UV-B absorbing compounds in Pinus spp. pollen indicate plastic responses to solar radiation

Mari Jokerud[1,2], Alistair Seddon[3], Joseph Chipperfield[2], Katherine Willis[4], and Vigdis Vandvik[3]

[1]Norwegian Institute for Nature Research (NINA)
[2]Norwegian Institute for Nature Research
[3]University of Bergen
[4]University of Oxford

May 5, 2020

Abstract

1. The abundance of UV-B absorbing compounds (for example p-Coumaric acid, pCA) within pollen-grain sporopollenin has been proposed as a proxy for reconstructing past changes in surface solar radiation, but drivers of the variation of these compounds in pollen grains remains poorly understood. 2. One important consideration is that the physiological response that results in the production and timing of pCA in pollen remains poorly understood. This calls for studies that explicitly tests the temporal resolution and plasticity of the response of pCA in pollen under field conditions. 3. To address this, we conducted two parallel experiments. First, we measured pCA in four Pinus spp. from Geneva in two consecutive years to investigate the impact of natural variation in ambient solar radiation, and differences in responses between species. Second, we covered pollen cones on Pinus sylvestris trees in Bergen with 90% shading cloth one month before dehiscence and compared the amount of pCA in pollen from shaded and sun-exposed cones from the same tree. 4. Between years, in Geneva, Pinus spp. produced 31% more pCA in 2014 than in 2013, with higher levels of solar radiation also observed in 2014. pCA content also showed strong species-level variation, largely reflecting differences in pollen size between species. Experimentally shaded Pinus sylvestris pollen produced 21% less pCA than fully exposed pollen. 5. Our work demonstrates a plastic response in the production of UV absorbing compounds (pCA) to inter-annual and experimentally induced variation in ambient solar radiation in Pinus spp. pollen. This supports pCA as a highly responsive proxy for early-season solar radiation. We also find strong species-level variation in pCA content in pine pollen, likely related to pollen grain size, which should be accounted for in reconstructions from sites with multiple Pinus species present.

Introduction

Plants are sessile organisms, and many plants use physical or chemical responses to defend against environmental stressors. For example, the phenolic compound p -coumaric acid (p CA) is implicated as a chemical defence mechanism with respect to exposure to solar radiation, especially the UV-B wavelengths (280-315 nm). This compound is stimulated through the phenylpropanoid pathway (PPP), and has been shown to be an effective absorber of UV-B radiation (Blokker et al., 2006). It is also an important building block for a number of essential plant compounds, including sporopollenin, the major biomolecule that constitutes the exine of pollen grains (Wehling et al., 1989, Blokker et al., 2006, Rozema et al., 2001a, Jungfermann et al., 1997). Indeed,p CA has been found in higher abundances in the pollen-based sporopollenin of plants exposed to higher levels of UV-B (Rozema et al., 2001b, Blokker et al., 2005). Since sporopollenin is also highly resistant to degradation under anoxic conditions, recent studies have proposed using the pollen chemical record preserved in sediments as a proxy for UV-B (Rozema et al., 2001b, Rozema et al., 2002, Fraser et

al., 2011, Mazza et al., 2000, Willis et al., 2011) or more generally for total solar irradiance (Jardine et al., 2016) on centennial timescales or longer. The development of such a tool would have major implications for long-term ecological research, providing an independent of solar radiation in the past, with a broad range of palaeoecological and palaeoclimatological applications (Magri, 2011).

To enable a robust application of the chemical signatures of plants to reconstruct past changes in solar radiation, an understanding of the drivers of variability of these compounds within pollen grains is required (Seddon et al., 2019). However, whilst a number of studies have demonstrated positive relationships between exposure to solar radiation and *p* CA along latitudinal and elevation gradients (Willis et al., 2011, Jardine et al., 2016, Watson et al., 2007, Lomax et al., 2012), the timing of the response of these compounds to UV-B radiation remains poorly understood. For example, a number of studies have assumed that*p* CA is a direct indicator of solar radiation during the growing season (Rozema et al., 2001a, Rozema et al., 2001b, Lomax et al., 2008), but this assertion has yet to be tested under field conditions. In fact, in some species, such as *Pinus sylvestris* , tapetal cells produce the membrane containing sporopollenin (including *p* CA) less than 11 days prior to pollen shed (Rowley et al., 2000) so significantly shorter-term plastic responses than those currently proposed are possible. Furthermore, the extent to which the *p* CA production is driven by conditions experienced locally on the plant (i.e. if variability is observed between branches or individual flowers shaded in different ways, or if the signal represents a fully-integrated whole-tree tree response) remains unknown. This has implications for how the proxy is understood from the point of view from the palaeorecord since it determines whether a signal derived *p* CA represents a short-term or growing-season response, as well as whether within-sample variability should be interpreted as reflecting the stand-, tree-, or within-canopy patterns. Therefore, determining the timing and extent of this plastic response remains an essential question in understanding how pollen responds to changes in solar radiation.

A second aspect that remains poorly understood is the variation in both the *p* CA content and in the response to environmental variation between individuals and species. For example, the widespread abundance of *Pinus* pollen grains found in sediments, combined with the fact that *Pinus* is a light-demanding species, means that *Pinus*has become a focus for research into reconstruction of solar irradiance using *p* CA. However, the pollen members of the European flora of*Pinus* spp. remain difficult to separate in palaeoecological reconstructions using traditional light microscopic methods, and if different species exhibit varying responses under equivalent radiation, then this could have implications for both dose-response relationship of*p* CA and reconstructions from pollen sampled from a sediment core. An obvious source of species-level variation in *p* CA may be pollen size. European pines vary considerably in pollen size (Beug, 1961), and it is very likely that species may contain differences in total*p* CA simply due to differences in their surface-area. In palaeoecological reconstructions, such variation may be accounted for simply by measuring and taking these size differences into account. Species-level variation in phenolic acid content due to inherited genetic differences in the ability to produce these acids, or in the response to variation in radiation, would be more challenging to account for using palaeoecological reconstructions. To date, the extent to which species-level variation can potentially affect the dose-response relationship between *p* CA and solar radiation remains poorly understood.

Here, we present the results of two studies which aimed to investigate the effect of short-term variations in ambient solar radiation on the abundance of *p* CA in *Pinus* pollen grains. Using a resampling of the same individuals over two consecutive years with natural variation in ambient solar radiation, our first objective is to investigate whether the abundance of *p* CA in pollen grains varies in response to natural variability in radiation, and if the level or response varies between *Pinus* species. Using shading cloths on 10 individuals of *Pinus sylvestris* , our second objective was to investigate whether the abundance of *p* CA in pollen grains varies in response to artificial reductions of ambient solar radiation at the level of individual male cones.

Material and Methods

Study design

To investigate inter-annual responses to natural variation in ambient solar radiation and species-level variation, pollen samples were collected during dehiscence season of 2013 (collection date: May 14th) and

2014 (May 26th) from the same *Pinus nigra* J.F. Arnold, *P. pinaster* Aiton, *P. sylvestris* L. and *P. uncinata*Ramond ex DC. (Table 1) trees in both years in Geneva Botanical Garden and Conservatory (N 46.2253, E 6.1465). The number of trees varied per species, depending on availability. Five fully sun-exposed branches were sampled on each tree, on the south facing side during or immediately after dehiscence. Pollen cones were placed in an individual paper bag, which again were placed within a plastic zip lock bag to minimize contamination and stored at room temperature in sealed plastic boxes.

An ambient solar-radiation exclusion experiment was set up on April 28th 2014 in order to investigate variations in *p*- coumaric acid at the flower level, ten freestanding/sun-exposed *Pinus sylvestris* trees 6–8 metres in height were selected from the Arboretum and Botanical Garden of Milde in Bergen, Norway (N 60.2557, E 5.2706). Two south facing branches with a distance of one to two metres between on each tree were used, one randomly allocated to the shade treatment and covered with a shade cloth fabric with 90% shade intensity, the other marked and left without any shade cloth under ambient solar radiation. Similar to a study by Fraser et al. (2011), which used the natural shading of forest canopy to screen out solar radiation, the shade cloths in our experiment screen out a proportion of total incoming solar radiation. Pollen was collected on May 28th 2014, when one to three male pollen cones from one branch tip were placed in an individual paper bag, which again was placed within a plastic zip lock bag to minimize contamination and stored at room temperature in sealed plastic boxes.

Measurement of p-coumaric acid in Pinus spp. pollen

To determine variation in *p* CA within pollen sporopollenin we used thermally assisted hydrolysis and methylation pyrolysis-gas chromatography and mass spectrometry (THM-py GC/MS). This method developed by Blokker et al. (2005) was adapted by introducing an internal standard where a solution of a known amount of nonadecanoic acid was applied to each pollen sample during the THM reaction (Seddon et al., 2017). Our sample preparation steps were as follows.

Pollen samples from one branch were submerged in distilled water in a petri dish and three replicates with a predefined number of pollen grains were then picked under a Leica DMIL 090-135.001 inverted microscope with a self-customised Pasteur pipette. *Pinus nigra, P. pinaster and P. uncinata* samples contained 150 pollen grains but due to the smaller pollen size and in order to avoid THM-GC/MS results close to the machine detection level, samples of *P. sylvestris* contained 200 pollen grains. The pollen samples were transferred to a microvial, centrifuged and dried overnight at 50^{o}C. We then added 2 µl of a 25% TMAH: Nonadecanoic acid: MEOH solution to the sample using a Hamilton Digital 1701RN 10µl syringe. The samples were centrifuged and left in room temperature for 30 minutes before they were placed in an oven at 70° for 2 hours. Measurements of *p* CA were made using an Hewlett Packard (HP) 7890 Gas Chromatographer with an HP 6890 detector. The pyrolysis unit is a GL Sciences ATAS-GL LINEX with a PAL Combi robotic auto sampler. To check for analytical consistency between runs, we ran samples with equal amounts of *p* CA and nonadecanoic acid after every second pollen sample. For all experiments, The pyrolysis heating programme was set to rise from 40 °C to 600 °C (maximum) at the maximum ramp rate (approximately 60 °C/s). The GC oven was programmed from 40 °C (6 minute hold time) to 130 °C at 15 °C/min followed by 250 °C at 8 °C/min, up to 320 °C at 15 °C/min and 1.5 min isothermal at 320 °C (Willis et al., 2011).

We measured a total of 108 samples for *p* CA using TMH-py GC/MS. In the cross-year study, we measured four species with one to three trees per species and three replicates per tree, resulting in 48 analysed samples. In the shading experiment we measured 60 samples; ten trees, two treatments, and three replicates (Table 1). *p* CA ratios were calculated by dividing the measured *p* CA peak area with measured nonadecanoic acid peak area.

Data analyses

We used hierarchical mixed-effect models to test for differences in i)*p* CA quantity between *Pinus* pollen produced in 2013 and 2014 and between *Pinus* species (inter-annual and inter-specific variability study), ii) *p* CA quantity between shaded and fully sun-exposed pollen of *Pinus sylvestris* (ambient solar radiation exclusion experiment). In both experiments tree was set to random effect, and differences in *p* CA abundance

were tested in samples from trees between (i) different years and (ii) shaded and unshaded treatments (both set as fixed effects). Parameters of the model were estimated using Bayesian inference. In both cases, our model essentially replicates a t-test with the trees set as a random effect to account for potential individual-level effects related to, for example, local adaptation (Bell et al., 2018). Bayesian inference was used to characterise uncertainty at different parts of the analytical process, and was based on three main components characterising variance in the pollen picking procedure, the GC-MS instrument, and the variance in the sample (see Fig. S1 in Supporting Information).

A Bayesian framework was used because of the challenges related to the precise quantification of pollen grains using the py-GC-MS technique, for which replication is realistically achievable to a relative standard deviation of ˜ 5% (Seddon et al., 2017). Our hierarchical modelling approach enables us to incorporate an additional sub-model to account for this uncertainty, and provides the first quantitative solution to a component-based proxy system model outlined in Seddon et al. (2019). Differences between years or treatment were based on Bayesian 95% credible intervals (CRIs), which contain 95% of the values from the posterior distribution of parameter estimates, and are analogous to frequentist 95% confidence intervals (Ellison, 2004). CRIs were calculated based on the posterior. For further details about the statistical model developed, see Supporting Information. All statistical analyses were performed in RStudio (RStudio Team, 2015) using the R statistical software package version 3.3.1 (R Core Team, 2017).

Ambient solar radiation data

The principal driver of *p* CA is thought to be variations in UV-B radiation, however as UV-B radiation is difficult to differentiate from other incoming radiation (e.g. PAR, UV-A) using only one location, we used solar radiation measurement (W/m^2) as a proxy for differences in the incoming solar radiation in inter-annual and inter-specific variability study from the IDAWEB meteorological data archive provided by MeteoSwiss (https://gate.meteoswiss.ch/idaweb/more.do). The measurement station in Geneva is located in Cointrin (N 46°15', E 6°08') which is approx. 2 km from the botanic garden. We used daily mean global radiation (W/m^2) data from June 1st 2012 to May 31th 2014 (Fig. 1 a). The daily measurements from January 1st 2004 to December 31th 2014 were summarised into monthly mean solar radiation and averaged across all years to calculate monthly anomalies (Fig. 1 b). We summarised total solar radiation (W/m^2) 28 days and 56 days prior to each collection date, May 14th 2013 and May 26th 2014. The following R packages were used to obtain these data: tidyverse (Wickham, 2017), scales (Wickham, 2016), lubridate (Garrett and Hadley, 2011) and for plotting we used ggplot2 (Wickham, 2009), grid (R Core Team, 2017) and cowplot (Wilke, 2016).

Results

Inter-annual and inter-specific variability in response to natural variation in ambient solar radiation

There was a substantial difference in *p* CA concentrations in pollen grains from the same individual tree samples in 2013 and 2014, and between *Pinus* spp. (Table 2 and Fig. 2 a). On average, pollen from 2014 contained 31% more *p* CA than pollen from the same trees in 2013. The 95% credible interval of the differences between the two years means did not cross zero, providing strong statistical support that *p* CA abundance was different between the years for the individuals of all species tested (*Pinus nigra, P. pinaster, P. sylvestris* and *P. uncinata*) (Fig. 2 b).

The measured solar radiation shows variation between the two collection years, particularly during the pollen development period, but not during the preceding growing season (Fig. 1). The total amount of solar radiation during the month (28 days) before pollen collection in May 2013 was 5214 W/m^2 compared to 6401 W/m^2 in 2014 and two months before was 9429 W/m^2 in 2013 and 12191 W/m^2 in 2014. Across both years, there was strong and consistent species-level variation, which can be partially accounted for by pollen size (Fig. 2 c, d). However, differences between species remained even after correcting for pollen size corrections, and *Pinus pinaster* still showed higher *p* CA ratios compared with the other species (Fig. 2 c, d).

Response to short-term experimental ambient solar radiation exclusion

We found a substantial difference in *p* CA between shaded and sun exposed *Pinus sylvestris* pollen grains (Table 3 and Fig. 3a, b). Shaded pollen produced 21% less *p* CA than the sun-exposed pollen, and the 95% credible interval for the differences of the two treatment means within each MCMC run did not cross zero, giving us strong statistical confidence in the observed difference between the two treatments (Fig. 3b). The individual trees show differences in how strongly they respond to the shading treatment; two trees barely have a reduction of *p* CA production whilst seven trees show a strong response to shading (Fig. 3c).

Discussion

The key finding in this paper is that *p-coumaric* acid in pollen grains responds to short-term changes in ambient solar radiation occurring in the last few-weeks to one-month prior to dehiscence. In the study investigating responses to natural variation in radiation between years, an average of 31% more *p* CA was produced in 2014 compared to 2013, with an equivalent difference in intensity of 29% in the equivalent pollen-development periods. In the ambient solar-radiation exclusion experiment the shade cloth was installed one month prior to pollen dehiscence (i.e., covering the pollen development period) and this resulted in a clear reduction (21%) in the amount of *p* CA. As the shading cloth treatment was conducted for individual branches within trees, this experiment also builds on previous preliminary evidence that this plastic response to short-term reduction in solar radiation can occur locally within the tree, at the cone bud level (Rozema et al., 2009). The inter-annual study was conducted across several *Pinus* species, and also suggests consistent species-specific differences above and beyond those explained by pollen-size variability alone.

These results support and enhance current understanding of pollen formation in *Pinus* spp. Although the pollen cone buds begin growing in August the previous year, the pollen grains within the bud start developing two to three months before dehiscence. During the last stages in pollen-grain development, the peritapetal membrane that contains sporopollenin (e.g. *p* CA) is produced and covers the exine (Dickinson and Bell, 1972, Rowley et al., 2000). The peritapetal membrane most likely plays an important role in production of*p* CA, and this final stage happens less than 11 days before flowering (Rowley et al., 2000). We find that there was no difference in incoming solar radiation in Geneva during the bud growing period of 2013 and 2014, but there was a large difference in solar radiation during the pollen development period, in both one month and two months prior to flowering (1187 and 2762 W/m^2, respectively, see Fig. 1). A short-term response within the pollen development period is thus the most likely way to explain the considerable difference in *p* CA between the two years.

Results from the inter-annual and inter-specific variability study also point to potentially important variation at the species level. For example, *Pinus pinaster* contained up to twice the amount of*p* CA compared with the other species. One obvious explanation for these differences is pollen size. In general, *P. pinaster* pollen grains are approximately two times larger in biovolume than the other species. Indeed, when we correct for this, using Beug (1961)'s reported average pollen size, the difference between species is reduced (Fig. 2 d). This effect is most obvious for *P. pinaster* and *P. nigra.* However, after size is corrected for some species-level variation still remains, hinting at other potential genetic differences resulting in differences in *p* CA production or in response to solar radiation. However, in this study we were limited by the number of individuals found within the Geneva botanic garden. More individuals of each of the different species tested here would be required for more robust inferences around species effects.

These findings have implications for the usage of *p* CA as a chemical proxy in palaeoecological reconstructions. First, our findings indicate that the production of *p* CA is a plastic, short-term response to the environment, which implies that pollen grains from a given tree sampled across multiple years should accurately reflect changes in the local solar radiation signal during the dehiscence season. Second, pollen-chemistry measurements taken from sediment cores will represent an integrated early-seasonal radiation flux across the range of years represented in a given sediment sample. Depending on sedimentation rate and temporal resolution, a typical sample from the pollen record represents anywhere between 5 and 20 years, and any between-year environmental variation is therefore averaged out, giving us confidence that we can observe long-term trends (e.g. Willis et al. (2011)). Finally, the potential for species-level variation observed in our study implies that in sediment cores sampled in an area with known occurrence of several species of pine may make chemical

reconstructions more complex. This is especially the case if these species vary greatly in pollen size (for example, in Spain *Pinus nigra, P. pinaster*and *P. sylvestris* have overlapping range areas (Debreczy et al., 2011). Given these complexities, we expect that applying size corrections to *p* CA ratios could improve the accuracy of the reconstruction of past solar radiation, especially if specific size-correction factors for a given site can be established.

One important consideration is that, in this study, we were unable to disentangle the possible effects of different parts of the incoming solar spectrum (e.g. UV-B, UV-A or PAR) on *p* CA production. For example, we were unable to obtain measurements for ground-based UV-B, UV-A or PAR intensities at the Geneva botanic garden, whilst the shading cloths in our solar-radiation exclusion experiment did not only filter UV-B radiation. Furthermore, temperature and humidity may have differed in the shading treatments in our exclusion experiment but our experimental design did not allow us to account for these potentially confounding factors.

However, despite these limitations, several lines of evidence suggest the most likely driver of the variations in *p* CA is variation in UV-B radiation. Firstly, experimental studies reveal increased sporopollenin-based *p* CA in response to increased UV-B radiation, also in experiments where PAR was kept constant (Blokker et al., 2006, Blokker et al., 2005, Rozema et al., 2001b). Second, increases in*p* CA have been observed across both latitudinal (Willis et al., 2011, Jardine et al., 2016) and elevational gradient studies (Watson et al., 2007, Lomax et al., 2012), but the relationship between UV-B and PAR is decoupled between elevation and latitude gradients (although in these broader-scale studies potential species-level effects remain difficult to disentangle). We suggest that further experimental studies, which focus on determining the quantitative dose-response relationship in *Pinus* pollen of *p* CA to different solar radiation wavelengths, are required.

In summary, we investigated the effect of short-term changes in ambient solar radiation on the production in UV-B absorbing phenolic acids (*p*- Coumaric acid; *p* CA) in *Pinus* spp. pollen, and also species-level variation. Our results in both studies show a plastic response in *p* CA production in *Pinus* spp. pollen due to short-term changes in early-season solar radiation. *p* CA content show strong species-level variability, largely reflecting differences in pollen size between species. Based on these underlying and species-specific plastic responses, we expect that the integrated signal obtained from pollen grains in sediment cores (i.e. pollen grains representative of multiple years) will be able to detect longer term changes of past solar radiation. Our findings thus support the usage of*p* CA as a solar radiation proxy in palaeoecological reconstructions as an indicator of seasonal changes in solar radiation, and suggests directions for how the method can be refined, e.g., by exploring in more detail the timing of the response, the exact spectra of solar irradiance that drive the response, and sources and consequences of species-level variation.

Acknowledgements

We thank Linn C. Krüger for assistance with pollen-picking, Professor Daniel Jeanmonod for permission to sample pollen from Conservatoire et Jardin botaniques, Ville de Genève and H. John B. Birks for comments. The daily-mean ambient solar radiation data and daily-mean pine pollen dehiscence data have been provided by MeteoSwiss, the Swiss Federal Office of Meteorology and Climatology. Funding for this project was provided by the Norwegian Research Council FRIMEDBIO programme to the PARASOL project (Project number 214359). AWRS was also supported by the Norwegian Research Council FRIPRO project PollChem (Project number 249844).

Conflict of interest

The authors have no competing interests to declare.

Authors contribution

KJW and VV developed the "PAlynological Reconstructions of pAst SOLar radiation" (PARASOL) project, AWRS, MJ, and VV conceived the research idea and experimental design for this study, AWRS and MJ conducted the experiment, AWRS collected pollen from Bergen, MJ collected pollen from Geneva, MJ carried out the lab work, AWRS and MJ analysed the data, JC contributed on the statistical analyses, MJ wrote

the paper, AWRS, KJW, and VV commented critically on drafts of the paper. All authors approved the final version.

Data availability statement

Data supporting the manuscript results were archived in the Dryad Digital Repository (https://doi.org/10.5061/dryad.7sqv9s4nv).

References

BELL, B. A., FLETCHER, W. J., RYAN, P., SEDDON, A. W. R., WOGELIUS, R. A. & ILMEN, R. 2018. UV-B-absorbing compounds in modern Cedrus atlantica pollen: The potential for a summer UV-B proxy for Northwest Africa. *Holocene,* 28**,** 1382-1394.

BEUG, H.-J. 1961. *Leitfaden der Pollenbestimmung für Mitteleuropa und angrenzende Gebiete,* Stuttgart, Gustav Fischer.

BLOKKER, P., BOELEN, P., BROEKMAN, R. & ROZEMA, J. 2006. The occurrence of p-coumaric acid and ferulic acid in fossil plant materials and their use as UV-proxy. *Plant Ecology,* 182**,** 197-207.

BLOKKER, P., YELOFF, D., BOELEN, P., BROEKMAN, R. A. & ROZEMA, J. 2005. 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. *Analytical Chemistry,* 77**,** 6026-6031.

DEBRECZY, Z., RÁCZ, I. & MUSIAL, K. 2011. *Conifers around the world : conifers of the temperate zones and adjacent regions : Vol. 1,*Budapest, Dendro Press.

DICKINSON, H. G. & BELL, P. R. 1972. The role of the tapetum in the formation of sporopollenin-containing structures during microsporogenesis in Pinus banksiana. *Planta,* 107**,**205-215.

ELLISON, A. M. 2004. Bayesian inference in ecology. *Ecology Letters,* 7**,** 509-520.

FRASER, W. T., SEPHTON, M. A., WATSON, J. S., SELF, S., LOMAX, B. H., JAMES, D. I., WELLMAN, C. H., CALLAGHAN, T. V. & BEERLING, D. J. 2011. UV-B absorbing pigments in spores: biochemical responses to shade in a high-latitude birch forest and implications for sporopollenin-based proxies of past environmental change. *Polar Research,*30**,** 6.

GARRETT, G. & HADLEY, W. 2011. Dates and Times Made Easy with lubridate. *Journal of Statistical Software,* 40**,** 1-25.

JARDINE, P. E., FRASER, W. T., LOMAX, B. H., SEPHTON, M. A., SHANAHAN, T. M., MILLER, C. S. & GOSLING, W. D. 2016. Pollen and spores as biological recorders of past ultraviolet irradiance. *Scientific Reports,* 6**,** 39269.

JUNGFERMANN, C., AHLERS, F., GROTE, M., GUBATZ, S., STEUERNAGEL, S., THOM, I., WETZELS, G. & WIERMANN, R. 1997. Solution of sporopollenin and reaggregation of a sporopollenin-like material: A new approach in the sporopollenin research. *Journal of Plant Physiology,*151**,** 513-519.

LOMAX, B. H., FRASER, W. T., HARRINGTON, G., BLACKMORE, S., SEPHTON, M. A. & HARRIS, N. B. W. 2012. A novel palaeoaltimetry proxy based on spore and pollen wall chemistry. *Earth and Planetary Science Letters,* 353**,** 22-28.

LOMAX, B. H., FRASER, W. T., SEPHTON, M. A., CALLAGHAN, T. V., SELF, S., HARFOOT, M., PYLE, J. A., WELLMAN, C. H. & BEERLING, D. J. 2008. Plant spore walls as a record of long-term changes in ultraviolet-B radiation.*Nature Geoscience,* 1**,** 592-596.

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

MAZZA, C. A., BOCCALANDRO, H. E., GIORDANO, C. V., BATTISTA, D., SCOPEL, A. L. & BALLARÉ, C. L. 2000. Functional Significance and Induction by Solar Radiation of Ultraviolet-Absorbing Sunscreens in Field-Grown Soybean Crops. *Plant Physiology,* 122**,** 117.

R CORE TEAM 2017. R: A language and environment for statistical computing Vienna, Austria: R Foundation for Statistical Computing.

ROWLEY, J. R., SKVARLA, J. J. & WALLES, B. 2000. Microsporogenesis inPinus sylvestris L. VIII. Tapetal and late pollen grain development.*Plant Systematics and Evolution,* 225**,** 201-224.

ROZEMA, J., BLOKKER, P., FUERTES, M. A. M. & BROEKMAN, R. 2009. UV-B absorbing compounds in present-day and fossil pollen, spores, cuticles, seed coats and wood: evaluation of a proxy for solar UV radiation.*Photochemical & Photobiological Sciences,* 8**,** 1233-1243.

ROZEMA, J., BROEKMAN, R. A., BLOKKER, P., MEIJKAMP, B. B., DE BAKKER, N., VAN DE STAAIJ, J., VAN BEEM, A., ARIESE, F. & KARS, S. M. 2001a. 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 and Photobiology B-Biology,*62**,** 108-117.

ROZEMA, J., NOORDIJK, A. J., BROEKMAN, R. A., VAN BEEM, A., MEIJKAMP, B. M., DE BAKKER, N. V. J., VAN DE STAAIJ, J. W. M., STROETENGA, M., BOHNCKE, S. J. P., KONERT, M., KARS, S., PEAT, H., SMITH, R. I. L. & CONVEY, P. 2001b. (Poly)phenolic compounds in pollen and spores of Antarctic plants as indicators of solar UV-B – A new proxy for the reconstruction of past solar UV-B? *Plant Ecology,* 154**,**9-26.

ROZEMA, J., VAN GEEL, B., BJORN, L. O., LEAN, J. & MADRONICH, S. 2002. Paleoclimate: Toward solving the UV puzzle. *Science,*296**,** 1621-1622.

RSTUDIO TEAM 2015. RStudio: Integrated Development for R. *RStudio, Inc., Boston, MA URL http://www.rstudio.com/.*

SEDDON, A. W. R., FESTI, D., ROBSON, T. M. & ZIMMERMANN, B. 2019. 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.*Photochemical & Photobiological Sciences,* 18**,** 275-294.

SEDDON, A. W. R., JOKERUD, M., BARTH, T., BIRKS, H. J. B., KRÜGER, L. C., VANDVIK, V. & WILLIS, K. J. 2017. Improved quantification of UV-B absorbing compounds in Pinus sylvestris L. pollen grains using an internal standard methodology. *Review of Palaeobotany and Palynology,* 247**,** 97-104.

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

WEHLING, K., NIESTER, C., BOON, J. J., WILLEMSE, M. T. M. & WIERMANN, R. 1989. p-Coumaric acid - a monomer in the sporopollenin skeleton.*Planta,* 179**,** 376-380.

WICKHAM, H. 2009. ggplot2 : elegant graphics for data analysis. New York: Springer.

WICKHAM, H. 2016. scales: Scale Functions for Visualization. *R package version 0.4.0,* https://CRAN.R-project.org/package=scales.

WICKHAM, H. 2017. tidyverse: Easily Install and Load the 'Tidyverse'. R package version 1.2.1. https://CRAN.R-project.org/package=tidyverse.

WILKE, C. O. 2016. cowplot: Streamlined Plot Theme and Plot Annotations for 'ggplot2'. *R package version 0.6.2,*https://CRAN.R-project.org/package=cowplot.

WILLIS, K. J., FEURDEAN, A., BIRKS, H. J. B., BJUNE, A. E., BREMAN, E., BROEKMAN, R., GRYTNES, J. A., NEW, M., SINGARAYER, J. S. & ROZEMA, J. 2011. Quantification of UV-B flux through time using UV-B-absorbing compounds contained in fossil Pinus sporopollenin. *New Phytologist,* 192**,** 553-560.

Table 1. Number of species and number of individuals sampled in 2013 and 2014.

Species	**Year**	**Individuals**	**Replicates**	**No. samples**
P. nigra	2013	3	3	9
P. pinaster	2013	1	3	3
P. sylvestris	2013	2	3	6
P. uncinata	2013	2	3	6
P. nigra	2014	3	3	9
P. pinaster	2014	1	3	3
P. sylvestris	2014	2	3	6
P. uncinata	2014	2	3	6
Total samples		**16**	**3**	**48**

Table 2. Posterior distribution estimates of mean, standard deviation and the 95% credible intervals in the two-year comparison of*p* -Coumaric acid in *Pinus* spp. pollen.

Year	**Mean**	**SD**	**CRI 95%**
2014 pollen	0,0135	0,0038	0,0083 - 0,0209
2013 pollen	0,0103	0,0029	0,0063 - 0,0158
Difference	0,0032	0,0013	0,0014 - 0,00583

Table 3. Posterior distribution estimates of mean, standard deviation and the 95% credible intervals in the shading experiment of*p* -Coumaric acid in *Pinus sylvestris* pollen.

Treatment	**Mean**	**SD**	**CRI 95%**
Sun exposed pollen	0,00668	0,00059	0,00559 - 0,00792
Shaded pollen	0,00531	0,00046	0,00445 - 0,00623
Difference	0,00138	0,00044	0,000588 - 0,0022

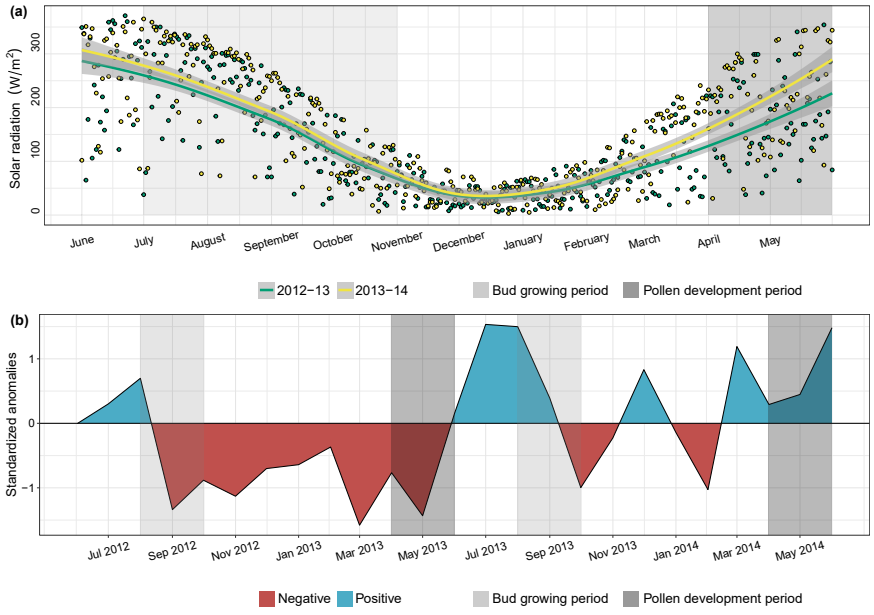
Figure captions

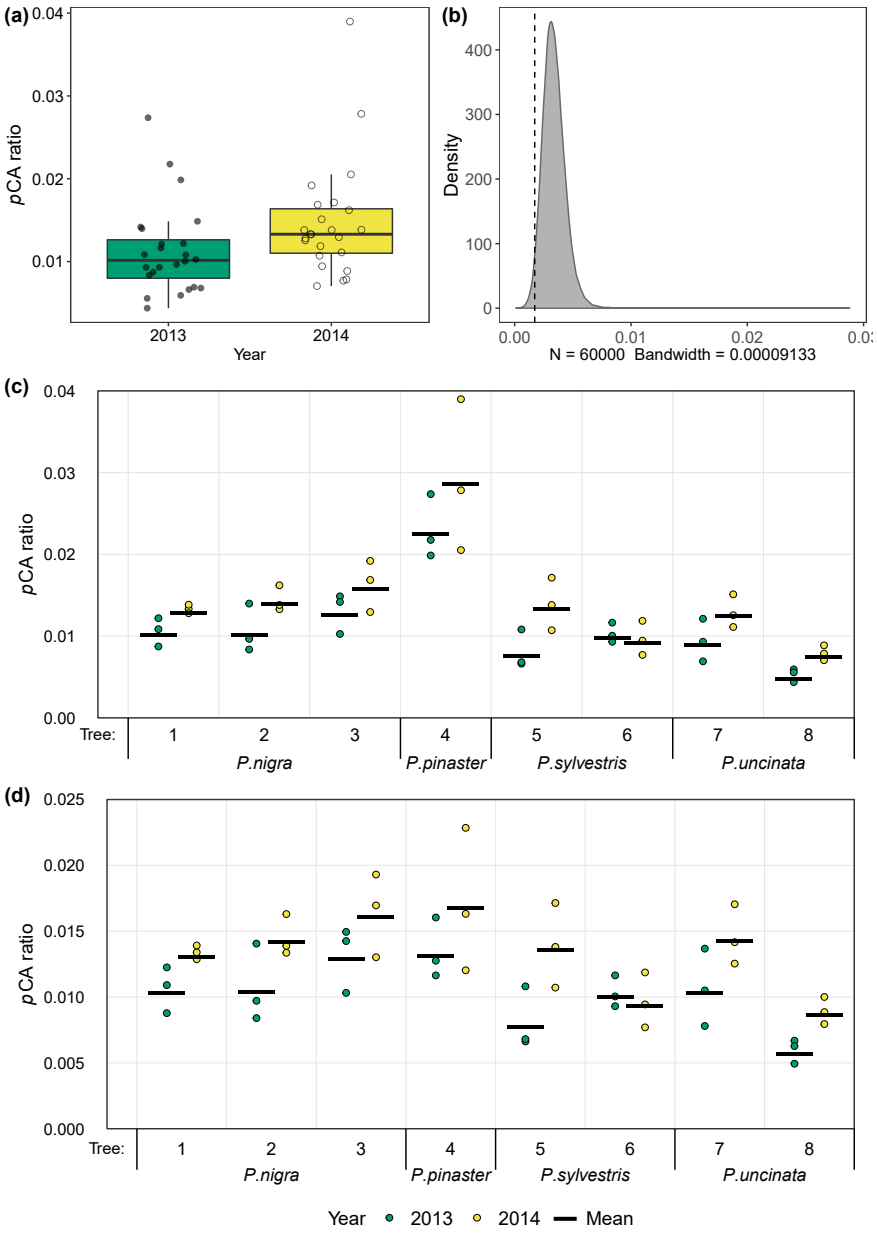
Fig. 1 (a) Daily solar radiation in Geneva, green line and circles is time period June 2012-2013 and yellow line and open circles is June 2013-2014, both are fitted with a loess smoothed line with a 95% confidence region. (b) Monthly standardised anomalies in Geneva, red area is negative and blue are positive anomalies. The pollen development period (dark grey area) occur two months before dehiscence (April-May) and growing season (light grey area) is the formation and growth period of male pollen cone buds (July-October).

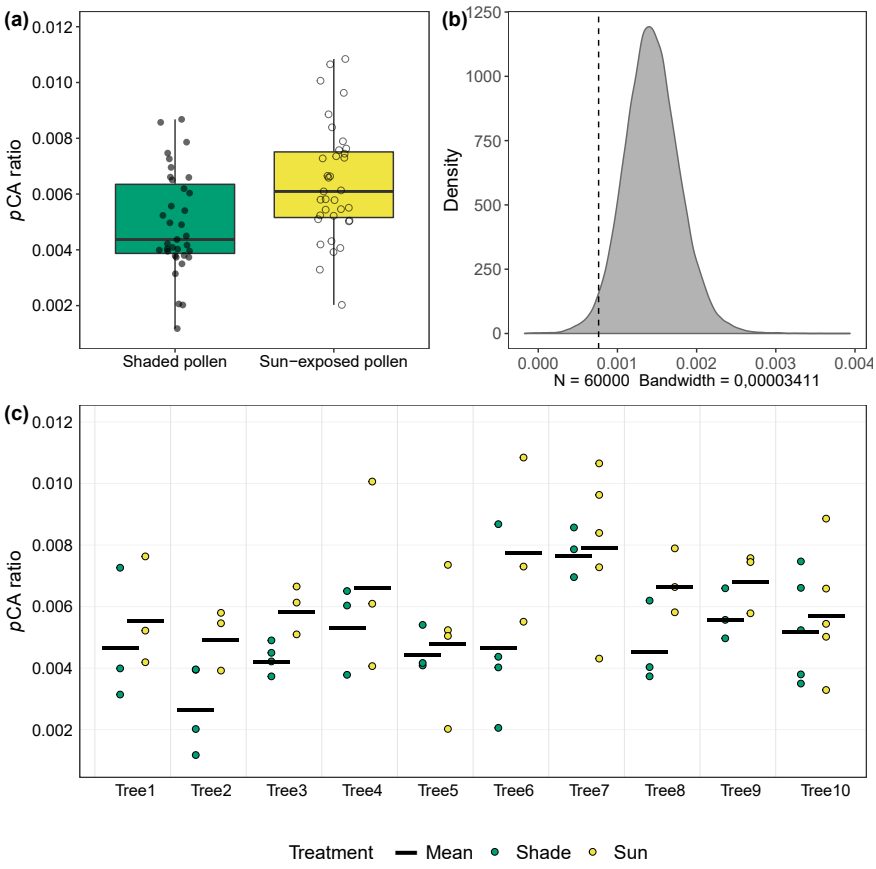
Fig. 2 (a) Boxplot of Pinus spp. pollen from 2013 (green) and 2014 (yellow). The box shows 1st and 3rd quartile, horizontal line within is the median and the whiskers show minimum and maximum values. The points represent samples of Pinus nigra, P. pinaster, P. sylvestris and P. uncinata analysed for p-Coumaric acid (pCA). (b) Posterior estimates of difference in pCA between years (grey area). When the 95% credible interval (black dotted line) does not overlap zero, the amount of pCA in the different years are credibly different. (c) Scatter plot of Pinus spp. showing the response of pCA production to different solar radiation in Geneva. (d) Scatter plot of Pinus spp. when pollen-grain size is accounted for, showing the response of

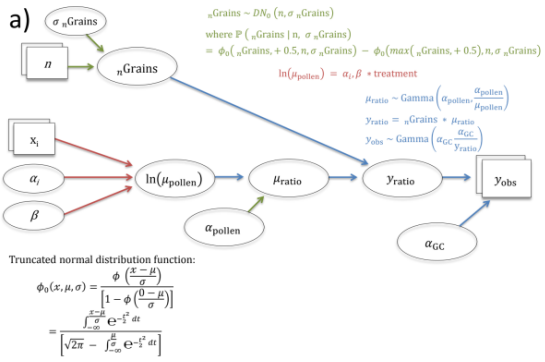
pCA production to different solar radiation in Geneva. Pinus spp. pollen in 2013 (green circles) and 2014 (yellow circles), black line is the mean for each species.

Fig. 3 (a) Boxplot of shaded (green) and sun-exposed (yellow) Pinus sylvestris pollen. The box shows 1st and 3rd quartile, horizontal line within is the median and the whiskers show minimum and maximum values, excluding two outliers. The points represent all samples analysed for p-Coumaric acid (pCA). (b) Posterior estimates of difference in sun exposure treatments (grey area). When the 95% credible interval (black dotted line) does not overlap zero, the amount of pCA in the different years are credibly different. (c) Scatterplot of individual trees showing the response of the shaded (green circles) and sun-exposed (yellow circles) Pinus sylvestris pollen, black line is the mean for each tree.



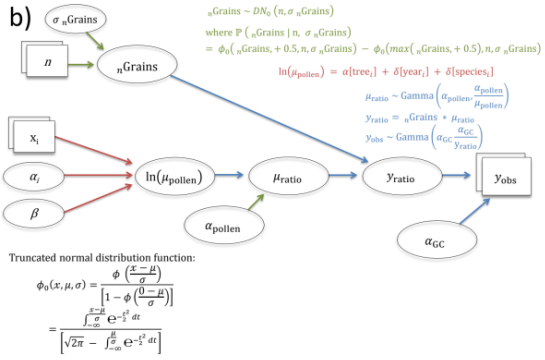






Truncated normal distribution function:

$$\phi_0(x, \mu, \sigma) = \frac{\phi\left(\frac{x-\mu}{\sigma}\right)}{\left[1 - \phi\left(\frac{0-\mu}{\sigma}\right)\right]} = \frac{\int_{-\infty}^{\frac{x-\mu}{\sigma}} e^{-\frac{t^2}{2}}\, dt}{\left[\sqrt{2\pi} - \int_{-\infty}^{\frac{0}{\sigma}} e^{-\frac{t^2}{2}}\, dt\right]}$$



Truncated normal distribution function:

$$\phi_0(x, \mu, \sigma) = \frac{\phi\left(\frac{x-\mu}{\sigma}\right)}{\left[1 - \phi\left(\frac{0-\mu}{\sigma}\right)\right]} = \frac{\int_{-\infty}^{\frac{x-\mu}{\sigma}} e^{-\frac{t^2}{2}}\, dt}{\left[\sqrt{2\pi} - \int_{-\infty}^{\frac{0}{\sigma}} e^{-\frac{t^2}{2}}\, dt\right]}$$

1