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Is Pollen Morphology of *Salix polaris* Affected by Enhanced UV-B Irradiation? Results from a Field Experiment in High Arctic Tundra

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Abstract

This study tested the hypothesis that the thickness of the pollen wall will increase in response to enhanced UV-B irradiation, by examining the effect of enhanced UV-B irradiance on the pollen morphology of *Salix polaris* Wahlenb. grown in a field experiment on the Arctic tundra of Svalbard. Measurements of pollen morphology were conducted by light microscopy on plants grown at two sites, Adventdalen and Isdammen. *Salix* vegetation was grown under control, enhanced UV-A, and two enhanced UV-B (simulating 15 and 30% reduction in the thickness of the stratospheric ozone layer) treatments. At the Adventdalen site, pollen wall thickness significantly increased under enhanced UV-A and UV-B treatments compared with the control. A thicker pollen wall helps to prevent damage by UV-B radiation of the DNA of the pollen. In contrast, plants at the Isdammen site did not exhibit any significant pollen morphological response to the enhanced UV treatments. The inconsistency in plant response to enhanced UV treatments between the two sites may be explained by greater habitat heterogeneity at the Isdammen site; abiotic soil conditions including nutrient and water availability may also have an influence on pollen morphology.

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Introduction

Early concerns about potential stratospheric ozone depletion (e.g. Johnston, 1971), and the first detection of a depleted Antarctic ozone layer by Farman et al. (1985) triggered a considerable body of research on the effects of UV-B radiation on plants (e.g. Caldwell et al., 1995). This research showed the reproductive parts of plants such as anthers and ovules are shielded from solar UV-B radiation (e.g. Flint and Caldwell, 1984). It has been known for a long time that the wall of pollen grains has a function of absorbing UV radiation, and protecting the genetic material held within the pollen grain from mutation (e.g. Stadler and Uber, 1942). The pollen wall is effective at screening out more than 80% of incident ultraviolet radiation (Tevini, 1993; Demchik and Day, 1996). Despite this, a considerable fraction of incident UV-B radiation (<20%) may be transmitted by the wall into the pollen cell (Demchik and Day, 1996). In most long-lived pollen grains, DNA is in a dehydrated state, which is particularly sensitive to UV-B irradiance (Musil, 1995). The pollen wall is composed of sporopollenin, a lignin-like compound containing phenolic acids (Rozema et al., 2001a, 2001b; Blokker et al., 2006). One of the functions of phenolics is the absorption of UV radiation, and a laboratory experiment by Rozema et al. (2001a) showed absorbance at 280–320 nm of the sporopollenin fraction in the wall of *Vicia faba* pollen grains significantly increased in response to enhanced UV-B irradiance (Table 1).

If the chemistry of the pollen wall has been observed to change under increased UV-B irradiance, then it would be expected that the pollen wall would also show visible, physical change (as a result of increased sporopollenin deposition by the tapetum during pollen development in the parent plant; p. 20, Rozema et al., 2001b), and experiments on soybean (*Glycine max*) by Koti et al. (2004) indicated that the ornamentation of the pollen exine (outer wall) was visibly affected by enhanced UV-B

irradiation, though this effect was not quantified. This study tests the hypothesis that the thickness of the pollen exine will increase in response to enhanced UV-B irradiation by examining the effect of enhanced UV-B irradiance on the pollen morphology of *Salix polaris* Wahlenb. grown in a long-term (2 year) field experiment on the High Arctic tundra of Svalbard. Arctic tundra is a relevant environment for such a study, as the severe climate may exacerbate the effects of enhanced UV-B irradiance (Rozema et al., 2006), and *S. polaris* is a relevant species to study as its male reproductive parts including anthers, thecae, and ripe pollen grains are exposed to sunlight. Female flowers of *Salix polaris* are wind pollinated, and the reduction of solar UV-B radiation by a thicker pollen wall and UV-B absorbing compounds may prevent damage to the genetic material contained in the pollen grain.

Most experiments examining the effects of UV-B have used indoor studies in growth chambers and greenhouses. These studies have shortcomings, including the simulation of unrealistically low levels of photosynthetically active radiation (PAR) and UV-B, in addition to the spectral composition of UV. In contrast, long-term outdoor field experiments in natural ecosystems offer a more realistic opportunity to answer ecological questions (Caldwell et al., 1994; Rozema et al., 1997). Polar regions in particular, may be affected by increased solar UV-B because of the depletion of stratospheric ozone over both the Antarctic and Arctic for long periods each year (http://jwocky.gsfc.nasa.gov/ozone/today_v8.html; <http://ozonewatch.gsfc.nasa.gov/>). Terrestrial Antarctic and Arctic plants appear to be well protected against increased levels of solar UV-B irradiance, since little or no damage resulting from enhanced UV-B has been detected in field experiments (Rozema et al. 2006).

In addition to the context of the direct effects of increased UV-B irradiance on terrestrial biota, the results of this study are also pertinent to the understanding of the past dynamics of both

the stratospheric ozone layer and the global climate system. Pollen exines are preserved extremely well in conditions of frost, waterlogging, or high acidity (Moore et al., 1991); soil cores and lake sediments in the Arctic and Antarctic therefore have the potential to be unique archives of past UV irradiance. Reconstructions of past UV-B irradiance from measurements of pollen morphology can answer questions relating to the existence of past ozone depletion over the poles, the causes of such ozone depletion, and the natural variation in stratospheric ozone and solar UV-B. Furthermore, solar UV-C radiation stimulates the formation of stratospheric ozone; solar activity therefore inversely correlates with surface UV-B irradiance (Lean, 2000). As absorption of solar UV radiation is the primary energy input to the stratosphere, knowledge of past variations in UV-B irradiance and the status of the stratospheric ozone layer may give clues as to the past relationship between solar variability and the Earth's climate (Rozema et al., 2002).

Methods

FIELD METHODS

The Adventdalen site is a flat valley floor on glaciofluvial and fluvial deposits with *Salix polaris* and the mosses *Polytrichum hyperboreum* and *Sanionia uncinata* as the dominant tundra plant species (Table 1). The Adventdalen site is ca. 5 m above the water level surface of the stream channel flowing through the center of the Advent valley (200 m distance). The site is characterized by moist open tundra vegetation on glaciofluvial and fluvial deposits, mainly sandur (Kristiansen and Sollid, 1987). The Isdammen site is located near to the Isdammen water reservoir of Longyearbyen on a mountain slope, ca. 30 m above sea level on marine deposits (Kristiansen and Sollid, 1987). The Isdammen site is drier and more exposed to wind than the Adventdalen site; and the soil at Isdammen has a lower organic matter content.

The tundra plants, e.g. *Salix polaris* and *Cassiope tetragona*, are small, and their shoots are rarely longer than 10–15 cm (Johnstone and Henry, 1997). We assumed the 50 × 50 cm plots to contain representative parts of the tundra vegetation. The spatial distribution of the UV lamp plots was initially chosen such that *Salix* and *Cassiope* (Isdammen) or *Salix* and *Polytrichum* (Adventdalen) were well represented, within the aim of a wider research project to assess the effects of enhanced UV-B radiation on the ecology and physiology of these tundra species. Treatment and control (see below) plots were randomized. Total vegetation cover of the UV lamp plots at Isdammen and Adventdalen was 80% and 65–85%, respectively.

In June 2002, 16 mini-UV lamp sets were installed over the tundra vegetation at both Isdammen and Adventdalen, each covering an area of 50 × 50 cm. At each site, four treatments were

applied (each containing four lamp sets): (1) control (C) with wooden bars replacing the fluorescent UV-B tubes; (2) (UV-A) where Mylar foil blocked UV-B and UV-C, but transmitted UV-A radiation emitted by the lamps; (3) (UVB₁) where cellulose acetate foil blocked UV-C radiation, transmitting UV-B (and UV-A) simulating 15% stratospheric ozone depletion; (4) (UVB₂) with a longer lamp burning time than UVB₁, simulating 30% stratospheric ozone depletion. Lamp spectra and biologically effective irradiances, in addition to details of the UV-B dosimetry and electronics of the lamp switch control system are reported by Boelen et al. (2006, pp. 147–149). In 2002 and 2003, the UV supplementation systems operated from mid June until late August–early September. At that time, small male and female flower buds for the next tundra spring were also observed to have formed. Ripe *S. polaris* male catkins were collected in late July–early August 2003, thus allowing exposure to the experimental treatments during the complete tundra spring and summer period.

LABORATORY METHODS

Pollen from *Salix* male catkins was dusted into glass vials and mounted in glycerin oil on glass microscope slides. At ×1250 magnification (estimate based on the specifications of the lenses), the dimensions of pollen in equatorial view (width, W; thickness of pollen wall on width axis, ω) were measured using an eyepiece fitted with a calibrated vernier scale. The dimensions measured are shown in Figure 1. The smallest measurable unit on the vernier scale was equivalent to 0.03 μm. Twenty-five pollen grains were measured in all replicates (with the exception of one replicate from the Isdammen site, where fifteen pollen grains were measured). Pollen width (W) was measured to enable the calculation of the ratio of pollen grain width:wall thickness along width axis (W:ω). It was expected that this ratio may be a better representation of morphological change than absolute values of wall thickness (ω, μm) as the recorded thickness of the pollen wall and the size of the pollen grain will vary with focal depth under the microscope.

STATISTICAL ANALYSES

Kolmogorov–Smirnov tests indicated that much of the data did not have a normal distribution (see below), and non-parametric tests (Kruskal-Wallis, Mann-Whitney U) were therefore used to determine whether enhanced UV had an effect on pollen morphology. Tests were applied using SPSS 11.0 software.

Results

For each pollen grain, two morphological parameters were calculated: (1) ω (μm); and (2) W:ω. The mean values of each morphological parameter measured are shown for each UV treatment at each site in Figures 2a and 2b. To determine whether the data were normally distributed, the Kolmogorov-Smirnov test was applied, and showed that the data from both the Adventdalen and Isdammen sites were not normally distributed ($p < 0.05$). The non-parametric Kruskal-Wallis test was therefore used to determine whether there was significant variation between treatments at each site. There were significant differences between UV treatments ($p = 0.038$) for ω at the Adventdalen site, and for the related ratio W:ω at both the Adventdalen and Isdammen sites ($p < 0.001$). In contrast, there were no significant differences between UV treatments for ω at the Isdammen site ($p = 0.181$).

For the parameters and sites which showed significant differences between treatments, the Mann-Whitney U test was

TABLE 1

Absorbance of acetolysis resistant residue (sporopollenin) obtained from pollen grains of *Vicia faba* exposed to 0 and enhanced UV-B radiation (full details in Rozema et al., 2001a). The absorbance of the residue in glycerol suspension was scanned from 280 to 320 nm and integrated; mean values of three replications with standard error of the mean. The effect of enhanced UV-B was significant at $p < 0.05$.

UV-B treatment (kJ m ⁻² day ⁻¹ UV-B _{BE})	UV-B absorbance (280–320 nm)	S.E.M
0	14.1	2.3
10.6	27.6	3.1

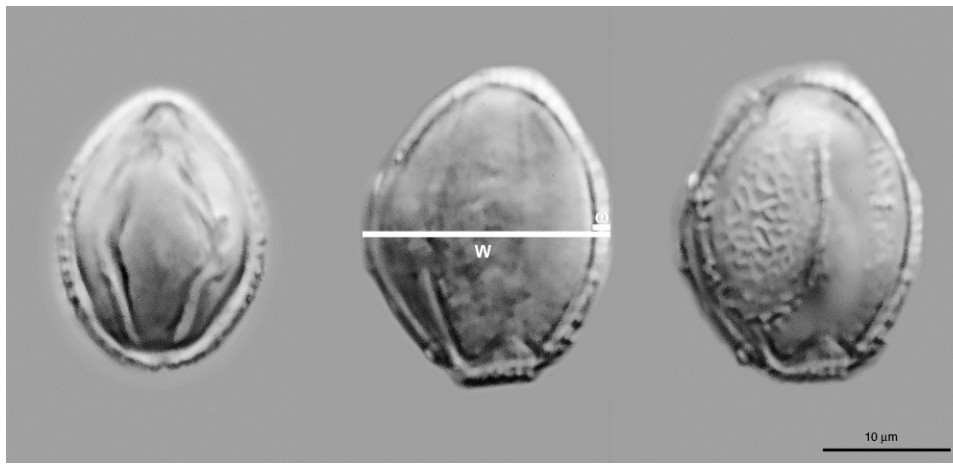


FIGURE 1. *Salix polaris* pollen grains (equatorial view). W, pollen grain width; ω , pollen grain wall thickness.

used to test for significant differences between the control and enhanced UV treatments (Fig. 2). Figure 2 shows that there are significant differences between the control and all three enhanced UV treatments for ω and $W:\omega$ at the Adventdalen site, with the UVA, UVB₁, and UVB₂ treatments all producing a mean increase of wall thickness $\sim 0.07 \mu\text{m}$ (6.4%). Figure 2 suggests that this is due to an increased thickness of the pollen wall in response to enhanced UV irradiation. There was also a significant difference in

$W:\omega$ when the UVB₁ treatment was applied at the Isdammen site. Figure 2 shows this is due to the UVB₁ treatment pollen having a reduced $W:\omega$, related to an increase in the thickness of the pollen wall. At the Adventdalen site, where there were significant differences between the control and all three enhanced UV treatments for ω and $W:\omega$, further Mann Whitney U tests showed no significant differences could be distinguished between the enhanced UV treatments for ω and $W:\omega$.

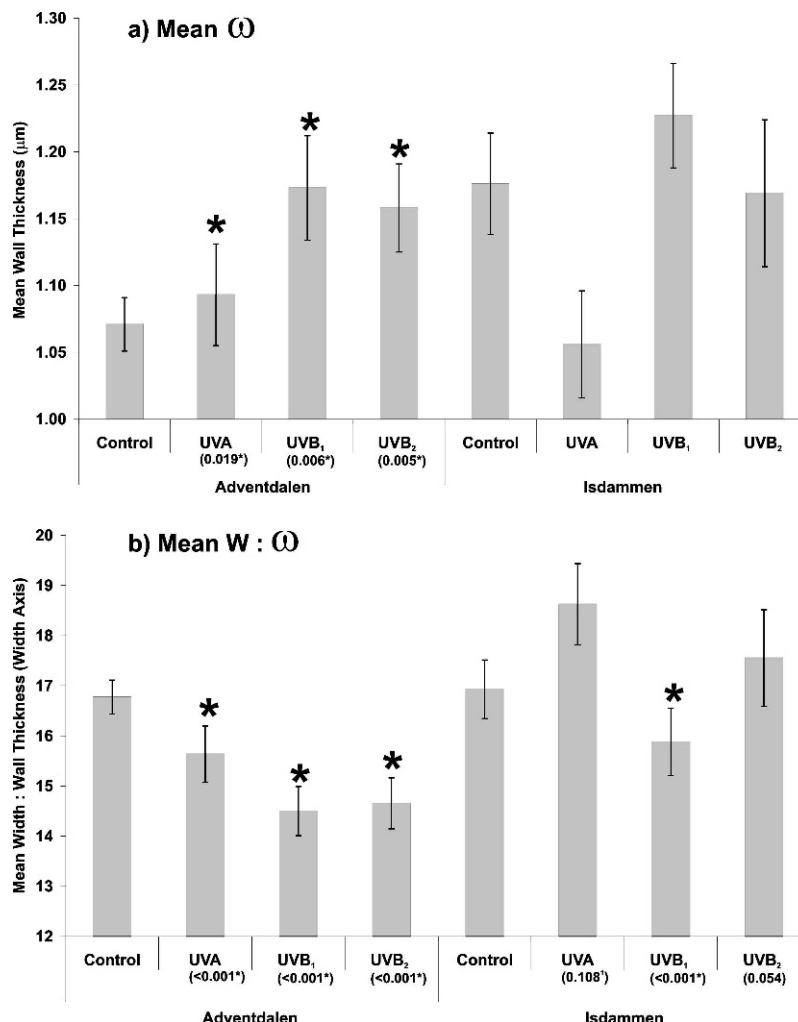


FIGURE 2. Mean measurement parameters for control and enhanced UV treatments for the two sites Adventdalen and Isdammen: (a) Mean wall thickness, ω (μm); (b) ratio of pollen grain width:wall thickness ($W:\omega$). Error bars ± 1 SE. Probabilities (p) of Mann-Whitney U tests of differences between the control and enhanced UV treatments are shown in brackets. There are significant differences between the control and enhanced UV treatment if $p < 0.050$, denoted by *. p calculated using a Monte Carlo 1-tailed test. $n = 100$ in all cases except ¹, where $n = 90$.

Discussion

At the Adventdalen site, there was a clear plant response to enhanced UV irradiance; with the UVA, UVB₁, and UVB₂ treatments all producing a mean increase of wall thickness $\sim 0.07 \mu\text{m}$ (6.4%). A thicker pollen cell wall will help to reduce penetration of solar UV-B radiation that may damage the DNA contained by the pollen grains. There was no discernible difference in ω and W: ω between the three UV treatments, probably owing to the precision of the measurement method (the smallest measurable unit was $0.03 \mu\text{m}$). The clear plant response to enhanced UV irradiance at the Adventdalen site is an important finding as it shows increased pollen wall thickness (in addition to an increased content of polyphenolic UV-B absorbing compounds) represents an effective defense against damage to the DNA in the pollen grains by solar UV-B. This contrasts with the earlier results of Rozema et al. (2001a), who examined *Vicia faba* pollen grown in a climatically controlled greenhouse experiment; light and scanning electron microscope observations indicated that the pollen wall thickness was not affected by increased UV-B irradiance ($10.6 \text{ kJ m}^{-2} \text{ day}^{-1}$). The results of Rozema et al. (2001a) were not quantified however, and the present study is the first attempt to quantify the morphological characteristics of pollen from plants grown under enhanced UV-B conditions.

Although the pollen morphology of *S. polaris* grown at Adventdalen showed a significant response to the UV treatments, the pollen morphology of plants at Isdammen did not exhibit any significant differences with the control (with the exception of W: ω for the UVB₁ treatment). To check that the order of measurement was the reason for the observed inconsistency in the plant response to enhanced UV irradiance, 10 slides were remeasured in random order (the 'observed' measurements), and the mean ω of each slide was compared to the original measurements (in non-random order, the 'expected' measurements). A χ^2 of 0.086 ($p = 0.9$, 9 d.f.) between the two sets of observations shows the order of measurement did not have a significant effect on the results, but there was a 90% probability that a deviation of $0.086 \mu\text{m}$ would occur in repeated measurements; giving an idea of the precision of the measurement method.

Could differences in the environmental conditions between the two sites be the cause of the inconsistency in the plant response to enhanced UV irradiance? The geography of the two sites differs: Adventdalen is situated on a flat valley floor with glaciofluvial and fluvial deposits, the soil is relatively moist and has a high organic content; in contrast, the drier Isdammen site is on a slope composed of marine deposits (Kristiansen and Sollid, 1987). These abiotic differences may be reflected in the vegetation composition of the two sites. Vegetation surveys conducted during the field experiment showed that Isdammen has a higher plant diversity than Adventdalen (15 species compared to 10 at Adventdalen), and possesses generally greater vegetation cover, with 80% cover compared to 65–85% at Adventdalen (Rozema et al., 2006). The vegetation surveys also suggested that the plant cover by individual species at the Adventdalen site was homogenous between plots, while at the Isdammen site, tundra plant species were heterogeneously distributed (Table 3 in Rozema et al., 2006).

How could habitat heterogeneity cause the inconsistency in the plant response to enhanced UV irradiance? Experiments on agricultural plants have shown that differences in local abiotic environment can result in the production of pollen with varying morphology. Temperature has been shown to affect both the thickness of the pollen grain wall (Kawecka, 1926) and the ornamentation of the exine (Porch and Jahn, 2001). Soil conditions have also been shown to affect pollen size. Mineral

nutrition (N, P) affects the pollen grain diameter and chemical composition of *Cucurbita pepo*, with pollen from plants treated with increased N showing an increase in diameter of $2 \mu\text{m}$ compared with the control (Lau and Stephenson, 1993, 1994). Water availability may also affect pollen morphology, and reduced pollen size resulting from dry soil conditions has been suggested by Stanley and Linskens (1974) to be related to a general depression of synthesis and growth during the onset of meiosis. Furthermore, Porch and Jahn (2001) demonstrated that water-stressed *Phaseolus* plants produced pollen lacking a cellulose intine (inner wall).

The results of this study suggest the effect of soil, and possibly other environmental conditions on pollen morphology of a number of taxa should be quantified before further work can be conducted on the effects of UV on pollen morphology. Furthermore, as the increase in pollen wall thickness at the Adventdalen site was indistinguishable between UV-A and UV-B treatments, further work needs to be conducted to assess the component of plant response due to UV-B alone. Once this has been achieved, dose-response relationships between solar UV-B and pollen wall thickness (ω or W: ω) may allow the development of a proxy of past UV conditions: the use of biological proxies to reconstruct UV-B irradiance may help to test and validate relations among solar irradiance, ozone, and climate change during past centuries (Rozema et al., 2002).

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