



Differential sensitivity of canola (*Brassica napus*) seedlings to ultraviolet-B radiation, water stress and abscisic acid

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ARTICLE INFO

Article history:

Received 16 July 2008
Received in revised form
23 December 2008
Accepted 4 March 2009

Keywords:

Abscisic acid
Brassica napus (canola)
Chemical attributes
Growth
Ultraviolet-B radiation
Water stress

ABSTRACT

Responses of canola (*Brassica napus* L.) seedlings to three ultraviolet (UV)-B levels [0 (zero), 5 (ambient) and 10 (enhanced) $\text{kJ m}^{-2} \text{d}^{-1}$], two watering regimes (well-watered and water-stressed), and two abscisic acid (ABA) levels (with and without application) were investigated. Overall, enhanced UVB and water stress negatively affected plant growth and physiology, but ABA had very little effect. Enhanced UVB decreased stem height, leaf area, plant dry matter, water use efficiency and wax content, but increased concentrations of chlorophyll *a*, carotenoids and flavonoids, and ethylene evolution. Water stress reduced stem height and diameter, leaf area, plant dry matter, leaf weight ratio and shoot:root weight ratio under zero and ambient UVB. Water stress also reduced chlorophyll *a* and carotenoids in plants exposed to enhanced UVB. ABA with watering regime had significant interactive effects only on leaf dry matter and wax content. We found that enhanced UVB and water stress adversely affected *B. napus* seedlings. Interaction between these two factors affected plant performance. In this interaction, ABA had little significant role. Also, optimum vegetative growth and biomass were achieved under ambient UVB.

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1. Introduction

Solar electromagnetic radiation contains UV in the range 200–400 nm, and this range includes UVB (280–320 nm) radiation (Frohnmeyer and Staiger, 2003). Due to release of anthropogenic pollutants, such as chlorofluorocarbons, the thickness of the ozone layer has decreased in the last 60 years (Pyle, 1996). Ozone depletion has resulted in increased UVB radiation reaching the Earth's surface with serious implications for all organisms (McKenzie et al., 2007). Enhanced UVB radiation has many effects on plant morphology, physiology and development, and its impacts on growth and development are seen in many plant species (Frohnmeyer and Staiger, 2003). UVB radiation targets three important features of plant cells: the genetic system (e.g., DNA), photosynthesis, and membranes (Björn, 1996). Also, UVB radiation has been shown to increase (Poulson et al., 2006), decrease (Qaderi et al., 2007) or have no effect on chlorophyll content (Cechin et al., 2007). Much of UVB radiation is attenuated in leaves by leaf cuticles, by UV-absorbing compounds produced and deposited in leaf epidermal cells or hairs (Manetas, 2003) or by antioxidant systems (Jenkins and Brown,

2007). The physiological relevance of UV-absorbing compounds as UVB “sunscreen” has previously been shown (Landry et al., 1995; Bieza and Lois, 2001). However, the effects of UV-absorbing compounds are species specific, as either increase (Poulson et al., 2006) or no change (Cechin et al., 2007) in their concentration have been reported. UVB radiation enhances ethylene evolution (A.-H.-Mackerness et al., 1999), which is also increased in response to water stress (Ingram and Bartels, 1996).

Water stress negatively affects many plant processes, such as photosynthesis, transpiration, stomatal conductance, and metabolite accumulation (Larcher, 2003; Ohashi et al., 2006), and causes substantial reductions in plant productivity (Yordanov et al., 2000; Reddy et al., 2004). Plant responses to water stress include morphological and biochemical changes, resulting in acclimation in non-severe cases, and damage and loss of plant parts, in severe cases (Chaves et al., 2002).

Abscisic acid is widely recognized as the key triggering agent of stomatal closure, but it may have other roles to play in the adaptation of plants to UVB radiation (Yang et al., 2000, 2005) or water stress (Quarrie, 1984; Reddy et al., 2004; Yang et al., 2005; Qaderi et al., 2006). Earlier studies have shown the importance of endogenous ABA in limiting ethylene production (Yang and Hoffman, 1984). The restriction of ethylene production may be a widespread function of ABA, and that endogenous ABA may often function to maintain rather than inhibit plant growth (Sharp, 2002).

A recent study (Duan et al., 2008) has shown the interactive effects of UVB, drought and ABA on growth and biomass allocation

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of a tree species. Plants exposed to UVB have been shown to be more tolerant to water stress (Nogués et al., 1998; Poulson et al., 2006) and ABA is involved in many aspects of a plant's response to water stress (Yang et al., 2005). Thus, the main objective of this study was to investigate if exogenous ABA could influence the interactive effects of UVB and water stress in *B. napus*. Since water-stressed plants should have higher levels of ABA than the well-watered plants, we hypothesized that the effects of ABA could be greater for the well-watered plants than for the water-stressed plants.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of canola (*Brassica napus* L. cv. 46A65, Pioneer Hi-Bred Ltd., Chatham, ON, Canada) were soaked in water overnight, then sown in 10 cm-diameter pots containing media mixture (parts: peat moss, 2; Perlite, 1; Vermiculite, 1; and Terragreen, a crushed baked clay medium, 0.25). The pots were maintained in a growth chamber (Enconaire, Model: GC 50 BH, Winnipeg, MB, Canada) set to 24/18 °C, 16 h light/8 h dark, at the University of Calgary. Inside the growth chamber, light was provided by a mixture of Philips 60 W incandescent lamps (Philips Electronics Ltd., Markham, ON, Canada), and cool white fluorescent tubes (Philips F72T12/CW/VHO, Philips Lighting Company, Somerset, NJ, USA). Relative humidity was between 60 and 70%. Photosynthetically active photon flux density (PPFD), measured at the apex of the plants with a Quantum LI-185B radiometer/photometer (LI-COR, Inc., Lincoln, NE, USA), was 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$. In order to keep the light intensity constant, distance between plants and light banks was adjusted.

2.2. UVB irradiation, water stress and ABA treatments

One-week-old seedlings were exposed to various treatments (see below). For the UVB irradiation treatments, four UVB fluorescent tubes (UVB 313EL, Q-Panel, Cleveland, OH, USA) were used. The space below the tubes was transversely divided into three independent compartments, using barrier of white glossy cardboard. Each section was supplied with 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD and one of the following biologically effective doses of UVB (UVB_{BE}): zero ($0 \text{ kJ m}^{-2} \text{ d}^{-1}$), ambient ($5 \text{ kJ m}^{-2} \text{ d}^{-1}$, as control) and enhanced ($10 \text{ kJ m}^{-2} \text{ d}^{-1}$). UVB_{BE} of $5 \text{ kJ m}^{-2} \text{ d}^{-1}$ was chosen as a control because this level is within the range of natural solar UVB radiation measured in the summer for Calgary, Canada (WOUDC, 2001). For the zero UVB, UV lamps were wrapped with two layers of 0.127 mm polyester (Mylar D) film (Grafix Plastics, Cleveland, OH, USA), which absorbs all radiation below 320 nm. For the ambient and enhanced UVB, UV-lamps were filtered with two and one layer of 0.127 mm cellulose diacetate film (JCS Industries Inc., La Mirada, CA, USA) respectively, that filters radiation below 280 nm. All filters were changed once per week. UVB_{BE} levels were measured by a PMA2100 photometer/radiometer, which was calibrated against a National Institute of Standards and Technology (NIST) traceable standard (Solar Light Co., Inc., Philadelphia, PA, USA). UVB_{BE} was estimated using Caldwell (1971) generalized plant damage action spectrum normalized to 300 nm. Daily UVB radiation was for 9.5 h, from 8:00 to 17:30 h, for the entire period of the experiment. To minimize positional effects, plants were rotated within the sections every 2 days.

One half of the plants, under each UVB condition, were watered to field capacity (well-watered) every day and the other half were watered after the leaves showed visible signs of wilting (water-stressed). These watering regimes were continued until the conclusion of the experiment. One half of both well-watered and water-stressed plants were treated with ABA [(±)-2-cis-4-trans-

abscisic acid; Sigma, Mississauga, Canada], which was applied to the growing point (shoot apex) of each plant, using a micropipette, every other day. For each plant, each application consisted of 10 μg of ABA dissolved in 10 μL of ethanol plus 90 μL of double distilled water.

The experiments, which were performed twice, consisted of 12 treatments with the following combinations: three UVB levels: zero, ambient and enhanced; two watering regimes: well-watered and water-stressed; and two ABA levels: +ABA and -ABA. Each treatment had six plant replications – three used for growth characteristics and three for physiological and chemical attributes – that were grown for 21 days.

2.3. Growth measurement

Plant growth parameters, such as stem height, stem diameter (first internode), leaf area, leaf dry weight, stem dry weight, and root dry weight were measured. Then, total plant dry weight, and growth indices, such as specific leaf weight [SLW (g m^{-2}) = leaf dry weight:leaf area], leaf weight ratio [LWR = leaf dry weight:total plant dry weight], leaf area ratio [LAR ($\text{cm}^2 \text{g}^{-1}$) = leaf area:total plant dry weight], and shoot:root ratio [SRR = shoot dry weight:root dry weight] were calculated. Leaf area was obtained by means of a ΔT area meter (Delta-T Devices Ltd., Burwell, Cambridge, UK), and all dry weights were determined using a portable electronic digital balance (OHAUS, Model No. CT10, OHAUS Cooperation, Florham Park, NJ, USA), after plant materials were dried in the oven for 72 h at 60 °C. For each of the above parameters, three replications were used.

2.4. Measurement of gas exchange

Gas exchange was measured with an infra-red gas analyzer (IRGA, CI-310 Portable Photosynthesis System, CID, Inc., Camas, WA, USA) between 10:00 and 14:00 h. Prior to measurements, the IRGA was calibrated with a known CO_2 concentration ($370 \mu\text{mol mol}^{-1}$). A steady air stream flow was provided to the sample chamber at the rate of 300 mL min^{-1} . An infrared temperature sensor was used to monitor leaf temperature, which was maintained at 24 ± 0.6 °C. Net CO_2 assimilation ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and transpiration ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) were determined, using three fully expanded leaves from each treatment, at 600 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ PPFD and $370 \mu\text{mol mol}^{-1} \text{ CO}_2$, and the values for these two parameters were obtained on the basis of total leaf area within the leaf chamber of IRGA. Water use efficiency ($\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$) was calculated by dividing net CO_2 assimilation by transpiration.

2.5. Analysis of photosynthetic pigments

Concentrations of chlorophyll and carotenoids were determined on the basis of leaf surface area. From each treatment, three replications of 1.1 cm^2 leaf surface (four small discs) were placed in each of three 12 mL vials containing 5 mL of dimethyl sulphoxide (Hiscox and Israelstam, 1979). The samples were incubated at room temperature in dark, for 24 h, for a complete extraction of chlorophyll and carotenoids, and then, from each replication, a 1 mL of extract was used. The absorbance of the extract was measured using a UV-vis spectrophotometer (Model Ultrospec 3100 pro, Biochrom Ltd., Cambridge, UK) at 664, 648 and 470 nm, against a solution of dimethyl sulphoxide. Then, the concentrations ($\mu\text{g cm}^{-2}$) of Chl *a*, Chl *b* and carotenoids were calculated, respectively (Chappelle et al., 1992).

2.6. Measurement of UV-absorbing compounds

From each treatment, three replications of 0.55 cm^2 leaf surface (two small discs) were transferred to 5 mL solution of methanol:

Table 1
Analysis of variance for canola (*Brassica napus* cv. 46A65) seedlings grown under three ultraviolet (UV)-B levels [0 (zero), 5 (ambient) and 10 (enhanced) $\text{kJ m}^{-2} \text{d}^{-1}$] and two watering regimes (well-watered and water-stressed), either with or without abscisic acid application. *F* values for UVB levels, watering regime, abscisic acid application and their interaction on growth parameters and dry matter accumulation of 21-day-old seedlings.

Source	Plant growth parameter			Dry matter accumulation			
	Stem height	Stem diameter	Leaf area	Leaf	Stem	Root	Total
UVB level (UVB)	5.6*	1.6	12.3**	37.9***	7.2**	12.6**	21.9***
Water regime (WR)	7.4*	18.2**	45.9***	128.5***	29.1***	0.0	55.0***
Abscisic acid (ABA)	13.3**	0.2	1.6	3.1	2.5	0.0	1.9
UVB × WR	4.2*	1.2	7.9**	27.1***	7.8**	5.0*	16.3***
UVB × ABA	0.0	0.0	0.1	0.3	0.1	0.4	0.2
WR × ABA	1.6	1.0	1.9	5.1*	4.4	0.6	3.9
UVB × WR × ABA	0.5	0.1	0.8	1.2	0.0	0.5	0.5

Significant values: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

water: hydrochloric acid (79:20:1, v/v/v) in each of three 12 mL vials (Robberecht and Caldwell, 1986), and left for 24 h in the dark. Then, from each replication, a 1 mL of extract was used to determine the concentration of UV-absorbing compounds, such as anthocyanins and flavonoids, with the same spectrophotometer used for the measurement of photosynthetic pigments. Absorbance was measured at 657, 530 and 300 nm against a solution of MeOH:H₂O:HCl. Then, the concentrations of anthocyanins and flavonoids were calculated (Chimphango et al., 2003). Because flavonoids, with 300 nm absorbance, were the major compounds of the extraction, the absorbance for UV-absorbing compounds was reported at 300 nm, and expressed on the basis of leaf surface area ($A_{300} \text{ cm}^{-2}$).

2.7. Wax extraction

For wax extraction, a modification of the method as described by Qaderi et al. (2002) was used. Shortly after detaching leaves from each treatment (three replications), their fresh weights were determined. Then, the samples were immersed in 20 mL chloroform and

gently stirred for 30 s. The obtained solution was evaporated in the fumehood to dryness. The residue was diluted with small amount of chloroform (amount required) and transferred to a pre-weighed 2 mL vial, and left to dry. Then, the amount of extracted wax was determined and expressed on the basis of fresh weight ($\mu\text{g mg}^{-1}$).

2.8. Ethylene evolution

A modification of the method as described by Emery et al. (1994) was used. Leaves from each treatment (ca. 0.1 g fresh weight) were taken (three replications) and incubated in 3-mL syringes for 20 min. Then, a 1 mL of trapped gas was collected in a 1 mL syringe and injected into a Photovac 10S plus gas chromatograph (Photovac, Markham, ON, Canada), equipped with a photoionization detector and 40/60 carbopack B column (Supelco Canada, Oakville, ON, Canada). The amount of ethylene was calculated on fresh weight basis ($\text{pmol g FW}^{-1} \text{ h}^{-1}$).

2.9. Statistical analysis

Overall effects of UVB radiation, watering regime, ABA and their interactions on morphological, physiological, and chemical attributes of *B. napus* were determined by means of a three-way analysis of variance (ANOVA). A one-way ANOVA was performed to determine treatment differences for each parameter. All one-way ANOVAs were accompanied by Fisher Pairwise Comparison test at 5% level (Zar, 1999; Minitab Inc., 2004).

3. Results

3.1. Plant growth

Overall, plants that were grown under enhanced UVB, experienced water stress or received exogenous ABA had reduced stem height ($P < 0.05$). Differences among UVB treatments, between watering regimes and ABA levels, and the two-way interaction between UVB × WR (watering regime) were significant (Table 1). At both ABA levels, enhanced UVB significantly reduced stem height of the well-watered plants, compared to the zero UVB. Water-stressed plants were significantly shorter than the well-watered plants grown under zero UVB and received no ABA. ABA reduced stem height in the well-watered plants grown under zero UVB (Fig. 1). Water stress decreased stem diameter ($P < 0.05$), which was significant between the two watering regimes (Table 1). Stems of well-watered plants, without exogenous ABA, were significantly thicker than those of water-stressed plants with the same ABA treatment, under zero and ambient UVB (Fig. 1). Enhanced UVB and water stress reduced leaf area ($P < 0.05$). Differences among UVB levels, between watering regimes and the two-way interaction between UVB × WR were significant (Table 1). Regardless of ABA application, the well-watered plants grown under ambient

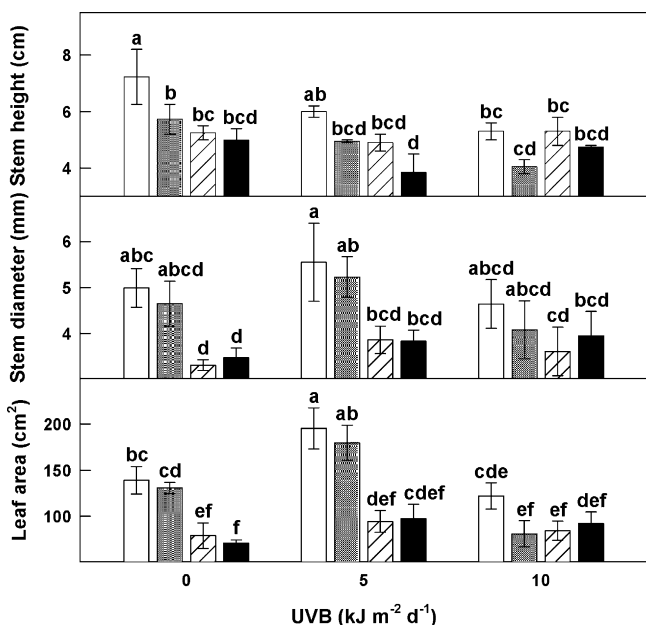


Fig. 1. Effects of UVB, water stress and ABA on stem elongation, stem diameter and leaf area of canola (*Brassica napus*) seedlings. Plants were grown for 21 days under three UVB levels [0 (zero), 5 (ambient) and 10 (enhanced) $\text{kJ m}^{-2} \text{d}^{-1}$], and exposed to two watering regimes and two ABA applications as following: well-watered without ABA application (open bars), well-watered with ABA application (shaded bars), water-stressed without ABA application (hatched bars), and water-stressed with ABA application (closed bars). Data are means \pm SE of six samples from two experiments. Bars surmounted by different letters are significantly different ($P < 0.05$) according to Fisher's Pairwise Comparison test.

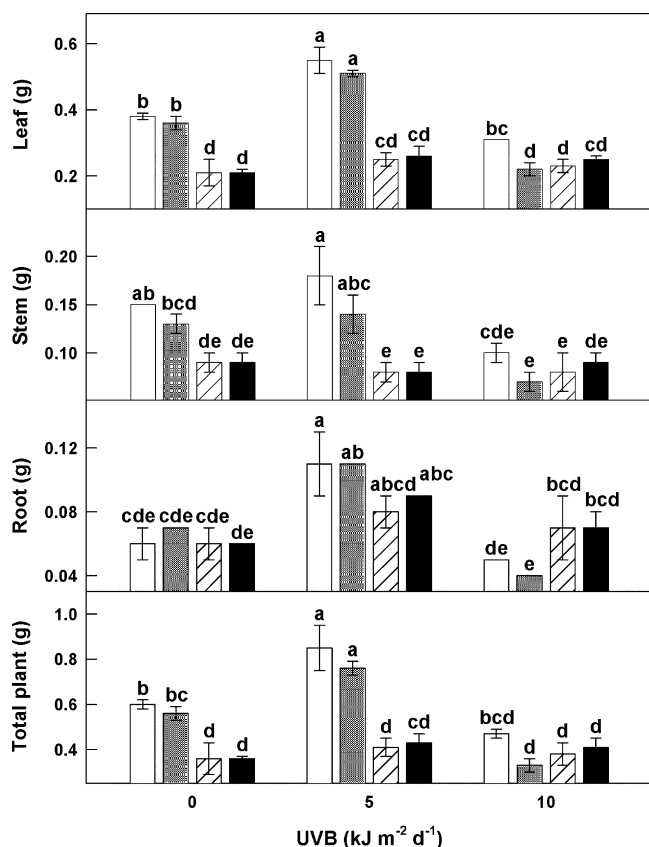


Fig. 2. Effects of UVB, water stress and ABA on leaf, stem and root dry matters of canola (*Brassica napus*) seedlings. Otherwise, as for Fig. 1.

UVB produced larger leaves than plants with the same watering regime grown under other UVB treatments. Water stress significantly reduced leaf area under zero and ambient UVB, but did not under enhanced UVB (Fig. 1).

3.2. Dry matter accumulation

Overall, enhanced UVB and water stress reduced leaf dry matter ($P < 0.05$). Differences among UVB treatments, between watering regimes and the two-way interaction between UVB \times WR and WR \times ABA were significant (Table 1). At both ABA levels, the well-watered plants grown under enhanced UVB had significantly lower leaf dry matter compared to those grown under ambient UVB. Water stress significantly reduced leaf dry matter under zero and ambient UVB at both ABA levels, but under enhanced UVB only at no ABA application. In the well-watered plants, under enhanced UVB, leaf dry matter was significantly lower with exogenous ABA than those with no ABA (Fig. 2). Enhanced UVB and water stress also reduced stem dry matter ($P < 0.05$). In general, differences among UVB treatments, between watering regimes and the two-way interaction between UVB \times WR were significant (Table 1). In the well-watered plants at both ABA levels, enhanced UVB significantly reduced stem dry matter, compared to the zero and ambient UVB. Water stress significantly reduced stem dry matter under zero UVB with no exogenous ABA, and under ambient UVB at both ABA levels (Fig. 2). Root dry matter was significantly lower for plants grown under enhanced and zero UVB, compared to those grown under ambient UVB ($P < 0.05$). Differences among UVB treatments and the two-way interaction between UVB \times WR were significant (Table 1). Under conditions of enhanced UVB and ABA application, water-stressed plants had significantly higher root dry matter than the well-watered plants (Fig. 2). Overall, plants grown

Table 2

Analysis of variance for canola (*Brassica napus* cv. 46A65) seedlings grown under three ultraviolet (UV)-B levels [0 (zero), 5 (ambient) and 10 (enhanced) $\text{kJ m}^{-2} \text{d}^{-1}$] and two watering regimes (well-watered and water-stressed), either with or without abscisic acid application. *F* values for UVB levels, watering regime, abscisic acid application and their interaction on growth indices and gas exchange of 21-day-old seedlings.

Source	Growth index				Gas exchange		
	SLW	LWR	LAR	SRR	A_N	E	WUE
UVB	0.3	1.8	1.2	4.8*	0.2	1.4	4.4*
WR	0.1	40.6***	5.8*	63.2***	6.1*	2.2	3.9
ABA	0.2	0.0	0.3	1.3	0.1	0.3	0.3
UVB \times WR	0.3	0.1	0.6	0.4	2.2	0.5	3.4
UVB \times ABA	0.2	0.1	0.2	0.7	0.1	0.1	0.5
WR \times ABA	0.0	0.5	0.2	0.7	0.0	0.0	0.0
UVB \times WR \times ABA	0.3	0.8	0.6	0.8	0.1	0.1	0.0

Significant values: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. SLW, specific leaf weight; LWR, leaf weight ratio; LAR, leaf area ratio; SRR, shoot:root weight ratio; A_N , net CO_2 assimilation; E, transpiration; WUE, water use efficiency.

under enhanced UVB or experienced water stress had reduced total dry matter ($P < 0.05$). Differences among UVB treatments, between watering regimes and the two-way interaction between UVB \times WR were significant (Table 1). Regardless of ABA application, the well-watered plants, grown under ambient UVB, produced significantly greater total dry matter than those grown under zero and enhanced UVB. Water stress significantly decreased total plant dry matter under zero and ambient UVB, but did not under enhanced UVB (Fig. 2).

3.3. Growth indices

UVB, watering regime or ABA did not affect specific leaf weight (SLW), as none of these factors and their interactions was significant (Table 2; Fig. 3). Water stress reduced leaf weight ratio (LWR) ($P < 0.05$), which was significant between the two watering regimes (Table 2). Water stress significantly decreased LWR for plants grown under zero and ambient UVB and received exogenous ABA, and for plants grown under enhanced UVB at both ABA levels (Fig. 3). Water stress decreased leaf area ratio (LAR) ($P < 0.05$), which was significant between the two watering regimes (Table 2). However, LAR between the corresponding treatments of UVB or watering regime was not significant on the basis of one-way ANOVA (Fig. 3). Plants grown under zero and enhanced UVB or exposed to water stress had greater shoot:root weight ratio (SRR) ($P < 0.05$). Overall, differences among UVB treatments and between watering regimes were significant (Table 2). Well-watered plants, without exogenous ABA, under zero UVB had significantly higher SRR than plants from the same treatment under ambient UVB. Water-stressed plants had significantly lower SRR than the well-watered plants, under zero UVB, without exogenous ABA, and under ambient and enhanced UVB at both ABA levels (Fig. 3).

3.4. Gas exchange

Plants that were grown under water stress condition exhibited reduced net CO_2 assimilation ($P < 0.05$). Differences were significant only between watering regimes (Table 2), but they were not significant among treatments on the basis of one-way ANOVA (Fig. 4). Neither UVB nor watering regime and ABA application affected transpiration, as none of these factors and their interactions was significant (Table 2; Fig. 4). Water use efficiency decreased as UVB increased ($P < 0.05$). Overall, differences among UVB treatments were significant (Table 2). In the well-watered plants at both ABA levels, enhanced UVB significantly decreased water use efficiency, compared to the zero UVB (Fig. 4).

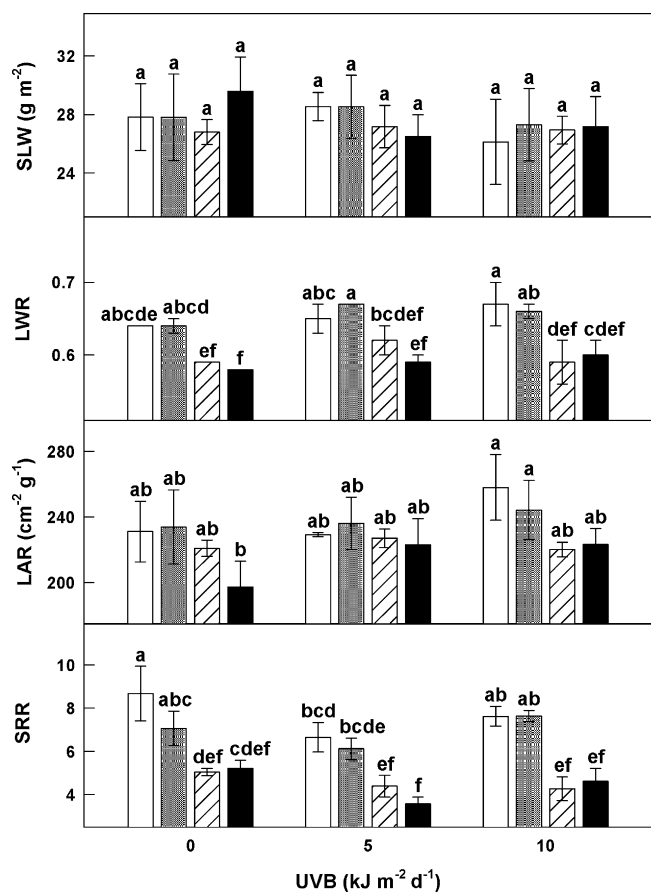


Fig. 3. Effects of UVB, water stress and ABA on various growth indices in canola (*Brassica napus*) seedlings. SLW, specific leaf weight; LWR, leaf weight ratio; LAR, leaf area ratio; SRR, shoot:root weight ratio. Otherwise, as for Fig. 1.

3.5. Photosynthetic pigments

Overall, plants grown under zero UVB had relatively lower concentration of Chl *a* compared to those grown under other UVB levels ($P < 0.05$). Differences in Chl *a* concentration were significant only among UVB treatments (Table 3). Well-watered plants, without exogenous ABA application, produced significantly higher Chl *a* under ambient and enhanced UVB than under zero UVB. Only under enhanced UVB, water stress significantly decreased Chl *a* than well watering, for plants that received no exogenous ABA (Fig. 5). The concentration of Chl *b* was not affected by UVB, watering regime or ABA application, as none of these factors and their interactions was significant (Table 3; Fig. 5). Plants grown under ambient or enhanced UVB produced higher concentrations of carotenoids than those grown under zero UVB ($P < 0.05$). Differences among UVB

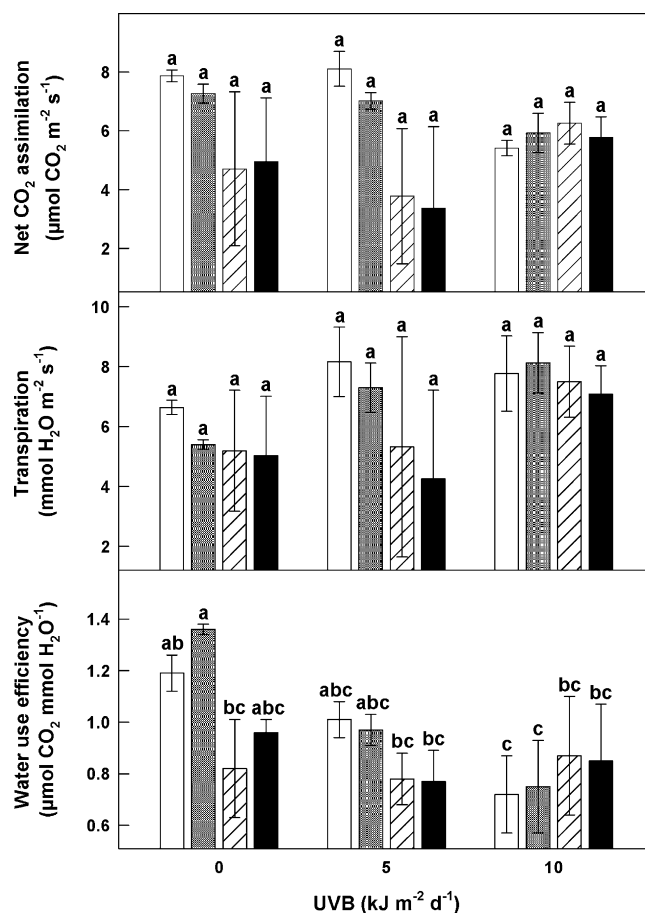


Fig. 4. Effects of UVB, water stress and ABA on net CO₂ assimilation, transpiration and water use efficiency in canola (*Brassica napus*) seedlings. Otherwise, as for Fig. 1.

treatments and the two-way interaction between UVB \times WR were significant (Table 3). Ambient UVB, in the well-watered plants with ABA, and both ambient and enhanced UVB, in the well-watered plants without exogenous ABA, significantly increased carotenoids than zero UVB in plants with the same watering regime and ABA application. Similar to Chl *a*, the concentration of carotenoids was significantly higher for the well-watered plants than for the water-stressed plants, under conditions of enhanced UVB and no ABA application (Fig. 5). Neither UVB nor watering regime and ABA application influenced Chl *a*:*b* ratio, as none of these factors and their interactions was significant (Table 3; Fig. 5).

3.6. UV-absorbing compounds

Regardless of watering regime and ABA application, plants grown under ambient and enhanced UVB produced significantly

Table 3
Analysis of variance for canola (*Brassica napus* cv. 46A65) seedlings grown under three ultraviolet (UV)-B levels [0 (zero), 5 (ambient) and 10 (enhanced) $\text{kJ m}^{-2} \text{d}^{-1}$] and two watering regimes (well-watered and water-stressed), either with or without abscisic acid application. *F* values for UVB levels, watering regime, abscisic acid application and their interaction on photosynthetic pigments and other chemical components of 21-day-old seedlings.

Source	Photosynthetic pigment				Chemical component		
	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Carotenoids	Chlorophyll <i>a</i> : <i>b</i>	Flavonoids	Wax	Ethylene
UVB	8.3**	1.6	10.7**	0.1	29.9***	3.5	4.7*
WR	2.3	0.2	2.6	0.2	0.3	2.3	1.9
ABA	1.3	0.3	0.1	0.0	0.3	0.2	0.0
UVB \times WR	3.5	0.5	5.6*	0.0	0.4	1.4	0.6
UVB \times ABA	0.1	0.0	0.3	0.0	0.1	1.3	0.9
WR \times ABA	0.5	0.6	1.0	0.0	0.6	7.0*	0.0
UVB \times WR \times ABA	1.3	0.4	1.2	0.1	0.5	2.1	0.2

Significant values: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

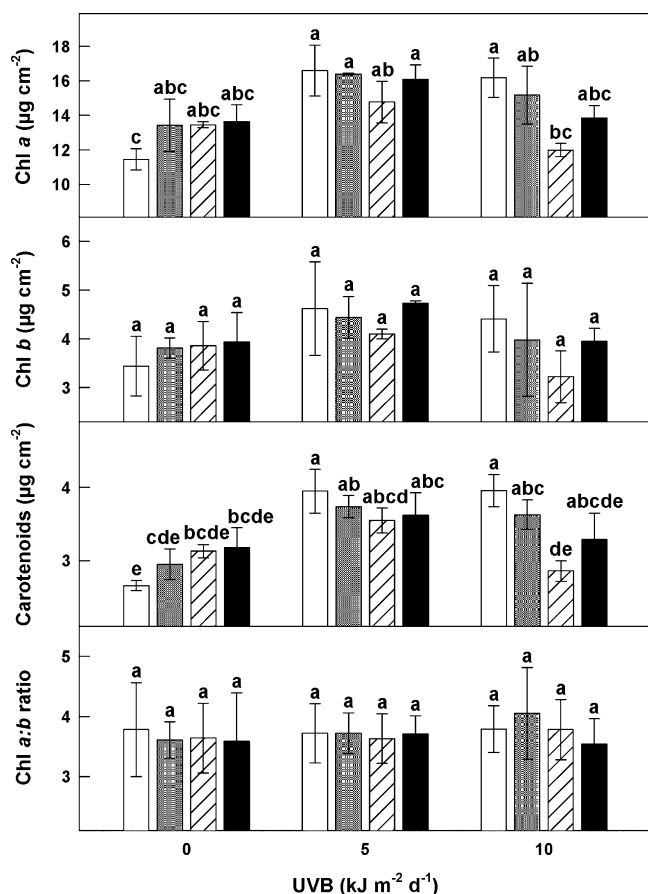


Fig. 5. Effects of UVB, water stress and ABA on the concentrations of chlorophyll *a*, chlorophyll *b* and carotenoids and the ratio of Chl *a*:*b* in leaves of canola (*Brassica napus*) seedlings. Otherwise, as for Fig. 1.

more flavonoids than plants grown under zero UVB (Fig. 6). Only differences among UVB treatments were significant (Table 3).

3.7. Epicuticular wax

Overall, wax content was not significantly affected by UVB, watering regime or ABA application. However, the two-way interaction between WR \times ABA was significant (Table 3). On the basis of one-way ANOVA, water-stressed plants, with ABA, grown under zero UVB had higher wax content than plants with the same watering regime and ABA application grown under enhanced UVB. Also, under zero UVB, water stress significantly increased wax content for plants that received exogenous ABA than for plants with the same ABA application that received water to field capacity (Fig. 6).

3.8. Ethylene evolution

Overall, enhanced UVB increased ethylene evolution ($P < 0.05$). Only differences among UVB treatments were significant (Table 3). In the water-stressed plants that received no exogenous ABA, enhanced UVB significantly increased ethylene than the ambient UVB (Fig. 6).

4. Discussion

We investigated the combined effects of UVB and water stress on growth and physiology of *B. napus* and the possible role of ABA in the interactive effects of these environmental factors. In our study, enhanced UVB reduced stem height and leaf area and

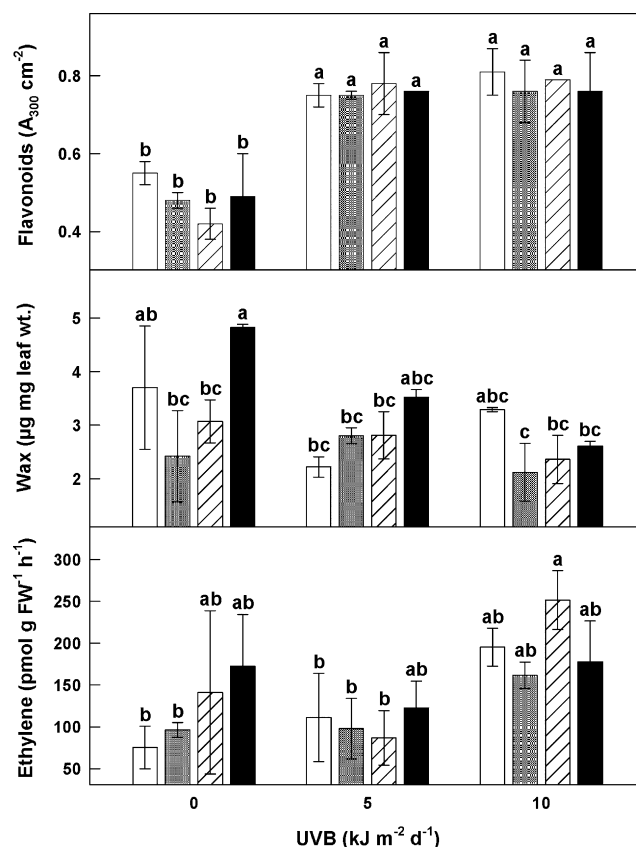


Fig. 6. Effects of UVB, water stress and ABA on flavonoids, wax and ethylene in leaves of canola (*Brassica napus*) seedlings. Otherwise, as for Fig. 1.

resulted in decreased dry matter of individual plant organs and whole plant. Water stress also reduced plant growth and dry matter, and the reduction was more pronounced under zero and ambient UVB, especially in plants that received no exogenous ABA (Table 1; Figs. 1 and 2). In this study, seedling height was the only parameter, which was significantly affected by all applied factors.

Although exogenous ABA reduced seedling height in all combinations of UVB and watering regime, the reduction was more apparent in the well-watered plants than the water-stressed ones (Fig. 1). This indicates that plants that have already been affected by water stress are less responsive to ABA than those that received water to field capacity. This inhibitory role of ABA on growth has previously been reported in other species (Watts et al., 1981; Cacho et al., 1995).

In earlier studies, UVB radiation either decreased plant growth and biomass (Gao et al., 2003; Hofmann et al., 2003; Qaderi and Reid, 2005) or increased certain plant parameters, such as chlorophyll content in some cultivars of lettuce (*Lactuca sativa* L.) (Smith et al., 2000). Earlier studies have also shown that water stress caused slower growth rate, which was because of inhibition of cell expansion and reduction in carbon assimilation that affected carbon partitioning (Osório et al., 1998; Hsiao and Xu, 2000). In our study, seedlings of *B. napus* produced more dry matter under ambient UVB than under zero UVB, but enhanced UVB decreased dry mass. The results indicate that *B. napus* exhibited some adaptive mechanisms to the ambient UVB, which could be best for their vegetative growth.

Reduced plant growth and dry matter under zero and enhanced UVB as well as under water stress could have been related to changes in other plant parameters. For example, in the well-watered plants that received no exogenous ABA, SRR was significantly higher under zero UVB compared to that under ambient

UVB (Fig. 3). This shows the effects of UVB on root systems and indicates that root mass decreases in the absence of UVB. Water stress reduced LWR and SRR, and the reduction was more pronounced with increased UVB levels (Fig. 3). Reduced LWR indicates that, under water stress, plants produced smaller leaves and, in turn, lowered biomass, whereas decreased SRR shows a relatively higher root dry mass under water stress, compared to the whole weight of the plant.

Decreased plant biomass, under enhanced UVB, might have been related to relatively decreased water use efficiency under this UVB level (Fig. 4). Well-watered plants that received no exogenous ABA had increased concentrations of Chl *a* and carotenoids under ambient and enhanced UVB than under zero UVB (Fig. 5). Cechin et al. (2006) reported that, in sunflower (*Helianthus annuus* L.), during water stress photosynthetic rate declined, but there were no changes in chlorophyll concentration. In our study, although the well-watered plants from the ambient and enhanced UVB had relatively more photosynthetic pigments than those from the zero UVB, only plants from the ambient UVB produced significantly more dry matter than plants from the zero UVB level (Figs. 2 and 5). This shows that plants under enhanced UVB might have allocated more photosynthates for other physiological activities, including defense mechanisms, rather than utilizing them for the accumulation of dry matter. Our argument can be supported by the relatively higher flavonoids under enhanced UVB than those under zero UVB level (see Fig. 6).

As pointed out earlier, plants under ambient and enhanced UVB had significantly higher flavonoids than those under zero UVB. Higher flavonoids could act as a protectant against UVB irradiation for plants grown under these two UVB levels. The UVB 'sunscreen' action of flavonoids has previously been shown (Bieza and Lois, 2001). Increased flavonoids and phenolic compounds under UVB radiation have also been reported by other researchers (Warren et al., 2003; Poulson et al., 2006; Jenkins and Brown, 2007).

We previously reported that, in *B. napus*, ambient UVB increased wax content compared to the low UVB (Qaderi and Reid, 2005). However, the current study revealed that enhanced UVB, compared to the zero UVB, significantly decreased wax content in the water-stressed plants that received exogenous ABA. Differences in wax content between these two studies might have been related to the experimental setup. In our 2005 study, we exposed plants, which were grown inside Plexiglas cabinets, to two UVB levels and two CO₂ concentrations. All plants received water to field capacity and no ABA was applied on them. The results of present study show that, besides UVB, watering regime and ABA can affect the accumulation of epicuticular wax in this species.

Role of ethylene cannot be ruled out in the decrease of dry matter under enhanced UVB, as highest ethylene was evolved by plants grown under this UVB treatment (Fig. 6), confirming previous reports on other species (A.-H.-Mackerness et al., 1999). Enhanced ethylene evolution might have been a result of response to stress (Dodd and Davies, 2004), which might reduce plant growth and dry matter. Relatively higher ethylene evolution was apparent in the water-stressed plants, except for those grown under ambient UVB with no exogenous ABA (Fig. 6). Both increased (Ingram and Bartels, 1996) and decreased (Balota et al., 2004) ethylene production rates under water stress have been reported. There has been some debate about ethylene evolution in response to drought (De Wit et al., 1990), as its production can be regulated by other environmental factors. Nevertheless, increased ethylene production has often been associated with reduced growth. In our study, water stress reduced plant growth and dry matter, and it is possible that higher ethylene might be one of the many factors leading to less growth.

In our study, along with the factor effects, the two-way interaction between UVB × WR and WR × ABA were also significant for

seven and two plant characteristics, respectively (Tables 1–3). For instance, well-watered plants were tallest under zero UVB, followed by plants grown under ambient and enhanced UVB. Water stress decreased plant height under zero and ambient UVB, but slightly increased it under enhanced UVB (Fig. 1). However, well-watered plants produced highest dry matter under ambient UVB, followed by those grown under zero and enhanced UVB. A sharp decrease in dry matter was apparent for water-stressed plants under zero and ambient UVB, but not under enhanced UVB (Fig. 2).

In our study, enhanced UVB increased plant tolerance to water stress, confirming previous findings by others. A study by Nogués et al. (1998) showed that UVB radiation delayed and reduced the harmful effects of water stress in pea (*Pisum sativum* L.) plants. Also, Hofmann et al. (2003) reported that interaction between UVB and water stress was beneficial for white clover (*Trifolium repens* L.), and suggested that the direction and extent of interaction between UVB and water stress depends on the duration of stress. Alexieva et al. (2001) noted that, in *P. sativum* and wheat (*Triticum aestivum* L.), interaction between UVB and water stress to induce protective mechanism was synergistic. Also, our results revealed that plant growth that was negatively affected by enhanced UVB (compared to the ambient level) was not further inhibited by water stress. This suggests that the effects of UVB and water stress on plant growth were not additive.

We mentioned earlier that the two-way interaction between WR × ABA was significant for leaf dry matter and wax content (Tables 1 and 3). Under enhanced UVB, well-watered plants with no exogenous ABA produced higher leaf dry matter than those with ABA application, whereas water-stressed plants showed no changes in dry matter with exogenous ABA (Fig. 2). Also, under zero UVB, with exogenous ABA, water-stressed plants had higher wax content than the well-watered plants, whereas with no ABA application there was no significant change in wax content between the well-watered and water-stressed plants (Fig. 6).

Watts et al. (1981) reported that the effects of water stress and exogenous application of ABA on plant development were similar since ABA acts as an essential mediator in triggering plant responses to dehydration (Botella et al., 2005). Although the role of ABA in the adaptation of plants to water stress has been documented (Quarrie, 1984), its role in the interactive effects of UVB and water stress was less marked in our study.

In summary, our study revealed that enhanced UVB and water stress adversely affected *B. napus* seedlings, and interaction between these two factors altered plant performance. However, exogenous ABA did not play a significant role in regulating interaction between UVB and watering regime. This study also showed that vegetative growth of *B. napus* was best under ambient UVB. Decreased growth under zero and enhanced UVB resulted in reduced biomass in this species.

Acknowledgements

We thank the Natural Sciences and Engineering Research Council (NSERC) of Canada for financial support through Discovery grants to D.M. Reid and C.C. Chinnappa. A sabbatical leave provided to M.H. Sangtarash by the University of Sistan and Baluchestan of Iran is greatly appreciated. The authors also thank Ms. Bonnie Smith for help with growth chamber setup.

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