

Effects of UV-B Radiation on Oxalate Content of Silver Beet Leaves

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Abstract

Silver beet (*Beta vulgaris* var. *cicla*) a common vegetable in New Zealand is known to contain high levels of oxalates in the leaves. Silver beet plants were grown in a field trial under glass and perspex sheets which filtered sunlight reaching the plants. After eight weeks of growth, the plants were harvested and the total, soluble and insoluble oxalate content of the leaves of the plants grown under the two filter treatments and a no-frame control were measured. Perspex allowed the transmission of UV-A, UV-B and photosynthetically active radiation (PAR), whereas glass excluded UV-B radiation. No significant differences between the perspex treatment and the no-frame control were observed when the data was compared on a wet matter (WM) or dry matter (DM) basis. Shielding the growing plants with glass significantly reduced the total oxalate and soluble oxalates to 83 and 84% respectively when compared to the perspex and no-frame treatments.

Keywords: silver beet, total, soluble and insoluble oxalates, UV spectrum

1. Introduction

An absolute requirement for the growth of plants is sunlight, as photosynthetic pigments absorb visible wavelengths and use the energy for carbon fixation in photosynthesis (Larson, 1988). However, the ultraviolet portion of the electro-magnetic spectrum, particularly UV-B (280-315 nm), can have damaging effects of reducing photosynthesis, increasing reactive oxygen species (ROS) production and decreasing growth (Asada, 1999; Booij-James et al., 2000; Hofmann et al., 2001; Hofmann et al., 2003; Jordan, 2002). Up to 90% of UV-B radiation is filtered out by the ozone layer (WHO, 2010), but due to ozone depletion and other anthropogenic and natural factors incident summer UV-B radiation in New Zealand is approximately 40% higher than at corresponding latitudes in the northern hemisphere (NIWA, 2006). Plants are adapted both to utilise high light conditions and protect themselves from its damaging effects. One way in which they can protect themselves is through the production of antioxidants such as ascorbate (Smirnoff & Pallanca, 1996), which functions to counteract the negative effects of ROS (Hofmann et al., 2000).

Silver beet (*Beta vulgaris* var. *cicla*), also known as Swiss chard, is a common vegetable plant consumed in New Zealand and throughout the temperate regions of the world (Simpson et al., 2009). Silver beet leaves contain high levels of vitamins A, B and C, phosphorus, iron and calcium (Pyo et al., 2004), but due to high levels of oxalate, a high proportion of the calcium is sequestered as insoluble calcium oxalate and is therefore unavailable to humans. Previously reported levels of total oxalates in silver beet range from 332.3 to 795.0 mg/100 g wet matter (WM) (Awadalla, 1985; Santamaria, 1999; Savage et al., 2000; Savage et al., 2004).

Oxalates exist in two forms, soluble and insoluble. Oxalic acid forms water-soluble salts with potassium, sodium and ammonium ions, and insoluble salts with calcium, magnesium and iron ions (Noonan & Savage, 1999; Radek & Savage, 2008). The soluble form appears to be of most concern in terms of health effects as insoluble oxalate is not absorbed and is simply excreted in the faeces. High oxalate concentrations in green leafy vegetables such as silver beet are a concern because they can lead to the development of kidney stones when the oxalate is excreted by the kidneys (Noonan & Savage, 1999). Reduction of oxalate in the diet can be made by avoiding certain foods, cooking high oxalate foods in such a way so as to reduce the levels of oxalates actually consumed (Savage et al., 2000) or alternatively by manipulating the levels of oxalate allowed to accumulate when growing crop plants. However, limited research has been conducted in the latter area.

Ascorbate plays a strong role in photosynthesis and photoprotection, and is increased in plants in response to UV radiation exposure (Grace & Logan, 1996; Logan et al., 1996; Smirnoff & Pallanca, 1996). Ascorbate has been shown to be the main precursor of oxalate in plants (Franceschi & Horner, 1979; Kostman et al., 2001) and increasing ascorbate concentrations also increase oxalate levels (Franceschi & Horner, 1979; Guo et al., 2005). Oxalate is well-known for its roles in plant protection, as ingestion of oxalate can be toxic at high concentrations (James & Butcher, 1972; Massonie, 1980; Yoshihara et al., 1980) and calcium oxalate crystals formed in plants can act as a physical obstacle or deterrent to grazing animals and insects (Franceschi & Nakata, 2005). There may be an additional role for oxalate in photosynthesis. Oxalate is oxidised to H_2O_2 and CO_2 by oxalate oxidase, and experiments in which ^{14}C -labelled L-ascorbic acid was fed to plants suggests that this may be a significant source of CO_2 for plants (Loewus, 1999). Based on results from studies on the oxalate-accumulating redwood sorrel (*Oxalis oregana*) by Yang and Loewus (1975) and other plants by Nuss and Loewus (1978) and Bjorkman and Powles (1981), a pathway involving oxalate as a reservoir for CO_2 for photosynthesis was proposed by Loewus (1999). In this model, it was proposed that oxalate oxidase may be light-regulated to release CO_2 during incidences of high light exposure for optimal photosynthesis. H_2O_2 , also released in the process, then it replenishes the oxalic acid pool through cleavage of ascorbic acid, whose synthesis is presumably under homeostatic control.

Little is known about the effects of UV-B radiation on oxalate accumulation. Preliminary unpublished trials indicated that the leaves of silver beet contained higher levels of oxalate when grown outside in full sun compared to being grown in a glasshouse. It is appreciated that growth of plants in a greenhouse is very different compared to growth in the field, for example plants in a greenhouse generally grow more rapidly as the night time temperatures tend to be higher. One of the most significant environmental differences is that plants grown in a greenhouse are protected by the roof glass from certain fractions of sunlight. Therefore the current trial was performed in the field using two different filter materials supported on frames, in order to measure the effects of different UV wavebands on oxalate accumulation in the growing leaves.

2. Materials and Methods

2.1 Growth of the Plants

Silver beet seedlings (cultivar Fordhook giant, Egmont Seed Company Ltd, New Plymouth, NZ) were grown in a Wakanui silt loam soil at the Horticulture Research Area, Lincoln University, Canterbury, New Zealand ($43^{\circ}38'S$, $172^{\circ}27'E$) at 19 m above sea level. The Wakanui silt loam had a good base fertility and no manures or chemical fertilisers were used. The plots were surrounded by shelter trees and plants were irrigated as required. In each row four plants were randomly allocated to each of the following treatments. Glass and perspex sheets were placed randomly over four adjacent plants in each row to comprise the treatments for this experiment. These materials were chosen because they absorb different UV radiation wavelengths. The 1220 x 610 x 4.5 mm sheets of glass and Perspex (polymethylmethacrylate) were supported on a metal frame box so that the sheets were 300 mm above the plants; this was raised to 500 mm as the plants grew taller. In addition, two no-frame control treatments of four plants were included in each row that received unfiltered sunlight. The plants at the end of each row and the outside rows served as border plants. Each row was separated by a guard row of the same plants and the trial was set up in a randomized complete block design.

2.2 Harvesting

The leaves of the silver beet plants were harvested randomly from each plant of each the four replicate plots after eight weeks of growth, when the plants had reached a height of 500 mm. Representative samples of the leaves from each treatment were freeze dried in a Cuddon Freeze Dryer (Model No. E. D. 5.3) to a final moisture content of 1-5%. Each freeze-dried sample was ground to a fine powder using a Sunbeam Multi Grinder (Model no. EMO 400 Sunbeam Corporation Limited, NSW, Australia). The dry matter (DM) content of the leaves was determined using an AOAC standard method (AOAC, 2002-method 925.10).

2.3 Oxalate Analysis

The oxalate content of each sample was determined using the method described by Savage et al. (2000). Three separate 0.5 g samples of dried ground silver beet leaves were placed in a 100 ml flask, 40 ml nanopure water (Arium 611uv, Sartorius Ltd. Germany) was added and incubated in a water bath at $80^{\circ}C$ for 15 min to extract soluble oxalates. Total oxalates were extracted using 40 ml 0.2 M HCL at $80^{\circ}C$ for 15 min. The extracts were allowed to cool and then transferred quantitatively into 100 ml volumetric flasks and made up to volume. The extracts were centrifuged at 2889 rcf for 15 min. The supernatant was filtered through a 0.45 mm cellulose nitrate filter. The chromatographic separation was carried out using a 300 x 7.8 mm Rezex ROA ion exclusion organic acid column (Phenomenex, Torrance, CA, USA) attached to a cation H^+ guard column (BioRad,

Richmond, California, USA). The analytical column was held at 25°C. The equipment consisted of an auto sampler (Hitachi AS-2000, Hitachi Ltd., Kyoto, Japan), a ternary Spectra-Physics, SP 8800 HPLC pump (Spectra-Physics, San Jose, California, USA), a Waters U6K injector (Waters Inc., Marlborough, Massachusetts, USA), a UV/VIS detector Spectra-Physics SP8450 (Spectra-Physics, San Jose, California, USA), set on 210 nm. Data capture and processing were carried out using a peak simple chromatography data system (SSI Scientific Systems Inc, State College, PA, USA). The mobile phase used was an aqueous solution of 25 mM sulphuric acid. Samples (20 µl) were injected onto the column and eluted at a flow rate of 0.6 ml/min. Insoluble oxalate content was calculated by difference (Holloway et al., 1989). The oxalate content of the leaves harvested from each treatment was calculated on a wet matter (WM) and a dry matter (DM) basis.

2.4 Analysis of the Filter Materials

Samples of the filter materials were analysed in triplicate for transmittance in a UV-Visible Spectrophotometer (Model T60 PG Instruments, NZ) and analysed using accompanying UVWin Spectrophotometer Software (version 5.0.5).

2.5 Statistical Analysis

The data was analysed using REML variance components analysis using GenStat version 13 for Windows 7 (VSN International Ltd., Hemel Hempstead, Hertfordshire, UK) to access the effect of the different UV filtering materials on the oxalate content of the silver beet leaves. The data was presented as the means of 4 determinations ± S. E.

3. Results and Discussion

The sheets placed above the plants filtered the sunlight the plants received each day from around 9 am to 3 pm, and at other times the plants received some unfiltered sunlight through the uncovered sides when the sun was at a low angle in the sky. The weekly averages of ambient biologically-weighted UV-B radiation (Flint & Caldwell, 2003) over the trial period ranged from 14.0-35.3 kJ/m²/day. Figure 1 shows the percentage of transmittance of each of the filter materials used. All treatments transmitted photosynthetically active radiation (PAR, wavelength range 400-700 nm). Glass excluded 95% of the UV-B fraction while perspex transmitted all UV-B wavelengths.

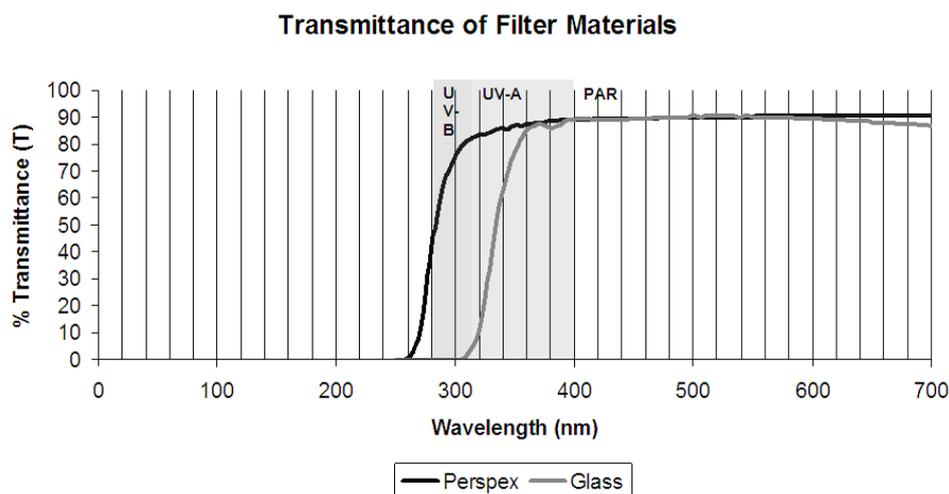


Figure 1. Transmittance through each of the filter materials

The oxalate data are presented in Table 1 on a WM and DM basis. The data presented on a WM basis shows the data as it would normally be eaten, while data presented on a DM basis shows the differences in oxalate accumulation between each of the three treatments as UV radiation is known to decrease biomass accumulation and yield (Kalbin et al., 2001; Krizek, Britz, & Mirecki, 1998; Mazza et al., 1999).

The filter material had a significant effect on total and soluble oxalate accumulation on both a WM and a DM basis, but no significant effect on the insoluble oxalate content of the leaves. The level of soluble and total oxalates decreased significantly in the glass treatment to approximately 82-85% of the perspex control values. The mean total, soluble and insoluble oxalate contents of the no-frame control plots were not significantly

different from the perspex treatments. The no-frame control was used to expose the silver beet plants to field conditions, and as it gave similar results to the perspex treatment, any differences from these two treatments to the glass treatment are therefore due to UV-B radiation. There were no significant differences in dry matter content between treatments (mean DM content of 12.56 g/100 g WM).

Table 1. Mean total, soluble and insoluble oxalate content (mg/100 g DM) of silver beet leaves grown under different filtering materials

Treatment	Dry matter content	Total oxalate		Soluble oxalate		Insoluble oxalate	
	(g/100 g WM)	(mg/100 g WM)	(mg/100 g DM)	(mg/100 g WM)	(mg/100 g DM)	(mg/100 g WM)	(mg/100 g DM)
		(% of perspex value)	(% of perspex value)	(% of perspex value)	(% of perspex value)	(% of perspex value)	(% of perspex value)
No-frame control	12.6 ^a	826.4 ^a (103.0)	6613 ^a (102.6)	742.4 ^a (103.5)	5934 ^a (103.5)	84.1 ^a (93.7)	648.4 ^a (90.0)
Perspex	12.5 ^a	802.1 ^a	6447 ^a	717.6 ^{ab}	5733 ^a	89.8 ^a	720.1 ^a
Glass	12.5 ^a	674.6 ^b (84.1)	5253 ^b (81.5)	606.4 ^{bc} (84.5)	4737 ^b (82.6)	65.4 ^a (72.8)	545.1 ^a (75.7)
SE (average)	0.53	43.6	128.8	46.7	243.7	15.0	110.4

Data in each column with a different superscript are significantly different ($p < 0.05$).

The decrease in oxalate levels in the glass treatment may be due to decreased UV-B-induced production of ascorbate, the main precursor of oxalate (Franceschi & Horner, 1979; Kostman et al., 2001). It is also possible that the reduced UV-B intensity affected oxalate oxidase activity and reduced the proposed 'C2' pathway of photosynthesis (Loewus, 1999), and as a result the oxalic acid pool was not increased.

The total oxalate contents in this study ranged from 674.6 to 826.4 mg/100 g WM and 5253 to 6613 mg/100 g DM (Table 1). The soluble oxalate content was between 606.4 and 742.4 mg/100 g WM and 779.2 and 896.8 mg/100 g DM, while the insoluble oxalate content ranged from 65.38 to 89.82 mg/100 g WM and 545.1 to 720.1 mg/100 g DM (Table 1). The results are within the range of values previously reported for silver beet (Awadalla, 1985; Santamaria, 1999; Savage et al., 2000; Savage et al., 2004).

4. Conclusions

Growing silver beet plants in conditions where the plants are protected from direct sunlight by glass, for example in a glasshouse, may reduce the amount of total and soluble oxalate synthesised in the leaves. This may be of interest to those at risk of developing kidney stones who wish to avoid consuming oxalate-rich foods.

This research has highlighted areas for further study, namely the mechanisms of UV effects on oxalate production and the role of oxalate in photosynthesis. Measurement of oxalate, ascorbate as well as other antioxidants and biomass, and the use of clonal plants and C¹⁴-labels, may be of particular use in determining the effect of UV radiation on oxalate production in plants.

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