UV irradiance as a major influence on growth, development and secondary products of commercial importance in Lollo Rosso lettuce ‘Revolution’ grown under polyethylene films

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Abstract

The growth and production of anthocyanin, flavonoid and phenolic compounds were evaluated in Lollo Rosso lettuce ‘Revolution’ grown continuously under films varying in their ability to transmit UV radiation (completely transparent to UV, transparent above 320, 350, 370 and 380 nm and completely opaque to UV radiation). Plants were grown from seed under UV transparent and UV blocking films and destructively harvested 3–4 weeks after transplanting. Plants under a complete UV blocking film (UV400) produced up to 2.2 times more total above ground dry weight than plants under the UV transparent film. In contrast, anthocyanin content in plants under the UV blocking film was approximately eight times lower than in plants under a UV transparent film. Furthermore, there was a curvilinear relationship between the anthocyanin content and UV wavelength cutoff such that above 370 nm there was no further reduction in anthocyanin content. Fluorescence measurements indicated that photosynthetic performance index was 15% higher under the presence of UVB and UV A (UV280) than under the presence of UVA (UV320) and 53% higher than in the absence of UV radiation suggesting protection of the photosynthetic apparatus possibly by phenolic compounds. These findings are of particular importance as the potential of UV transmitting films to increase secondary compounds may offer the opportunity to produce plants commercially with increased health benefits compared to those grown under conventional films.

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1. Introduction

One of the most exciting developments in commercial protected cropping in recent years has been the introduction of spectral filters incorporated into horticultural polyethylene films, which block specific wavebands. Reduction in plant height has been observed in a range of species under polyethylene films with low transmission of FR light, offering an alternative method of producing compact high quality plants without using chemical growth regulators (Haeringen et al., 1998; Rajapakse et al., 1999; Runkle and Heins, 2001; Rajapakse and Li, 2004; Fletcher et al., 2005). A new generation of UV transparent and UV blocking plastic films has also been developed which have a number of potential applications for protected cropping. Plastic films which are opaque to UV offer an environmentally friendly way of controlling pests and diseases (Doukas and Payne, 2007). Recent research has shown that the incidence of whitefly was significantly reduced under UV blocking films compared to standard films (Doukas, 2002; Doukas and Payne, 2007). Currently, commercial production under plastic film structures is based on standard horticultural films (or glass) that block some of the UV radiation. In contrast, plastic films with high UV transmission may offer a way of increasing secondary products and therefore potential health benefits in response to increased UV radiation. Little research has been carried out, however, to quantify the effects of UV radiation on crop quality.

UV radiation can be regarded as a stress factor which is capable of significantly affecting plant growth characteristics. Plant height, leaf area, leaf length have been showed to decrease, whereas leaf thickness was increased in response to UVB radiation (Teramura, 1983; Tevini and Teramura, 1989; Rozema et
radiation. According to Caldwell et al. (1994), UVB radiation and UV A mitigate damage caused specifically by UVB (320–400 nm) irradiance. It seems that ambient levels of alistically high UV radiation levels and inappropriate levels of supplementary UV radiation are difficult to interpret due to unre-field conditions, using films that sequentially block specific bands within UV region of the spectrum. The potential for increasing secondary product levels by using a highly UV transparent film compared to standard horticultural UVI/EVA film is assessed.

2. Materials and methods

2.1. Growth conditions and plant husbandry

Two experiments were conducted during the summers of 2005 and 2006 at the School of Biological Sciences Laboratories Field Unit, Shinfield, Reading, UK. Each experiment was carried out in a suite of 10 tunnels, measuring 3 m × 2.7 m × 6.8 m (W × H × L), specifically designed for studying experimental cladding materials. Six plastics that block progressively at 20-nm intervals across the UV region were supplied by British Polyethylene Industries Agri, Stockton-on-Tees, UK. The spectral transmission of each film, measured using a Bentham Spectroradiometer (M 300 EA monochromator, Bentham Instruments Ltd., Reading, Berkshire, UK) (Fig. 1).

Seeds of Lollo Rosso ‘Revolution’ (Elsoms Seeds Ltd., Lincolnshire, UK) were sown in plug trays containing peat-based compost (Bulrush Horticulture Ltd., UK) and then immediately transferred to each tunnel covered with one of the experimental films. After a period of 4 weeks they were transplanted into either 1-m peat-filled grow bags (Bulrush Horticulture Ltd., UK) (2005), or into 0.5-m peat-filled grow bags (2006) at a density of 5 and 3 plants per bag, respectively. Six grow bags were transferred into each tunnel.

Plants were irrigated with a standard commercial feed 3:1:1 (N, P, K) (Sinclair Horticultural, Lincoln, UK) applied through a drip irrigation system (Field Ltd., Kent, UK) (four drippers per peat bag) via a Dosatron (Dosatron International, Bordeaux, France) set to provide an electrical conductivity of 1.4 ms. Temperature in each tunnel was measured using T-type thermocouples inserted into aspirated screens. Tube solarimeters (in-house construction, Szczek et al., 1964), suspended 10 cm below the film, were used for measuring total radiation in each tunnel. Air temperature and radiation in each tunnel were measured every 30 s and half-hourly averages were recorded in a data-logger (DT-500, Data Electronics, Cambridge, UK).

![Spectral transmission of UV blocking and UV transparent polyethylene films.](image)
2.2. Experimental design

The experiment was set out as two blocks in which treatments (plastic films) were randomly allocated. In 2005, five films were used and replicated twice (UV280, UV320, UV370, UV380 and UV400) whereas six films were used in 2006 (UV280, UV320, UV380 replicated twice and UV350, UV370 and UV400 replicated once). The experiment was repeated three times, twice in 2005 and once in 2006. Six grow bags were randomly allocated to three plots within each tunnel. Two plants per grow bag were used for growth measurements and one plant per grow bag for secondary product analysis \((n = 24 \text{ in 2005 and } n = 12 \text{ in 2006). Data were analyzed using the ANOVA procedure of the Genstat statistical package (version 8) (VSN International Ltd., Oxford, UK).}

2.3. Growth and physiological measurements

Plants were harvested for analysis 26, 29 and 24 days after transplanting in June 2005, July 2005 and July 2006, respectively. Leaf number and fresh and dry leaf weight were recorded. Dry weight measurements were carried out after drying to constant weight in a ventilated oven at 70 °C. Chlorophyll fluorescence was measured on four different sunny days from 25 July 2006 to 29 July 2006 using a portable fluorometer (Handy PEA, Hansatech Instruments Ltd., Kings Lynn, UK). One leaf (same age) was chosen per plant from each of the six grow bags in each tunnel. A total of 12 measurements per treatment were made (UV280, UV320 and UV380). Fluorescence measurements included: maximum quantum yield of PS II \(\varphi_P\), number of active PSII reaction centres per quantity of light absorbed \(N_{PSII}\), and the efficiency of charge separation \(E_{CS}\).

2.4. Secondary product measurement

Sample preparation and extraction: Six lettuce plants from each tunnel were chosen for analysis (one per peat bag). Each sample was taken from one plant by removing the distal ends (4 cm) of five to six leaves (Voipio and Autio, 1995). These leaves were blended in a food processor and a 1-g sub-sample was extracted with 10 ml of methanol:HCl (99:1) and placed in a vial (Voipio and Autio, 1995). The mixture was stirred for 1–2 min with a hand held stirrer. The supernatant was then placed in a screw-cap vial and placed in a cold room (2 °C) overnight to facilitate extraction. An aliquot of the filtered extract was used the next day to determine total anthocyanins, flavonoids and phenolics levels.

Determination of total phenolics, flavonoids and anthocyanin: Total soluble phenolics in the extract were determined spectrophotometrically (CECIL 1000 series Cecil Instruments Ltd., Cambridge, UK) using a modified Folin-Ciocalteu colorimetric method (Singleton et al., 1999; Wang and Lin, 2000; Meyers et al., 2003). Results were expressed as mg of gallic acid equivalent (GAE) per 100 g of fresh weight. Total flavonoid content of the extracts was determined spectrophotometrically using a modified colorimetric method described by Jia et al. (1999), modified by Meyers et al. (2003). Results were expressed as mg of catechin equivalents per 100 g fresh weight. Total anthocyanin content was determined using the pH differential method. Anthocyanin content was estimated in a spectrophotometer at 510 and 700 nm buffers at pH 1.0 and 4.5 (Wrolstad, 1976; Giusti and Wrolstad, 2001; Meyers et al., 2003). All values were estimated as cyanidin-3-glucoside using a molar extinction coefficient of 26,900 (Wrolstad, 1976; Giusti et al., 1999).

2.5. Spectral transmission and experimental conditions

The UV400 film was opaque to radiation of the UV waveband, whilst, the UV280 film showed the highest transmission to UV spectrum (Fig. 1). The UV280 and UV320 films had 81% and 39% transmission, respectively in the UV region, whilst, UV350, UV370, UV380 and UV400 had 22%, 10%, 3.5% and 0% transmission, respectively (Table 1). All the films used in this study exhibited the same PAR (400–700 nm) transmission except for the UV370 film which had a slightly reduced transmission (between 4% and 7% lower than the other films). There was no significant effect of the film on temperature or light measured within the tunnels. Therefore, observed effects over the experimental period were most likely to be due to differences in UV light spectrum rather than to differences in other environmental variables (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PAR transmission (% of control)</th>
<th>UV transmission (% of control)</th>
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<tbody>
<tr>
<td></td>
<td>(400–700 nm)</td>
<td>(280–400 nm)</td>
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<tr>
<td>UV280</td>
<td>86</td>
<td>81</td>
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<tr>
<td>UV320</td>
<td>86</td>
<td>39</td>
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<td>UV350</td>
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<td>UV380</td>
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<td>3.5</td>
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<tr>
<td>UV400</td>
<td>84</td>
<td>0</td>
</tr>
</tbody>
</table>

*Only used in 2006 season.
3. Results

3.1. Effect of UV on growth

Lettuce showed a strong response to UV radiation. In reduced UV radiation vegetative growth was increased compared to plants in high UV radiation levels (Fig. 2). No significant interaction between vegetative growth and planting month was observed in 2005 and in both years total above ground dry weight in lettuce plants under UV350, UV370, UV380 and UV400 was higher than in plants under the UV280 and UV320 ($P < 0.001$). Plants under a complete UV blocking film (UV400) produced 40% and 122% more total above ground dry weight in 2005 and 2006, respectively, than plants under the UV transparent treatment (UV280) (Fig. 2A and B). Plants under the UV320 (standard horticultural UVI/EV A film) produced 10% and 34% higher total above ground dry weight than in plants under the UV280 film in 2005 and 2006, respectively (Fig. 2A and B). Leaf number was also proportionally increased in plants grown under treatment films that blocked UV radiation. Plants under the UV400 film produced 28% and 66% more leaves in 2005 and 2006, respectively than plants in the UV280 treatment (Fig. 2C and D). Total above ground dry weight was linearly related to UV wavelength cutoff of individual films (Fig. 3).

No significant differences were observed in the ratio of variable and maximum photochemical efficiency of PSII ($F_v/F_m$) of plants grown under UV380 compared to those plants grown under the UV280 and UV320 films (Fig. 4A). Significant differences were, however observed, in photosynthetic performance index (PIABS). Plants under a UV transparent film (UV280) had 15% and 53% higher performance index than plants under a less UV transparent film (UV320) and a UV blocking film (UV380), respectively (Fig. 4B).

3.2. Effect of UV on phenolic compounds

Total anthocyanin content of lettuce was higher in plants grown under the UV280 and UV320 films than in plants under the UV350, UV370, UV380 and UV400 films ($P < 0.001$) (Fig. 5A and B). In 2006, anthocyanin content was 50% higher under a UV transparent film (UV280) compared to plants under the standard horticultural UVI/EVA film (UV320) (Fig. 5B). The lowest anthocyanin content was observed in plants under the UV400 film which was up to eight times lower than plants under the UV transparent film UV280 film (2006) (Fig. 5B).

The total flavonoids followed a similar pattern to anthocyanins across the different film treatments. In general, the higher the UV radiation transmitted through the film, the higher the flavonoid content. Plants under the UV transparent film (UV280) had 63% and 92% higher flavonoid contents than plants...
under the UV blocking film (UV400) in June and July plantings (2005), respectively (Fig. 5C). Plants under the UV transparent (UV280) film had 20% higher flavonoid content than plants under the commercial film (UV320) (Fig. 5D).

The total phenolic content was also highest in plants from the UV280 and UV320 films and declined progressively in plants grown under the UV350, UV370, UV380 and UV400 films \( (P < 0.001) \) (Fig. 5F). Complete blocking of UV radiation (UV400) reduced phenolic content in lettuce plants by 50% compared to commercial film (UV320 treatment) and by 60% compared to UV transparent film (UV280) in the July 2005 planting (Fig. 5E). In 2006 the highly UV transparent film (UV280) increased total phenolic content by 20% compared to the UV320 film (standard horticultural UVI/EVA film) (Fig. 5F).

The month of planting (2005) had a significant effect on secondary product content \( (P < 0.001) \) (Fig. 5A, C and E). In general secondary product content was lower during June and higher during July, probably due to lower radiation levels in June.

Secondary products were highly correlated with the degree of UV radiation cutoff. A curvilinear relationship could be fit-
Fig. 6. (A) The curvilinear relationship between anthocyanin content and wavelength cutoff of individual treatment films. (B) The curvilinear relationship between phenolic content and wavelength cutoff (nm) of individual treatment films. $R^2$ values and standard error bars are shown.

4. Discussion

The Lolo Rosso lettuce ‘Revolution’ used in this study showed a clear response to an increase in the proportion of UV light by increasing secondary metabolites and reducing growth. Total anthocyanins were greatest when both UVA and UVB were present (UV280 film). Where UVB was excluded and UVA was transmitted, as in the UV320 film, anthocyanin content was reduced. This means that a highly UV transparent film (UV280) is capable of increasing anthocyanin content compared to a standard horticultural UV/EVA film (UV320). Excluding both UVA and UVB by the use of the UV400 film further reduced anthocyanin content by up to eight times. Voipio and Autio (1995), reported increases in anthocyanin content of lettuce plants under supplementary UV A radiation. The present study indicates that both UVB and UV A are involved in anthocyanin photo-induction. These observations agree with the previously reported studies of Krizek et al. (1998), who found that excluding UVA and UVB significantly reduced anthocyanin content of lettuce compared to plants grown in the presence of UV A and UVB radiation. However, in this study, we have shown that total above ground dry weight is positively correlated with the degree of UV radiation cutoff transmitted by the films. This finding indicates that the presence of ambient levels of UVB and UVA decreased growth of lettuce and agree with previously reported studies of Krizek et al. (1997, 1998) who found increases in cucumber and lettuce fresh weight when UVA and UVB were absent. Diaz et al. (2006) also reported a reduction in fresh weight of lettuce under films that transmit UV radiation. Krizek et al. (1998) suggested that the growth reduction in an UV environment could be due to damage to the photosynthetic apparatus. UV radiation can cause damage to photosynthetic apparatus by damaging photosystem II (Stapleton, 1992; Rozema et al., 1997; Mazza et al., 2000). Increases in flavonoids after exposure to UVB light have also been reported in *Brassica napus* by Olsson et al. (1998). Ryan et al. (1998) reported greater accumulation of flavonoids in response to UVB radiation. The present study is in agreement with Krizek et al. (1998), who found high rates of accumulation of UV-absorbing compounds in lettuce grown in the presence of UVA and UVB radiation.

We have shown in this study that total above ground dry weight is positively correlated with the degree of UV radiation cutoff transmitted by the films. This finding indicates that the presence of ambient levels of UVB and UVA decreased growth of lettuce and agree with previously reported studies of Krizek et al. (1997, 1998) who found increases in cucumber and lettuce fresh weight when UVA and UVB were absent. Diaz et al. (2006) also reported a reduction in fresh weight of lettuce under films that transmit UV radiation. Krizek et al. (1998) suggested that the growth reduction in an UV environment could be due to damage to the photosynthetic apparatus. UV radiation can cause damage to photosynthetic apparatus by damaging photosystem II (Stapleton, 1992; Rozema et al., 1997), and high levels of UVB radiation have been shown to reduce photosynthesis in pea (Nogués et al., 1999). In this study, however, we have shown that lettuce plants under ambient levels of UV are not subject to stress compared to plants under a non-UV environment, as no suppression on the $F_v/F_m$ ratio was observed. Photosynthetic performance index (PIABS), however, indicated an increased photosynthetic efficiency in plants exposed to UVB and UVA. This could be as a result of the increased anthocyanin levels protecting plants against photoinhibition.

Since growth reduction under UV environment was accompanied by accumulation of anthocyanins, flavonoids and phenolics, it may be that the plant diverts energy produced by photosynthesis to synthesize these compounds to protect itself from UV damage. Further studies, however, are needed in which photosynthetic rate will be measured directly to test whether growth inhibition is due to a high cost of photo-protection. In addition, accumulation of anthocyanins in the leaf epidermis may have caused internal shading which can lead to a reduction of light available to chlorophyll (Steyn et al., 2002; Neill and Gould, 2003). This possible light reduction may have also contributed to growth reduction.

5. Conclusion

The key findings of this study are the high levels of secondary products under UV transparent films. Plants in the presence of UVB and UVA (UV280) appeared not to be stressed and this may be because they accumulate secondary products which effec-
tively protect the photosynthetic apparatus. Our results indicate that the quality of Lollo Rosso (in terms of phenolic compounds) can be enhanced under a highly UV transparent film (UV280) compared to the standard horticultural UV/EVA film (UV320). These findings are of particular importance for growers as these films are commercially available.

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References


