

## Symposium-in-Print: Ultraviolet Radiation and Terrestrial Ecosystems

### The Use of Wavelength-selective Plastic Cladding Materials in Horticulture: Understanding of Crop and Fungal Responses Through the Assessment of Biological Spectral Weighting Functions

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#### ABSTRACT

Plant responses to light spectral quality can be exploited to deliver a range of agronomically desirable end points in protected crops. This can be achieved using plastics with specific spectral properties as crop covers. We have studied the responses of a range of crops to plastics that have either (a) increased transmission of UV compared with standard horticultural covers, (b) decreased transmission of UV or (c) increased the ratio of red (R) : far-red (FR) radiation. Both the UV-transparent and R : FR increasing films reduced leaf area and biomass, offering potential alternatives to chemical growth regulators. The UV-opaque film increased growth, but while this may be useful in some crops, there were trade-offs with elements of quality, such as pigmentation and taste. UV manipulation may also influence disease control. Increasing UV inhibited not only the pathogenic fungus *Botrytis cinerea* but also the disease biocontrol agent *Trichoderma harzianum*. Unlike *B. cinerea*, *T. harzianum* was highly sensitive to UV-A radiation. These fungal responses and those for plant growth in the growth room and the field under different plastics are analyzed in terms of alternative biological spectral weighting functions (BSWF). The role of BSWF in assessing general patterns of response to UV modification in horticulture is also discussed.

#### INTRODUCTION

The exploitation of plant photomorphogenic responses to light spectral balance in crop production remains rather limited. However, the known responses to red (R) : far-red (FR), blue, UV-A and UV-B have the potential to deliver a range of agronomically desirable end points. Limited application of R : FR manipulation has shown that this potential can be fulfilled, but other plant responses, especially to UV wavelengths remain largely unexploited. The studies we described here are focused on understanding the effects of spectral modification on a range of crops. We have quantified a range of crop responses, as well as those of pathogenic fungi and potential biocontrol agents. This broad approach is essential to obtain an overview of responses to spectral modification and the potential commercial trade-offs between different responses.

Experiments extend from field trials growing a range of crops using semi-commercial-scale production systems through to controlled environment and *in vitro* irradiations. The latter are designed to place the field studies within a solid understanding of the photobiological mechanisms underlying crop responses. We believe that this is necessary to allow more general predictions of the effects of spectral modification than are possible from field experiments at a specific location. This may be especially important when modifying UV wavelengths when it might be expected that crop responses could vary with changes in incident UV radiation due to season, latitude or local climatic factors such as clouds. However, attempting to derive general predictions for responses to UV modification requires careful consideration of biological spectral weighting functions (BSWF). As a result, we analyzed our data not just in terms of applied photobiology but also in terms of this key issue in plant photobiology.

#### Plant photomorphogenesis and its potential exploitation

Photomorphogenic responses to the balance of R and FR light are probably best understood, at scales of organization from the cellular and molecular biology of phytochrome photoreceptors through to the physiology and ecology (1,2). Plant photomorphogenic responses to R : FR ratio are also exploited in crop production. The use of copper sulfate solutions as a selective FR filter proved that it was possible to induce commercially useful growth modifications (3), but this technology was not viable in a commercial context. In

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**Abbreviations:** BSWF, biological spectral weighting function; CE, controlled environment; LD<sub>90</sub>, lethal dose for 90% of spores; PAR, photosynthetically active radiation (400–700 nm); PDA, potato dextrose agar; PAS300, UV radiation weighted using the BSWF of Caldwell *et al.*; R : FR, ratio of red to far-red radiation; RAF, radiation amplification factor; STC, Stockbridge Technology Centre; UV, ultraviolet radiation (200–400 nm); UV-A, ultraviolet-A radiation (320–400 nm); UV-B, ultraviolet-B radiation (280–320 nm); UV<sub>F&C</sub>, UV radiation weighted using the BSWF of Flint and Caldwell; UV<sub>QUAITE</sub>, UV radiation weighted using the BSWF of Quaitte *et al.*

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the past few years, plastic films that modify the R:FR ratio have become available, and their use investigated in a range of horticultural crops (*e.g.* 4–7). Plant responses to blue (400–500 nm) and UV-A radiation (320–400 nm) are also increasingly understood. It is clear that plants possess a range of photoreceptors responding to blue/UV-A and that these play a role in a variety of plant processes, including circadian rhythms, stomatal opening, phototropism, induction of pigment synthesis and other elements of photomorphogenesis (8). Supplementary blue light may be a useful tool in horticulture (7,9) and spectrally modifying plastics that have a transmission maximum in the blue are marketed for use in horticulture. However, these plastics largely seek to exploit the light responses not of plants but of pathogenic fungi. It is known that blue light (or a high ratio of blue to UV radiation) is inhibitory to the sporulation of many important phytopathogens, including downy mildews (10). Commercially available blue cladding films typically have low transmission of UV-A and photosynthetically active radiation (PAR: 400–700 nm) above 500 nm, resulting in low total PAR transmission (as low as 60% in some samples). The low PAR transmission of some blue films can result in poor yield and crop quality that must be set against any positive effects on disease control.

Horticultural cladding films are also produced with modified transmission in the UV. The standard films most commonly used in horticulture typically show declining transmission with decreasing UV wavelength, with little or no transmission below *ca* 350–360 nm. Thus, crops grown under such films receive near-zero UV-B (280–320 nm) and reduced UV-A. A number of UV-opaque films are marketed with more or less sharp cut-offs at 400 nm, providing a crop environment with near-zero UV-A as well as zero UV-B. Such plastics are marketed on their ability to contribute to pest and disease control (reviewed in 11). UV-opaque films can reduce crop diseases caused by a range of fungi that use UV as an environmental cue for sporulation. These include major pathogens, such as *Botrytis cinerea* (12). Zero-UV environments can substantially inhibit the sporulation of such fungi, resulting in slower epidemic development, reduced disease spread and overall reduced crop disease (13–15).

UV-opaque films act against crop pests by interfering with dispersal and foraging behavior in species that use UV in their visual systems (11). This approach has been shown to achieve commercial control of thrips and whitefly in some cropping systems (16,17). Direct crop responses to the exclusion of all UV radiation by UV-opaque films are poorly defined. The light environment under UV-opaque films differs from that under standard horticultural films in the removal of longer wavelength UV-A, and plant responses to this component of the UV spectrum remain poorly defined (18,19). However, results from the few published experiments suggest that solar UV-A has significant effects on growth, morphology and tissue chemistry in a range of species (*e.g.* 20–22). Thus, it is likely that complete exclusion of UV-A radiation would result in significant changes compared with standard horticultural plastic.

Plastics with increased UV transmission would also be expected to induce significant crop responses. These would be due partly to increases in UV-A radiation (see above) and partly to the UV-B component of the spectrum reaching the crop. Plant responses to UV-B radiation (280–320 nm) have been intensively studied, although largely in the context of stratospheric ozone depletion (reviewed in 18,23). However, a large number of experiments where solar UV-B has been attenuated using wavelength-selective filters, such as polyester, show a range of significant responses across many species and locations.

Responses to UV-B attenuation include increased growth and yield (20–22,24,25), altered pigmentation (20,21,26) and changes in herbivory and disease (27,28). Studies using UV-B lamps also suggest that UV-B manipulation could provide agronomically useful responses, such as control of growth, improved leaf color, altered pigment content and increased concentration of essential oils in some herbs (*e.g.* 29). As far as we are aware, UV-B supplementation has not been used in commercial horticulture, but the use of UV-transparent plastics offers an alternative route to delivering the same end points.

## MATERIALS AND METHODS

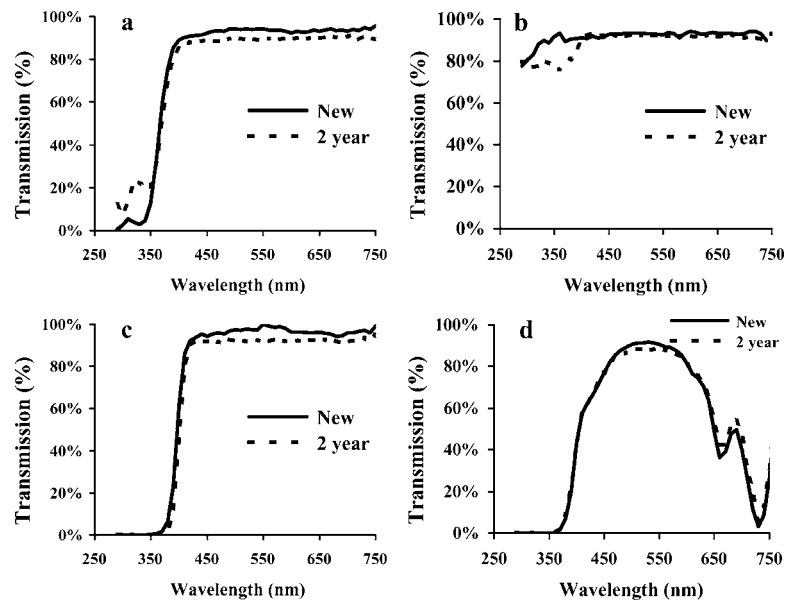
**Crop-scale experiments: The facility.** All crop-scale experiments were carried out at Stockbridge Technology Centre (STC: 53N 1W) using a series of commercial high-tunnel structures (Haygrove Tunnels Ltd., Ledbury, UK). Each spectral filter structure covers 740 m<sup>2</sup> over four individual bays, each measuring 3 m high × 6 m wide × 30 m long. The experiments cover a wide range of horticultural crops, but here we will concentrate only on those involving lettuce (*Lactuca sativa*), which, as well as being a commercially relevant protected crop in the UK, also provides a convenient model species.

**Plastics.** In our crop-scale experiments, we make use of a range of four commercially produced plastic cladding films (all supplied by bpi agri Ltd., Stockton on Tees, UK). In all cases, the base film is 150- $\mu$ m-thick polyethylene, with specific additives conferring specific spectral transmission properties. The control is a standard commercial horticultural cladding film that had a PAR transmission of 93% when new (Fig. 1a). Transmission in the UV declines rapidly with decreasing wavelength from 90% at 400 nm to less than 10% below 350 nm. Total UV-A transmission is approximately 50%. Transmission in the UV-B is less than 5% and effectively zero below 300 nm (Fig. 1a). Two films with modified UV transmission are used. The UV-opaque film has a total PAR transmission of 95% but a total UV-A transmission of only 10% and its UV-B transmission is zero (Fig. 1b). Transmission in the UV is zero below 375 nm but increases to around 60% at 400 nm (Fig. 1b).

When solar UV is weighted using Caldwell's generalized plant action spectra (=PAS300: 290–313 nm (30)) or the DNA damage action spectrum of Quate *et al.* (=UV<sub>QUAITE</sub>: 290–400 nm (31)) the transmission of the UV-opaque film is zero. Transmission of the biologically effective radiation calculated using recent BSWF of Flint and Caldwell (=UV<sub>F&C</sub>: 290–400 nm (19)) is approximately 7%. The UV-transparent film has a transmission greater than 80% across the whole of the solar UV range from 290 to 400 nm (Fig. 1c). Total transmissions in the PAR and UV-A are 94% and 90%, respectively. Unweighted UV-B transmission is 85% but, due to lower transmission at shorter UV-B wavelengths, transmission weighted using PAS300 or UV<sub>QUAITE</sub> is typically 80–85%, that of UV<sub>F&C</sub> approximately 90%.

The final spectrally modifying film is designed to increase the ratio of R:FR radiation. Its PAR transmission (*ca* 75%) is lower than in the other three films due to reduced transmission in both the blue and the red (Fig. 1d). Despite the general reduction in the transmission of red light, this is small compared with that in the FR; transmission between 650 and 670 nm averages 38% but that between 720 and 740 nm averages only 4% (Fig. 1d). As a result, the ratio R:FR transmission is approximately 8.8. As well as modifying red to FR, this film has UV transmissions very similar to those of the UV-opaque film (total transmission of UV-A and UV-B being 10% and 0%, respectively; Fig. 1d).

The transmission properties of the films have been relatively stable over two growing seasons under UK conditions. Transmission in the PAR fell by 7% in standard film, 7% in UV-opaque film, and 4% in UV-transparent film. The R–FR-modifying film was unusual in that PAR transmission actually increased slightly with age due to increased transmission at the red end of the spectrum. The mean transmission between 650 and 670 nm increased from 38% to 43%, while transmission between 720 and 740 nm increased from 4% to 7%. As a result, the ratio of R:FR transmission decreased from 8.8 to 5.9. There were no clear changes in the balance of PAR wavelengths in any other film (Fig. 1a). In the UV, transmission fell for all films except the standard. In the UV-transparent film, overall UV transmission fell from 83% to 80%, while in the standard film, UV transmission increased from 40% to 51%. This increase in transmission was especially evident in the UV-B (3% and 17% transmission, respectively, in new and 2-year-old films).



**Figure 1.** Spectral transmissions for the four plastics used in the field experiments (a) standard horticultural film, (b) UV-transparent film, (c) UV-opaque film and (d) R:FR-modifying film. Data are presented for new film (solid lines) and film used for 2 years in the field (dotted lines). Spectral transmissions were determined for triplicate samples (minimum  $4\text{ cm}^2$ ) using a 75-W Xenon arc lamp (LOT Oriol, Leatherhead, UK), a 10-cm integrating sphere and a scanning spectroradiometer with a double monochromator (Macam Photometrics, Livingston, UK). Variation between samples was negligible, and standard errors were too small to present.

**Plant material.** Studies of responses of lettuce to spectral modification used two different stages of crop development, which relate to two different types of protected crop in the UK. First, the propagation crop relates to the commercial production of lettuce seedlings under protection up to the 3–5 leaf stage, when they are sold to growers for transplantation into the field. Second, plants were grown to yield under the protection of the plastics. Plants at the propagation stage used plants of *Lactuca sativa* L. cv. Challenge (Syngenta seeds), raised for 14 days from sowing using standard UK commercial practices (Crystal Heart Salads, Holme-on-Spalding-Moor, UK). Seeds were germinated in  $4\text{-cm}^3$  peat blocks (Fison B2 Blocking Compost, Fisons, UK) at  $16 \pm 3^\circ\text{C}$  in the dark for 4 days before being transferred to commercial glass for a further 10 days. At 14 days, plants were transferred to STC and equally distributed under the four filter treatments for a period of 14 days, at which point they were harvested and fresh weights were determined.

Shoot dry weights were obtained after drying at  $75^\circ\text{C}$  until a constant mass was reached. Studies of responses to spectral modification throughout the growth period used *Lactuca sativa* L. cv. Constance (Rijk Zwaan Ltd., Netherlands), which is a red-leafed lollo rosso form. Seeds were germinated in peat blocks (as above) under commercial glass at  $30 \pm 5^\circ\text{C}/15 \pm 5^\circ\text{C}$  day/night temperature under natural daylight (no supplementary lighting) for 3 weeks before being transferred into the tunnels at a spacing of  $30\text{ cm} \times 30\text{ cm}$  for 5 weeks. Plants were then harvested and fresh weights were determined. Shoot dry weights were obtained after drying at  $75^\circ\text{C}$  until a constant mass was reached. Experiments at both the propagation stage and with plants grown to commercial harvest have been repeated six and four times, respectively, between May 2003 and September 2004.

All experiments have been repeated over time, with 10–15 replicate plants per treatment in each experiment. Individual experiments have been analyzed using one-way analysis of variance. Most significant effects have proved to be consistent over time, but because there is only a single, large structure per plastic, such simple analysis may be prone to positional effects. Thus, the position of plants within the structures was varied randomly between experiments and all the analysis presented here uses the repeats as replicates ( $n = 4\text{--}6$ ), with the mean value for each treatment/experiment being used as a single datum in one-way analysis of variance.

**Taste test.** An initial assessment of the taste of lettuce grown under the four different plastics was carried out by a 14-member taste panel, comprising 7 males and 7 females, with ages ranging from 26 to 52 years. Lettuce samples, less than 90 min after harvest, were presented blindly and in random order, and tasters were provided with mineral water as a palate cleanser between samples. The tasters rated the samples on a 1–5 scale, where 1 = very bitter and 5 = very sweet.

**Controlled environment studies: Plant responses to UV.** *Lactuca sativa* cv. Rex RZ and Constance RZ (both Rijk Zwaan, The Netherlands) were sown in 40-cell tray inserts with Levington M3 compost and were propagated under a light/dark regime of 16 h/8 h at  $25 \pm 2^\circ\text{C}$  and a PAR

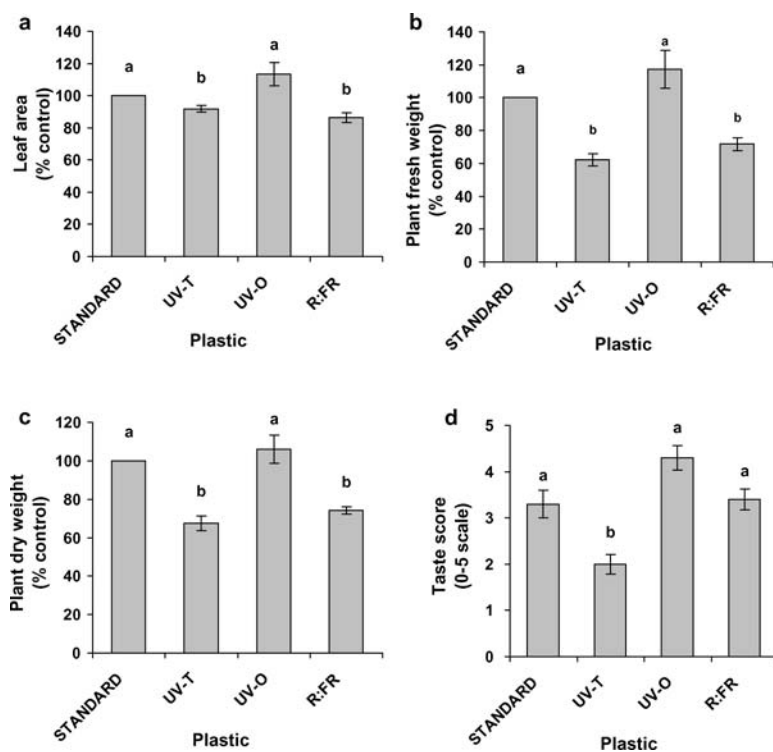
background of  $500\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$  provided from 400-W metal halide lamps (Osram HQI-BT 400 W; Osram Ltd., St Helens, UK). Six days after initial sowing, when the first leaf had emerged, plants were exposed to one of five different UV radiation treatments. The UV treatments were provided much as we have described before (32). Briefly, background UV-A was provided by the metal halide lamps plus six Q Panel UVA-340 tubes (Q-Panel Laboratory Products, Bolton, UK) filtered with clear polyester, PE (Lee filters, Andover, UK). UV-B was provided by six UV-B tubes (Philips TL40/12-RS; Starna Ltd., Romford, UK) filtered with 0.13-mm-thick cellulose diacetate, CA (Clarifoil, Courtaulds Ltd., Derby, UK).

The UV-B was manipulated by wrapping UV tubes with a white cotton fabric that altered irradiance but not the UV spectrum. The range of UV doses varied between zero and  $12\ \text{kJ m}^{-2}\ \text{day}^{-1}$  PAS300, that is up to 2.5-times the current ambient maximum at  $54^\circ\text{N}$ . We used PAS300 as our baseline BSWF because it has been widely used in the past, but we also analyzed our results in terms of  $\text{UV}_{\text{QUAITE}}$  and  $\text{UV}_{\text{F\&C}}$ . The treatments provided equated to a dose range of  $0\text{--}16\ \text{kJ m}^{-2}\ \text{day}^{-1}$   $\text{UV}_{\text{QUAITE}}$  and  $4\text{--}20\ \text{kJ m}^{-2}\ \text{day}^{-1}$   $\text{UV}_{\text{F\&C}}$ . All treatments were quantified using a double scanning spectroradiometer (model SR991-v7; Macam Photometrics, Livingston, UK). Plants were then harvested after 18 days total growth and leaf areas determined using a LI-COR LI-3000A area meter (LI-COR Inc., Lincoln, NE). There were 10–15 replicate plants per treatment and data have been analyzed using one-way analysis of variance and linear regression using SPSS v 11.5 (SPSS Inc., Chicago).

**Fungal responses to UV: *Botrytis cinerea* and *Trichoderma harzianum*.** The UV responses of *Botrytis cinerea*, a major pathogen of a wide range of crops, and its potential biological control agent, the fungus *Trichoderma harzianum*, have been studied. Both fungi were maintained in culture on potato dextrose agar (PDA) in 80-mm Petri dishes at  $18^\circ\text{C}$  for *B. cinerea* and  $25^\circ\text{C}$  for *T. harzianum*. For experimental use, conidia were washed from the cultures in 0.05% Tween, and the spore concentration adjusted to  $2.5 \times 10^6$  spores  $\text{mL}^{-1}$ . A 100- $\mu\text{L}$  aliquot of the spore suspension was then placed into a 50-mm Petri dish containing PDA and spread across the surface of the plate.

The Petri dishes were irradiated using custom-built lamp arrays in a temperature-controlled dark room held at  $20 \pm 2^\circ\text{C}$ . The lamp systems also incorporated a cooling base plate, which held the temperature of the agar at  $18 \pm 2^\circ\text{C}$ , and the base plate had 16 defined locations, each capable of holding a 50-mm Petri dish. By using combinations of plastic films and metal-mesh filters, which attenuated the UV without altering the spectrum, a series of treatments were provided simultaneously, and these were used to generate dose–response relationships. All experiments also included foil-wrapped Petri dishes as an internal dark control.

For UV-A irradiations, four unfiltered Philips TLD-30/12 tubes (Starna Ltd., Romford, UK) were positioned 50 mm above the Petri dishes. For UV-B irradiation, two Philips TL40/12-RS tubes (as above) were positioned 50 mm above the Petri dishes. In these experiments, the 16 Petri dishes were generally divided into two groups of eight, allowing



**Figure 2.** The growth of lettuce under spectrally modifying films in the field. (a) Leaf area in propagation lettuce, (b) harvestable fresh weight in lettuce cv. Constance grown to yield under the films, (c) above-ground dry weight in lettuce cv. Constance grown to yield under the films and (d) taste assessment of lettuce cv. Constance grown to yield under the films. In (a–c), data are means of six (a) or four (b,c) repeat experiments  $\pm 1$  SE. In (d), data are means  $\pm 1$  SE of 15 replicate plants in one field experiment. In all cases, treatments that do not share a common letter are significantly different in one-way analysis of variance and *post hoc* comparisons using Tukey's HSD.

simultaneous irradiation with seven doses (plus a dark control) from two filter treatments, either 0.13-mm cellulose diacetate (Clarifoil, Courtaulds Ltd., Derby, UK) to remove all wavelengths below 290 nm, clear polyester (Lee filters, Andover, UK) to remove all wavelengths below 320 nm, or unfiltered. Irradiances between 250 and 800 nm at the height of the surface of the Petri dish were measured for each treatment using a double monochromator scanning spectroradiometer (as above). Treatments were expressed in terms of two BSWF,  $UV_{QUAITE}$  and  $UV_{F&C}$ . We used  $UV_{QUAITE}$  as the baseline for defining treatments because this is thought to be quite close to a general BSWF for fungi (33,34). The treatments provided equated to  $0\text{--}60 \text{ kJ m}^{-2} \text{ day}^{-1} UV_{QUAITE}$  and  $0\text{--}30 \text{ kJ m}^{-2} \text{ day}^{-1} UV_{F&C}$ .

Following irradiation, Petri dishes were sealed and foil wrapped, then incubated as per initial culturing procedures for each organism for a period of 24 h. Following incubation, spore germination was measured by examining each Petri dish surface under a light microscope at  $20\times$  magnification. Five fields of view were examined for each dish and conidia were categorized as germinated or nongerminated. A conidium was classed as germinated if the hyphal tube length was at least that of the spore's diameter. All experiments have been repeated five times and data analyzed using repeats as replicates. In addition, fungal dose responses have been analyzed using the approach we described before (34).

## RESULTS

### Crop growth, yield and quality

**Lettuce.** Over the six repeats of the propagation experiments between May 2003 and September 2004, both the UV-transparent and R:FR-modifying films invariably caused small but significant reductions in the leaf area of seedlings of lettuce cv. Constance (Fig. 2a). Averaged across the repeats, UV-transparent film significantly ( $P = 0.005$ ) reduced leaf area by 8% and the R:FR-modifying film by 14% ( $P = 0.003$ ; Fig. 2a). Responses to the UV-opaque film were less consistent and, while leaf area of plants under this film were significantly greater (26–32%) than under the standard film in three of the six experiments, the mean effect over the six repeats (+13%) was not significant. However, leaf area was consistently and significantly higher under UV-opaque than UV-transparent film (mean =  $23\% \pm 7\%$ ,  $P = 0.013$ ; Fig. 2a).

The reductions in leaf area under UV-transparent and R:FR-modifying films were entirely due to highly significant reductions in leaf length; the films had no effect on leaf width. Leaves of lettuce seedlings produced under the UV-transparent film were significantly (typically 20–25%;  $P = 0.003$ ) thicker than leaves of plants from the standard film. No other film caused any significant change in leaf thickness. Changes in above-ground fresh and dry weight were consistent with those in leaf area (data not presented).

Over the four repeats of the experiments in which lettuce was grown to yield, both the UV-transparent and R:FR-modifying films invariably caused significant reductions in both harvestable fresh-weight yield and above-ground dry weight in lettuce cv. Constance. Averaged across the repeats, UV-transparent film significantly reduced fresh weight by 38% ( $P = 0.019$ ; Fig. 2b) and dry weight by 32% ( $P = 0.012$ ; Fig. 2c). These reductions were not significantly different from those caused by the R:FR-modifying film (28% and 26% for fresh and dry weight, respectively; Fig. 2b,c). Fresh and dry weights tended to be higher under the UV-opaque film than the standard film (mean  $\pm$  SE were  $27\% \pm 9\%$  and  $11 \pm 8\%$ , respectively), but changes in response to UV-opaque film were quite variable and were not significantly different when averaged across all repeats.

Fresh and dry weights under the UV-opaque film were both significantly greater than those under the UV-transparent film ( $P = 0.007$  and  $P = 0.008$ , respectively). The UV-modifying films significantly altered the taste as measured by the taste panel (Fig. 2d), with UV-transparent film resulting in a significantly lower taste score (*i.e.* more bitter/less sweet) than any other treatment ( $P < 0.01$  in all cases).

### Controlled environment dose responses

**Growth responses in lettuce.** Leaf area of seedlings of both lettuce cultivars was progressively reduced with increasing UV dose

(Fig. 3) and, in both, the maximum reduction was approximately 40% compared with the control. A single, highly significant ( $P < 0.001$ ) linear dose response fitted to data for both cultivars explained 89% of the variation in the data.

### UV effects on pathogenic and biocontrol fungi

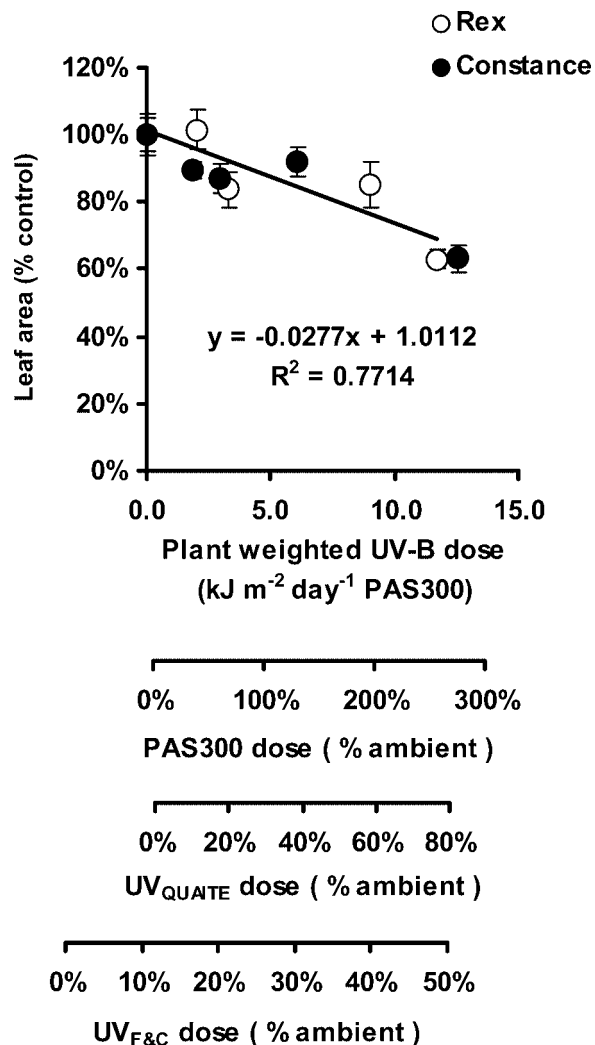
For *B. cinerea*, it is possible to fit a single dose response for all spore mortality data from the different lamp/filter combinations expressing doses weighted by  $UV_{QUAITE}$ . This inverse sigmoid UV dose response explained 96% of the variation in these combined data sets ( $P < 0.001$ ; Fig. 4a). This dose response indicates no significant effect at  $UV_{QUAITE}$  doses less than approximately  $1\text{--}2\text{ kJ m}^{-2}$ , while doses greater than approx.  $8\text{ kJ m}^{-2}$  resulted in complete kill. The calculated lethal dose for 90% of spores ( $LD_{90}$ ) for conidiospore mortality in *B. cinerea* was  $5.5\text{ kJ m}^{-2}$ . UV-A treatments of up to  $1000\text{ kJ m}^{-2}$  unweighted (from specific UV-A sources or polyester-filtered UV-B sources) caused no significant mortality of *B. cinerea* conidia. As a result, it was not possible to integrate all treatments in to a single dose response using  $UV_{F\&C}$  because this BSWF would predict far greater UV-A responses than was actually measured in this fungus (Fig. 4b). It is possible to fit a significant dose response using  $UV_{F\&C}$  if the UV-A data are excluded, but this model remains inferior to  $UV_{QUAITE}$  for this fungus.

In contrast with *B. cinerea*, it was not possible to fit a single  $UV_{QUAITE}$  dose response to data from all lamp/filter combinations for the biocontrol agent *T. harzianum*. Data for all UV-B lamp treatments (unfiltered, CA and PE filtered) but not UV-A lamps could be integrated in to a single inverse sigmoid  $UV_{QUAITE}$  dose response that explained 94% of the variation in these data ( $P < 0.001$ ; Fig. 4c). This UV-B response for *T. harzianum* indicates no significant effect at  $UV_{QUAITE}$  doses less than approximately  $2\text{ kJ m}^{-2}$  and complete kill at doses greater than approximately  $14\text{ kJ m}^{-2}$ . The calculated  $LD_{90}$  for *T. harzianum* was  $8.8\text{ kJ m}^{-2}$ . A separate  $UV_{QUAITE}$  dose response fitted to the UV-A was also highly significant ( $r^2 = 0.97$ ,  $P < 0.001$ ; Fig. 4c) but the calculated  $LD_{90}$  of  $0.7\text{ kJ m}^{-2}$  suggests that  $UV_{QUAITE}$  underestimates the relative UV-A response of *T. harzianum* by approximately one order of magnitude.

Expressing doses as  $UV_{F\&C}$  brings all treatments closer to a single dose response curve, although this explains only 62% of the variation in the data, with the effects of the UV-A treatment overestimated and those of unfiltered UV-B lamps underestimated (Fig. 4d). The calculated  $UV_{F\&C}$   $LD_{90}$  for *T. harzianum* is  $8.2\text{ kJ m}^{-2}$ . Expressed as an unweighted UV-A dose response, there is little mortality of *T. harzianum* conidiospores below approx.  $400\text{ kJ m}^{-2}$ , but once this threshold is exceeded, there is a rapid increase in mortality with increasing dose. The calculated  $LD_{90}$  for this UV-A response is  $970\text{ kJ m}^{-2}$ .

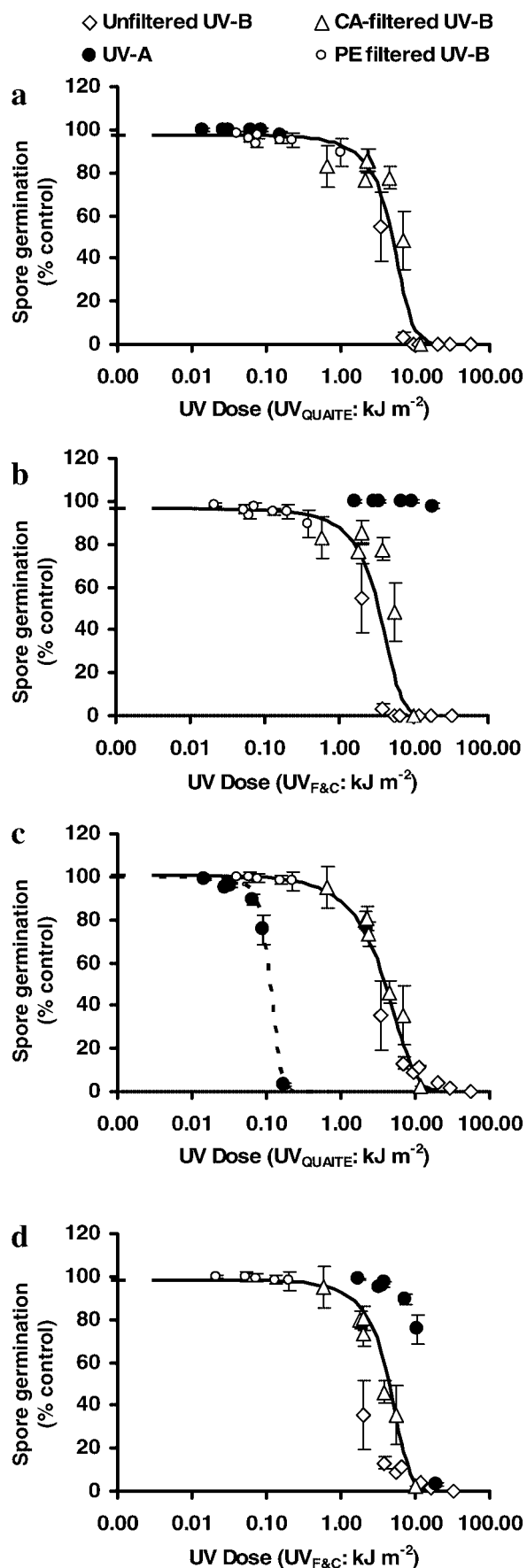
## DISCUSSION

Our studies show that UV-transparent cladding film has the potential to deliver commercially useful growth regulation. In lettuce, our model species, UV-transparent film was as effective as the R:FR-modifying film in reducing growth (Fig. 2). Conversely, growth was stimulated under UV-opaque film compared with the standard film. The capacity for UV modification within the ambient range to control growth is not surprising given the basic photobiological information on the exclusion of UV-B (20–28)



**Figure 3.** UV dose response for seedlings of two lettuce cultivars under controlled environment conditions. All points are means of 10–15 replicates  $\pm 1$  SE. The line is a linear regression fitted to both data sets ( $r^2 = 0.88$ ;  $P < 0.01$ ). UV treatments are expressed as absolute PAS300 doses and also relative to maximum daily field dose at the field site for three different BSWF. Note that the zero for the  $UV_{F\&C}$  BSWF is displaced relative to PAS300 and  $UV_{QUAITE}$  because of the contribution of longer wavelength background UV-A to  $UV_{F\&C}$  but not the other two BSWF, *i.e.* zero PAS300 or  $UV_{QUAITE}$  is not zero  $UV_{F\&C}$ .

and the more limited data on UV-A exclusion (20–22). That basic photobiological literature also suggests strongly that plant responses to UV manipulation would vary between species or genotype. However, across the range of responses and crops that have shown significant effects of either UV-transparent or R:FR films, 18% have occurred only with the former, 24% only with the latter and 58% with both films, so there is little to suggest that growth responses to UV-transparent film would be more species specific than those to R:FR modification. Also, there is little evidence that the effects of UV manipulation are more variable over time than those to R:FR modification. For example, both UV-transparent and the R:FR-modifying film significantly reduced growth in all six repeat experiments with propagation lettuce and all four with lettuce grown to yield, although it should be noted that no experiments have been carried on between October and April when UV doses would be expected to be lower. Although the



data presented here are for lettuce, the R:FR increasing and UV-transparent film also limit growth in a range of ornamental species, and are being considered as commercial alternatives to chemical growth regulators in such species.

Increasing crop quality is a major priority in commercial horticulture and the known effects of UV radiation on plant chemistry have the potential to contribute to this objective in a number of ways. In our current studies, manipulating UV certainly influenced the taste of lettuce, with increasing UV leading to a more intense, bitter taste (Fig. 2d). Increasing UV may increase the volatile oil content of some herbs (29). However, in our studies that include five species of herbs, only one (peppermint: *Mentha piperita*) showed any significant increase in oil concentration in responses to UV-modifying films over two growing seasons. The chemical composition of the herb oils were also not affected by spectral modification in any species (unpublished results from this laboratory).

UV modification certainly has substantial effects on crop pigmentation and, as in previous studies with lettuce (21) and some other crops (35), we have observed clear increases in the pigmentation of red-leafed lettuce with increasing UV. As well as being directly relevant to commercial grading in this crop, such increases in anthocyanins are typical of the effects of modification of solar UV on a range of pigments and antioxidants (36), many of which have a nutritional role in the human diet (37,38). The use of UV spectral modification may offer an alternative to metabolic engineering (38) to improve the nutritional quality of some crops grown under protection.

The effects of UV manipulation on the management of crop disease in protected crops remain unclear. It seems likely that disease will be influenced by a range of interacting responses to UV, including altered plant growth, leading to altered canopy microclimate, altered host resistance, changes in the survival of fungal spores and, perhaps, changes in sporulation. Our *in vitro* data suggest that using plastics that modify the UV regimes of the crop may also influence the biological control of disease. It is clear that fungi used in disease biocontrol are vulnerable to damage by solar UV, as is the case with fungi used in insect biocontrol (39,40). However, unlike pest control, in disease biocontrol, interactions are between two microorganisms and, in the *B. cinerea*/*T. harzianum* system, both are sensitive to UV damage.

The calculated UV<sub>QUAITE</sub> dose responses show that *B. cinerea* is rather more sensitive to UV-B than *T. harzianum*, although exposed conidiospores of both species would be predicted to suffer complete mortality within 1 day in the field during sunny summer conditions in the UK. However, while UV<sub>QUAITE</sub> may be a good approximation of the BSWF for many fungi (33,34), as it is here for *B. cinerea*, it is clear that it does not fully describe the response of *T. harzianum* to solar UV. Neither UV<sub>QUAITE</sub> nor UV<sub>F&C</sub> appear to be ideal BSWF for *T. harzianum*, although, using UV<sub>F&C</sub> as a starting point, we estimate that decreasing the weighting of our UV-A treatment by approximately one order of magnitude relative to that of unfiltered UV-B lamps would bring all treatments onto

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**Figure 4.** UV dose response for *Botrytis cinerea* (a,b) and *Trichoderma harzianum* (c,d) expressed in terms of UV<sub>QUAITE</sub> (a,c) or UV<sub>F&C</sub> (b,d). All points are means of five repeated experiments  $\pm 1$  SE. The dose-response curves have been fitted as inverse sigmoid curves using nonlinear regression in SPSS v11.5 (SPSS Inc., Chicago).

a single dose response, a relatively small change given the errors inherent in defining action spectra (19).

The contrasting spectral responses of *B. cinerea* and *T. harzianum* were only revealed by the inclusion of a specific UV-A treatment. Had only UV-B treatments been used, UV<sub>QUAITE</sub> would have appeared suitable for both species, and the fitted dose responses leads to the prediction that *T. harzianum* is rather more resistant to UV damage than *B. cinerea*. This conclusion is markedly altered if the UV-A response of *T. harzianum* is considered because this predicts that this species is more vulnerable than *B. cinerea* to exposure to the full spectrum of sunlight. This has practical implications because the greater sensitivity of *T. harzianum* to long-wave UV-A leaves it more vulnerable than *B. cinerea* to radiation transmitted through our standard horticultural plastic. We would predict that switching from standard plastics to UV-opaque film would be favorable for biocontrol of *B. cinerea* by *T. harzianum*, and this hypothesis will be tested in the field facility in future work.

The interpretation of our plant responses to UV modification may be influenced by assumptions over BSWF, but our data can also be analyzed to provide an assessment of alternative BSWF. This can be done in two ways. First, the relative magnitude of our measured responses to standard and UV-opaque filters in relation to UV-transparent film can be compared with those predicted by different BSWF (19). Using spectral data for early July at STC, PAS300 and UV<sub>QUAITE</sub> would suggest that the entire response to UV attenuation would occur with standard film, with UV-opaque film inducing no additional effect. By contrast, UV<sub>F&C</sub> suggests that responses to the UV reduction under standard film would be around 60% of the response under the UV-opaque film. In the field studies, the effect of the standard film compared with UV-transparent film was 78% of the total (UV-transparent compared with UV-opaque) for propagation lettuce and 75% for lettuce grown to yield.

However, this approach can only predict the relative effect of the two wavebands, not the magnitude of the response. A second approach to analyzing our data in relation to BSWF is to utilize the controlled environment dose response curves for the growth of lettuce seedlings. As always, extrapolation from controlled environment (CE) room experiments to the field requires caution. It is possible that the lower background PAR in our CE room studies compared with the field might exaggerate plant responses to UV, resulting in an overestimation of the slope of the dose response. However, the slope of the dose responses determined here for leaf area in lettuce appears to be comparable with those we determined for pea at approximately 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR (32) and also to those derived from other published dose responses.

Comparing UV-transparent with standard film under doses typical of the field experiments, the dose responses predict decreases in leaf area in the range of 5–10% with PAS300, 15–25% with UV<sub>QUAITE</sub> and 25–35% with UV<sub>F&C</sub>. These predictions compare with a measured increase in the leaf area of lettuce seedlings under standard film of 4–15% (mean = 9%  $\pm$  2%). Using the same approach to comparing UV-opaque film with the standard predicts no measurable effect on leaf area with PAS300 and UV<sub>QUAITE</sub>. By contrast, UV<sub>F&C</sub> predicts leaf area increases up to 15–20% under UV-opaque film. This compares with measured increases in leaf area of lettuce seedlings of 13  $\pm$  7% under UV-opaque compared with standard film.

Taken together, these two approaches show that

- i. UV<sub>F&C</sub> is the best of the three BSWF at predicting the balance of responses to longer and shorter UV, but while

PAS300 and UV<sub>QUAITE</sub> certainly underestimate responses to UV-A, UV<sub>F&C</sub> appears to overweight its relative effect.

- ii. UV<sub>F&C</sub> tends to overestimate the relative effectiveness of solar radiation relative to the UV radiation provided from lamps in the growth room experiments.

Interpreting this comparison requires careful consideration of the contrasting UV spectra of the field and controlled environment rooms. The maximum growth reduction that occurred in controlled environment studies, around 40%, would be interpreted as resulting from a range from zero to 2.5 $\times$  UK maximum PAS300 but from only 50% of the field range with UV<sub>F&C</sub> (Fig. 2). This is a function of the contrasting UV spectra of sunlight and the growth rooms, resulting in so-called enhancement errors (30). The UV-B sources used in the growth room (Philips TL40/12–RS), in common with other sources used in UV-B research (*e.g.* Q-Panel UVB-313), are relatively enriched at shorter UV-B compared with sunlight and so are highly efficient sources of plant effective radiation weighted using PAS300. For example, our spectroradiometric measurements show that TL40 lamps filtered with cellulose diacetate produce approximately 210 mW PAS300 per W of unweighted total UV. Expressed in these terms, summer sunlight produced only 2.9 mW PAS300 per W unweighted total UV.

The relative efficiency of lamps and sunlight are very different for an action spectrum such as UV<sub>F&C</sub>, which is strongly influenced by the UV-A content of sunlight. For our summer sunlight spectra, sunlight produced approximately 21.4 mW UV<sub>F&C</sub> per W unweighted UV, while cellulose diacetate-filtered TL40 produces approximately 180 mW UV<sub>F&C</sub> per W unweighted UV. While these enhancement errors are due to the effectiveness attributed to UV-A in UV<sub>F&C</sub>, this UV-A weighting also results in far better predictions of the overall effects of UV-modifying plastics in these experiments. Overall, our data supports the general validity of UV<sub>F&C</sub> but suggests that this new BSWF slightly overweights the relative effect of UV-A. We estimate that decreasing the mean weighting of UV-A wavelengths in UV<sub>F&C</sub> by as little as one order of magnitude would be sufficient to bring predictions close to our measured responses for both standard and UV-opaque films.

The issue of enhancement errors associated with different BSWF are not specific to our growth-room studies. Flint and Caldwell (19) point out the implications for field supplementations of this difference in the efficiency of UV-B lamps in producing plant-weighted radiation. However, the same arguments apply to glass-house or controlled-environment studies where UV-B lamps are the sole source of biologically effective radiation (30,41). Assuming cellulose diacetate-filtered TL40 lamps as the sole UV source, we estimate that PAS300 doses in excess of 30 kJ  $\text{m}^{-2} \text{day}^{-1}$  would be necessary to reach the summer, clear-sky maximum dose of UV<sub>F&C</sub> at mid latitudes. Thus, data from existing controlled-environment UV-B studies will require significant reinterpretation in the light of UV<sub>F&C</sub>. In particular, responses previously attributed to above ambient doses (designed to mimic some degree of ozone depletion) will have been produced by UV<sub>F&C</sub> doses well within the ambient range.

Assumptions over BSWF are also significant in applied research, where it is highly desirable to be able to make broad assessments of the likely effects of different UV-modifying plastics over a range of locations and seasons. In principle, such assessments could be calculated by analogy with the calculation of radiation amplification factors (RAF) in ozone-depletion research, by convoluting the sunlight UV spectrum for a given latitude and time of year with the spectral transmission of the plastic and with a BSWF. However,

just as with the calculation of RAF (19), such calculations are profoundly affected by choice of BSWF. On the basis of these experiments, we concur with the conclusions of Flint and Caldwell (19,42) that the most appropriate BSWF for plant responses, and probably also those of fungi, will have a significant tail in the UV-A, with the weighting intermediate between  $UV_{F\&C}$  and  $UV_{QUAITE}$ .

The characterization of the most appropriate BSWF for the responses of plants and associated organisms is as useful in applied UV research as it is in studies dealing with the photoecological consequences of stratospheric ozone depletion. A thorough understanding of BSWF may allow manufacturers of spectrally modifying plastics to improve the properties of their films. Equally, as we have sought to show here, experiments designed in the context of horticultural research also have the capacity to deliver information on the value of different BSWF for plant responses. Common areas of interest in applied and fundamental research are not limited to BSWF but extend into topics such as plant–herbivore interactions and the role of UV in regulating plant chemical composition. In our view, studies focusing on the application of plant UV responses in crop production have the ability to take this area of research forward in new directions, building on the great body of information obtained through research focused on ozone depletion.

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