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Article

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DRAFT

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

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Abstract/Summary (to be done)

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 UK, 80% of the strawberry crop is now grown under polythene (Wagstaffe et al, 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.

While the standard polythene film used for tunnels screens a proportion of the UV, films which differ in their ability to transmit UV light whilst retaining unaltered the available photosynthetically active radiation (PAR) are now available. 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 are 20% higher when plants are cultivated under UV transparent compared with UV blocking plastic films (Luthria et al., 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 (Hannum 2004; Beattie et al, 2005). For example, in France, based on average content and on consumption data, it has been shown that strawberry and lettuce are among 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 including temperature (Wang and Zheng, 2001), carbon dioxide concentration (Gil Et al, 1997; Wang et al., 2003), climate (Kalt et al, 2001), growing media (Wang and Lin, 2003), ripening stage and genotype (Wang and Lin, 2000; Moyer etal, 2002). However the effect of cultivation under different UV regimes has not been determined.

The main aim of the present 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 (Garcia-Macias 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.

Materials and Methods

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 Lollo Biondo type, green leaf, cv. Bergamo) was grown from seed as described previously (Garcia Macias 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 day cycle (picking on day 3), raspberries were picked on a two day cycle and blueberries were picked on a seven day cycle.

Comment [a1]: Matt correct ?

Comment [a2]: Matt can you add something about the commercial and fully ripe criteria for picking in year 1 then about whole crop in year 2

All plants were grown in blocks in a multi-span, open-sided tunnel (7 spans of 6.5 x 75m). 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 described previously (Garcia-Macias 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 380nm. 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 400nm.

Chemicals. Folin-Ciocalteu reagent, quercetin, ellagic acid, quercetin, gallic acid and caffeic acid were purchased from Sigma-Aldrich Company Ltd. (Poole, UK), and anthocyanidins from Polyphenols Laboratories (Sandnes, Norway).

Extraction. Lettuce was extracted on the day of harvest as described (Garcia-Macias et al, 2007). Soft fruit was blended in a food processor on the day of harvest and kept at -20^{o}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^{o}C in the dark, the extracts vacuum filtered through Whatman No.1 (11 µm), and kept at -20^{o}C until analysis.

Determinations. The total phenolic content, expressed as gallic acid equivalents (GAE), was determined by the Folin-Ciocalteu method (Singleton and Rossi, 1965) and flavonoids and phenolic acids by High-Performance Liquid Chromatography, all as described previously (Garcia-Macias et al, 2007).

Comment [a3]: Paulina: still correct ?

Statistical Analysis. The program used for statistical analysis was Genstat. Differences among the means were compared between treatments using one-way analysis of variance. Differences at $P<0.05$ were considered to be significant.

Comment [a4]: Paulina correct ?

Results and Discussion

Lettuce. In the red lettuce, Lollo Rosso, as found previously (Garcia-Macias 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 enormously raise phenolic levels in Lollo Rosso lettuce suggests that the phenolics in Lollo Rosso are not accumulated as a protection against UV damage, but are the manifestation of an unspecified adaptive response to non-damaging levels of UV. 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 (Garcia-Macias et al, 2007; Tsormpatsidis et al, 2007) may not be apparent among other lettuce cultivars, either of the green or red types.

Soft fruit. 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 (Table 2). With both cultivars of strawberry tested, the June-bearer Elsanta and the ever-bearer Everest, a reduction in total phenolics, anthocyanin and ellagic acid could be observed when crops were grown under UV blocking film, but the effect was not observed with all crops under all conditions. 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 2). Again with blueberry, there was no consistent effect of the UV transparency of the plastic film (Table 2).

Thus 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 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 varieties, which are likely to have been bred *inter alia* for stability of colour (and thus anthocyanin levels) under a variety of climatic conditions, and 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 levels, and higher UV irradiance levels than those experienced in the present experiments may lead to enhanced phenolic contents in soft fruit.

The soft fruit has a much lower surface area: volume ratio than the lettuce, and this may at least partly explain the lack of effect in soft fruit compared to Lollo Rosso lettuce. The depth of penetration of UV light into strongly coloured fruit is probably very limited, and consequently anthocyanin biosynthesis in most of the fruit would be unaffected by UV light even if it has an effect on the outer tissues of the fruit. The situation with Lollo Biondo is different since the lack of response indicates that the lettuce lacks the required enzymes for anthocyanin biosynthesis.

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 health-beneficial phenolics as 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), and we now show that these advantages are not accompanied by a phytochemical penalty.

Acknowledgements

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Table 1 Phenolic content of lettuce

Filter	Total Phenolics	Anthocyanin	Luteolin	Quercetin
	mg GAE / g FW	µg cyanidin / g FW	µg / g FW	µg / g FW
Lollo Rosso				
26 July				
Block	0.75 ± 0.03^{a}	15.71 ± 0.89^{a}	4.47 ± 1.63^{a}	33.18 ± 2.27^{a}
Low	0.88 ± 0.06^{a}	33.50 ± 1.53^{b}	8.05 ± 0.24^{b}	56.08 ± 7.51^{a}
Window	1.50 ± 0.13^{b}	127.09 ± 21.05^{c}	26.42 ± 3.43^{c}	213.03 ± 34.6^{b}
Lollo Biondo				
25 July				
Block	0.62 ± 0.06^{a}	n.d.	n.d.	16.49 ± 3.33^{a}
Low	0.66 ± 0.02^{a}	n.d.	n.d.	17.58 ± 2.11^{a,b}
Window	0.65 ± 0.03^{a}	n.d.	n.d.	27.44 ± 2.26^{b}

n.d. = not detected

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

	Film	Total Phenolics	Anthocyanin [1]	Phenolic acid [2]
Strawberry				
Elsanta		mg GAE / g FW	μg / g FW	μg / g FW
Year 1				
Crop 1 15 June Commercial ripeness	Block	2.98 ± 0.12[a]	146.5 ± 3.0[a]	198.5 ± 25.9[a]
	Low	3.46 ± 0.14[b]	170.1 ± 7.4[b]	263.6 ± 27.1[b,c]
	Window	3.36 ± 0.03[b,d]	162.9 ± 8.6[b]	239.2 ± 3.4[b]
Fully ripe	Block	2.77 ± 0.21[c]	209.4 ± 1.5[c]	255.4 ± 31.6[b,c]
	Low	3.28 ± 0.05[d]	249.0 ± 10.7[d]	346.6 ± 40.0[d]
	Window	3.01 ± 0.11[a]	219.0 ± 2.8[c,d]	295.7 ± 9.2[c]
Crop 2 3 July Commercial ripeness	Block	2.83 ± 0.15[a]	202.9 ± 7.8[a]	425.7 ± 17.3[a]
	Low	2.96 ± 0.03[b]	187.4 ± 6.0[b]	490.8 ± 7.8[b]
	Window	3.28 ± 0.18[c]	199.5 ± 16.6[a,b]	534.5 ± 57.6[b]
Fully ripe	Block	2.34 ± 0.05[d]	261.9 ± 8.7[c]	312.2 ± 12.2[c]
	Low	2.88 ± 0.10[a,b]	297.5 ± 5.5[d]	392.8 ± 31.0[d]
	Window	2.64 ± 0.06[e]	268.7 ± 8.8[c]	351.2 ± 30.0[d]
Year 2				
Crop 1 25 June	Block	2.80 ± 0.14[a]	220.5 ± 2.0[a]	406.6 ± 19.7[a]
	Low	3.07 ± 0.09[a,b]	226.63 ± 3.8[a,b]	501.8 ± 1.0[b]
	Window	3.24 ± 0.05[b]	241.3 ± 7.9[b]	510.8 ± 23.6[b]
Crop 2 23 July	Block	2.89 ± 0.13[a]	298.1 ± 16.8[a]	583.6 ± 45.7[a]
	Low	2.99 ± 0.02[a]	296.6 ± 1.8[a]	640.6 ± 18.0[a]
	Window	3.16 ± 0.13[b]	315.5 ± 28.9[a]	646.4 ± 73.6[a]
Everest 13 August	Block	3.07 ± 0.06[a]	329.6 ± 16.7[a]	172.0 ± 12.7[a]
	Low	3.47 ± 0.14[b]	343.8 ± 10.8[a]	187.7 ± 10.8[a,b]
	Window	3.67 ± 0.06[b]	353.3 ± 9.3[a]	199.5 ± 15.1[b]
Raspberry				
Year 1				
Tulameen 11 July	Block	1.34 ± 0.07[a]	357.0 ± 8.3[a]	485.8 ± 13.4[a]
	Low	1.45 ± 0.10[a,b]	311.2 ± 20.9[b]	489.8 ± 9.5[a]
	Window	1.35 ± 0.10[b]	364.4 ± 31.2[a]	483.6 ± 22.3[a]
Joan Squire 7 September	Block	2.13 ± 0.21[a]	384.7 ± 13.5[a]	554.7 ± 111.3 [a]
	Low	2.17± 0.29[a]	362.9 ± 7.1[b]	603.0 ± 144.5 [a]
	Window	2.25 ± 0.22[a]	393.5 ± 16.7[a]	606.3 ± 92.7[a]

Blueberry				
Year 1	Block	2.65 ± 0.17[a]	1052.3 ± 154.5[a]	1510.1 ± 30.6[a]
31 July	Low	3.07 ± 0.08[b]	1132.7 ± 113.2[a]	1524.6 ± 26.8[a]
	Window	3.25 ± 0.07[c]	1507.9 ± 54.4[b]	1543.4 ± 26.6[a]
Year 2	Block	2.93 ± 0.19[a]	553.9 ± 53.6[a]	833.0 ± 20.9[a]
17 July	Low	3.46 ± 0.17[b]	778.4 ± 123.7[b]	861.9 ± 14.5[a,b]
	Window	2.88 ± 0.29[a]	596.4 ± 151.9[a]	872.3 ± 23.5[b]

[1] Anthocyanin was determined as µg pelargonidin / g FW in strawberry, µg cyanidin / g FW in raspberry and µg malvidin / g FW in blueberry.

[2] Phenolic acid was determined as ellagic acid in strawberry and raspberry, and is expressed as µg ellagic acid / g FW in strawberry and raspberry, and determined as hydroxycinnamic acids in blueberry, and expressed as µg caffeic acid equivalents/g FW in blueberry.