Project title:	Hormetic UVC Treatments for Control of Plant Diseases on Protected Edibles
Project number:	PE 023
Project leader:	Dr Gilbert Shama, Loughborough University
Report:	Annual report, August 2015
Previous reports:	None
Key staff:	George Scott, Loughborough University Matevž Rupar, The University of Nottingham Professor Matt Dickinson, The University of Nottingham Dr Gilbert Shama, Loughborough University
Location of project:	The University of Nottingham, Sutton Bonington Campus, Plant Science.
Industry Representative:	Philip Pearson, APS Salads, Aston Way, Middlewich, Cheshire, CW10 0HS. James Bean, Crystal Heart Salads, Eastrington Road, Sandholme, Brough, North Humberside, HU15 2XS.
Date project commenced:	1st September 2014
Expected completion date:	31st August 2017

DISCLAIMER

While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with 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 interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

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

George Scott

Doctoral Researcher

Loughborough University

Signature ..... Date .....

Report authorised by:

Dr Gilbert Shama

Reader

Loughborough University

Signature ..... Date .....

CONTENTS

GROWER SUMMARY

Headlines

- Post-harvest treatments of tomato fruit with a high intensity, pulsed UV source show induced disease resistance against *Botrytis cinerea* and delayed ripening. Treatment time is reduced by 98-99 % in comparison to low intensity, conventional UV sources.
- Preliminary studies indicate UV treatments of tomato and lettuce foliage induce resistance against *B. cinerea*.

Background

Hormesis is a dose-response phenomenon where low doses of a stressor bring about a positive response in the organism undergoing treatment. The benefits of UV hormesis have been known for over 20 years. A broad range of benefits are observed from increased nutritional content to disease resistance and reduced chlorophyll degradation. To date, the majority of studies have been performed using conventional low pressure mercury UVC sources on post-harvest produce. Commercial application of these treatments has, in part, been prevented due to the lengthy exposure times necessitated. Treatment can require exposure times of several minutes. High intensity, pulsed UV sources, however, have been developed which hold the potential of drastically reducing treatment times and making UV treatment a commercial possibility. However, it is necessary to demonstrate that such sources have the ability to induce disease resistance and delayed ripening on tomato fruit through post-harvest treatments (Objective 1).

Recently, exposure of foliage to UV has been shown to induce resistance against downy mildew and grey mould on *Arabidopsis thaliana*. The horticultural application of such treatments, however, have not been explored. We, therefore, aim to research pre-harvest UV treatments to induce resistance on both tomato and lettuce crops (Objectives 2 & 3). Utilisation of UV treatments in commercial situations may allow an alternate to traditional chemical-based disease control and provide a residue-free alternative to other inducers of disease resistance.

Summary

Objective 1 - Validation of the High Intensity Pulsed UV Source

Tomato fruit of the cv. Meccano were treated at both the mature green and ripe stage. An established conventional UV treatment was performed alongside a number of pulsed treatments. This was to allow comparison of the sources and monitoring of induced disease

resistance against *B. cinerea* and demonstrate delayed ripening. Both conventional and pulsed sources successfully induced resistance against *B. cinerea* on mature green and ripe fruit following artificial inoculation. Ripe fruit showed the requirement for increased levels of UV exposure to effectively induce resistance with the optimal treatment of 24 pulses giving a 37 % reduction in disease, Table 1. Mature green fruit showed a lower optimal treatment of 16 pulses giving a total treatment time of 10 seconds yielding a 97 % disease reduction, Table 2. The ability to induce resistance to *B. cinerea* at both the mature green and ripe stages shows that post-harvest UV treatment could be adopted by growers who harvest at differing fruit maturities. The majority of previously published research was focused only on fruit at the mature green stage.

Table 1: The mean area underneath the disease progression curve (AUDPC) and disease reduction for ripe fruit of the cv. Meccano treated with both conventional and pulsed UV.

Treatment	Total treatment time (s)	AUDPC	Disease reduction (%)
Control	0.00	40.62 ±10.47	-
Conventional	370.00	36.99 ±9.04	8.94
8 Pulses	5.00	31.89 ±16.71	21.49
16 Pulses	10.00	30.14 ±15.11	25.81
24 Pulses*	15.00	25.61 ±15.70	36.96

* Indicates a significant difference to the control at the $p < 0.05$ level by ANOVA

Table 2: The mean area underneath the disease progression curve (AUDPC) and disease reduction for mature green fruit of the cv. Meccano treated with both conventional and pulsed UV.

Treatment	Total treatment time (s)	AUDPC	Disease reduction (%)
Control	0.00	73.24 ±10.54	-
Conventional*	370.00	51.08 ±18.98	30.25
8 Pulses*	5.00	59.87 ±11.72	18.26
16 Pulses*	10.00	41.95 ±15.33	42.72
24 Pulses*	15.00	42.49 ±21.62	41.98

* Indicates a significant difference to the control at the $p < 0.05$ level by ANOVA

The effects of UV treatment on ripening were only monitored for mature green fruit. Fruit colour measurements were taken from tissue directly facing the UV sources and at 90 degrees from the source to assess the requirement for complete surface exposure. Delayed ripening was most efficiently induced with a 16 pulse treatment giving a 41 % difference in tomato colour index, Table 3. Tomato colour index increases with ripening. Little change was observed for tissue at 90 degrees from the source and thus it can be concluded that the tomato requires direct exposure for delayed ripening, Figure 1.

Table 3: The change in tomato colour index (TCI) and percentage difference from control of mature green fruit from the cv. Meccano after ten days of storage following treatment with conventional and pulsed UV sources.

Treatment	Direct		90 ^{o}	
	Change in TCI	Difference (%)	Change in TCI	Difference (%)
Control	259.22		267.51	
Conventional	174.73	- 32.60	268.32	+ 0.30
8 pulses	235.85	- 9.02	326.86	+ 22.18
16 pulses	155.15*	- 41.15	271.85	+ 1.62
24 pulses	182.78	- 29.49	257.10	- 3.75

* Indicates a significant difference to the control at the $p < 0.05$ level by ANOVA

We have shown here that the use of a pulsed source rich in UV can induce disease resistance against *B. cinerea* on both mature green and ripe tomatoes. Furthermore, a delay in ripening on mature green tomatoes was also observed. The use of a high intensity pulsed source can reduce treatment time by 97-99 %.The use of such a source has the potential for integration into post-harvest production lines to reduce losses through disease. Moreover, the observed delayed ripening would allow increased storage or transportation times.

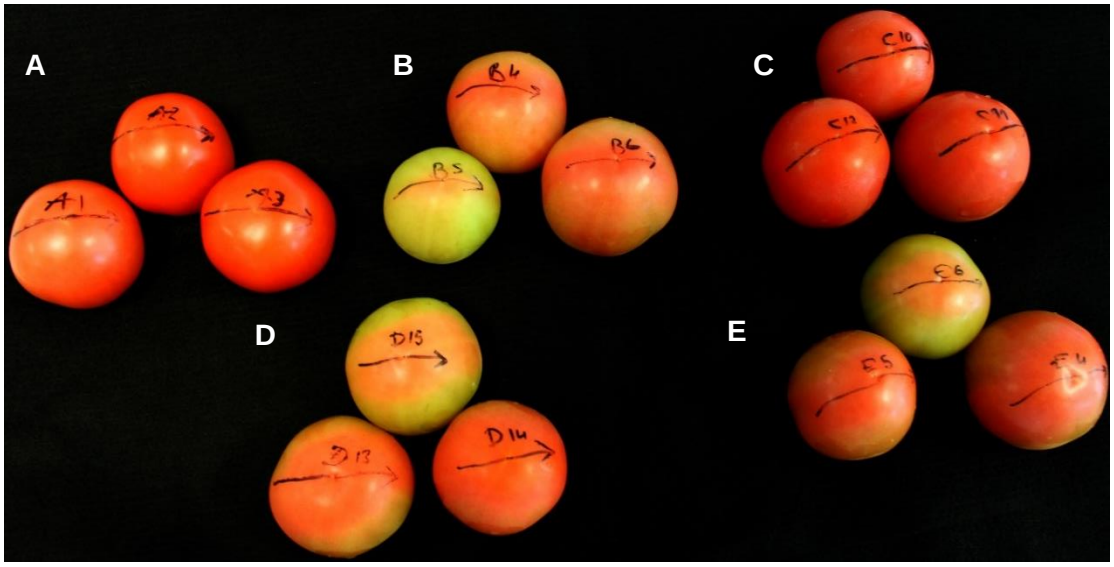


Figure 1: A representative sample from the fruits treated post-harvest showing: **A)** Control fruit. **B)** Conventional treatment with the low pressure mercury source. **C)** An 8 pulse treatment. **D)** A 16 pulse treatment and **E)** A 24 pulse treatment. Black lines on the fruit run parallel to the direction of UV source exposure which highlights the dependency of full surface exposure for delayed ripening.

Objective 2 - Foliar UV treatments of Tomato

No previous work has been carried out on the induction of resistance on tomato through exposure of the foliage to UV. The first step was, therefore, to find the point at which damage was observed on plants exposed to both conventional and pulsed UV sources. This was performed at two developmental stages; early vegetative and early flowering. Damage was observed above 0.5 kJ/m^2 for the conventional source and at 20 pulses. Hormetic treatments will, therefore, fall below these thresholds. During preliminary studies we have shown induced resistance against *B. cinerea* on a number of occasions. Further research, however, is required before the level and longevity of resistance can be determined.

Objective 3 - Foliar UV treatments of Lettuce

Damage assessments for lettuce were carried out at the 3-5 true leaf and early head formation developmental stages. Damage was observed above 2.25 kJ/m^2 and 45 pulses for the conventional and pulsed sources, respectively. Early indications also point towards the successful induction of disease resistance against *B. cinerea.*

Financial Benefits

Calculation of financial benefits are not possible at this time.

Action Points

There are no immediate action points.

SCIENCE SECTION

Objective 1 - Validation of Pulsed UV Source

Introduction

UV hormesis is a dose response phenomenon where small doses of UV bring about a positive reaction in the target organism. The positive effects of UV on fresh produce have been known for over 20 years and have shown to be effective on orange, strawberry and sweet potato to mention a few (Shama & Alderson, 2005, Ben-Yehoshua *et al.,* 1992, Pombo *et al.,* 2011, Ranganna *et al.,* 1997). The effects include a wide range of responses including pathogen resistance, delayed senescence, delayed ripening, increased nutritional content and reduced chilling injury (Charles *et al.*, 2008, Costa et al,. 2006, Stevens *et al.,* 1998, Eicholz *et al.,* 2011, Pongprasert *et al.,* 2011). The focus in this study is on the induction of disease resistance.

To date, induction of disease resistance has been focused primarily on post-harvest treatment of fresh produce with numerous experiments aimed at monitoring disease progression. One must be careful when reviewing the literature, however, as a number investigations have relied on initiation of disease through natural inoculum or have performed inoculations pre-treatment. This may create some confusion as it may fail to truly attribute the level of disease reduction to the UV induced effects alone. This is because we cannot account for the direct effect of UV on the inoculum which may be present on the fruit surface during treatment.

There are a number of studies whose experimental design allow the quantification of resistance induced by UV hormesis. As with other elicitors of induced resistance UV does not provide complete control of disease with reductions in severity and incidence of disease ranging from 10 - 91 % (Nigro *et al.,* 1998, Charles *et al.,* 2008). Levels of resistance have been shown to be affected by not only the number of days post-treatment that a fruit is inoculated but also by the day post inoculation that disease is observed (Charles *et al.,* 2008 & Ben-Yehoshua *et al.,* 1992). Furthermore; harvest date, cultivar, developmental stage, levels of visible light after treatment and target organ have all been shown to influence the efficacy of induced defences (D'Hallewin *et al.,* 1999, Stevens *et al.,* 1997, Vicente *et al.,* 2005, Stevens *et al.,* 1998 & Petit *et al.,* 2009).

UV-induced disease resistance is achieved in the fruit through alterations in the physical structure of fruit, secondary metabolism and regulation of defence genes. Firstly, physical modifications such as cell wall reinforcement, through suberin and lignin deposition, hinder fungal movement and therefore prevent disease progression (Charles *et al.,* 2009).

Secondly, the changes in secondary metabolism can include the upregulated biosynthesis of many phenolic compounds. These include the flavonols and anthocyanins which act not only as antioxidants but also absorb potentially damaging wavelengths of light. Moreover, many of the secondary metabolites act as phytoalexins exhibiting direct antimicrobial activity. Furthermore, their antioxidant capacity also increases the dietary value of the fruit for the consumer. Finally, the upregulation or priming of defence-related genes also occurs following UV treatment. These genes can include those involved directly in challenging pathogens such as chitinases but also those involved in defence signalling pathways.

UV treatments to date have been focused primarily on the use of UVC from conventional i.e. low pressure mercury sources that necessitate exposure times of several minutes for effective induction of resistance. An important objective here is to validate the use of a high intensity pulsed UV source for the induction of disease resistance against *Botrytis cinerea* through post-harvest fruit treatment with the intention of extending its application to pre-harvest, whole plant treatments.

Materials and methods

Both mature green and ripe tomato fruit from commercial cultivar Meccano were obtained from APS Salads via same day delivery and treated upon arrival. Prior to treatment colour measurements were taken to assess the effects on ripening. Mature green fruit were measured with a calibrated CR-200 Chroma meter (Konica, Minolta) in l*a*b* mode. Readings were taken at a single point directly facing the source and at a 90° axial rotation from that point. A second colour measurement was taken using the same reference points at 10 days post treatment. This was used to calculate the change in TCI over 10 days. Tomato colour measurements were transformed into the tomato colour index and the first reading was subtracted from the second to calculate change in TCI and therefore ripening progression, Figure 2.

All treatments were carried out in an enclosed gantry to protect users from UV light. A UV protective face shield was worn at all times and along with LaserShield (NoIR Laser Company) glasses while using the pulsed source. Conventional treatments were carried out with the source UVI 12OU2G11 CP15/469 (UV-Technik) with principal emission at 254 nm. The source was housed within anodised aluminium parabolic reflectors with a removable cover to protect the user between treatments. The conventional source was switched on at least 30 minutes before treatment and not terminated until the end of the experiment to allow constant emission. Pulsed treatments were carried out with the RT-847 cabinet along with RC-802 controller and LH-840 ozone-free B lamp (XENON).

An established conventional UVC treatment of 3.7 kJ/m^2 delivered at 2000µW/cm^2 (Charles *et al.,* 2008) was used as a benchmark to assess the efficacy of induced disease resistance from the pulsed source. Fruit were positioned 10 cm from the pulsed source and treated with a range of pulses (P). For both sources fruit received exposure on two sides through 180° axial rotation. Following treatment fruit were immediately incubated in the dark at 13 °C to prevent photoreversal. Fruit were stored in humidity boxes lined with damp paper and raised by a double layer of plastic mesh. At 10 days after treatment fruit were inoculated; this was shown to be the optimum point of UVC induced disease resistance by Charles *et al.,* 2008.

Fruit were surface sterilised in 1 % sodium hypochlorite and rinsed three times in sterile distilled water and allowed to air dry. A calibrated spore solution was made from a 10 day old culture of *B. cinerea.* Fruit were then wounded with a sterile hypodermic needle to the depth of 3mm. Ripe fruits were inoculated with 5 µl of 1x10^5 spores. Mature green fruits, however, were inoculated with 5 µl of 1x10^6 spores. Total lesion diameter was then measured with digital Vernier callipers at 3 and 4 days post inoculation. Lesion sizes were then used for the calculation of the area under the disease progression curve (AUDPC); a method used in both epidemiology and resistance breeding for the calculation of disease progression, Figure 2, (Simko & Piepho, 2011). Statistical analysis was performed via one-way ANOVA in SPSS.

$$\mathrm{TCI} = \frac{2000(a)}{\sqrt{l(a^2 + b^2)}} \qquad \mathrm{AUDPC} = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} (t_{i+1} - t_i)$$

Figure 2: Formulae for the calculation of TCI and AUDPC. For TCI calculation l= lightness, a= red-green and b = blue-yellow. For AUDPC n= total number of

Results

The areas under the disease progression curve data were then evaluated using ANOVA in SPSS to highlight any differences in disease progression. For the ripe fruit homogeneity of variance could not be met p = 0.039; Levenes' test of homogeneity of variances. The Welch robust test for the equality of means was, therefore, performed giving result *F*(4,34.5) = 2.666, p = 0.044. Games-Howell post-hoc testing for non-homogenous data was performed and gave one significant difference between the control group and P24, p = 0.046. For the mature green tomatoes, again, homogeneity of variance could not be met, p = 0.006. The procedures stated above were followed. Welch testing gave a statistic of *F*(4,34.4) = 12.651, p = 0.000. All treatments were significantly different to the control group and

conventional, P8, P16 and P24 showed p values of 0.008, 0.028, <0.001, and <0.001, respectively. A statistically significant differences was also observed between the treatment P8 and P16 at p = 0.014. See Table 4 for results summary.

Table 4: The mean area under the disease progression curve (AUDPC) and standard deviation of fruit treated, at both ripe and mature green stage, with 3.7kJ/m2 of UVC from a low pressure mercury source and a varying number of pulses (P) from a pulsed source rich UV.

Stage	Control$^{(A)}$	3.7$^{(B)}$	P8$^{(C)}$	P16$^{(D)}$	P24$^{(E)}$
Ripe	40.62^{E} ±10.47	36.99 ±9.04	31.89 ±16.71	30.14 ±15.11	25.61^{A} ±15.70
Mature green	73.24^{BCDE} ±10.54	51.08^{A} ±18.98	59.87^{AD} ±11.72	41.95^{AC} ±15.33	42.49^{A} ±21.62

Superscript labelling indicates significant results, at the p< 0.05 level, and the group to which the difference was identified.

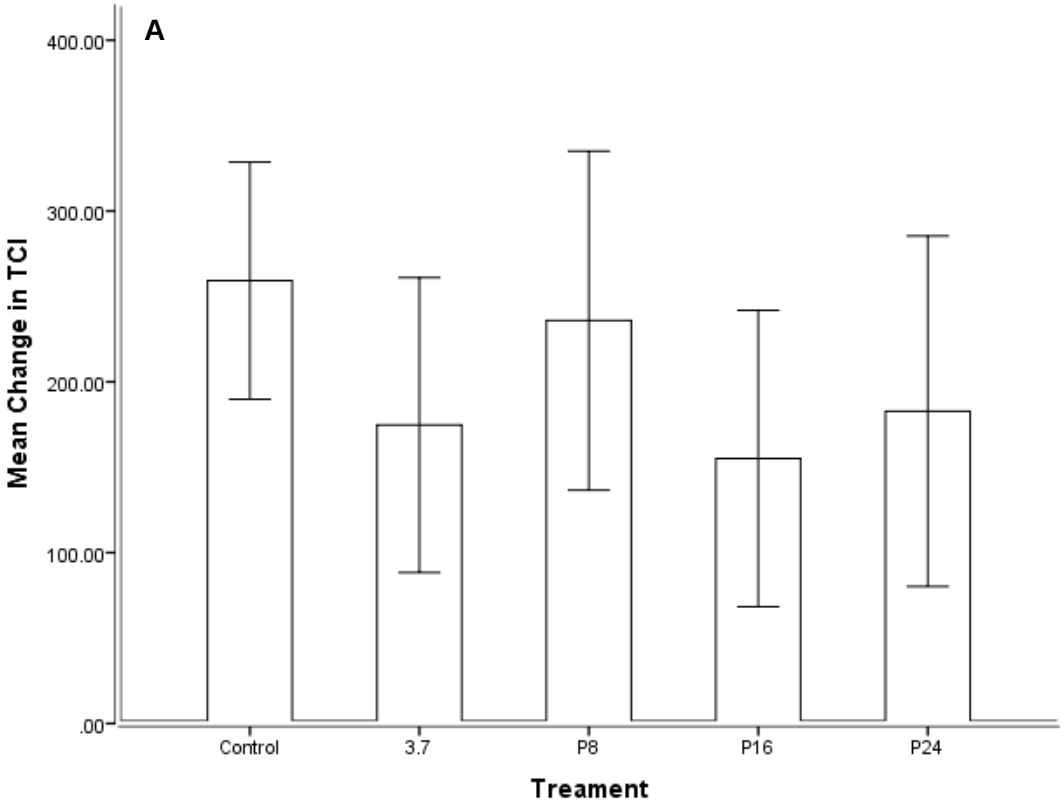
All treatments on both developmental stages of fruit showed reductions in disease progression. The greatest reductions were observed for the mature green fruit. The optimal treatment observed was P16 with a 43 % reduction in disease progression. P24, however, showed similar results with a 42 % reduction. The conventional treatment showed a 30 % reduction followed by P8 at 18 %. Ripe fruit showed a differing optimal treatment of P24 which showed a 37 % decrease in comparison to the conventional treatment with only a 9 % decrease in mean disease progression. P16 and P8 showed 26 and 22 % reductions.

ANOVA analysis was then performed for tomato colour index (TCI) data which identified a difference between treatment groups, $F(4,70) = 3.60$, $p = 0.01$, for the area directly facing the UV source. Post- hoc testing with Tukey's HSD identified only a single group, P16, which was significantly different from the control, $p = 0.018$. No significant difference was found in the change of TCI for measurements taken at 90° from the tissue directly facing the source $F(4,70) = 1.88$, $p = 0.124$.

Reductions in the change of TCI were observed for all treated groups for measurements taken directly facing the UV sources, Figure 3A. The smallest mean change in TCI was observed for P16 at 155.15 with a mean TCI at 10 days post treatment of 23.12. This was followed by the conventional and P24 treatments with observed mean changes of 174.73 and 182.78 and a TCI at 10 days post treatment of 46.99 and 52.56, respectively. P8

treatment showed the largest change in TCI for a treatment at 235.85 and a final TCI 104.31. Control fruits showed a mean change of 259.22 and a final TCI of 134.34. Conventional and 8, 16 and 24 pulse treatments showed a 32.60, 9.02, 41.15 and 29.49 % reduction in TCI change, respectively.

For readings taken at 90° from the tissue directly facing the source little difference was observed in the changes in TCI, Figure 3B. These were 267.51, 268.32, 271.85 and 257.10 for the control, conventional, P16 and P24 treatments. P8, however, showed a larger increase in the mean change in TCI at 326.86. Final TCI measurements at 10 days post treatment (DPT) were 141.03, 139.41, 138.23 and 126.88 for control, conventional, P16 and P24 treatments, respectively. The final TCI measurement for P8 was 182.00.



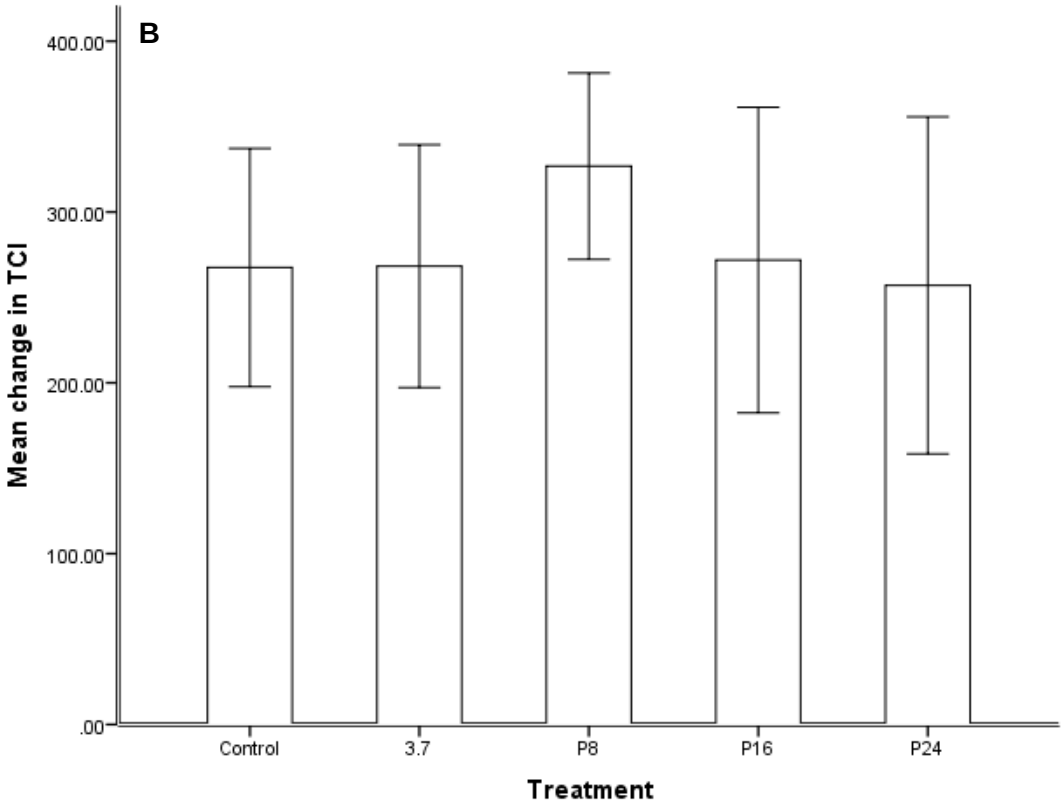


Figure 3: The mean change in the TCI, tomato colour index, of fruit from the commercial cv. Meccano. Measurements were taken prior to treatment and 10 DPT before inoculation with *B. cinerea*. Error bars show ± 1 standard deviation. **A)** The mean change in TCI from tissue directly facing the UV source. **B)** Tissue at 90° from that directly facing the source.

Discussion

Mature green fruit showed a reduction in disease progression, measured as area under the disease progression curve, of 30 % for the conventional treatment and 19 and 43 % for the P8 and P16 treatments, respectively. The P8 and P16 treatments equate to treatment times of 2.5 – 5 seconds, respectively, in comparison to the conventional treatment which, when delivered at 2000 µW/cm^2, is 185 seconds. Each treatment was repeated twice on each fruit through 180 ^{0} axial rotation of the fruit, and thus total treatment time is double of that stated. As the reduction in disease progression for the conventional source falls between that observed for P8 and P16 this equates to a reduction in treatment time of 97 – 99 %. The successful validation of the pulsed source on tomato fruit will aid with the commercial application of UV hormesis through the vastly reduced treatment times and also supports its extended application to pre-harvest foliar treatments in Objectives 2 and 3.

Objective 2 - Pre-harvest UV Treatment of Tomato

Introduction

To date the majority of laboratory experiments on the induction of UV hormesis have been focused on its application to preventing post-harvest spoilage of fruit. Post-harvest UV hormesis has shown vast potential applications on fruits with beneficial effects from reduced chilling injury and chlorophyll degradation to delayed ripening and disease resistance. The commercial application of such treatments have, however, been prevented due to the long exposure times; up to several minutes. In Objective 1 the use of a high intensity pulsed source was validated for use in the induction of UV hormesis which, in for delayed ripening and disease resistance on tomato, can reduce treatment time by 97- 99 %.

Recently the induction of disease resistance has been shown through whole plant UV treatments (Stefanato *et al.,* 2009, Kunz *et al.,* 2008, Reglinski *et al.,* 2013). Kunz *et al.,* 2008 showed UVC treatment of *Arabidopsis thaliana* at 0.5 kJ/m^2 reduced the disease severity of *Hyaloperonospora parisitica,* the causative agent of downy mildew, by approximately 84 %. Disease resistance was assayed at 1, 3 7 DPT and was shown to be most effective at 1 DPT. Moreover, Stefanato *et al.,* 2009, showed the induction of *B. cinerea* resistance, also on *A. thaliana*, through UVC treatment and induced production of the phytoalexin camalexin.

For *Diplodia pinea,* the causative agent of dieback on *Pinus radiata* incidence and susceptibility was also shown to be reduced following UVC treatment of 1.2 kJ/m^2 (Reglinski *et al.,* 2013). Single treatments were performed either 1, 3 or 6 weeks before inoculation

with treatment 1 week before inoculation showing the greatest resistance. Multiple treatments at 6, 3 and 1 week before inoculation showed the greatest reduction in disease incidence and severity. The application of pre-harvest UV hormesis through foliar treatments has, however, with the exception of Reglinski *et al.,* 2013 not been explored with horticultural relevance. Pre-harvest UV induced resistance has, however, been shown to induce disease resistance and systemic delayed ripening through the treatment of fruit on the truss (Obande *et al.*, 2011).

The aim here is to explore the use of conventional and pulsed UV sources as inducers of disease resistance through the foliar treatments of tomato. Initially the point of visible damage will be determined. Where visible damage is not evident treatments will be assayed for disease control against a number of diseases including the pathogens *B. cinerea, Passalora fulva, Oidium neolycopersici,* tomato mosaic virus and tomato spotted wilt virus. Fungal pathogens will be used for initial resistance assays. Following initial observations of resistance the length and periods of optimal resistance will be explored. This will then be used to determine optimal treatment plans and the applicability of the respective sources within a horticultural setting.

Materials and methods

All plants were grown under glass at The University of Nottingham's Sutton Bonington Campus. Tomatoes were germinated and grown for approximately 1 month in Levington® M3 Pot and Bedding High Nutrient compost in 50 mm propagation trays. Plants were grown under a 16 hr minimum photoperiod with venting above 18 °C. Plants were re-potted as necessary. For damage assessment conventional treatments were performed between 5 and 1 kJ/m^2 in 1 kJ/m^2 increments delivered at 2000 µW/cm for plants at the 4-5 leaf and 7-10 leaf stage of the cv. Shirley. Symptoms were observed visually at 2 DPT and a simple qualitative assessment for the presence or absence of disease was performed. The second round of damage observations were performed on plants at the 7-10 leaf stage at 1, 0.5 and 0.1 kJ/m^2. Pulsed UV treatments were carried out at 20 cm from the distal leaf of the plant between 5 and 45 pulses in 5 pulse increments at the 7-10 leaf stage.

Results

Physical damage was observed for cv. Shirley at all treatments above 1 kJ/m^2 from the conventional source on plants at both the 4 - 5 and 7 - 10 leaf stage. For plants at the 7 - 10 leaf stage damage was observed at 0.5 but not at 0.1 kJ/m^2, see Table 5 for summary of results. The pulsed source showed damage at 20 pulses and above for plants at the 7 - 10 leaf stage, see Table 6.

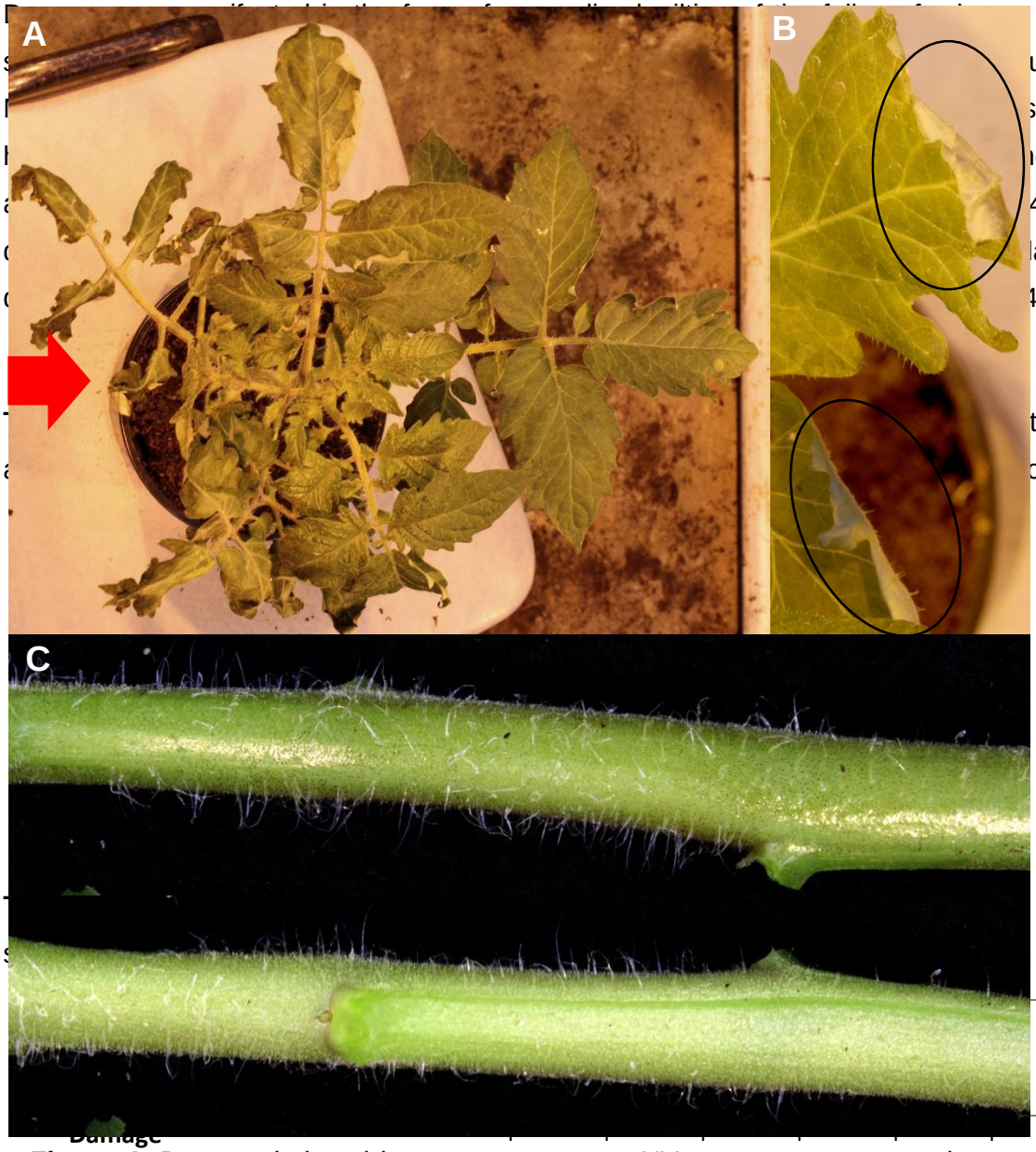


Figure 4: Damage induced by over exposure to UV sources on tomato plants cv. Shirley. **A)** The influence of source positioning on damage elicited to the plant and example of heavy damage. Red arrow indicates the side of the plant closest to the source. **B)** The mild leaf curling symptoms that develop at the lower exposure treatments that cause damage. **C)** Two sides of a treated plants stem. The top side was facing the source and shows distortion of trichromes on the stem and “shiny” appearance. The bottom stem was facing away from the source.

Discussion

Damage was observed on 7 - 10 leaf tomato plants of the cv. Shirley at treatments above 0.5 kJ/m^2 from the low pressure mercury source when delivered at 2000µW/cm^2. Pulsed treatments were damaging from 20 pulses and above when delivered from 20 cm. It should, therefore, be considered that any truly hormetic exposure will be lower than that for which obvious visual symptoms of damage are observed. Damage exhibited itself in a similar manner from both sources with wilting of both leaves and petioles. Glossy appearances on the leaf and stem surface were also observed and were accompanied by damage to the trichromes.

Horticulturally relevant resistant assays were attempted but the failure to achieve disease on a number of occasions, inability to fully control humidity within the glasshouse environment, scale of the experiments required and laborious methods deemed them inappropriate for the timescale of the project. Bioassays were trialled including stem- and leaf- based assays. Stem bioassays showed signs of induced resistance induction the nature of disease progression, however, was unnatural and did not lead to the formation of stem lesions. Moreover, it did not allow a scale measurement of disease progression. Leaf based bioassays are now being developed for the monitoring resistance.

Preliminary results from in situ resistance assays gave valuable information on the point of induced resistance against *B. cinerea.* Resistance was observed at both 0.2 and 0.4 kJ/m^2 and at 5, 10 and 15 pulses with 0.2 kJ/m^2 and 10 pulses showing the greatest reductions in disease. Data obtained from these preliminary results can now be extrapolated into the leaf based resistance assays which will allow the faster completion of work as multiple pathogens can be assayed for resistance at once.

To conclude, UV treatments from both the pulsed and conventional source show a promising ability for inducing disease resistance against *B. cinerea.* The work will be continued through the investigation into optimal dose and also the longevity of the protection for each of the pathogens under study. The study will then be replicated on a second cultivar of tomato cv. Moneymaker due to its previous use as a commercial cultivar and similar physiology to those currently being used without the broad range of pathogen resistance observed for modern commercial cultivars

Objective 3 – Pre-harvest UV Treatment of Lettuce

Introduction

Until recently the focus of UV research on lettuce has been twofold with postharvest applications for extension of shelf life and surface decontamination of minimally processed lettuce and pre-harvest research into the effects of restoring natural UV levels through the use of UV-permeable housing for crops grown under protection (Allende & Artes, 2003, Allende *et al.,* 2006, Tsormpatsidis *et al.,* 2008). The former was mainly concerned with Enterobacteria associated with human pathology but did show a reduction in *Erwinia carotovora* a soft rot causing phytopathogen (Allende *et al.,* 2006). The results, however, do not mitigate the direct germicidal effects of UVC, as only natural microbial populations were monitored, and induced resistance cannot be inferred.

Research on the use of UV permeable sheeting and supplementary UVB lighting for protected lettuce crops has shown a number of induced effects such as the production of a more compact plant, reduction in biomass, changes in colouration and a reduced incidence of diseases caused by *Bremia lactucae* and *B. cinerea* (Paul *et al.,* 2012, Wargent *et al.,* 2005). Park *et al.,* 2007 treated lettuce with 1.65 kJ/m^2 of UVB per day for 10 days and observed that an increase in red colouration correlated with accumulation of anthocyanins.

Recently, UVC induced disease resistance has been shown on lettuce by Ouhibi *et al.,* 2014. A treatment of 0.85 kJ/m^2 gave post-harvest resistance against *B. cinerea* and *Sclerotinia minor* with 20 and 34 % reductions in lesion size at 4 DPI, respectively. One would expect the application doses shown to be similar for both pre and post-harvest treatments.

Here, it is intended to extrapolate and build upon this data to show the scope and longevity of the protection from two contrasting UV sources; a low pressure mercury source and a high intensity pulsed source. Resistance against *B. cinerea, Rhizoctonia solani, B. lactucae, Sclerotinia sclerotiorum,* lettuce big vein and tomato spotted wilt viruses will be tested. The longevity and optimal resistance for each of the pathogens will be used to calculate potential treatment plans for use within commercial settings.

Materials and methods

Lettuce were germinated in rockwool propagation cubes until emergence of their first true leaves and then transferred to an NFT system under natural light conditions. Day and night temperatures were 12 - 14 °C and 2 - 6 °C, respectively. Vents were opened above 10 °C in the evening and 4 °C during the day. Lettuce of the commercial cultivar Amica were subjected to treatment with both pulsed and conventional sources. Pulsed treatments were

delivered from 40 cm and conventional treatments were delivered at 2000 µW/cm^2 from directly above the lettuce plant. Treatments were performed at both 3-5 true leaf stage and early head formation for the pulsed source and only the former for the conventional. Damage was visually inspected at 5 DPT and recorded qualitatively as simply the presence or absence of damage.

Results

Damage was found to be induced by high intensity pulsed treatments greater than 45 pulses at both the early head formation and 3 - 5 leaf stages, see Table 7. Treatments above 2.25kJ/m^2 from the low intensity mercury source at the 3-5 leaf stage, Table 8. Mature leaves showed the greatest susceptibility to damage which manifested itself as dry brown lesions, Figure 5. Damage from lower exposure levels could be observed as vascular discoloration observed as a yellow/brown hue, Figure 6.

Table 7: Summary of treatments from the low pressure mercury UVC source and their ability to cause damage on lettuce cv. Amica at the 3-5 true leaf stage

Treatment (kJ/m^2)	**0.75**	**1.5**	**2.25**	**3**	**3.75**	**4.5**	**5.25**
Damage	-	-	+	+	+	+	+

Table 8: Summary of treatments from the high intensity pulsed source and their ability to cause damage on lettuce cv. Amica at the 3-5 true leaf stage and early head formation

No. Pulses	**15**	**30**	**45**	**60**	**75**	**90**	**105**
3-5 leaves	-	-	+	+	+	+	+
Early head formation	-	-	+	+	+	+	NT

Figure 5: A lettuce, cv. Amica, at early head formation treated with 75 pulses of high intensity UV exhibiting severe damage to its mature leaves which is manifested as dry brown lesions.

Figure 6: A lettuce from a plant at early head formation treated with 45 pulses showing veins with a yellow/brown hue as a symptom of mild damage caused

Discussion

Damage was induced by the conventional source above 2.25 kJ/m^2 when delivered at 2000 µW/cm^2 and 45 pulses from the high intensity source from 40cm. This is manifested as dry brown lesions and vascular discolouration, see Figure 5 and 6. In preliminary results, during bioassay development, a 15 % reduction in *B. cinerea* lesion development from a 20 pulse treatment was observed. Conventional treatments have yet to be assayed for resistance. Their success has previously been shown, however, as a post-harvest treatment by Ouhibi *et al.,* 2014. This was achieved by a treatment of 0.85 kJ/m^2 which is supported by our observation that damage is caused above 2.25 kJ/m^2.

A leaf bioassay has been developed, with amendment, based on the method of Laboh, 2009 for the inoculation of *B. cinerea*. This assay has the potential to be further adjusted for use with *B. lactucae, R. solani* and *S. sclerotiorum*. Its suitability for the use with viral pathogens has yet to be established.

To conclude, preliminary results obtained here for the induction of disease resistance with the pulsed source and the methods published by Ouhibi *et al.,* 2014 will be extrapolated and used to further study the spectrum of pathogens that UV provides protection against. The longevity of disease resistance will also be examined. This work will be supported through investigations into the nature of UV induced resistance in lettuce.

Conclusions

Post-harvest fruit treatments:

- The high intensity, pulsed UV source was shown to successfully induce both disease resistance and delayed ripening on tomato fruit of the cv. Meccano.
- The pulsed source gave a 97-99 % reduction in treatment time when achieving similar levels of induced resistance in comparison to a conventional UVC source.

Pre-harvest foliar treatments:

- The point at which damage is inflicted for both tomato and lettuce crops has been determined for both pulsed and conventional sources at two contrasting developmental stages.
- Preliminary work indicates the successful induction of resistance against *B. cinerea* on both tomato and lettuce with both the conventional and pulsed UV sources.

Future work:

- Longevity of induced resistance and the impact of repeated treatments on an array of further pathogens on both tomato and lettuce.
- Assess the potential as a curative treatment.
- Effect of treatment on physiological and consumer properties of the plant.

Knowledge and Technology Transfer

Project meetings:

- Initiation meeting, Sutton Bonington, 16th March 2015

Conferences:

- Molecular Biology of Plant Pathogens, UWE, poster presentation, 9th April 2015

References

Allende, A., & Artés, F. (2003). UV-C Radiation as a Novel Technique for Keeping Quality of Fresh Processed “Lollo Rosso” Lettuce. Food Research International 36:739–746.

Allende, A., McEvoy, J. L., Luo, Y., Artes, F., & Wang, C. Y. (2006). Effectiveness of Two-Sided UV-C Treatments in Inhibiting Natural Microflora and Extending the Shelf-Life of Minimally Processed “Red Oak Leaf” Lettuce. Food Microbiology 23:241–249.

Ben-Yehoshua, S., Rodov, V., Kim, J.J. & Carmeli, S. (1992). Preformed and Induced Antifungal Materials of Citrus Fruits in Relation to the Enhancement of Decay Resistance by Heat and Ultraviolet Treatments. Journal of Agricultural and Food Chemistry 40:1217–1221.

Charles, M.T., Goulet, A. & Arul, J. (2008). Physiological basis of UV-C induced resistance to *Botrytis cinerea* in tomato fruit. IV. Biochemical modification of structural barriers. Postharvest Biology and Technology 47:41–53.

Charles, M.T., Tano, K., Asselin, A., & Arul, J. (2009). Physiological Basis of UV-C Induced Resistance to *Botrytis cinerea* in Tomato Fruit. V. Constitutive Defence Enzymes and Inducible Pathogenesis-Related Proteins. Postharvest Biology and Technology 5:414–424.

Costa, L., Vicente, A.R., Civello, P.M., Chaves, A.R. & Martinez, G.A. (2006). UV-C treatment delays postharvest senescence in broccoli florets. Postharvest Biology and Technology 39:204–210.

D'Hallewin, G., Schirra, M., Manueddu, E., Piga, A. & Ben-Yehoshua (1999). Scoparone and Scopoletin Accumulation and Ultraviolet-C Induced Resistance to Postharvest Decay in Oranges as Influences by Harvest Date. Journal of the American Society for Horticultural Science 124:702-707.

Eicholz, I., Huyskens-Keil, S., Keller, A., Ulrich, D., Kroh, L.W., et al. (2011). UV-B-Induced Changes of Volatile Metabolites and Phenolic Compounds in Blueberries (*Vaccinium corymbosum* L.). Food Chemistry, 126:60–64.

Kunz, B.A, Dando, P.K., Grice, D.M., Mohr, P.G., Schenk, P.M. et al. (2008). UV-Induced DNA Damage Promotes Resistance to the Biotrophic Pathogen *Hyaloperonospora parasitica* in *Arabidopsis*. Plant Physiology 148:1021–1031.

Laboh, R. (2009). Control of Botrytis cinerea on Lettuce (*Lactuca sativa* L.) Using Biocontrol and Transgenes. Thesis (The University of Nottingham).

Nigro, F., Ippolito, A. & Lima, G. (1998). Use of UV-C Light to Reduce Botrytis Storage Rot of Table Grapes. Postharvest Biology and Technology 13:171–181.

Obande, M.A., Tucker, G.A. & Shama, G. (2011). Effect of Preharvest UV-C Treatment of Tomatoes (*Solanum lycopersicion* Mill.) on Ripening and Pathogen Resistance. Postharvest Biology and Technology 62:188-192.

Ouhibi, C., Attia, H., Nicot, P., Urban, L., Lachaal, M., et al. (2014). Effect of UV-C Radiation on Resistance of Romaine Lettuce (*Lactuca sativa* L.) Against *Botrytis cinerea* and *Sclerotinia minor*. Journal of Phytopathology 163:578-582.

Park, J.S., Choung, M.G., Kim, J.B., Hahn, B.S., Kim, J.B., et al. (2007). Genes Up-Regulated During Red Coloration in UV-B Irradiated Lettuce Leaves. Plant Cell Reports 26:507–516.

Paul, N.D., Moore, J.P., McPherson, M., Lambourne, C., Croft, P., et al. (2012). Ecological

Responses to UV Radiation: Interactions between the Biological Effects of UV on Plants and on Associated Organisms. Physiologia Plantarum 145:565–581.

Petit, A.N., Bailieul, F., Vaillant-Gaveau, N., Jacquencs, L., Conreux, A., et al. (2009). Low Responsiveness of Grapevine Flowers and Berries at Fruit Set to UV-C Irradiation. Journal of Experimental Botany 60:1155–1162.

Pombo, M.A., Rosli, H.G., Martinez, G.A. & Civello, P.M. (2011). UV-C Treatment Affects the Expression and Activity of Defense Genes in Strawberry Fruit (*Fragaria* × *ananassa*, Duch.). Postharvest Biology and Technology 59:94–102.

Pongprasert, N., Sekozawa, Y., Sugaya, S. & Gemma, H. (2011). The Role and Mode of Action of UV-C Hormesis in Reducing Cellular Oxidative Stress and the Consequential Chilling Injury of Banana Fruit Peel. International Food Research Journal 18:741–749.

Ranganna, B., Kushalappa, a. C. & Raghavan, G.S.V. (1997). Ultraviolet irradiance to control dry rot and soft rot of potato in storage. Canadian Journal of Plant Pathology 19:30–35.

Reglinski, T., Taylor, J. T., Ah-Chee, A., Northcott, G., & Spiers, M. (2013). Biochemical Responses to Ultraviolet-C Radiation and Methyl Jasmonate in *Pinus radiata* Seedlings that Accompany Induced Resistance to *Diplodia pinea*. Plant Pathology 62:851–858.

Shama, G. & Alderson, P. (2005). UV Hormesis in Fruits: A Concept Ripe for Commercialisation. Trends in Food Science and Technology 16:128–136.

Stefanato, F.L., Abou-Mansour, E., Buchala, A., Kretschmer, M., Mosbach, A., et al. (2009). The ABC Transporter BcatrB from *Botrytis cinerea* exports Camalexin and is a Virulence Factor on *Arabidopsis thaliana*. Plant Journal 58:499–510.

Stevens, C., Khan, V.A., Lu, J.Y., Wilson, C.L., Pusey, P.L., et al. (1997). Integration of Ultraviolet (UV-C) Light with Yeast Treatment for Control of Postharvest Storage Rots of Fruits and Vegetables. Biological Control 10:98–103.

Stevens, C., Liu, J., Khan, V.A., Lu, J.Y., Wilson, C.L., et al. (1998). Application of Hormetic UV-C for Delayed Ripening and Reduction of *Rhizopus* Soft Rot in Tomatoes:

The Effect of Tomatine on Storage Rot Development. Journal of Phytopathology 146:211–221.

Tsormpatsidis, E., Henbest, R.G.C., Davis, F.J., Battey, N.H., Hadley, P. et al. (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.

Vicente, A.R., Pineda, C., Lemoine, L., Civello, P.M., Martinex, G.A., et al. (2005). UV-C Treatments Reduce Decay, Retain Quality and Alleviate Chilling Injury in Pepper. Postharvest Biology and Technology 35:69–78.

Wargent, J.J., Taylor, A. & Paul, N.D. (2005). UV Supplementation for Growth Regulation and Disease Control. ISHS Acta Horticulturae 711:333-338.

