolysis steps[28,42]. However, this study did not reveal whether the chemical structure of the sporopollenin changed as a result of these procedures. To address this issue FTIR spectroscopy was recently used to investigate the oxidation effect on *Lycopodium clavatum* spores, in addition to pollen from eight angiosperm taxa[104]. This study showed that aggressive nitric acid treatments (with samples exposed for > 10 minutes duration) had clear degradation effects on sporopollenin structure (leading to total destruction of the pollen and spores at high temperatures). However, they found that whilst acetolysis at 90ºC removes non-fossilisable components of the sporomorphs within 1-2 minutes treatment, FTIR spectra then remain relatively stable for up to 240 minutes, suggesting that hot acetolysis treatment leaves the sporomorph exine relatively unchanged. Thus, processing methods are important consideration, particularly on modern grains where other components of sporomorphs are removed by chemical procedures, and when quantitative calibration between modern and fossil pollen and spores are in development.

5. Observation model

601 Detection of UV-B absorbing compounds has been the area of research to experience the
602 most progress. Initial work involved using spectrophotometry to assess UV-B absorbance of
603 different components of the pollen grain following the sequential extraction of soluble (e.g.
604 pollen grain intine) and insoluble (sporopollenin) fractions (Table 1)[28,42]. Since then, work to
605 quantify UV-B absorbing compounds has progressed on two main fronts, using THM-GC-
606 MS[33] and vibrational methods using Fourier Transform Infrared Spectroscopy (FTIR)[31,35].
607 Each approach has specific advantages to estimate the abundance of UV-B absorbing
608 compounds. We consider them both in the remainder of this section (Figure 3).
609
610 THM-GC-MS involves using a strong base reagent (e.g. tetramethylammonium hydroxide,
611 TMAH) to hydrolyse the constituents within the sporopollenin and then subsequently
612 methylate and pyrolyse the products[33,35]. Compounds within the analyte are then separated as
613 the different molecules (with different chemical properties, e.g. molecule size, polarity) pass
614 through the gas-chromatograpy (GC) column, before their molecular mass is determined
615 using mass spectrometry (MS). The THM and pyrolysis step is essential to enable phenolic
616 compounds such as *para*-coumaric acid to be separated within the GC-column phase. The
617 method has been used in a variety of applications since the protocol for quantifying UV-B
618 absorbing compounds was developed, including for analyzing variations in UV-B absorbing
619 compounds in spores across elevation gradients[35], a latitudinal transect and subsequent
620 reconstruction using *Pinus* spp.[29], and various experimental studies[34].
621

Figure 3: The two main approaches currently used to quantify UV-B absorbing compounds in sporomorphs. Main advantages/ disadvantages in green/ red respectively.



The main advantage of this method is that UV-B absorbing compounds can be precisely described by comparing against either analytical standards or detailed reference libraries. In addition to precise fingerprinting of different compounds, the method also enables an approximation of absolute quantification of the compounds within a sample. Furthermore, although UV-B absorbing compounds such as *para*-coumaric acid are highly susceptible to contamination, which can cause additional noise and uncertainty in quantification estimates, using a standardization procedure can minimize these effects. For example, one study tested a variety of methods for improving analytical precision of *para*-coumaric acid in *Pinus* spp[44]. They showed that using an internal standard such as vanillic acid, where a known quantity of a standard compound is added to the sample prior to analysis to aid in quantification, provides an almost doubling of the analytical precision compared to when either an external standard or no standard method was used. Interestingly, relative standardization against sporopollenin-based long-chain fatty acids did not improve analytical precision, indicating that the abundance of these compounds are not stable, or there are variable reaction efficiencies of these long-chain fatty acids, from sample to sample[35]. The chemical reagents used in the THM reaction are highly stressful on the GC column, resulting in rapid peak tailing and reduced sensitivity after approximately only 100 samples. Thus, one particular benefit of a standardization approach is that it ensures that it enables robust comparison of batches of samples measured at different time periods or from different laboratories.

Although there are advantages to more precise quantification of UV-B absorbing compounds using THM-GC-MS, a major limitation relates to the large number of pollen grains or spores required to result in a statistically significant measurement. The approach is also time consuming, a batch of ten samples and associated calibration and blank samples can be realistically run over a two-day period. Therefore, an alternative approach based on vibrational spectroscopy has been developed for the measurement of pollen chemistry[31,35,58]. In general, vibrational spectroscopies, such as Fourier Transform Infrared (FTIR), are rapid, non-destructive and highly sensitive biophysical methods that provide precise signatures of the overall biochemical composition of a sample.

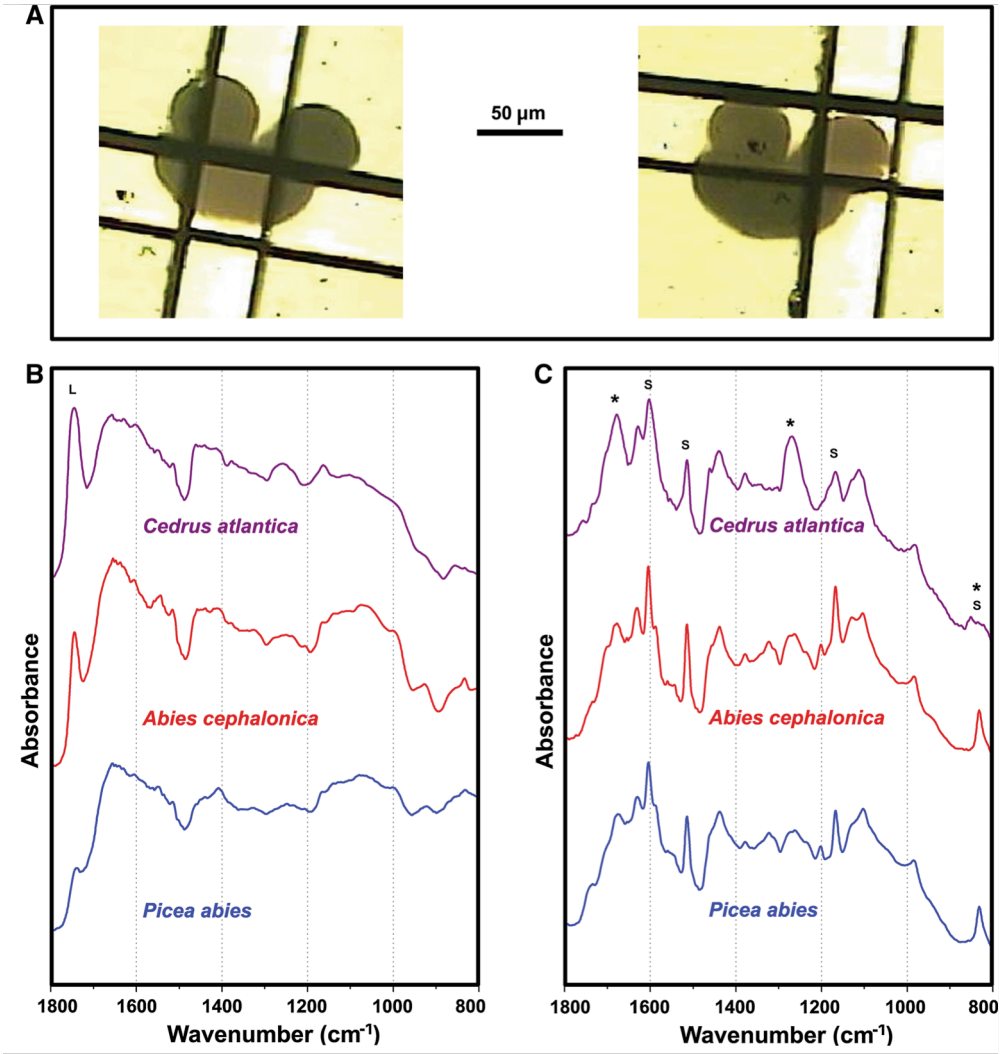


Figure 4. Comparison of FTIR microspectroscopy spectra of three species of the family within Pinaceae (reprinted from Zimmerman et a. 2015, ref. [59]). A) Optical microscope images of the measured *Abies cephalonica* pollen grain substructures, corpus (left) and saccus (right), on 3 mm ZnSe slide, with depicted 40×40 µm aperture. B) µFTIR spectra of corpus regions, and C) saccus regions of *Cedrus atlantica*, *Abies cephalonica*, and *Picea abies*. For better viewing the spectra are offset. The marked bands are associated with lipids (L) and sporopollenins (S); the spectral regions of interest are denoted with asterisks. Wavenumber (x axis) refers to the frequencies of the infrared radiation absorbed by the sample.

FTIR has been developed concurrently with THM-GC-MS for analysis of UV-B absorbing compounds and can provide a solution to some of the disadvantages experienced when using THM-GC-MS[10,31]. FTIR involves irradiating a sample with a broadband source of infrared light and then measuring absorbance, transmittance or reflectance of the infrared light. The wavenumbers (or frequencies) of the IR radiation absorbed by the sample are related to the frequencies of molecular vibrations within the constituent sample, so an infrared spectrum can be used to provide identifiable spectral features that are directly related to chemical composition (Figures 3,4). In the case of measurement of pollen and spores, FTIR provides precise signatures of the overall biochemical characterisation of a sample, including the specific signals of lipids, proteins, carbohydrates, water, and cell-wall biopolymers such as

cellulose and sporopollenins[109]. For example, FTIR spectra of pollen show specific signals of phenylpropanoids, the UV-B absorbing building blocks of sporopollenins, with distinctive bands associated with the vibrations of aromatic rings at specific wavenumbers in the FTIR spectra (1605, 1510, 1171, 853, 833 and 816 cm^{-1}). Based on these signals, the content of derivatives of para-coumaric, ferulic and sinapic acid can be determined[58]. This approach is much faster than THM-GC-MS. A standard bulk-sample FTIR spectroscopy analysis (with approximately 1 mg of pollen per sample)[68,110,111] can run up to approximately 100 samples per day (in triplicate measurements), whilst FTIR microspectroscopy (producing FTIR spectra of an individual pollen grain) can measure approximately 30 grains per hour depending on experimental settings[112,113]. In general, FTIR spectroscopy is a very versatile method and offers a number of different measurement settings.

One disadvantage of FTIR is that it can only measure UV-B-absorbing compounds relative to other parts of the spectrum. FTIR measurements of UV-B-absorbing compounds in plant spores and pollen are based on the assumption that a broad hydroxyl peak (at approx. 3300 cm^{-1}, related to OH stretching) is stable across all samples for a given pollen type. The peak related to the aromatic UV-B-absorbing compounds (at approx. 1510 cm^{-1}, related to phenyl ring vibrations) is then normalised by the hydroxyl peak to provide an estimate of the abundance[31,35]. Although the results of these FTIR studies are encouraging[10,114], it should be noted that the studies were based on relatively limited sample sets that lacked direct measurement of UV-B irradiation as reference values. Therefore, it is hard to assess if the normalization procedure is universally valid. A number of compounds commonly present in pollen and spores also show a strong hydroxyl peak, such as carbohydrates, proteins, and water. As a result, it can be expected that the hydroxyl-peak absorbance will strongly depend on moisture content of pollen, which can be influenced by the storage conditions as well as atmospheric conditions during measurement. In fact, compared to pollen-nutrient reserves in the form of lipids and carbohydrates, which can show strong variation depending on environmental conditions[110,111], the content of sporopollenins, and indirectly UV-B absorbing compounds, is relatively stable. For example, signals of sporopollenins (and proteins) were used recently for normalization of FTIR spectra and estimation of lipid variation in pollen grains of a number of Pinaceae species[68].

An additional complication for single grain FTIR analysis is that pollen grains can be subject to scattering effects[59,115]. Infrared light used in FTIR has a wavelength between 2 and 25 µm, which is similar in size to a number of smaller pollen grains. This means that reproducibility of single grain measurements can be difficult to achieve. Scattering is less of a problem in larger pollen grains such as the Pinaceae, but studies indicate that the chemical composition

of pollen grains can vary in different parts of the pollen grains[58,59]. For example, in Pinaceae, proteins and lipids accumulate in the corpus, whilst sporopollenins are observed mainly in the sacci (Figure 4). This can complicate single-grain measurements since different spectra can be obtained under axial and polar views[59,113]. These problems can be addressed by numerical correction methods, such as analytical Mie solutions[116] and spectral averaging[113], or by experimental settings, such as measurement in the embedding matrix[112]or multigrain measurement[58].

6. Synthesis and recommendations

Proxy-system modelling provides a framework to undertake a systematic evaluation of each stage of the UV-B proxy based on the chemistry of pollen and spores (Table 3). What is clear from this assessment is that, whilst considerable achievements have been made in quantification and measurement of UV-B absorbing compounds (i.e. the observation model), a number of key uncertainties exist in both the archive and sensor. Although improving understanding of two key components of the archive model (e.g. sporopollenin chemical stability and preservation; source-area effects) remains challenging, it is possible to take a number of careful steps to reduce the impacts of these factors prior to analysis. For example, the degree of sporopollenin preservation can be estimated through detailed assessment of sporopollenin colour[101], and this analysis can be used to select sites with minimal diagenetic effects on sporopollenin preservation. Similarly, careful site selection, combined with detailed age-depth sedimentation models calculated using large numbers of radiocarbon dates, are likely to be the most effective method to reduce uncertainty in the archive model in the near-term. Since many of the main challenges related to the archive model are not necessarily specific to reconstructions of received UV-B irradiance using pollen chemistry, a focus on this is not the most efficient way of making progress for researchers with specific expertise in understanding UV-B effects.

Our assessment reveals four major uncertainties related to the sporomorph-sensor model including: (i) species-specific dose-response relationships; (ii) whether results are transferable across taxa; (iii) the critical developmental stage at which sporomorph chemistry is sensitive to UV-B exposure; and (iv) the sensitivity and effects of other wavelengths of solar radiation on sporomorph chemistry. We suggest that solving these key challenges are most likely to result in the fastest and most significant gains in improving reconstruction precision and accuracy for pollen-chemistry reconstructions of UV-B irradiance. Addressing these questions would provide important new data to help resolve the apparent disagreements between UV-B ecologists and palaeoecologists[8].

742 **Table 3:** Prospects and challenges for a a proxy-system model used to reconstruct the UV-B radiation
743 received by plants using pollen and spore chemistry. The levels of current understanding of each
744 component of the proxy-system model are denoted as: good (+++), reasonable (++) or poor (+) based
745 on our assessment described in the main text.
746

	Current status of understanding	Key areas of achievement	Key knowledge gaps
Observation	+++	- Quantification of UV-B-absorbing compounds possible using complimentary techniques: vibrational spectroscopy and THM-GC-MS	- Whether quantification of pCA/ FA response is appropriate for all taxa (THM-GC-MS) - Whether standardization procedures are applicable across all taxa (Vibrational approaches) - Scattering and complications arising from single grain measurements (Vibrational approaches)
Archive	++	- Sporopollenins show relative stability through geological time and various stages of thermal maturity. Thermal diagenetic effects are less important below 250-300ºC. - Some pollen processing methods (acetolysis) appear to have limited effects on sporopollenin chemistry	- Integrating understanding from other palynological and paleobiological proxies to improve experimental design in the archive model has not commonly been considered so far - Consideration of chemical processing method when developing calibration models to compared between modern and fossil/ sub-fossil pollen and spores
Sensor	+	- UV-B absorbing compounds do increase across a range of experimental types and taxa when the plants are exposed to UV-B radiation	- Variations between species within different genera/ family, and across broader sections of the phylogenetic tree - Timing of the response/ plasticity - Sensitivity to different wavelengths and interactions with other variables

747
748

749 One interesting observation about the four challenges related to the sensor model is that they
750 remain relevant to researchers into UV-B impacts on plants on more recent timescales[117]. An
751 advantage arising from this overlap between neo-ecological and palaeoecological research is
752 that it is possible to collaborate and share research methods. Following the interest in
753 detailing the potential effects of CFC-induced stratospheric ozone depletion on plants after
754 the 1980s, a large body of research built up to develop a set of sophisticated methodologies
755 for assessing UV-B responses under higher UV-B conditions in both laboratory and field-
756 experimental settings. In conjunction with this, the field of researchers investigating the
757 contemporary effects of UV-B on plants has matured to develop a set of standardized, best
758 practice methods for conducting UV-B research[27]. Surprisingly, the palaeoecological

759 community has generally under-used these approaches so far (but see reference[34]). We argue
760 that there is much benefit to be gained from taking an interdisciplinary approach to address
761 the critical knowledge gaps outlined above.
762
763 Any progress made in the research challenges outlined above will result in a number of
764 exciting prospects for a UV-B proxy based on the sporopollenin of pollen or spores, for
765 palaeoclimate (e.g., reconstructing ozone and/ or solar variability in the past); in palaeocology
766 (e.g. investigating the responses of organisms and ecosystems to solar forcing and ozone
767 variability on different timescales), and in 'deep-time' palaeobiological research (e.g.
768 investigating the effects of UV-B radiation on origination and extinction rates related to
769 tectonic processes)[118]. Furthermore, addressing the key knowledge gaps identified here can
770 result further new exciting opportunities. For example, if phenolic compounds in pollen do
771 indeed respond to wavelengths other than UV-B (section 3.4), in addition to changing ozone,
772 for example, the proxy may be used to provide independent reconstructions of orbitally-
773 forced solar variability. Reconstructions from long sediment sequences could then be tuned to
774 Milankovitch oscillations, which would enable more precise age-depth modelling under
775 conditions when radiometric dates are less useful (e.g. beyond the range of radiocarbon
776 dating)[10].
777
778 The benefits from addressing these questions are also not only limited to understanding past
779 UV-B irradiance and the link to ecological, evolutionary, and palaeoclimatic changes in the
780 past. The four research challenges we have identified also represent fundamental questions
781 related to species responses to environmental change (e.g. sensitivity to environmental stress,
782 the relative importance of ecological plasticity)[76,77]. Thus, sporomorph-chemistry responses to
783 UV-B irradiance can represent an interesting model system for understanding general
784 responses of plants to environmental distress[117].
785
786 We conclude by arguing that palaeo-UV-B research based on the sporopollenin found in
787 pollen and spores is an exciting area, with broad knowledge gaps related to how plants
788 respond to environmental change. We stress that we prefer to see the issues raised in here to
789 be viewed as challenges and not as long-term problems; we intend this perspective to provide
790 a guideline for both researchers already involved in this field, and also researchers that are
791 interested in contributing to generation of new knowledge surrounding key sensitivities and
792 responses of the proxy. As interest in the proxy from both palaeoecologists and from UV-B
793 ecologists grows, there is an opportunity to make novel insights into the chemical responses
794 of pollen and plants, and the associated changes in UV-B radiation in the past.
795

7. References

1 IARC, *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Lyon, France, 2012, vol. 100D.

2 A. B. Britt, DNA damage and repair in plants, *Annual Review of Plant Physiology and Plant Molecular Biology*, 1996, **47**, 75–100.

3 A. Sancar and G. B. Sancar, DNA-Repair Enzymes, *Annual Reviews of Biochemistry*, 1988, **57**, 29–67.

4 J. Rozema, J. van de Staaij, L. O. Björn and M. Caldwell, UV-B as an environmental factor in plant life: Stress and regulation, *Trends in Ecology & Evolution*, 1997, **12**, 22–28.

5 S. Weber, Light-driven enzymatic catalysis of DNA repair: a review of recent biophysical studies on photolyase, *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 2005, **1707**, 1–23.

6 K. J. Willis, K. D. Bennett and H. J. B. Birks, Variability in thermal and UV-B energy fluxes through time and their influence on plant diversity and speciation, *J Biogeogr*, 2009, **36**, 1630–1644.

7 J. F. Bornman, P. W. Barnes, S. A. Robinson, C. L. Ballaré, S. D. Flint and M. M. Caldwell, Solar ultraviolet radiation and ozone depletion-driven climate change: effects on terrestrial ecosystems, *Photochem. Photobiol. Sci.*, 2015, **14**, 88–107.

8 A. F. Bais, R. M. Lucas, J. F. Bornman, C. E. Williamson, B. Sulzberger, A. T. Austin, S. R. Wilson, A. L. Andrady, G. Bernhard, R. L. McKenzie, P. J. Aucamp, S. Madronich, R. E. Neale, S. Yazar, A. R. Young, F. R. de Gruijl, M. Norval, Y. Takizawa, P. W. Barnes, T. M. Robson, S. A. Robinson, C. L. Ballaré, S. D. Flint, P. J. Neale, S. Hylander, K. C. Rose, S. Å. Wängberg, D. P. Häder, R. C. Worrest, R. G. Zepp, N. D. Paul, R. M. Cory, K. R. Solomon, J. Longstreth, K. K. Pandey, H. H. Redhwi, A. Torikai and A. M. Heikkilä, Environmental effects of ozone depletion, UV radiation and interactions with climate change: UNEP Environmental Effects Assessment Panel, update 2017, *Photochem. Photobiol. Sci.*, 2018, **17**, 127–179.

9 L. O. Björn, S. Widell and T. Wang, Evolution of UV-B regulation and protection in plants, *Adv. Space Res.*, 2002, **30**, 1557–1562.

10 P. E. Jardine, W. T. Fraser, B. H. Lomax, M. A. Sephton, T. M. Shanahan, C. S. Miller and W. D. Gosling, Pollen and spores as biological recorders of past ultraviolet irradiance, *Scientific Reports*, 2016, **6**, 39269 10.1038/srep39269

11 J. Rozema, B. van Geel, L. O. Björn, J. Lean and S. Madronich, Toward Solving the UV Puzzle, *Science*, 2002, **296**, 1621–1622.

12 D. J. Beerling, M. Harfoot, B. Lomax and J. A. Pyle, The stability of the stratospheric ozone layer during the end-Permian eruption of the Siberian Traps, *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, 2007, **365**, 1843–1866.

13 C. V. Looy, R. J. Twitchett, D. L. Dilcher, J. H. A. Van Konijnenbyrg-van Cittert and H. Visscher, Life in the end-Permian dead zone, *Proceedings of the Academy of Natural Sciences of the United States of America*, 2001, **98**, 7879–7883.

14 H. Visscher, C. V. Looy, M. E. Collinson, H. Brinkhuis, J. H. A. Van Konijnenbyrg-van Cittert, W. M. Kürschner and M. A. Sephton, Environmental mutagenesis during the end-Permian ecological crisis, *Proceedings of the Academy of Natural Sciences of the United States of America*, 2004, **101**, 12952–12956.

15 C. B. Foster and S. A. Afonin, Abnormal pollen grains: an outcome of deteriorating atmospheric conditions around the Permian–Triassic boundary, *Journal of the Geological Society, London*, 2005, **162**, 653–659.

16 J. R. Flenley, Why is pollen yellow? And why are there so many species in the tropical rain forest?, *J Biogeogr*, 2011, **38**, 809–816.

17 B. H. Lomax, W. T. Fraser, G. Harrington, S. Blackmore, M. A. Sephton and N. B. W. Harris, A novel palaeoaltimetry proxy based on spore and pollen wall chemistry,

Earth and Planetary Science Letters, 2012, **353-354**, 22–28.

18 R. L. McKenzie, P. J. Aucamp, A. F. Bais, L. O. Björn, M. Ilyas and S. Madronich, Ozone depletion and climate change: impacts on UV radiation, *Photochem. Photobiol. Sci.*, 2011, **10**, 182–198.

19 A. F. Bais, R. L. McKenzie, G. Bernhard, P. J. Aucamp, M. Ilyas, S. Madronich and K. Tourpali, Ozone depletion and climate change: impacts on UV radiation, *Photochem. Photobiol. Sci.*, 2015, **14**, 19–52.

20 C. E. Williamson, R. G. Zepp, R. M. Lucas, S. Madronich, A. T. Austin, C. L. Ballaré, M. Norval, B. Sulzberger, A. F. Bais, R. L. McKenzie, S. A. Robinson, D.-P. Häder, N. D. Paul and J. F. Bornman, Solar ultraviolet radiation in a changing climate, *Nature Climate Change*, 2014, **4**, 434–441.

21 J. R. McConnell, A. Burke, N. W. Dunbar, P. Köhler, J. L. Thomas, M. M. Arienzo, N. J. Chellman, O. J. Maselli, M. Sigl, J. F. Adkins, D. Baggenstos, J. F. Burkhart, E. J. Brook, C. Buizert, J. Cole-Dai, T. J. Fudge, G. Knorr, H.-F. Graf, M. M. Grieman, N. Iverson, K. C. McGwire, R. Mulvaney, G. Paris, R. H. Rhodes, E. S. Saltzman, J. P. Severinghaus, J. P. Steffensen, K. C. Taylor and G. Winckler, Synchronous volcanic eruptions and abrupt climate change ~17.7 ka plausibly linked by stratospheric ozone depletion., *Proceedings of the National Academy of Sciences of the United States of America*, 2017, **114**, 10035–10040.

22 J. Beer and B. van Geel, in *Natural Climate Variability and Global Warming: A Holocene Perspective*, eds. R. W. Battarbee and H. Binney, Blackwell, Chichester, UK, 2008, pp. 138–162.

23 P. Moffa-Sánchez, A. Born, I. R. Hall, D. J. R. Thornalley and S. Barker, Solar forcing of North Atlantic surface temperature and salinity over the past millennium, *Nature Geoscience*, 2014, **7**, 275–278.

24 V. E. Fioletov, G. E. Bodeker, A. J. Miller, R. D. McPeters and R. Stolarski, Global and zonal total ozone variations estimated from ground-based and satellite measurements: 1964-2000, *J. Geophys. Res. Atmos.*, 2002, **107**, 4647.

25 D. A. Hodgson, W. Vyverman, E. Verleyen, P. R. Leavitt, K. Sabbe, A. H. Squier and B. J. Keely, Late Pleistocene record of elevated UV radiation in an Antarctic lake, *Earth and Planetary Science Letters*, 2005, **236**, 765–772.

26 Q. Chen, Y. Nie, X. Liu, L. Xu and S. D. Emslie, An 800-year ultraviolet radiation record inferred from sedimentary pigments in the Ross Sea area, East Antarctica, *Boreas*, 2015, **44**, 693–705.

27 P. J. Aphalo, A. Albert, L. O. Björn, A. McLeod, T. M. Robson and E. Rosenqvist, Eds., *Beyond the visible: a handbook of best practice in plant UV photobiology. COST Action FA0906 UV4growth*, University of Helsinki, Division of Plant Biology, Helsinki, 2012.

28 J. Rozema, A. J. Noordijk, R. A. Broekman, A. van Beem, B. M. Meijkamp, N. V. J. de Bakker, J. W. M. van de Staaij, M. Stroetenga, S. J. P. Bohncke, M. Konert, S. Kars, H. Peat, R. I. L. Smith and P. Convey, (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*, 2001, **154**, 9–26.

29 K. J. Willis, A. Feurdean, H. J. B. Birks, A. E. Bjune, E. Breman, R. Broekman, J.-A. Grytnes, M. New, J. S. Singarayer and J. Rozema, Quantification of UV-B flux through time using UV-B-absorbing compounds contained in fossil *Pinus* sporopollenin, *New Phytologist*, 2011, **192**, 553–560.

30 W. T. Fraser, M. A. Sephton, J. S. Watson, S. Self, B. H. Lomax, D. I. James, C. H. Wellman, T. V. Callaghan and D. J. Beerling, 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*, 2011, **30**, 6026.

31 B. H. Lomax, W. T. Fraser, M. A. Sephton, T. V. Callaghan, S. Self, M. Harfoot, J. A. Pyle, C. H. Wellman and D. J. Beerling, Plant spore walls as a record of long-term

changes in ultraviolet-B radiation, *Nature Geoscience*, 2008, **1**, 592–596.
32 P. Blokker, P. Boelen, R. Broekman and J. Rozema, The occurrence of p-coumaric acid and ferulic acid in fossil plant materials and their use as UV-proxy, *Plant Ecology*, 2006, **182**, 197–207.
33 P. Blokker, D. Yeloff, P. Boelen, R. A. Broekman and J. Rozema, Development of a Proxy for Past Surface UV-B Irradiation: A Thermally Assisted Hydrolysis and Methylation py-GC/MS Method for the Analysis of Pollen and Spores, *Anal. Chem.*, 2005, **77**, 6026–6031.
34 J. Rozema, P. Blokker, M. A. Mayoral Fuertes and R. Broekman, UV-B absorbing compounds in present-day and fossil pollen, spores, cuticles, seed coats and wood: evaluation of a proxy for solar UV radiation, *Photochem. Photobiol. Sci.*, 2009, **8**, 1233–12.
35 J. S. Watson, M. A. Sephton, S. V. Sephton, S. Self, W. T. Fraser, B. H. Lomax, I. Gilmour, C. H. Wellman and D. J. Beerling, Rapid determination of spore chemistry using thermochemolysis gas chromatography-mass spectrometry and micro-Fourier transform infrared spectroscopy, *Photochem. Photobiol. Sci.*, 2007, **6**, 689.
36 W. T. Fraser, B. H. Lomax, P. E. Jardine, W. D. Gosling and M. A. Sephton, Pollen and spores as a passive monitor of ultraviolet radiation, *Front. Ecol. Evol.*, 2014, **2**, 437.
37 J. W. de Leeuw, G. J. M. Versteegh and P. F. van Bergen, in *Plants and Climate Change*, Springer Netherlands, Dordrecht, 2006, vol. 182, pp. 209–233.
38 S. D. Flint and M. M. Caldwell, Partial Inhibition of In Vitro Pollen Germination by Simulated Solar Ultraviolet-B Radiation, *Ecology*, 1984, **65**, 792–795.
39 N. Tuteja, M. B. Singh, M. K. Misra, P. L. Bhalla and R. Tuteja, Molecular Mechanisms of DNA Damage and Repair: Progress in Plants, *Critical Reviews in Biochemistry and Molecular Biology*, 2008, **36**, 337–397.
40 É. Hideg, M. A. K. Jansen and Å. Strid, UV-B exposure, ROS, and stress: inseparable companions or loosely linked associates?, *Trends in Plant Science*, 2013, **18**, 107–115.
41 W. T. Fraser, A. C. Scott, A. E. S. Forbes, I. J. Glasspool, R. E. Plotnick, F. Kenig and B. H. Lomax, Evolutionary stasis of sporopollenin biochemistry revealed by unaltered Pennsylvanian spores, *New Phytologist*, 2012, **196**, 397–401.
42 J. Rozema, R. A. Broekman, P. Blokker, B. B. Meijkamp, N. de Bakker, J. van de Staaij, A. van Beem, F. Ariese and S. M. Kars, UV-B absorbance and UV-B absorbing compounds (para-coumaric acid) in pollen and sporopollenin: the perspective to track historic UV-B levels, *Journal of Photochemistry & Photobiology, B: Biology*, 2001, **62**, 108–117.
43 M. Jokerud, Plastic response in *Pinus* spp., determining the temporal window of response and species-level variation of UV-B absorbing compounds to short-term variation in UV-B radiation. Advances in developing a pollen-based UV-B proxy using THM py-GC/MS. PhD Thesis, University of Bergen, Norway, 2017.
44 A. W. R. Seddon, M. Jokerud, T. Barth, H. J. B. Birks, L. C. Krüger, V. Vandvik and K. J. Willis, Improved quantification of UV-B absorbing compounds in *Pinus sylvestris* L. pollen grains using an internal standard methodology, *Rev Palaeobot Palyno*, 2017, **247**, 97–104.
45 B. A. Bell, W. J. Fletcher, P. Ryan, A. W. Seddon, R. A. Wogelius and R. Ilmen, UV-B-absorbing compounds in modern *Cedrus atlantica* pollen: The potential for a summer UV-B proxy for Northwest Africa, *The Holocene*, 2018, **49**, 095968361877707–13.
46 B. C. Thomas, B. D. Goracke and S. M. Dalton, Atmospheric constituents and surface-level UVB: Implications for a paleoaltimetry proxy and attempts to reconstruct UV exposure during volcanic episodes, *Earth and Planetary Science Letters*, 2016, **453**, 141–151.
47 M. N. Evans, S. E. Tolwinski-Ward, D. M. Thompson and K. J. Anchukaitis, Applications of proxy system modeling in high resolution paleoclimatology, *Quaternary Sci Rev*, 2013, **76**, 16–28.

48 S. T. Jackson, Representation of flora and vegetation in Quaternary fossil assemblages: known and unknown knowns and unknowns, *Quaternary Sci Rev*, 2012, **49**, 1–15.
49 M. N. Evans, Toward forward modeling for paleoclimatic proxy signal calibration: A case study with oxygen isotopic composition of tropical woods, *Geochemistry Geophysics Geosystems*, 2007, **8**, Q07008.
50 M. E. Mann, Z. Zhang, M. K. Hughes, R. S. Bradley, S. K. Miller, S. Rutherford and F. Ni, Proxy-based reconstructions of hemispheric and global surface temperature variations over the past two millennia, *Proceedings of the National Academy of Sciences of the United States of America,* 2008, **105**, 13252–13257.
51 D. M. Thompson, T. R. Ault, M. N. Evans, J. E. Cole and J. Emile-Geay, Comparison of observed and simulated tropical climate trends using a forward model of coral $\delta^{18}O$, *Geophys. Res. Lett.*, 2011, **38**, L14706.
52 J. Verdebout, A European satellite-derived UV climatology available for impact studies, *Radiation Protection Dosimetry*, 2004, **111**, 407–411.
53 J. Laskar, P. Robutel, F. Joutel, M. Gastineau, A. C. M. Correia and B. Levrard, A long-term numerical solution for the insolation quantities of the Earth, *A&A*, 2004, **428**, 261–285.
54 L. Rizzini, J.-J. Favory, C. Cloix, D. Faggionato, A. O'Hara, E. Kaiserli, R. Baumeister, E. Schaefer, F. Nagy, G. I. Jenkins and R. Ulm, Perception of UV-B by the Arabidopsis UVR8 Protein, *Science*, 2011, **332**, 103–106.
55 J. M. Christie, A. S. Arvai, K. J. Baxter, M. Heilmann, A. J. Pratt, A. O'Hara, S. M. Kelly, M. Hothorn, B. O. Smith, K. Hitomi, G. I. Jenkins and E. D. Getzoff, Plant UVR8 Photoreceptor Senses UV-B by Tryptophan-Mediated Disruption of Cross-Dimer Salt Bridges, *Science*, 2012, **335**, 1492–1496.
56 S. Wallace, C. C. Chater, Y. Kamisugi, A. C. Cuming, C. H. Wellman, D. J. Beerling and A. J. Fleming, Conservation of Male Sterility 2function during spore and pollen wall development supports an evolutionarily early recruitment of a core component in the sporopollenin biosynthetic pathway, *New Phytologist*, 2014, **205**, 390–401.
57 B. H. Lomax and W. T. Fraser, Palaeoproxies: botanical monitors and recorders of atmospheric change, *Palaeontology*, 2015, **58**, 759–768.
58 M. Bağcıoğlu, B. Zimmermann and A. Kohler, A Multiscale Vibrational Spectroscopic Approach for Identification and Biochemical Characterization of Pollen, *PLoS ONE*, 2015, **10**, e0137899–19.
59 B. Zimmermann, M. Bağcıoğlu, C. Sandt and A. Kohler, Vibrational microspectroscopy enables chemical characterization of single pollen grains as well as comparative analysis of plant species based on pollen ultrastructure, *Planta*, 2015, **242**, 1237–1250.
60 T. M. Robson and P. J. Aphalo, Species-specific effect of UV-B radiation on the temporal pattern of leaf growth, *Physiologia Plantarum*, 2012, **144**, 146–160.
61 J. Torabinejad, M. M. Caldwell, S. D. Flint and S. Durham, Susceptibility of pollen to UV-B radiation: an assay of 34 taxa, *American Journal of Botany*, 1998, **85**, 360–369.
62 K. Klem, P. Holub, M. Štroch, J. Nezval, V. Špunda, J. Tříska, M. A. K. Jansen, T. M. Robson and O. Urban, Ultraviolet and photosynthetically active radiation can both induce photoprotective capacity allowing barley to overcome high radiation stress, *Plant Physiology and Biochemistry*, 2015, **93**, 74–83.
63 T. Kotilainen, R. Tegelberg, R. Julkunen-Tiitto, A. Lindfors and P. J. Aphalo, Metabolite specific effects of solar UV-A and UV-B on alder and birch leaf phenolics, *Global Change Biology*, 2008, **14**, 1294–1304.
64 M. Schreiner, M. Wiesner-Reinhold, S. Baldermann, F.S. Hanschen and S. Neugart, in Plant UV Biology., ed. B. Jordan, CABI publishers, 2017, ch. 4, pp. 39-57.
65 P. W. Barnes, T. M. Robson, M. A. Tobler, I. N. Bottger and S. D. Flint, *Plant UV Biology*, CABI publishers, 2017.
66 S. T. Andersen, Influence of Climatic Variation on Pollen Season Severity in Wind-Pollinated Trees and Herbs, *Grana*, 1980, **19**, 47–52.

67 J. N. Owens, The reproductive biology of lodgepole pine, 2006, FGC extension note, 07, Prepared for Forest Genetics Council of British Columbia.
68 B. Zimmermann and A. Kohler, Infrared Spectroscopy of Pollen Identifies Plant Species and Genus as Well as Environmental Conditions, *PLoS ONE*, 2014, **9**, e95417.
69 D. Treutter, Managing Phenol Contents in Crop Plants by Phytochemical Farming and Breeding—Visions and Constraints, *IJMS*, 2010, **11**, 807–857.
70 Q.-W. Wang, C. Kamiyama, J. Hidema and K. Hikosaka, Ultraviolet-B-induced DNA damage and ultraviolet-B tolerance mechanisms in species with different functional groups coexisting in subalpine moorlands, *Oecologia*, 2016, **181**, 1069–1082.
71 Y. O. Kim and E. J. Lee, Comparison of phenolic compounds and the effects of invasive and native species in East Asia: support for the novel weapons hypothesis, *Ecol Res*, 2011, **26**, 87–94.
72 H. Wang, X. Ma, L. Zhang, J. Zou and E. Siemann, UV-B has larger negative impacts on invasive populations of Triadica sebiferabut ozone impacts do not vary, *Journal of Plant Ecology*, 2016, **9**, 61-68.
73 M. Beckmann, M. Hock, H. Bruelheide and A. Erfmeier, The role of UV-B radiation in the invasion of *Hieracium pilosella*-A comparison of German and New Zealand plants, *Environmental and Experimental Botany*, 2012, **75**, 173–180.
74 M. Hock, M. Beckmann, R. R. Hofmann, H. Bruelheide and A. Erfmeier, Effects of UV-B radiation on germination characteristics in invasive plants in New Zealand, *NB*, 2015, **26**, 21–37.
75 T. Václavík, M. Beckmann, A. F. Cord and A. M. Bindewald, Effects of UV-B radiation on leaf hair traits of invasive plants—Combining historical herbarium records with novel remote sensing data, *PLoS ONE*, 2017, **12**, e0175671–18.
76 F. Valladares, S. Matesanz, F. Guilhaumon, M. B. Araujo, L. Balaguer, M. Benito-Garzón, W. Cornwell, E. Gianoli, M. van Kleunen, D. E. Naya, A. B. Nicotra, H. Poorter and M. A. Zavala, The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change, *Ecol Lett*, 2014, **17**, 1351–1364.
77 M. Benito-Garzón, R. Alía, T. M. Robson and M. A. Zavala, Intra-specific variability and plasticity influence potential tree species distributions under climate change, *Global Ecol Biogeogr*, 2011, **20**, 766–778.
78 S. M. Hartikainen, A. Jach, A. Grané and T. M. Robson, Assessing scale-wise similarity of curves with a thick pen: As illustrated through comparisons of spectral irradiance, *Ecol Evol*, 2018, **1**, 21–13.
79 S. D. Flint and M. M. Caldwell, A biological spectral weighting function for ozone depletion research with higher plants, *Physiologia Plantarum*, 2003, **117**, 137–144.
80 S. D. Flint and M. M. Caldwell, Field testing of UV biological spectral weighting functions for higher plants, *Physiologia Plantarum*, 2003, **117**, 145–153.
81 T. Kotilainen, T. Venäläinen, R. Tegelberg, A. Lindfors, R. Julkunen-Tiitto, S. Sutinen, R. B. O'Hara and P. J. Aphalo, Assessment of UV Biological Spectral Weighting Functions for Phenolic Metabolites and Growth Responses in Silver Birch Seedlings, *Photochemistry and Photobiology*, 2009, **85**, 1346–1355.
82 R. Julkunen-Tiitto, H. Häggman, P. J. Aphalo, A. Lavola, R. Tegelberg and T. Veteli, Growth and defense in deciduous trees and shrubs under UV-B, *Environmental Pollution*, 2005, **137**, 404–414.
83 P. Krauss, C. Markstädter and M. Riederer, Attenuation of UV radiation by plant cuticles from woody species, *Plant, Cell & Environment*, 1997, **20**, 1079–1085.
84 J. Rozema, M. Tosserams, H. J. M. Nelissen, L. van Heerwaarden, R. A. Broekman and N. Flierman, Stratospheric ozone reduction and ecosystem processes: enhanced UV-B radiation affects chemical quality and decomposition of leaves of the dune grassland species Calamagrostis epigeios, *Plant Ecology*, 1997, **128**, 285–294.
85 E. A. Tripp, Y. Zhuang, M. Schreiber, H. Stone and A. E. Berardi, Evolutionary and ecological drivers of plant flavonoids across a large latitudinal gradient, *Molecular*

Phylogenetics and Evolution, 2018, **128**, 147–161.
86 L. Jaakola and A. Hohtola, Effect of latitude on flavonoid biosynthesis in plants, *Plant, Cell & Environment*, 2010, **160**, 1239–1247.
87 M. Tattini, C. Galardi, P. Pinelli, R. Massai, D. Remorini and G. Agati, Differential accumulation of flavonoids and hydroxycinnamates in leaves of Ligustrum vulgare under excess light and drought stress, *New Phytologist*, 2004, **163**, 547–561.
88 F. Sato, *Plant Secondary Metabolism* in *eLS*, John Wiley & Sons, Ltd, Chichester, UK, 2014.
89 T. M. Robson, S. M. Hartikainen and P. J. Aphalo, How does solar ultraviolet-B radiation improve drought tolerance of silver birch (*Betula pendula* Roth.) seedlings?, *Plant, Cell & Environment*, 2014, **38**, 953–967.
90 L. von Post, Einige Südschwedischen Quellmoore, *Bulletin of the Geological Institute of Uppsala University*, 1916.
91 L. von Post, Skogsträdpollen i sydsvenska torvmosselager-följder, *Förhandlingar Skandinavia Naturforskermøte*, 1918, 43–465.
92 H. J. B. Birks and B. E. Berglund, One hundred years of Quaternary pollen analysis 1916–2016, *Veg Hist Archaeobot*, 2018, **27**, 271–309.
93 A. Dawson, C. J. Paciorek, J. S. McLachlan, S. Goring, J. W. Williams and S. T. Jackson, Quantifying pollen-vegetation relationships to reconstruct ancient forests using 19th-century forest composition and pollen data, *Quaternary Sci Rev*, 2016, **137**, 156–175.
94 S. Sugita, Pollen Representation of Vegetation in Quaternary Sediments - Theory and Method in Patchy Vegetation, *J Ecol*, 1994, **82**, 881–897.
95 I. C. Prentice, Pollen Representation, Source Area, and Basin Size - Toward a Unified Theory of Pollen Analysis, *Quaternary Res*, 1985, **23**, 76–86.
96 M. Blaauw and E. Heegaard, in *Tracking Environmental Change Using Lake Sediments, Volume 5: Data Handling and Numerical Techniques*, eds. H. J. B. Birks, A. F. Lotter, S. Juggins and J. P. Smol, Dordrecht: Springer, 2012, pp. 379–413.
97 M. Blaauw and J. A. Christen, Flexible paleoclimate age-depth models using an autoregressive gamma process, *Bayesian Anal.*, 2011, **6**, 457–474.
98 M. B. Davis, Palynology after Y2K - Understanding the source area of pollen in sediments, *Annu. Rev. Earth Planet. Sci.*, 2000, **28**, 1–18.
99 S. Sugita, S. Hicks and H. Sormunen, Absolute pollen productivity and pollen-vegetation relationships in northern Finland, *J. Quaternary Sci.*, 2009, **25**, 724–736.
100 H. Seppä and H. J. B. Birks, July mean temperature and annual precipitation trends during the Holocene in the Fennoscandian tree-line area: pollen-based climate reconstructions, *The Holocene*, 2001, **11**, 527–539.
101 W. T. Fraser, J. S. Watson, M. A. Sephton, B. H. Lomax, G. Harrington, W. D. Gosling and S. Self, Changes in spore chemistry and appearance with increasing maturity, *Rev Palaeobot Palyno*, 2014, **201**, 41–46.
102 K. D. Bennett and K. J. Willis, in *Tracking Environmental Change Using Lake Sediments*, *Volume 3: Terrestrial, Algal, and Siliceous Indicators* eds. J. P. Smol, H. J. B. Birks, W. M. Last, R. S. Kluwer Academic Publishers, Dordrecht, 2002, vol. 3.
103 A. Traverse, *Paleopalynology*, Massachusetts, 1988.
104 P. E. Jardine, W. T. Fraser, B. H. Lomax and W. D. Gosling, The impact of oxidation on spore and pollen chemistry, *Journal of Micropalaeontology*, 2015, **34**, 139–149.
105 G. D. Wood, A. M. Gabriel and J. C. Lawson, in *Palynology Principles and Applications*, eds. J. Jansonius and D. C. McGregor, American Association of Stratigraphic Palynologists Foundation, Dallas, 1996.
106 N. G. Johnson, Early Silurian palynomorphs from the tuscarora formation in central Pennsylvania and their paleobotanical and geological significance, *Rev Palaeobot Palyno*, 1985, **45**, 307–359.
107 V. Lebreton, E. Messager, L. Marquer and J. Renault-Miskovsky, A neotaphonomic experiment in pollen oxidation and its implications for archaeopalynology, *Rev Palaeobot Palyno*, 2010, **162**, 29–38.

108 A. R. Hemsley, A. C. Scott, P. J. Barrie and W. G. Chaloner, Studies of fossil and modern spore wall biomacromolecules using ^{13}C solid state NMR, *Annals of Botany*, 1996, **78**, 83–94.

109 B. Zimmermann, Characterization of pollen by vibrational spectroscopy, *Applied spectroscopy*, 2010, **64**, 1364–1373.

110 B. Zimmermann, M. Bağcıoğlu, V. Tafinstseva, A. Kohler, M. Ohlson and S. Fjellheim, A high-throughput FTIR spectroscopy approach to assess adaptive variation in the chemical composition of pollen, *Ecol Evol*, 2017, **7**, 10839–10849.

111 M. Bağcıoğlu, A. Kohler, S. Seifert, J. Kneipp and B. Zimmermann, Monitoring of plant-environment interactions by high-throughput FTIR spectroscopy of pollen, *Methods in Ecology and Evolution*, 2016, **8**, 870–880.

112 B. Zimmerman, V. Tafintseva, M. Bağcıoğlu, M. Høegh Berdahl and A. Kohler, Analysis of Allergenic Pollen by FTIR Microspectroscopy, *Anal. Chem.*, 2015, **88**, 803–811.

113 B. Zimmermann, Chemical characterization and identification of Pinaceae pollen by infrared microspectroscopy, *Planta*, 2017, **247**, 171–180.

114 P. E. Jardine, F. A. J. Abernethy, B. H. Lomax, W. D. Gosling and W. T. Fraser, Shedding light on sporopollenin chemistry, with reference to UV reconstructions, *Rev Palaeobot Palyno*, 2016, 1–28.

115 R. Blümel, R. Lukacs, B. Zimmermann, M. Bağcıoğlu and A. Kohler, Observation of Mie ripples in the synchrotron Fourier transform infrared spectra of spheroidal pollen grains, *J. Opt. Soc. Am. A*, 2018, **35**, 1769–11.

116 R. Lukacs, R. Blümel, B. Zimmerman, M. Bağcıoğlu and A. Kohler, Recovery of absorbance spectra of micrometer-sized biological and inanimate particles, *Analyst*, 2015, **140**, 3273–3284.

117 P. W. Barnes, M. A. K. Jansen, G. I. Jenkins, F. Vandenbussche, C.C. Brelsford, A.K. Banas, W. Bilger, A. Castagna, D. Festi, A. Gaberščik, M. Germ, A. Golob, M.-T. Hauser, L. Llorens, J. Martinez-Abaigar, L.O. Morales, S. Neugart, M. Pieristè, N. Rai, L. Ryan, M. Santin, A.W.R. Seddon, J. Stelzner, E. Tavridou, J. Łabuz, and T. M. Robson, The importance and direction of current and future plant-UV research, *UV4Plants Bulletin*, 2018, 3, 19-32.

118 D. Magri, Past UV-B flux from fossil pollen: prospects for climate, environment and evolution, *New Phytologist*, 2011, **192**, 310–312.