

Morphological and physiological responses of canola (*Brassica napus*) siliquas and seeds to UVB and CO₂ under controlled environment conditions

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Abstract

Combined effects of UVB radiation and CO₂ concentration on plant reproductive parts have received little attention. We studied morphological and physiological responses of siliquas and seeds of canola (*Brassica napus* L. cv. 46A65) to UVB and CO₂ under four controlled experimental conditions: UVB radiation (4.2 kJ m⁻² d⁻¹) with ambient level of CO₂ (370 μmol mol⁻¹) (control); UVB radiation (4.2 kJ m⁻² d⁻¹) with elevated level of CO₂ (740 μmol mol⁻¹); no UVB radiation (0 kJ m⁻² d⁻¹) with ambient level of CO₂ (370 μmol mol⁻¹); and no UVB radiation (0 kJ m⁻² d⁻¹) with elevated level of CO₂ (740 μmol mol⁻¹). UVB radiation affected the outer appearance of siliquas, such as colour, as well as their anatomical structures. At both CO₂ levels, the UVB radiation of 4.2 kJ m⁻² d⁻¹ reduced the size of seeds, which had different surface patterns than those from no UVB radiation. At both CO₂ levels, 4.2 kJ m⁻² d⁻¹ of UVB decreased net CO₂ assimilation (A_N) and water use efficiency (WUE), but had no effect on transpiration (E). Elevated CO₂ increased A_N and WUE, but decreased E, under both UVB conditions. At both CO₂ levels, the UVB radiation of 4.2 kJ m⁻² d⁻¹ decreased chlorophyll fluorescence, total chlorophyll (Chl), Chl *a* and Chl *b*, but had no effect on the ratio of Chl *a/b* and the concentration of UV-screening pigments. Elevated CO₂ increased total Chl and the concentration of UV-screening pigments under 4.2 kJ m⁻² d⁻¹ of UVB radiation. Neither UVB nor CO₂ affected wax content of siliqua surface. Many significant relationships were found between the above-mentioned parameters. This study revealed that UVB radiation exerts an adverse effect on canola siliquas and seeds, and some of the detrimental effects of UVB on these reproductive parts can partially be mitigated by CO₂.

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1. Introduction

Changes in global climate are inevitable, with two factors being important components: enhancement of ultraviolet-B radiation (Caldwell et al., 2003) and elevation of atmospheric CO₂ (Ainsworth and Long, 2005). The amount of solar UVB radiation (280–320 nm) reaching the Earth's surface has increased as a result of ozone depletion by anthropogenic gases (Madronich et al., 1998). Also, the level of atmospheric CO₂ has increased about 38 percent from the start of the industrial revolution, and the current level of CO₂ (372 μmol mol⁻¹) may surpass 700 μmol mol⁻¹ by the end of this century (Long et al., 2004). These two aspects of climate change have been studied extensively (see Kakani et al., 2003; Long et al., 2004).

Plant growth and development are affected by solar UVB radiation and atmospheric CO₂. In general, the effects of atmospheric CO₂ are beneficial and those of UVB radiation are detrimental (Rozema et al., 1997). The effects vary among species (Visser et al., 1997; Dai and Upadhyaya, 2002). Some plants respond positively to the ambient levels of UVB radiation, but most negatively. For example, ambient UVB radiation improved survival and increased height growth of cucumber (*Cucumis sativus*) (Teklemariam and Blake, 2003) or required for normal development of oil glands in sweet basil (*Ocimum basilicum*) (Ioannidis et al., 2002), but reduced plant height in some tropical dicotyledon species (Searles et al., 1995), or reduced biomass accumulation and grain yield in barley (*Hordeum vulgare*) (Mazza et al., 1999). Enhanced levels of UVB radiation can commonly negatively affect plant physiological processes and growth (Day and Neale, 2002). Decrease in plant growth is thought to result from damage to DNA (Rousseaux et al., 1999) and photosynthetic apparatus.

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tus (Greenberg et al., 1996). Elevated levels of CO₂ generally increase photosynthesis, growth and plant productivity (Long et al., 2004). Plants can adapt or tolerate to low levels of UVB radiation, but are impaired by higher levels (Tevini, 2000). Various defense mechanisms have been developed in plants to protect them against UVB radiation (Laakso and Huttunen, 1998; Stratmann, 2003). These mechanisms include smaller and thicker leaves (Bornman and Vogelmann, 1991), higher content of epicuticular waxes (Steinmüller and Tevini, 1985), and higher levels of UV-screening pigments (Cockell and Knowland, 1999). The sensitivity of crops to UVB radiation can be determined by the production of UV-screening pigments and the extent of repair mechanisms (Saile-Mark and Tevini, 1997), which differ within and among species (Visser et al., 1997).

Plant growth and physiological responses to either UVB radiation (see Tevini, 2000; Day and Neale, 2002) or CO₂ (see Kimball, 1983; Jablonski et al., 2002) have been documented on a variety of species. However, there have been growing interests to study plant responses to multiple factors and to consider the combined effects of UVB and CO₂ on plants (see Wand et al., 1996; Tosserams et al., 2001; Kakani et al., 2004; Qaderi and Reid, 2005). In such an attempt, Teramura et al. (1990) have shown that elevated level of CO₂ increased total plant biomass and seed yield in wheat, rice and soybean, but under concurrent enhanced UVB radiation, these effects were eliminated in the first two, and remained in the latter. Their findings indicate that UVB radiation can modify the positive effects of CO₂ on photosynthesis and yield, but the combined effects of these factors are species specific. In particular, the effects of UVB radiation on photosynthesis vary among species. In some species, UVB radiation can negatively affect leaf photosynthesis (Ambasht and Agrawal, 1995; Pal et al., 1999), but in some others, it has little or no effect (Fiscus and Booker, 1995; Searles et al., 2001). To date, most of the studies, which investigated the effects of UVB radiation on seed yield, have dealt with plant vegetative parts, whereas plant reproductive parts have received little attention. Thus, this aspect should be explored further.

Canola (*Brassica napus*) is a major oilseed crop in Canada. Environmental factors, such as UVB radiation and atmospheric CO₂, can affect its growth and physiological processes. In our previous study, we found that canola height and seed yield were positively affected by CO₂ and were negatively affected by UVB radiation. However, UVB radiation did not affect canola leaf photosynthesis, chlorophyll fluorescence, and some other morphological and physiological parameters (Qaderi and Reid, 2005). Therefore, we were interested in elucidating how plants that are exposed to UVB radiation produce lower seed yield than those of unexposed plants if there is little or no difference in some of the essential growth factors that affect plant reproductive efforts.

We hypothesized that seed yield can be affected directly by siliques at the later growth stages, when plants lose most of their leaves and become more dependant on green stems and siliques to perform photosynthesis, which differs under different levels of UVB radiation. Our objective was to determine changes in morphological, anatomical, physiological and chemical responses of canola siliques and seeds to UVB radiation and CO₂ concen-

tration at relatively lower photosynthetically active photon flux density (PPFD) under controlled environment conditions. Since this study was an extension of our previous study carried out a year ago (Qaderi and Reid, 2005), we conducted experiments under similar growth conditions as used previously.

2. Materials and methods

2.1. Plant material and growth conditions

All experiments were conducted twice under controlled environment conditions at the University of Calgary, Canada. Canola (*B. napus* L. cv. 46A65, Pioneer Hi-Bred Ltd., Chatham, Ont., Canada) plants were grown in 0.3 L plant starter cell paks (Agri-Growth International Inc., Edmonton, AB, Canada) containing a soil mixture of peat moss, Perlite, Vermiculite and Terragreen (2:1:1:0.25, v/v/v/v). Plants were grown in a growth chamber (Model PGR15, Conviron, Controlled Environments Ltd., Winnipeg, MB, Canada) with day and night temperatures of 24 and 18 °C, respectively, and relative humidity of 60–70%. Light was provided by a mix of cool white fluorescent tubes (Philips F72T12/CW/VHO, Philips Lighting Company, Somerset, NJ, USA) and Philips 60 W incandescent lamps (Philips Electronics Ltd., Markham, Ont., Canada) on a 16 h photoperiod. The photosynthetically active photon flux density (PPFD), measured with a quantum LI-185B radiometer/photometer (LI-COR, Inc., Lincoln, NE, USA), was 260 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the shoot apex. The irradiance, which was similar to that of our previous experiments, was kept constant by adjusting distance between plants and light banks.

2.2. UVB irradiation and CO₂ concentration

Similar to our previous studies, we wanted to examine the effects of UVB radiation and CO₂ concentration on older reproductive plants and reproductive yield, but not on young plants. Therefore, equal sizes of 30-day-old plants, which were about to flower, were transferred to 1 L pots (Kord Products Inc., Toronto, Ont., Canada) and were placed under four experimental conditions: (a) UVB radiation ($4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$) with ambient level of CO₂ ($370 \mu\text{mol mol}^{-1}$) (control), (b) UVB radiation ($4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$) with elevated level of CO₂ ($740 \mu\text{mol mol}^{-1}$), (c) no UVB radiation ($0 \text{ kJ m}^{-2} \text{ d}^{-1}$) with ambient level of CO₂ ($370 \mu\text{mol mol}^{-1}$), and (d) no UVB radiation ($0 \text{ kJ m}^{-2} \text{ d}^{-1}$) with elevated level of CO₂ ($740 \mu\text{mol mol}^{-1}$). Many previous studies (e.g., Searles et al., 1995; Saile-Mark and Tevini, 1997; Mazza et al., 1999; Zavala and Botto, 2002) that examined the effects of ambient UVB radiation on plants have used only two levels (+UVB and -UVB). This protocol was followed in this experiment. The experiment was conducted in two Conviron growth chambers, each containing two equal size Plexiglas cabinets with 110 cm height, 75 cm width and 70 cm depth. In each cabinet, the top surface was made of Acrylite OP-4 (ultraviolet transmitting) and the upright sides were made of Acrylite FF (ultraviolet absorbing) (GE Polymershapes, Calgary, AB, Canada). Within each chamber, one cabinet was supplied with $370 \mu\text{mol mol}^{-1}$ CO₂ and the other with $740 \mu\text{mol mol}^{-1}$ CO₂.

Only one of the chambers was supplemented with UVB radiation. Ultraviolet radiation was supplied by fluorescent lamps (UVB 313EL, Q-Panel, Cleveland, OH, USA), which were placed on the top of cabinets. The lamps were first preburnt for 96 h to stabilize the UVB output, and then wrapped in 125 μm cellulose diacetate (JCS Industries Inc., La Mirada, CA, USA) to filter radiation below 280 nm. Before use, filters were exposed to UVB radiation for 10 h to stabilize transmittance (Adamse and Britz, 1992). Filters were changed once per week. From flowering to seed maturation, daily UVB radiation for 8 h, from 08:00 to 16:00 h, was $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$, which represents a medium duration. The chamber with no UVB was identical to the one with UVB, except that the plants received *ca.* $0.03 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB radiation. Biologically effective UVB radiation (UVB_{BE}) was measured with a PMA2100 photometer/radiometer, which was calibrated against a National Institute of Standards and Technology traceable standard (Solar Light Co., Inc., Philadelphia, PA, USA). The UVB_{BE} was estimated using Caldwell's (1971) generalized plant damage action spectrum normalized to 300 nm. The total daily dose of UVB_{BE} used in this experiment is within the range of natural solar UVB levels ($3.1\text{--}5.4 \text{ kJ m}^{-2} \text{ d}^{-1}$) measured (June–August, 1992–2001) for Alberta (WOUDC, 2001).

Inside the cabinets, photoperiod, day and night temperatures, relative humidity, and the photosynthetically active photon flux density were the same as the initial growth conditions (see above). Plants were watered with tap water daily and fertilized with N–P–K (20:20:20) weekly. To minimize positional effects, plants were rotated twice a week.

2.3. Siliqua and seed collection

From five plants grown under 0 and $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB radiation at ambient or elevated CO₂, maturing siliquas and seeds were harvested 25 days after anthesis (DAA) to determine photosynthetic and UV-screening pigments, and surface and transverse structures of siliquas and seeds. At the end of the experiment, mature siliquas were harvested from 10 intact plants grown under each of the 4 conditions to determine siliqua and seed characteristics.

2.4. Siliqua and seed characteristics

Five fully matured siliquas were selected randomly from each of 10 plants grown under each of the 4 conditions. Seeds were removed from each siliqua by shaking, and separated into sound (fully developed) and aborted (not developed) categories. From each siliqua, one sound seed was selected randomly. The seeds were weighed by means of a Sartorius electrobalance (Model H51, Sartorius GmbH, Goettingen, Germany). To determine the weight of testas and embryos, seeds were soaked in 96-well plates containing water and kept in the dark at room temperature for 48 h. The naturally separated testas were air dried and weighed, and their weight was subtracted from the total seed weight to obtain the embryo weight. The weight of seed components was reported in milligrams.

2.5. Measurement of gas exchange parameters

Net CO₂ assimilation (A_N) and transpiration (E) were determined for maturing siliquas (25 DAA) developed under 0 and $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB radiation at ambient or elevated CO₂ concentration under $260 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PPFD by means of an infra-red gas analyzer (IRGA, CI-310 Portable Photosynthesis System, CID, Inc., Camas, WA, USA). Prior to measurements, the IRGA was calibrated with a known CO₂ concentration. Three replications were used for each measurement. Values for net CO₂ assimilation ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) and water-use efficiency (WUE) ($\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$) were calculated based on the total siliqua surface area within the IRGA chamber.

2.6. Measurement of chlorophyll fluorescence

Chlorophyll fluorescence was measured at the surface of maturing siliquas with a modulated chlorophyll fluorometer (Model OS1-FL, Opti-Sciences, Tyngsboro, MA, USA). Fluorescence readings were taken on four siliquas from each growth condition. The quantum yield of Photosystem II (PS II) electron transport during steady state photosynthesis (Y) was measured in light-adapted siliquas under light condition, whereas the ratio of variable to maximal fluorescence (F_v/F_m) was measured in dark-adapted siliquas that had been enclosed in aluminum foil for 30 min. The saturating light pulse was delivered at $260 \mu\text{mol m}^{-2} \text{ s}^{-1}$ for 0.8 s.

2.7. Analysis of photosynthetic and UV-screening pigments

Chlorophyll (Chl) and UV-screening pigments were determined in maturing siliquas, which were harvested from plants grown under 0 and $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB radiation at ambient or elevated CO₂ concentration. Three samples of 0.1 g tissue of siliqua wall were weighed and placed in a vial with 5 mL of dimethyl sulphoxide. The samples were heated in a water bath at 65 °C for 60 min, for a complete extraction of chlorophyll (Hiscox and Israelstam, 1979). The absorbance of the extracts was measured at 645 and 663 nm against a dimethyl sulphoxide blank with a Ultrospec 3100 *pro* UV/VIS spectrophotometer (Model: Ultrospec 3100 *pro*, Biochrom Ltd., Cambridge, UK) and the concentrations of chlorophyll (Chl) *a* and *b* were estimated using the equations of Arnon (1949). UV-screening pigments were extracted from three fresh siliqua discs (0.55 cm^2) with 5 mL of MeOH:H₂O:HCl (79:20:1, v/v/v) solution in 15-mL test tubes (Robberecht and Caldwell, 1986). Samples were heated in a water bath at 85 °C for 40 min, cooled to room temperature and centrifuged for 15 min at $2000 \times g$ (IEC Model CL centrifuge, International Equipment Company, Needham Heights, MA, USA). From each sample, the supernatant was transferred to a 25-mL test tube, brought up to 15 mL with the extraction medium and 1 mL was used to determine concentration of UV-screening pigments by measuring absorbance at 300 nm (A_{300}). Due to the accumulation of flavonoids and other UV-screening compounds, absorbance at 300 nm has often been used to measure the UV-screening capacity of plant tissues (Day,

1993; Liakoura et al., 2001; Dai et al., 2004; Griffen et al., 2004). The absorbance was expressed on a unit disc area.

2.8. Extraction of siliqua surface wax

Siliqua surface wax was extracted using a modification of the method as described by Qaderi et al. (2002). For extraction of siliqua surface wax, five replicates of single siliqua were taken from each growth condition and their fresh weight was determined by means of a Sartorius electrobalance shortly after detaching from plants. Each siliqua was immersed in 20 mL trichloromethane for 30. The siliquas were let to dry for few minutes, split longitudinally into two equal halves and their surface area was measured by means of a ΔT area meter (Delta-T Devices Ltd., Burwell, Cambridge, UK). The solutions obtained were filtered, evaporated to dryness and the resulting yields were determined gravimetrically. The wax content was expressed on the basis of either siliqua surface area ($\mu\text{g mm}^{-2}$) or siliqua fresh weight ($\mu\text{g mg}^{-1}$).

2.9. Light microscopy of morphological features

The surfaces of maturing siliquas that developed under 0 and $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB radiation at ambient or elevated CO_2 concentration were examined by means of a Leica M-Series stereo-microscope (Model Leica MZ125, Leica Microsystems Ltd., Wetzlar, Germany) and their images were captured by means of a Canon PowerShot S45 digital camera (Canon Inc., Tokyo, Japan) attached to the microscope.

2.10. Light microscopy of anatomical structures

Maturing siliquas that developed under 0 and $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB radiation at ambient or elevated CO_2 were harvested (25 DAA) and hand sectioned to study the pigmented areas as a result of the UVB treatment. Free-hand sections were prepared according to the method detailed by Yeung (1998) using double-sided stainless steel razor blades. The fresh hand-sections were stained using toluidine blue O (Yeung, 1998). All sections were viewed under an Aristoplan photomicroscope (Leitz, Wetzlar, Germany) and the images were captured using a Leica DFC 480 digital camera (Leica Microsystems AG, Wetzlar, Germany).

2.11. Scanning electron microscopy of seeds

Surfaces of seeds that were matured under 0 and $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB radiation at ambient or elevated CO_2 concentration were examined by means of a Philips XL30 ESEM-TMP scanning electron microscope (FEI Company, Portland, OR, USA), at accelerating voltage of 20 kV. To view the fine structures with SEM, seeds were attached to specimen stubs using double-coated tape and dried to critical point. The specimens were coated with gold using a Technics Hummer I sputter coater (Anatech Ltd., Union City, CA, USA) under a 50 mTorr vacuum, a current of 10 mA, and an argon environment for 3.5 min.

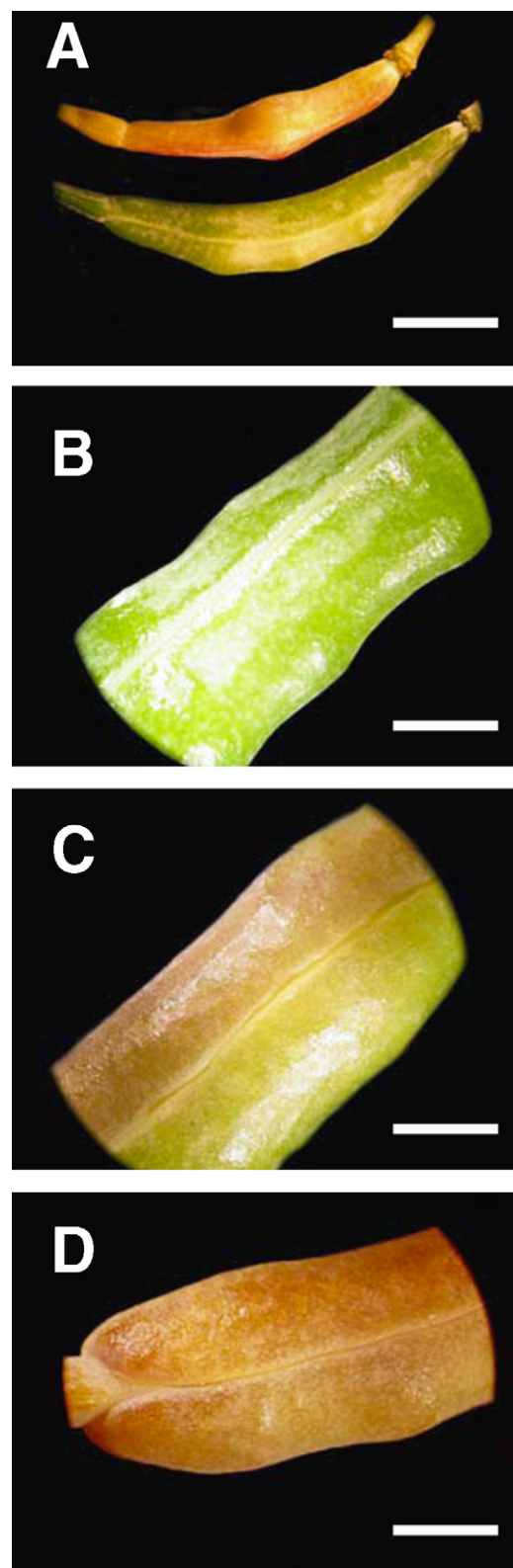


Fig. 1. Surface views of maturing siliquas of canola (*Brassica napus* cv. 46A65) developed under two levels of UVB radiation. (A) Upper, an orange siliqua from $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB; lower, a green siliqua from $0 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB, (B) a siliqua from $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB with less exposure, (C) a siliqua from $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB with half exposure, and (D) a siliqua from $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB with full exposure. Scale bars: (A) 6.4 mm; (B–D) 2.2 mm.

2.12. Statistical analyses

Morphological, physiological and chemical characteristics of siliquas and seeds that developed under 0 and $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB radiation at ambient or elevated CO_2 concentration were analysed by means of a two-way analysis of variance (ANOVA), followed by a one-way ANOVA. All one-way ANOVAs were accompanied by Tukey's multiple comparison test to determine differences among treatments for each characteristic (Zar, 1999; Minitab Inc., 2004). Also, Pearson's correlation (r) was used to show the relationship between each of the morphological, physiological and chemical characteristics (Zar, 1999; Minitab Inc., 2004).

3. Results

3.1. Structural and physical characteristics of siliquas and seeds

UVB radiation affected the outer appearance of siliquas (Fig. 1A). Only comparisons between the control ($4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB at ambient CO_2) and the UVB radiation of $0 \text{ kJ m}^{-2} \text{ d}^{-1}$ at ambient CO_2 are reported here, as there were no significant effects of CO_2 . Colour of some siliquas, which were under more intense UVB radiation, was gradually changed from green to orange (Fig. 1B–D), was accompanied by collapsing of the epidermal layer (see Fig. 2C and D). Anatomical structures of siliqua wall were also affected by UVB radiation (Fig. 2). Transverse sections of the siliqua wall revealed differences in cell size and shape between maturing siliquas developed under 0 and $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB radiation at ambient or elevated CO_2 . Only comparisons between the control ($4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB at ambient CO_2) and the UVB radiation of $0 \text{ kJ m}^{-2} \text{ d}^{-1}$ at ambient CO_2 are reported here. The epidermal layer was intact under $0 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB (Fig. 2A) or under $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB, when there was less exposure (Figs. 1B and 2B), and partially intact, when there was half exposure (Figs. 1C and 2C), but collapsed when there was full exposure (Figs. 1D and 2D). The siliqua wall from the UVB radiation of $0 \text{ kJ m}^{-2} \text{ d}^{-1}$ had fewer cell layers with few larger cells in each layer (Fig. 2A), whereas those from the UVB radiation of $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$, under half or full exposure, had more cell layers with many smaller cells in each layer (Fig. 2C and D). Also, under $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB radiation, sub-epidermal cells of the exposed siliqua wall were elongated (Fig. 2D) compared to those under $0 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB radiation (Fig. 2A).

Scanning electron microscopy of seed surface showed some differences between seeds matured under 0 and $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB radiation at both CO_2 concentrations. Here, we show comparisons between the control ($4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB at ambient CO_2) and the UVB radiation of $0 \text{ kJ m}^{-2} \text{ d}^{-1}$ at ambient CO_2 . Seeds that matured under $0 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB radiation had fewer depressions (Fig. 3A) than those matured under $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB radiation (Fig. 3B). The depressions were wide and shallow for seeds matured under $0 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB radiation (Fig. 3C), but narrow and deep for those matured under $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB radiation (Fig. 3D). A detailed

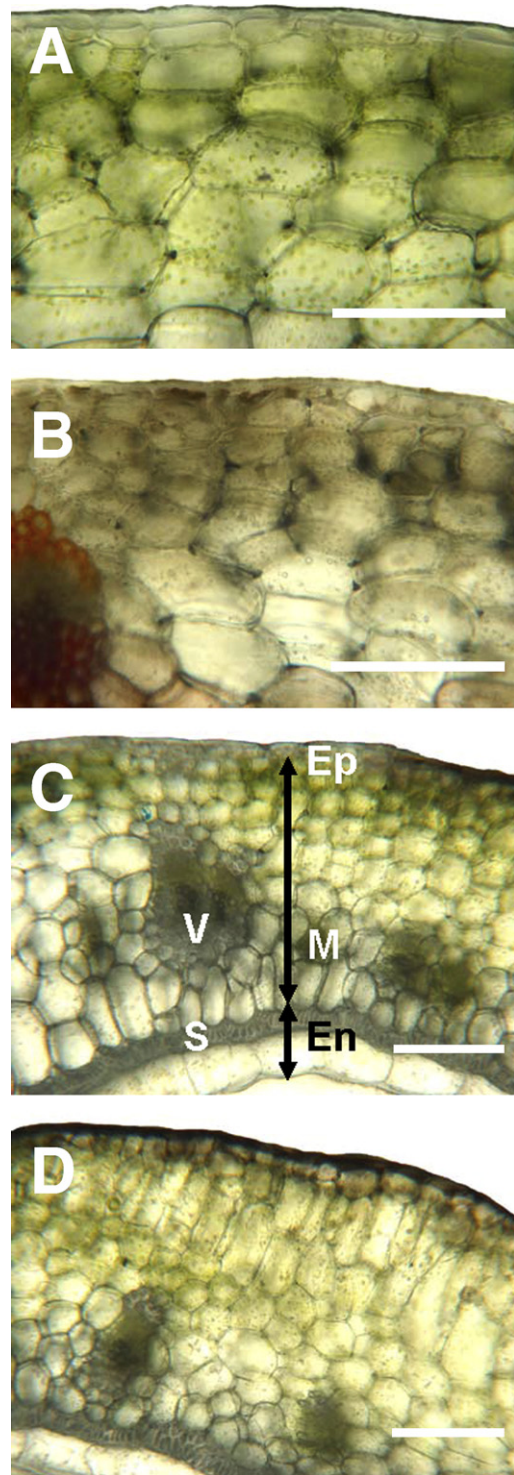


Fig. 2. Transverse views of free-hand sections of siliqua wall from maturing siliquas of canola (*B. napus* cv. 46A65) developed under two levels of UVB radiation that were captured by a photomicroscope. (A) A section from $0 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB, (B) a section from $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB with less exposure, (C) a section from $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB with half exposure, and (D) a section from $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB with full exposure. The epidermis (Ep), mesocarp (M), vascular bundle (V), sclerenchyma (S) and endocarp (En) are shown. Scale bars: $50 \mu\text{m}$.

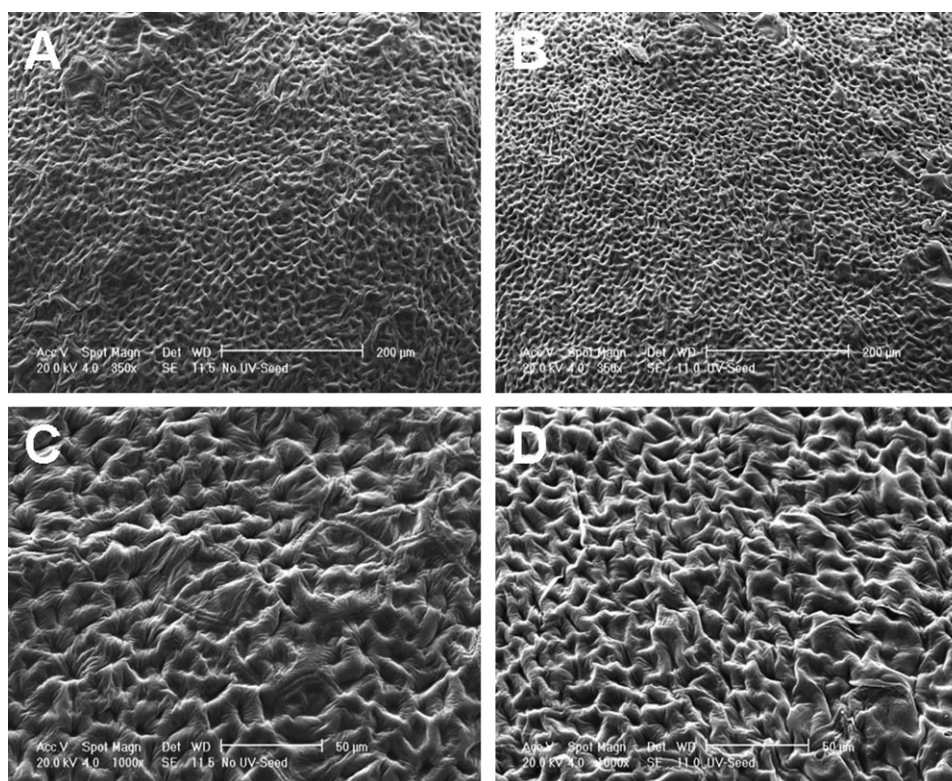


Fig. 3. Scanning electron microscopy of canola (*B. napus* cv. 46A65) seeds matured under two levels of UVB radiation. (A and C) a seed matured under $0 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB, and (B and D) a seed matured under $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB. Magnification: (A and B) $350\times$; (C and D) $1000\times$.

measurement of depressions in several micrographs showed that the number of surface depressions was an average of 6.2 mm^{-2} for seeds matured under $0 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB, whereas it was 7.8 mm^{-2} for seeds matured under $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB radiation. This difference accounted for about 26% more surface depressions for seeds from the UVB radiation of $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$.

UVB radiation significantly decreased the weight of intact seeds and embryos, but had no effect on the weight of testas (Table 1). Compared with the control ($4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB at ambient CO_2), seeds (intact seed or embryo) that matured under $0 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB at ambient or elevated CO_2 were heavier (Table 1). The testa/embryo weight ratio was not affected by CO_2 . The two-way interaction between UVB and CO_2 was not significant for seed components (Table 1).

3.2. Gas exchange parameters

Both UVB radiation and CO_2 concentration affected net CO_2 assimilation (A_N) and water use efficiency (WUE) (Table 2). UVB radiation significantly decreased A_N and WUE at both ambient and elevated CO_2 , but had no effect on transpiration (E). Compared with the control ($4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB at ambient CO_2), siliques that developed under $0 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB at ambient or elevated CO_2 had higher A_N and WUE, and only those from elevated CO_2 had lower E . Elevated CO_2 significantly increased A_N and WUE, but decreased E , under both UVB radiations (Table 2). Compared with the control ($4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB at ambient CO_2), siliques that developed under $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB at elevated CO_2 had higher

Table 1

Physical characteristics of canola (*Brassica napus* cv. 46A65) seeds matured under two levels of UVB radiation and two levels of CO_2 concentration in controlled environment chambers

UVB ($\text{kJ m}^{-2} \text{ d}^{-1}$)	CO_2 ($\mu\text{mol mol}^{-1}$)	Seed component			
		Intact seed (mg)	Testa (mg)	Embryo (mg)	Testa/embryo weight ratio
0.0	370	4.35 ± 0.12 a	0.64 ± 0.03 a	3.71 ± 0.11 a	0.17 ± 0.01 a
	740	4.55 ± 0.10 a	0.68 ± 0.02 a	3.87 ± 0.09 a	0.18 ± 0.01 a
4.2	370	3.63 ± 0.11 b	0.59 ± 0.02 a	3.03 ± 0.10 b	0.20 ± 0.01 a
	740	4.01 ± 0.09 b	0.66 ± 0.03 a	3.35 ± 0.08 b	0.20 ± 0.01 a
P (UVB)		<0.001	0.231	<0.001	0.004
P (CO_2)		0.008	0.035	0.016	0.643
P (UVB \times CO_2)		0.399	0.610	0.421	0.923

Values are mean \pm S.E. ($n = 80$). Values followed by different letters within columns are significantly different ($P < 0.05$) according to Tukey's multiple comparison test. Weights were determined for completely matured seeds after harvest.

Table 2
Net CO₂ assimilation (A_N), transpiration (E) and water use efficiency (WUE) for maturing siliquas of canola (*B. napus* cv. 46A65) grown under two levels of UVB radiation and two levels of CO₂ concentration in controlled environment chambers

UVB (kJ m ⁻² d ⁻¹)	CO ₂ (μmol mol ⁻¹)	Physiological parameter		
		A_N (μmol CO ₂ m ⁻² s ⁻¹)	E (mmol H ₂ O m ⁻² s ⁻¹)	WUE (μmol CO ₂ mmol H ₂ O ⁻¹)
0.0	370	2.14 ± 0.09 b	0.81 ± 0.02 a	2.64 ± 0.06 c
	740	3.13 ± 0.08 a	0.63 ± 0.01 b	4.94 ± 0.05 a
4.2	370	1.82 ± 0.04 c	0.84 ± 0.03 a	2.17 ± 0.09 d
	740	2.35 ± 0.05 b	0.69 ± 0.02 b	3.43 ± 0.07 b
P (UVB)		<0.001	0.091	<0.001
P (CO ₂)		<0.001	<0.001	<0.001
P (UVB × CO ₂)		0.010	0.606	<0.001

Values are mean ± S.E. ($n=6$). Values followed by different letters within columns are significantly different ($P<0.05$) according to Tukey's multiple comparison test. Measurements were performed on the maturing siliquas 25 days after anthesis (DAA).

A_N and WUE, but lower E . The two-way interaction between UVB and CO₂ was significant for A_N and WUE (Table 2), suggesting that these parameters were highest under 0 kJ m⁻² d⁻¹ of UVB at elevated CO₂ and lowest under 4.2 kJ m⁻² d⁻¹ of UVB at ambient CO₂.

3.3. Chlorophyll fluorescence

UVB radiation significantly decreased the ratio of variable to maximal fluorescence under steady-state photosynthetic conditions (Y) and the ratio of variable to maximal fluorescence in dark-adapted siliquas (F_v/F_m) ($P<0.001$) (Fig. 4). Compared with the control (4.2 kJ m⁻² d⁻¹ of UVB at ambient CO₂), siliquas that developed under 0 kJ m⁻² d⁻¹ of UVB at ambient or elevated CO₂ had higher chlorophyll fluorescence (Y and F_v/F_m). Elevated CO₂ did not affect F_v/F_m under both UVB radiations, but increased Y under 0 kJ m⁻² d⁻¹ of UVB ($P<0.001$) (Fig. 4). The two-way interaction between UVB and CO₂ was not significant for chlorophyll fluorescence ($P>0.05$).

3.4. Photosynthetic and UV-screening pigments

UVB radiation significantly decreased total chlorophyll (Chl), Chl *a* and Chl *b* at both ambient and elevated CO₂, but had no effect on the ratio of Chl *a/b* and the concentration of UV-screening pigments (Table 3). Compared with the control (4.2 kJ m⁻² d⁻¹ of UVB at ambient CO₂), siliquas that devel-

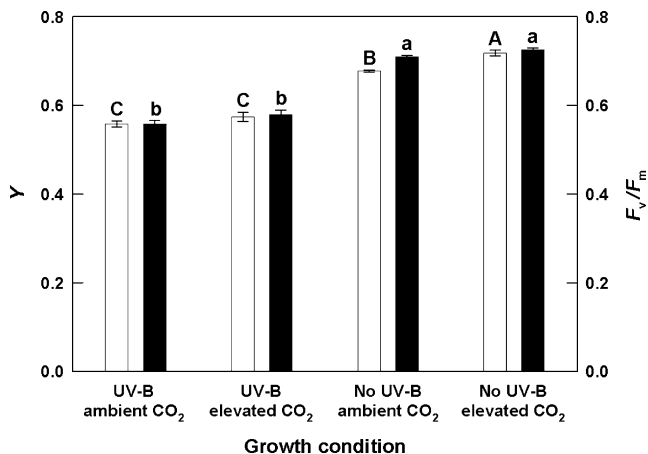


Fig. 4. Chlorophyll fluorescence (Y and F_v/F_m) for 25-day-old (days after anthesis) maturing siliquas of canola (*B. napus* cv. 46A65) developed under two levels of UVB radiation and two levels of CO₂ concentration in controlled environment chambers. Open bars represent Y and closed bars F_v/F_m . Bars are mean ± S.E. ($n=8$). Bars surmounted by different upper-case or lower-case letters within chlorophyll fluorescence parameters (Y and F_v/F_m , respectively) are significantly different ($P<0.05$) according to Tukey's multiple comparison test.

oped under 0 kJ m⁻² d⁻¹ of UVB at ambient or elevated CO₂ had higher total Chl, Chl *a* and Chl *b*. Elevated CO₂ increased total Chl and the concentration of UV-screening pigments under 4.2 kJ m⁻² d⁻¹ of UVB radiation. Compared with the control (4.2 kJ m⁻² d⁻¹ of UVB at ambient CO₂), siliquas that devel-

Table 3
Chlorophyll (μg mL⁻¹ extract) and UV-screening pigments (A_{300} , cm⁻²) in maturing siliquas of canola (*B. napus* cv. 46A65) grown under two levels of UVB radiation and two levels of CO₂ concentration in controlled environment chambers

UVB (kJ m ⁻² d ⁻¹)	CO ₂ (μmol mol ⁻¹)	Pigment				
		Total chlorophyll	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Chlorophyll <i>a:b</i>	UV-screening pig.
0.0	370	0.27 ± 0.012 ab	0.21 ± 0.009 ab	0.06 ± 0.003 ab	3.56 ± 0.067 a	0.23 ± 0.016 ab
	740	0.31 ± 0.021 a	0.25 ± 0.018 a	0.07 ± 0.004 a	3.61 ± 0.203 a	0.23 ± 0.007 ab
4.2	370	0.15 ± 0.006 c	0.12 ± 0.005 c	0.03 ± 0.001 c	3.75 ± 0.047 a	0.15 ± 0.012 b
	740	0.22 ± 0.017 b	0.17 ± 0.013 bc	0.05 ± 0.006 bc	3.74 ± 0.330 a	0.25 ± 0.034 a
P (UVB)		<0.001	<0.001	<0.001	0.451	0.168
P (CO ₂)		0.006	0.008	0.015	0.929	0.038
P (UVB × CO ₂)		0.356	0.391	0.381	0.892	0.044

Values are mean ± S.E. ($n=6$). Values followed by different letters within columns are significantly different ($P<0.05$) according to Tukey's multiple comparison test. Measurements were performed on the maturing siliquas 25 days after anthesis (DAA).

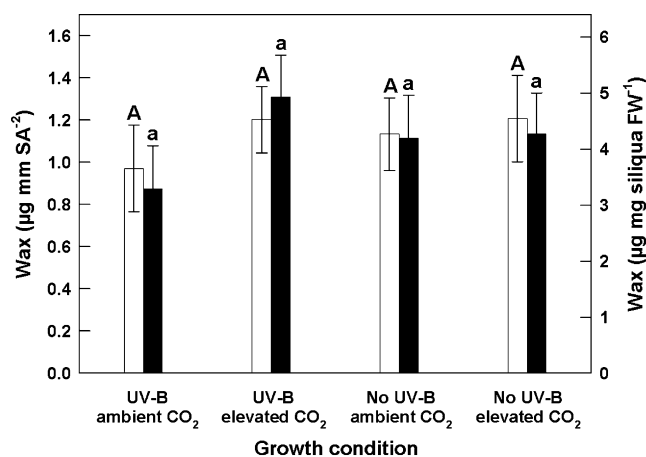


Fig. 5. Wax content of maturing siliques of canola (*B. napus* cv. 46A65) developed under two levels of UVB radiation and two levels of CO₂ concentration in controlled environment chambers. Wax content, obtained from siliques after 25 days of anthesis (DAA), is shown on the basis of either siliqua area (SA) (open bars) or siliqua fresh weight (FW) (closed bars). Bars are mean \pm S.E. ($n=10$). Bars surmounted by different upper-case or lower-case letters within wax contents (SA⁻¹ and siliqua FW⁻¹, respectively) are significantly different ($P<0.05$) according to Tukey's multiple comparison test.

oped under 4.2 kJ m⁻² d⁻¹ of UVB at elevated CO₂ had higher total Chl and UV-screening pigments. The two-way interaction between UVB and CO₂ was not significant for the photosynthetic pigments, but was significant for the UV-screening pigments (Table 3), suggesting that the concentration of UV-screening pigments was highest under 4.2 kJ m⁻² d⁻¹ of UVB at elevated CO₂ and lowest under the same UVB treatment at ambient CO₂.

3.5. Siliqua surface wax

No significant differences were found among treatments in wax content either on the basis of siliqua area or siliqua fresh weight ($P>0.05$) (Fig. 5). The two-way interaction between UVB and CO₂ was not significant for the wax content ($P>0.05$).

3.6. Relationship between morphological, physiological and chemical characteristics

Pearson's correlation analysis revealed many significant relationships between different morphological, physiological and chemical parameters of siliques and seeds that developed under 0 and 4.2 kJ m⁻² d⁻¹ of UVB radiation at ambient or elevated CO₂. For instance, the intact seed weight was positively correlated with total Chl ($r=0.999$, $P=0.001$), Chl *a* ($r=0.999$, $P=0.001$), Chl *b* ($r=1.000$, $P<0.001$) and embryo weight ($r=0.999$, $P=0.001$). The embryo weight had positive correlations with F_v/F_m ($r=0.962$, $P=0.038$), total Chl ($r=0.996$, $P=0.004$), Chl *a* ($r=0.995$, $P=0.005$) and Chl *b* ($r=0.998$, $P=0.002$). Correlation between the testa weight and the wax content, on per area basis, was positive ($r=0.979$, $P=0.021$). Also, correlation between the weight ratio of testa/embryo and Chl *a/b* was positive ($r=0.996$, $P=0.004$).

4. Discussion

Previously, we reported that plant height and seed yield of canola can be affected negatively by UVB radiation (4.2 kJ m⁻² d⁻¹), particularly at the ambient level of CO₂ (370 $\mu\text{mol mol}^{-1}$). Also, we demonstrated that elevated level of CO₂ (740 $\mu\text{mol mol}^{-1}$) can partially ameliorate some of the adverse effects of UVB on seed yield, but it cannot bring the yield to the level produced by plants in the absence of UVB radiation (0 kJ m⁻² d⁻¹) at ambient CO₂ (Qaderi and Reid, 2005). Most previous studies that discussed the effects of UVB radiation on seed yield considered plant vegetative parts and their related morphological and physiological changes, which may affect yield (see Day and Neale, 2002; Kakani et al., 2003). Only a few studies considered the effects of UVB radiation on reproductive parts (Grammatikopoulos et al., 1998; Sampson and Cane, 1999; Griffen et al., 2004; Koti et al., 2004). In this study, therefore, we explored the effects of UVB radiation on siliques, and its impacts on seed yield during reproductive stage of canola.

Visual observation and microscopy examination of siliques revealed some differences between siliques that developed under 0 kJ m⁻² d⁻¹ of UVB and those developed under 4.2 kJ m⁻² d⁻¹ of UVB radiation (Figs. 1 and 2). Over time changes in siliqua colour indicate that the effects of UVB radiation may be cumulative and that during changing plant developmental stages, the detrimental effects of UVB increase in severity. If under enhanced and prolonged UVB radiation, siliques are not protected well by wax layer and UV-screening pigments, chlorophyll degradation and collapsing of epidermal cells may occur and, as a result, siliques lose their green colour and cannot assimilate CO₂ properly. In this situation, damage to photosynthetic functions can be translated into lower yield (Tevini, 2000). However, multi layers of photosynthetic cells (Fig. 2C and D) may allow compensatory adjustments to the UV-induced damage, as found in pea leaves following UVB exposure (Day and Vogelmann, 1995). Many narrow and deep depressions on the surfaces of UVB exposed seeds (Fig. 3B and D) may help to protect them against the detrimental effects of UVB radiation by extending or diverting the light pathway. Lower weight of intact seeds and embryos matured under 4.2 kJ m⁻² d⁻¹ of UVB than those matured under 0 kJ m⁻² d⁻¹ of UVB (Table 1) suggests that the defense layers of siliques were not strong enough to fully protect developing seeds from the harmful effects of incoming UVB radiation, even at elevated CO₂.

Regardless of CO₂ concentration, UVB radiation decreased A_N and WUE (Table 2). The adverse effects were greater at elevated CO₂ (24.9 and 30.6%, respectively) than at ambient CO₂ (14.9 and 17.8%, respectively). Also, the positive effects of elevated CO₂ on A_N and WUE were lower under 4.2 kJ m⁻² d⁻¹ of UVB radiation (Table 2). At elevated CO₂, A_N and WUE were increased by 31.6 and 46.6%, respectively, under 0 kJ m⁻² d⁻¹ of UVB, and by 22.5 and 36.7%, respectively, under 4.2 kJ m⁻² d⁻¹ of UVB radiation. These findings indicate the negative effects of UVB radiation on siliqua photosynthesis and the production of assimilates. Lower A_N for siliques that developed under 4.2 kJ m⁻² d⁻¹ of UVB radiation

could be due to damage to chloroplasts and changes in photosynthetic apparatus (Sullivan and Rozema, 1999), which might have caused reduced seed size. Also, lower chlorophyll fluorescence for siliques that developed under $4.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ of UVB at both ambient and elevated CO_2 (Fig. 4) indicates that UVB radiation might have damaged the D1 and D2 proteins of PS II (Olsson et al., 2000) and degraded chlorophyll, which might have resulted in reduced quantum efficiency or lower photosynthetic capacity (Sullivan, 1997). In the case of silique photosynthesis, chlorophyll has a crucial role in the production of assimilates, because during this reproductive stage, it is essential for photosynthesis (Jones, 1992). It has been shown that siliques can participate in photosynthesis at the later stages of plant life when leaves turn yellow and do not function properly (Chongo and McVetty, 2001). Thus, changes in chemical properties of siliques may play a direct role on seed yield. As a result of decreased chlorophyll (total Chl, Chl *a* and Chl *b*) in the UVB exposed siliques (Table 3), less assimilates might have been translocated to the developing seeds (Major et al., 1978), and manifested themselves in small seeds. On the basis of Pearson's correlation, the weights of intact seeds and embryos were positively correlated with total Chl ($r=0.999$ and 0.996 , respectively), Chl *a* ($r=0.999$ and 0.995 , respectively), and Chl *b* ($r=1.000$ and 0.998 , respectively) of the siliques. These strong relationships reveal the importance of silique chlorophyll on seed weight.

It is most likely that UV-screening pigments (Table 3) and surface wax content (Fig. 5) did not play a significant role in the protection of siliques and, to some extent, seeds against UVB radiation. However, an increased production of epicuticular wax (Qaderi and Reid, 2005) and UV-screening pigments in vegetative parts of this species have been reported previously (Cen and Bornman, 1993; Olsson et al., 1998; Qaderi and Reid, 2005).

In summary, UVB radiation can exert a significantly negative impact on the performance of canola silique, and, in turn, affect seed quality. However, under the highly controlled and consequently somewhat artificial conditions of our growth chambers, elevated CO_2 can partially mitigate some of the adverse effects of UVB on this reproductive organ. This topic requires further investigation in order to reach a firm conclusion about the effects of UVB radiation on the reproductive efforts of plants at the ambient or elevated levels of CO_2 . Therefore, multi-factorial experiments should be designed to incorporate UVB radiation, as a factor, to the role of silique on seed quality and quantity. Multi-factorial studies, conducted under natural environmental conditions, may refine our understanding in the effects of global climate change on plants.

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