Influence of UV-B radiation and Cd\(^{2+}\) on chlorophyll fluorescence, growth and nutrient content in *Brassica napus*

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Abstract

The possible interaction of two stresses, UV-B radiation and cadmium, applied simultaneously, was investigated in *Brassica napus* L. cv. Paroll with respect to chlorophyll fluorescence, growth and uptake of selected elements. Plants were grown in nutrient solution containing CdCl\(_2\), \((0, 0.5, 2\) or \(5 \) \(\mu\)M) and irradiated with photosynthetically active radiation (PAR, 400–700 nm, \(800 \text{ mol m}^{-2} \text{s}^{-1}\)) with or without supplemental ultraviolet-B radiation (UV-B, 280–320 nm, \(15 \text{ kJ m}^{-2} \text{d}^{-1}\), weighted irradiance). After 14 d of treatment, the most pronounced effects were found at 2 and \(5 \) \(\mu\)M CdCl\(_2\) with and without supplemental UV-B radiation. Exposure to cadmium significantly increased the amount of Cd in both roots and shoots. In addition, increases occurred in the concentration of Fe, Zn, Cu, and P in roots, while K was reduced. In shoots the S content rose significantly both in the presence and absence of UV-B radiation, while significant increases in Mg, Ca, P, Cu, and K occurred only in plants exposed to Cd and UV-B radiation. Manganese decreased significantly under the combined exposure treatment. The rise in S content may have been due to stimulated glutathione and phytochelatin synthesis. Cadmium exposure significantly decreased root dry weight, leaf area, total chlorophyll content, carotenoid content, and the photochemical quantum yield of photosynthesis. As an estimation of energy dissipation processes in photosynthesis, non-photochemical quenching (\(q_{\text{NPQ}}\)) was measured using a pulse amplitude modulated fluorometer. The \(q_{\text{NPQ}}\) increased with increasing Cd, while the combination of cadmium and UV-B reduced the \(q_{\text{NPQ}}\) compared to that in plants exposed only to cadmium or UV-B radiation. The chlorophyll \(a:b\) ratio showed a reduction with UV-B at no or low Cd concentrations (0, 0.5 \(\mu\)M CdCl\(_2\)), but not at the higher Cd concentrations used (2 \(\mu\)M, 5 \(\mu\)M CdCl\(_2\)). Thus in some instances there appeared to be a UV-B and Cd interaction, while in others plant response could be attributed to either treatment alone.

Key words: *Brassica napus*, cadmium, ultraviolet-B radiation.

Introduction

Living organisms, including plants, are often exposed to several stress factors simultaneously and two of them, UV-B radiation and cadmium, were chosen for this investigation.

Cadmium is an increasing problem in soil, due to industrial pollution, circulation of sewage sludge and the use of commercial phosphorous fertilizers. However, different bedrocks also give rise to soil containing varying amounts of cadmium. The sedimentary bedrocks alum shale and sandstone contain high amounts of cadmium (Eriksson et al., 1995).

Abbreviations: \(F_m\), maximal fluorescence; \(F_{m\infty}\), maximal fluorescence under illumination; \(F_o\), initial fluorescence; \(F_t\), fluorescence yield at a given time, \(t\); \(F_v\), variable fluorescence \((F_m-F_o)\); GSH, reduced glutathione; HL, high light \((800 \text{ mol m}^{-2} \text{s}^{-1} \text{PAR} (400–700 \text{nm}))\); LED, light emitting diode; PAM, pulse amplitude modulated fluorometer; PSII, photosystem II; \(q_{\text{NPQ}}\), non-photochemical quenching, \((F_m-F_{m\infty})/F_{m\infty}\); \(\Phi_{\text{ps}}\), photochemical quantum yield, \((F_m-F_v)/F_{m\infty}\); SLA, specific leaf area; SNK, Student–Newman–Keul (post-test); UV-B, ultraviolet-B radiation (280–320 nm); UV-B\(_{\text{BE}}\), biologically effective UV-B radiation.

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In plants, cadmium may depress growth due to inhibition of chlorophyll synthesis (Stobart et al., 1985) and photosynthesis (Greger and Ögren, 1991), depending upon concentration and conditions. In sugarbeet elevated cadmium levels (5 μM, 50 μM) resulted in decreased nutrient uptake and transport (Greger and Lindberg, 1987). Cadmium irreversibly replaces other metal ions in essential metalloenzymes (Jackson et al., 1990). It also enhances the level of lipid peroxides and decreases the activity of antioxidant enzymes in *Phaseolus vulgaris* (Somasekaraiah et al., 1992).

Plants have developed various mechanisms to decrease the potential deleterious effects of elevated Cd levels. In most species, cadmium is trapped in the roots and according to Lindberg and Wingstrand (1985) the Cd content in sugarbeet may be 5–10 times higher in roots than in shoots. In soybean plants, only 2% of the accumulated Cd was transported to the leaves, but during seed-filling 8% of the absorbed Cd was transported to the seeds (Catalado et al., 1981). Some plants, for example, *Brassica juncea*, can be used as bioaccumulators due to their ability to accumulate Cd (Salt et al., 1995).

The basic strategy of Cd tolerance in plants is the reduction in cytosolic concentration of free Cd, which is achieved by various mechanisms. Possible mechanisms involve association with the cell wall, sequestration in the vacuole together with organic acids and complexation with reduced glutathione (GSH) in the cytosol (Vögeli-Lange and Wagner, 1996, and references therein). When plants are exposed to high Cd levels or to long-term exposure, complexation with certain polypeptides is induced. Cadmium, among other heavy metals, induces these metal-binding polypeptides, called phytochelatins (Steffens, 1990). Phytochelatins are synthesized from GSH and γ-glutamylcysteine, probably in the cytosol, where they bind to Cd and form a Cd complex, which is then transported into the vacuole where it is accumulated (Vögeli-Lange and Wagner, 1990). Various transport mechanisms into the vacuole have been suggested (Salt and Wagner, 1993; Ortiz et al., 1995; Rauser, 1995).

During the last decade the stratospheric ozone layer has decreased in thickness due to pollutants such as freons and nitrogen oxides. The increasing greenhouse effect also appears to provide favourable conditions for the breakdown of ozone molecules by decreasing the temperature in the stratosphere (Pyle, 1997). The resultant thinner ozone layer facilitates penetration of ultraviolet-B radiation (UV-B, 280–320 nm) to the Earth’s surface.

Plant response to UV-B radiation has been investigated in several plant species (see the review by Bornman and Teramura, 1993), but there are few general rules that apply to all plants. This is often a consequence of plant diversity and experimental conditions.

The possible interaction of cadmium and UV-B radiation was studied because of the increasing importance in agriculture of these two factors and because multiple environmental effects are often overlooked. An additional reason for the choice of the two potential stressors is that, in future studies, the possible interaction between Cd and UV-B radiation through the glutathione pool, which is involved in both phytochelatin and phenolic pathways (Steffens, 1990; Smith et al., 1990), will be investigated. Both cadmium and UV-B radiation have been reported to negatively influence photosynthesis, which is intimately related to productivity and crop yield. In earlier experiments with spruce seedlings the combined stress of UV-B radiation and cadmium resulted in a reduction of CO₂ assimilation and total chlorophyll (Dubé and Bornman, 1992).

In the present paper the influence of increasing Cd levels and UV-B radiation on *Brassica napus* on (a) the mineral content in shoots and roots, (b) growth parameters such as leaf area and dry weight, (c) pigment content, and (d) regulatory processes affecting photosynthesis was tested. The cadmium concentrations used in this investigation are quite low compared to many other studies, which makes it more relevant when comparing the results to real soil conditions. According to Stoeppler (1992) typical cadmium levels in non-polluted soils are ≤0.01–0.5 mg kg⁻¹ and ≤0.2–50 mg kg⁻¹ in polluted soils. The amount of UV-B radiation used, although relatively high, approached natural conditions found in certain regions (Bachelet et al., 1991).

**Materials and methods**

**Plant material and growth conditions**

Oilseed rape (*Brassica napus* L. cv. Paroll) was grown hydroponically in black containers with 11 of continuously aerated nutrient solution per plant. The nutrient solution (pH 5.5) consisted of macronutrients (mM): 5.0 KNO₃, 2.25 Ca(NO₃)₂, 4H₂O, 0.5 MgSO₄, 0.5 KH₂PO₄, 0.5 Na₂HPO₄, 0.625 NH₄Cl; and micronutrients (μM): 20.0 Fe-EDTA, 5.0 MnSO₄·H₂O, 4.0 ZnSO₄·7H₂O, 30.0 H₃BO₃, 0.75 CuCl₂, and 0.5 Na₂MoO₄. The solutions were changed twice a week. The plants were grown in a greenhouse chamber in which they received 800 μmol m⁻² s⁻¹ of photosynthetically active radiation, 400–700 nm (PAR), during a photoperiod of 12 h; night temperature was 20/18 °C. The visible light was supplied by a bank of Osram Power Star dysprosium lamps (Awake 400 W/D, Germany). UV-B radiation was supplied by Q-Panel (UV–313, Largo, Gothenburg) sunlamps. The radiation from the UV-B lamps was filtered through Plexiglas (FBL.2458, Ro¨hm Gmbh, Chemische Fabrik, Germany) and ozone layer facilitates penetration of ultraviolet-B radiation (UV-B, 280–320 nm) to the Earth’s surface. In polluted areas, the resultant thinner ozone layer facilitates penetration of ultraviolet-B radiation (UV-B, 280–320 nm) to the Earth’s surface.
to give a biologically effective radiation (UV-B\textsubscript{eq}) of 15 \text{ kJ m\textsuperscript{-2} d\textsuperscript{-1}}. UV-B radiation was measured with a spectroradiometer (Model 752, Optronic laboratories, Orlando, FL, USA) and the UV-B\textsubscript{eq} was calculated using a UV-dosage model according to Björn and Murphy (1985) and the generalized plant action spectrum by Caldwell (1971), normalized to unity at 300 nm.

Cadmium was added as CdCl\textsubscript{2} in four concentrations; 0, 0.5, 2, and 5 \text{ \mu M}. Five plants from each cadmium concentration were irradiated with PAR only and another five from each concentration, with PAR + UV-B. The plants treated with different cadmium concentrations were randomized within each light treatment. The plants were treated for 14 d before they were harvested.

Measurements were carried out on the first pair of leaves. All parameters were measured at harvest time except for the chlorophyll fluorescence which was monitored three times during treatment; 3, 10 and 14 d after the beginning of the experiment. The experiment was repeated three times.

**Cadmium and nutrient analysis**

Cadmium and nutrient content in shoot and root were determined on dried material (105 °C, 48 h). If plant dry matter exceeded 0.5 g, the sample was milled and 0.3 g taken for analysis. The samples were dissolved in HNO\textsubscript{3} (65%) according to the microwave technique. For element analysis, inductively coupled plasma emission (ICP-AES) was used (JY 238 glass. The trends were, however, similar in all three experiments to the microwave technique. For element analysis, inductively coupled plasma emission (ICP-AES) was used (JY 238 ULTRACE, Paris, France). The following elements were determined:

- Ca, Cd, Cu, Fe, K, Mg, Mn, P, S, and Zn.

**Leaf and root parameters**

Four leaf discs (diameter 4.2 mm) per plant were dried at 105 °C for 48 h and then weighed. Roots were also dried and weighed. Leaf area was calculated from photocopies of the same leaves that were used for chlorophyll fluorescence analyses and pigment determination. The area was expressed in cm\textsuperscript{2} or normalized to g dry weight (specific leaf area, SLA).

**Pigment analysis**

The total chlorophyll and bulk carotenoid content of four leaf discs (diameter 4.2 mm) from each treatment were extracted with 80% acetone and centrifuged at 1600 g for 10 min. All pigment extraction was done in the dark with the samples kept on ice. The absorbance of the supernatant was measured using a dual-wavelength/double beam spectrophotometer (Shimadzu, UV–3000). Chlorophyll \textsubscript{a} was measured at 663.2 nm, Chlor \textsubscript{b} at 646.8 nm and carotenoids at 470 nm. The results were analysed using a computer program based on the equations of Wellburn (1994) and then normalized to leaf dry weight. The extraction volume was 10 ml.

The same procedure was followed for the extraction of UV-screening pigments except that extraction was done in methanol/water: HCl (79:20:1, by vol.) according to Robberecht and Caldwell (1986). An absorption spectrum was taken between 230–530 nm and values at 310 and 420 nm were expressed on a leaf area basis. The content of UV-screening pigments was assessed at 310 nm (Cen and Bornman, 1993; Reuber \textit{et al.}, 1996). The peak at 420 nm was included for analysis, since increasing CdCl\textsubscript{2} had a profound effect on absorbance at this wavelength.

**Chlorophyll fluorescence**

Chlorophyll fluorescence of photosystem II (PSII) was measured with a pulse amplitude modulated fluorometer (PAM 101, 102, 103, H Walz, Effeltrich, Germany). The measurements were done at room temperature (20 °C) in the dark. Plants were first adapted for 30 min in total darkness. The initial fluorescence (\textit{F}<\textit{o>}) was determined by the measuring beam of the PAM fluorometer (PAM 101). The maximal fluorescence (\textit{F}<\textit{m>}) was determined at the beginning of each measurement using a saturating pulse (2000 \text{ \mu mol m\textsuperscript{-2} s\textsuperscript{-1}}). FL 103, H Walz, Effeltrich, Germany). Actinic light was obtained from an LED (70 \text{ \mu mol m\textsuperscript{-2} s\textsuperscript{-1}}). The variable fluorescence (\textit{F}<\textit{v>}) was taken from the formula, \textit{F}<\textit{v>} = \textit{F}<\textit{m>}–\textit{F}<\textit{o>}, and the following fluorescence quenching parameters were used:

\[ q_{NPQ} = \frac{(\textit{F}<\textit{m>} – \textit{F}<\textit{v>} – \textit{F}<\textit{o}>)}{\textit{F}<\textit{m}>}; \]

\[ \phi_{PSI} = \frac{(\textit{F}<\textit{m>} – \textit{F}<\textit{v}>)}{\textit{F}<\textit{m}>}; \]

\[ \textit{F}<\textit{v}> = \frac{(\textit{F}<\textit{m>} – \textit{F}<\textit{o}>)}{\textit{F}<\textit{m}>}; \]

\[ \textit{F}<\textit{o}> = \textit{F}<\textit{m}>– \textit{F}<\textit{v}>. \]

The variable fluorescence was measured after dark-adaptation, while \textit{F}<\textit{m>} was measured on an illuminated sample. \textit{F}<\textit{o>} is the fluorescence yield at a given time, \textit{t} (Horton and Bowyer, 1990; Schreiber and Bilger, 1987).

**Results**

**Cadmium and nutrient content**

The Cd content in both roots and shoots increased significantly with exposure to increasing cadmium concentrations (Table 1). In both roots and shoots the Cd content increased to the same extent in HL and HL + UV-B plants, showing that the plants did not respond differently when exposed to UV-B with respect to Cd uptake. In roots, the content of Fe, Zn, P, and Cu also increased significantly, while the amount of K decreased with increasing Cd concentration. UV-B radiation had no additional influence on the content of the measured nutrients in roots. Mg, Ca, P, Cu, and K increased in shoots with increasing cadmium concentration, but the increase was only significant when plants received UV-B radiation. In contrast, Mn decreased significantly under the combination of UV-B and Cd. Results of plants exposed to two stress factors appeared to show less variation. The shoots of cadmium treated plants (2 and 5 \text{ \mu M CdCl\textsubscript{2}}) significantly increased their S content under both irradiation conditions.

**Leaf and root parameters**

After 14 d of treatment the leaf area of the first leaf pair was significantly smaller at higher CdCl\textsubscript{2} concentration (Fig. 1). Cadmium concentrations above 2 \text{ \mu M} had no further decreasing effect on the leaf area. No significant differences in leaf dry weight or SLA were found
Table 1. Cd, Mg, S, Fe, Zn, Ca, Mn, P, Cu, and K concentrations in roots and shoots of Brassica napus after 14 d of Cd and light treatment (HL or HL + UV-B).

Values within each subset, followed by different letters were significantly different at \( P < 0.05 \). The letters next to the values refer to each subset of data within each treatment and can be compared only vertically, and not across columns. \( n = 5 \).

<table>
<thead>
<tr>
<th>[CdCl(_2)] (( \mu )M)</th>
<th>Content (( \mu )mol g(^{-1}) DW)</th>
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<tbody>
<tr>
<td>Cd</td>
<td>Mg</td>
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<tr>
<td>Root high light treatment</td>
<td></td>
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<tr>
<td>0.0</td>
<td>0.01 a</td>
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<tr>
<td>0.5</td>
<td>1.25 a</td>
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<tr>
<td>2.0</td>
<td>10.3 b</td>
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<tr>
<td>5.0</td>
<td>17.8 c</td>
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| Root UV-B treatment |
|---------------------|-------------|
| 0.0                  | 0.00 a      | 145 a      | 272 a    | 69.1 a   | 4.62 a   | 335 a    | 5.04 ab  | 463 a  | 0.89 a  |
| 0.5                  | 1.39 a      | 153 a      | 313 a    | 76.2 a   | 5.61 a   | 238 a    | 7.57 a   | 393 a  | 1.04 a  |
| 2.0                  | 11.0 b      | 132 a      | 319 a    | 349 b    | 15.2 b   | 252 a    | 2.92 ab  | 516 b  | 3.28 b  |
| 5.0                  | 17.6 c      | 121 a      | 328 a    | 365 b    | 10.7 c   | 261 a    | 1.16 b   | 521 b  | 3.35 b  |

<table>
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<th>Shoot high light treatment</th>
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<tr>
<td>0.0</td>
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<td>0.5</td>
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| Shoot UV-B treatment |
|---------------------|-------------|
| 0.0                  | 0.00 a      | 126 a      | 342 a    | 1.37 a   | 2.98 a   | 704 a    | 2.59 a   | 223 a  | 0.23 a  |
| 0.5                  | 0.32 a      | 137 a      | 387 a    | 1.40 a   | 3.02 a   | 716 a    | 2.48 a   | 251 ac | 0.28 a  |
| 2.0                  | 2.34 b      | 194 b      | 826 b    | 1.06 a   | 4.43 a   | 908 b    | 2.23 ab  | 296 b  | 0.85 b  |
| 5.0                  | 3.71 c      | 193 b      | 847 b    | 0.88 a   | 3.30 a   | 950 b    | 1.93 b   | 285 bc | 0.61 c  |

Fig. 1. Leaf area after 14 d of treatment with different Cd concentrations ± UV-B. Error bars represent SE of the mean, \( n = 5 \), \( P < 0.05 \).

between treatments (results not shown). However, root dry weight significantly decreased with increasing cadmium concentration (Fig. 2).

Pigment content

Total chlorophyll content g\(^{-1}\) dry weight decreased by 89% in leaves of plants receiving 2 and 5 \( \mu \)M CdCl\(_2\) compared to controls and plants receiving 0.5 \( \mu \)M CdCl\(_2\) (Fig. 3A, B). UV-B had no significant effect upon pigment content in this case. Chlorophyll \( a \) was also most reduced at the two higher Cd concentrations (92% relative to controls without Cd). The percentage decrease in Chl \( a \) at the higher CdCl\(_2\) concentrations compared to 0 and 0.5 \( \mu \)M, was considerably larger than that for Chl \( b \) (79% relative to controls without Cd). UV-B radiation decreased the Chl \( a:b \) ratio when plants received no or low CdCl\(_2\) amounts (0 and 0.5 \( \mu \)M; Fig. 3C). Bulk carotenoids showed a similar pattern to that of the chlorophylls in that there was a significant reduction of pigment content with 2 and 5 \( \mu \)M CdCl\(_2\), although there were no differences between UV and HL treatments (Fig. 3D). As an estimate of UV-screening pigments, absorbance values at 310 nm were representatively chosen. UV-B
radiation significantly increased the amount of these pigments, but cadmium did not exert any influence (Fig. 4). Peaks at 420 nm were also considered, since a marked decrease in absorbance at this wavelength was observed with increasing cadmium concentration, while UV-B had no effect (Fig. 4).

**Chlorophyll fluorescence**

The potential photochemical yield after dark adaptation, $F_v/F_m$, which may be used as a stress indicator (Björkman and Demmig, 1987), was monitored after 3, 10 and 14 d of treatment. After 10 d $F_v/F_m$ had decreased significantly by 33% in plants exposed to 5 μM CdCl$_2$ relative to controls (0 μM CdCl$_2$) under HL and by 25% for plants under HL + UV-B (Table 2). No statistically significant change was noted in $F_v/F_m$ after 3 d, although a decreasing trend with increasing Cd was observed. The fluorescence maximum ($F_m$) was most affected and showed a decrease at 2 and 5 μM CdCl$_2$. The decrease in $F_m$ was low in the beginning of the experiment but after 10 d the significant...
difference between 0 and 5 \( \mu \text{M} \) CdCl\(_2\) was 73\% (HL) and 65\% (HL+UV-B). After 14 d no large changes occurred compared with the 10 d analyses. Cadmium showed a tendency to increase \( F_0 \) in the beginning of the treatment but after 10 d the effect was reversed.

Results of the non-photochemical quenching are presented only for plants exposed to the full 14 d treatment. After 3 and 10 d the \( q_{\text{NPQ}} \) from leaves of plants exposed to 2 and 5 \( \mu \text{M} \) CdCl\(_2\) increased with time both under HL and HL+UV-B and the yields showed the same decreasing trend as that after 14 d. The \( q_{\text{NPQ}} \) increased when plants were exposed to 2 or 5 \( \mu \text{M} \) CdCl\(_2\) for 14 d compared to control plants (0 \( \mu \text{M} \) CdCl\(_2\)) and plants receiving 0.5 \( \mu \text{M} \) CdCl\(_2\) (Fig. 5). UV-B radiation decreased the non-photochemical quenching in plants exposed to 2 or 5 \( \mu \text{M} \) CdCl\(_2\) compared to HL-irradiated plants. The photochemical yield was lower at 2 and 5 \( \mu \text{M} \) CdCl\(_2\) under both HL and HL+UV-B compared to 0 and 0.5 \( \mu \text{M} \) CdCl\(_2\) exposure.

**Discussion**

Exposure to 2 or 5 \( \mu \text{M} \) CdCl\(_2\) reduced both root and leaf growth (Figs 1, 2). Growth reductions due to Cd may result from decreased photosynthesis and impaired nutrient uptake and transport. Cadmium taken up by the plant often accumulates in the roots (Cutler and Rains, 1974; Catalado et al., 1981) where changes in nutrient levels are most pronounced, especially in the fine roots (Gussarsson, 1994). In our experiment, root dry weight decreased with increasing Cd concentration (Fig. 2).

Cadmium exposure of the *Brassica napus* plants led to substantial changes in nutrient composition in both roots and shoots (Table 1). Roots grown in the presence of Cd contained less K than control roots. In sugarbeet, cadmium inhibits both K\(^+\) stimulation of the K\(^+\) ATPase and K\(^+\) uptake (Lindberg and Wingstrand, 1985). The increase in Fe, Zn and Cu content in roots of plants exposed to Cd may have led to an increased phytochelatin synthesis. In addition, it is known that Cu also can bind to Cd-induced phytochelatins (Maitani et al., 1996). These authors also found Fe-binding phytochelatins. The increased levels of these ions in our experiment with *Brassica napus* might have been due to accumulation, because of their chelation to phytochelatins.

Treatment with 2 or 5 \( \mu \text{M} \) CdCl\(_2\) also affected shoots. The content of S in shoots treated with 2 or 5 \( \mu \text{M} \) CdCl\(_2\) increased. This could have been caused by an increased demand for S due to glutathione and phytochelatin synthesis. Plants exposed to cadmium and UV-B showed higher amounts of Mg, Ca, P, Cu, and K at 2 and 5 \( \mu \text{M} \) CdCl\(_2\) compared to control plants. According to Gregor and Lindberg (1987) cadmium inhibits Mg transport to the shoots of sugarbeet, while Ca transport is facilitated. Leaf area decrease (Fig. 1) and there was also a loss of chlorophyll (Fig. 3) and apparent production of anthocyanin (visually). UV-B-irradiated plants were more anthocyanin-coloured than control plants, which may indicate a greater stress. A decrease in leaf area could be due to competition between Cd and essential cations, leading to deficiency of these ions (Gussarsson et al., 1996). However, ICP analysis only showed a decrease in K ions in roots exposed to cadmium when compared to controls (Table 1).

Cadmium decreased the content of both chlorophylls and carotenoids (Fig. 3). According to Stobart et al. (1985), the decrease in chlorophyll is due to inhibition of protochlorophyllide reduction and inhibition of aminole-
vulinic acid synthesis. Dube and Bornman (1992) also observed a decrease in chlorophyll content in spruce when Cd was present. UV-B radiation often has a negative effect on chlorophyll content (Strid and Porra, 1992), but in this study no effect due to UV-B was found.

In this study the chlorophyll $a:b$ ratio decreased when the plants were irradiated with UV-B (Fig. 3C), which may have been due to a faster breakdown or decreased synthesis of Chl $a$ compared to Chl $b$, although Chl $b$ also decreased. UV-B decreased the Chl $a:b$ ratio when the plants were exposed to zero or low cadmium concentrations, but at higher Cd concentrations the ratio was not influenced by UV-B, which may indicate relatively little interaction of the two stresses on the Chl $a:b$ ratio.

UV-B had no influence on the amount of carotenoids, although carotenoids have been shown to increase (Middleton and Teramura, 1993) or decrease (Tevini and Teramura, 1989) depending on species, growth conditions, etc. The amount of UV-screening pigments increased after UV-B radiation (Fig. 4), which is consistent with earlier experiments (Middleton and Teramura, 1993), while Cd had no influence on these pigments. One possibility considered was that the UV-screening pigments might decrease when Cd was present due to the synthesis of phytochelatins. GSH is thought to act as a signal transducer of the UV-B stimuli in the induction of UV-screening pigments. When Kalbin et al. (1997) exposed pea plants to UV-B radiation the content of GSH increased 4.5-fold. They also observed that UV-B stimulated mRNA encoding for chalcone synthase and phenylalanine ammonia lyase, which are key enzymes in UV-screening pigment synthesis. GSH is also a precursor of phytochelatin, induced by cadmium, and thus possible competition for GSH may lead to a decreased induction of UV-screening pigments or a reduced ability to chelate cadmium ions. However, this hypothesis will need further detailed analysis at both lower and higher Cd levels. Vectors on chlorophyll content (Strid and Porra, 1992), but in this study no effect due to UV-B was found.

The ratio $F_0/F_m$ is often used as a stress indicator and describes the potential yield of the photochemical reaction and has a mean value of 0.83 under optimal conditions (Björkman and Demmig, 1987). This ratio decreased with increasing cadmium concentration (2$\mu$M, 5$\mu$M CdCl$_2$) implying that the plants were under stress (Table 2). The fluorescence parameters were monitored three times, during which $F_0$ decreased after 10 d treatment compared to 3 d, but increased to a small extent after 14 d (Table 2). These results showed that at 3 d the plants were stressed and after another 4 d they apparently had acclimated to the stress conditions. Different environmental stresses increase $F_0$, probably due to decreased efficiency of energy transfer from the antenna chlorophyll $a$ to the reaction centres and/or inactivation of PSII reaction centres (Briantais et al., 1986). However, in some instances non-functional PSII centres may act as dissipative sinks, thereby decreasing $F_0$ (Oquist et al., 1992), which may have accounted for the decreasing trend, although this was statistically not significant (Table 2), after 10 and 14 d (effect for HL + UV-B after 14 d).

Cadmium influenced photosystem II, seen as an increased non-photochemical quenching (Fig. 5). Most of the decrease in fluorescence with an increased $q_{NPQ}$ is attributed to heat dissipation (Horton and Bowyer, 1990).
In the present study this was most pronounced under HL, and increasing Cd concentrations. According to Krupa et al. (1993), increased $q_{NPQ}$ is due to Cd poisoning of the Calvin cycle, which results in limitation of ATP and NADPH consumption, causing a high pH-gradient and a limited electron transport. On the other hand, the marked decrease in amplitude of the $q_{NPQ}$ curves for UV-B radiation may have been due to Cd and an inhibition of the activity of violaxanthin de-epoxidase. This could explain the lowered $q_{NPQ}$ seen when plants were treated with Cd+UV-B compared to Cd only. Impairment of the water-oxidizing system due to replacement of Mn$^{2+}$ and Ca$^{2+}$ ions may occur (Baszynski et al., 1980), as well as that of the electron transport system on the reducing side of PSII upon Cd exposure (Atal et al., 1991). The decreased photochemical yield observed with 2 and 5 $\mu$M CdCl$_2$ compared to 0 $\mu$M, did not appear to be markedly affected by the addition of UV-B radiation. In the present study, with regard to the partial reactions of photosynthesis, plant response was probed using modulated fluorometry which gives information only from the upper leaf tissue and thus cannot give a mean estimate of the whole leaf performance. However, the changes within the upper palisade of a leaf can still be taken as an indication of plant response to an external stress.

In conclusion, Cd decreased growth by reducing root dry weight and leaf area. With regard to photosynthesis, photochemical yield and Chl content were negatively affected. At the same time there were indications of a stress response in the increased $q_{NPQ}$ relative to plants not subjected to Cd. Many of these plant responses are likely to have been regulatory rather than indicating actual damage. The ion content, particularly in roots, was influenced by Cd, shown as an increase in content of Cu, Fe, P, and Zn, while K decreased. In shoots only the S content was significantly raised.

The most pronounced effect of the combined stress factors, Cd+UV-B, was seen in a decreased $q_{NPQ}$ and Chl $a:b$ ratio. The addition of UV-B to Cd-treated plants reduced the variability of data from this study, which led, in shoots, to the detection of significantly increased levels of Ca, Cu, K, Mg, and P, and decreased level of Mn, with increasing Cd concentration, only when UV-B was present. To date, little information exists on plant response to the combination of Cd and UV-B (Dubé and Bornman, 1992).

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References


UV-B and cadmium


