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Effects of Solar Ultraviolet Radiation at High Altitude on the Physiology and the Biochemistry of a Terricolous Lichen (*Cetraria islandica* (L.) Ach.)

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Abstract

The field experiment described, is based on the selective screening of UV-B and UV-A solar radiation by means of filters. After a 3 months exposure period to sunlight at an altitude of 2100 m, photosynthetic and respiratory activities, phenolic compound concentrations, and chlorophyll and carotenoid concentrations of *Cetraria islandica* thalli were analysed. The screening of the UV-B&A band provoked a 52% significant average decrease in 8 phenolic compound concentrations in the thalli of this lichen species, which can be explained by the screening out of the UV-B band (–53% in average, all 12 phenolic compounds analysed being affected). Screening of the UV-A band had no effect on average concentration of phenolic compounds. Chlorophyll and carotenoid concentrations as well as dark respiratory and gross photosynthetic (on a chlorophyll basis) rates were not significantly modified by screening of the UV-B&A band. Gross photosynthetic (on a chlorophyll basis) and dark respiratory rates were not significantly affected by the screening of the UV-B band. Because of questionable "control filter effects" on chlorophyll and carotenoid concentrations, which were

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probably due to an infection, it was not possible to differentiate between UV-B and UV-A band effects for these parameters. Unexpectedly, irrigation had no effects on the biochemistry and the physiology of this lichen.

In addition to discussing the fundamental interest of such an investigation, as well as the practical problems encountered in this field experiment, these results demonstrate the effect of UV-B radiation on the phenolic compound biosynthesis of a lichen species, and strengthen the hypothesis regarding the UV-B protective role of lichen aromatic substances. Since the screening of the UV-B&A band had no significant effects on photosynthetic pigments (chlorophylls and carotenoids), and gross photosynthetic (on a chlorophyll basis) and dark respiratory rates, this suggests that phenolic compounds in the thalli of *Cetraria islandica* displayed a considerable constitutive resistance to ultraviolet damage.

Keywords: Altitude, control filter, UV-B filter, UV-B&A filter, irrigation, photosynthesis, dark respiration, phenolic compounds, chlorophyll, carotenoid, photobiont, mycobiont, *Cetraria islandica*

Abbreviations

UV = ultraviolet; UV-C = radiation between 200 and 280 nm; UV-B = radiation between 280 and 320 nm; UV-A = radiation between 320 and 400 nm; PAR = photosynthetically active radiation between 400 and 700 nm; dw = dry weight; HPLC = high performance liquid chromatography; RI = retention index; SE = standard error; ns = no significant difference between the experimental group and the control group; AU = absorbance units.

1. Introduction

Due to the depletion of stratospheric ozone (Kerr, 1991, 1992, 1993), there is a potential for increased UV-B radiation to reach the earth (Blumthaler and Ambach, 1990).

Lichens have interesting features for testing UV responses. This particular plant group is common in high altitude ecosystems, has a slow growth rate and lacks a cuticule and epidermis. Lichens would therefore need to employ biochemical resistance strategies if they are to protect themselves against the high solar fluxes prevailing at high altitudes. Since cryptogams play a major role in terrestrial ecosystems at high latitudes (Callaghan et al., 1992), studies of these plants would be of particular importance when considering arctic and sub-arctic ecosystems and their dynamics related to climate change (Sonesson et al., 1995).

Some laboratory experiments have demonstrated that the concentrations of phenolic compounds in lichens increases with increasing light intensities

(Rundel, 1969; Fahselt, 1981; Stephenson and Rundel, 1979; Mateos et al., 1993). The two most common lichen substances, usnic acid and parietine, have a strong UV absorbance (Rundel, 1969; Hill and Woolhouse, 1966). Pigmentation could protect the photobiont against UV radiation, particularly for high mountain lichens (Kappen, 1973). In the same way, some researchers (Anon, 1991; Galloway, 1992; Quilhot et al., 1994) believe that lichens are a potentially useful tool in monitoring the long-term effects of ozone thinning. Experimental data regarding the protective screening role of these phenolic compounds against UV-B radiation is rare and speculative, based on both field observations (Heide-Jorgenson and Johnsen, 1995; Johnsen and Heide-Jorgenson, 1995) and laboratory experimentation (Sonesson et al., 1995).

With respects to UV-B damage, existing data describing the effect of light on the concentration of photosynthetically active pigments (chlorophyll a and b and carotenoids) is restricted to the effect of the visible part of the solar spectrum (Czeczuga, 1981). Additionally, in lichens, nothing is known of UV-B effects on physiological parameters such as photosynthesis and respiration.

The field experiment presented in this paper was undertaken as a means of contributing to our understanding of the sensitivity of a mountain terricolous lichen (*Cetraria islandica*) to UV-B and UV-A radiation, under realistic ecological conditions. A novel UV selective screening system was used under natural light to provide different solar UV regimes. *Cetraria islandica* has a fruticose shrub-like thallus with flat lobes and was chosen as a model because of its wide distribution in terms of its elevational range as well as its variable pigmentation with altitude.

2. Material and Methods

Collection and transplantation of lichen mats

Cetraria islandica mats (20 × 20 × 5 cm) were collected from a low altitude site (Pellafol, Isère, France, 1200 m altitude, north side exposure). Five lichen mats were selected based on homogeneity of appearance and were collected on the same day, thereby making the assumption that they were similar from a biochemical and physiological point of view. After rinsing the lichen mats with water they were placed on a screen (a wooden frame fitted with a plastic netting of narrow-mesh size), and held in place by plastic netting of wide-mesh size. These samples were transplanted to the experimental site (Lautaret Pass, Hautes-Alpes, France, 45°02 N) at 2058 m altitude and on a south side exposure.

Growth conditions

It has been shown that in the dessicated state, both green algal and cyanobacterial lichens are unaffected by high light intensities (Adams III et al., 1993). Lichen mats were thus watered three times a day (at 5, 13 and 21h00 Universal Time = solar time at the Greenwich Meridian) for 5 minutes at each time, throughout the duration of the experiment (17/06 to 26/09/95). Each irrigation period allowed lichen mats to reach 130% (on a dw basis) thallus water content (maximum thallus water content for this lichen species is 160%: Bachereau, 1993). Measurements of thallus water content have shown that the screening plates used in this experiment acted as a physical barrier against rainfall and even water vapor (Bachereau, unpublished data). Thus, all plant groups were in non-limiting standardized hydrous conditions with a minimally physiologically-active period each day, the exception being the "south side ni (no irrigation)" group which acted as a control to assess the effect of irrigation. Wind-breaks were set up in order to protect plants from dessication and high winds.

Experimental set-up

The experimental system of a series of selective exclusions was set up in the Lautaret Pass Alpine Garden. It used natural light, and was placed at an high altitude, allowing for samples to be exposed to a high, but natural UV level (a 30% increase as compared to sea level irradiance).

All groups were exposed to a south side exposure. The selective screening of UV-B and UV-A was achieved by the use of two selective test filters: a UV-B&A filter (plexiglass PVCG, supplied by "Air & Eau Systèmes", Ludres, France) and a UV-B filter (window glass supplied by Castorama, St Martin d'Hères, France). In addition, a control filter (+UV-B&A) (plexiglass GS2458 supplied by "Air & Eau Systèmes", Ludres, France) with high UV transmittance permitted us to detect any microclimatic induced variation caused by the plate itself (control filter effect). The dimensions of all three plates were $1000 \times 1000 \times 3$ mm.

In total, 3 separate artificial ecological situations were created which were combined in pairs (see Table 1) – one experimental group where the radiation was screened (test filter, low UV level) and a control group where the radiation was not screened (control filter, high UV level), permitting us to analyse the screening effect of 3 spectral bands (UV-B&A, UV-B and UV-A). UV transmittance of each plate was analysed by spectrophotometry (HITACHI U2000). Fig. 1 shows the Visible and UV transmission spectra of each filter. Concerning the UV-B&A band, the test filter (UV-B&A filter)

had a 0% UV-B transmission and a 0.25% UV-A transmission as compared to the control group (+UV-B&A). Concerning the UV-B band, the test filter (UV-B filter) had a 1.8% UV-B transmission and a 78% UV-A transmission as compared to the control group (+UV-B&A). This 22% UV-A absorbance was considered as insignificant. Concerning the UV-A band, the test filter (UV-B&A filter) had a 0% UV-B transmission and a 0.32% UV-A transmission as compared to the control group (UV-B filter). UV-B&A filter presents a slightly lower Visible transmittance (around 600 nm) as compared to both other filters, but this difference was considered as insignificant since Visible light intensities were not a limiting factor in our experimental conditions.

In addition, two other groups were added, one without a plate (the "south side" treatment) acting as a control group to study a possible "control filter effect", the other without a plate and without an irrigation treatment (the "south side ni" treatment) to study the significance of irrigation on lichen responses. Fig. 2 shows the position and orientation of the screening plate position above lichen mats.

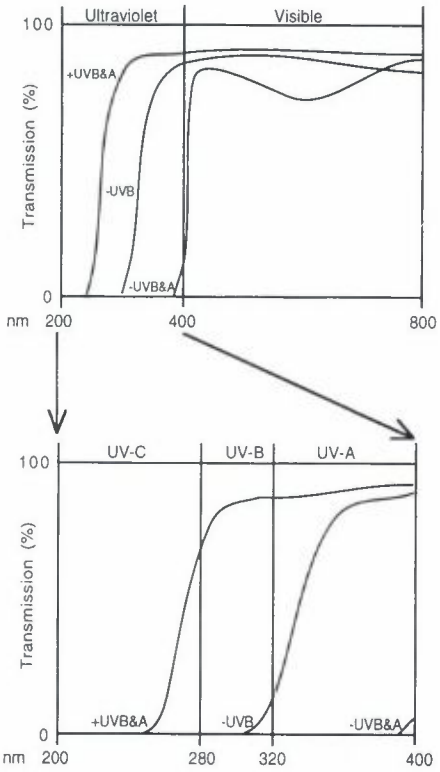

Figure 1. Transmission spectra of control filter (+UVB&A), UVB&A filter (–UVB&A) and UVB filter (–UVB).

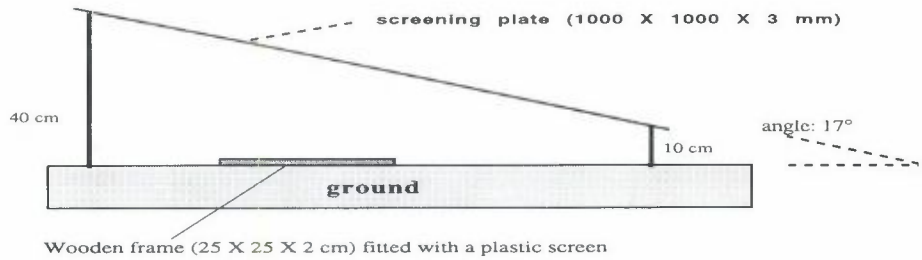

Figure 2. Side-view of positioning of screening plate over lichen mats.

Table 1. Calculation of irrigation and control filter effects and the screening effect of each spectral band studied.

Results under each ecological situation for x factor (numerical example)					
	n.i.: No irrigat.	With irrigation treatment			
Groups	1:n.i.South side	2: South side	3: Control filter	4: UV-B&A filter	5: UV-B filter
x factor value	100	100	100	150	125

Irrigation effect, control filter effect and the screening effect of each spectral band studied, as a % of the control group[1]					
Effects studied	Irrigation	Control filter	–UV-B&A	–UV-B	–UV-A
Initial calc.	((2–1)/1)*100	((3–2)/2)*100	a: ((4–3)/3)*100	b: ((5–3)/3)*100	a–b
x factor effect	0	0	50	25	25
Modified calc.[2]	((2–1)/1)*100	((3–2)/2)*100	a: ((4–2)/2)*100	b: ((5–2)/2)*100	a–b
x factor effect	0	0	50	25	25

[1]Except for UV-A effect which was the difference between UV-B&A effect and UV-B effect; [2]for UV-B&A and UV-B effects; control group n° 3 was replaced by n° 2.

By working under natural light and using selective filters, visible light levels were not modified, thereby avoiding an overestimation of UV effects through the limitation of photoreactivation mechanisms, a common side-effect in green-house and laboratory experiments. Equally, discriminating between UV-B and UV-A bands avoids an overestimation of UV-B effects by including possible UV-A effects.

Microclimatic measurements were undertaken to check for any differences between the control group (no screening) and the experimental group (screening), for each of the spectral bands studied. The results showed that amongst the 3 types of plates used, for any given experimental/control pairs, the differential temperature (average of continuous recording for a 3 months period) did not exceed 0.2°C, and the differential visible light (400–800 nm) level did not go beyond 10% transmittance. When comparing microclimatic conditions between treatments with plates and treatments without plates, the average greenhouse effect developing under the plates did not exceed 0.5°C, and the minimum (average between 800 and 400 nm) visible light transmittance was 80%.

After a 3 months treatment period (101 days), samples were collected, dried in the dark at 20°C for 72 hours, and then stored in a freezer at –30°C.

Control filter and UV-B filter groups were suspected to be infected by a pathogenic organism as indicated by low values for chlorophyll and carotenoid concentrations and the unusual net photosynthetic curves obtained. Fig. 3 reveals a very shallow slope for the first photosynthetic slopes (P1) in these two groups, indicative of poor photosynthetic rates. It was not possible therefore to discriminate between UV-B and UV-A screening effects on chlorophyll and carotenoid concentrations. Moreover, the original control group (under the control filter) paired with the two UV filters had to be replaced by the "south side" group. However, since climatic conditions have been shown to be very similar with or without a plate, it seems reasonable to use "south side" as a control for the two UV filters. This hypothesis is consistent with our results for the growth of *Pisum sativum* (Bachereau and Asta, 1997), which was studied under identical experimental regimes: there was no significant "control filter" effect observed on the total dw growth of this species after a 30 days treatment period.

Table 1 shows the methods used for the calculation of the effects of irrigation, control filter and screening of each spectral band. By contrast to other effects, the calculation of UV-A screening effects is not the difference between the experimental group and the control group, expressed as a % of the control group. As a matter of fact, this method would lead to a mathematical artefact. Thus, this UV-A effect is simply the difference between UV-B&A screening effect and UV-B screening effect.

Dry weight determination

Water loss of samples was measured after samples were kept for 30 minutes in a oven set at 105°C (Bachereau, 1993).

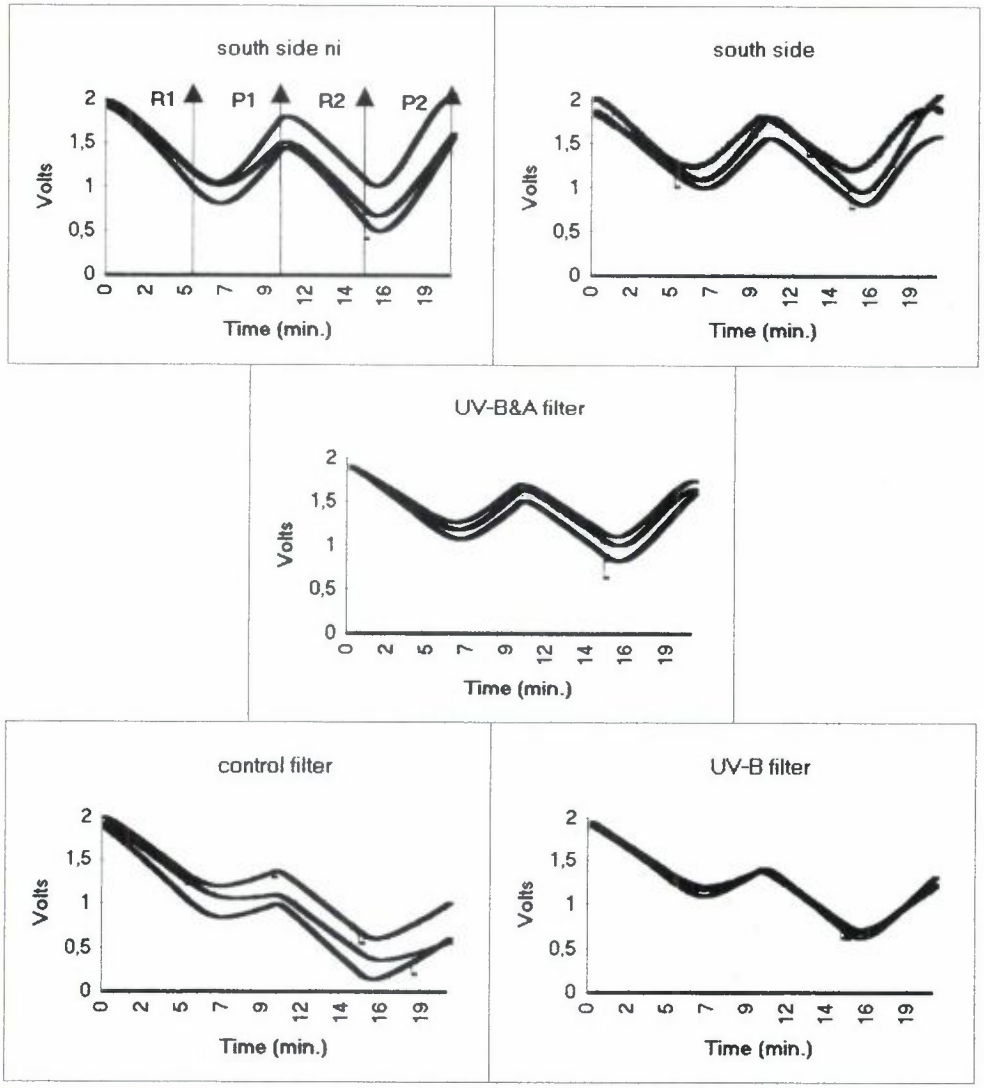

Figure 3. Net photosynthetic (P1&2) and dark respiratory (R1&2) curves of *Cetraria islandica* for the five different treatments, after a 3 months treatment period. Three replicates curves were obtained per group using a Clark-type oxygen electrode as illustrated above. Ordinate: 1 Volt = 145 nmoles O_2.

Laboratory analytical tests

Physiological and biochemical analyses were successively completed on the same sample in the following order: photosynthetic and respiratory activities,

phenolic compound extractions and chlorophyll and carotenoid extractions. Between each analysis, samples were dried and stored in a freezer at –30°C.

It has been found that the apical 10 mm regions of *Cladonia stellaris* and *C. rangiferina* thalli can account for approximately 50% of their photosynthetic capabilities (Moser et al., 1981). A gradual reduction in photosynthetic and respiratory rates (Carstairs and Oechel, 1978; Nash et al., 1980), and phenolic compounds concentrations (Fedoseev and Yakimov, 1960; Golojuch and Lawrey, 1988) has also been measured from the tips to the bases of lichen thalli. In view of these findings, physiological and biochemical analyses were therefore limited to the tips (first 15 mm) of the thalli of *Cetraria islandica*.

Photosynthetic and respiratory activities

100 mg dw of fragmented thallus (1–2 mm) was pre-hydrated in 10 ml deionised water, for 10 minutes, at 20°C, in the dark. The aim of this step was to allow the lichen to recover from a period of inactivity as well as for the thalli to become fully hydrated, thereby avoiding modification of the analysis medium volume by thallus water uptake.

Once fully hydrated the thalli were placed in the analysis medium: 1 ml deionised water (pH = 5.5) at 20°C, with continuous automatic stirring. Before use, fresh deionised water was allowed to equilibrate with room temperature and ambiant CO_2 level for at least 30 minutes with the aid of a magnetic stirrer. The subsequent cycle of analysis lasted 20 minutes and was divided into 4 stages (5 minutes each): dark/light/dark/light period. The first cycle dark/light was repeated once to be sure that figures were obtained on physiologically active lichens. Thus, for each sample (3 independent samples/group) analysed, the figures for respiration and photosynthesis were the mean of 2 replicates. Dark respiratory rates were estimated by oxygen uptake during the dark periods. The net photosynthetic response was analysed during light periods, under high light intensities, with irradiance (light source: halogenous lamp, 150W/15V, FORT, model GLI154) levels of 3600 µmoles PAR/m^2/s reaching the lichen thalli. Irradiance was measured (with an Hansatech QTP1 probe sensor) directly into the analysis medium. Evolution of oxygen in the medium was recorded with a Clark-type oxygen electrode (Hansatech DW1) connected to an O_2 control box (Hansatech CB1D) and recording software (Hansatech Minirec software and IF2 interface card). Gross photosynthesis (real photosynthesis) was obtained by adding measured dark respiratory and net photosynthetic responses. Then, gross photosynthesis was expressed on a chlorophyll basis.

HPLC analysis

Phenolic compound extraction

The procedure used was adapted from Feige et al. (1993). Fragmented thalli (1–2 mm) were soaked in 100% acetone (50 mg dw/ml), in the dark, for 15 minutes, with continuous stirring. The colourless extract was then filtered through a 0.2 µm membrane. This procedure allowed for enough phenolic compounds to be extracted for their subsequent quantification, while avoiding chlorophyll and carotenoid extractions.

Chromatographic conditions

RP HPLC (Reversed Phase High Performance Liquid Chromatography) with gradient elution: Instruments: Dionex 4500i (eluant degas module and gradient pump), Shimadzu SIL-9A (Autoinjector), Shimadzu SPD 6A (UV detector), Shimadzu Workstation Class CR10 (software); column: Spherisorb 5 µm ODS 2, 250 × 4.6 mm, at ambient temperature; mobile phase: A/Ultra-pure water with 1% Orthophosphoric acid; B/Methanol (HPLC grade); gradient profile: linear in 44 min., 30% B to 100% B, then 100% B in isocraty for 18 min; flow rate: 0.7 ml/minute; sample size: 150 µl; UV detection set at 254 nm; analysis time: 62 minutes; internal standards: benzoic acid and dioctylphthalate. RI = ((Rt of peak − Rt of benzoic acid)/Rt of dioctylphthalate) × 100.

Chlorophyll and carotenoid extractions

The extraction procedure of pigments used was adapted from Barnes et al. (1992). Pigments were extracted with pure DMSO (dimethylsulfoxyde) (25 mg dw/ml DMSO) at 35°C, for 2 hours, in the dark, without stirring. Concentrations were analysed by multiwavelengths spectrophotometry (in nm): 750 (turbidity), 664.9 and 648.2 (Chls a&b) and 460 (Carotenoids).

Statistical methodology

There were 3–4 independent replicates of each sample group. The results were expressed as the percentage of variation in the experimental groups as compared to the control groups. The difference between both groups, for each ecological factor studied, was analysed with the non parametric Mann-Whitney test ($p < 0.05$).

3. Results

Phenolic compound concentrations

The thalli of *Cetraria islandica*, exposed to direct sunlight (south side ni, south side and control filter groups), demonstrated a visible increase in their pigmentation. They were brown-green before transplantation, turning brown-

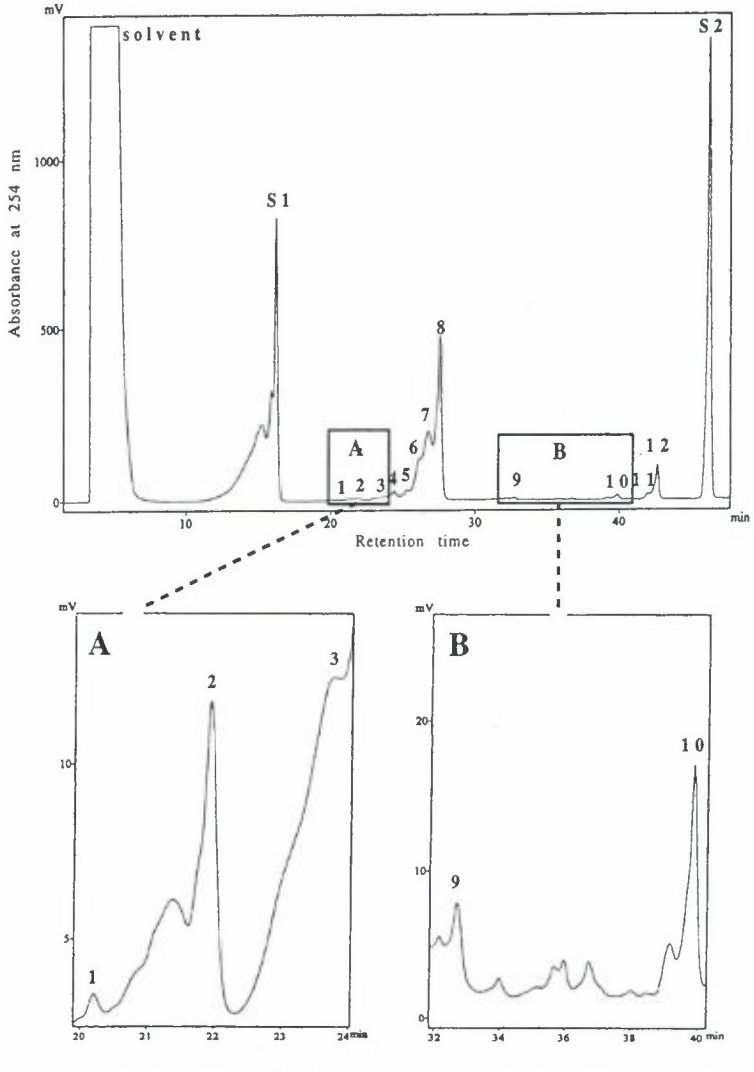

Figure 4. HPLC chromatogram of *Cetraria islandica* acetone extract. Internal standards: benzoic acid (S1), dioctylphthalate (S2). 12 phenolic compounds were separated (n° 1 to 12). Ordinate: 1 Volt = 1.25 AU at 254 nm.

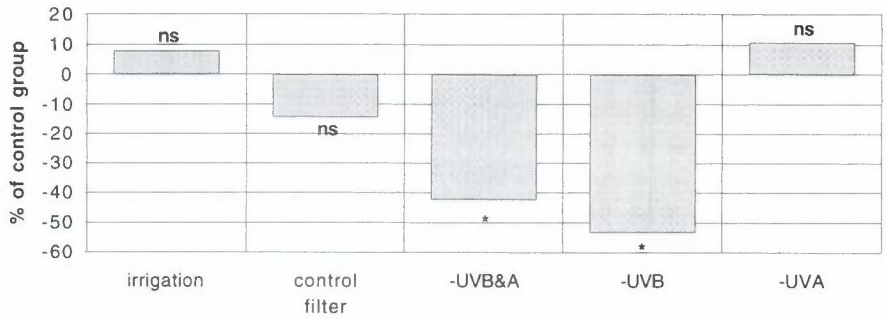

Figure 5. Average effect of irrigation, control filter and screening of each spectral band studied, as a % of the control group, on phenolic compound concentrations (n =12) of *Cetraria islandica*, after a 3 months treatment period. *: $p<0.05$.

black after a three months exposure period. Thalli under UV filters (UV-B&A filter and UV-B filter), on the other hand, retained their brown-green colour throughout the experimental period.

The HPLC protocol used has permitted us to separate 12 phenolic compounds (Fig. 4). Seven aromatic compounds having already been isolated in this species (Culberson, 1969, 1970; Culberson et al., 1977). The 12 phenolic compounds analysed have not yet been identified. RI values are given in Table 2. Fig. 5 illustrates the mean effect of each experimental treatment on all 12 phenolic compound concentrations. Fig. 6 illustrates the effect of each experimental treatment for each of the 12 phenolic compounds analysed.

The average concentration (3 replicates) of all of the phenolic compounds (sum of the height of all 12 peaks) measured for all 5 treatments was 175 AU (at 254 nm)/g dw (total content of the corresponding 20 ml extraction volume). SE express as a % of the mean ranged from 3 to 13. After the 3 months treatment, UV-B&A band screening provoked a statistically significant 43% average decrease in phenolic compound concentration. This effect is significant

Table 2. Mean (18 replicates) and SE (expressed as a % of the mean value) of RI value for each of the 12 phenolic compounds analysed by HPLC in *Cetraria islandica*, with benzoic acid and dioctylphthalate as standards.

Phenolic compound n°	1	2	3	4	5	6	7	8	9	10	11	12
Mean RI	8.6	12.3	16.3	17.7	19.7	21.8	23.1	24.5	35.7	51.0	55.6	57.1
SE	0.22	0.27	0.34	0.23	0.17	0.35	0.32	0.20	0.16	0.08	0.12	0.05

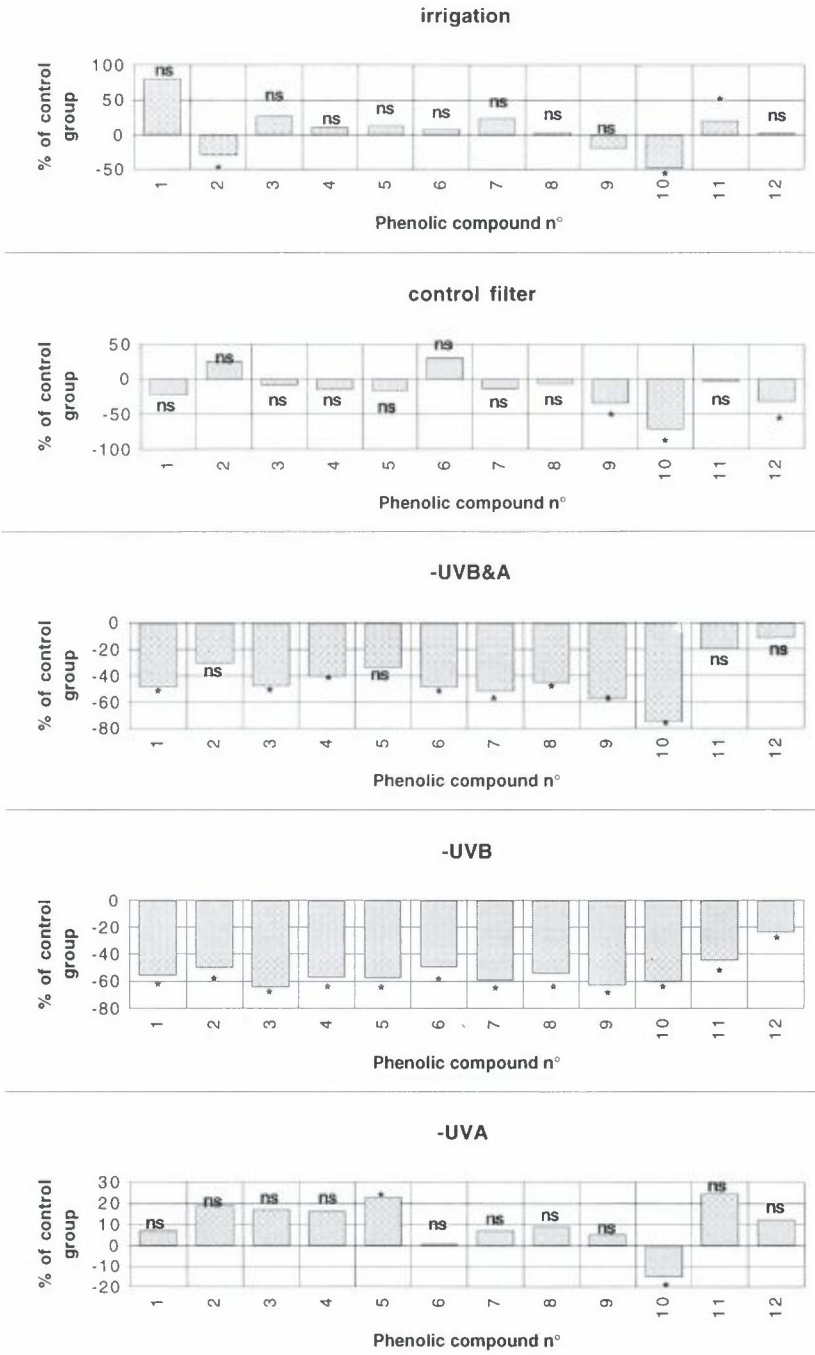

Figure 6. Effect of irrigation, control filter and screening of each spectral band studied, as a % of the control group, on the concentration of each phenolic compound analyzed in *Cetraria islandica*, after a 3 months treatment period. *: $p<0.05$.

for 8 compounds (n°'s 1, 3, 4, 6, 7, 8, 9 and 10). Where a significance level of 5% is imposed, the average decrease was 52% with a range from –40% to –75%. Screening out of the UV-B band induced a 53% average decrease in phenolic compound concentrations, all 12 compounds being significantly affected (–24% to –64%).

Irrigation, UV-A band screening, as well as the control filter treatment, had no significant effects on the average concentration of the combined concentrations of all phenolic compounds. A detailed analysis, however, reveals that individual compounds were sensitive to different treatments: 3 compounds (n°'s 2, 10 and 11) being sensitive to the irrigation treatment (–29, –48 and +21%, respectively), 2 compounds (n°'s 5 and 10) being sensitive to UV-A band screening (+23% and –15% respectively), and 3 compounds (n°'s 9, 10 and 12) being sensitive to the control filter treatment (–35%, –71% and –33%, respectively).

Chlorophyll concentrations (Fig. 7)

The average concentration (4 replicates) of chlorophyll a+b measured for all treatments was 1308 µg/g dw (1459 µg/g dw if control group was not included). SE express the % of the mean ranged from 3 to 5. The chlorophyll a/b ratio was not modified by any of the treatments. We observed, however, the presence of a significant "control filter effect" (–26% for chl "b" and –47% for chl "a"). Screening of the UV-B&A band as well as the irrigation treatment had no significant effects on chlorophyll concentrations.

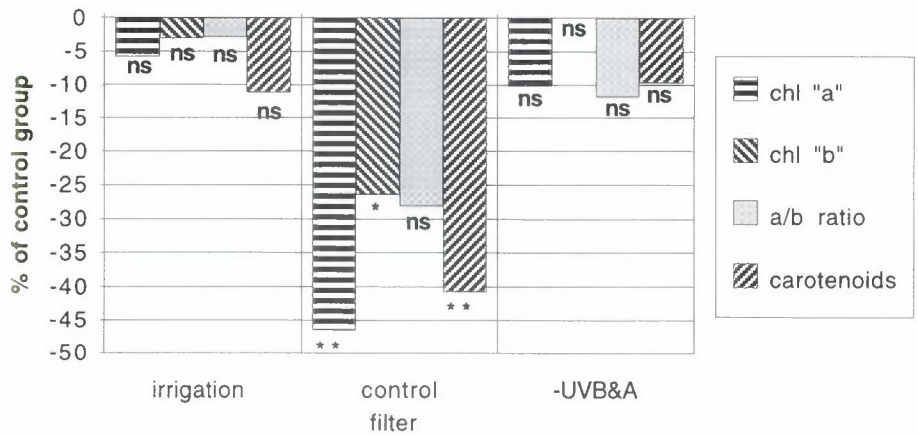

Figure 7. Effect of irrigation, control filter and screening of the UV-B&A band, as a % of the control group, on the chlorophyll and carotenoid concentrations of *Cetraria islandica*, after a 3 months treatment period. **: $p<0.02$; *: $p<0.04$.

Carotenoid concentrations (Fig. 7)

The mean carotenoid concentration (4 replicates) measured was 93 AU (at 460 nm)/g dw (104 AU (at 460 nm)/g dw if control group was not included). SE express the % of the mean ranged from 2 to 10. Here again, we observed the presence of a significant "control filter effect" (–41%). The screening of the UV-B&A band as well as the irrigation treatment had no significant effect on carotenoid concentrations.

Respiratory and photosynthetic rates (Fig. 8)

The average dark respiration (3 replicates) measured was 207 nmoles O_2/min/g dw. SE express as a % of the mean ranged from 3 to 10. Respiratory activities were not significantly modified by any of the treatments.

The average gross photosynthesis (3 replicates) measured was 314 nmoles O_2/min/mg total Chls. SE express the % of the mean ranged from 4 to 19. Photosynthetic activities were not significantly modified by any of the treatments.

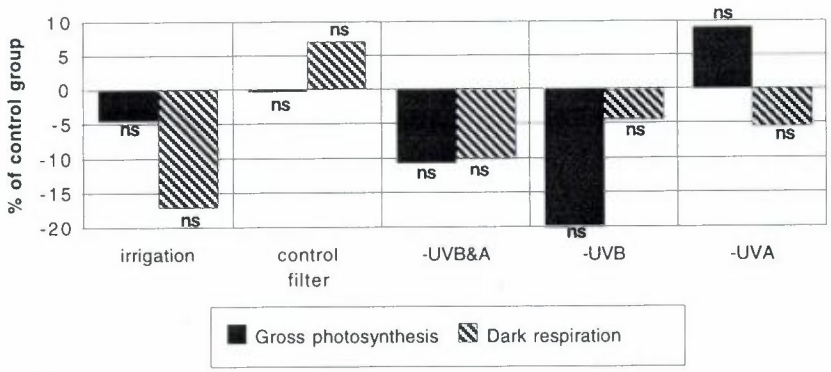

Figure 8. Effect of irrigation, control filter and screening of each spectral band studied, as a % of the control group, on the gross photosynthetic (on the chlorophyll basis) and dark respiration rates of *Cetraria islandica*, after a 3 months treatment period.

4. Discussion

Results can be summarized by the effect of solar UV-B&A radiation on the biochemistry and the physiology of *Cetraria islandica* (Fig. 9). The screening of the UV-B&A band induced a 52% average decrease in the concentration of 8

phenolic compounds, while there were no significant effects on chlorophyll and carotenoid contents as well as photosynthetic and dark respiratory rates. With regards to phenolic compound contents as well as gross photosynthetic and dark respiratory rates, it was possible to estimate the screening effects of UV-B radiation, which were very similar to those of the UV-B&A band.

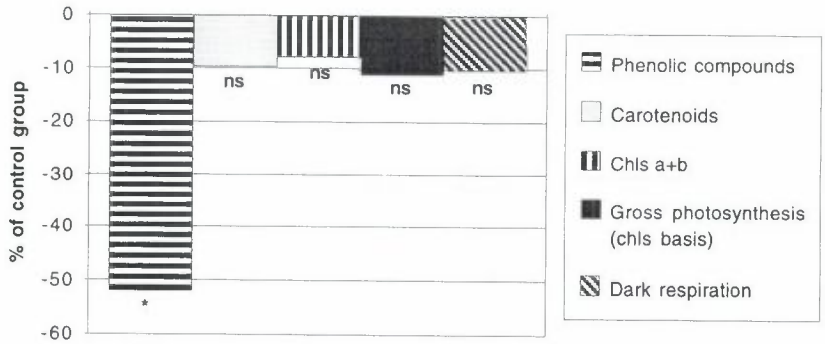

Figure 9. Screening effects of UV-B&A radiation, as a % of the control group, on the physiology and biochemistry of *Cetraria islandica*, after a 3 months treatment period. *:$p<0.05$.

The "control filter effect"

Measurements have shown that microclimates (air temperature and visible light) were very similar in each pair control group/experimental group. These slight climatic differences were almost certainly too small to induce any significant physiological or biochemical modifications. Therefore, it would be unlikely to observe a control filter effect due to the variability of these two climatic factors. As explained earlier, this hypothesis was consistent with our results concerning the growth of *Pisum sativum* (Bachereau and Asta, 1997). Nevertheless, it was unclear why we observed such a significant "control filter effect" on some parameters (chlorophyll and carotenoid contents) while other parameters (phenolic compound concentrations as well as gross photosynthetic on a chlorophyll basis and dark respiratory rates) were not affected.

It is worth noting that only parameters (chlorophyll and carotenoid contents) linked to the photobiont were modified by this "control filter effect", the mycobiont (phenolic compound production and dark respiratory rates), on the other hand, appearing insensitive. Because this "control filter effect" was expressed by a decrease in chlorophyll and carotenoid concentrations, we suspected an external cause such as an infection by a pathogenic organism on the lichen mats. These lower chlorophyll concentrations resulted in unusual net

photosynthetic curves (Fig. 3). Interpretation of the photosynthetic slopes indicated that 2 groups (control filter and UV-B filter) had a much reduced "first photosynthetic slope" (P1), indicative of poor photosynthetic rates. In contrast to P2 (the second photosynthetic slope) which is less strongly modified, P1 slope would appear as a good indicator of the health condition of the lichen. As there were no significant differences between any experimental/control pairs for gross photosynthesis expressed on a chlorophyll basis, this suggests that reduced P1 curves were solely due to the lower chlorophyll concentrations of the infected cells, photosynthetic efficiency of the remaining healthy cells being not affected.

If an infection did, in fact, play a significant role, and as the lichen mats under the control filter acted as a control group for the UV-B&A filter and the UV-B filter groups, using this group in the calculation of chlorophyll and carotenoid concentrations, would certainly lead to an overestimation of the photobiont's response to UV-B radiation.

The effects of this unexpected infection at first referred to as "control filter effects" highlighted the practical problems scientists can potentially encounter when undertaking experiments in the field, and emphasized the need to check the physiological integrity of plant samples before and after experimentation. This is particularly important when working with lichens because a weakened or even a dead lichen (i.e. either the algal or fungal symbiont) does not necessarily look different from an alive lichen (unpublished data), at least not for some time.

Biochemical and physiological responses to UV radiation

The average screening effect of the UV-B&A band on phenolic compound concentrations (–52%; 8 sensitive molecules) can be explained by the screening of the UV-B band (–53%; 12 sensitive molecules), the screening of the UV-A band having no significant effects (with the exception of 2 molecules, +23%, –15%). This data clearly demonstrates the stimulation effect of UV-B radiation on the biosynthesis of phenolic compounds since UV-B exclusion induced a lower phenolic compound biosynthesis. The number of UV-B sensitive molecules vary slightly depending on the spectral band studied (UV-B&A filter or UV-B filter). This difference could be explained by the natural variability of pigments concentrations in lichens (Golojuch and Lawrey, 1988), since the 4 molecules which were not UV-B&A-sensitive had an error risk close to the 5% level (P = 27, 13, 13 and 27%). For comparison, the average error risk for the 9 non-sensitive molecules to irrigation were 52%.

With no doubts, the darkening of thalli which were exposed to direct sunlight can be attributed to an increased phenolic compound synthesis

(Kappen, 1973). It is highly probable that the acetone-soluble compounds analysed account for only a small part of the lichen response to UV-B radiation, because the acetone-insoluble polymerized compounds responsible for the strong increase in pigmentation observed in the field, were not measured by our procedure. The dark thalli, in fact, did not loose their colour following the acetone extraction.

Chlorophyll and carotenoid concentrations as well as gross photosynthetic (on a chlorophyll basis) and dark respiratory rates were not significantly affected by the screening of the UV-B&A band. Gross photosynthetic (on a chlorophyll basis) and dark respiratory rates were not significantly affected by the screening of the UV-B band. The combined carotenoid response to UV-B&A screening does not suggest any photoprotective role for this pool. However, the function of carotenoids as collectors of light energy driving photosynthetic processes and their role in protecting the photosynthetic apparatus from the destructive effects of light and O_2, are well established (Siefermann-Harms, 1987). One can assume that the majority of carotenoids play an important role in plants as light-trapping antennae under low light conditions, whereas a few other carotenoids can protect chlorophyll pigments from photodestruction under high light conditions. Thus, the average response of the carotenoid pool could hide specific molecular variations. It would be interesting to undertake detailed HPLC analyses to investigate the response of each individual carotenoid compound in this lichen species.

Biochemical and physiological responses to irrigation

Unexpectedly, there were no differences in biochemical and physiological responses to the irrigation treatment. This suggests that this artificial water supply had counterbalanced the lower water availability under the filters without inducing any side-effects on the physiological activity of lichens, as compared to natural conditions.

5. Conclusion

In addition to expressing the fundamental interest and feasibility, as well as practical problems, of a field experiment, these results demonstrate the effect of UV-B radiation on the phenolic compound biosynthesis of a lichen, and strengthen the hypothesis of the UV-B protective role of lichen aromatic substances. Since the screening of the UV-B&A band had no significant effects on photosynthetic pigments (chlorophylls and carotenoids) as well as gross photosynthetic (on a chlorophyll basis) and dark respiratory rates, this

suggests that phenolic compounds in the thalli of *Cetraria islandica* provided considerable resistance to ultraviolet damage. Dark respiration not being affected by the treatment, we can state that the mycobiont is not sensitive to UV-B radiation. Since the photobiont from two groups was probably infected, it was not possible to discriminate between UV-B and UV-A band screening effects for the related parameters.

All of the parameters studied are indicators of photobiont sensitivity with the exception of respiration which is mainly due to the mycobiont. *A priori*, the case of the phenolic compounds is not simple because these compounds are produced exclusively by the mycobiont (Mosbach, 1973). It has been demonstrated that the algal partner in the lichen does not regulate the chemotype of the fungal partner (Culberson and Ahmadjian, 1980; Culberson et al., 1985). But, we know that carbon for the lichen is furnished primarily by the photosynthetic activity of the algal partner (Elix, 1996; Mosbach, 1973). Moreover, since these secondary products are supposed to protect the photobiont from high light damage, we could reasonably assume that phenolic compound concentrations are indicators of photobiont sensitivity. Nevertheless, this hypothesis has yet to be demonstrated.

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