ANNUAL REPORT

To:
Horticultural Development Council
Bradbourne House
Stable Block
East Malling
Kent
ME19 6DZ

THE USE OF SPECTRAL MODIFICATION TO CONTROL PLANT GROWTH AND QUALITY- A BIOLOGICAL FRAMEWORK FOR PREDICTING CROP RESPONSES

December 2006

Commercial – In Confidence

Project title:
HDC STUDENTSHIP: THE USE OF SPECTRAL MODIFICATION TO CONTROL PLANT GROWTH AND QUALITY- A BIOLOGICAL FRAMEWORK FOR PREDICTING CROP RESPONSES

Project number: CP 26

Project leader Dr N. D. Paul
Dept of Biological Sciences, Institute of Environmental and Natural Sciences, Lancaster University, Lancaster LA1 4YQ.

Report: Annual Report

Date commenced: 1 October 2004

Duration: 3 years

The results and conclusions in this report are based on a series of crop scale observations, crop trials and more detailed field- and laboratory-based experiments. The conditions under which the studies were carried out and the results have been reported with detail and accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with the interpretation of the results especially if they are used as the basis for commercial product recommendations.

Authentication

I declare that this work was done under my supervision according to the procedures described herein and that this report represents a true and accurate record of the results obtained.

N. D. Paul BSc PhD
Dept of Biological Sciences,
Institute of Environmental and Natural Sciences,
Lancaster University, Lancaster LA1 4YQ
Tel: 01534 593406; Fax: 01534 843854
e-mail: n.paul@lancaster.ac.uk

Date……

Practical section for growers

- The overall aim of this PhD studentship is to understand in detail the mechanisms that lead to the regulation of growth in a range of crops that occurs under spectrally modified plastics. The project is conducted in close collaboration with crop-scale studies under CP19 and PC221, and information from the projects work together to define responses to smart plastics.
- The primary focus of the project in its second year has been lettuce. This was driven partly by the suitability of this crop for investigating growth responses, and also because one key outcome of CP19 has been the adoption of UV-transparent covers for propagation crops of both lettuce and brassicas. In addition, some work has been carried out using *Arabidopsis thaliana*, the classic model plant species, which has allowed the underlying mechanisms of response to be studied in more detail.
- CP19 has shown that propagation under UV-transparent plastic produces seedlings that are more compact, with thicker leaves and that such seedlings go to give higher yields in the field. We showed that the same changes in plant form can be produced using UV lamps in controlled environment rooms, and we used lamps to define the dose response for growth responses to UV light.
- We used the controlled environment system to show that reduced leaf growth occurred because leaf cells expanded less, not because there was less cell division. We investigated mechanisms that led to reduced expansion, and showed that this due to the UV treatment making leaves stiffer. As well as being a key driver in regulating leaf growth, this increased leaf stiffness may contribute to the better field performance of plants propagated under the UV-transparent film. We also showed that increased leaf stiffness could be attributed to increased activity of a specific type of enzyme under UV treatment.
- We considered other mechanisms by which propagation under UV-transparent films could make plants better able to withstand "transplant shock". Using material grown under project CP19, we showed that plants propagated under plastics that transmitted less UV had lower concentration of protective pigments at the end of the propagation stage. We used chlorophyll fluorescence to show that such plants suffered more "stress" in the first day or two are transplanting to the field.
- We also investigated how UV affected the production of leaf colour in lollo rosso lettuce, since CP19 has shown that good colour depends on the crop receiving the full UV-component of sunlight i.e. by growing under the UV-transparent film. In controlled environment rooms we defined the UV dose response to pigment (anthocyanin) production, and using Arabidopsis, we are studying the basis of this response in more detail. In collaboration with CP19, we confirmed that UV was important for colour development in both "double red" and "triple red" lettuce varieties, but that the response was stronger in "double reds".
- A poster summarising this HDC studentship was presented at the House of Commons.

Discussion.

The results obtained to date in this PhD project have delivered a far greater understanding of the mechanisms of growth response to UV manipulations that have been of commercial interest in CP19. It is clear that in lettuce, growth regulation can be attributed to a series of steps, as follows:

- UV irradiation induces cell wall peroxidise activity at a much earlier stage of leaf development than occurs in the absence of UV.

- Increased cell wall peroxidise activity leads to cell wall stiffening, and this, in turn, reduces cell expansion.
- Reduced cell expansion is the primary driver of reduced leaf expansion, there is no evidence of reduced cell division.

This series of events may not be complete, for example other cell wall enzymes might be involved in changes in cell wall properties, but it is the most complete description of how UV regulates leaf growth available for any plant species. Changes in cell wall properties may be valuable in propagation crops not only by contributing to reduced growth but because "stronger leaves" may be less prone to physical damage during transplanting and due to initial wind damage in the field. By analogy with previous studies, such changes might also contribute to improved shelf-life in baby-leaf salads[7, 8]. The data obtained has also clarified UV-induction of foliar pigments, both the visible anthocyanins that contribute commercial leaf colour in red-leaved lettuce, and the protective pigments that play a key role in protecting leaves from high light (UV and longer wavelengths) in the field. The key role of UV in inducing such pigments is well known, including the possible "master switch" controlling UV responses, but we have shown here that induction may play a key role in protecting plants propagated under protection during the first days after transplanting to the field.

Work in the final year will develop established lines of research. On the basis of discussions with growers, the use of UV lamps for growth regulation in the very early stages of propagation, to limit hypocotyl elongation for example, will be considered, based on the CE-based studies that have formed the back-bone of these studies. This work will not directly define commercially applicable approaches, but will demonstrate whether the concept of using UV lights in this way is feasible. We will also continue to investigate the basic mechanisms of growth regulation by UV, in particular whether the understanding that can be obtained from more fundamental approaches using a model plant can contribute to the development of new approaches to crop growth regulation.

Science section

Project background

The cultivation of crops under simple plastic covered structures is now commonplace in UK horticulture because of its potential to extend growing seasons, control harvests and improve the quality of produce. In recent years advances in technology have allowed the manufacture of novel materials, so called "spectral filters" or "smart plastics" that 'fine-tune' the growing environment still further, by manipulating the intensity and wavelength of light reaching the crop.

HDC projects CP19 and PC221 have been carrying out a programme of research in to the use of "smart plastics" for a range of crops under UK conditions. Those projects have demonstrated a number of commercially useful effects, but, for the most part, have not been able to deliver any underpinning science relating to the mechanisms of the observed responses. This studentship was designed to complement CP19 and PC221 by concentrating on the mechanisms of one response to smart plastics that has already attracted commercial interest, the use of specific plastics to regulated growth in propagation crops. There are two approaches to growth regulation using spectral modification. The first approach is to increase the ratio of red to far red light (R:FR), delivered commercially by using films that selectively absorb far red light (e.g. solatrol film in the CP19 crop-scale studies). Increasing R:FR has been quite widely studied as a means of growth regulation, and CP19 showed that propagation under solatrol produced more compact plants in lettuce, brassicas and some bedding species, but that there was marked between-species variation. The other approach to growth regulation using spectral modification is to increase the irradiances of UV radiation reaching the crop. Conventional horticultural plastics transmit no UV-B (280-315nm) and little UV-A (315-400nm) below approximately 350nm. Using plastics that transmit the full solar UV spectrum has been shown in CP19 to deliver good growth regulation, and this appears to be especially beneficial in propagation vegetable crops, such as lettuce and brassicas. Indeed, results from CP19 have already led some UK propagators to switch to UV-transparent claddings.

The first year of the project was broadly focussed, considering both ornamentals (bedding species) and vegetable crops (propagation lettuce) and both UV and R:FR approaches to growth regulation using spectral modification. One initial aim of this PhD was to understand the basis of species-to-species variation in response to increased R:FR, with the working hypothesis that variation might be linked to the innate sun or shade adaptation of particular species, data from CP19 and from elsewhere revealed that there is also substantial variation in response between cultivars of the same species (e.g. fuchsia). These data undermined the use of bedding species as key species for investigating mechanisms of response. This, plus the fact that UV-transparent films were already being adopted commercially for propagation vegetable crops, and a body of data from CP19 and PC 221 that such films increased the final commercial yield of these crops after transplanting to the field, led the project to be focussed on the effects of UV manipulation on plant growth, using lettuce as the model species. Since CP19 had also highlighted the effects of UV on pigmentation in lettuce cultivars such as lollo rosso, this aspect of UV response was also studied.

The background literature on plant UV responses was reviewed in detail in the first year report on this project. In brief, it is known that increasing UV-B tends to reduce stem elongation and leaf expansion, and increase leaf thickness and branching, with these responses being broadly consistent across a range of plant species ([e.g. 1, 2]). However, the fundamental mechanism underlying these growth responses remains far from clear.

Different workers have reported that reduced leaf expansion could be attributed to reduced cell division or reduced cell expansion, or both, and it is not clear whether this variation in the literature is due to genuine biological variation or different experimental approaches. Therefore, these mechanisms were studied in detail in the second year of this project, underpinned with investigations of related mechanisms such as altered leaf biophysical properties and cell wall enzyme activity. Mechanistic studies were performed in controlled environment (CE) rooms, with defined UV treatments provided by specific UV light sources, against a background of photosynthetically active radiation provided by metal halide lamps.

All the data presented here has been obtained by Mr Jason Wargent, the PhD student who is supported by this HDC-funded studentship.

Materials and methods

Lettuce seedlings were sown in 40 cell tray inserts with Levington M3 compost and were propagated under a light/dark regime of 16hr/8hr at 25 $\pm$ 2°C and a PAR background of 500 µmol m^{-2} s^{-1} provided from 400W metal halide lamps. 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[3]. Briefly, background UV-A was provided by the metal halide lamps plus six Q Panel 340 UV-A tubes filtered with clear polyester (Lee filters, Andover, UK). UV-B was provided from six Philips TL40 UV-B tubes filtered with 0.13mm thick cellulose diacetate (Clarifoil, Courtaulds Ltd., Derby, UK). The UV-B was manipulated by wrapping UV tubes with a white cotton fabric which altered irradiance but not UV spectrum. The range of UV doses varied between zero and 12 kJ m^{-2} d^{-1} PAS300, that is up to 2.5x the current ambient maximum at 54°N (Note that, in terms of other commonly used action spectra, the treatments provided equated to a dose range of 0-16 kJ m^{-2} d^{-1} UV_{QUAITE} and 4-20 kJ m^{-2} d^{-1} $UV_{F\&C}$). All treatments were quantified using a double scanning spectroradiometer (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 USA). There were 10-15 replicate plants per treatment and data have been analysed using one-way ANOVA and linear regression using in SPSS v 11.5 (SPSS Inc., Chicago).
Epidermal cell size and number were measured using a light microscope attached to am image analysis system (Image Pro-Plus version 4.5, Media Cybernetics UK, Wokingham, UK). Leaf biophysical properties were determined in collaboration with Dr Roland Ennos, University of Manchester, using an Instron device. Cell wall peroxidise activity was quantified using the procedures of Moore *et al.*[4], with modifications developed in this project to optimise the method for lettuce.
Pigmentation analysis method followed closely to that of González *et al.*[3], Briefly, after six days of UV irradiation, the 2nd true leaf of each replicate was homogenised with acidified methanol and centrifuged for 15 minutes. Each sample was then subject to spectrophotometric analysis, with absorbance values recorded at 300nm (for general UV absorbing compounds) and at 524nm (in order to quantify anthocyanin content.

Results

Mechanisms of growth regulation by UV manipulations

It was confirmed that leaf area reduction in lettuce seedlings was a function of UV-dose, and that this held for at least two types of lettuce (Figure 1a). The dose response was relatively well described by a linear regression and the magnitude of reductions at a given dose appeared broadly comparable to the reductions observed under plastics in CP19, e.g.

around 20% under summer UK conditions. Controlled environment studies also confirmed that increasing UV radiation led to increased leaf thickness (Figure 1b), as observed in the field studies.

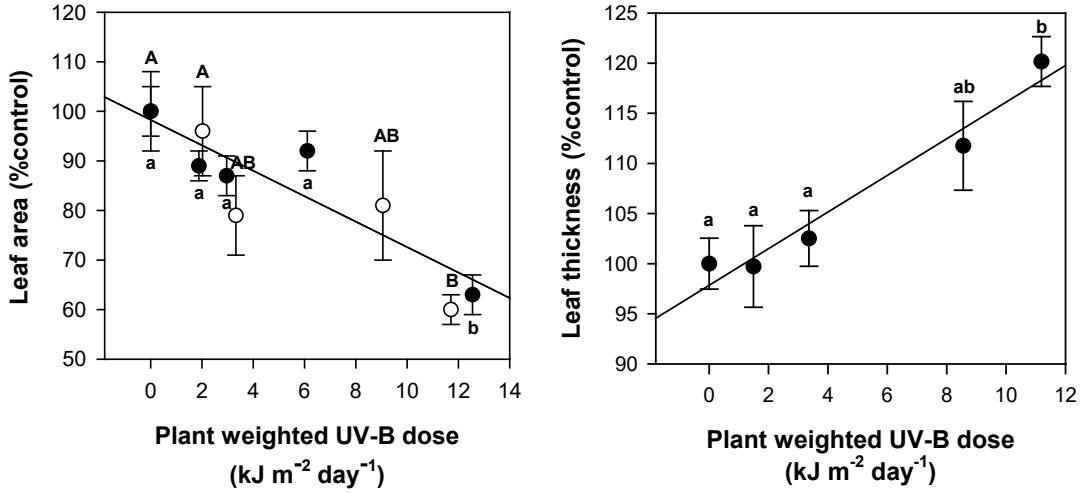

Figure 1. The effects of UV dose on (a) Leaf area of lettuce cultivars 'Constance' (●) and 'Rex' (○) plants and (b) leaf thickness in "Constance". Data are represented as % control against weighted UV dose. Data are means ± SE, and means with different letters are significantly different ($P < 0.05$).

The close similarities between data observed in the field and controlled environments provide confidence that the responses seen under experimental conditions are pertinent to the crop environment. Controlled environments allowed investigation of responses that have not been studied in the field, for example it was shown that increasing UV increased root:shoot ratio (Figure 2a), and also increased concentrations of protective pigments ("sunscreens") in the leaves (Figure 2b), both of which may contribute to the good performance of seedlings after transplanting.

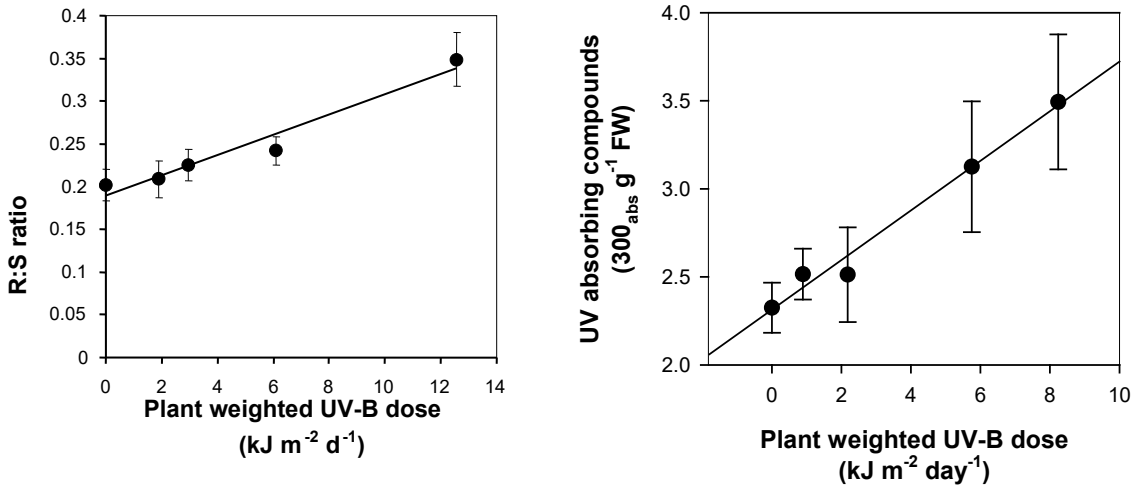

Figure 2. The effects of UV dose on (a) leaf thickness and (b) root:shoot ratio of 'Rex' plants, Data are represented as % control against weighted UV dose. . Data are means ± SE, and means with different letters are significantly different ($P < 0.01$)

Studies of the mechanisms of leaf growth reduction were made in parallel using material produced under defined UV conditions in the CE rooms and also from the crop-scale studies under plastics carried-out under CP19. It was clear both sets of investigations that

reduced leaf expansion could be attributed to significantly reduced cell expansion (shown for the CE studies in Figure 3a) while cell number was not altered by UV (Figure 3b)

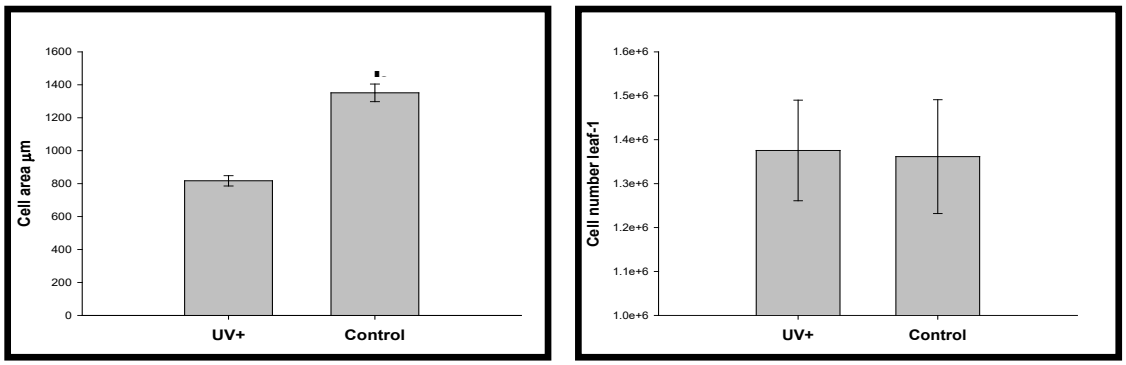

Figure 3. The effects of UV treatment on (a) epidermal cell area and (b) epidermal cell number in seedlings of lettuce cv. "Constance". . Data are means ± SE, and means with different letters are significantly different ($P < 0.01$)

The confirmation that reduced leaf growth in lettuce was due to a reduction in cell expansion with increasing UV focussed attention on the biophysical properties of cell walls, which is the key determinant of cell expansion. It was confirmed that leaf extensibility was significantly lower in seedlings grown in the presence of UV (Figure 4). This effect occurred during the first few days of leaf expansion, consistent with the observed pattern of change in leaf growth.

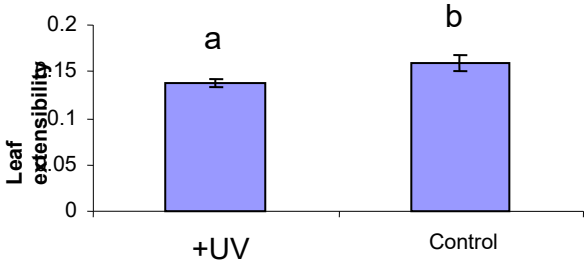

Figure 4. The effects of UV treatment leaf extensibility in seedlings of lettuce cv. "Constance". . Data are means ± SE, and means with different letters are significantly different ($P < 0.05$)

Cell wall stiffening could be due to a variety of factors, but a likely candidate was the up-regulation of cell wall peroxidases, and these have now been studied in material from both the CE room studies and field experiments. Both in CE studies (Figure 5) and the field (data not presented) it was confirmed that exposure to increasing UV radiation leads to significantly increased cell wall peroxidise activity in the first few days after leaf emergence. This is consistent with the observed pattern of cell wall stiffening and reduced leaf expansion.

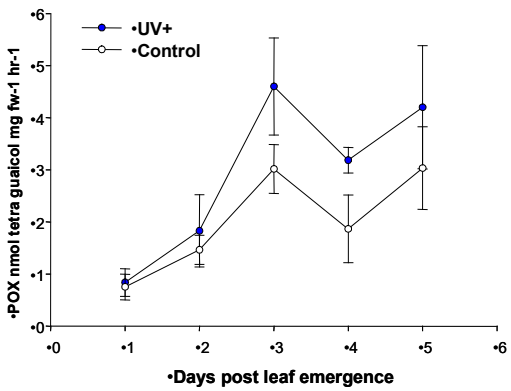

Figure 5. The effects of UV treatment cell wall peroxidise activity in seedlings of lettuce cv. "Constance". . Data are means ± SE, and means with different letters are significantly different ($P < 0.05$)

Other data

Although understanding growth regulation has been the main focus of research during year 2, other areas have also been investigated, partly to deliver improved understanding of changes seen under plastics in the crop-scale studies, and partly to deliver more fundamental "underpinning" science pertinent to crop responses to manipulation of UV radiation.

The former body of work has been a close collaboration between Jason Wargent, and the HDC research fellow employed on PC221 (Dr Jason Moore). Together, they have quantified the physiological responses of lettuce in the first few days after being transplanted from the propagation environment, using plants propagated under contrasting plastics. Briefly, they showed that the concentration of protective flavonoid pigments in the leaves of plants was strongly determined by UV in the propagation environment (i.e. plastics transmitting less UV produced plants with lower flavonoids concentrations). Even in the propagation environment, differences in flavonoids concentration were associated with differences in chlorophyll fluorescence and CO_2 fixation, with plants propagated under the UV-transparent film generally performing better, as measured by both these parameters. In the first one-two days after transplanting, all plants showed some degree of "transplant shock" (measured by a fall in the chlorophyll fluorescence parameter Fv/Fm), but this shock was less in plants with higher flavonoids concentrations (i.e. from the UV-transparent film). Long-term performance of transplants is likely to be a function of many factors, (CP19 data certainly suggests that leaf thickness at the end of the propagation phase is an important factor), and changes in protective pigments also seem to be important for maintaining plant function in the initial post-transplantation period.

The more fundamental, underpinning science has used the model plant *Arabidopsis thaliana* to investigate the mechanisms of growth responses to UV in more detail than is possible with lettuce. In particular, a gene (uvr8) has been identified in *A. thaliana* that is considered to be the "master-switch" for the induction of leaf pigments by UV[5, 6]. If this gene is also responsible for growth responses, then this understanding might allow responses produced by UV to be mimicked using other approaches, offering new routes to growth regulation. To date, we have shown that mutants lacking uvr8 activity are more vulnerable to UV-induced growth reductions, but only at rather high UV doses. It is not clear whether this mechanism is responsible for growth responses observed in crop scale studies in CP19, but we have begun to use the understanding obtained using *Arabidopsis thaliana* to assess natural plant signalling compounds for regulating plant growth. Initial studies show that at least one such compound delivers effective growth regulation, but whether this fully mimics the desirable features of UV-induced growth regulation (e.g. increased R:S and pigment accumulation) remains unclear.

Discussion.

The results obtained to date in this PhD project have delivered a far greater understanding of the mechanisms of growth response to UV manipulations that have been of commercial interest in CP19. It is clear that in lettuce, growth regulation can be attributed to a series of steps, as follows:

- UV irradiation induces cell wall peroxidise activity at a much earlier stage of leaf development than occurs in the absence of UV.
- Increased cell wall peroxidise activity leads to cell wall stiffening, and this, in turn, reduces cell expansion.

- Reduced cell expansion is the primary driver of reduced leaf expansion, there is no evidence of reduced cell division.

This series of events may not be complete, for example other cell wall enzymes might be involved in changes in cell wall properties, but it is the most complete description of how UV regulates leaf growth available for any plant species. Changes in cell wall properties may be valuable in propagation crops not only by contributing to reduced growth but because "stronger leaves" may be less prone to physical damage during transplanting and due to initial wind damage in the field. By analogy with previous studies, such changes might also contribute to improved shelf-life in baby-leaf salads[7, 8]. The data obtained has also clarified UV-induction of foliar pigments, both the visible anthocyanins that contribute commercial leaf colour in red-leaved lettuce, and the protective pigments that play a key role in protecting leaves from high light (UV and longer wavelengths) in the field. The key role of UV in inducing such pigments is well known, including the possible "master switch" controlling UV responses, but we have shown here that induction may play a key role in protecting plants propagated under protection during the first days after transplanting to the field.

Work in the final year will develop established lines of research. On the basis of discussions with growers, the use of UV lamps for growth regulation in the very early stages of propagation, to limit hypocotyl elongation for example, will be considered, based on the CE-based studies that have formed the back-bone of these studies. This work will not directly define commercially applicable approaches, but will demonstrate whether the concept of using UV lights in this way is feasible. We will also continue to investigate the basic mechanisms of growth regulation by UV, in particular whether the understanding that can be obtained from more fundamental approaches using a model plant can contribute to the development of new approaches to crop growth regulation.

In addition to the formal programme of research, the student supported by the project will continue to interact with growers, directly and via grower conferences. Of course, the final year of this contract will also be the final year of the PhD it supports, and a key activity will be the preparation and writing of the student's PhD thesis.

References cited

1. Paul, N. D. & Gwynn-Jones, D. Ecological roles of solar UV radiation: towards an integrated approach. Trends in Ecology & Evolution 18, 48-55 (2003).
2. Searles, P. S., Flint, S. D. & Caldwell, M. M. A meta analysis of plant field studies simulating stratospheric ozone depletion. Oecologia 127, 1-10 (2001).
3. Gonzalez, R., Mepsted, R., Wellburn, A. R. & Paul, N. D. Non-photosynthetic mechanisms of growth reduction in pea (Pisum sativum L.) exposed to UV-B radiation. Plant Cell and Environment 21, 23-32 (1998).
4. Moore, J. P., Taylor, J. E., Paul, N. D. & Whittaker, J. B. Reduced leaf expansion as a cost of systemic induced resistance to herbivory. Functional Ecology 17, 75-81 (2003).
5. Brown, B. A. et al. A UV-B-specific signaling component orchestrates plant UV protection. Proceedings of the National Academy of Sciences of the United States of America 102, 18225-18230 (2005).
6. Kliebenstein, D. J., Lim, J. E., Landry, L. G. & Last, R. L. Arabidopsis UVR8 regulates ultraviolet-B signal transduction and tolerance and contains sequence similarity to human Regulator of Chromatin Condensation 1. Plant Physiology 130, 234-243 (2002).
7. Clarkson, G. J. J., Rothwell, S. D. & Taylor, G. End of day harvest extends shelf life. Hortscience 40, 1431-1435 (2005).

8. Clarkson, G. J. J., O'Byrne, E. E., Rothwell, S. D. & Taylor, G. Identifying traits to improve postharvest processability in baby leaf salad. Postharvest Biology and Technology 30, 287-298 (2003).

