Enhanced UV-B and Elevated CO₂ Impacts Sub-Arctic Shrub Berry Abundance, Quality and Seed Germination

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Abstract This study investigated the effects of long-term-enhanced UV-B, and combined UV-B with elevated CO₂ on dwarf shrub berry characteristics in a sub-arctic heath community. Germination of *Vaccinium myrtillus* was enhanced in seeds produced at elevated UV-B, but seed numbers and berry size were unaffected. Elevated UV-B and CO₂ stimulated the abundance of *V. myrtillus* berries, whilst UV-B alone stimulated the berry abundance of *V. vitis-idaea* and *Empetrum hermaphroditum*. Enhanced UV-B reduced concentrations of several polyphenolics in *V. myrtillus* berries, whilst elevated CO₂ increased quercetin glycosides in *V. myrtillus*, and syringetin glycosides and anthocyanins in *E. hermaphroditum* berries. UV-B × CO₂ interactions were found for total anthocyanins, delphinidin-3-hexoside and peonidin-3-pentoside in *V. myrtillus* berries but not *E. hermaphroditum*. Results suggest positive impacts of UV-B on the germination of *V. myrtillus* and species-specific impacts of UV-B × elevated CO₂ on berry abundance and quality. The findings have relevance and implications for human and animal consumers plus seed dispersal and seedling establishment.

Keywords CO₂ · Elevated · UV-B · Enhanced · Arctic · Reproduction · Berry · Abundance · Secondary metabolites

INTRODUCTION

UV-B radiation is a biologically important component of the solar spectrum and is known to increase when the

ozone layer thins (Allen et al. 1998). Ozone depletion events will continue into the future at high latitudes, regardless of the success of the 1987 Montreal Protocol (Manney et al. 2011). In addition to variations in UV-B due to ozone depletion, UV-B fluxes are affected by cloud cover, vegetation canopies, topography and aspect (Paul and Gwynn-Jones 2003). It has also been suggested that future changes in UV-B will occur due to the influence of climate change on cloud cover (McKenzie et al. 2011).

Concern about the ecological consequences of ozone depletion in the late 1980s generated a large number of publications on the effects of enhanced UV-B on terrestrial ecosystems. Over the past two decades these ranged from simple growth room studies of individual plant effects, to outdoor manipulations where UV-B was either screened (Ballaré et al. 2001; Day et al. 2001; Phoenix et al. 2002; Robson et al. 2003; Albert et al. 2011) or artificially enhanced via fluorescent light frames. Outdoor UV-B enhancement experiments were initiated across the globe and utilised a range of lighting systems from simple square wave (on/off) step-wise control to sophisticated temporal and seasonally modulated systems (Björn et al. 1997; Markham et al. 1998; Phoenix et al. 2001; Musil et al. 2002; Caldwell et al. 2003; Rozema et al. 2006 etc.). UV-B enhancement experiments were most relevant to high latitude regions in relation to ozone depletion impacts. However, there has been growing interest in the biological role of UV-B in both the ecological and the agricultural contexts (e.g. Jansen et al. 2008), and continued reporting from ozone depletion and other studies have provided valuable ecological insight into the role of UV-B as a biological factor.

During the early 1990s, it was also appreciated that atmospheric CO₂ concentrations were rising and more recently it has been suggested that concentrations will

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increase from the 2007 level of 379 ppm to between 445 and 710 ppm by 2050 (IPCC 2007). The study described herein was established in this context at Abisko in Northern Sweden and responded to concerns by looking at the interactive effects of enhanced UV-B in combination with elevated CO₂. Early UV-B × CO₂ studies focussed mainly on short-term plant growth responses in glasshouses (Teramura et al. 1990; Wand et al. 1996; Tosserams et al. 2001; Qaderi and Reid 2005). Recent studies have also investigated food crops and their reproductive physiology in response to UV-B and CO₂ (Koti et al. 2005; Qaderi et al. 2007; Singh et al. 2010). In this study, the reproductive characteristics of the dominant dwarf shrubs in a sub-arctic heath were considered in relation to enhanced UV-B and elevated CO₂ treatment.

UV-B alone has been studied in the context of seed production and viability (van de Staaij et al. 1997; Wang et al. 2008; Lake et al. 2009) and also as a potential tool to manipulate or beneficially enhance the secondary metabolite chemistry of fruit and vegetables (see review by Jansen et al. 2008). For example, research has been conducted on UV-B impacts on grape berries due to commercial interest in fruit characteristics and resultant wine quality. Solar radiation increased the quercetin glycoside (flavonol) concentration in their skin (Price et al. 1995). Berli et al. (2008) further showed that polyphenolics, anthocyanins and resveratrol were stimulated in grapes when ripened in the presence of solar UV-B, suggesting a significant role for UV-B as a regulator of fruit phenolic chemistry during reproduction.

Studies on the effects of elevated CO₂ on plant reproduction are also numerous, and mainly focus on crops or the seeds of forage plants (e.g. Jablonski et al. 2002; Hikosaka et al. 2011). Elevated CO₂ has the potential to increase the size of berries, because of increased photosynthesis and more effective carbohydrate accumulation, as described by Wang et al. (2003) in field-grown strawberries. In addition, elevated CO₂ in strawberries also stimulated the anthocyanin, flavonoid and phenolic contents of the fruit. This suggests a potential beneficial role for elevated CO₂ on fruit production, although Gonçalves et al. (2009) found no effects of elevated CO₂ on grape anthocyanin and tannin content in red wine vines.

Where elevated UV-B and CO₂ are studied together they have the capacity to interact affecting reproductive characteristics. For example, Wand et al. (1996) studied the short-term effects of UV-B and elevated CO₂ on the reproduction of the winter ephemeral forb, *Dimorphotheca pluvialis*. Reproductive phenology was delayed and reproductive biomass reduced at elevated CO₂. However, UV-B stimulated reproduction significantly at ambient CO₂ but not at elevated CO₂. Qaderi et al. (2007) suggested that UV-B was detrimental to siliques and seeds, but that some

of these negative effects were ameliorated by elevated CO₂. Schultz (2000) reviewed the effects of enhanced UV-B radiation (in conjunction with separate elevated CO₂ experiments) on grape varieties. He found that UV-B increased levels of flavonoids, amino acids and carotenoid pigments in fruit skins. Singh et al. (2010) completed further study on how multiple stresses (including enhanced UV-B) affected the tropical legume *Vigna unguiculata* (cowpea), showing that UV-B (in combination with elevated temperatures) caused the greatest negative impact on the reproductive capacity of this species, and that genotype dependent responses interacted with elevated CO₂.

The only study that has looked at the simultaneous effects of elevated CO₂ and enhanced UV-B on fruit in natural communities was conducted at the same experimental site used in the current study (Gwynn-Jones et al. 1997). Three years' exposure to elevated CO₂ had no effects on berry production. However, enhanced UV-B stimulated *Vaccinium myrtillus* berry abundance (expressed per m⁻²). Phoenix et al. (2001) also observed a stimulation of flower abundance, berry abundance and total berry fresh weight (expressed per m⁻²) in the dwarf shrub *V. myrtillus* with UV-B. They also highlighted large inter-annual variation and found significant responses to UV-B in particular years. For the competing species within this community, *Empetrum hermaphroditum*, no significant impacts of UV-B (Gwynn-Jones et al. 1997; Phoenix et al. 2001) and CO₂ (Gwynn-Jones et al. 1997) were apparent on fruit abundance.

Dwarf shrub berries are particularly valued by the human populations at Northern latitudes as an autumn harvest, but are also consumed by a wide range of animals (Anderson 1985). From a human perspective, the fruit contain high concentrations of flavonoids and anthocyanins (Heinonen et al. 1998; Faria et al. 2005; Heinonen 2007)—which can scavenge cancer-causing free-radicals (Martin-Aragón et al. 1998; Taruscio et al. 2004) and reduce the oxidative stress caused by these compounds in animals (Johnson and Felton 2001). There is therefore commercial interest in the chemistry of such berries, particularly if they have potential health benefits to both humans and animals via polyphenolic antioxidants (Meyer et al. 1997; Jansen et al. 2008). For example, there is already laboratory evidence suggesting that the consumption of *V. myrtillus* berry flavonoids by small mammals can increase the antioxidant capacity of their blood plasma which could promote their fitness (Talavera et al. 2006).

This study investigated the long-term effects (15 years treatment) of enhanced UV-B on seed germination of *V. myrtillus* L. (bilberry) and *E. hermaphroditum* L. (mountain crowberry) in seeds collected in 2006. This was followed by an investigation of long-term (17 years)-enhanced UV-B combined with elevated CO₂ treatment on

berry size, quality and abundance (expressed per m⁻²) of the dominant dwarf shrub species in 2009 [*V. myrtillus*, *E. hermaphroditum*, *V. vitis-idaea* L. (lingonberry)]. Data collection for some elements was practicable only in specific years when there were sufficient berries across the two replicated experiments to permit valid analyses. Based on previous study (Gwynn-Jones et al. 1997), it was hypothesised that elevated CO₂ would not affect berry size and abundance. However, UV-B was expected to stimulate berry abundance but not berry size. Enhanced UV-B exposure of plants was also expected to produce seeds that have lower germination success. Both the UV-B and the elevated CO₂ were hypothesised to stimulate berry tissue polyphenolic compounds. The ecological implications of the results are considered in relation to fruit consumption and seed dispersal by animals.

MATERIALS AND METHODS

Experimental Site and Treatments

Experiments simulating enhanced UV-B and elevated CO₂ were conducted at Abisko Scientific Research Station in Northern Sweden (68.35°N, 18.82°E). The vegetation studied was sub-arctic *Empetrum-Vaccinium myrtillus* forest heath community as described by Sonesson and Lundberg (1974). For this study, data collection was dictated by the availability of replicable samples across the two experiments, we employed: (1) an experiment with an enhanced UV-B treatment only and (2) an experiment with combined UV-B and elevated CO₂ treatments.

Enhanced UV-B Treatment (Experiment 1)

Vaccinium myrtillus and *E. hermaphroditum* berries were collected in 2006 to investigate seed germination characteristics from an experiment following 15 years of enhanced UV-B treatment (1991–2006). Vegetation was exposed to enhanced UV-B treatment simulating ~15 % ozone depletion conditions under cloudless skies (see Johanson et al. 1995a, b). Six fluorescent tubes (Q-PANEL UVB-313, Cleveland, OH, USA) housed in bespoke lamp holders were suspended above vegetation by aluminium frames (2.5 × 1.3 × 1.5-m high). Four of the frames represented a control and maintained vegetation under ambient UV-B conditions using window glass to cover the UV-B lamps and absorb all radiation <320 nm. Four treatment frames exposed vegetation to enhanced UV-B conditions with lamps filtered with UV transparent Perspex and cellulose diacetate filters to absorb radiation <280 nm but allow UV-B exposure (see Johanson et al. 1995a, b for full experimental details). Treatments started during

snowmelt (late April 1991) and ran until mid-September of every growing season. Lamps were connected to timers providing square wave enhancement of UV-B, which was seasonally adjusted (every 2 weeks). For a summary of site climatic data (temperature, precipitation and photosynthetically active radiation) from 1991 to 2009, see supplementary data provided (Tables S1, S2 and S3).

Enhanced UV-B and Elevated CO₂ Treatment (Experiment 2)

We separately assessed berry numbers, size and quality from a further experiment investigating looking at enhanced UV-B effects in combination with elevated CO₂ (1993–2009; i.e. Ambient conditions; +UV-B; +CO₂; +UV-B +CO₂, *n* = 4). In combination with UV-B treatments (see above for details), 16 open-top chambers (OTCs) made of UV transmitting Perspex (0.85-m diameter, 0.5-m height and vegetation area 0.73 m²) were placed on ambient and enhanced UV-B plots. The OTCs used for this study also elevated air temperature by 1 °C as described by Gwynn-Jones et al. (1997) (details of local climatological conditions are provided as supplementary data, Tables S1, S2 and S3).

The vegetation within these OTCs was exposed to either ambient air or air enriched with CO₂ to elevated atmospheric levels of 600 ppm, against an ambient level of 360–386 ppm (Gwynn-Jones et al. 1997). Examples of CO₂ concentration measurements within the elevated CO₂ plots are provided as supplementary data (see Fig. S1).

Assessing Seed Germination Responses to UV-B Treatment (from Experiment 1)

A representative and spatially randomised field sample (~25 g) of mature *E. hermaphroditum* and *V. myrtillus* berries were harvested from the ambient and enhanced UV-B treatment plots (*n* = 4) from the 1st to the 11th of August 2006. The berry samples were immediately air dried at room temperature for 72 h and transferred to airtight sealed containers for transport to the UK. On arrival, the berries were re-hydrated in distilled water for 36 h. Seeds were extracted from berry mesocarp using fine tweezers and transferred to a Petri dish which contained a moistened disc of filter paper, maintained at saturation with distilled water. Fifty seeds obtained from several berries were distributed evenly on each filter paper disc. The procedure was repeated so that six sub-replicate Petri dish germination vessels were generated per experimental treatment plot. The germination vessels were then sealed in zip-lock bags to prevent moisture loss and transferred to a controlled temperature facility to facilitate seed stratification.

During the period of warm seed stratification required for *E. hermaphroditum* germination (Baskin et al. 2002) fungal growth problems arose, so this part of the study was discontinued. The germination of *V. myrtillus* required only a 20-week period of cold stratification ($\sim 0^{\circ}\text{C}$) (Baskin and Baskin 1998) and this species remained unaffected by fungus. Following stratification, the germination vessels were removed from their zip-lock bags, which allowed air to circulate within them, and transferred to a controlled environment chamber for a germination trial that ran until $>25\%$ of seeds had germinated in all treatments (54 days). The controlled environment chamber provided a diurnal cycle of 14 h of $190\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ light at 20°C and 10 h in darkness at 10°C .

The filter papers were kept moist throughout with periodic additions of distilled water. Every 4 days during the trial, seeds were examined under magnification to observe germination incidence and to check for any potential fungal development.

Assessing Berry Abundance and Size in Response to UV-B and CO_2 (from Experiment 2)

Total berry number for each species (including *V. vitis-idaea*) was counted within each plot (ripe and unripe). Percentage species cover of the dominant dwarf shrubs (*V. myrtillus*, *E. hermaphroditum*, *V. vitis-idaea*) was measured in order to correct berry abundance data for species abundance; abundance data were presented as number of berries per m^2 cover of the species assessed. Cover was determined using a quadrat frame ($0.5 \times 0.5\text{ m}$) placed within the chambers and the individual % cover from 100 squares within the quadrat was determined by eye. Total % cover was determined as the sum of all values from the 100 squares assessed.

Vaccinium myrtillus and *E. hermaphroditum* ripe berries were also collected (27th July–9th Aug 2009) to determine berry size. Six berries per plot were collected and measured for diameter by passing them through a Perspex ruler with precision drilled holes (4.5–10.5 mm at 0.5-mm interval).

Further ripe berries were also freeze dried at -50°C for 48 h (MODULYOD 230, Thermo Electron Corporation, Holbrook, USA) for tissue quality analyses. For this element experimental samples were collected over a 2-week timescale to reduce temporal variation in fruit quality. We selected a short harvesting period as the stage of berry development is important for consistency in chemical analyses, as has been shown in other studies on red berries (e.g. Macheix et al. 1990). Phenolic compounds, for example, usually accumulate in berries as they mature (Heinonen et al. 1998). In our study, we only sampled ripe berries, determined by colour, at the same developmental stage (when berry colour was fully established) for

chemical analysis. Using colour reduced sampling variation but it should be emphasised that there were no visible differences in berry appearance or size according to treatments. We acknowledge that some berries will have ripened after the sampling period used. For our third species (*V. vitis-idaea*) this 2-week time period was too short to collect sufficient berries of similar ripeness (based on colour) from across the experimental site, hence this species was not studied in detail.

Freeze-dried berries were next dissected and their seeds were extracted and counted, the remaining exocarp (skin) and mesocarp together (hereafter collectively described as ‘berry tissue’) were subsequently assessed for methanol soluble polyphenolic content. For all species studied, protocols were developed from previous study at the same site (Gwynn-Jones et al. 1997; Phoenix et al. 2001). In agreement with this previous study the experimental treatments did not appear to affect plant phenology, berry development or ripening.

Berry Tissue Polyphenols

A broad range of polyphenols were studied to provide an insight into secondary metabolite responses to the treatments. The extraction of phenolics from berry tissue of *V. myrtillus* and *E. hermaphroditum* was conducted using the same berry samples as the seed number and berry size measurements. A bulked (plot basis) 100–200 mg sample of de-seeded berry tissue (exocarp plus mesocarp) was weighed, then extracted for phenolic compounds by grinding in 2 ml 70 % aqueous methanol for 2 min per sample and stored at 5°C . Extract was centrifuged for 5 min at 14.5 k rpm and the supernatant collected with sediment pellets rinsed with 0.5 ml of 70 % aqueous methanol, centrifuged at 14.5 k rpm and supernatant pooled. This was heated at 80°C for 10 min to denature oxidising enzymes. Methanol was removed by vacuum centrifugation with application of heat.

Evaporated solute was made up to 1 ml in water and centrifuged for 5 min at 14.5 k rpm and samples (500 μl) were partially purified with Waters Sep-PAK (500 mg) C_{18} reverse-phase cartridges, dried down under air and re-suspended in 500 μl 50 % aqueous methanol. Hydroxycinnamic acids, anthocyanins and flavonols were analysed by reverse-phase HPLC with an on-line photodiode array detector (PDA). The HPLC system used consisted of a WATERS 717 plus Autosampler, WATERS 515 HPLC pump, WATERS 996 Photodiode Array Detector and a PC with Empower software. Phenols were separated on a Waters C_{18} reversed-phase Nova-Pak cartridge (4.0 mm, $8.0 \times 100\text{ mm}$). Mobile phase A consisted of purified water–acetic acid (95:5, v/v) and mobile phase B of HPLC grade methanol. Initial conditions were A:B (100:0, v/v)

and the percentage of mobile phase B was increased linearly to 100 % over 50 min with a flow rate of 2 ml min⁻¹. The sample injection volume was 60 µl. UV and light absorption was monitored over the wavelength range of 240–600 nm, to enable identification of anthocyanins with characteristic absorption profiles in the 500–550 nm range. Compounds were quantified by integrating the areas underneath the curve for absorbance at 340 nm for hydroxycinnamates and flavonols and 280 nm for anthocyanins. Peaks associated with individual compounds were distinguished according to retention times (R_t) and peak maxima (nm), and were quantified against standards.

The area under the curve of peaks for hydroxycinnamates, flavonols and anthocyanins were converted to chlorogenic/coumaric acid, quercetin and peonidin equivalents (µg per peak), respectively, using appropriate response factors (2.65×10^{-6} for chlorogenic at 340 nm, 7×10^{-7} for coumaric acid at 340 nm, 2.09×10^{-6} for quercetin at 340 nm and 1.8×10^{-6} cyanidin at 280 nm).

Compounds were identified by HPLC/MSⁿ with a LC–MS system (Thermo Electron Corporation, Waltham, USA) comprising a Finnigan Surveyor PDA Plus detector, a Finnigan LTQ linear ion trap with ESI source and a Waters C₁₈ reversed-phase Nova-Pak column (4 µm, 3.9 × 100 mm). The auto-sampler tray temperature was kept at 5 °C and the column temperature was maintained at 30 °C. Sample injection volume was 10 µl, the detection wavelength was set at 240–600 nm and the flow rate was 1 ml min⁻¹, with 100 µl min⁻¹ going to the mass spectrometer. The mobile phase consisted of purified water–formic acid (A; 100:0.1, v/v) and HPLC grade MeOH–formic acid (B; 100:0.1, v/v). The initial condition was A: B (5:95, v/v), and the percentage of B increased linearly to 50 % over 45 min.

Mass spectra were acquired in negative ionisation mode. Ionisation parameters were optimised by infusion of chlorogenic acid standard at a constant rate into the LC flow. Interface and MSD parameters were as follows: sheath gas 30 au, auxiliary gas 15 U, spray voltage 4 kV, capillary temperature 320 °C, capillary voltage –1 V and tube lens offset –68 V. Initial MS/MS fragmentation was carried out at normalised collision energy 35 % and isolation width 2.0 (m/z). Compounds were identified by comparison with their molecular mass and with fragmentation patterns of commercial standards for phenolic acids, flavonol aglycones and anthocyanins obtained from Sigma-Aldrich, UK and PhytoLab, Vestenbergsgreuth, Germany.

Statistical Analyses

Seed germination responses were derived from Petri dish germination vessels, which were sub-replicated six times for each of the eight experimental plots. Data were

averaged to obtain a single mean value for each plot at each observation time point and were recorded at 4-day intervals over 36 days from the initial point of germination giving 11 time points overall. This yielded separate data for (1) germination incidence (as a running total for each time point during the trial) and (2) germination rate over time (expressed as the number of new seedlings germinating per day). Daily germination rates were obtained from counts of newly germinated seedlings at each observation point, divided according to the period between observations. All data were tested for experimental treatment effects by repeated measures analysis in a generalised linear mixed effects model (GLMM) using the statistical programme R v2.12.2 (R Development Core Team 2011). The model was Poisson corrected to account for the distribution of the data. Sampling time point and plot were nested, respectively, as mixed (random) effects within the model.

Berry abundance analyses of *V. myrtillus*, *E. hermaphroditum* and *V. vitis-idaea* were conducted using the statistical programme R v2.12.2 (R Development Core Team 2011). Main factor effects and interaction (UV-B × CO₂) were determined using a generalised linear model (GLM) or GLMM, with species percentage cover defined as a random (uncontrolled) experimental variable within the model for berry abundances. Data were logarithmically transformed where necessary to gain homogeneity of variances and the model then tested for the treatment effects of UV-B or CO₂ on berry number. Probability plots using the Kolmogorov–Smirnov normality test of each vegetation type were conducted and the area (% cover) of the species considered to ensure that they were not affected by treatment. Differences at $P < 0.05$ level were considered significant.

Berry tissue polyphenolic content was analysed using a nested ANOVA to partition variability both within and between the replicate plots, using Minitab version 14 (Minitab Ltd., Coventry, UK). However, seed number and HPLC polyphenolic data for statistical analyses were assessed using a mean value basis per plot. Data were first assessed using the Kolmogorov–Smirnov normality test to test for deviation from normal distribution and tests for equality of variances were conducted before using balanced ANOVAs (Minitab Ltd., Coventry, UK).

RESULTS

V. Myrtillus Seed Germination in Response to UV-B

Enhanced UV-B had a significant positive effect on *V. myrtillus* seed germination. Seeds from the UV-B treatment germinated earlier and more rapidly, with twice the abundance overall than those from the ambient UV-B

Fig. 1 Time-series line plots showing *V. myrtillus* germination responses over 36 days, according to experimental UV-B treatment (ambient UV-B versus elevated UV-B): **a** mean total number of germinated seedlings per treatment, **b** mean daily seed germination rate per treatment

Table 1 Summary of GLMM repeated measures analyses on time-series analyses for *V. myrtillus* germination at 11 time points during 54 days of experimentation

Model	AIC	<i>f</i>	<i>P</i>	<i>df_d</i>	<i>df_n</i>
<i>V. myrtillus</i> total germination success					
UV-B treatment	558.2	71.6	***	1	75
<i>V. myrtillus</i> daily germination rate					
UV-B treatment	90.2	5.4	*	1	75

Experimental responses were tested according to UV-B treatment (ambient UV-B versus elevated UV-B). Akaike information criterion (AIC), degrees of freedom for numerator and denominator (*df_n*, *df_d*), *f* and *P* values for significance given: *** <0.001, * <0.05

treatment (Fig. 1). The repeated measures analysis of these data by GLMM determined that over the trial period the enhanced UV-B treatment exerted a highly significant effect on the running total for germination success ($P < 0.001$) and a significant effect on germination rate ($P = 0.02$) (Table 1), as calculated at 4-day intervals when observations were made of newly emerging seedlings. The reduced significance for germination rate is likely to derive from the high initial rate of germination in seeds from the UV-B treatments (Fig. 1). Owing to high initial germination rates, proportionally fewer un-germinated seeds from the UV-B treatment remained during the rest of the trial period, such that germination rates inevitably declined to levels similar to those of the ambient UV-B treatment.

Enhanced UV-B and Elevated CO₂ Impacts on Berry Number, Diameter and Seed Size

Vaccinium myrtillus berry abundance was significantly increased by both the elevated CO₂ and the enhanced UV-B; however, the combined treatments had no greater effect than either treatment in isolation (Fig. 2a; Table 2). UV-B alone promoted berry abundance in the related species *V. vitis-idaea* and in *E. hermaphroditum*, but no effects of elevated CO₂ nor interactions were observed (Fig. 2b, c; Table 2). No

significant treatment effects were observed for berry diameter or seed number in any of the species studied (Table 3).

Berry Tissue Polyphenolic Content

There was a consistent and significant negative effect of UV-B on the quantity of polyphenolics in the berry tissue of *V. myrtillus* (Fig. 3; Table 4). Total anthocyanin, chlorogenic acid, delphinidin-3-hexoside and petunidin-3-pentoside were all reduced at enhanced UV-B. The only effect of elevated CO₂ for this species was an increase in quercetin glycosides. There were also UV × CO₂ interactive effects apparent for the anthocyanins delphinidin-3-hexoside and petunidin-3-pentoside (Table 4).

In *E. hermaphroditum*, there was a significant positive effect of elevated CO₂ on syringetin glycosides and the five measured anthocyanins (delphinidin-3-hexoside, cyanidin-3-hexoside, petunidin-3-hexoside, malvidin-3-pentoside, malvidin-3-hexoside, Fig. 4; Table 5). However, when all data were combined there was no significant ($P > 0.05$) effect on total anthocyanin content (Table 5). There were also no further significant treatment effects (UV-B) or interactions (UV-B × CO₂) on the berry tissue of *E. hermaphroditum*.

DISCUSSION

The berries of the dwarf shrub species studied are widely distributed at high latitudes in the Northern hemisphere and valued from a human perspective (Anderson 1985; Heinonen 2007). They also represent an important food source for animals and birds, and are a very rich source of phenolic antioxidants (Prior et al. 1998), especially anthocyanins (Heinonen 2007), which provide nutritional benefit to both humans and animals. Although mainly clonal in growth, these species can effectively reproduce via seed (Szmidi et al. 2002) and dispersal is achieved via consumption by bears, foxes, small mammals and bird

Fig. 2 Berry abundance responses during August 2009 to community experimental treatment [enhanced UV-B, elevated CO₂, UV-B and CO₂ against ambient (control) conditions] for 17 field seasons. Species shown are: **a** *V. myrtillus*, **b** *V. vitis-idaea* and **c** *E. hermaphroditum*. Mean and standard error given according to experimental treatment. Statistical significance: * $P < 0.05$, *** $P < 0.001$. See Table 2 for further statistical information

Table 2 Responses of species (*V. myrtillus*, *E. hermaphroditum* and *V. vitis-idaea*) 2009 berry abundance to community experimental treatment [enhanced UV-B, elevated CO₂, UV-B and CO₂ against ambient (control) conditions] over 17 field seasons

Model	Log-likelihood	z	AIC	P	df_d	df_n
<i>V. myrtillus</i> berry abundance						
UV-B × CO ₂	−54.8	−4.9	119.6	***	12	15
UV-B	−70.2	2.2	146.5	*	14	15
CO ₂	−70.0	−2.4	146.0	*	12	15
<i>E. hermaphroditum</i> berry abundance						
UV-B × CO ₂	−63.1	0.9	136.2	n/s	12	15
UV-B	−63.5	−2.4	133.1	n/s	14	15
CO ₂	−64.7	1.9	135.4	n/s	12	15
<i>V. vitis-idaea</i> berry abundance						
UV-B × CO ₂	−32.5	1.0	75.0	n/s	8	15
UV-B	−36.4	3.12	78.7	**	12	15
CO ₂	−41.5	−0.1	89.1	n/s	14	15

Statistical significance of response variable according to Poisson-corrected GLMM, with individual species percentage cover defined as a random experimental variable. Log-likelihood, Akaike information criterion (AIC), degrees of freedom for numerator and denominator (df_n , df_d), z and P values given. Significant P values in the table are highlighted in bold, according to * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ levels of statistical significance

consumers. Ptarmigan and grouse in particular facilitate seedling recruitment into disturbed areas (Ritchie 1956; Tybirk et al. 2000).

Seed Germination Responses to UV-B

This study initially examined the effects of enhanced UV-B on seed germination of the dominant dwarf shrub species

V. myrtillus. The results indicated that UV-B had a strongly positive effect on germination, in terms of both the rate and the overall success (Fig. 1; Table 1). Graae et al. (2011) suggest that *V. myrtillus* seeds already germinate effectively under natural conditions and data herein suggest that UV-B exposure of the parent could further promote germination success. Positive effects of UV-B on reproductive output have already been reported in the literature including increased berry production of sub-arctic heath vegetation (Gwynn-Jones et al. 1997; Phoenix et al. 2001); positive effects on seed production in a Mediterranean shrub species (Stephanou and Manetas 1998); promotion of reproductive development with ambient UV in two Antarctic vascular plant species (Day et al. 1999, 2001); positive effects of UV on seed production and germination of an European alpine forb (van de Staaij et al. 1997). The mechanisms responsible for this wide range of positive reproductive responses to UV-B are unclear. However, increased germination success in this study may derive from investment in reproductive resources, which may facilitate the survival of a species under stressful conditions (Harper and Ogden 1970).

Although reproductive output has been considered in response to UV-B, there has been limited work to date examining the effects of enhanced parental UV-B exposure on seed germination and seedling viability, particularly in species from natural ecosystems. A study on the Mediterranean shrub *Cistus creticus*, found seed production was increased by enhanced UV-B (Stephanou and Manetas 1998) but there was no effect on germination. Germination rates were reduced by UV-B during a similar study on the perennial South African forb

Table 3 Diameters and seed numbers of *V. myrtillus* and *E. hermaphroditum* berries collected from plants grown in a community at enhanced UV-B, elevated CO₂, UV-B and CO₂ plus ambient (control) conditions following 17 field seasons of exposure

Growth measures	Control	UV-B	CO ₂	UV-B/CO ₂	UV-B effect		CO ₂ effect		UV-B × CO ₂ interaction		Plot	
					<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
<i>V. myrtillus</i>												
Berry diameter (mm)	7.9 ± 0.2	8.6 ± 0.2	7.8 ± 0.1	8.1 ± 0.2	3.03	0.107	1.42	0.256	0.41	0.533	3.49	***
Seed number	53.9 ± 9.9	63.3 ± 6.2	56.4 ± 12.4	52.6 ± 10.9	0.08	0.787	0.16	0.692	0.42	0.531		
<i>E. hermaphroditum</i>												
Berry diameter (mm)	7.2 ± 0.3	7.5 ± 0.2	7.7 ± 0.2	7.4 ± 0.2	0.00	0.989	0.16	0.696	0.84	0.378	3.33	***
Seed number	6.7 ± 0.3	7.1 ± 0.3	6.9 ± 0.2	6.9 ± 0.6	0.24	0.634	0.00	0.968	0.17	0.688		

Samples were collected in August 2009 and show mean ± standard error of the mean (SEM) showing variation within the data set. Significant *P* values in the table are highlighted in bold, according to *** *P* < 0.001 level of statistical significance. Significant plot effects are evident for both the species

Fig. 3 UV-B or CO₂ treatment effects on flavonoid concentrations for *V. myrtillus* berries collected in August 2009 following community experimental treatment [enhanced UV-B, elevated CO₂, UV-B and CO₂ against an ambient (control) conditions] following 17 field seasons. Mean and standard error given according to the experimental treatment. See Table 4 for further statistical information

Table 4 Responses of *V. myrtillus* berry tissue flavonoid concentration to plant community experimental treatment [enhanced UV-B, elevated CO₂ conditions, UV-B and CO₂ against ambient (control) conditions] following 17 field seasons

Effect	<i>df_d</i>	Total Anthocyanins		Chlorogenic acid		Quercetin glycosides		Delphinidin-3-hexoside		Cyanidin-3-hexoside		Petunidin-3-pentoside		Peonidin-3-pentosidein		Malvidin-3-hexoside/pentoside	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
UV-B	14	4.91	0.04*	5.22	0.04*	1.27	0.28	5.25	0.04*	0.13	0.72	6.01	0.03*	2.49	0.13	0.51	0.49
CO ₂	14	1.47	0.25	1.24	0.28	4.95	0.04*	4.05	0.07	0.62	0.44	0.55	0.47	0.10	0.76	0.36	0.56
UV-B × CO ₂	12	2.31	0.15	0.06	0.80	1.09	0.32	6.42	0.03*	4.05	0.06	3.43	0.08	5.62	0.04*	3.27	0.09

Statistical significance of log-transformed response variable data according to GLM, with denominator degrees of freedom (*df_d*), *F* and *P* values given. Significant *P* values in the table are highlighted in bold, according to * *P* < 0.05 level of statistical significance

D. sinuata (Musil 1996). Oilseed rape was investigated by Demchik and Day (1996) who found that parental UV-B exposure detrimentally affected seed germination,

but only under varying degrees of UV-B exposure during the germination period, which may have directly inhibited germination.

Fig. 4 Significant CO₂ treatment effects on flavonoid concentrations for *E. hermaphroditum* berries in August 2009 following community experimental treatment [enhanced UV-B, elevated CO₂, UV-B and CO₂ against an ambient (control) conditions] following 17 field

seasons. Mean and standard error given according to the experimental treatment. No data are present for UV-B effects as no significant impacts ($P > 0.05$) were observed. See Table 5 for further statistical information

Table 5 Responses of *E. hermaphroditum* berry tissue flavonoid concentration to community experimental treatment [enhanced UV-B, elevated CO₂ conditions, UV-B and CO₂ against an ambient (control) conditions] following 17 field seasons

Effect	df_d	Total anthocyanin		P-Coumaroyl quinic acid		Syringetin glycosides		Delphinidin-3-hexoside		Cyanidin-3-hexoside		Petunidin-3-hexoside		Malvidin-3-pentoside		Malvidin-3-hexoside	
		F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P
UV-B	14	0.09	0.77	0.84	0.78	2.57	0.13	0.55	0.47	0.98	0.34	1.87	0.19	2.34	0.15	1.41	0.25
CO ₂	14	0.24	0.63	1.67	0.21	9.09	<0.01**	10.6	<0.01**	11.5	<0.01**	11.3	<0.01**	10.4	<0.01**	9.53	<0.01**
UV-B × CO ₂	12	1.48	0.25	0.00	0.99	1.67	0.22	0.40	0.53	1.74	0.22	0.60	0.45	0.77	0.40	0.38	0.55

Statistical significance of log-transformed response variable data according to GLM, with denominator degrees of freedom (*df_d*), *F* and *P* values given. Significant *P* values in the table are highlighted in bold, according to ** $P < 0.01$ level of statistical significance

Although our experiments did not directly expose the seeds to UV-B during germination (as is likely to happen under field conditions), the findings nevertheless demonstrate investment by *V. myrtillus* to seed fertility, which is attributable to UV-B exposure. This apparently positive effect could potentially be counteracted under field conditions, because direct UV-B exposure may inhibit germination (Sullivan and Teramura 1988).

The results shown represent the first evidence of a change in the normal reproductive functioning of *V. myrtillus* at the seed level due to UV-B exposure (Fig. 1; Table 1). This deciduous dwarf shrub species appears better adapted than evergreen competitors to exploit seedling dispersal as an adaptive reproductive strategy (Tolvalen and Laine 1997). Therefore, this as an adaptive response mechanism in *V. myrtillus* to UV-B underlines the ecological significance of these findings for competitive dynamics within the sub-arctic heath community.

However, further ecological factors including the availability of suitable disturbed patches (see Graae et al. 2011) may dictate the success of seedling establishment.

Enhanced UV-B and Elevated CO₂ Impacts on Berry Abundance, Size and Tissue Quality

The long-term treatment effects of enhanced UV-B and elevated CO₂ on the berry reproductive characteristics and associated polyphenolics were also studied. Data collection focussed on berries collected from a single season (2009) when berry numbers were consistently high. Large inter-annual variation of berry production is recognised in this system (Phoenix et al. 2001). The berry abundances of the dwarf shrub species in this study were also lower than the values presented by Phoenix et al. (2001) for previous years at the same point. Furthermore, data could not be collected in the subsequent seasons 2010 and 2011 as there

were insufficient berries within all plots for fully replicated analyses.

In line with previous study on the same species (Gwynn-Jones et al. 1997; Phoenix et al. 2001) and other species in the Mediterranean (Stephanou and Manetas 1998), flower/ berry abundance was stimulated by enhanced UV-B in both of the *Vaccinium* species studied (Fig. 2; Table 2). This response also occurred with *E. hermaphroditum*, a finding not reported in prior studies. Such dwarf shrub species predominantly propagate by clonal growth (Eriksson 1989). However, under stressful conditions resource allocation prioritises species/genotype survival by promoting sexual reproduction (Zvereva and Kozlov 2005). Hence, long-term UV-B stress impacts on *Vaccinium* species and *E. hermaphroditum* may be inducing berry production in a similar way but the underlying mechanism is unknown.

Vaccinium myrtillus berry abundance was also higher at elevated CO₂ (Fig. 2; Table 2), which agrees with previous study on strawberries (Wang et al. 2003). However, in this study, the response is considered to be via the effects of elevated CO₂ on resource availability rather than a stimulation of flower production. Berries represent a local sink for carbohydrates, and berry abortion, at least in grapes, is determined by photoassimilate availability (Caspari et al. 1998). Previous elevated CO₂ research has suggested that plants can maintain higher rates of photosynthesis in the longer term at elevated CO₂ but the additional photoassimilates do not often manifest in increased vegetative growth (Körner 2006). Reproductive sinks therefore represent an obvious destination for any additional photoassimilates produced at elevated CO₂.

No effects of elevated CO₂ were observed on the berry abundance of the competing species *V. vitis-idaea* and *E. hermaphroditum* (Fig. 2; Table 2), which is in line with previous research (Gwynn-Jones et al. 1997). Both these species are evergreen and this may explain why they respond differently to elevated CO₂ compared to the deciduous species *V. myrtillus*. Evergreen species such as *E. hermaphroditum* are slow growing but very competitive species (Tybirk et al. 2000). They favour asexual vegetative layering for expansion but can produce large numbers of berries (Szmidt et al. 2002). Shevtsova et al. (1995) further noted that only the deciduous species *V. myrtillus* was sensitive to climate in terms of sexual reproduction.

Changes in dwarf shrub berry abundance in response to UV-B or CO₂ were not reflected by changes in size. Berry diameter and mean number of seeds per berry for *V. myrtillus* and *E. hermaphroditum* were unaffected by treatment (Table 3). These findings differ from those of laboratory based studies on seeds where UV-B reduced seed size and numbers (e.g. Lake et al. 2009; Singh et al. 2010).

UV-B is known to induce photoprotective responses in plants, with increases in leaf flavonoids being the

commonest effect reported in meta-analyses (Searles et al. 2001; Newsham and Robinson 2009). However, past study on sub-arctic dwarf shrub vegetation suggests that enhanced UV-B did not stimulate the antioxidant capacity of the leaves (Taulavuori et al. 1998). This study was the first to investigate berry polyphenolic responses to enhanced UV-B and elevated CO₂. For *V. myrtillus* only, UV-B significantly reduced berry concentrations of total anthocyanins, delphinidin-3-hexoside and petunidin-3-pentoside and chlorogenic acid at enhanced UV-B, whilst there were no significant effects ($P > 0.05$) on all other compounds studied (Fig. 3; Table 4). This contrasts with previous study that has shown a stimulation of tomato polyphenolics in response to UV-B (Giuntini et al. 2008). Our study therefore represents the first study to date that highlights negative UV-B impacts on fruit tissue polyphenolics. We emphasize that these findings were not the consequence of any sampling bias as berry collection was randomised across treatments, and berries were also not visibly different in size or colour.

Higher levels of polyphenolics in dwarf shrub berry tissue may be expected at elevated CO₂, based on previous study on field-grown strawberries showing higher phenolic acids, flavonoids, anthocyanins and antioxidant capacity (Wang et al. 2003). Increased photoassimilate supply could stimulate more essential polyphenolics (Peñuelas and Estiarte 1998; Gonçalves et al. 2009). Results for *E. hermaphroditum* agree with this hypothesis, showing higher levels of syringetin glycosides and the five characterised anthocyanins (Fig. 4; Table 5; delphinidin-3-hexoside, cyanidin-3-hexoside, petunidin-3-hexoside, malvidin-3-pentoside, malvidin-3-hexoside). By contrast, effects of elevated CO₂ on *V. myrtillus* were limited with only an increase in quercetin glycoside observed but this particular response was in agreement with Wang et al. (2003) working on strawberries. Hence overall, consumers of *E. hermaphroditum* may gain higher antioxidant intake at elevated CO₂, whilst UV-B reduces the polyphenolic content of *V. myrtillus*.

CONCLUSION

This study has shown important species-specific responses to UV-B and CO₂ in the quality and abundance of berries in dwarf shrub species with a wide geographical distribution. However, the mechanisms explaining the responses are unclear and merit further investigation. Our data confirm previous observations on berry abundance (Gwynn-Jones et al. 1997; Phoenix et al. 2001) but provide significant added value by further investigating seed germination and berry polyphenolics. The findings suggest possible implications for the feeding behaviour of Arctic

frugivorous birds and animals with potentially important consequences for the dispersal and relative recruitment capacity of the competing species that make up this plant community. It is already known that some European bird species show preferential feeding towards berries with higher antioxidant contents (Catoni et al. 2008), which could have important implications for the palatability and, therefore seed dispersal, of these species. Specific effects of UV-B on the germination of *V. myrtillus* in particular years could have implications for plant community dynamics, biodiversity and ecosystem functioning. Furthermore, our findings have cultural and nutritional relevance for the people of the Northern regions as berries from the species studied are widely recognised and exploited as a valuable food resource for humans.

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