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Short communication

Phenolic contents of lettuce, strawberry, raspberry, and blueberry crops cultivated under plastic films varying in ultraviolet transparency

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ABSTRACT

The levels of health-related phytochemicals were determined in lettuce leaf and in strawberry, raspberry and blueberry fruits grown in near-commercial conditions under plastic films of three different UV transparencies. In the red lettuce Lollo Rosso, total phenolics, anthocyanin, luteolin and quercetin levels were all raised by changing from a UV blocking film to a film of low UV transparency, and to a film of high UV transparency. The related green lettuce, Lollo Biondo, cultivated under the same conditions, showed virtually no phytochemical responses to the same variation in UV levels. Overall, the phenolic levels of strawberries, raspberries, and blueberries were unresponsive to the UV transparency of the plastic film under which the crops were grown. The significance of these findings is discussed in relation to the nutritional quality of soft fruit and salad crops which are increasingly being grown commercially under plastic tunnels.

1. Introduction

In temperate climates, soft fruit and salad crops are increasingly being grown commercially under the protective cover provided by plastic tunnels. For example in the United Kingdom, 80% of the strawberry crop is now grown under polythene (Wagstaffe, Hadley, & Battey, 2008). The quality of light reaching plants is well known to affect plant growth and development, and the employment of plastic films would therefore be expected to affect crop quality and production. The standard polythene film used for tunnels screens a proportion of the UV. However, films are now available which differ in their ability to transmit UV light whilst retaining, unaltered, the available photosynthetically active radiation. The effect of altering the level of UV light in growing systems has been found to affect the pigment and colourless phytochemical contents of some crop plants. For example, the total phenolic and phenolic acid contents of tomato fruits were 20% higher when plants were cultivated under UV-transparent compared with UV blocking plastic films (Luthria, Mukhopadhyay, & Krizek, 2006).

Soft fruit and salads are significant sources of anthocyanins and other flavonoids, which are important dietary components helping to reduce the risk of chronic disease (Beattie, Crozier, & Duthie, 2005; Hannum, 2004). For example, in France, strawberry and lettuce are amongst the main sources of polyphenols from fruit and vegetables, respectively (Brat et al., 2006). The levels of secondary compounds in soft fruit have previously been shown to be influenced by environmental factors (Kalt, Howell, Duy, Forney, & McDonald, 2001; Wang & Zheng, 2001), but the effect of cultivation under different UV regimes has not been determined.

The main aim of this paper is to determine the levels of health-related phytochemicals in strawberry, raspberry and blueberry fruit grown under plastic films of three different UV transparencies. We also extend previous work on the phenolic composition of lettuce (García-Macías et al., 2007; Tsormpatsidis et al., 2008) grown under these conditions, comparing red with non-red leaf lettuce. All crops were grown and harvested in near-commercial conditions in order to make the results relevant to commercial production.

2. Materials and methods

2.1. Plant material and growing conditions

All plants were grown in 2006 (year 1) and 2007 (year 2) at the Shinfield Field Unit (University of Reading, UK). Lettuce (*Lactuca sativa* L. Lollo Rosso type, red leaf, cv. Revolution; and

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Lollo Biondo type, green leaf, cv. Bergamo) was grown from seed as previously (García-Macías et al., 2007). Strawberries (*Fragaria* x *ananassa* Duch. cvs. Elsanta, Everest) were grown in peat bags and raspberries (*Rubus idaeus* L. cvs. Tulameen, Joan Squire) and blueberries (*Vaccinium corymbosum* L. cv. Bluecrop) were grown in pots of substrate specific to each crop (Bulrush Horticulture Ltd., UK). Strawberry and raspberry plants were irrigated, using a fertiliser mix optimised for peat-based soft fruit growing and blueberries were fed with a standard commercial blueberry fertiliser (Hortifeeds, Lincoln, UK). The irrigation timing, fertiliser concentration and pH were all controlled automatically. Harvesting was carried out, based on commercial picking standards. Strawberries were picked on a three and four day cycle (picking on day 3/4) in 2006 and a three day cycle in 2007. In 2006, since the harvesting was less frequent than in some commercial practice, fruit was sorted into two classes ('commercial' and 'fully' ripe), based on visual colour. This allowed a sample which was similar to a commercial crop to be analysed alongside a sample which would allow for the potential for increased phenolic content to result in darker (and therefore visually more ripe) fruit. In 2007, to simulate commercial practice, all strawberries were included as a single sample. Raspberries were picked on a two day cycle and blueberries were picked on a seven day cycle.

All plants were grown in blocks in a multi-span, open-sided tunnel (seven spans of 6.5 × 75 m). Three experimental blocks per treatment were laid out as equally sized areas down the length of each of three tunnel spans. The spectral properties of the films were as previously reported (García-Macías et al., 2007). All three films contained the infra-red reducing and light diffusing components of Luminance THB (British Polythene Industries PLC, Greenock, UK). The UV block film blocked between 94% and 99% of light in UV-B wavelengths and 96–99% of light in UV-A wavelengths up to 380 nm. The UV Low film, which represented standard polythene film used commercially, blocked between 74% and 87% of light in UV-B wavelengths and between 23% and 78% of light in UV-A wavelengths. The UV Window film transmitted between 60% and 78% of UV wavelengths between 260 and 400 nm.

2.2. Analytical methods

Anthocyanidins were purchased from Polyphenols Laboratories (Sandnes, Norway), and other reagents from Sigma–Aldrich (Poole, UK).

Lettuce was extracted on the day of harvest, as previously described (García-Macías et al., 2007). Soft fruit was blended in a food processor on the day of harvest and kept at −20 °C until extraction. Frozen purée was thawed and 1 g was extracted with 20 ml of acidified methanol (1% HCl). The mixture was left for 20 h at 6 °C in the dark, the extracts vacuum-filtered through Whatman No. 1 (11 μm), and kept at −20 °C prior to analysis.

The total phenolic content, expressed as gallic acid equivalents (GAE), was determined by the Folin–Ciocalteu method (Singleton & Rossi, 1965) and flavonoids and phenolic acids by HPLC using a HP 1050 chromatograph equipped with a DAD. HCl was added to the extract to 2.5 M final concentration, and extracts were heated for 1 h at 90 °C prior to analysis then allowed to cool in ice, and filtered through a 0.22 μm syringe filter. HPLC analysis was performed with a Prodigy ODS3 column (250 × 4.6 mm, 5 μm particle size) from Phenomenex (Macclesfield, UK) equipped with a 5 μm ODS guard column (4.0 × 4.6 mm). The solvent flow rate was 1 ml/min, and the column was allowed to equilibrate for 15 min between injections (50 μl). Anthocyanins, quercetin, luteolin, ellagic acid and hydroxycinnamic acids were identified by retention time, co-chromatography with external standards, and UV spectra: quercetin and luteolin at 360 nm, caffeic acid derivatives at 320 nm, anthocyanins at 520 nm, and ellagic acid at 260 nm. The mobile phase for separation consisted of (A) 1% formic acid and (B) methanol, using a gradient starting with 25% B for 10 min, increasing to 27% B at 12 min, 31% B at 20 min, 31% B at 30 min, 38% B at 40 min, 47% B at 44 min, 60% B at 46 min, and 65% at 54 min and then reverting to 10% at 60 min.

Differences amongst the means were compared between treatments using one-way analysis of variance. Values provided are the means of three independent replicates ± standard error of the mean (SEM). Different letters in the same column per crop indicate significant differences ($P < 0.05$).

3. Results and discussion

In the red lettuce, Lollo Rosso, as found previously (García-Macías et al., 2007) total phenolics, anthocyanin, luteolin and quercetin levels were all raised by moving from UV block to UV low and to UV window (Table 1). On the other hand, the related green lettuce, Lollo Biondo, cultivated under the same conditions, showed virtually no phytochemical responses to the same variation in UV levels (Table 1). The fact that Lollo Biondo lettuce does not greatly raise phenolic levels in response to UV conditions, that greatly raise phenolic levels in Lollo Rosso lettuce, suggests that the phenolics in Lollo Rosso are not accumulated as a protection against UV damage, instead they may be the manifestation of an as-yet unspecified adaptive response to non-damaging levels of UV (see Edreva, 2005), which has been selected for in-breeding Lollo Rosso. The commercial implication of this suggestion is that the dramatic increase in health-beneficial phenolic compounds noted previously with Lollo Rosso grown under UV-transparent plastic (García-Macías et al., 2007; Tsormpatsidis et al., 2008) may not be apparent amongst other lettuce cultivars, either of the green or red types.

Overall, the phenolic levels of strawberries, raspberries, and blueberries were unresponsive to the UV transparency of the plastic film under which the crops were grown (Tables 2–4). With both cultivars of strawberry tested, the June-bearer Elsanta and the ever-bearer Everest, UV blocking film reduced total phenolics, anthocyanin and ellagic acid in some of the crops, but the effect was not observed with all crops under all conditions (Table 2). With neither of the two cultivars of raspberry, the summer-fruiting cv. Tulameen, and the fall-fruiting cv. Joan Squire, did the UV transparency of the films have any effect on the phenolic contents of the fruit (Table 3). Again with blueberry, there was no consistent effect of the UV transparency of the plastic film (Table 4). The simplest biological explanation for the relative unresponsiveness to UV of soft fruit compared with leaves is as follows. In leaves, phenolics help protect the leaf photosynthetic apparatus against UV damage, but in fruit, phenolics interact with seed dispersers. Moreover, soft fruit has a much lower surface area:volume ratio than a leaf, and

Table 1
Phenolic content of lettuce. Lollo Rosso was harvested on 26 July, and Lollo Biondo on 25 July.

Filter	Total phenolics mg GAE/g FW	Anthocyanin μg cyanidin/g FW	Luteolin μg/g FW	Quercetin μg/g FW
Lollo Rosso				
Block	0.75 ± 0.03[a]	15.7 ± 0.89[a]	4.47 ± 1.63[a]	33.2 ± 2.27[a]
Low	0.88 ± 0.06[a]	33.5 ± 1.53[b]	8.05 ± 0.24[b]	56.1 ± 7.51[a]
Window	1.50 ± 0.13[b]	127 ± 21.1[c]	26.4 ± 3.43[c]	213 ± 34.6[b]
Lollo Biondo				
Block	0.62 ± 0.06[a]	N.D.	N.D.	16.5 ± 3.33[a]
Low	0.66 ± 0.02[a]	N.D.	N.D.	17.6 ± 2.11[a,b]
Window	0.65 ± 0.03[a]	N.D.	N.D.	27.4 ± 2.26[b]

N.D. – not detected.

Table 2
Phenolic contents of strawberry grown under plastic films of three different UV transparencies. The crops were harvested at the dates shown, as described in Section 2.

	Film	Total phenolics mg GAE/g FW	Anthocyanin µg pelargonidin/g FW	Phenolic acid µg ellagic acid/g FW
Elsanta				
Year 1				
Crop 1	Block	2.98 ± 0.12[a]	147 ± 3.0[a]	199 ± 25.9[a]
15 June	Low	3.46 ± 0.14[b]	170 ± 7.4[b]	264 ± 27.1[b,c]
Commercial ripeness	Window	3.36 ± 0.03[b,d]	163 ± 8.6[b]	239 ± 3.4[b]
Fully ripe	Block	2.77 ± 0.21[c]	209 ± 1.5[c]	255 ± 31.6[b,c]
	Low	3.28 ± 0.05[d]	249 ± 10.7[d]	347 ± 40.0[d]
	Window	3.01 ± 0.11[a]	219 ± 2.8[c,d]	296 ± 9.2[c]
Crop 2	Block	2.83 ± 0.15[a]	203 ± 7.8[a]	426 ± 17.3[a]
3 July	Low	2.96 ± 0.03[b]	187 ± 6.0[b]	491 ± 7.8[b]
Commercial ripeness	Window	3.28 ± 0.18[c]	200 ± 16.6[a,b]	535 ± 57.6[b]
Fully ripe	Block	2.34 ± 0.05[d]	262 ± 8.7[c]	312 ± 12.2[c]
	Low	2.88 ± 0.10[a,b]	298 ± 5.5[d]	393 ± 31.0[d]
	Window	2.64 ± 0.06[e]	269 ± 8.8[c]	351 ± 30.0[d]
Year 2				
Crop 1	Block	2.80 ± 0.14[a]	221 ± 2.0[a]	407 ± 19.7[a]
25 June	Low	3.07 ± 0.09[a,b]	227 ± 3.8[a,b]	502 ± 1.0[b]
	Window	3.24 ± 0.05[b]	241 ± 7.9[b]	511 ± 23.6[b]
Crop 2	Block	2.89 ± 0.13[a]	298 ± 16.8[a]	584 ± 45.7[a]
23 July	Low	2.99 ± 0.02[a]	297 ± 1.8[a]	641 ± 18.0[a]
	Window	3.16 ± 0.13[b]	316 ± 28.9[a]	646 ± 73.6[a]
Everest	Block	3.07 ± 0.06[a]	330 ± 16.7[a]	172 ± 12.7[a]
13 August	Low	3.47 ± 0.14[b]	344 ± 10.8[a]	188 ± 10.8[a,b]
	Window	3.67 ± 0.06[b]	353 ± 9.3[a]	200 ± 15.1[b]

Table 3
Phenolic contents of raspberry grown under plastic films of three different UV transparencies. The crops were harvested at the dates shown, as described in Section 2.

	Film	Total phenolics mg GAE/g FW	Anthocyanin µg cyanidin/g FW	Phenolic acid µg ellagic acid/g FW
Year 1				
Tulameen	Block	1.34 ± 0.07[a]	357 ± 8.3[a]	486 ± 13.4[a]
11 July	Low	1.45 ± 0.10[a,b]	311 ± 20.9[b]	490 ± 9.5[a]
	Window	1.35 ± 0.10[b]	364 ± 31.2[a]	484 ± 22.3[a]
Joan Squire	Block	2.13 ± 0.21[a]	385 ± 13.5[a]	555 ± 111 [a]
7 September	Low	2.17 ± 0.29[a]	363 ± 7.1[b]	603 ± 145 [a]
	Window	2.25 ± 0.22[a]	394 ± 16.7[a]	606 ± 92.7[a]

Table 4
Phenolic contents of blueberry grown under plastic films of three different UV transparencies. The crops were harvested at the dates shown, as described in Section 2.

	Film	Total phenolics mg GAE/g FW	Anthocyanin µg malvidin/g FW	Phenolic acid µg caffeic acid equivalents/g FW
Year 1				
31 July	Block	2.65 ± 0.17[a]	1052 ± 155[a]	1510 ± 30.6[a]
	Low	3.07 ± 0.08[b]	1133 ± 113[a]	1525 ± 26.8[a]
	Window	3.25 ± 0.07[c]	1508 ± 54.4[b]	1543 ± 26.6[a]
Year 2				
17 July	Block	2.93 ± 0.19[a]	554 ± 53.6[a]	833 ± 20.9[a]
	Low	3.46 ± 0.17[b]	778 ± 124[b]	862 ± 14.5[a,b]
	Window	2.88 ± 0.29[a]	596 ± 152[a]	872 ± 23.5[b]

the depth of UV penetration into strongly coloured fruit is probably very limited.

We find that the soft fruit tested resemble the green lettuce type, Lollo Biondo, and differ from the red lettuce type, Lollo Rosso, and the tomato fruits examined elsewhere (Luthria et al., 2006), in that overall, the soft fruit are unresponsive to the range of ambient UV levels provided by plastic films of differing UV transparency. The generality of this finding needs to be qualified by two considerations. First, the cultivars chosen for the present study are commercial cultivars, which are likely to have been bred *inter alia* for stability of colour (and thus anthocyanin levels) under a variety of climatic conditions. Other varieties, more variable and therefore less useful commercially, may be more responsive to UV. Second, the UK climate is not noted for high solar UV irradiance, and higher UV intensities than those experienced in the present experiments may lead to enhanced phenolic contents in soft fruit.

The implication of the present experiments for commercial production of soft fruit is that, at least for temperate climates, such as that of the UK, crops grown in tunnels under polythene film that partially (or completely) blocks UV, are as rich in those health-beneficial phenolics which we have determined as are crops grown under UV-transparent film. Cultivation of soft fruit crops under polythene films provides advantages of extended season and enhanced crop quality (Wagstaffe et al., 2008). For the compounds which we determined, these advantages are not accompanied by a phytochemical penalty.

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References

Beattie, J., Crozier, A., & Duthie, G. G. (2005). Potential health benefits of berries. *Current Nutrition and Food Science, 1*, 71–86.

Brat, P., George, S., Bellamy, A., Du Chaffaut, L., Scalbert, A., Mennen, L., et al. (2006). Daily polyphenol intake in France from fruit and vegetables. *Journal of Nutrition, 136*, 2368–2373.

Edreva, A. (2005). The importance of non-photosynthetic pigments and cinnamic acid derivatives in photoprotection. *Agriculture, Ecosystems and Environment, 106*, 135–146.

García-Macías, P., Ordidge, M., Vysini, E., Waroonphan, S., Battey, N. H., Gordon, M. H., et al. (2007). Changes in the flavonoid and phenolic acid contents and antioxidant activity of red leaf lettuce (Lollo Rosso) due to cultivation under plastic films varying in ultraviolet transparency. *Journal of Agricultural and Food Chemistry, 55*, 10168–10172.

Hannum, S. M. (2004). Potential impact of strawberries on human health: A review of the science. *Critical Reviews in Food Science and Nutrition, 44*, 1–17.

Kalt, W., Howell, A., Duy, J. C., Forney, C. F., & McDonald, J. E. (2001). Horticultural factors affecting antioxidant capacity of blueberries and other small fruit. *HortTechnology, 11*, 523–528.

Luthria, D. L., Mukhopadhyay, S., & Krizek, D. T. (2006). Content of total phenolics and phenolic acids in tomato (*Lycopersicon exculentum* Mill.) fruits as influenced by cultivar and solar UV radiation. *Journal of Food Composition and Analysis, 19*, 771–777.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Tsormpatsidis, E., Henbest, R. G. C., Davis, F. J., Battey, N. H., Hadley, P., & Wagstaffe, A. (2008). UV irradiance as a major influence on growth, development and secondary products of commercial importance in Lollo Rosso lettuce 'Revolution' grown under polyethylene films. *Environmental and Experimental Botany, 63*, 232–239.

Wagstaffe, A., Hadley, P., & Battey, N.H. (2008). Tunnel production of strawberry in the UK: a review. In F. Takeda, D.T. Handley, & E.B. Poling, *Proceedings of the 2007 North American Strawberry Symposium* (pp. 23–28). Kemptville, Canada: North American Strawberry Growers Association.

Wang, S. Y., & Zheng, W. (2001). Effect of plant growth temperature on antioxidant capacity in strawberry. *Journal of Agricultural and Food Chemistry, 49*, 4977–4982.