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Response of sugar beet plants to ultraviolet-B (280–320 nm) radiation and *Cercospora* leaf spot disease

Ioannis Panagopoulos, Janet F. Bornman and Lars Olof Björn

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Sugar beet (*Beta vulgaris* L.) plants injected with *Cercospora beticola* Sacc. as well as non-infected plants were grown under visible light with or without ultraviolet-B (UV-B, 280–320 nm) radiation for 40 days. An interaction between UV-B radiation and *Cercospora* leaf spot disease was observed, resulting in a large reduction in leaf chlorophyll content, dry weight of leaf laminae, petioles and storage roots. Lipid peroxidation in leaves also increased the most under the combined treatments. This was also true for ultraweak luminescence from both adaxial and abaxial leaf surfaces. However, no correlation between lipid peroxidation and ultraweak luminescence was observed. Ultraviolet-B radiation given alone appeared to have either a stimulating effect, giving an increase in dry weight of laminae and reducing lipid peroxidation, or no effect. This lack of effect was seen in the absence of change in dry weight of storage roots and chlorophyll content relative to controls. The study demonstrated a harmful interaction between UV-B radiation and *Cercospora* leaf spot disease on sugar beet.

Key words – *Beta vulgaris*, *Cercospora beticola*, infection, lipid peroxidation, sugar beet, ultraviolet-B radiation, ultraweak luminescence.

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Introduction

Numerous studies have demonstrated that increased levels of UV-B radiation can alter plant growth and physiological processes (Teramura 1983, Tevini and Teramura 1989). However, with regard to the effects of UV-B radiation on the growth and physiology of plants infected with fungi, bacteria or viruses, not much information has been documented. Semeniuk and Stewart (1981) reported on the effect of UV-B radiation on the infection process, and studies have also been carried out on the effect of UV-B radiation on virus localization in inoculated leaves and on local lesion development (Semeniuk and Goth 1980). Recently a study on post-infectional and pre-infectional effects of UV-B radiation was reported (Orth et al. 1990).

Cercospora leaf spot disease is one of the most widespread diseases of sugar beet, resulting in the destruction of the leaves of up to 70% and losses up to 44% in

root weight; in extreme conditions it may cause total loss of the crop (Lebrun 1988). The pathogenic role of the fungus, *Cercospora beticola* Sacc., is the disruption of the plasma membrane, tonoplast, and chloroplast membranes (Steinkamp et al. 1979), due to production of singlet oxygen and superoxide radicals via a photosensitizing agent produced in the fungus (Daub 1982, Daub and Hangarter 1983).

The work presented here had a two-fold purpose: (i) to evaluate the interaction between *Cercospora* leaf spot disease and UV-B radiation on sugar beet plants by determining the dry weight of leaf laminae, petioles and storage roots (the economically important part of the plant) and (ii) to examine the effects of these stresses on the peroxidative processes in the sugar beet plant. The oxidative deterioration of polyunsaturated lipids was evaluated by measuring lipid peroxidation, whereas the occurrence of short-lived electronically excited molecular species and/or levels of activated forms of oxygen

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was determined indirectly by measuring the ultraweak luminescence. Generally, high levels of ultraweak luminescence indicate a higher level of short-lived electronically excited molecular species and/or levels of activated forms of oxygen.

Abbreviations- MDA, malondialdehyde; RL, red light; TBA, thiobarbituric acid; UL, ultraweak luminescence; UV-B, ultraviolet-B radiation (280–320 nm).

Materials and methods

Plant material

In order to avoid differential action UV-B radiation on plants with different genomic background, regenerated plants of *Beta vulgaris* L. from meristem culture were used. The plants (kindly supplied by Hillesthög AB, Landskrona, Sweden) were clonally propagated according to Miedema (1982) from a single donor plant (a male fertile monogerm diploid from an inbred line). Adventitious shoots were formed on a solid medium containing half-strength Murashige and Skoog mineral nutrients (MS; Murashige and Skoog 1962) supplemented with 92.5 mM sucrose and 10 μ M benzyladenine (BA). The shoots were multiplied by axillary bud proliferation on a solid medium containing: (i) half-strength MS mineral nutrients, (ii) half-strength MS organic compounds (0.28 mM myo-inositol, 2.03 μ M nicotine acid, 0.15 μ M thiamine-HCl, 1.22 μ M pyridoxine HCl and 13.32 μ M glycine), (iii) 92.5 mM sucrose and (iv) 1 μ M BA. The shoots were later placed for rooting on a solid medium having the same composition as the medium for shoot proliferation but with 10 μ M indolyl butyric acid instead of BA. All the media were solidified with 8 g l⁻¹ agar (Agar Agar Kadoya, ICN Biomedicals B.V., NL), adjusted to pH 5.8 and autoclaved at 120°C for 15 min.

During the micropropagation procedure the cultures received a photon flux density (400–700 nm) of ca 30 μ mol m⁻² s⁻¹ (Osram L36W/77 Fluoro, Germany) with a 16 h photoperiod at 22±2°C. Finally the rooted plants were transferred to soil and placed in a climate chamber at 17–22°C and a photon flux density of ca 106 μ mol m⁻² s⁻¹ (16 h photoperiod) for 7 to 10 days. Lamps were from General Electric (General Electric F96PG17, CWX Power Groove Deluxe CW, Nela Park, Cleveland, OH, USA). Our material was taken from these rooted plants. Only plants 7–9 cm in height were used.

Inoculation

The inoculum was kindly supplied by Hillesthög AB, Landskrona, Sweden. Six strains of *Cercospora beticola* Sacc. collected in Sweden, France, Italy (2 strains), Germany and Austria were grown separately on V-8 juice agar medium (Miller 1955), solidified with 2% agar (Agar Agar Kadoya, ICN Biomedicals B.V., NL), for 14 days in a growth chamber at 23°C and a 16 h

photoperiod. The photon flux density was ca 67 μ mol m⁻² s⁻¹ and the light was from 400 W high pressure metal halogen lamps (HGMI/1400/DH, 83510 E40) together with Tungsram bulbs (100 W, Long life, Rough service). Prior to inoculation, agar plates from all the strains were mixed with water in a blender, and the spore concentration in the suspension was counted in a Bürker haemocytometer. For the inoculum, a suspension of 1×10⁵ conidia ml⁻¹ was used. The suspension was sprayed over the plants, using a handspray such that all leaves (both sides) were inoculated. The inoculated plants were placed under a plastic cover with 100% relative humidity for 3 days and a temperature of ca 25°C. The plants were inoculated twice: 7 to 8 days before they were placed under experimental conditions (see below) and once more, 20 days after they had been placed under the experimental conditions.

Light conditions

According to the treatments given, 4 groups of sugar beet plants were used: (i) non-infected plants subjected to visible light (control plants), (ii) non-infected plants subjected to UV-B radiation and visible light (UV-B plants), (iii) plants infected with *Cercospora beticola* and exposed to visible light (infected plants) and (iv) those infected with *Cercospora beticola* and exposed to UV-B radiation and visible light (UV-B+infection plants).

Visible light was given from Osram power star lamps (HQI-E 400W/D, q×3, Germany), which gave a photon flux density of ca 500 μ mol m⁻² s⁻¹ (400–700 nm). The photoperiod was 16 h. Ultraviolet-B radiation (280–320 nm) from Philips lamps (FS40 UVB), USA was filtered through Plexiglas (FBL. 2458, Röhm GMBH, Chemische Fabrik, Germany; 3.0 mm thick) together with 0.08 mm cellulose diacetate, which removed radiation below 280 nm. The plants received UV-B radiation for 4 h daily between 10 00 and 14 00. The exposure to UV-B radiation was based on calculations by Björn and Murphy (1985) and on the generalized plant action spectrum to UV-B radiation (Caldwell 1971). The biologically effective radiation (UV-B_{BE}) was 6.91 kJ m⁻² day⁻¹. This amount simulated a 9% reduction in the present amount of stratospheric ozone for Lund, Sweden (55.7°N, 13.4°E), on 15 July on a cloudless day, with aerosol level zero. All values were normalized to 300 nm.

Apart from photosynthetic photon flux density (400–700 nm) plants subjected to visible light received ca 48 μ mol m⁻² s⁻¹ UV-A radiation (320–400 nm) emitted by Osram lamps and those subjected to UV-B radiation and visible light received ca 50 μ mol m⁻² s⁻¹ UV-A radiation (320–400 nm).

Radiation was measured with a spectroradiometer (Optronix Laboratories, Model 742, Orlando, FL, USA) interfaced with a microcomputer (Hewlett Packard 85).

Fifteen to 20 plants were placed under each experimental condition and the experiment was performed twice.

Growth measurements

After 40 days the plants were harvested, and dry weight was determined after 24 h at 120°C. The results were expressed as the average per plant (\pm standard error).

Chlorophyll analysis

Chlorophyll was extracted in 80% acetone using quartz sand and a pestle and mortar. The extract was centrifuged (1 600 g, for 10 min) and the supernatant used for chlorophyll determination (Aminco DW-2A spectrophotometer in the split-beam mode) using the equations of Lichtenthaler (1987).

Ultraweak luminescence

Ultraweak luminescence (UL) has been used previously to monitor damage induced by UV-B radiation (Panagopoulos et al. 1989, 1990). The apparatus for the detection of UL has been described in the aforementioned references.

The plants were kept in the dark for 24 h to remove delayed chlorophyll fluorescence, and leaves were detached just before measurements started. Ultraweak luminescence was measured from both upper and lower leaf surfaces. Seven to 8 leaves were sampled, and 60 to 80 data points were collected, each representing the number of photons recorded during 20 s. In order to check whether UV-B radiation had an indirect effect on the chemistry of active forms of oxygen in the storage roots, we measured also UL from the surface of the storage roots. These roots were carefully washed and remained in the dark for at least 8 h before measurement.

Lipid peroxidation

The thiobarbituric acid (TBA) test was used. The assay was done according to Heath and Packer (1968) as modified by Dhindsa et al. (1981). Leaf samples (0.2–0.25 g fresh weight) were homogenized in 5 ml 0.1% TCA using a pestle and mortar. The homogenate was centrifuged at 1 600 g for 15 min. To an 1 ml aliquot of the supernatant, 4 ml 20% TCA containing 0.5% TBA were added. The mixture was heated at 95°C for 30 min and then quickly cooled in an ice-bath. The cooled mixture was centrifuged at 1 600 g for 10 min and absorbances of the supernatant at 532 and 600 nm were read. Concentrations of MDA were calculated using its absorption coefficient of $1.55 \times 10^5 M^{-1} \text{cm}^{-1}$ (Heath and Packer 1968) after the subtraction of non-specific absorbance (600 nm) from the absorbance at 532 nm.

Statistical analysis

For the comparison between two treatments, the Mann and Whitney (*U*) test was used (Mann and Whitney 1947) at a level of significance of $P \leq 0.05$.

Results

Plant growth

Beta vulgaris plants grown under the different experimental conditions showed different growth. In plants infected with *Cercospora beticola*, two leaf categories were observed, those with visible spots and those without. The former leaves were the older ones, whereas the latter were younger and situated towards the center of the crown. No distinction between those leaves were made for the growth parameters described below since whole plants were analyzed; on the other hand, only infected leaves with visible spots were used for all the other analyses.

Sugar beet plants subjected to both UV-B radiation and *Cercospora* had the lowest dry weights for all plant parts (Tab. 1). Dry weight of leaf laminae, petioles and

Tab. 1. Dry weight of leaf laminae, petioles and storage roots from sugar beet plants under different experimental conditions. Values are averages and are expressed as g per plant \pm SE; n denotes number of samples. Means in each column followed by the same letter are not significantly different at $P \leq 0.05$ according to the Mann and Whitney (*U*) test.

| Experimental condition | Leaf laminae | | Petioles | | Storage roots | |
|------------------------|----------------------------|-----|-----------------------------|-----|------------------------------|-----|
| | g | % | g | % | g | % |
| Control | 4.20 \pm 0.20a (n=8) | 100 | 1.20 \pm 0.10a,b (n=8) | 100 | 4.49 \pm 0.28a,b (n=13) | 100 |
| UV-B | 5.00 \pm 0.30b (n=9) | 119 | 1.40 \pm 0.10a (n=9) | 117 | 4.55 \pm 0.33a (n=15) | 101 |
| Infection | 4.30 \pm 0.20a (n=14) | 102 | 1.00 \pm 0.07b (n=14) | 83 | 4.26 \pm 0.26b (n=31) | 95 |
| UV-B+infection | 2.85 \pm 0.30c (n=14) | 68 | 0.74 \pm 0.08c (n=14) | 62 | 3.46 \pm 0.23c (n=28) | 77 |

Tab. 2. Chlorophyll content in leaves of non-infected plants and those infected with *Cercospora beticola*. Values are averages and are expressed as $\mu\text{g Chl (g FW)}^{-1} \pm \text{SE}$; n denotes number of samples. Means in each column followed by the same letter are not significantly different at $P \leq 0.05$ according to the Mann and Whitney (*U*) test.

| Experimental condition | Chl _{tot} | | Chl <i>a</i> | | Chl <i>b</i> | | Chl <i>a/b</i> |
|------------------------|---------------------------|-----|---------------------------|-----|---------------------------|-----|----------------|
| | $\mu\text{g (g FW)}^{-1}$ | % | $\mu\text{g (g FW)}^{-1}$ | % | $\mu\text{g (g FW)}^{-1}$ | % | |
| Control | 1620 ± 74a (n=10) | 100 | 1210 ± 48a (n=10) | 100 | 410 ± 42a (n=10) | 100 | 2.99a,b |
| UV-B | 1520 ± 117a (n=9) | 94 | 1160 ± 87a (n=9) | 96 | 357 ± 30a (n=9) | 88 | 3.24a |
| Infection | 314 ± 10b (n=8) | 19 | 243 ± 6b (n=8) | 20 | 71 ± 4b (n=8) | 18 | 3.42c |
| UV-B+infection | 307 ± 15b (n=8) | 19 | 232 ± 12b (n=8) | 19 | 75 ± 4b (n=8) | 18 | 3.09b |

storage root was reduced by 32, 38 and 23%, respectively, compared to the dry weight of the control plants (Tab. 1). On the other hand, non-infected plants subjected to UV-B and visible light had increased leaf laminae dry weight by 19% ($P \leq 0.05$) relative to control plants (Tab. 1).

Chlorophyll determination

Total Chl, Chl *a*, Chl *b* and Chl *a/b* ratios in leaves from sugar beet plants under different treatments are shown in Tab. 2. No statistical difference between control and UV-B plants was observed in chlorophyll contents of leaves. Under visible light the most marked change in Chl content occurred in infected leaves, with a ca 80% reduction as compared to controls. This trend was seen in similiary infected leaves under UV-B treatment (also ca 80% reduction in Chl content).

Lipid peroxidation

The lowest value of lipid peroxidation was detected in leaves from UV-B plants (22% lower compared to leaves from control plants, $P \leq 0.05$; Tab. 3). Under visible radiation alone, lipid peroxidation of infected leaves of plants was 57% greater than controls. How-

Tab. 3. Lipid peroxidation in leaves of plants under the experimental conditions. Values are averages and are expressed as $\text{nmol MDA (g FW)}^{-1} \pm \text{SE}$; n denotes number of samples. Means followed by a different letter are significantly different at $P \leq 0.05$ according to the Mann and Whitney (*U*) test.

| Experimental condition | MDA, nmol (g FW) ⁻¹ | % |
|------------------------|--------------------------------|-----|
| Control | 64.7 ± 4.8a (n=10) | 100 |
| UV-B | 50.3 ± 2.6b (n=9) | 78 |
| Infection | 101.7 ± 3.1c (n=8) | 157 |
| UV-B+infection | 131.4 ± 3.3d (n=8) | 203 |

ever, still higher values were observed for infected leaves subjected to UV-B radiation in addition to visible radiation.

Ultraweak luminescence

The effect of different treatments on UL was measured from both the adaxial and abaxial leaf surfaces. Since the trends for the parameters measured were similar, only the results for the adaxial surface are shown (Fig. 1). Ultraweak luminescence was highest in infected leaves from both visible and UV-B radiation conditions, and there was no apparent interaction between infection and added UV-B stress (Fig. 1). The red light (RL) component was ca 50% of the total UL in all cases.

Ultraweak luminescence and the RL component were also tested from the surface of storage roots (Fig. 2). The highest UL was observed from roots of infected plants with or without exposure to UV-B radiation. In absolute values, the RL component was constant for all treatments.

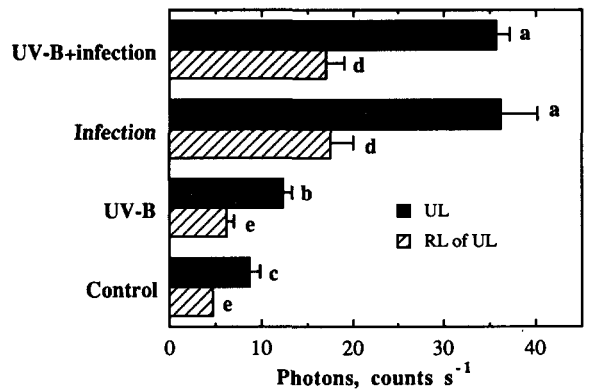


Fig. 1. The effect of different experimental conditions on ultraweak luminescence (UL) and the red portion of this luminescence (RL of UL) measured from the adaxial surface of leaves of *Beta vulgaris*. Each bar is the mean of 7 to 8 measurements. Bars followed by the same letter are not significantly different at $P \leq 0.05$ according to the Mann and Whitney (*U*) test.

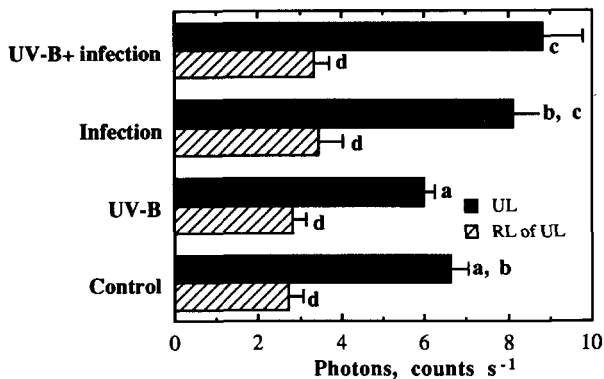


Fig. 2. The effect of different experimental conditions on ultra-weak luminescence (UL) and the red portion of this luminescence (RL of UL) measured from the surface of the storage roots of *Beta vulgaris*. Each bar is the mean of 5 measurements. Bars followed by the same letter are not significantly different at $P \leq 0.05$ according to the Mann and Whitney (U) test.

Discussion

The results presented in this study show that UV-B radiation increases the severity of *Cercospora* leaf spot disease in sugar beet plants. Since the plants were clonally propagated from a single donor plant, and thus had identical genomic background, the probability of a differential effect of UV-B radiation on different genomes was excluded. In order to determine whether the effect of UV-B radiation on the severity of the disease was related to UV-B sensitivity of the sugar beet plants, non-infected plants were also exposed to UV-B radiation. Unlike earlier investigations where UV-B radiation reduced shoot and root growth and chlorophyll content of sugar beet (Ambler et al. 1978, Panagopoulos et al. 1990), in the present study UV-B radiation had either a stimulating effect by increasing dry weight of leaf laminae (Tab. 1) or no effect on parameters such as dry weight of storage roots (Tab. 1), chlorophyll content (Tab. 2) and UL from storage roots (Fig. 2). This may have been due to the different line used in this study and/or different light conditions. Although it was previously hypothesized that lipid peroxidation may increase under UV-B radiation (Panagopoulos et al. 1990), those experiments were carried out with a much lower background of visible light than was the case for the present study. This may partly explain the rather surprising result of a decrease in lipid peroxidation under UV-B radiation (Tab. 3). Kramer et al. (1991) showed that UV-B radiation increased lipid peroxidation in two cucumber cultivars. However, the biological effective dose of UV-B radiation was twice as high as in the present study.

The slightly higher UL for leaves of UV-B plants compared to that for leaves of control plants (Fig. 1) indicated a higher level of activated forms of oxygen under UV-B radiation, which could not be correlated

with the effect of UV-B radiation either on plant growth (Tab. 1), chlorophyll content (Tab. 2) or lipid peroxidation (Tab. 3).

There was an interaction between UV-B radiation and *Cercospora* leaf spot disease, resulting in a more harmful action than either treatment alone. Thus the reduction of dry weight for leaf laminae, petioles and storage roots of UV-B+infection plants was greater than the sum of either UV-B radiation or infection stress alone (Tab. 1). The same result was found for lipid peroxidation (Tab. 3), although the results were complicated by the fact that UV-B radiation decreased the lipid peroxidation. This did not, however, hold true for Chl content, where infection by *Cercospora beticola* seemed to be the determining stress (Tab. 3).

Although it is known that lipid peroxidation is a source of UL (Cadenas 1984), in the present study UL of leaves (Fig. 1) and the comparison with lipid peroxidation (Tab. 4) revealed that lipid peroxidation was not the main source of UL. For example, in leaves from infected plants exposed to UV-B radiation the lipid peroxidation was much higher than in leaves from infected plants under visible light. However, the UL was about the same for both cases. With regard to UL from the surface of storage roots (Fig. 2), the results suggest an indirect effect of the treatments on these roots. It is possible that one or more activated products could be transported to the roots. The combination of UV-B radiation and infection may have increased the production of these products and/or their transport.

In this study the combined stress of UV-B radiation and *Cercospora* leaf spot disease appeared to increase the severity of the disease. The results of this interaction may be enhanced even more under conditions favouring the spread of the disease.

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