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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.

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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

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GROWER SUMMARY

Headline

- Post-harvest treatments of tomato fruit with a high intensity, pulsed polychromatic light source (HIPPL), rich in UV-C, show disease control against *Botrytis cinerea* and delayed ripening through delayed colour and texture changes.
- Treatment time is reduced by 97.3% for the HIPPL source in comparison to a conventional low intensity UV-C (LIUV) source.
- Post-harvest HIPPL and LIUV treatments of tomato fruit control disease against *Penicillium expansum* on ripe tomatoes.
- The molecular mechanisms underpinning HIPPL and LIUV hormesis on tomato fruit are extremely similar. Disease control is achieved through induced resistance. Down-regulation of genes involved in ethylene biosynthesis enzyme (*ACO1*) and polygalacturonase is observed. Increased expression of jasmonic acid (*OPR3*) and salicylic acid (*P4*) biosynthesis enzymes and markers are observed. Up-regulation of a pathogenesis related proteins (*CHI9* and GLUB) is also observed.
- Post-harvest HIPPL and LIUV treatments of tomato fruit elicit a local response when fruit are treated from either the blossom end, calyx or side. Full surface exposure is, therefore, required.
- The HIPPL induced resistance and delayed ripening on tomato fruit is not solely due to UV-C. UV-C emissions, however, account for approx. 50 % of the observed induced resistance and delayed ripening.
- Pre-harvest HIPPL and LIUV treatments showed the potential to control *Botrytis cinerea* on lettuce (cv. Temira) in a lighting and temperature controlled environment; reducing disease progression by 21.4 and 21.0 %, respectively.
- LIUV treatment of tomato seeds decreased disease progression and disease incidence of *B. cinerea* on flowering plants by approx. 10 %.
- Biostimulation of seedling growth is observed following LIUV treatment of tomato seeds. Biostimulation is observed for both roots and shoots. Root growth, however, is stimulated to a greater extent.

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-C hormesis have been known for nearly 30 years. A broad range of benefits are observed from increased nutritional content to disease resistance and reduced chlorophyll degradation. To date, the

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majority of studies have been performed using conventional low pressure, low intensity UV-C (LIUV) sources on post-harvest produce. Commercial application of these treatments has, in part, been prevented due to the lengthy exposure times that are required: conventional treatments of tomato fruit take in excess of six minutes. High intensity, pulsed polychromatic light sources (HIPPL), rich in UV-C, however, have been developed which hold the potential of drastically reducing treatment times and making such treatments 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.

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 LIUV and HIPPL treatments to induce resistance on both tomato and lettuce crops. Utilisation of such treatments in commercial situations may allow an alternative 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 Polychromatic Light Source as an Inducer of Hormesis on Tomato Fruit

Tomato fruit of the cv. Mecano were treated at both the mature green and ripe stage. An established LIUV treatment was performed alongside a number of HIPPL treatments. This was to allow a comparison of the sources' ability to induce both disease resistance against *Botrytis cinerea* and *Penicillium expansum,* and delay ripening. Both LIUV and HIPPL sources successfully controlled disease, to comparable levels, against *B. cinerea* on mature green fruit following artificial inoculation. Disease progression on ripe fruit, for *B. cinerea* and *P. expansum*, was inhibited to a greater extent by the HIPPL source. Furthermore, ripening as measured through both colour change and texture, was delayed by the HIPPL source to comparative levels to that observed for the LIUV source.

Both ripe and mature green fruit showed optimal HIPPL treatments of 16 pulses giving a total treatment time of 10 seconds yielding a 97.3 % reduction in treatment time in comparison to the LIUV treatment. The ability to induce resistance to *B. cinerea* at both the mature green and ripe stages shows that post-harvest HIPPL treatment could be adopted by growers who harvest at differing fruit maturities. The majority of previously published research was focused on fruit at the mature green stage.

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Objective 2 – Comparing the Molecular Mechanisms Underpinning LIUV and HIPPL Hormesis on Tomato Fruit

Utilising quantitative PCR we have found that the molecular mechanisms leading to induced resistance and delayed ripening for both the LIUV and HIPPL source are extremely similar. Both sources show an upregulation of both salicylic acid and jasmonic acid biosynthesis enzymes or markers. Furthermore, 24 hours after treatment a transient peak in ethylene biosynthesis enzyme *AC01* is observed. At 10 days after treatment and 12 hours after inoculation with *B. cinerea,* however*,* a reduction in *ACO1* is seen. The upregulation of pathogenesis related (PR) proteins, involved in the plant's defence response, is observed for both LIUV and HIPPL treatments. Interestingly upregulation of PR protein transcripts, associated with defence against biotrophic and necrotrophic pathogens and plant pests, was observed. This may indicate that LIUV and HIPPL treatments can protect against a wide range of pathogens and pests. Finally, polygalacturonase was downregulated and changes to secondary metabolism were observed. These include downregulation of flavonols and upregulation of carotene-hydroxylase and phenylalanine ammonia lyase.

Objective 3 - Assessing the Importance of Direct Tissue Exposure and Fruit Orientation during LIUV and HIPPL Treatment

Multiple treatment orientations were attempted including treatments from the side, blossom end and calyx. Both the LIUV and HIPPL induced disease resistance and delayed ripening are local responses in tomato fruit. Fruit would, therefore, require full surface exposure.

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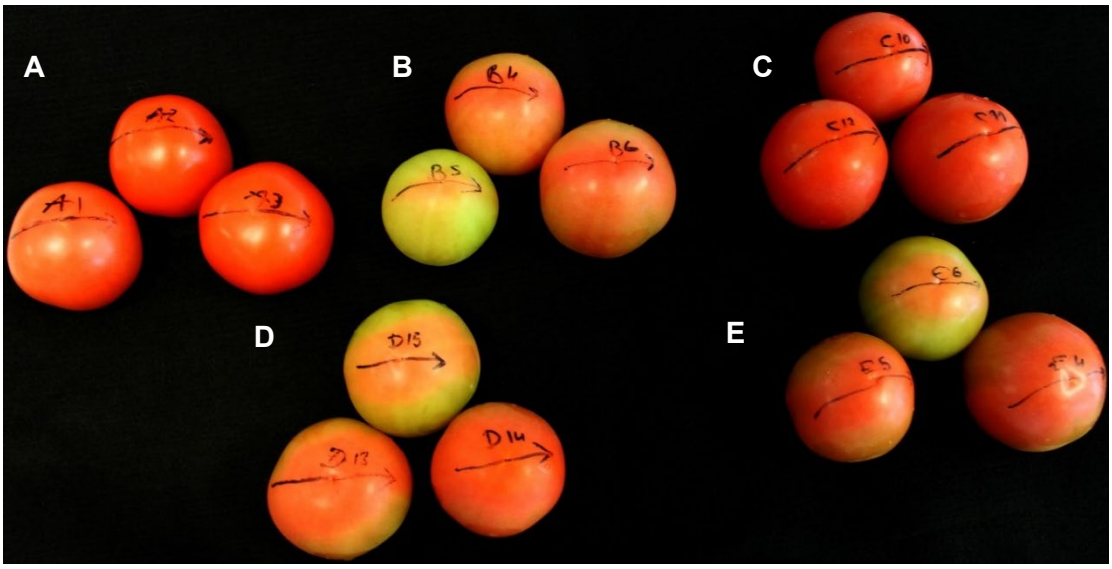

A representative sample from the fruits treated post-harvest showing: **A)** Control fruit. **B)** Conventional treatment with the low intensity UV-C (LIUV) source. **C)** An 8 pulse high-intensity, pulsed polychromatic light (HIPPL) treatment. **D)** A 16 pulse HIPPL treatment and **E)** A 24 pulse HIPPL 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. (Scott *et al.,* 2017)

Objective 4 - Assessing the Importance of UV-C, B and A and visible light within the High Intensity Pulsed Polychromatic Light Source, for Inducing the Hormetic Effects Observed on Tomato Fruit cv. Mecano

HIPPL treatments were performed with or without UV-C filtering glass. Disease resistance and colour progression was delayed both with and without UV-C. Disease resistance and delayed ripening without the presence of UV-C, however, were reduced by approximately 50 %. This indicated that although UV-C is not essential to maintain such short treatment times, UV-C is required to achieve the full benefits of treatment.

Objective 5 – Pre-harvest Foliar LIUV and HIPPL Treatments of Lettuce

Foliar LIUV and HIPPL treatments of lettuce were performed on two commercial butterhead varieties, Amica and Temira, grown in a temperature-controlled glasshouse with assimilation lighting during the winter months. Damage assessments and disease control bioassays were carried out at the 3-5 true leaf and early, mid and late head formation developmental stages. No conclusions could be drawn from the data.

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Objective 6- Low-Dose Foliar LIUV and HIPPL Treatments of Lettuce

To avoid any unwanted damage to crops, low dose LIUV and HIPPL treatments, which were shown to not be damaging at any point during the year were tested. Unfortunately, both single and multiple applications of such low dose treatments were prone to variation. It was, therefore, decided that experiments should be performed in a controlled environment.

Objective 7- LIUV and HIPPL Treatments of Lettuce in a Controlled Environment

Lettuce plants of the cvs. Amica and Temira were grown in a light and temperature controlled environment with no natural lighting. Plants were grown to the 8-true leaf stage and then treated with either HIPPL or LIUV. Plants were assessed for damage and then inoculated with *B. cinerea* using a leaf disc bioassay on the second day following treatment. Amica plants were more susceptible to damage from both the LIUV and HIPPL source. Only Temira showed statistically significant levels of disease control with the 0.64 kJ/m^2 LIUV and a 48-pulse HIPPL treatments reducing disease progression by 21.0 and 21.4 %, respectively. Further investigation is required.

Objective 8- LIUV Seed Treatments of Tomato to Control *B. cinerea*

Seeds were treated with either 0, 2, 4 or 6 kJ/m^2 LIUV. Inoculations were performed on the plant through the application of a calibrated spore solution onto a petiole stub. All treatments reduce disease progression on flowering plants. The 4 kJ/m^2, however, was shown to be the most successful and statistically significant with a reduction in both disease incidence and progression of approx. 10 %.

Objective 9-Effects of LIUV Tomato Seed Treatment on Germination and Early Seedling Growth

To determine any potential detrimental effects of UV-C, germination and early plant development and growth were monitored. The 4 kJ/m^2 treatment was used along with two higher treatments of 8 and 12 kJ/m. Interestingly, we observed biostimulation of seedling growth following the 8 kJ/m treatment. Germination speed and synchronicity was increased along with a significant increase in root, hypocotyl, and cotyledon dry mass. Furthermore, no differences were observed in root or hypocotyl length indicating an increase in volume. A significant increase in root mass fraction was also observed for the 8 kJ/m^2 treatment

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indicating that root growth is stimulated to a greater extent than that of shoot growth. This may lead to increased efficiency in water and nutrient uptake, further investigation is required. Moreover, biostimulation of root growth does not appear to negatively impact the shoots where a significant increase in dry mass was also observed.

Financial Benefits

Calculation of financial benefits is not possible at this time.

Action Points

There are no immediate action points.

SCIENCE SECTION

Objective 1 - Validation of the High-Intensity Pulsed Polychromatic UV-C Source

Introduction

UV-C hormesis is a dose response phenomenon where small doses of UV-C bring about a positive reaction in the target organism. The positive effects of UV-C on fresh produce have been known for over 30 years and have shown to be effective on orange, strawberry and sweet potato to mention a just a few species (Ben-Yehoshua *et al.,* 1992; Ranganna *et al.,* 1997; Shama & Alderson, 2005; Pombo *et al.,* 2011). The effects include a wide range of responses including pathogen resistance, delayed senescence, delayed ripening, increased nutritional content and reduced chilling injury (Stevens *et al.,* 1998; Costa *et al*,. 2006; Charles *et al.*, 2008; 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 of investigations have relied on initiation of disease through natural inoculation 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-C induced effects alone. This is because the direct effect of UV-C on the

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inoculum, which may be present on the fruit surface during treatment, cannot be accounted for.

There are a number of studies whose experimental design allow the quantification of resistance induced by UV-C hormesis. As with other elicitors of induced resistance UV-C 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 (Ben-Yehoshua *et al.,* 1992; Charles *et al.,* 2008). 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 (Stevens *et al.,* 1997; Stevens *et al.,* 1998; D'Hallewin *et al.,* 1999; Vicente *et al.,* 2005; Petit *et al.,* 2009).

UV-C induced disease resistance is achieved in tomato 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, which 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 to absorb potentially damaging wavelengths of light, but also as antioxidants. 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-C treatment. These genes can include those involved directly in challenging pathogens such as chitinases but also those involved in defence signalling pathways.

UV-C treatments to date have been focused primarily on the use of UV-C 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 polychromatic light source, rich in UV-C, 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.

Aims

The aims of this study were to investigate whether HIPPL sources were able to delay colour and texture changes during ripening and induce resistance against *B. cinerea* on mature

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green tomato. Treatments were also conducted with a LIUV source as a basis for comparison. Additionally, treatments using both types of source, HIPPL and LIUV, were conducted to assess their ability to induce disease resistance against *B. cinerea* and *Penicillium expansum* on red-ripe fruit, as an increasing number of tomato growers are harvesting at this stage due high consumer demand.

Materials and Methods

Fruit Production

Tomato fruit, cv. Mecano, were grown in the commercial glasshouse at APS Salads (UK), picked at the mature green developmental stage and delivered at ambient temperature to the University of Nottingham within 24 h of harvesting. Fruit were sorted to remove fruit showing deviation from the desired developmental stage, size deviations or surface damage.

Colour and Texture Analysis

Mature green fruit were measured with a calibrated CR-200 Chroma meter (Konica Minolta, UK) 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 DPT. This was used to calculate the change in tomato colour index (TCI) over 10 days. Fruit firmness was measures with a TA.XT plus texture analyser (Stable Micro Systems, UK)

Low Intensity UV-C and High-Intensity, Pulsed Polychromatic Light Treatment

An established conventional LIUV treatment of 3.7 kJ/m^2 delivered at 20 W m^2 was used as a benchmark to assess the efficacy of induced disease resistance from the pulsed source (Charles *et al.,* 2008a). Fruit were positioned 10 cm from the pulsed source and treated with a range of pulses. Through extrapolation of the manufacturer's data an estimated 4.6 kJ/m^2/pulse was delivered at fruit level.

For both sources, fruit received exposure on two sides through 180° axial rotation. Following treatment, fruit were immediately stored in the dark until sterilisation. For sterilisation, tomatoes were immersed in 2 % Sodium hypochlorite (Sigma-Aldrich, UK) for approximately 5 – 10 seconds to prevent growth of naturally occurring microorganisms during the incubation period. Fruit were then rinsed three times in sterile distilled water (SDW), dried and immediately incubated in the dark at 13 °C to prevent photoreversal. Fruit were stored in

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humidity boxes lined with damp paper and raised by a double layer of plastic mesh at≥ 98 % RH (figure 1.1). At 10 days after treatment fruit were inoculated to allow for the induction of defence responses; this was shown to be the optimum point of UV-C induced disease resistance by Charles *et al.,* 2008a.

Figure 1.1: An example of treated fruit in a humidity box with moist tissue lining and plastic mesh raising tomatoes from direct contact with the tissue paper.

Pathogen Maintenance, Spore Preparation and Inoculation

A *Botrytis cinerea* culture, originally isolated from a plant of the genus *Rosa*, was supplied from The University of Nottingham's collection. Cultures were grown at room temperature on potato dextrose agar (Sigma-Aldrich, UK) supplemented with Penicillin G sodium salt (Sigma-Aldrich, UK) at 33 mg/L and Streptomycin sulphate salt (Sigma-Aldrich, UK) at 133 mg/L. A calibrated spore solution was made from 10 day old cultures 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 $1x10^5$/ml spores. Green fruits, however, were inoculated with 5 µl of $1x10^6$ spores due to decreased levels of susceptibility observed in preliminary work. Total lesion diameter including all sunken lesions, splitting and tissue maceration was measured with digital Vernier callipers at 3 and 4 DPI. For *Penicillium expansum* inoculations on ripe fruit a culture was also obtained from The University of Nottingham and cultured as stated for *B. cinerea.* Spores

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were isolated from 7 day old cultures and fruit were inoculated with 5 µl of spores at a concentration of $1x10^6$/mL.

Experimental Design and Data Analysis

All data presented here were collected from two independent replicate experiments. For experiments concerned with tomato colour change and *B. cinerea* disease resistance, fifteen fruit per treatment group per replicate experiment were used. For experiments concerned with *Penicillium expansum* resistance and texture analysis, 10 fruits per treatment group per experiment were used.

Tomato colour measurements were transformed into the tomato colour index (TCI) and the first reading was subtracted from the second to calculate change in TCI and therefore ripening progression (Lopez Camelo & Gomez, 2004; Corcuff, *et al.,* 2012; Hobson, 1987). The formulae for calculating TCI can be found in equation 1.1. Lesion size measurements were 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 (Eq. 1.2).

$$\mathrm{TCI} = \frac{2000(a)}{\sqrt{L(a^2 + b^2)}}$$

Equation 1.1: Tomato colour index (TCI) formula where L= lightness, a= red-green and b = blue-yellow values (Hobson, 1987).

$$\mathrm{AUDPC} = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} (t_{i+1} - t_i)$$

Equation 1.2: Area Underneath the Disease Progression Curve formula where n= total number of observations, i= observation, y= disease score and t= time (Jeger and Viljanen-Rollinson, 2001).

Statistical analysis was performed using statistical software package SPSS 22 (IBM). One-way ANOVA with Tukey's post-hoc testing was performed. Where the homogeneity of variances assumption could not be met, Welch's robust ANOVA was performed followed by the Games-Howell post-hoc test. Statistical significance is here defined as $p \leq 0.05$. Dates for

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experimental replicates can be found in table 1.1. Data from independent experimental replicates can be found in appendix 1.

Table 1.1: Experimental replicate dates for investigations on the validation of the pulsed polychromatic light source as an inducer of hormesis.

Experiment	Replicate	Date
Disease resistance on mature green fruit	1	22nd May 2015
	2	25th August 2015
Disease resistance on red ripe fruit	1	25th August 2015
	2	1st December 2015
Delayed ripening measured by colour change	1	22nd May 2015
	2	25th August 2015
Testing the necessity for direct tissue exposure for resistance	1	6th October 2015
	2	3rd November 2015

Results and discussion

Delayed Ripening

The induction of delayed ripening in mature green tomatoes is an established beneficial effect following hormetic UV-C treatment (Stevens et al., 1998a; Corcuff et al., 2012). Furthermore, colour is the key external indicator for ripening progression on tomato fruit (Lopez Camelo and Gomez, 2004). Changes in TCI were used to monitor the progression in ripening; with lower TCI values indicating a greener tomato.

The 3.7 kJ/m^2 UV-C, 16 and 24 HIPPL treatments showed significantly lower ripening progression, Δ TCI, in comparison to the control (Figure 1.2). The 3.7 kJ/m2 and 16 pulse HIPPL treatments reduced change in TCI over 10 days by 43.1 and 50.1 %, respectively. This data supports the successful induction of delayed ripening with either HIPPL or LIUV. Fruit treated with 8 pulses, however, did not ripen at a rate significantly different from the control. Representative samples of tomato fruit are shown in Figure 1.3.

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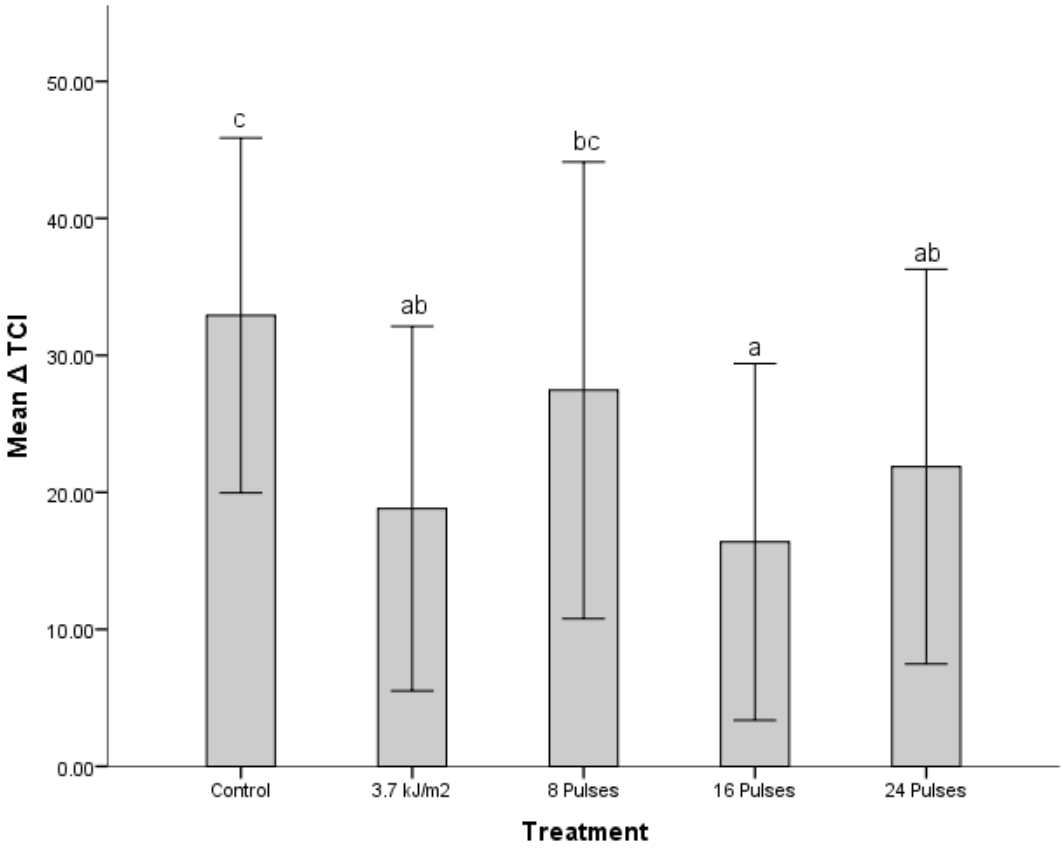

Figure 1.2: The Change in tomato colour index (TCI) over 10 days following treatment with either 3.7 kJ/m^2 of low intensity UV-C (LIUV) or 8, 16 and 24 pulses from a high intensity pulsed polychromatic light source (HIPPL). Results are from two independent replicate experiments; n=30, IRE=2.

Figure 1.3: Representative samples of tomato fruit of the cultivar Mecano at 10 days post treatment. Groups show the control fruit (**A**), the 3.7 kJ/m2 LIUV treatment with peak emissions at 254nm (**B**) and fruit treated with the high intensity pulsed polychromatic light (HIPPL) light source at 8 (**C**), 16 (**D**) and 24 (**E**) pulses (Scott *et al.,* 2017).

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This data contradicts recent work by Pataro *et al*. (2015) who observed no effect for either LIUV or HIPPL treatments on the ripening of tomato fruit of cv. San Marzano. The HIPPL source used by Pataro *et al.* (2015) gave comparable pulse length (360 µs) and spectral emission (200 to 1100 nm) to that produced by the source used here. The spectral irradiance, i.e. intensity of specific wavelengths, however, may have differed to the source used in this study as the information on this was not provided. Furthermore, different experimental protocols used by Pataro *et al.,* (2015) may have led to the failure to detect a significant difference in colour change for LIUV and HIPPL treated fruits. Specifically, the use of a 14 / 10 h day and night light cycle during fruit storage may have affected the induction of delayed ripening.

To further investigate the effects of HIPPL treatment on fruit ripening, fruit texture was monitored following treatment. During preliminary work fruit texture was measured at 7 day intervals and it was found that there was a difference in fruit firmness in HIPPL-and LIUV-treated fruit at 21 days following treatment (Appendix 1). Unfortunately, due to a discontinuation in the production of the cv. Mecano during this period, it was not possible to repeat the initial experiments in full. Evidence, however, is available to show a significant difference in the texture change of tomato fruit at 21 DPT.

Both the LIUV and HIPPL treatments (16 pulses) show a significant reduction in the softening of tomato fruit over the 21 days of storage when compared to the control. The control exhibited a mean change in texture of 15.83 N whereas the HIPPL and LIUV treated fruit were approximately 5 Newtons firmer at 11.27 and 11.42 N, respectively (Figure 1.4).

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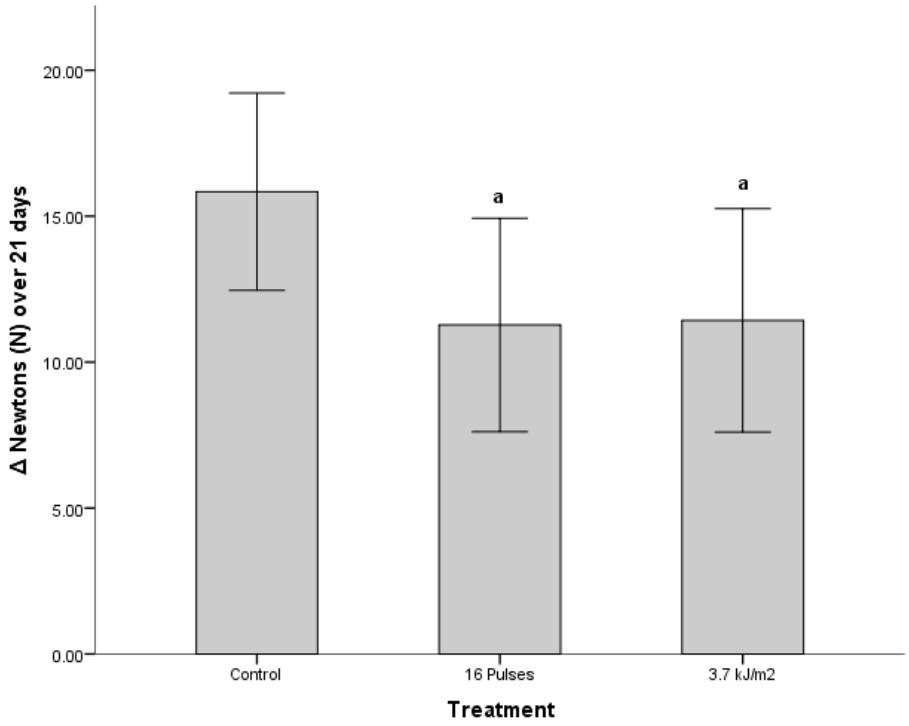

Figure 1.4: The Change (Δ) in firmness Newtons (N) over 21 days following treatment with a low intensity UV-C (LIUV) and high intensity pulsed polychromatic light (HIPPL) sources. Results are from two independent replicate experiments; n=20, IRE=2.

Disease Resistance

LIUV has previously been shown to induce disease resistance against *B. cinerea* on tomato fruit (Charles et al., 2008a). The possibility of inducing resistance with HIPPL was, therefore, investigated. HIPPL- and LIUV-treated fruit showed reductions in mean AUDPCs indicating reduced disease progression (Table 1.2). Welch's ANOVA showed that disease progression for all treated groups was significantly lower than the control. No significant differences were observed between HIPPL treatments and the LIUV treatment. However, a significant difference between the AUDPCs of the 8 and 16 pulse treatments was observed showing increased disease resistance for the 16 pulse treatment. These results show that HIPPL can induce resistance to *B. cinerea* on mature green tomatoes to similar levels to that of LIUV treatment. This is in contrast to the results obtained by Marquenie *et al.* (2003) who reported no effect of pulsed light on the disease progression of *B. cinerea* on strawberries, *Fragaria ananassa*. This could be due to the employment of a different plant species or to differences in the spectral emission of the HIPPL sources. The HIPPL source used by Marquenie *et al.* (2003) produced 30 µs pulses at 15 pulses per second (15 Hz). The source in this study, however, produces 360 µs pulses at 3.2 pulses per second. Furthermore, the authors

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reported that the percentage of light falling within the UV region was 50 % of a 7 J pulse in contrast to the output obtained here (1 % of a 505 J pulse).

The 16 pulse treatment, employed here, provides comparable levels of disease resistance to the 3.7 kJ/m^2 LIUV treatment with 41.5 % and 38.1 % reductions in AUDPC, respectively. The total duration of the treatment times for both the HIPPL and LIUV sources are 10 s and 370 s, respectively. This equates to a 97.3 % reduction in exposure time or a 37-fold increase in the number of tomatoes that could be treated with HIPPL compared to a LIUV treatment. Such a reduction could help overcome one of the factors - lengthy treatment times - that has militated against the adoption of LIUV hormesis in commercial horticulture.

Table 1.2: Area underneath the disease progression curve (AUDPC) from mature green fruit cv. Mecano treated with a conventional low intensity UV-C (LIUV) source, with peak emissions at 254 nm, and a high intensity pulsed polychromatic light (HIPPL) source. Inoculations were performed with *B. cinerea* at 10 d post treatment; n = 30, IRE=2 (Scott *et al,* 2017).

Treatment	Treatment time (s)	Mean AUDPC	Standard deviation	Mean AUDPC Reduction (%)
Control	0	70.74	14.00	-
3.7 kJ/m^2	370	43.76^{ab}	25.13	38.14
8 Pulses	5	56.05^{b}	16.82	20.76
16 Pulses	10	41.21^{a}	17.09	41.74
24 Pulses	15	45.15^{ab}	22.91	36.17

Superscript labelling indicates statistical significance. Means sharing the same superscript are not significantly different from each other at $p < 0.05$.

The majority of studies on LIUV-induced disease resistance have been carried out postharvest on mature green tomatoes. Treatment at this stage is not entirely relevant for the UK tomato industry where tomatoes are picked when at the red ripe stage to meet consumer preferences. Induced resistance against *B. cinerea* and *P. expansum* on red ripe tomatoes was, therefore, investigated.

LIUV treated fruit did not show significantly reduced disease progression against *B. cinerea* (Table 1.3). Moreover, an 8-pulse HIPPL treatment did result in a slight reduction of disease progression but it was not statistically significant. Both 16 and 24-pulse HIPPL treatments,

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however, did significantly reduce the AUDPC in comparison to the control. Variation in the induction of hormetic responses for the HIPPL and LIUV sources is not unexpected due to the differences in spectral emission, the intensity of dose delivery and fractionation of the dose with HIPPL sources.

Similar results were observed for *P. expansum* as those for *B. cinerea* resistance assays on ripe fruit. The 16 pulse HIPPL treatment showed a greater reduction in AUDPC when compared to the 3.7 kJ/m^2 with reductions in disease progression of 18.2 and 13.5 %, respectively, figure 1.4.

Table 1.3. Area Underneath the Disease Progression Curve (AUDPC) for ripe fruit cv. Mecano treated with a conventional low intensity UV-C (LIUV) source with peak emissions at 254 nm and a high intensity pulsed polychromatic light (HIPPL) source, followed by inoculation with *B. cinerea* at 10 d post treatment; n = 30, IRE=2 (Scott *et al,* 2017)..

Treatment	Treatment time (s)	Mean AUDPC	Standard deviation	Mean Disease Reduction (%)
Control	0	57.98^{b}	20.00	-
3.7 kJ/m^2	370	50.20^{ab}	12.66	13.43
8 Pulses	5	48.12^{ab}	18.98	17.00
16 Pulses	10	41.43^{a}	20.04	28.54
24 Pulses	15	41.65^{a}	19.84	28.15

Superscript labelling indicates statistical significance. Means sharing the same superscript are not significantly different from each other at $p < 0.05$.

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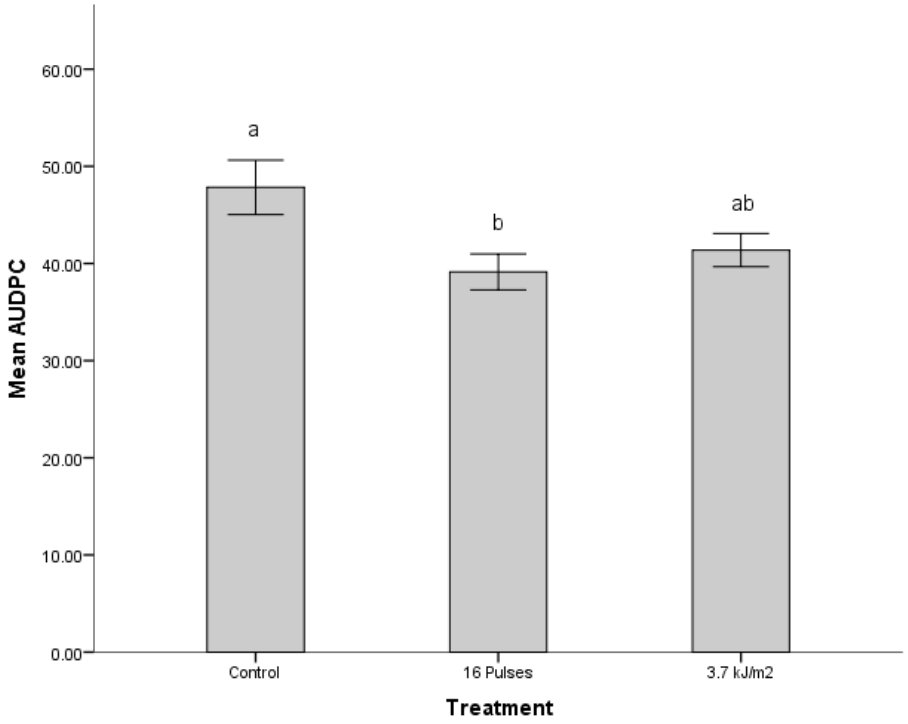

Figure 1.4 Area underneath the disease progression curve (AUDPC) from ripe fruit cv. Mecano treated with a conventional low intensity UV-C (LIUV) source at 3.7 kJ/m^2 , with peak emissions at 254 nm, and an high intensity pulsed polychromatic light (HIPPL) source. Inoculations were performed with *P. expansum* at 10 d post treatment; n = 30, IRE=3.

Summary and Conclusions

Despite the existence of a considerable body of experimental evidence the adoption of UV-C hormesis into the horticultural sector has not occurred. This is in part due to the long exposure times necessitated to induce its beneficial effects such as disease resistance and delayed ripening, among others. With conventional low pressure, low intensity mercury UV-C sources, treatment can take in the region of 6 minutes per fruit. The HIPPL source, rich in germicidal UV-C, has been shown here to both induce disease resistance and delayed ripening with a significant reduction in treatment time of 97.3 %.

Moreover, on mature green fruit, levels of resistance and delayed ripening were approximately equivalent to that delivered by the established LIUV treatment of 3.7 kJ/m^2 (Charles *et al.,* 2008a). The monitoring of ripening by both colour and texture analysis indicated that a 16 pulse HIPPL treatment delayed both to similar levels to that of the LIUV treatment.

In addition to the investigations on mature green fruit, induced disease resistance was also monitored on ripe fruit. Fruit, generally, becomes more susceptible to disease as it ripens as its nutrients become more easily available to pathogens. Furthermore, it is this developmental

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state that is of the greatest interest to commercial growers in the UK as fruit is harvested at the ripe stage due to improved consumer qualities. The majority of pathogen-related problems are observed in storage before shipment to the supermarkets.

A 16 pulse HIPPL treatment significantly reduced disease progression of both *B. cinerea* and *P. expansum* on red ripe fruit; a feature not exhibited by the established LIUV treatment. The LIUV treatment, however, did provide some level of resistance.

Following the findings of this study there are a number of questions that remain unanswered. These include the necessity for direct tissue exposure during treatment, and the importance of fruit orientation during treatment for both LIUV and HIPPL sources. Moreover, are the mechanisms underpinning hormesis for HIPPL similar to that of LIUV, and finally, what is the extent to which the UV-C plays a role in the induction of the defence response from the HIPPL source?

Objective 2 – Comparing the Molecular Mechanisms Underpinning LIUV and HIPPL Hormesis on Tomato Fruit

Introduction

In our previous study (objective 1) we found that a 16-pulse treatment at 4.6 kJ/m^2/pulse of HIPPL induced both delayed ripening and disease resistance on tomato fruit at comparable levels to a 3.7 kJ/m^2 treatment with LIUV (Scott *et al.,* 2017a). The use of HIPPL reduced treatment times from 350 s to 10 s when LIUV treatments were delivered at 20 W m^{-2}.

One of the major benefits of HIPPL and LIUV hormesis is that of induced resistance. Resistance is achieved four-fold through phytoalexin production, delayed ripening and senescence, the production of pathogenesis related (PR) proteins and the production of physical barriers that slow pathogen progression (Ben-Yehoshua *et al*., 1992; D'Hallewin *et al.,* 1999; D'Hallewin *et al.,* 2000; Mercier *et al.,* 2000; Romanazzi *et al.,* 2006; Charles *et al.,* 2008a; Charles *et al.,* 2009). PR proteins that have been shown to be induced or increase in concentration following LIUV treatment include chitinases and β-1,3-glucanases (Charles *et al.,* 2009). Such PR proteins interact directly with pathogens and cleave their respective substrates leading to loss of pathogen viability.

Upon treatment with biotic and abiotic factors, defence-related genes can either be constitutively upregulated or primed locally or systemically, as reviewed by Goellner &

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Conrath, (2008); Walters & Fountian (2009) and Walters *et al.,* (2013). Priming in plants plays an important role in both induced systemic resistance (ISR) and systemic acquired resistance (SAR) (Conrath *et al.,* 2015). The first instance of gene priming was observed via exogenous dichloroisonicotinic or salicylic acid (SA) application to parsley (*Petroselinum crispum*) cell culture (Kauss *et al.,* 1992). Priming allows the host to upregulate/downregulate defence-related genes, in response to biotic or abiotic stress, at a faster pace and to a greater extent (Conrath *et al.,* 2015). Such a response is facilitated through changes in epigenetic control including DNA methylation and histone modification; two processes involved in chromatin remodelling (Dowen *et al.,* 2012; Espinas *et al*, 2016).

A further benefit of hormesis in tomato fruit is that of increased nutritional content through changes in secondary metabolism. Changes to secondary metabolism have been observed on a wide range of LIUV-treated fruit including blueberries (*Vaccinium corymbosum*), grapefruit (*Citrus paradisi*) and mango (*Mangifera indica*) -to mention but a few (D'Hallewin. *et al.,* 2000; Gonzalez-Aguilar *et al.,* 2007; Perkins-Veazie *et al.,* 2008). Both HIPPL and LIUV treatments significantly increase total carotenoid and phenolic content as well as the antioxidant activities of tomato fruit (Liu *et al.,* 2009; Liu *et al.,* 2012; Pataro *et al.,* 2015). To date, however, little is known of the molecular mechanisms underpinning HIPPL hormesis in tomato fruit.

Aims

The aim of this investigation was to explore whether the LIUV and HIPPL treatments induce disease resistance through similar changes in gene expression and to identify which of the main defence signalling pathways (SA, JA and ET) are involved. Secondly, gene expression profiles were monitored following inoculation to determine whether genes undergo gene priming following treatment.

Materials and Methods

Fruit Production, LIUV and HIPPL treatment and inoculation

Fruit production, treatment and inoculation was performed as stated in objective 1.

Sampling, RNA Extraction and Reverse Transcription

A No.2 cork borer (6.25 mm outer diameter) was used to take a 50-75 mg sample of pericarp from tissue directly facing the light sources. Samples were placed into microcentrifuge tubes

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and immediately frozen in liquid nitrogen. Samples were stored at -80 °C until use. Twenty-four hours before tissue homogenisation a single 4 mm steel bead (Qiagen) was cooled in liquid nitrogen and added to each microcentrifugetube. Samples were placed into a Tissuelyser II (Qiagen) block and stored at -80 °C overnight. Samples were homogenised using two runs of a Tissuelyser II (Qiagen) at 30Hz for 1 minute. Homogenised samples were stored at -80 °C until RNA extraction.

RNA was extracted with the RNeasy Plant Mini Kit (Qiagen) following the manufacturers guidelines. An on-column DNase treatment was performed with the RNASE free DNASE kit (Qiagen). A further off column DNase step was performed with the TURBO™ DNase kit (Ambion) following the manufacturers guidelines. RNA purity and yield was assessed via NanoDrop (Thermo Scientific). All samples were then diluted to a concentration of ≤ 50 ng/µl. A 20 µl Reverse transcription reaction was then performed using the High-Capacity RNA-to-cDNA kit (Applied Biosystems) following the manufacturers protocol. The resulting cDNA was stored at -20 °C until use.

qPCR

Two technical replicates were performed for each sample. Each 10 µl reaction contained 5 µl of 2x Fast SYBR® Green master mix (Applied Biosystems) and 2 µl of template cDNA. Primer concentrations and annealing temperatures were as stated in table 1. Reactions were run on a LightCycler 480 ® (Roche) with a two-step amplification cycle. The cycle was as follows; a pre-incubation of 10 minutes at 95 °C followed by 40 cycles of 95 °C for 5 s and the anneal for 45 s. Ct values were calculated utilising the second derivative maximum method. A melting curve was run between 90 °C and 60 °C following the amplification to allow for checking of product specificity. Primers were optimised utilising a pooled sample and a 5-point 5-fold dilution series from which efficiency was calculated (Eq. 2.1). Specificity of products from each primer pair were confirmed by sequencing and NCIB basic local alignment search tool (BLAST) analysis.

$$\boldsymbol{AE = D^{(\frac{-1}{\nabla})}}$$

Equation 2.1: Amplification efficiency showing efficiency (AE), fold dilution (D) and gradient of the logarithmically plotted dilution curve (∇) (Pfaffl, 2004).

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Table 2.1: Information on the primers used in qPCR

Target gene	Reference	Accession	Product Tm (°C)	Conc. (nm)	Anneal (°C)	Efficiency (%)	Sequence 5'-3'
ACT	Aime *et al.,* 2008	U60480	75.4	100	60	81.0	F: AGGCACACAGGTGTTATGGT R: AGCAACTCGAAGCTCATTGT
ACO1	Van de Poel *et al.,* 2012	X04792	76.4	500	60	85.8	F: ACAAACAGACGGGACACGAA R: CCTCTGCCTCTTTTTCAACC
CHI9	Aime *et al.,* 2008	Z15140	78.5	50	58	80.0	F: GAAATTGCTGCTTTCCTTGC R: CTCCAATGGCTCTTCCACAT
CRTRB	Tiecher *et al.,* 2013	SGN-U568606	77.8	500	60	101.4	F: TTGGGCGAGATGGGCACAC R: TGGCGAAAACGTCGTTCAGC
FLS	Tiecher *et al.,* 2013	GI 225321931	71.7	250	60	97.3	F: ATGGAGGCAGCTGGTGGTGAA R: CAGGCCTTGGACATGGTGGATA
GLUB	Aime *et al.,* 2008	M80608	75.8	100	60	79.3	F: TCTTGCCCCATTTCAAGTTC R: TGCACGTGTATCCCTCAAAA
OPR3	Blanco-Ulate *et al.,* 2013	Solyc07g007870	76.8	300	60	86.0	F: TGGGTTTCCTCATGTGCCAG R: GCAGCTCCAGCAGGTTGATA
PAL	Bovy *et al.,* 2002	M83314.1	74.0	500	60	96.3	F: ATTGGGAAATGGCTGCTGATT R: TCAACATTTGCAATGGATGCA
PG	Xie *et al.,* 2014	X05656.1	74.6	250	58	78.5	F: ATACAACAGTTTTCAGCAGTTCAAGT R: GGTTTTCCACTTTCCCCTACTAA
PR1a	Aime *et al.,* 2008	AJ011520	80.9	250	58	78.9	F: TCTTGTGAGGCCCAAAATTC R: ATAGTCTGGCCTCTCGGACA

Experimental design and data analysis

Data was collected from two independent replicate experiments. For each experiment three fruit per treatment group per time point were analysed; n=6. Fruit were sampled before treatment (baseline expression), at 24 HPT, 10 DPT and 12 HPI. Each gene of interest was run on its own 384 well plate (Roche) along with a 5-point, 5-fold dilution series that was used to calculate the efficiency of amplification (Eq 2.1). Following qPCR samples were checked for non-specific products (melt curve analysis), Ct values ≥ 35 and technical replicate standard deviations > 0.5. Samples exhibiting these characteristics were considered unsuitable for further analysis and the data was re-collected. Inter-plate calibration was performed with a pooled sample to correct for interpolate bias (Eq. 2.2). Amplification efficiency was then used to correct Ct values following Eq. 2.3. Technical replicates were

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then averaged before further analysis. All data for individual experimental repeats can be found in appendix 2.

$$Ct^{corrected} = Ct - Ct^{IPC} + \frac{1}{N}\sum_{i=1}^{N} Ct^{IPC}$$

Equation 2.2: Interplate calibration equation. The Ct for any given sample is Ct. The Ct value of the interpolate calibrator is Ct^{IPC} and N is equal to the number of plates that are being calibrated between (TATAABiocenter, 2012).

$$CtE = Ct \times \frac{Log10(AE)}{Log10(2)}$$

Equation 2.3: Efficiency correction of cycle threshold (Ct) values. CtE is the efficiency corrected Ct value and AE is the efficiency of amplification (Kubista & Sindelka, 2007).

Actin was used as reference gene as in previous UV-C studies and *B. cinerea* inoculation studies (Liu *et al.,* 2011; Virk *et al.,* 2012; Blanco-Ulate *et al.,* 2013;Tiecher *et al.,* 2013). Following efficiency correction actin was used to normalise the data giving ΔCt (Eq. 2.4). Data was normalised to baseline gene expression and fold change between treatment groups was calculated following Eq. 2.5. For experiments utilising theoretical copy number a copy number of 100 was assigned to the baseline (pre-treatment) gene expression levels and the further data was adjusted accordingly.

$$\Delta CtE = CtE(goi) - CtE(ref)$$

Equation 2.4: Normalisation of gene of interest with reference gene. CtE(goi) is the efficiency corrected Ct value for the gene of interest and CtE(ref) is the efficiency corrected Ct value for the reference gene (Pfaffl, 2004).

$$Fold\ change = 2^{-(\Delta CtET - \Delta CtEC)}$$

Equation 2.5: Calculation of fold change. ΔCtET is the normalised and efficiency corrected mean Ct value for the treatment group and ΔCtEC is the normalised and efficiency corrected mean Ct value of the control group (Livak & Schmittgen, 2001).

Statistical analysis was performed on the normalised Ct values (ΔCt) using statistical software package SPSS 22 (IBM). One-way ANOVA with Tukey's post-hoc testing was performed. Where the homogeneity of variances assumption could not be met Welch's robust ANOVA

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was performed followed by the Games-Howell post-hoc test. Statistical significance is defined as $p \leq 0.05$. All data from individual experimental replicates can be found in appendix 2.

Results and discussion

Expression profiles of genes involved in plant defence and ripening were analysed in HIPPL-treated fruit and compared with LIUV. The comparison was made over a time course starting with 24 HPT, 10 DPT, immediately before inoculation with *B. cinerea*, and at 12 HPI. The changes in expression at each time point were calculated relative to baseline expression in samples taken before the treatment.

Phytohormones and disease resistance

Ethylene (ET) is a plant hormone and a major contributor to the control of ripening. Its perception also plays a role in ripening-related susceptibility to *B. cinerea* in tomato fruit (Cantu *et al.,* 2009). ACO (1-aminocyclopropane-1-carboxylic acid oxidase) is involved in the final oxygen dependant step converting ACC (1-aminocyclopropane-1-carboxylic acid) to ethylene (Hamilton *et al.,* 1991; Dong *et al.,* 1992). ACO1 is one of five identified ACO enzymes involved in ethylene biosynthesis in tomato (Hamilton *et al.,* 1991; Bouzayen *et al.,* 1993; Sell & Hehl, 2005). In this study the expression of *ACO1* in control fruit increased during the 10 day storage by approximately 8-fold, which is consistent with *ACO1* increase during the normal ripening (van de Poel *et al.,* 2012).

Expression of *ACO1* in treated fruit was shown to be significantly different from that of the control at 24 HPT. Expression levels for HIPPL- and LIUV-treated fruit were both 3.1-fold higher than that of the control. At 10 DPT and 12 HPI, however, the levels of *ACO1* were not significantly different across groups. Expression in control fruit however, was approximately 1.2 to 2.2-fold higher than treated fruit, figure 2.1.

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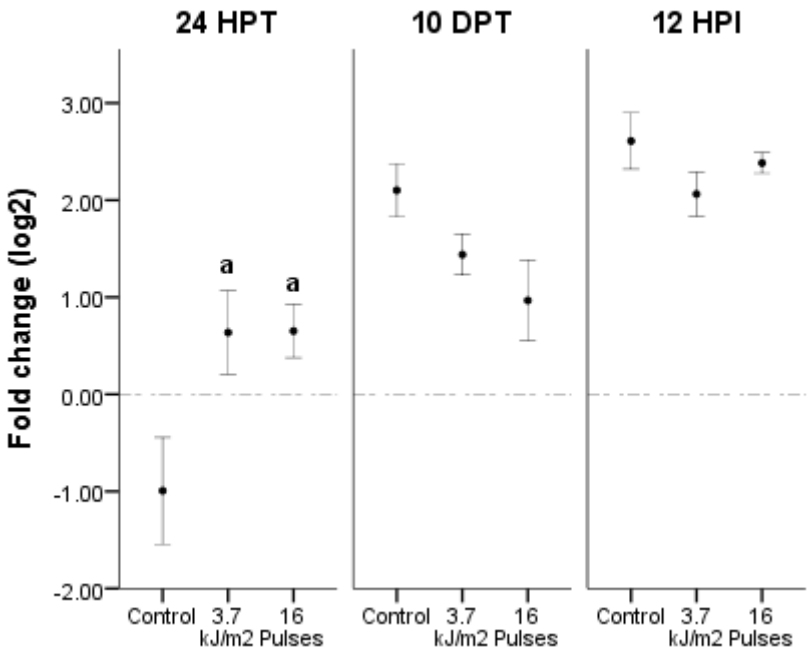

Figure 2.1: Relative expression of *ACO1* (1-aminocyclopropane-1-carboxylic acid oxidase 1), a bottleneck enzyme in ethylene biosynthesis, following treatment with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C source (LIUV). Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). Labelling indicates statistical significance, within a given time point, where groups sharing labels are not significantly different at $p< 0.05$. N=6, IRE=2. Bars show ± 1S.E.M. (Scott *et al.,* 2017b)

Our results are consistent with that of Maharaj et al., (1999) who observed a transient peak in ethylene production at 3 and 5 days after LIUV treatment followed by a lag in ethylene production and a lower maximum in ethylene level from seven days following treatment. Similarly, Teicher et al., (2013) found that *ACO* was upregulated in both the exocarp and mesocarp of tomato fruit treated with LIUV at 24 HPT while at 7 DPT expression of *ACO* in the control was greater than that of the LIUV treated fruit.

Jasmonic Acid (JA) is a phytohormone whose major roles include the plant's adaptation to herbivorous pests and necrotrophic plant pathogens (Spoel & Dong 2012). OPR3 (12-Oxophytodienoate reductase 3) is an enzyme involved in jasmonate biosynthesis (Schaller *et al.,* 2000).

In HIPPL treated fruit we detected a slight downregulation of *OPR3* (<2-fold) at 24 HPT, figure 2.2. Expression in control fruit remained at the baseline levels. After 10 days of storage (10 DPT) a significant increase in *OPR3* expression was observed at 3.8 and 3.9-fold for HIPPL and LIUV treatments in comparison to the control. Following inoculation (12 HPI) *OPR3* expression increased in all groups. Expression, however, was still significantly higher in treated fruit at 2.1 and 2.2-fold greater in HIPPL and LIUV treated fruit. *OPR3* overexpression

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in both light treatments would result in JA levels and activation of JA inducible plant defences. The initial reduction in *OPR3* expression was analogous to the results observed by Liu *et al.,* (2011) who showed a 3.9-fold reduction in *OPR2* at 24 HPT following LIUV treatment; no further time points were monitored.

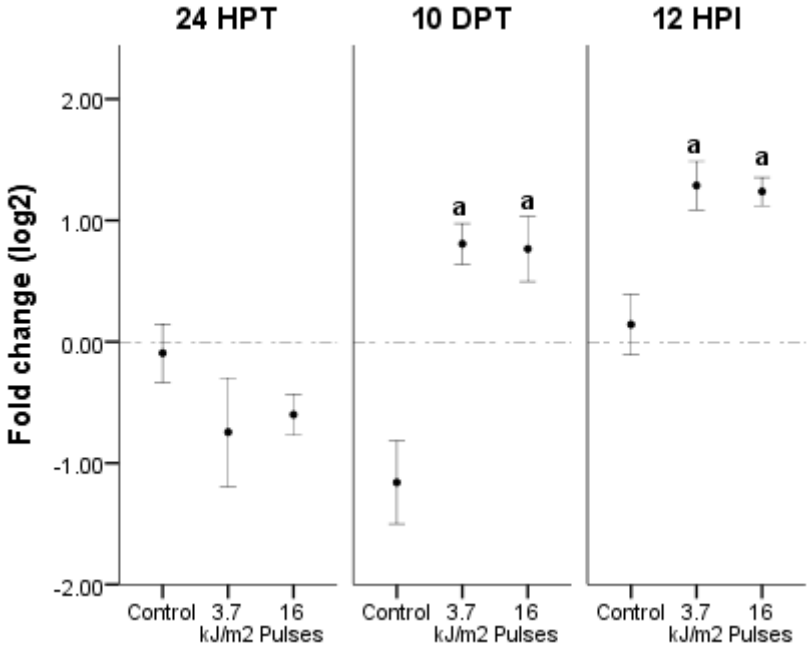

Figure 2.2: The relative expression of *OPR3* (12-Oxophytodienoate reductase 3) a jasmonate biosynthesis protein transcript following treatment with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). Labelling indicates statistical significance, within a given time point, where groups sharing labels are not significantly different at $p< 0.05$. N=6, IRE=2. Bars show ± 1S.E.M. (Scott *et al.,* 2017b)

Salicylic Acid (SA) is a phytohormone which plays a major role in defence against biotrophic pathogens, insect pests and abiotic stress and also DNA repair (Spoel & Dong 2012; Yan *et al.,* 2013; Song & Bent, 2014). There are at least two biosynthesis pathways for the production of SA (Lee *et al.,* 1995). It was, therefore, decided that an SA-inducible product would be monitored to infer changes to SA biosynthesis. P4 (PR1a) is a salicylic acid inducible PR protein and marker of SAR.

P4 expression was increased in comparison to the control at each of the time-points, figure 2.3. The differences, however, were only significant at 10 DPT and 12 HPI. *P4* levels in LIUV and HIPPL treated fruit were 50.3 and 55.5-fold and 38.0 and 35.5-fold higher than that of the control at 10 DPT and 12 HPI, respectively. Results indicate that HIPPL and LIUV treatments induce SA signalling upon treatment.

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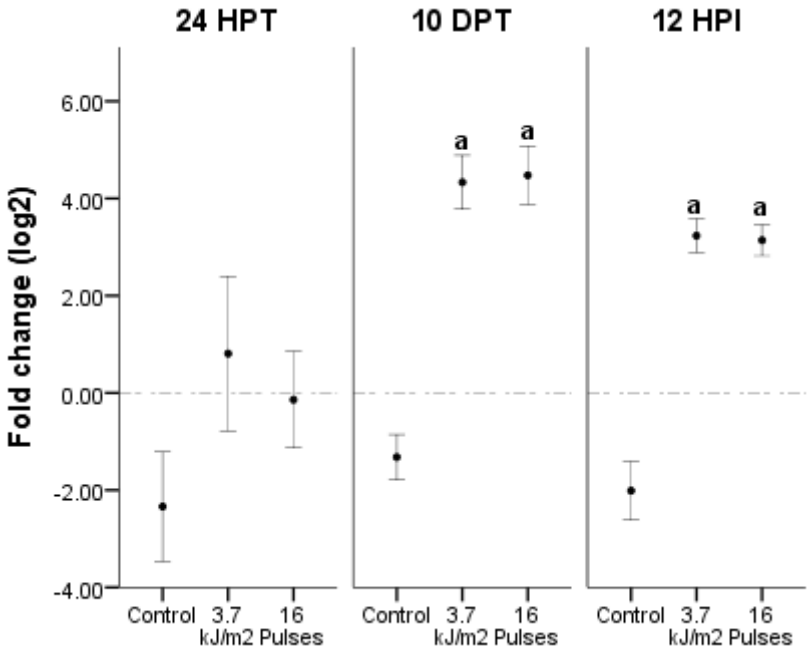

Figure 4.3: The relative expression of *P4* (*PR1a*) a salicylic acid inducible pathogenesis related protein and marker of systemic acquired resistance (SAR) following treatment with either 16 pulses from a high intensity pulsed polychromatic light source (HIPPL) or 3.7 kJ/m2 from a low intensity UV-C source (LIUV). Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). Labelling indicates statistical significance, within a given time point, where groups sharing labels are not significantly different at $p< 0.05$. N=6, IRE=2. Bars show ± 1S.E.M. (Scott *et al.,* 2017b)

β -1,3-Glucanases play many important roles in the plant from regulating germination to defending the plant from pathogen attack. Here we observed significant upregulation in the expression of a basic, intracellular 33 kDa ethylene inducible PR β-1,3,-Glucanase (*GluB*) (van Kan *et al.,* 1992; Aimee *et al.,* 2008).

Levels of *GluB* were similar in all groups at 24HPT, figure 2.4. At 10 DPT, however, expression of *GluB* was increased 32.4 and 40.1 –fold in HIPPL- and LIUV-treated tomato fruit, respectively. *GluB* expression increased by approx. 32-fold and 2-fold for control and treated samples following inoculation (12 HPI). Expression levels in both HIPPL and LIUV treated fruit remained significantly higher than the control in treated fruit with 2.1 and 2.2-fold differences, respectively. A similar pattern in protein expression was observed by Charles *et al.,* (2008b) on LIUV treated tomato fruit. They reported the induction of a basic 33.1 kDa β-1,3,-Glucanase which increased in concentration between 3 and 10 days after treatment and following inoculation with *B. cinerea*.

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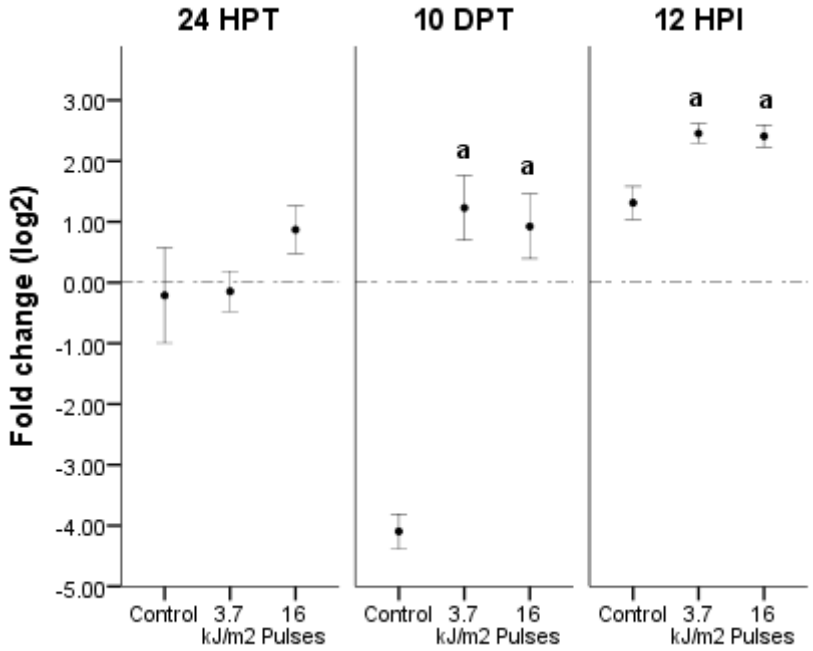

Figure 2.4: Relative expression of *GluB* (β-1,3,-Glucanase) an ethylene inducible pathogenesis related protein transcript following treatment with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). Labelling indicates statistical significance, within a given time point, where groups sharing labels are not significantly different at $p< 0.05$. N=6 IRE=2. Bars show ± 1S.E.M. (Scott *et al.,* 2017b)

PR chitinases are involved in the breakdown of glycosidic bonds in the cell wall of fungal pathogens. Here we are monitoring an ethylene, JA and wounding inducible chitinase CHI9 (chitinase I) (Diaz *et al.,* 2002; Wu & Bradford 2003). CHI9 is upregulated in response to plant pests including the whiteflies *Bemisia tabaci* and *Trialeurodes vaporariorum* and the necrotrophic pathogen *B. cinerea* (Puthoff *et al.,* 2010; Levy *et al.* 2015).

Expression profiles observed for *CHI9* were similar to profiles of *GluB*. At 24 HPT a slight increase in *CHI9* expression was detected in HIPPL- and LIUV-treated fruit while expression in control decreased below baseline, figure 2.5. At 10 DPT a statistically significant increase in expression can be seen with 10.0 and 7.3-fold differences between the control and LIUV and HIPPL treatments, respectively. This was approximately 2-fold above baseline.

Following inoculation (12 HPI) expression of *CHI9* only increased in the control fruit. The expression in treated samples, however, was still significantly greater than the control at 2.9 and 3.8-fold for the HIPPL and LIUV groups. Our results indicate that disease resistance due to increased chitinase expression is a mechanism shared by both light treatments. Two chitinases observed by Charles *et al.,* (2008b) also showed a similar pattern of expression to

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those observed here with little change in expression at 3 DPT but intensified expression at 10 DPT and also induced following inoculation as seen for the control fruit which showed approximately a 2-fold increase in expression following inoculation.

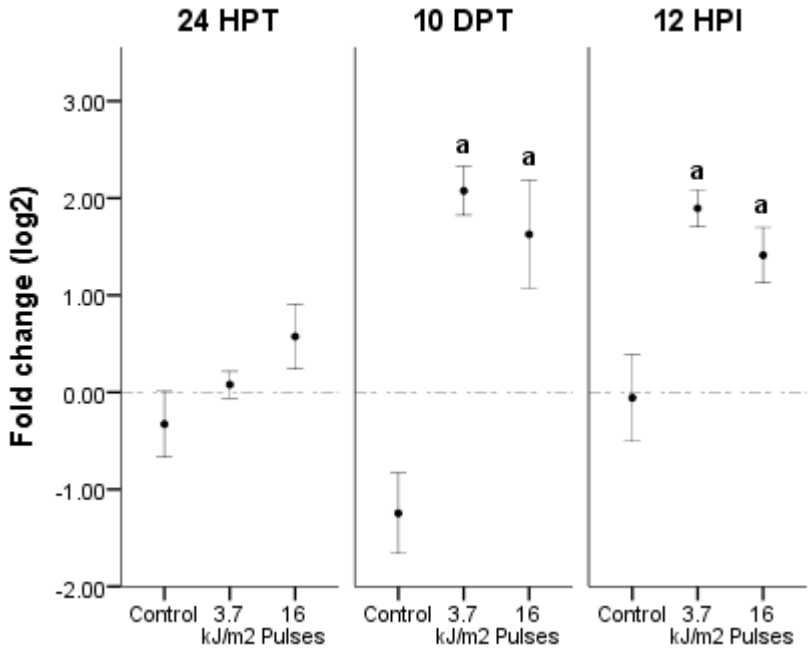

Figure 2.5: Relative expression of *CHI9* (Chitinase 9) a jasmonic acid induced pathogenesis related protein transcript following treatment with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). Labelling indicates statistical significance, within a given time point, where groups sharing labels are not significantly different at $p< 0.05$. N=6. Bars show ± 1S.E.M. (Scott *et al.,* 2017b)

Ripening and secondary metabolism

A delay in ripening through colour change and the softening of tomato fruit texture is a further benefit of LIUV hormesis which extends shelf life and reduces pathogen progression (Bennett *et al.,* 1993; Barka *et al.,* 2000). Polygalacturonase (PG) is one of the primary hydrolases involved in the breakdown of pectin in the cell wall during ripening (King & O'Donoghue, 1995). Furthermore, increased polygalacturonase activity increases tomato's susceptibility to *B. cinerea* (Bennett *et al.,* 1993).

At 24 HPT *PG* expression was at baseline levels in all groups, figure 2.6. The expression in all groups increased at 10 DPT. In HIPPL- and LIUV-treated fruit however, levels of *PG* were

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significantly lower than the control with 6.1 and 32.2-fold decreases, respectively. *PG* levels decreased in response to inoculation (12 HPI) with *B. cinerea* in all groups. Fruit from both treated groups however, still showed significantly lower expression than control fruit with 15.4 and 3.0-fold less *PG* in LIUV and HIPPL treated fruit, respectively. Expression of *PG* in HIPPL treated fruit supports our observations that control fruit were 14.6 and 22.4 % softer than HIPPL treated fruit at 14 and 21 days post treatment (unpublished data). Furthermore, Barka *et al.,* (2000) showed a reduction in PG activity following LIUV treatment.

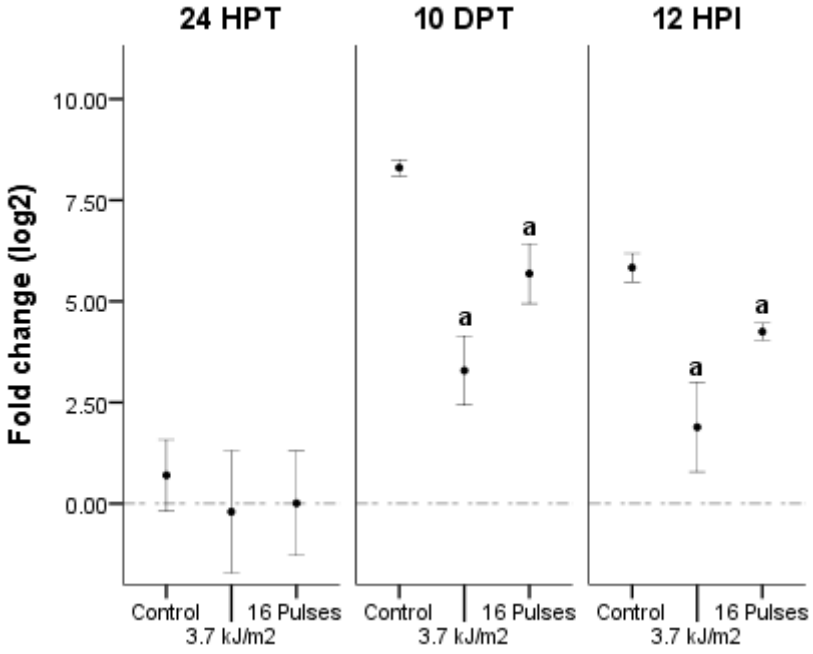

Figure 2.6: The relative expression of *PG* (polygalacturonase) following treatment with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). Labelling indicates statistical significance, within a given time point, where groups sharing labels are not significantly different at $p< 0.05$. N=6, IRE=2. Bars show ± 1S.E.M. (Scott *et al.,* 2017b)

Carotenoids are organic molecules responsible for the red, orange and yellow pigmentations found in flowers and fruits (Yuan *et al.,* 2015). The carotenoid β –carotene gives rise to the orange pigmentation in tomato fruits and are synthesised from the cyclisation of lycopene; the major carotenoid in tomato fruit which gives them their red colour (Pecker *et al.,* 1996; Tadmor *et al.,* 2005; Yuan *et al.,* 2015). Here we are monitoring the expression of β -carotene hydroxylase (*CRTR-B1*) involved in β –carotene modification which produces the xanthophylls zeaxanthin and lutein giving plant organs a yellow pigmentation (Galpaz *et al.,* 2006). These carotenoids are also found in the retina of the human eye, and their uptake through food can lower the risk of age-related macular degeneration of retina.

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We have shown a significant 1.7-fold increase in *CRTR-B1* expression in HIPPL and LIUV treated fruit 24 HPT, figure 2.7. At 10 DPT and 12 HPI, however, expression of *CRTR-B1* was not significantly different from that of the control. Analogous patterns of expression of CRTR-B1 along with zeaxanthin and lutein concentrations were observed by Teicher et al., (2013) who observed increases in both at 1 DPT following LIUV treatment and similar levels to the control at 7 DPT.

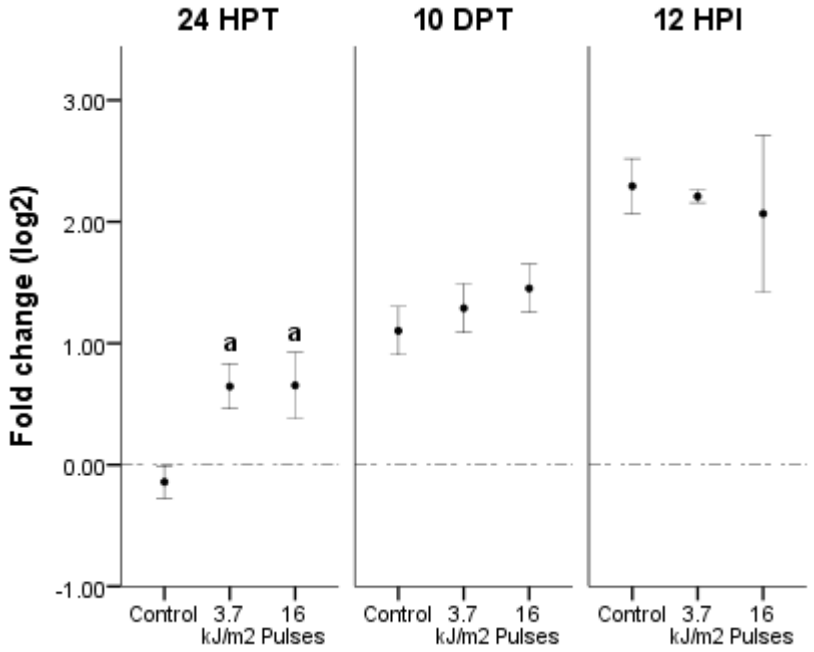

Figure 2.7: Relative expression of *CRTR-B1* (β -carotene hydroxylase) following treatment with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). Labelling indicates statistical significance, within a given time point, where groups sharing labels are not significantly different at $p< 0.05$. N=6, IRE=2. Bars show ± 1S.E.M. (Scott *et al.,* 2017b)

The total phenolic content of tomatoes has been shown to increase following treatment with LIUV (Liu *et al.,* 2009). Phenylalanine ammonia lyase (PAL) is involved in the biosynthesis of phenolic compounds, many of which are involved in pathogen defence acting as phytoalexins, free radicle absorption and light quenching (Pietta, 2000; Sourivong *et al.,* 2007; Lev-Yadun & Gould, 2009). It also plays an important role in salicylic acid biosynthesis.

Following treatment, expression of *PAL* was approximately at baseline levels in all groups at 24 HPT, figure 2.8. Following 10 days of storage and immediately before inoculation (10 DPT) a slight increase in expression of *PAL*, in comparison to the control, was observed for

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the treated fruit with a 1.4 and 1.5-fold increases for HIPPL and LIUV treatments, respectively. The differences, however, were not significant. Following inoculation (12 HPT) *PAL* expression was significantly greater for both HIPPL and LIUV with a 2.0 and 2.1-fold increase in comparison to the control, respectively. The results are in agreement with those of Teicher et al., (2013) who showed an approximately 2 to 3-fold increase in *PAL* following LIUV treatment in the mesocarp of tomato fruit at both 1 and 7 DPT. The exocarp, however, showed no increase in *PAL* at either 1 or 7 DPT. Expression of *PAL*, however, was not monitored following inoculation.

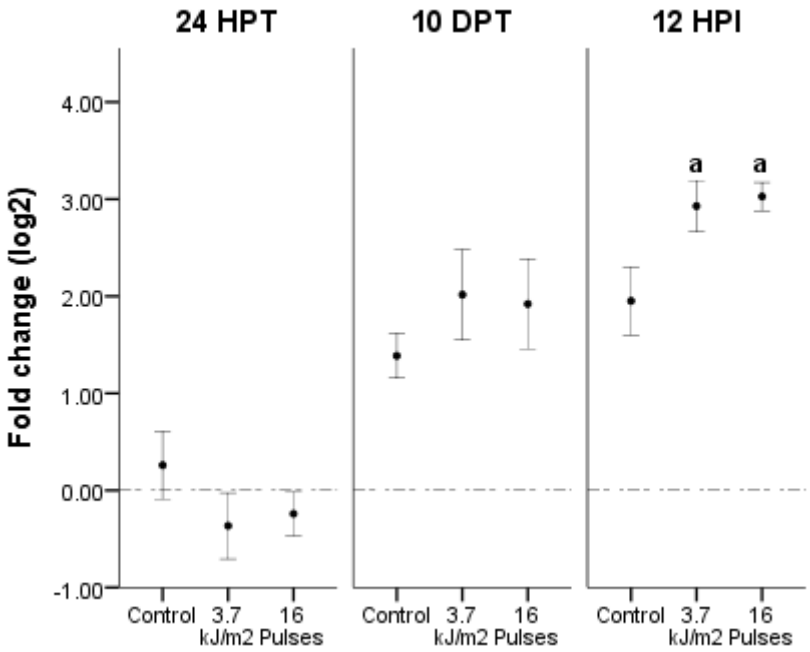

Figure 2.8: The relative expression of *PAL* (phenylalanine ammonia lyase) following treatment with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). Labelling indicates statistical significance, within a given time point, where groups sharing labels are not significantly different at $p< 0.05$. N=6, IRE=2. Bars show ± 1S.E.M. (Scott *et al.,* 2017b)

Flavonols are a group of phenolic flavonoid antioxidants which have recently been targeted for enrichment in genetically modified tomato for their health-promoting benefits (Choudhary *et al.,* 2016). Following LIUV treatment total phenolic and flavonoid concentrations have been shown to increase. Flavonol synthase (*FLS*) is directly involved in biosynthesis of flavonols, compounds with important role in plant-pathogen interaction due to their antioxidant properties.

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FLS expression was decreased at 24 HPT with 5.8 and 2.5- fold higher concentration in the control fruit when compared to the LIUV and HIPPL sources, respectively, figure 2.9. Only the LIUV treatment was significantly different from the control. At 10 DPT *FLS* expression further decreased with the HIPPL and LIUV treated fruit showing 100.3 and 109.1-fold differences when compared to the control. At 12 HPI *FLS* expression in the control fruit decreased by approx. 4-fold to baseline levels. Expression for both treatments increased to 8.9-fold and 10.8 below the control for HIPPL- and LIUV-treated fruit, respectively. This was still significantly lower than the control.

Downregulation of *FLS* would result in decreased biosynthesis of flavonols such as myricetin, quercetin and kaempferol. A previous study by Tiecher *et al.,* (2013) reported similar results in LIUV treated fruit where quercetin concentration was measured by by HPLC. They showed increased levels in both the exocarp and mesocarp at 1 DPT and an approximately 4-fold increase at 7 DPT in the control fruit compared to the control. Levels of quercetin when the fruit were ripe, however, were greater in LIUV-treated fruit. In contradiction to this, however, Tiecher *et al.,* (2013) showed approximately a 2.5-fold increase at 1 DPT and a 10-fold increase in *FLS* expression at 7 DPT following treatment with LIUV.

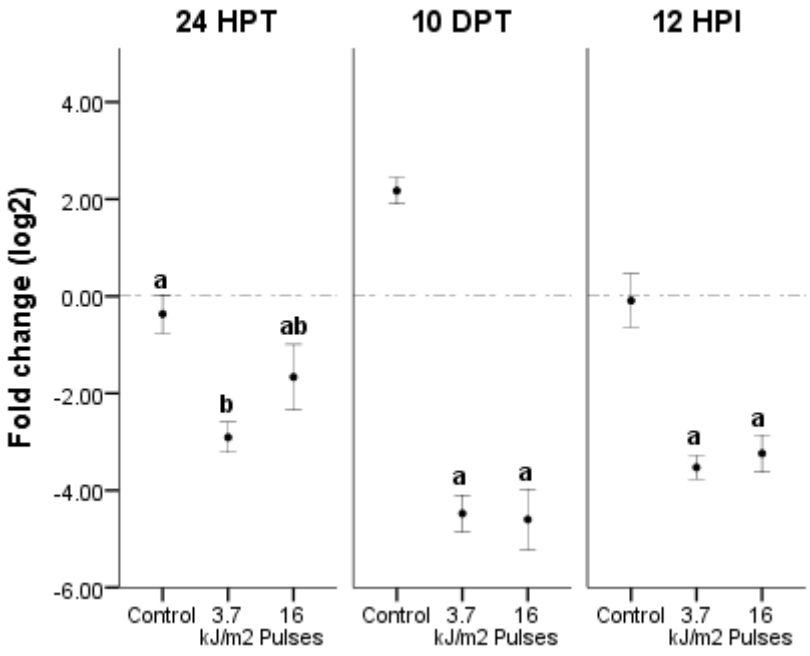

Figure 2.9: Relative expression of *FLS* (flavonol synthase) following treatment with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). Labelling indicates statistical significance, within a given time point, where groups sharing labels are not significantly different at $p< 0.05$. N=6. Bars show ± 1S.E.M. (Scott *et al.,* 2017b)

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Gene priming

It has been shown that biotic and abiotic inducers of disease resistance can prime plant defences; reducing the impact of the phytopathogens (Mur *et al.,* 1996; Latunde-Dada & Lucas, 2001; Cools & Ishii, 2002; Yang *et al.,* 2015). Defence priming is postulated to be an adaptive, low-cost defensive measure activated by a given priming stimulus**,** in our case HIPPL and LIUV treatments. In primed plants transcriptional responses are deployed in a faster, stronger or more sustained manner following the perception by the plant of a secondary stress (Martinez-Medina *et al.,* 2016). To test if priming occurred in our experiments we monitored gene expression at 10 DPT, immediately before inoculation, and 12 hours following the defence triggering stimulus; inoculation with *B. cinerea*.

Among the investigated genes only *PAL* exhibited a priming related expression profile in HIPPL- and LIUV-treated fruit, figure 2.10 and 2.8. A priming related expression profile can be identified as the lack of a significant difference between the control and treated samples before inoculation, as seen in figure 2.8. This is then followed by a greater change in gene expression following a stress stimulus, figure 2.10.

In other analysed genes the priming response was not identified due to significantly different expression levels between treated and control fruit before the inoculation (GLUB, CHI9, FLS, OPR3, PG & P4) or due to very similar expression profile across observed time points (ACO1 & CRTR-B1).

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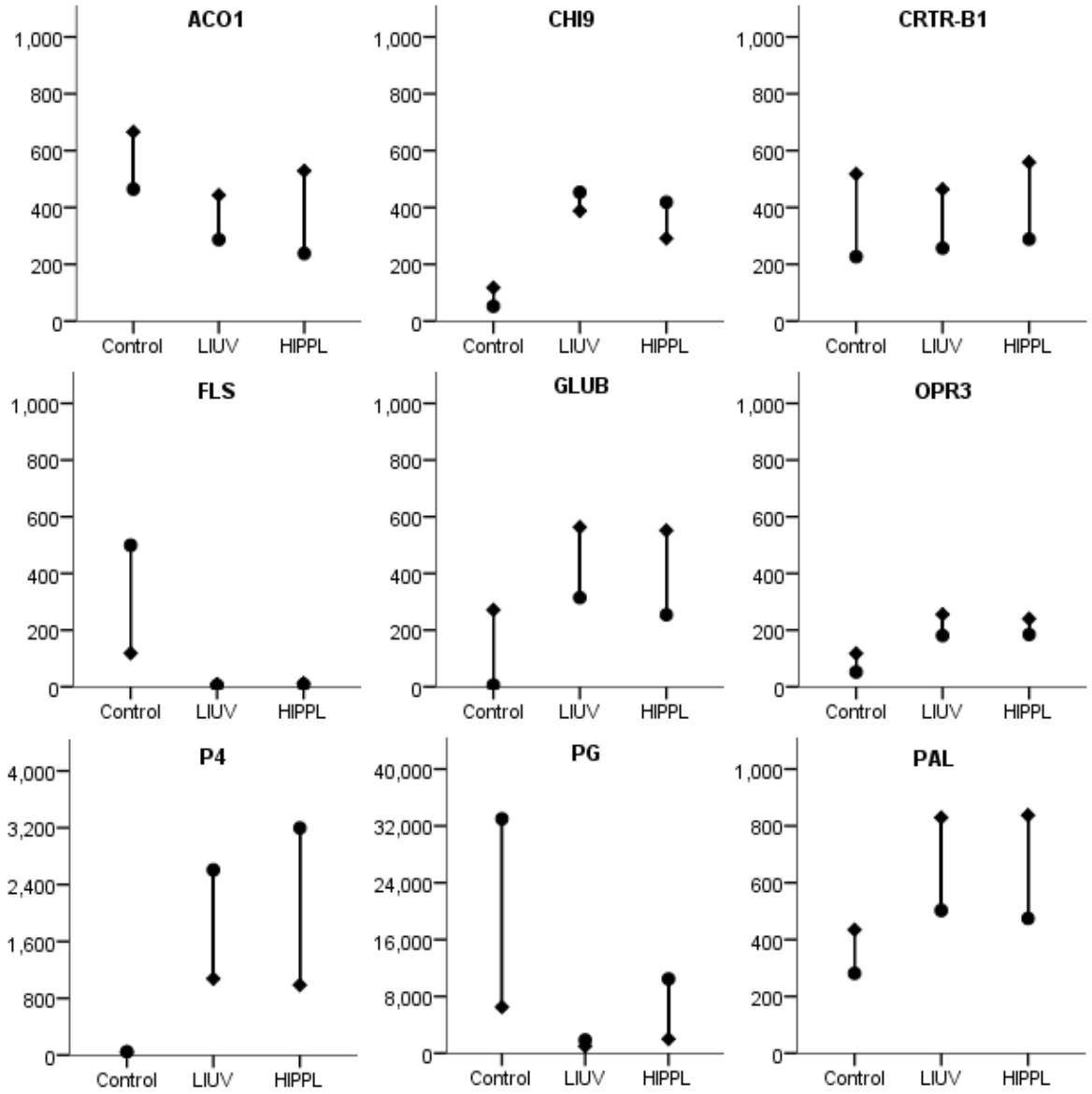

Figure 2.10: Gene expression levels shown as theoretical copy number (TCN) between samples taken at 10-days post treatment (●) and 12-hours post inoculation with *Botrytis cinerea* (◆). The vertical line denotes the magnitude of change. Fruit were treated with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C (LIUV) source and compared to untreated control Graphs show the following genes; ***ACO1*** (1-aminocyclopropane-1-carboxylic acid oxidase; a bottleneck enzyme in ethylene biosynthesis), ***GLUB*** (β-1,3,-Glucanase an ethylene inducible pathogenesis related protein) , ***CHI9*** (chitinase 9 a jasmonic acid pathogenesis related protein) ***CRTR-B1*** (β -carotene hydroxylase), ***FLS*** (flavonol synthase), ***OPR3*** (12-Oxophytodienoate reductase 3, a jasmonate acid biosynthesis protein), ***PAL*** (phenylalanine ammonia lyase), ***PG*** (polygalacturonase), ***P4*** (a salicylic acid inducible pathogenesis related protein). N=6, IRE=2. (Scott *et al.,* 2017b)

The observed HIPPL- and LIUV-induced resistance may, therefore, be mainly due to increased expression and/or accumulation of transcripts between treatment and the day of inoculation (10 DPT). This could result in a gradual increase in resistance following light treatment, similar to that observed by *Charles* et al. (2008) following LIUV treatment of

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tomatoes. Priming, however, may also play a role in the induction of resistance as an expression profile analogous to that of the priming response seen for *PAL.* It is also possible that the priming response for other genes monitored here may have occurred before or after 12 HPI and went unnoticed in our experimental design as such responses have shown greater levels of protein activity and gene expression > 3 hours following inoculation (Mur *et al.,* 1996, Latunde-Dada & Lucas, 2001, Cools & Ishii, 2002, Yang *et al.,* 2015). Further investigation is required to elucidate the full extent to which priming may play a role in LIUV an HIPPL induced resistance.

Summary and conclusions

In our previous study (Scott *et al.,* 2017) we showed that 16 pulses of HIPPL induced similar hormetic benefits to a 3.7 kJ/m^2 LIUV treatment on both mature green and ripe tomatoes. Utilising HIPPL reduced treatment times by 97.3 % to only 10 seconds. In this study, we have looked into similarities of the molecular mechanisms underpinning the delayed ripening and induced resistance observed following HIPPL and LIUV hormesis.

On the basis of genes monitored here we can now confirm that the HIPPL and LIUV sources elicit similar transcriptional changes following treatment. *GLUB*, *P4*, *CHI9* and *OPR3* were significantly upregulated at 10 DPT and 12 HPI. *PG* and *FLS* were significantly downregulated at 10 DPT and 12 HPI. *ACO1* and *CRT* were significantly upregulated 24 HPT whereas *PAL* was significantly upregulated at 12 HPI. Following inoculation, only *PAL* showed an expression profile analogous to that of a priming response.

Importantly we can infer that HIPPL induced resistance, similarly to that of LIUV, is due to the production of PR proteins including P4, B-1,3-Glucanase and Chitinase 9. Moreover, a reduction in polygalacturonase expression may contribute towards delayed ripening and reduced susceptibility to *B. cinerea* in HIPPL fruit; as shown for the former in LIUV-treated fruit by (Barka *et al.,* 2000).

Changes in the expression of phytohormone biosynthesis genes *OPR3* and *ACO1*, SA inducible gene *P4* elucidates that both LIUV and HIPPL treatments trigger multiple defence responses controlled by ET, JA and SA. The upregulation of ET and JA inducible *GLUB* and *CHI9* further supports this. This indicates that the benefits of HIPPL and LIUV hormesis may provide not only broad range pathogen resistance against both biotrophic and necrotrophic pathogens but also abiotic stressors. This is supported by previous work carried out on *Arabidopsis thaliana in* which it was observed that LIUV-induced resistance to both downy mildew (*Hyaloperonospora parisitica*) and grey mould (*B. cinerea*).

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Objective 3 - Assessing the Importance of Direct Tissue Exposure and Fruit Orientation during LIUV and HIPPL Treatment

Introduction

During the work carried out and described in objective 1, it was noted that following treatment the delay in colour change appeared to be most intense at the points of the fruit directly facing either the low-intensity UV-C (LIUV) or high-intensity, pulsed polychromatic light (HIPPL) light source. There is limited information on whether LIUV hormesis induces a systemic or local effect. What limited information is available seems to be dependent on the produce undergoing treatment. No information to date is available on the nature of induced resistance for the HIPPL source.

It has been previously observed by Mercier *et al.* (2000) that postharvest LIUV treatment induced a local response in carrot, *Daucus carota.* Mercier *et al.* (2000) showed that the local accumulation of a 24 kDa chitinase and phytoalexin 6-methoxymellein coincided with the induction of local resistance against *B. cinerea*. This, however, contradicts the results observed by Obande *et al.,* (2011) who showed the systemic induction of delayed ripening when treating tomato fruit preharvest on the truss. These differences may be caused by the difference in species, plant organ undergoing treatment or tissue developmental stage i.e. pre or post-harvest. Such differences are support by Petit *et al.,* (2009) who monitored biomarkers for resistance with Q-PCR. Petit *et al.,* (2009) showed that in grapevine, *Vitis vinifera*, the plants response to LIUV was both organ and developmental stage specific with no response to LIUV being observed in flowers at any developmental stage. Furthermore, berry developmental stage and size seemed to play a role in the level of response observed, with berries at fruit set showing weak changes in gene expression with increasing levels of responsiveness as the fruit grew. Finally, bunch-stems showed increased levels of two chitinases; the class I chitinases Chi1b and the class III chitinase CH3, PAL and stilbene synthase (STS) in comparison to flowers and berries. The only gene showing lower levels of transcription in bunch-stems in comparison to flowers and berries was the β-1,3-Glucanase.

In addition to the variation in responsiveness from both the developmental stage and organ treated, Stevens *et al.,* (2005) showed that alterations in treatment orientation may influence the propagation of a systemic signal and induction of systemic disease resistance. Stevens *et al.,* (2005) showed that treatment at the calyx followed by inoculation at the blossom end resulted in systemic disease resistance on apples (*Malus domestica*) against *Collectotrichum gloesporioides*, peaches (*Prunus persica*) against *Monilinia fructicola* and tangerines (*Citrus reticulate*) against *Penicillum digitatum*.

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Aims

Disease control and delayed ripening determined by colour change will be monitored to explore the response of tomato fruit to LIUV and HIPPL treatment to ascertain whether a local or systemic response occurs following treatments directed towards the side of fruit. Treatments will then be performed focused on either the blossom end or calyx to assess the importance of fruit orientation during treatment.

Materials and methods

Plant material was obtained and handled as stated in objective 1. Light treatments, pathogen maintenance, colour measurements and inoculum preparation were also performed as stated in objective 1.

Experimental design and data analysis

For the investigations concerned with the necessity for direct tissue exposure for delayed ripening, when fruit are treated from the side, 15 fruit per group were used in each experimental replicate (n=30). For disease control assays 10 fruit per group per independent replicate experiment were used (n=20). For investigations into blossom end and calyx-focused treatments 10 fruit per treatment group per experiment were used. For experiments concerning colour change, with blossom-end and calyx-directed treatments, two experimental replicates were performed (n=20). Only a single experimental replicate was performed for the experiments concerning disease resistance (n=10). Analysis was performed using statistical software package SPSS 22 (IBM). One-way ANOVA with Tukey's post-hoc testing was performed. Where the homogeneity of variances assumption could not be met Welch's robust ANOVA was performed followed by the Games-Howell post-hoc test. Statistical significance is here defined as $p \leq 0.05$. All data for individual experimental repeats can be found in appendix 3.

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Results and discussion

Exploring the necessity for direct tissue exposure when treating Fruit from the Side

To establish whether LIUV and HIPPL delayed ripening is a local response, Δ TCI was calculated for tissue directly facing the light sources and at 90 º from that directly exposed to the sources. For all groups the tissue at 90 º from the source showed no significant difference in ripening progression. When compared with directly exposed tissue, however, tissue at 90 º from the 16 and 24 pulse treatments showed a significantly greater progression in ripening to that of the directly exposed tissue (Figure 3.1). Tissue at 90 º for the LIUV treatment ripened faster than directly exposed tissue but was not statistically significant from directly exposed tissue or the control. The data presented here indicates that direct exposure to both LIUV and HIPPL is required for the induction of delayed ripening. Disease resistance was also monitored to determine whether direct tissue exposure is required for induction of hormesis. Unexposed tissue inoculations showed no reduction in AUDPC and similar levels of disease progression to that of the control (Figure 3.2). The directly exposed tissue, however, showed significant reductions following both HIPPL and LIUV treatment. It can therefore be stated that treatments employing HIPPL or LIUV sources require direct tissue exposure to successfully induce resistance to *B. cinerea*. This is in agreement with previous findings (Stevens *et al.,* 1998a; Charles *et al.,* 2008; Liu *et al.,* 2011) who routinely rotated the fruit during LIUV treatment to ensure that the entire surface area of the fruit was irradiated, although they did not specifically set out to show that failure to do so would not result in systemic resistance.

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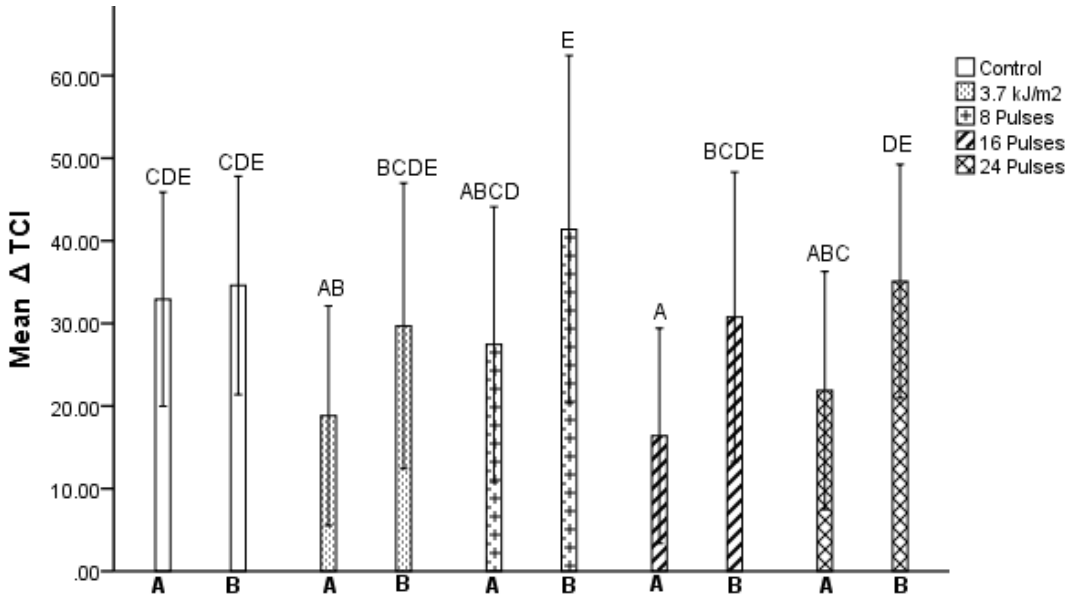

Figure 3.1: The Δ TCI (tomato colour index) from day 0 - 10 of mature green fruit from cv. Mecano. Fruit were treated with a hormetic LIUV treatment of 3.7 kJ/m^2 from a low intensity source with peak emissions at 254nm and three high intensity pulsed polychromatic light (HIPPL) treatments of 8, 16 and 24 pulses. TCI measurements were taken from tissue directly facing the light source (A) and at 90º from the source (B). Error bars show a single standard deviation; n = 30, IRE=2 (Scott *et al.,* 2017).

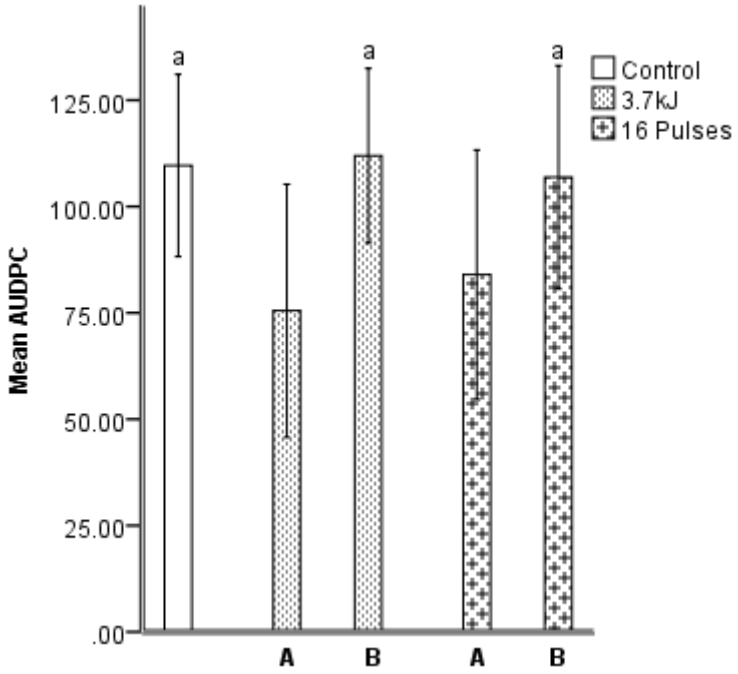

Figure 3.2: Area underneath the disease progression curve (AUDPC) of tomatoes, cv. Mecano, treated on a single side and inoculated with *B. cinerea* at 10 d post treatment (DPT). Fruit were treated with an established low intensity UV-C (LIUV) treatment of 3.7 kJ/m^2, peak emissions at 254 nm, and a high intensity pulsed polychromatic light (HIPPL) treatment of 16 pulses. Exposed tissue (**A**) or unexposed tissue (**B**). Error bars show ± 1 standard deviation; n = 20, IRE=2. Labelling indicates statistical significance. Means sharing the same label are not significantly different from each other at $p < 0.05$. (Scott *et al.,* 2017)

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These findings are supported by Mercier *et al.* (2000) who showed a local response in carrot following LIUV treatment. The results are contradictory to Obande *et al.,* (2011) who showed systemic delayed ripening following preharvest treatment of tomato fruit. As the cultivar used here and by Obande *et al.,* (2011) were the same, varietal differences cannot account for the dissimilarities in observation. The propagation of a systemic signal during preharvest treatments may, therefore, be down to differences in the developmental stage of the fruit, its attachment the plant or the unintended exposure of bunch-stems and other foliage to UV-C during fruit treatment. Petit *et al.,* (2009) showed that bunch-stems respond to UV-C to a greater extent to that of both fruit and flowers. This un-intended exposure of bunch-stems may have led to the propagation of a systemic response to treatment. Furthermore, fruit orientation during treatment may have also played a role in the production of a systemic response. This, however, is impossible to determine as information on the fruits' orientation is not given. Based on the information published by Stevens *et al.,* 2005 and the potential difference in treatment orientation from the study performed by Obande *et al.,* (2011) it was decided that the importance of fruit orientation during treatment should be explored.

Exploring the necessity for direct tissue exposure when treating fruit from the blossom end or calyx

As previously discussed, Stevens *et al.,* (2005) showed that treatment at the calyx resulted in systemic disease resistance on apples (*Malus domestica*), peaches (*Prunus persica*) and tangerines (*Citrus reticulate*). Alternative treatment orientations were, therefore, performed to establish whether directing treatments at either the blossom end or calyx would allow the translocation of a systemic signal to delay ripening.

Both the fruit receiving direct exposure to a 3.7 kJ/m^2 LIUV treatment and monitoring of colour at the calyx and blossom-end exhibited a significant reduction in colour change figures 3.3 A and C, respectively. The change in TCI was 59.8 and 59.9 % less for the calyx and blossom end-treated fruit, respectively. For fruit receiving indirect treatment the TCI changed to a greater extent to that of their respective controls. These differences, however, were not significant (Figure 3.3 B and D). This indicates that direct tissue exposure is required for the induction of delayed ripening LIUV on fruit treated from both the blossom-end and calyx.

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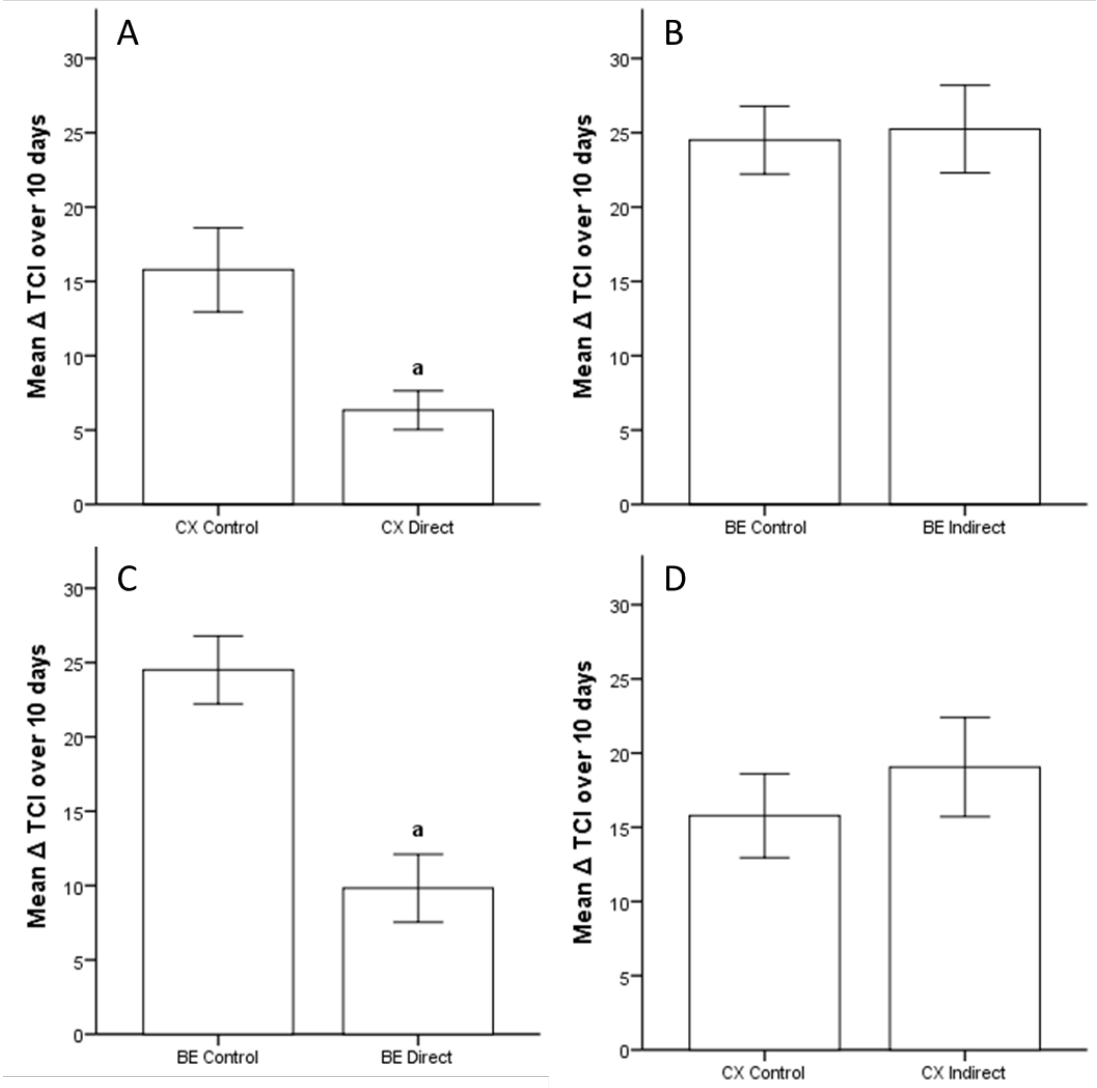

Figure 3.3: The change in tomato color index (TCI) over ten days of storage following a 3.7 kJ/m^2 treatment with a low intensity UV-C source (LIUV). **A)** Measurements taken from the calyx (CX) of control fruit and fruit treated at the CX. **B)** Measurements taken from the blossom end (BE) of control fruit and of fruit treated at the CX **C)** Measurements taken from the BE of control fruit and fruit treated at the BE. **D)** Measurements taken from the CX of control fruit and the CX of fruit treated at the BE. N=20, IRE=2. Bars show ± 1 S.E.M.

For the monitoring of induced disease resistance an identical pattern was observed with both direct treatments which showed a reduction in disease progression and indirect treatments resulting in similar or increased levels of disease in comparison to the control (Figure 3.4). Fruit inoculated and treated from the calyx showed a significant reduction in disease progression with a 47.0 % reduction in comparison to the control (Figure 3.4 A). Similarly, the fruit treated and inoculated at the blossom end showed a 33.7 % reduction in disease progression (figure 3.4 C). This reduction was not significant and further experimental replicates are required. The results from directly exposed tissue are in contrast to fruit receiving treatment from the calyx and inoculated at the blossom end, and treatments at the

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blossom end and inoculation at the calyx. Both of the treatments showed similar or slightly increased levels of disease progression in comparison with the control (Figures 3.4 B and D).

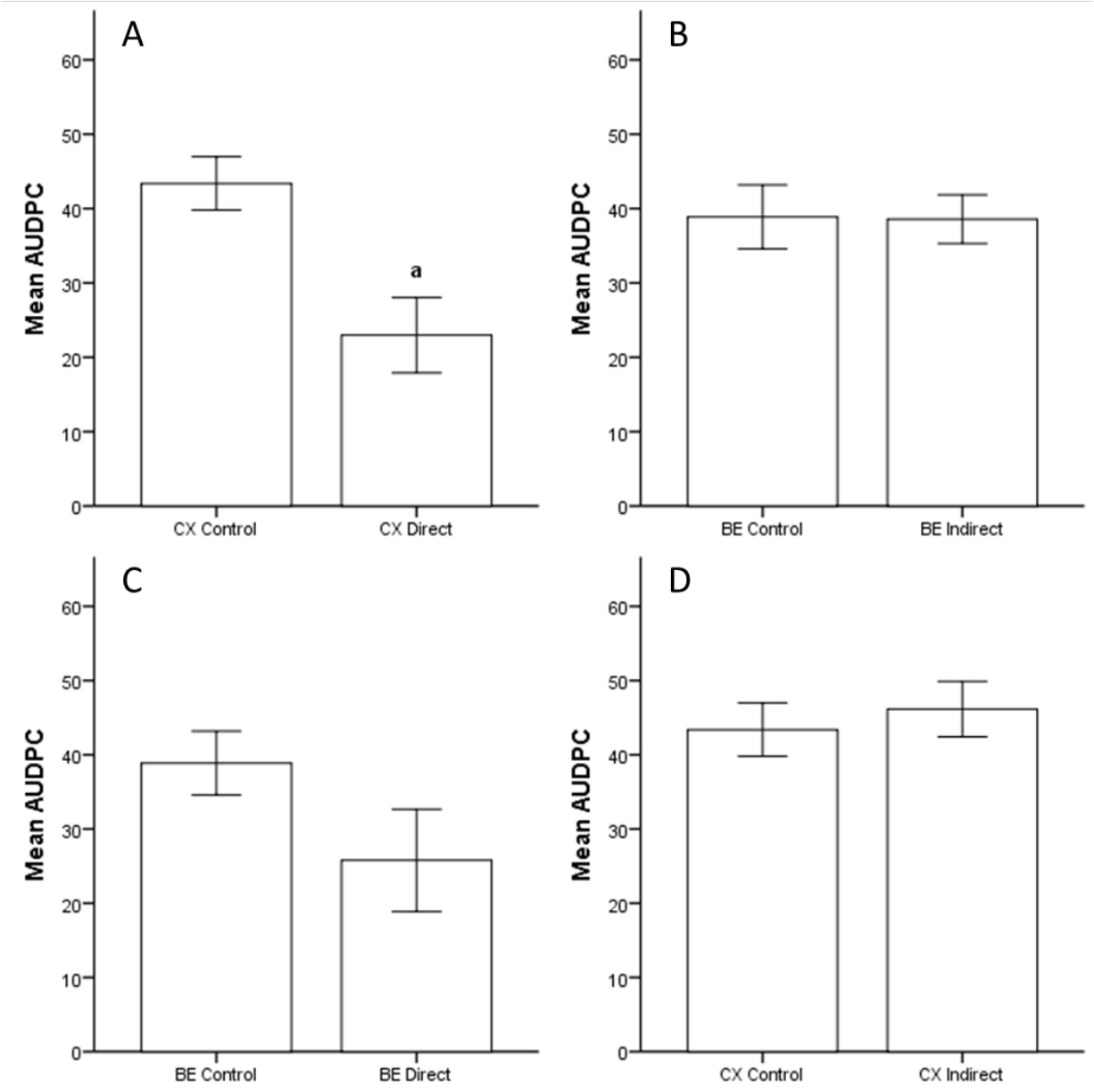

Figure 3.4: Area underneath the disease progression curve (AUDPC) from tomato fruit of the cv. Mecano following a 3.7 kJ/m^2 treatment with a low intensity UV-C source (LIUV) and inoculation with *B. cinerea*. **A)** Measurements taken from the calyx (CX) of control fruit and fruit treated at the CX. **B)** Measurements taken from the blossom end (BE) of control fruit and of fruit treated at the CX **C)** Measurements taken from the BE of control fruit and fruit treated at the BE. **D)** Measurements taken from the CX of control fruit and the CX of fruit treated at the BE. N=10, IRE=1. Bars show ± 1 S.E.M.

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Treatments with the HIPPL source yielded very similar results to that of those observed with treatments using LIUV for both the monitoring of disease resistance and delayed ripening measured by colour change. For tomatoes treated directly at the calyx and blossom end a delay in colour change was observed with reduction of 40.8 and 12.8 %, respectively (Figures 5.5 A and C). These differences, however, were not significantly different which may be due to increased deviation from the mean in the sample populations. Further experimental replicates are required.

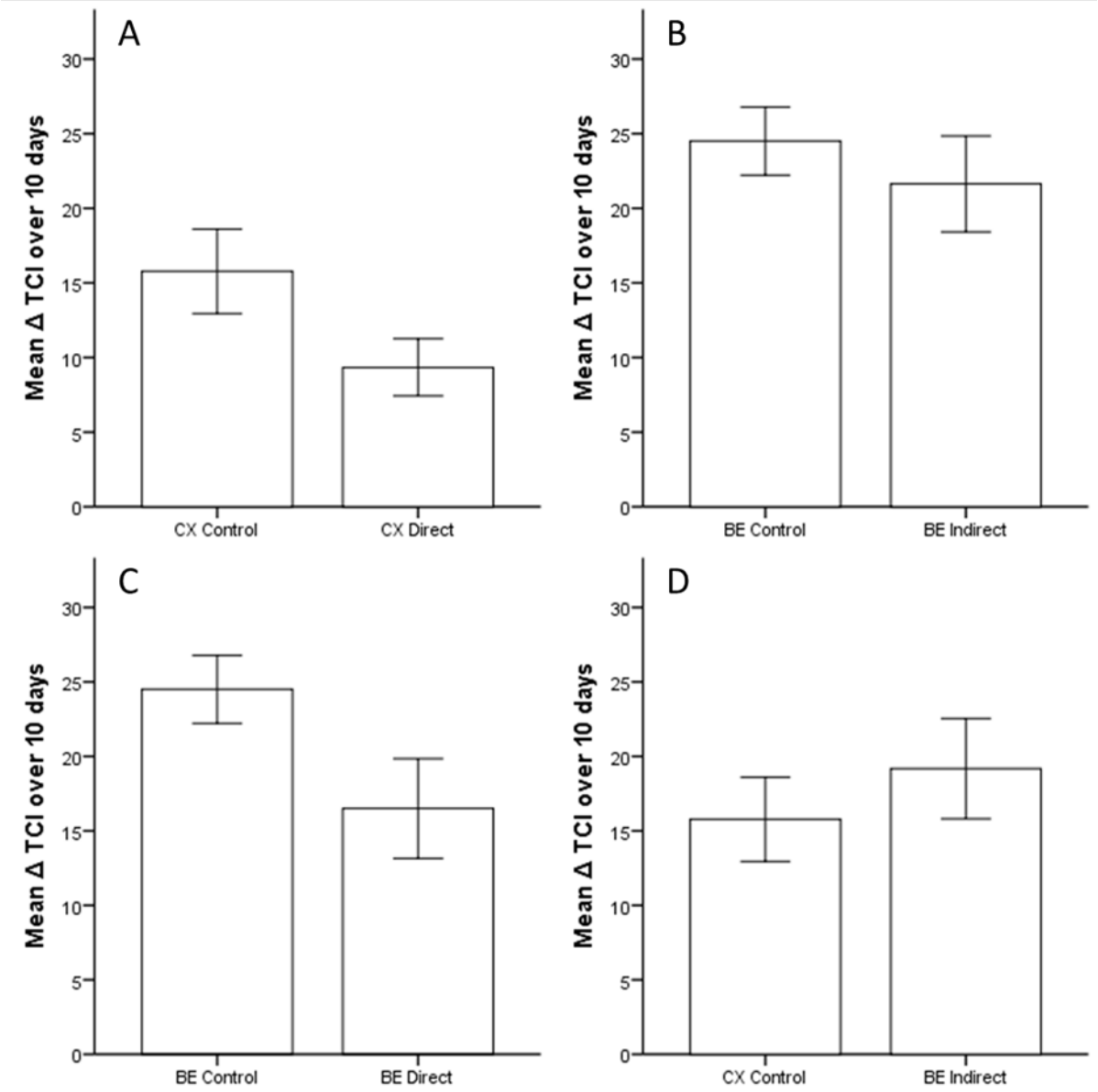

Figure 3.5: The change in tomato color index (TCI) over ten days of storage following a 16 pulse treatment with a high intensity pulsed polychromatic light (HIPPL) source. **A)** Measurements taken from the calyx (CX) of control fruit and fruit treated at the CX. **B)** Measurements taken from the blossom end (BE) of control fruit and of fruit treated at the CX **C)** Measurements taken from the BE of control fruit and fruit treated at the BE. **D)** Measurements taken from the CX of control fruit and the CX of fruit treated at the BE. N=20, IRE=2. Bars show ± 1 S.E.M.

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Unlike the LIUV source, a slight decrease in colour change was observed for the fruit treated at the calyx with colour monitored at the blossom end (Figure 3.5 B). This was not statistically significant and further testing would be required to determine this definitively. It is, however, likely to be due experimental variation as treatment from the blossom end while monitoring the calyx gave an increase in ripening in comparison to the control, figure 3.5 D, as has been seen for all LIUV treated fruit for both colour change and disease resistance, (Figures 3.4 and 3.3).

Treatment orientation also showed no difference in the acquisition of a systemic response for HIPPL-treated fruit when monitoring disease resistance. Again, both tissue directly treated at the blossom end and calyx showed reductions in disease progression when compared to the control. Reductions were 53.8 and 23.6 % for the calyx and blossom end respectively (Figures 3.6 A and C). The former was significantly different from the control. As was seen for LIUV-treated fruit, the reduction in disease progression when fruit are treated from the blossom end was not as great as that from the calyx. With reductions of 53.8 and 23.6 % for the HIPPL-treated fruit from the blossom end and calyx, respectively, and 47.0 and 33.7 % for LIUV-treated fruit. This may indicate that not only do specific plant organs vary in their sensitivity and reactivity to UV-C, as shown by Petit *et al.,* (2009), but there may also be a differential response in spatially separated tissues of those organs. This is supported by evidence from Stevens *et al.,* (2005) who also showed that levels of induced resistance varied depending on the orientation of treatment.

One could hypothesise that the tissue of the blossom end of fruit would contain fewer photoreceptors to those at the calyx, as its natural positioning is towards the ground. Alternatively, its cellular homeostasis may be geared away from any severe adaptation to changes in lighting conditions and may therefore respond to a lesser extent to treatment. The reduction in the level of delayed ripening when treated from the blossom end can be seen in figure 3.7 where treatments from the calyx (C) and side (A) produce islands of chlorophyll-rich tissue whereas the treatment from the blossom end (B) the production of carotenoids has become evident, but to a lesser extent to that of the untreated tissue at the calyx.

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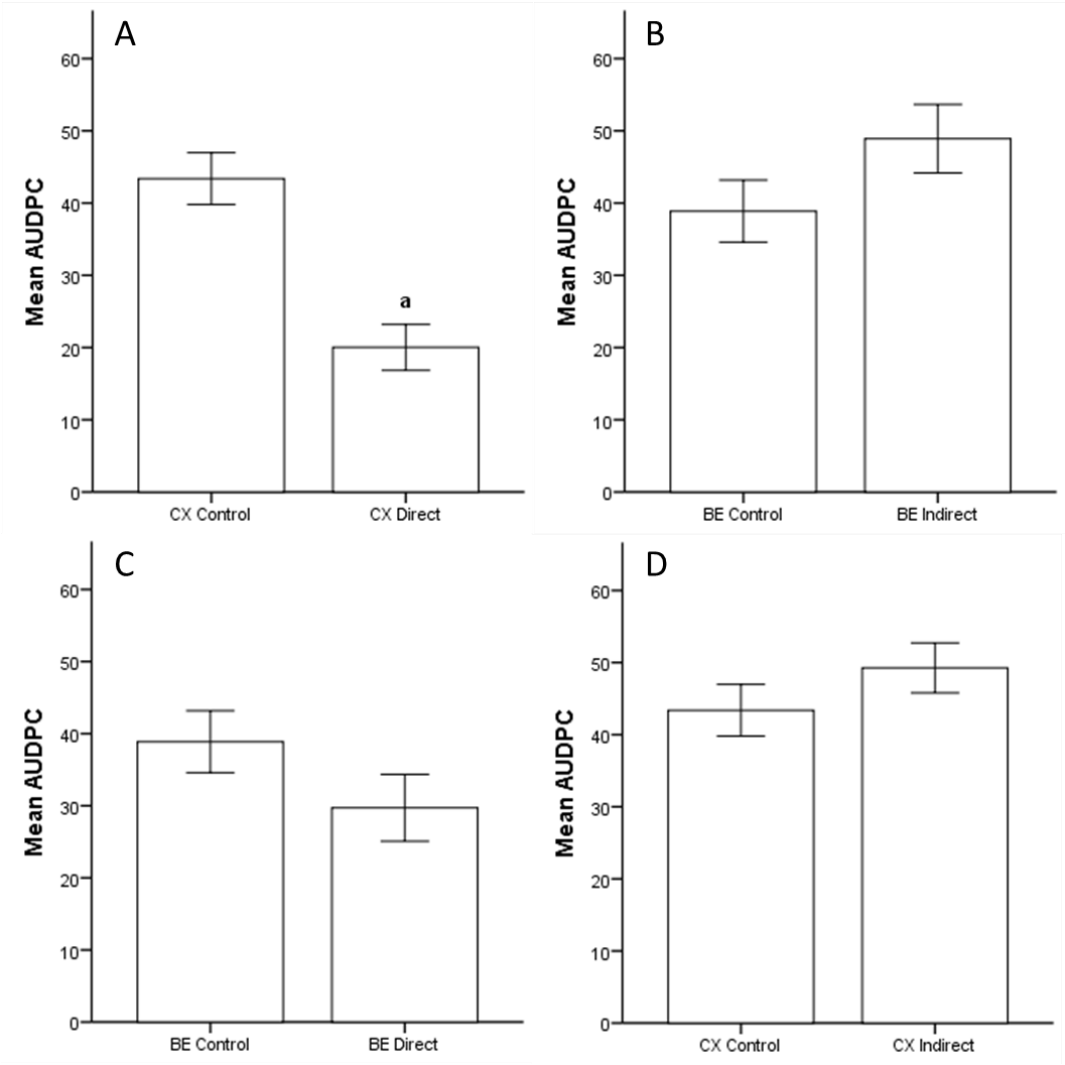

Figure 3.6: Area underneath the disease progression curve (AUDPC) from tomato fruit of the cv. Mecano following a 16-pulse treatment with a high intensity pulsed polychromatic light (HIPPL) source and inoculation with *B. cinerea*. **A)** Measurements taken from the calyx (CX) of control fruit and fruit treated at the CX. **B)** Measurements taken from the blossom end (BE) of control fruit and of fruit treated at the CX **C)** Measurements taken from the BE of control fruit and fruit treated at the BE. **D)** Measurements taken from the CX of control fruit and the CX of fruit treated at the BE. N=10, IRE=1. Bars show ± 1 S.E.M

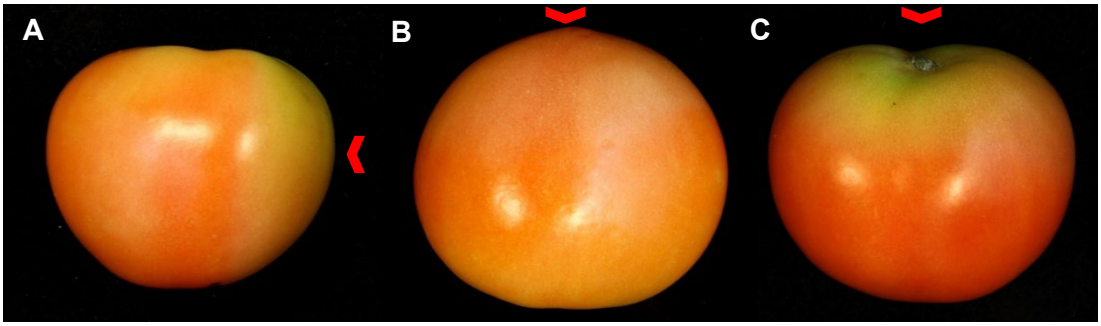

Figure 3.7: Representative samples of tomato fruit exposed to polychromatic light from different orientations. Fruit, cv. Mecano, were treated with 16 pulses of high-intensity, pulsed light (HIPPL) and photographed at 10 days post treatment. Red arrows indicate the positioning of the HIPPL source. **A**) Treatment from the side. **B**) Treatment from the blossom end. **C**) Treatment from the calyx (Scott *et al.,* 2017)

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Summary and conclusions

In this study preliminary observations from objective 1, in which uneven ripening progression was observed, were built upon. It was shown by Stevens *et al.,* (2005) that treatments of apples, peaches and tangerines from the calyx resulted in the induction of disease resistance in tissues not directly exposed to the source both the side of the fruit and at the blossom end.

Here, both LIUV and HIPPL sources showed the necessity for direct tissue exposure for both a delay in colour change and induced resistance against *B. cinerea* when treated from either the side or blossom end or calyx. The data collected for fruit treated from the side provides comprehensive evidence that direct exposure is a necessity for the induction of the full benefits of LIUV and HIPPL hormesis. The data collected for treatments directed at the blossom end and calyx, however, should be used with caution as only one experimental replicate was performed for monitoring disease resistance with a small sample size of 10 per treatment group. It was the intention to have total sample sizes of 30 fruit per treatment group. Unfortunately, this could not be achieved as the commercial production of cv. Mecano had ceased.

The data collected here directly contradicts that of Stevens *et al.,* (2005), however, a different fruit is being used which highlights the fact that different species, genera and cultivars may respond to LIUV and HIPPL treatments differently. Furthermore, the spatial location of tissues within plant organs may also respond differently to treatment. This is supported by work done by both Stevens *et al.,* (2005) and Petite *et al.,* (2009) who showed differing levels of resistance depending on the spatial local of the tissue undergoing treatment and also differing level of reactivity to UVC depending on the plant organ undergoing treatment, respectively. In depth analysis of the variation of response to spatially distinct tissues require much greater investigation.

When considering the data as a complete set, however, the evidence suggests that, for tomato fruit, direct tissue exposure is required for the induction of full hormetic benefits. This may provide complications when integrating either LIUV or HIPPL treatments into the commercial production line as the fruit would require either rotation or treatment from multiple sources suitably positioned. Further problems would arise with the treatment of high sugar, high value tomatoes that are sold on the vine. Such varieties cannot tolerate a large amount of physical manipulation before fruit would fall from the vine.

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Objective 4 - Assessing the Importance of UV-C, B and A and visible light within the High Intensity Pulsed Polychromatic Light Source, for Inducing the Hormetic Effects Observed on Tomato Fruit cv. Mecano

Introduction

In objective 1 it was established that high-intensity, pulsed polychromatic light (HIPPL) treatments can induce both disease resistance and delayed ripening on tomato fruit to similar levels to that observed with LIUV treatment. Unlike the low-intensity UV-C (LIUV) source, the HIPPL source emits broad spectrum (polychromatic) light. It is unclear to what extent wavelengths longer than UV-C contribute to HIPPL-induced resistance.

Previously published research suggests that both postharvest UV-B and UV-A treatments can lead to delayed ripening, delayed senescence and disease resistance. UV-B and UV-A can delay senescence in broccoli and UV-B can increase dietary value and colour of apple (Hagen *et al.,* 2007; Alamla *et al.,* 2009). Furthermore, a 20 kJ/m^2 UV-B treatment successfully reduced tissue softening and ripening during storage of tomato fruit (Liu *et al.,* 2011). Recent work on postharvest UV-B treatments of tomato fruit by Kasim & Kasim (2015), however, showed that 0.5 and 1.1 kJ/m^2 treatments showed an increase in the colour (L* value) and hue towards the end of the red spectrum following the 0.5 kJ/m^2 treatment. This suggests that fruit respond to UV-B in a dose responsive manner.

Little research has been performed on the effects of postharvest UV-A treatment on tomato fruit, and the published research showed no affect in tomato fruit following three treatments of 0.02, 0.5 and 2 mW/cm^2 (Maneerat *et al.,* 2003). There is, however, an immediate problem with the analysis of the data conducted by these authors. Their stated total treatment doses are presented as mW/cm^2 which is, in fact, a measure of light intensity. Total treatment energy (dose) is the product of intensity and treatment time, and should be given in joules (watts x seconds). A potential factor in these authors' inability to observe any effect on tomato fruit may be the use of a 25 º C storage temperature. During preliminary work it was noted that storage of the fruit at 21 º C following treatment failed to produce any visible signs of delayed ripening for the established 3.7 kJ/m^2 UV-C treatment. Whereas lower temperatures of 13 ºC showed visible signs of delayed ripening. Such induced effects may, therefore, be dependent on post-treatment environmental conditions. For example post-treatment exposure to visible light leads to photoreversal and the absence of any hormetic benefits.

It is established that storage of tomato fruits under visible light leads to increased ripening when measured in terms of colour change (Boe & Salunkhe, 1967). The effects of high intensity visible light and its ability to induce hormesis, however, have not been explored to a

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great extent. It is known that high intensity visible light can lead to the loss of viability of microrganisms such as *Escherchia coli, Porphyromonas gingivalis, Staphylococcus aureus* and *Fusobacterium nucleatum,* human pathogenic bacteria (Lipovsky *et al.,* 2008). By definition hormesis is a phenomenon where low doses of a stressor bring about a positive change in the organism undergoing treatment. As high intensity visible light can cause damage to a broad range of cells and organisms, including plants, the ability for visible light to induce hormesis remains viable and a possibility which requires investigation.

Further evidence supports high intensity visible light-induced hormesis in plants. Firstly, exposure of plants to high intensity visible light can lead to the production of reactive oxygen species (ROS), (Schmitt *et al.,* 2015). ROS production and signalling can play an important role in plant's adaptation to stress (Vidhyasekaran, 2015) and is one of the key potential components that may lead to UV-C-induced hormesis. ROS production is also noted as a potential mechanism for the bacteriocidal effect of visible light (Lubart *et al.,* 2011). Secondly, the hormetic or photobiomodulatory effects of visible light on wound healing have been observed in a number of studies; as reviewd by Tchanque-Fossuo *et al.,* (2016). The bacteriocidal effects of visible light, however, cannot be discounted. Finally, it has also been observed that growing plants under high intensity visible light, for intermittant periods, can induce disease resistance (Al-Jafar, 2016).

Aims

The aim of this study is to ascertain the relative contribution of UV-C, UV-B and UV-A in the HIPPL source for inducing both disease resistance and delayed ripening.

Materials and methods

Fruit were obtained and light treatments were performed as stated in objective 1. The addition of 5 mm Borofloat ® 33 UV-C filtering glass (Schott, UK) was used to filter UV-C from the HIPPL source. The glass was cut to 500 x 300 (+/-1.0) mm and placed into a bespoke frame. The frame was mounted onto the front of the HIPPL source. The optical transmission of the glass can be seen in table 4.1 and figure 4.1. Inoculum preparation and colour and firmness measurements were performed as stated in objective 1.

Table 4.1: Average optical transmittance for the major electromagnetic radiation groups emitted by the high intensity pulsed polychromatic light (HIPPL) source.

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Light group	Wavelengths (nm)	Average Optical Transmittance (%)
UV-C	100-279	<0.1
UV-B	280-324	36.4
UV-A	325-379	87.7
Visible light	380-700	92.0

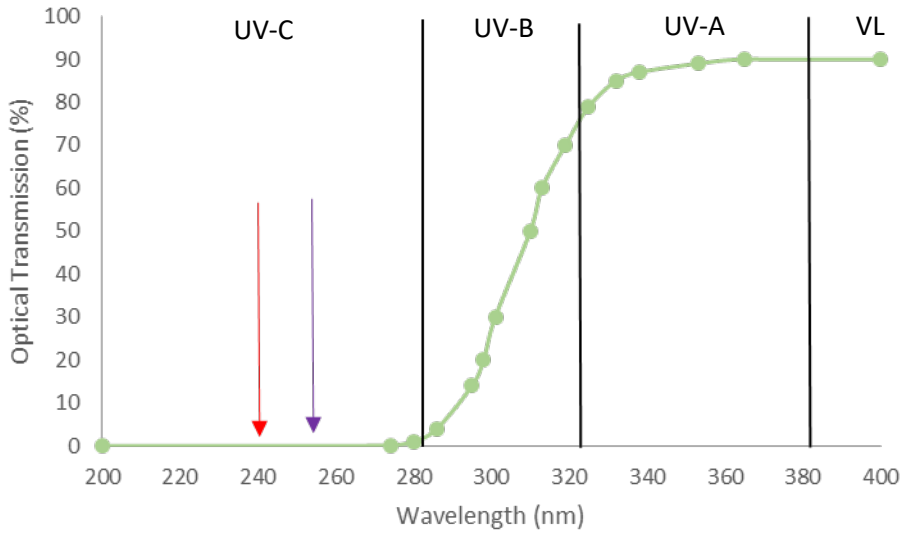

Figure 4.1: Optical transmission for the Borofloat ® 33 UV-C filtering glass (Schott). The red arrow indicates the optical cut off point (minimum wavelength) emmited byt the high intensity pulsed polychromatic light (HIPPL) source. The purple arrow indicates the standard peak emission for low intensity UV-C (LIUV) sources (254 nm). Vertical lines indicate the groups of electromagnetic radiation UV, groups C, B and A along with VL (visible light).

Experimental design data analysis

All data presented here were collected from two replicate experiments. Ten fruit per treatment group per experiment were used. Analysis was performed using statistical software package SPSS 22 (IBM). One-way ANOVA with Tukey's post-hoc testing was performed. Where the homogeneity of variances assumption could not be met, Welch's robust ANOVA was performed followed by the Games-Howell post-hoc test. Statistical significance is here defined as $p \leq 0.05$. All data for individual experimental repeats can be found in appendix 4.

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Results and discussion

HIPPL treatments, either with or without UV-C, significantly reduced disease progression of *B. cinerea* in comparison to the control (Figure 4.2). Full spectrum HIPPL treatments reduced disease progression by 50.5 % whereas treatments without UV-C only gave a 21.8 % reduction. This indicates that UV-C in the HIPPL source accounts for 56.8 % of the disease control.

HIPPL treatments with the UV-C filtered out also led to a delay in the ripening process following treatment (Figure 4.3). HIPPL and UV-C treatments led to a 44.1 and 16.6 % reduction in ripening progression, respectively. A similar contribution was also seen for the involvement of UV-C in the response to delayed ripening where UV-C in the pulsed source accounted for 62.4 % of the observed delayed ripening. For delayed ripening, however, the UV-C treatment was not significantly different to the control. Further experimental replicates are required. Unfortunately, further replications were not possible due to the ceasing in commercial production of the cv. Mecano during this time.

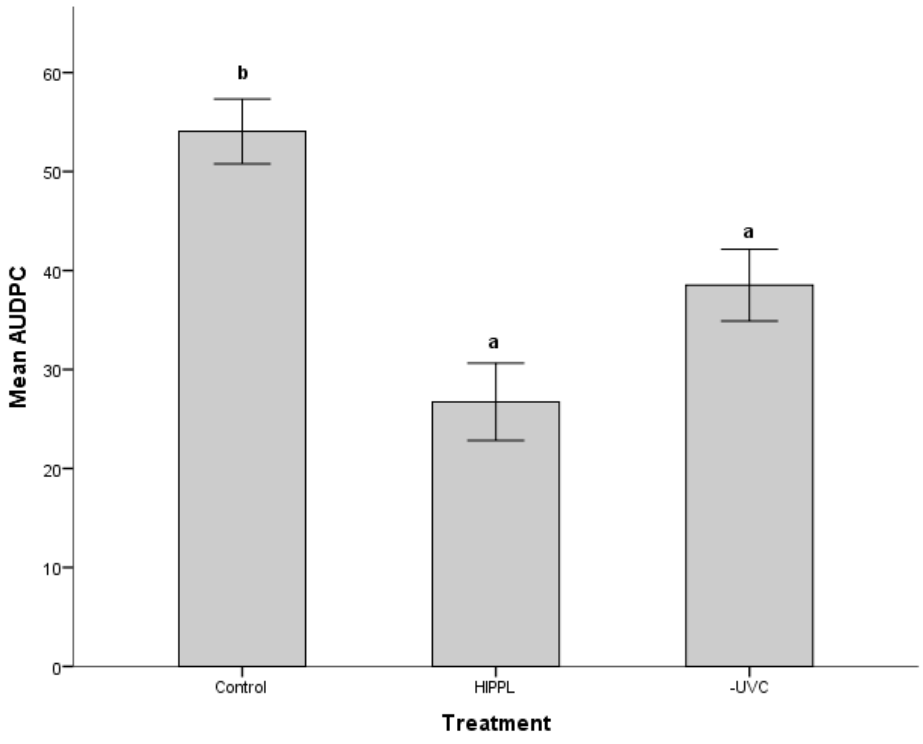

Figure 4.2: Area underneath the disease progression curve (AUDPC) from tomatoes of the cv. Mecano inoculated with *B. cinerea.* Inoculations were performed following treatment with 16 pulses of high intensity pulsed polychromatic light (HIPPL) and a 16 pulse HIPPL treatment utilising a UV-C filter (Schott) which removes wavelengths below 280 nm. N=20 and IRE=2. Error bars show ± 1 S.E.M.

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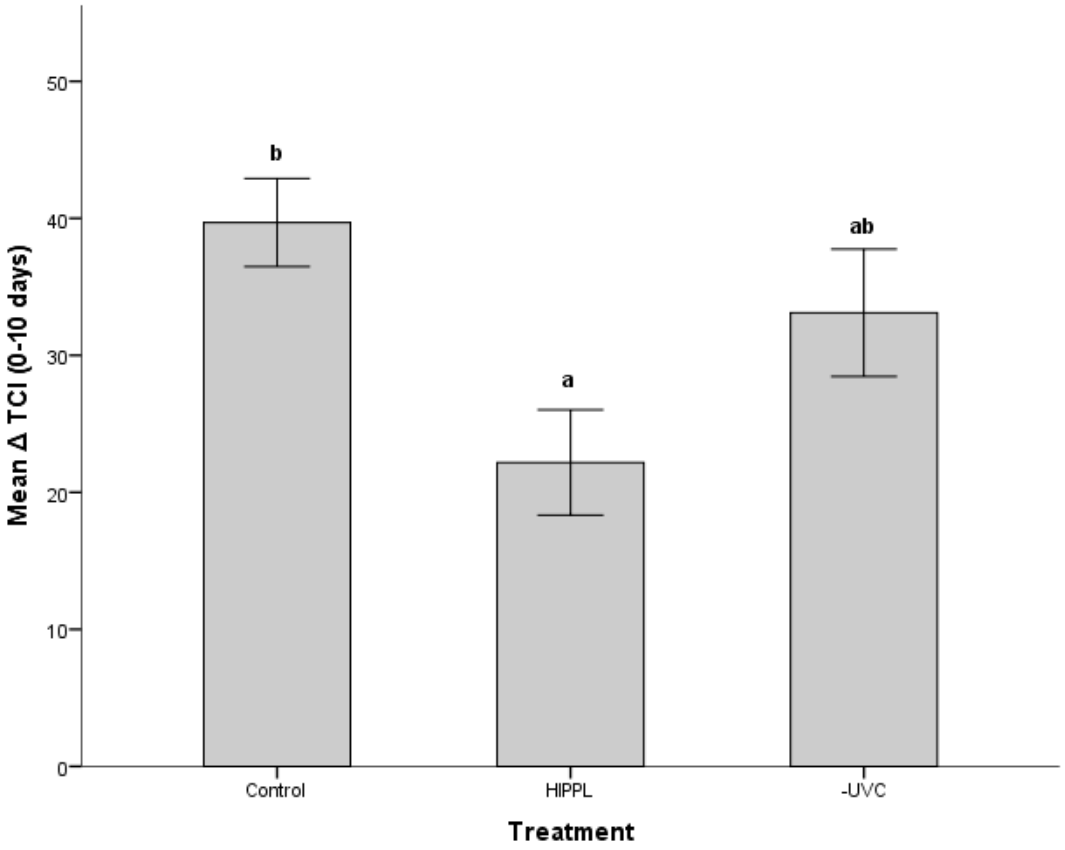

Figure 4.3: The change (Δ) in tomato colour index (TCI) over 10 days from tomatoes of the cv. Mecano following treatment with 16 pulses of high intensity pulsed polychromatic light (HIPPL) and a 16 pulse HIPPL treatment utilising a UV-C filter (Schott) which removes wavelengths below 280 nm. N=20 and IRE=2. Error bars show ± 1 S.E.M.

The HIPPL source emits a broad range of electromagnetic radiation from 240 – 1000 nm including UV-C, B and A. The ability of the HIPPL source to continue to induce resistance and delay ripening in the absence of UV-C is not unexpected. As previously discussed, UV-B has shown the potential to induce resistance and delay the ripening of tomato fruit (Liu *et al.,* 2011). Previously published work on postharvest UV-A treatment of tomatoes, however, showed no effects on tomato (Maneerat *et al.,* 2003). It is worth noting, however, that the intensity of dose delivery and total treatment dose of these treatments of the previous studies are not comparable to those delivered by the HIPPL source used here. Further investigation is required to elucidate whether postharvest UV-A treatments, delivered from LIUV and HIPPL sources, can elicit a hormetic response in tomato fruit.

It is difficult to determine the factors leading to such a high reliance on UV-C to induce the full effects of HIPPL treatments. It may be due to a number of factors including a peak in spectral irradiance in the germicidal UV region (Middleton, 2015). Furthermore, UV-C is the most biologically active region of the UV spectra. Smaller doses, therefore, would be required to stimulate a beneficial response. This can be highlighted by the much greater UV-B treatment

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that is required for successful induction of delayed ripening when treating light from this portion of the spectrum (20 kJ/m^2). This is in comparison to the much smaller (3.7 kJ/m^2) dose required for successful induction of UV-C hormesis (Charles *et al.,* 2008; Liu *et al.,* 2011).

There is no previously published literature noting hormesis or disease control from visible light treatments. Furthermore, there is literature stating the opposite; increased tomato ripening following exposure to visible light (Boe & Salunkhe, 1967). The intensity and duration of visible light delivery in Boe & Salunkhe (1967), however, differs drastically in comparison to that delivered by the HIPPL source. The intensity of the lighting delivered was approx. 10 lux, similar to that of fluorescent tube lighting in an office, and also delivered constantly over a 15 day period. Here, light is delivered in 320 µs pulses at extremely high intensities. We have estimated, from extrapolation of manufacturers data that 4.6 kJ/m^2/pulse of polychromatic light is delivered at fruit level. As the pulse duration is known it is possiblie to estimate that the intensity of the visible light (at 555 nm) from the HIPPL source is approximatley 9 million times greater than that of the lighting used by (Boe & Salunkhe, 1967). The effects of such high intensity lighting and its ability to induce hormesis have not been explored. Exposure of plants to high-intensity visible light, however, can lead to the production of ROS (Schmitt *et al.,* 2015). ROS production and signalling can plays an important role in plants adaptation to stress (Vidhyasekaran, 2015). It could therefore be hypothesised that hight-intensity visible light alone, could lead to the induction of hormesis. Further investigation is required to asses this hypothesis.

Summary and conclusions

In this study, it was found that UV-C was not essential for the induction of hormesis following HIPPL treatment. It did, however, account for 56.8 and 62.4 % of the observed disease resistance and delayed ripening, respectively. Differences in the spectral irradiance around the UV-C region of the HIPPL source and the greater biological action of UV-C may account for the large authority of UV-C in the HIPPL treatment. Further investigation is required to determine the extent to which UV-B, UV-A and visible light play in the elicitation of the hormetic response.

It was the intention of this study to further analyse the importance of UV-B and UV-A for the induction of hormesis in tomato fruit. Due to the cessation of production of the cv. Mecano by the industrial partners, in the final months of the project, it was decided that research into HIPPL postharvest tomato treatments would have to be suspended.

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Objective 5 – Pre-harvest foliar low-intensity UV-C and high-intensity, pulsed polychromatic light treatments of lettuce

Introduction

Until recently the focus of UV research on lettuce has been two fold, the first of which is postharvest UV-C treatments for elongation of shelf life and surface decontamination of minimally-processed lettuce. Secondly, preharvest research into the effects of restoring natural UV-B and UV-A 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 UV-C, as only natural microbial populations were monitored, and induced resistance cannot be inferred.

Research on the use of UV-permeable sheeting and supplementary UV-B lighting for protected lettuce crops has shown a number of induced effects such as the production of a more compact plant, reduction in mass, changes in colouration and a reduced incidence of diseases caused by *Bremia lactucae* and *Botrytis cinerea* (Paul *et al.,* 2012; Wargent *et al.,* 2005). Park *et al.* (2007) treated lettuce with 1.65 kJ/m^2 of UV-B per day for 10 days and observed that an increase in red colouration correlated with accumulation of anthocyanins. Phenolic compounds including anthocyanins and flavonoids have been shown to have direct antimicrobial effects. Furthermore, they observed upregulation of putative genes involved in signal transduction, disease, defence and secondary metabolism amongst others.

Recently, UV-C 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. Moreover, treated plants showed accumulation and significant increases in the levels of the ROS H_2O_2 at 4 DPI which may be playing a role in defence, aside from its role in cellular signalling, as the greatest difference in disease was observed at 4 DPI. One would expect the application of doses employed here to be similar for both pre and post-harvest treatments and could infer from this report that UV-C can successfully provide pre-harvest protection.

Aims

Pre-harvest LIUV treatments of lettuce were previously shown by Ouhibi *et al.* (2014) to have the potential to provide post-harvest disease resistance. In objective 1, it was observed that

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new high-intensity pulsed polychromatic light (HIPPL) sources can induce resistance on tomato fruit (cv. Mecano) following postharvest treatment. Here, it is intended to extend these findings and build upon this data to show the scope and longevity of the protection of the two contrasting light sources HIPPL and LIUV. Resistance assays against *B. cinerea, Rhizoctonia solani, B. lactucae* and *Sclerotinia sclerotiorum* will be performed. This data will be used to determine a treatment regime suitable for commercial glasshouses.

Materials and methods

Plant husbandry

Commercial butterhead lettuce varieties Amica and Temira (Enza Zaden) were germinated in 25 mm rockwool propagation cubes (Grodan) in the glasshouse until emergence of roots from the cubes (approx. 14 days). Seedlings were then placed into 3" Delta cubes (Grodan) and then transferred to an NFT system. Venting of the glasshouse was set to open above 20 °C in the day and 16 °C during the night. During the winter months LED assimilation lighting was used to extend the photoperiod to 16 hours.

LIUV and HIPPL treatments

Lettuce plants were subjected to treatment with both high-intensity pulsed polychromatic light (HIPPL) and conventional low-intensity UV-C sources (LIUV). Pulsed treatments were delivered from 40 cm distance from the source and conventional treatments were delivered at either 2000 μW/cm^2 or 1000 μW/cm^2 from the apical leaf. Treatments were performed at the 3-5 and 6-8 true leaf stages along with plants at early, mid and late head formation.

Damage assessments following HIPPL and LIUV treatment

Damage to the lettuce plants was visually inspected at 2 DPT or 5 DPT and recorded qualitatively as simply the presence or absence of damage. Damage manifested itself as dry, brown necrotic lesions and brown vascular discolouration; examples of which can be seen in figures 5.1 and 5.2.

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Figure 5.1: 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 5.2: A lettuce plant at early head formation treated with 45 pulses showing veins with a yellow/brown hue as a symptom of mild damage caused after UV treatment with the high intensity pulsed source.

Pathogen propagation, inoculum preparation and disease resistance assays

For calibrated spore solution inoculations 10 µl of spore solution was pipetted into the centre of the leaf disc. Inoculations utilising agar plugs were performed with a 3 mm cork-borer. Agar plugs were taken from 3-5 day old cultures, dependant on species, and placed into the centre

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of leaf disks. Cultures were grown and spore suspensions were made as described for objective 1.

A leaf disc bioassay based on the method of Laboh (2009), was used to assess disease resistance following treatment. Briefly, 20 mm leaf discs were cut with a cork borer and placed into 120 mm square Petri dishes with a maximum of 16 leaf discs per plate. Prior to this, the plates were filled with 25 ml of molecular grade agar (Oxoid) to prevent leaf desiccation and to provide humidity for pathogen growth.

Leaf discs were then inoculated with either *B. cinerea*, *R. solani* or *S. sclerotiorum*. At 2 and 3 days post inoculation (DPI) photos of the leaf discs were taken and analysed in Image-J. This allowed the calculation of the diseased area in mm^2. Taking measurements at multiple time points allowed the monitoring of disease progression over time. Data from the two time points were used to calculate the area under the disease progression curve; equation 1.2.

Experimental design and statistical analysis

The plants for each experiment were grown in a single NFT system with four gutters, thus maintaining equal nutrient supply to all plants in a single experiment. Plants were grown to their required size in the NFT system and a completely random design was used where treatments were randomly assigned to each plant in the experiment. Statistical analysis was performed either by ANOVA or Kruskal Wallis (non-parametric ANOVA) where the assumptions of normality and homogeneity of variance could not be met. Statistical significance is here defined as p=0.05. All the results from individual experimental replicates in this chapter can be found in appendix 5.

Results and discussion

LIUV and HIPPL damage thresholds

Damage to lettuce following both LIUV and HIPPL treatments was observed as dry brown lesions and vascular discolouration (Figures 5.1 and 5.2). Damage susceptibility for both LIUV- and HIPPL-treated lettuce varied throughout the year on the cv. Amica. During March, damage was seen above 60 pulses and 2.25 kJ/m^2 for the HIPPL and LIUV sources, respectively (Figure 5.3). For April, the beginning of the commercial growing season, 45 pulses was observed to cause damage to the plants; LIUV treatments were not tested (Figure 5.4). Between April and October no damage was observed for any of the treatments. Treatments, however, were all >45 pulses.

From October onwards a dramatic reduction in the damage threshold for both HIPPL and LIUV treatments was seen. HIPPL-treated plants exhibited damage for treatments ≥ 16

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pulses (Figure 5.5). Furthermore, LIUV treatments showed damage above 0.36 kJ/m^2. Up to this point in the LIUV trials doses of UV-C were applied at 2000 µW/cm^2 which gave a total treatment time of 18 seconds for a 0.36 kJ/m^2 treatment. As treatment times now had to be further reduced it was decided that treatments would be delivered at an intensity of 1000 µW/cm^2 to provide greater control of the dose application.

At this point it is unclear what was influencing the damage susceptibility to LIUV and HIPPL treatments. Potential factors include the number of daylight hours and sunlight intensity. Changes to daylight hours, light intensity and natural UV-B/UV-A exposure have been shown to lead lettuce plants to adapt to their environment and alter the levels of light-quenching phenolic pigments carried in their leaf (Romani *et al.,* 2002, Kang *et al.,* 2013). This, however, is not definitive due to the highly integrated nature of the plants further homeostatic responses to other environmental factors, including temperature and osmotic stress, which may also play a role in the plants variation in LIUV and HIPPL damage susceptibility.

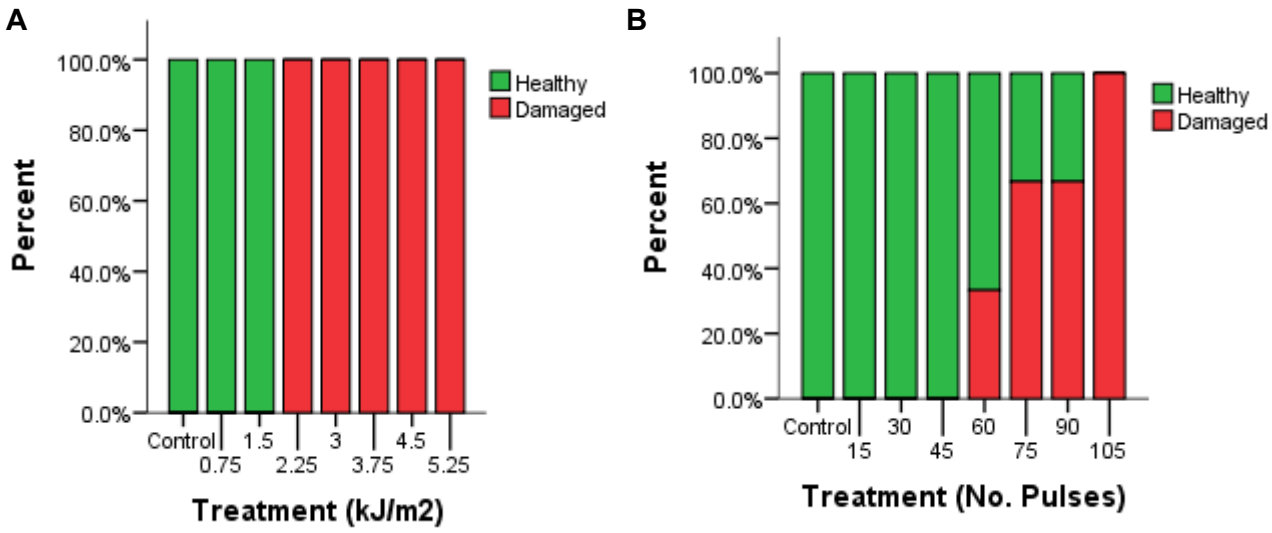

Figure 5.3: The percentage of healthy and damaged plants for the lettuce variety Amica treated during March 2015. Plants were either treated with a conventional low pressure mercury UV-C source (**A**) or a high intensity pulsed polychromatic source (**B**), n=3.

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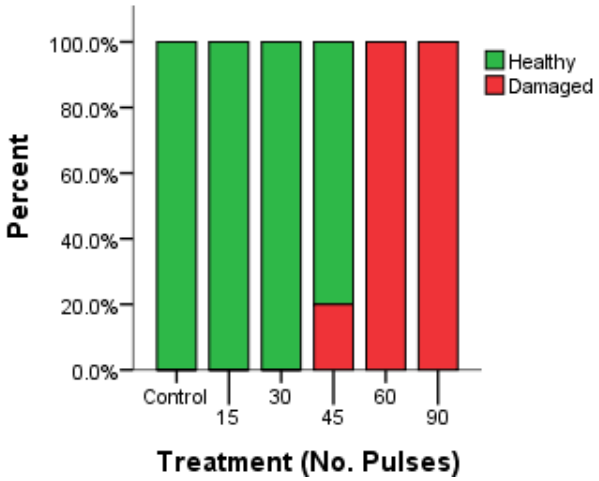

Figure 5.4: The percentage of healthy and damaged plants for the lettuce variety Amica treated during April 2015. Plants were treated with a high intensity pulsed polychromatic source, n=5.

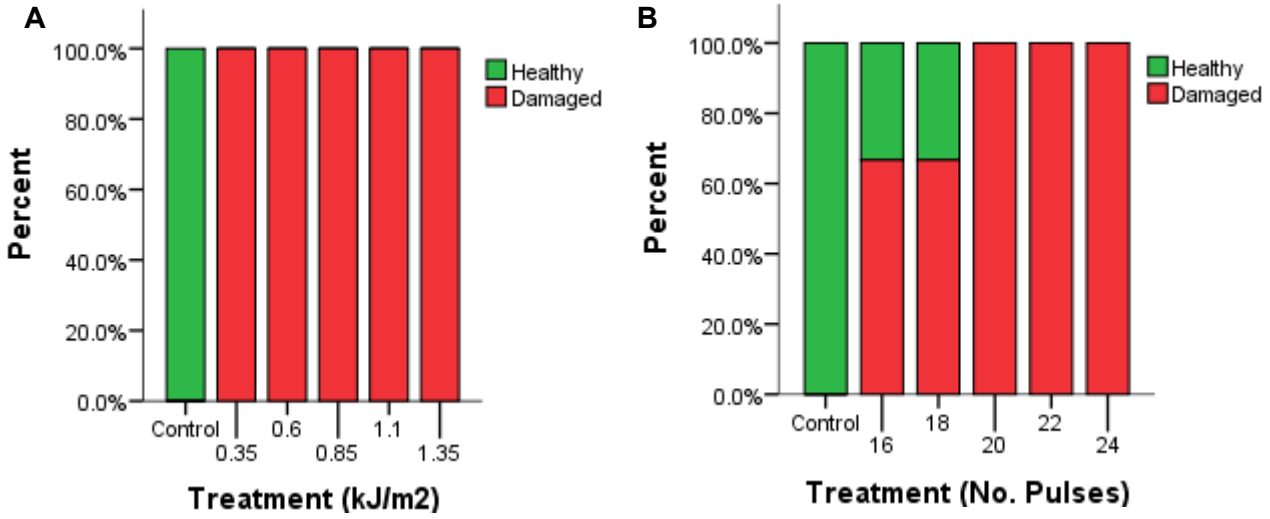

Figure 5.5: The percentage of healthy and damaged plants for the lettuce variety Amica treated during October 2015. Plants were either treated with a conventional low pressure mercury UV-C source (**A**) or a high intensity pulsed polychromatic source (**B**), n=3.

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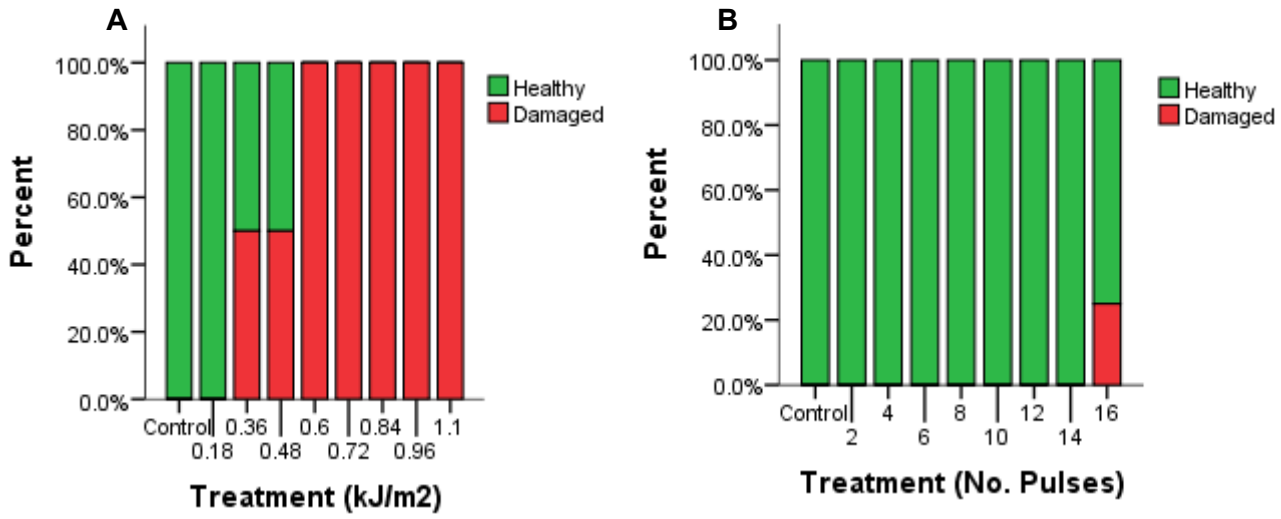

Figure 5.6: The percentage of healthy and damaged plants for the lettuce variety Amica treated during November 2015. Plants were either treated with a conventional low pressure mercury UV-C source (**A**) or a high intensity pulsed polychromatic source (**B**), n=4.

Development of disease control bioassays

During April 2015, preliminary resistance assays were performed on the cv. Amica. Lettuce at early head formation were treated with a range of 15 to 90 pulses, and then incubated in the dark for approx. 12 hours. At 6 days post treatment (DPT) inoculation was performed in situ onto the lettuce leaf with 3 mm agar plugs from 5 day old cultures of *B. cinerea*. Lesion diameter was measured with Vernier callipers. The 45-pulse treatment, which exhibited minor visible damage, showed an increase in lesion size suggesting physiological changes favouring the development of disease were occurring. A reduction in lesion size was observed at both 15 and 30 pulses. Measurement of lesions, however, was subject to error due to uneven development of disease and leaf topography making it difficult to accurately measure lesion size.

Due to the aforementioned problems, a bioassay was adapted from Laboh (2009) to increase accuracy of lesion measurement. Briefly, 20 mm leaf discs were cut from abscised lettuce leafs and placed on 120 mm square Petri dishes containing 1.2 % w/v of Agar Technical No.3 (Oxoid) amended with 20 mg/l of 6-benzylaminopurine, a plant growth regulator, to prevent leaf senescence.

For the next round of preliminary experiments, plants (cv. Amica) were grown to early head formation and treated with 5 to 30 pulses of HIPPL, incubated in the dark for 12 hours, and at 6 DPT leaf discs were then inoculated with 3mm agar plugs from 5-day old *B. cinerea*

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cultures. Measurements were taken at 2 and 3 DPI. All treatments showed a decrease in lesion size with a statistically significant reduction of 15 % for the 20 pulses treatment.

There were, however, a number of problems with the experimental procedure. Firstly, lesion development was still uneven due to the need to use multiple agar plates across the experiment (Figure 5.7 A). The use of agar plugs is inherently variable due to its lack of calibration and one cannot produce constant hyphal densities. Furthermore, addition of 6-benzylaminopurine to the agar may have additional bias on the data as it has previously been demonstrated to be an inducer of disease resistance (Mills *et al.,* 1986). The bioassay was, therefore, further adapted with the removal of 6-benzylaminopurine and the use of a calibrated spore solution following the procedures in objective 1.

Inoculations with 10 µl of spore solution were then tested at concentrations of $1x10^5$ and $1x10^6$ with or without the addition of 50 % potato dextrose broth (Sigma-Aldrich) and measured at 2 DPI. The use of a calibrated spore solution utilising 1 x 10^6 spores per ml amended with 50 % potato dextrose broth provided much greater homogeneity to the development of disease (Figure 5.7 B). Although inoculating leaves with spore solutions improved the within-group variation, there was still a degree of uneven lesion development.

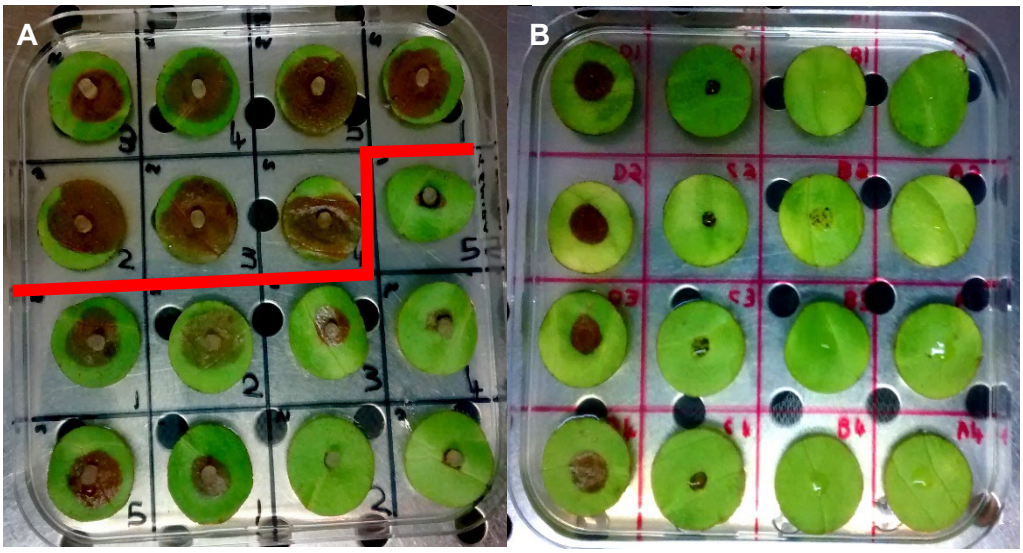

Figure 5.7: The Lettuce leaf bioassay adjusted from Laboh (2009). **A)** 3 mm plugs from 5 day old plates of *Botrytis cinerea* which exhibit a large variation in the size of lesion on untreated lettuce leaves. Red line indicates where agar plugs were taken from differing cultures. **B)** Inoculation with 10 µl of calibrated spore solutions of *B. cinerea* from 14 day old cultures. Columns from left to right show inoculation with calibrated spore solutions of $1x10^6$ and $1x10^5$ spores per ml with 50 % potato dextrose broth and $1x10^6$ and $1x10^5$ spores per ml in sterile distilled water.

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To improve the accuracy of lesion measurements in further experiments, photographs of the lesions were taken. Measurements of lesion size was then performed using the software package ImageJ (Image Processing and Analysis in Java). Image colour channels were split and the green channel was kept for image analysis; this allowed the greatest contrast between healthy and diseased tissues. Lesion measurement was performed utilising the wand tool set to 8-conntected and a threshold of 20. This allowed accurate determination of lesion area in mm^2.

Inoculation procedures for two following lettuce pathogens, *R. solani* and *S. sclerotiorum*, were then optimised. Unfortunately, calibrated spore solutions could not be used for either of the pathogens. *Sclerotinia sclerotiorum* only produces telemorphic ascospores following the formation of apothecia; introducing genetic variation to the inoculum (Hays *et al.,* 2010). Moreover, *R. solani* is only known to produce spores during its sexual cycle, again introducing genetic variability into the inoculum being applied. Furthermore, production of sexual spores from both *S. sclerotinia* and *R. solani* is highly laborious.

Due to these reasons, methods of creating a calibrated suspension of hyphae to inoculate the leaves were attempted. Briefly, the fungi were grown in liquid potato dextrose broth (Sigma Aldrich) until a visible mass of hyphae could be seen. Hyphae were then rinsed in sterile distilled water (SDW) and various weights of hyphae were added to either SDW or 50 % PDB. Hyphae were then homogenised with the table top homogeniser PCU-P2 (Polytron) and 10 µl was pipetted into the centre of leaves. Unfortunately, neither pathogens successfully produced lesions from the application of this method. Furthermore, problems with the efficacy of homogenisation of hyphae also led to “clumping” and problems with pipetting. It was therefore decided that agar plug inoculations would be performed for both of the remaining pathogens.

Both *R. solani* and *S. sclerotiorum* exhibited faster hyphal growth on PDA in comparison to that of *B. cinerea.* Hyphal plugs were therefore taken from 3 day old cultures. Cultures at 3 to 4 days old showed uneven hyphal densities on the agar plates. This was observed to be due to the initiation of the formation of resting bodies or sclerotia. Both pathogens produce sclerotia for long term survival in the soil.

For *R. solani* inoculations a method published by Fiddaman *et al.* (2000) was adapted. Agar plugs were placed in the centre of the underside of the leaf. It was observed that when placing agar plugs on the upper surface of leaves that disease did not progress well and often stalled. Observation of the initiation of disease suggested that hyphae entered through natural openings, such as stomata, which are present at greater levels on the underside of leaves. Inoculations with *S. sclerotiorum* were performed on the upper side of leaves. Example

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photographs of the final inoculation procedures and lesion development at 2 and 3 DPI can be seen in figure 5.8.

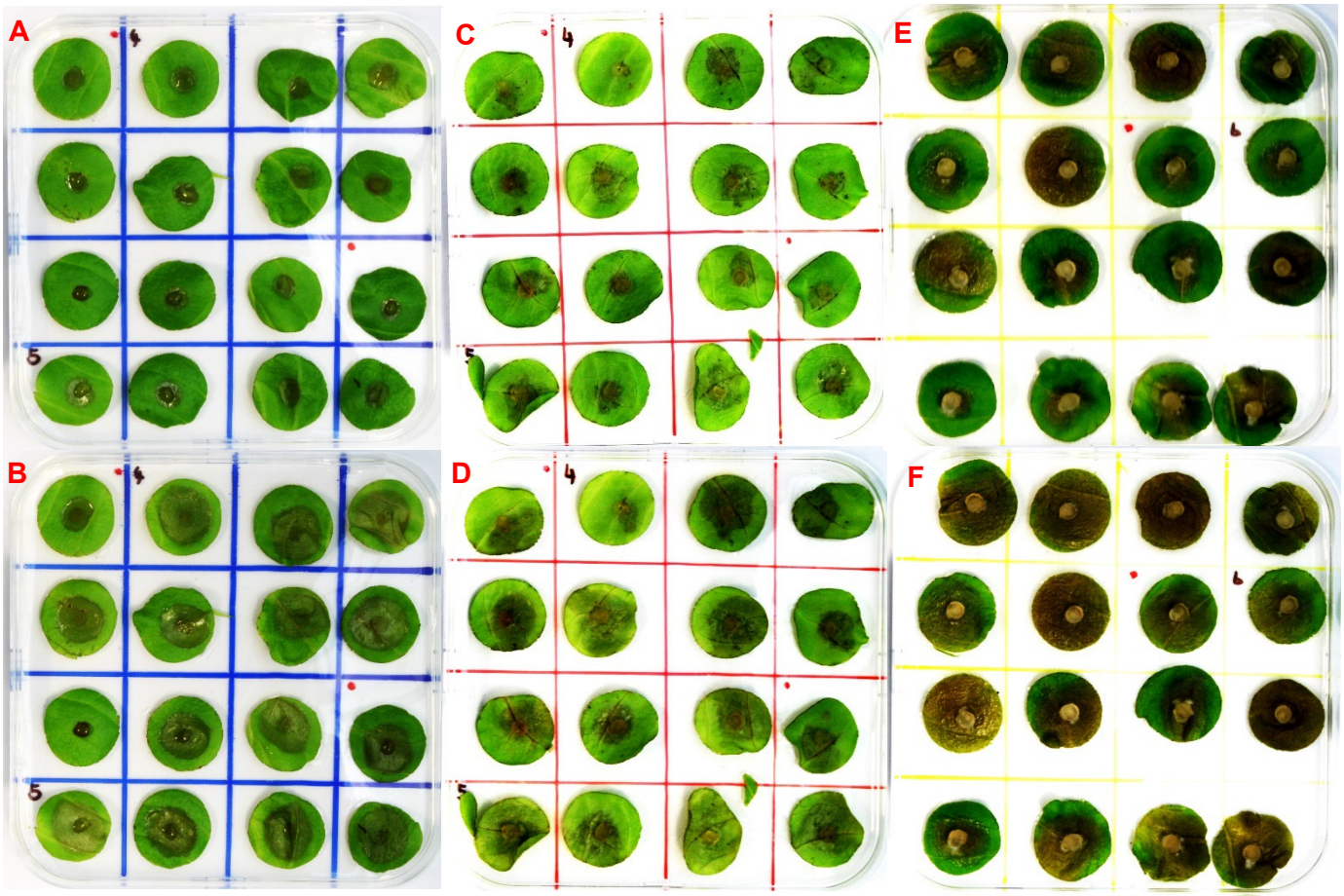

Figure 5.8: Examples of the leaf disc bioassay adapted from Laboh (2009). Images show *Botrytis cinerea* spore solution inoculations at 2 days post inoculation (DPI) (**A**) and 3 DPI (**B**). *Rhizoctonia solani* inoculations with 4mm agar plugs at 2 DPI (**C**) and 3 DPI (**D**). *Sclerotinia sclerotiorum* inoculations with 4mm agar plugs at 2 DPI (**E**) and 2 DPI (**F**).

Disease control bioassays

Disease resistance assays were performed with two main objectives in mind. The first of these was to determine the optimal LIUV and HIPPL treatment for controlling disease on the cultivars undergoing investigation. Secondly, to investigate the longevity of resistance, i.e. at what point is induced resistance at its peak level and how long does resistance last for. A

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large amount of variation was observed for both of the major objectives. None of the experiments utilising the adapted inoculation and lesion measurement techniques were significantly different from the control for both the HIPPL and LIUV source.

To summarise the findings of these experiments, the treatments for which the greatest reductions in disease progression were observed appeared to change across the year, as does the plants susceptibility to damage. The best HIPPL treatment for *B. cinerea* resistance (cv. Amica) dropped from 22 pulses in September to 16 pulses in October 2015 (Table 5.1). This variation was also observed for LIUV treatments 0.6 to 0.35 and 0.18 kJ/m^2 in September, October and November, respectively (Table 5.2). A similar pattern was observed for cv. Temira with the most successful HIPPL and LIUV treatments dropping from 18 pulses and 1.1 kJ/m^2 to 8 pulses and 0.12 kJ/m^2 in February (Tables 5.3 and 5.4).

The most effective treatments against *B. cinerea* also differed between cultivar. For example, in September the treatment was 22 pulses for cv. Amica but 16 pulses for cv. Temira (Tables 5.1 and 5.3). LIUV treatments with the greatest reduction also showed between cultivar variation with 0.6 kJ/m^2 for cv. Amica and 1.1 kJ/m^2 treatments for cv. Temira during September (Tables 5.2 and 5.4). This was further complicated by differences in the most effective treatments for the various pathogens under investigation. For cv. Amica the most effective HIPPL treatments against *B. cinerea, R. solani* and *S. sclerotiorum* were 12, 8 and 14 pulses in December, respectively (Table 5.1). This was also observed for the LIUV treatments with treatments at 0.3, 0.18 and 0.12 kJm^2 showing the greatest levels of disease control, respectively. Furthermore, the responsiveness to treatment also varies between cultivars with Amica showing an average reduction in *B. cinerea* disease progression of 14.47 % whereas Temira showed 21.1 % following pulsed treatments. Similarly, LIUV treatments showed 14.41 % and 31.3 % mean reductions for cv. Amica and Temira, respectively.

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Table 5.1: Experimental results from high intensity pulsed polychromatic light treated Amica plants. This includes the month and date treatment commenced, the growth stage of the plants, the treatment range under investigation, the point at which damage was observed and the day of inoculation post treatments (DPT). The results from inoculation studies are also included showing the most successful treatments and percentage reduction in disease for *Botrytis cinerea, Rhizoctonia solani* and *Sclerotinia sclerotiorum.*

Month	Date	No. true leaves	Range (pulses)	Damage threshold	Inoculation day (DPT)	Disease resistance					
						B. cinerea		*R. solani*		*S. sclerotiorum*	
						Pulses	%	Pulses	%	Pulses	%
March	20.03.2015	3-5	15-105	≥60	-	-	-	-	-	-	-
April	14.04.2015	EHF[1]	15-90	≥45	9	15	15.1	-	-	-	-
May	05.05.2015	EHF[1]	5-30	N/O[3]	6	20	8	-	-	-	-
September	10.09.15	3-5	16-24	N/O[3]	5	22	18.5	-	-	-	-
September	10.09.15	3-5	16-24	N/O[3]	8	NR[4]	NR[4]	-	-	-	-
October	08.10.15	LHF[2]	16-24	≥16	5	16[5]	2.8	-	-	-	-
November	18.11.15	3-5	2-16	≥16	2	14	21.7	10	17	-	-
December	09.12.15	8-10	8-16	N/O[3]	2	12	2.9	8	7.2	14	12.9
December	09.12.15	8-10	8-16	N/O[3]	5	14	10.3	10	14.1	8	17.8
December	09.12.15	8-10	8-16	N/O[3]	9	12	11.8	8	20.6	12	38.7
February	12.02.16	6-8	8-16	N/O[3]	2	10	44.1	12	37.7	-	-

[1]- Early head formation. [2]-Late head formation. [3]- No damage was observed. [4] – No reduction in disease progression was observed. [5] - Treatments caused damage to the plant. -Denotes that results were not collected.

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Table5.2. Experimental results from conventional UV-C light source treated Amica plants. This includes the month and date treatment commenced, the growth stage of the plants, the treatment range under investigation, the point at which damage was observed, the day of inoculation post treatments (DPT). The results from inoculation studies are also included showing the most successful treatments and percentage reduction in disease for *Botrytis cinerea, Rhizoctonia solani* and *Sclerotinia sclerotiorum.*

Month	Date	No. true leaves	Range (kJm^2)	Damage threshold	Inoculation day (DPT)	Disease resistance					
						B. cinerea		*R. solani*		*S. sclerotiorum*	
						kJm^2	%	kJm^2	%	kJm^2	%
March	20.03.2015	3-5	0.75-5.25	>1.5	-	-	-	-	-	-	-
September	10.09.15	3-5	0.35-1.35	N/O[2]	5	0.60	11.9	-	-	-	-
September	10.09.15	3-5	0.35-1.35	N/O[2]	8	0.35	16.7	-	-	-	-
October	08.10.15	LHF[1]	0.35-1.35	≥0.35	5	0.35[4]	9.0	-	-	-	-
November	18.11.15	3-5	0.18-0.96	≥0.35	2	0.18	21.8	0.35[4]	14.0	-	-
December	09.12.15	8-10	0.06-0.24	N/O[2]	2	NR[3]	NR[3]	0.18	2.23	0.18	8.8
December	09.12.15	8-10	0.06-0.24	N/O[2]	5	0.30	20.2	0.18	5.3	0.12	16.8
December	09.12.15	8-10	0.06-0.24	N/O[2]	9	0.12	8.3	0.12	8.8	0.12	2.0
February	12.02.16	6-8	0.06-0.30	N/O[2]	2	0.06	27.0	0.18	32.9	-	-

[1]- Early head formation. [2]- No damage was observed. [3] – No reduction in disease progression was observed. 4- Treatments caused damage to the plant. -Denotes that results were not collected

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Table 5.3 Experimental results from high intensity pulsed polychromatic light treated Temira plants. This includes the month and date treatment commenced, the growth stage of the plants, the treatment range under investigation, the point at which damage was observed, the day of inoculation post treatments (DPT). The results from inoculation studies are also included showing the most successful treatments and percentage reduction in disease for *Botrytis cinerea, Rhizoctonia solani* and *Sclerotinia sclerotiorum.*

Month	Date	No. true leaves	Range (pulses)	Damage threshold	Inoculation day (DPT)	Disease resistance					
						B. cinerea		*R. solani*		*S. sclerotiorum*	
						Pulses	%	Pulses	%	Pulses	%
May	05.05.2015	EHF[1]	5 to 30	N/O[3]	6	20	11.0	-	-	-	-
September	29.09.15	3-5	16-24	N/O[3]	3	18	7.3	-	-	-	-
September	29.09.15	3-5	16-24	N/O[3]	5	16	23.5	-	-	-	-
October	28.10.15	MHF[2]	8-20	N/O[3]	2	12	51.9	-	-	-	-
October	28.10.15	MHF[2]	8-20	N/O[3]	5	12	36.0	-	-	-	-
October	28.10.15	MHF[2]	8-20	N/O[3]	7	14	26.4	-	-	-	-
Feb	12.02.16	6-8	6-16	N/O[3]	2	8	40.6	14	37.8	-	-

[1]- Early head formation. [2]-Mid head formation. [3]- No damage was observed. -Denotes that results were not collected.

Table 5.4 Experimental results from conventional UV-C light source treated Temira plants. This includes the month and date treatment commenced, the growth stage of the plants, the treatment range under investigation, the point at which damage was observed, the day of inoculation post treatments (DPT). The results from inoculation studies are also included showing the most successful treatments and percentage reduction in disease for *Botrytis cinerea, Rhizoctonia solani* and *Sclerotinia sclerotiorum.*

Month	Date	No. true leaves	Range (kJm^2)	Damage threshold	Inoculation day (DPT)	Disease resistance					
						B. cinerea		*R. solani*		*S. sclerotiorum*	
						kJm^2	%	kJm^2	%	kJm^2	%
September	29.09.15	3-5	0.35-1.35	N/O[1]	3	1.1	9.7	-	-	-	-
September	29.09.15	3-5	0.35-1.35	N/O[1]	5	0.35	22.5	-	-	-	-
Feb	12.02.16	6-8	0.06-0.30	N/O[1]	2	0.24	31.3	0.24	53.8	-	-

[1]- No damage was observed. -Denotes that results were not collected.

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The doses that gave the lowest levels of disease progression across the year, as discussed above, appeared to fluctuate. To allow us to see how the most successful treatments changed across this year they were plotted against month. This was performed utilising the most complete set of resistance assay data from *B. cinerea* inoculations of cv. Amica.

Pulsed treatments show an increase in optimum treatment from 15 to 22 pulses between April and September and a drop to 10 in February (Figure 5.9). LIUV treated plants also show a homologous drop in dose from September, where the most effective treatment was 0.6 kJ/m^2, to 0.06 kJ/m^2 in February

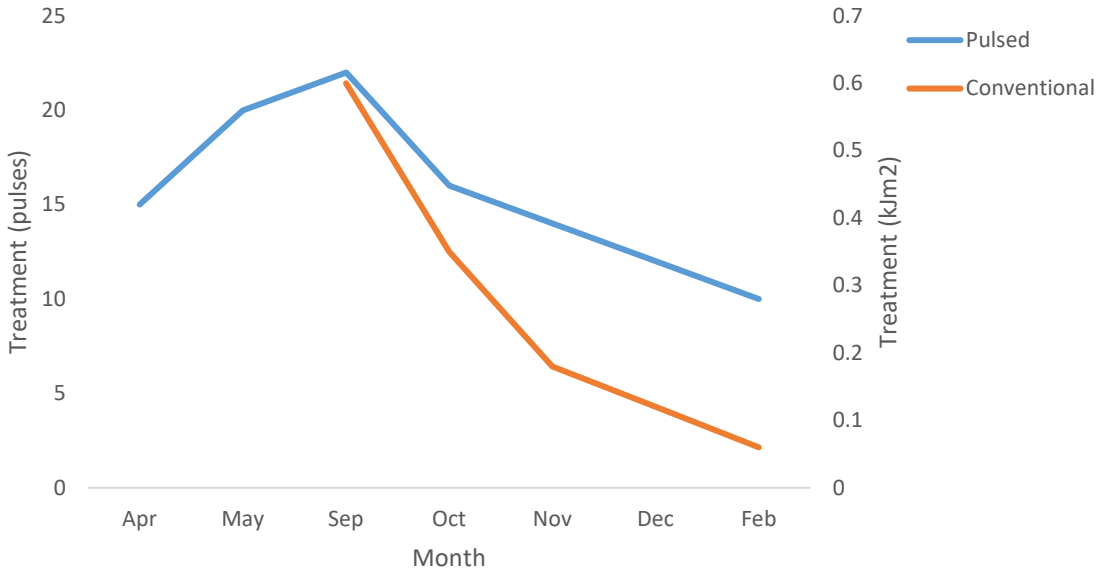

Figure 5.9: The pulsed and conventional treatments showing the greatest potential for reducing the disease progression of against *Botrytis cinerea* at differing months of the year for the cv. Amica

Such wide variation may indicate a complex situation where not only the environmental conditions effect the optimum treatment to induce resistance but also so do the cultivar and pathogen undergoing investigation. To attempt to remove a degree of the observed variation it was decided that the most successful trials would be repeated from 2015/2016 during 2017. The experiments would be performed in exactly the same manner that they were carried out previously. This was to attempt to mitigate any variation that may have been caused by day length and seasonal environmental fluctuations. It was, therefore, decided to repeat the experiments that were performed during February 2016 as all treatments on both cultivars showed a reduction in disease progression >27.0 % for both *B. cinerea* and *R. solani*.

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Treatments from February 2016 and February 2017

To allow for the potential seasonal variation observed in preliminary trials, treatments from a single month were replicated using both LIUV and HIPPL sources. This was to ascertain the extent to which the treatment ranges may differ between growing seasons and whether treatments would have to be adjusted between seasons. To summarise briefly, the experimental protocols; both varieties Amica and Temira were grown to 6-8 true leaves. They were then inoculated with at 2 DPT with *B. cinerea* due to the ease of inoculation and homogeneity of inoculation procedures. Lesion measurements were taken at 2 and 3 DPI with ImageJ and used to calculate the area underneath the disease progression curve.

No significant differences were found when analysing the combined experimental data (data not shown). When analysing the experimental replicates separately, variation in the treatment giving the optimum reduction in disease progression, again, showed variation for both varieties and light sources undergoing investigation. For Amica, during 2016 the LIUV treatment showing the greatest reduction in disease was 0.06 kJ/m^2 at 27.0 % (Figure 5.10A). During 2017, however, although all treatments showed a small reduction in disease progression, they were very small with the most successful treatment of 0.30 kJ/m^2 only reducing disease by 19.7 % (Figure 5.10B). Similarly, HIPPL treatments showed a large degree of disease reduction in 2016 with the treatment of 10 pulses giving a 44.1 % reduction (Figure 5.11A). In 2017, all treatments showed a reduction in disease progression. These were, however, very small with the treatment of 14 pulses only giving a 17.7 % reduction in disease progression (Figure 5.11B).

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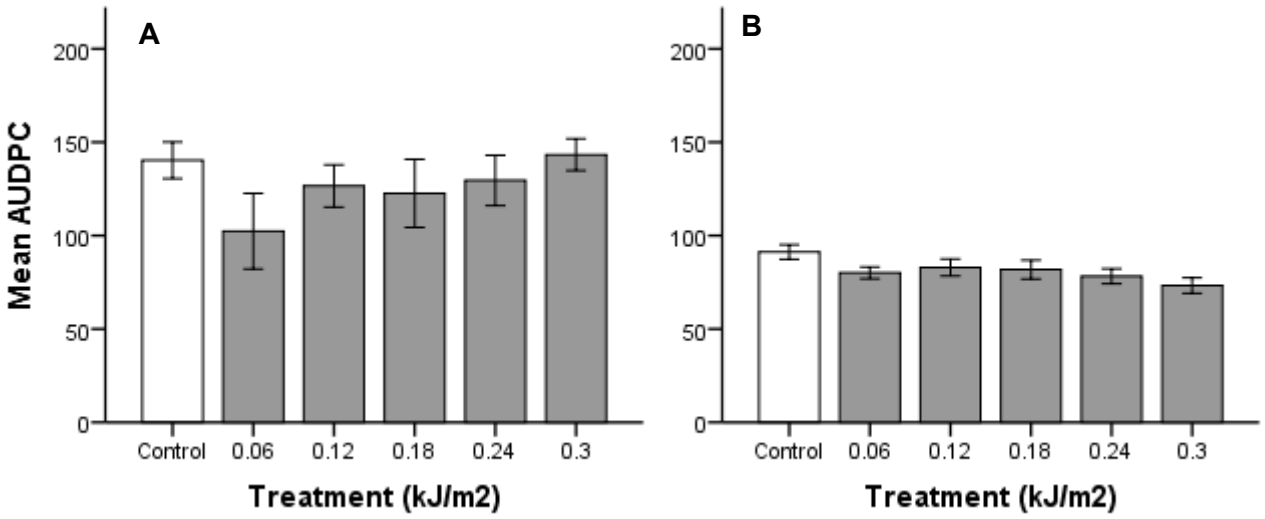

Figure 5.10: Area underneath the disease progression curve (AUDPC) for lettuce plants of the cv. Amica treated with a low intensity UV-C source at the 6-8 true leaf stage. Plants were inoculated at 2 days post treatment with *Botrytis cinerea* and disease was measured with ImageJ at 2 and 3 days post inoculation. Replicate experiments were performed in February 2016 (**A**) and February 2017 (**B**). Two technical replicates were performed for each biological replicate. N=5 and 7 for A and B, respectively. Error bars show ± 1 S.E.M.

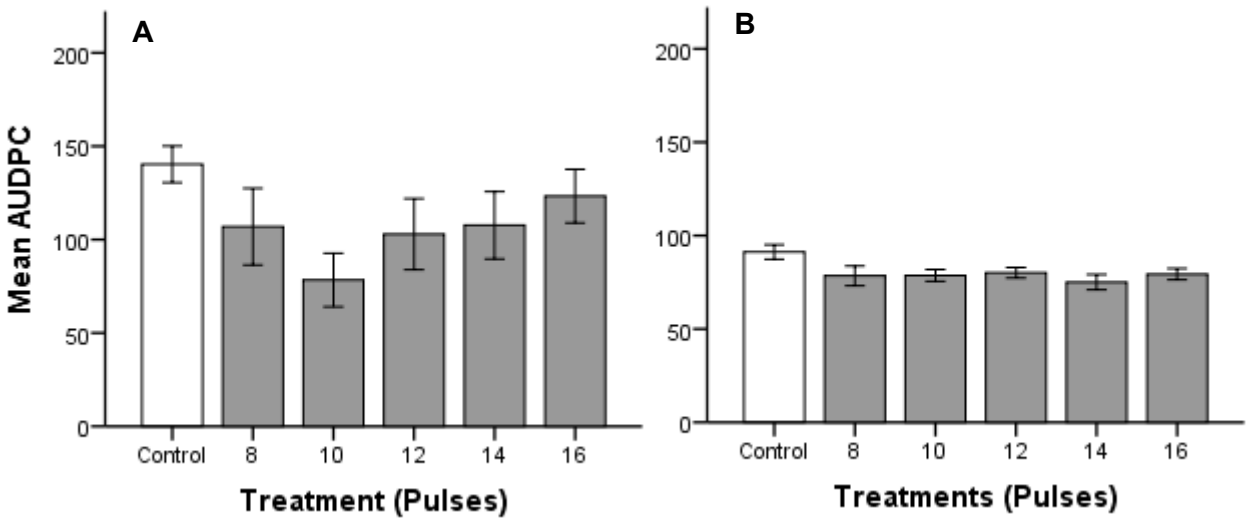

Figure 5.11: Area underneath the disease progression curve (AUDPC) for lettuce plants of the cv. Amica treated with a high-intensity pulsed polychromatic light source at the 6-8 true leaf stage. Plants were inoculated at 2 days post treatment with *Botrytis cinerea* and disease was measured with ImageJ at 2 and 3 days post inoculation. Replicate experiments were performed in February 2016 (**A**) and February 2017 (**B**). Two technical replicates were performed for each biological replicate. N=5 and 7 for A and B, respectively. Error bars show ± 1 S.E.M.

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For the cultivar Temira, a similar set of observations were made. The levels of disease reduction for LIUV treated plants were high in 2016. The treatment, 0.24 kJ/m^2 showed a 31.3 % reduction in disease progression (Figure 5.12A). For the experimental replicate performed in 2017, however, the 0.06 kJ/m^2 showed the greatest level of disease reduction (Figure 5.12B). Again, the levels of disease reduction during 2017 were much smaller than that observed for 2016 with the most successful treatment only showing a 12.7 % reduction. Experiments with HIPPL treated cv. Temira lettuce produced similar findings. In 2016 the greatest reduction in disease was seen for the 8-pulse treatment at 40.6 % (Figure 5.13A). In 2017, however, the 16-pulse treatment showed the highest level of reduction at 13.8 % (Figure 5.13B).

The differences between experimental replicates were homologous for all but the LIUV treatments on Temira. All experiments showed an increase in the most effective treatment and a reduction in the level of disease progression, including controls, when going from 2016 to 2017. The differences in the most successful treatment were large with a 5-fold and 40 % increase in dose observed for the LIUV and HIPPL treatments for the cv. Amica. Furthermore, the optimal HIPPL treatment for cv. Temira increased 2-fold. The LIUV treatments for cv. Temira, however, reduced from 0.24 to 0.06 kJ/m^2. The reductions for the 0.06 and 0.30 kJ/m^2 treatments, however, are extremely similar at 69.8 and 72.8, respectively.

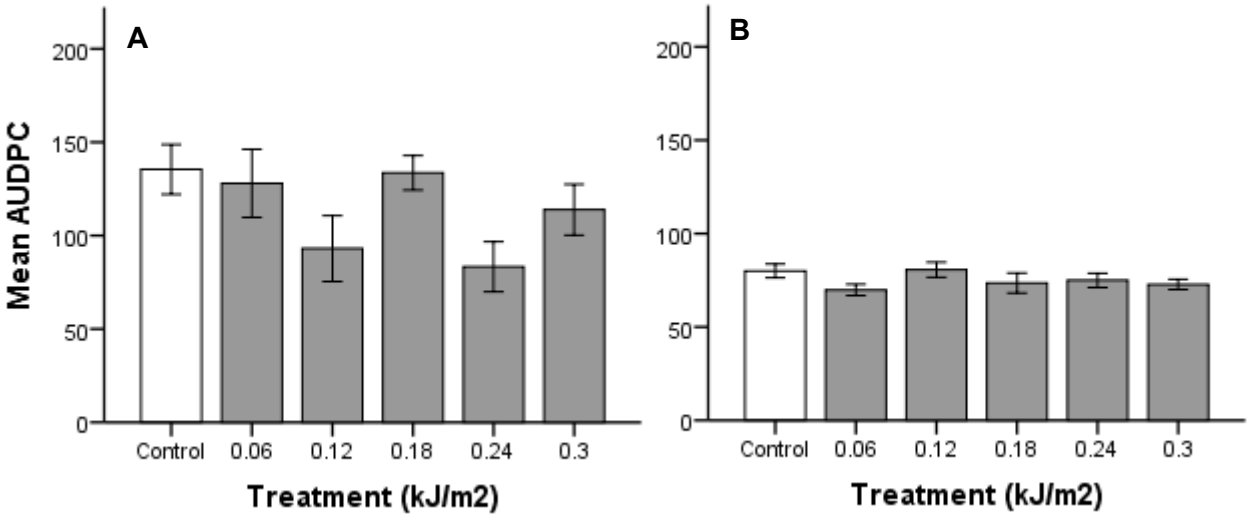

Figure 5.12: Area underneath the disease progression curve (AUDPC) for lettuce plants of the cv. Temira treated with a low intensity UV-C source at the 6-8 true leaf stage. Plants were inoculated at 2 days post treatment with *Botrytis cinerea* and disease was measured with ImageJ at 2 and 3 days post inoculation. Replicate experiments were performed in February 2016 (**A**) and February 2017 (**B**). Two technical replicates were performed for each biological replicate. N=5 and 7 for A and B, respectively. Error bars show ± 1 S.E.M.

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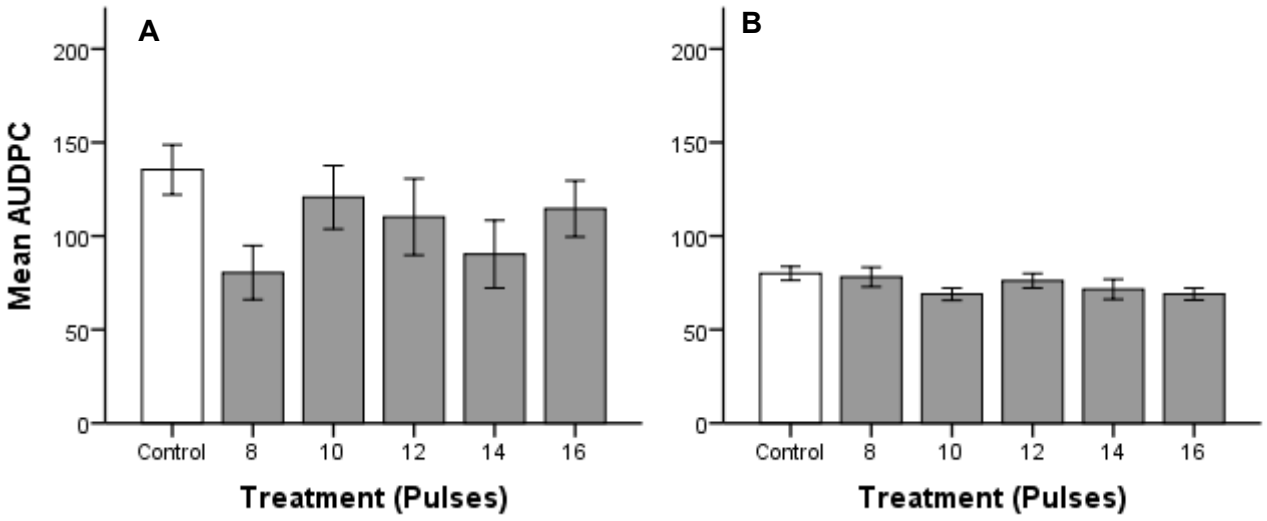

Figure 5.13: Area underneath the disease progression curve (AUDPC) for lettuce plants of the cv. Temira treated with a high-intensity pulsed polychromatic light source at the 6-8 true leaf stage. Plants were inoculated at 2 days post treatment with *Botrytis cinerea* and disease was measured with ImageJ at 2 and 3 days post inoculation. Replicate experiments were performed in February 2016 (**A**) and February 2017 (**B**). Two technical replicates were performed for each biological replicate. N=5 and 7 for A and B, respectively. Error bars show ± 1 S.E.M.

The observation of decreased sensitivity to treatment and reduced levels of disease progression may be explained by a number of factors. Firstly, an increased visible light intensity or increased levels of natural UV-B and UV-A during 2017 may have led to an upregulation of phenolic compounds, which can act as phytoalexins, and potential stimulation of a defence response. This may explain the reduction in disease progression observed in the control. Moreover, this may also explain a decrease in sensitivity to treatment and the observed increase in dose to achieve optimum levels of disease reduction. This may be due to the light-quenching function that phenolic compounds may hold. A natural increase in total phenolic compounds would thus reduce the total available light energy irradiating important cellular membranes such as the mitochondria and chloroplasts that readily produce ROS following light stress. A reduction in ROS production may stimulate a defence response, and the production of pathogenesis-related proteins, but may still lead to a production in phenolic compounds to adapt to increased light stress. This may explain why only a marginal decrease in disease development is observed.

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Conclusions and summary

Two commercial cultivars of butterhead lettuce, Amica and Temira, were grown in a temperature-controlled glasshouse. The first aim of the study was to ascertain the damage thresholds for LIUV and HIPPL treatments. Damage is induced by both HIPPL and LIUV sources manifests as dry brown lesions and vascular discolouration, see figure 5.1 and 5.2. The damage thresholds of the cv. Amica showed variation across the year decreasing from 60 to 16 pulses and 2.25 kJ/m^2 to 0.36 kJ/m^2 between April and October 2015 for the HIPPL and LIUV sources, respectively.

Treatments below the damage threshold were assessed for their ability to reduce disease progression. Due to complications in performing and measuring in situ inoculations, a leaf bioassay was developed, with adjustments, as published by Laboh (2009). This was used for the inoculation with *B. cinerea*, *R. solani* and *S. sclerotiorum*. As with the damage threshold, treatments for the reduction of disease progression were shown to be highly variable. Changes in treatment were observed for every variable tested, including the cultivar and pathogen undergoing investigation, the day after treatment that inoculations were performed and the date that the treatments were performed.

Postharvest LIUV treatments of lettuce, however, were shown to be successful by Ouhibi *et al.* (2014) with a treatment of 0.85 kJ/m^2. After in depth review of their research, however, little in the way of experimental design is discussed. Firstly, only a single treatment was performed and compared with the control group; with no reference as to why the dose employed had been chosen. We should, therefore, interpret the data with caution. Secondly, it is stated that the disease assay data is supplied from a sample size of 20. The number of independent replicate experiments, however, is not specified. This brings into question the reliability of their work and fails to answer the question whether their experiments can be successfully repeated.

From our results, although we cannot say with statistical confidence, we have an abundance of evidence that suggests that both LIUV and HIPPL can induce resistance against all three pathogens that were investigated. It remains, however, that a simple “silver bullet” treatment is not suitable when attempting to induce resistance on actively growing plants. If there is no single treatment that shows reduced disease progression across the year it could, therefore, be hypothesised that multiple low dose treatments delivered to the plant at regular intervals may be able to stimulate a defence response in plants; this, however, requires further investigation.

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Objective 6- Multiple low-dose foliar LIUV and HIPPL treatments of lettuce

Introduction

During objective five a large amount of variation was found in both the damage threshold to both LIUV and HIPPL treatments and the treatments which reduce disease progression. The primary factor affecting damage to the crop appeared to be linked to environmental conditions. As these were not being monitored in real time, however, it cannot be concluded what was causing the shift in the point of damage. It is however, likely that light intensity and hours of daylight play a significant role in determining the outcome. Furthermore, the treatments for reducing disease progression also appeared to be influenced by both the cultivar and also the pathogen being studied. It was decided at this point not to conduct experiments in a controlled environment as the main objective of the study was to produce a treatment regime that could be applied directly to a commercial glasshouse environment.

Aims

The aims of this investigation were to test the hypothesis that either a single or multiple low-level treatments induce disease resistance against *R. solani* on lettuce. Treatments were chosen based on the previous data (objective 5) and were shown not to be damaging at any point during the growing season.

Materials and Methods

Plant husbandry, LIUV and HIPPL treatment and inoculation

Lettuce of the cv. Amica and Temira were grown to the 6-8 leaf stage. The chosen low-dose treatments were 0.12 and 0.24 kJm^2 for the LIUV source and 7 and 14 pulses for the HIPPL source. Each of the higher treatments, for the LIUV and HIPPL sources, showed a level of reduced disease progression against each of the pathogens under investigation in objective five. Treatments were applied twice with either 2, 4 or 6 days between treatments (DBT). Treatments were performed as stated in objective five. Plants were treated and inoculated with *R. solani* utilising the detached lead bioassay as outlined in objective five. Inoculations

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were performed at 2 DPT as described Ouhibi *et al.,* (2015). *Rhizoctonia solani* was chosen as the pathogen to undergo study as it had shown the greatest level of responsiveness to treatment in previous studies. Lesion area was measured at 3 DPI only as at 2 DPI lesions were not sufficient to measure accurately.

Experimental design

Five biological and two technical repeats were used for each experimental replicate. Three replicate experiments were carried out, one in each of the months March, April and May 2016. A completely randomised design was used. The data from the three replicates was combined and statistically analysed in SPSS via One-Way ANOVA. Only data from the 2 and 6 DBT was used as the data from 4 DBT was not collected in the third replicate experiment. Single treatments were also not included in the statistical analysis. All the results from individual experimental replicates in this chapter can be found in appendix 6.

Results and discussion

Three replicate experiments were carried out, one in the month March, April and May 2016. All LIUV treatments showed slight reductions in mean lesion size at 2 DBT for the variety Amica (Figure 6.1A). All treatments, however, showed an increase in mean lesion area for treatments with 6 DBT. The LIUV treatment showing the greatest reduction was 0.12 kJ/m^2 giving a 13.24 % reduction at 2 DBT. None of the conventional treatments showed a significant difference from the control with p values of 0.362 and 0.916 for the 2 and 6 DBT treatments, respectively.

The pulsed treatments showed the same pattern with both treatments showing slight reductions when treatments were performed with 2 DBT and increases in mean lesion area when performed with 6 DBT (Figure 6.1C). The greatest reduction in lesion size was observed for the 14-pulse treatment at 3.6 %. The 7-pulse treatment also showed a similar reduction at 3.6 %. Again, statistical testing showed p values of 0.896 and 0.672 for the 2 DBT and 6 DBT treatments respectively.

For the variety Temira multiple LIUV treatments showed small reductions at both 2 and 6 DBT. The treatment showing the largest decrease in mean lesion area was the 0.24 kJ/m^2 treatment at 2 DBT showing a 7.5 % decrease (Figure 6.2A). With 6 DBT the treatment showing the greatest reduction in mean lesion size was 0.12 kJ/m^2 at 9.5 %.

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For HIPPL treatments, only 14 pulses applied with 2 DBT showed a reduction in mean lesion size at 9.9 % (Figure 6.2C). Statistical analysis, however, highlighted no significant differences and again showed particularly large p values. LIUV treatments gave p values of 0.722 and 0.587 for the 2 and 6 DBT trials, respectively. HIPPL treatments similarly showed high p values at 0.374 and 0.929 for the 2 and 6 DBT trials. A summary of the most successful treatments for each experimental replicate is given in tables 6.1, 6.2, 6.3 and 6.4.

The failure to significantly reduce disease here is not unexpected. The data from the experimental replicates shows that the most effective treatment regime, be that 2, 4 or 6 DBT, showed variation between each replicate experiment for both cultivars and light sources (Tables 6.1, 6.2, 6.3 and 6.4). For example, Temira plants treated with LIUV showed the most effective applications (DBTs) were 4, 6 and 2 for the replicates in March, April and May respectively (Table 6.3). Furthermore, the most successful treatment also changed. For example, for Amica plants the most successful pulsed treatments were 7, 7 and 14 at 4, 2 and 2 DBT, respectively, table 6.2.

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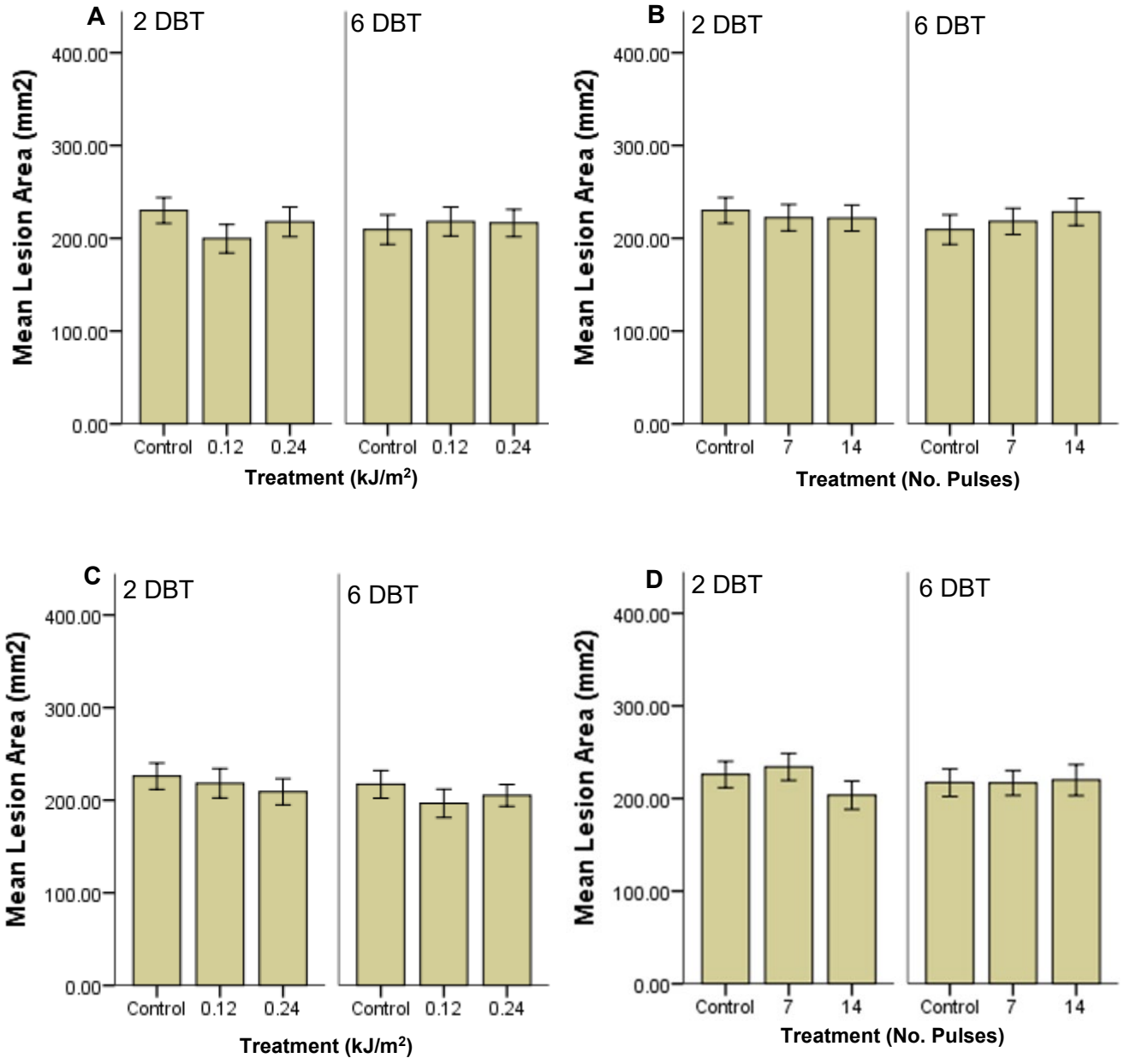

Figure 6.1: The combined experimental data from three replicate experiments utilising multiple low level treatments of the variety Amica. Experimental replicates were performed during March, April and May 2016. Plants were inoculated at 2 days post treatment with 4mm *R. solani* agar plugs and lesions were measured at 3 days after inoculation with ImageJ. Graphs show plants treated with either 2 or 6 days between treatments (DBT) (**A**) The mean lesion area of conventional UV-C treated plants. (**B**) The Lesion areas of conventional UV-C treated plants. (**C**) The mean lesion area of pulsed polychromatic treated plants. (**D**) The Lesion areas of pulsed polychromatic treated plants. Error bars on A and C show ± 1 standard error of the mean. N= 15, IRE=3.

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Interestingly, it is also clear that single treatments performed with high doses that were not damaging at any point of the year also showed little effectiveness in reliably reducing disease. For Amica plants treated with the pulsed polychromatic source, a single 14 pulse treatment showed a 19.3 %, 0.4 % and 4.4 % reduction in mean lesion area for the trials during March, April and May, respectively, table 6.2. For plants treated with the LIUV source a 0.24 kJm^2 dose showed no reduction in disease, 2.9 % and 3.2% reductions in lesion area for March, April and May, respectively (Table 6.1).

Temira plants treated with a single 14 pulse treatment showed a similar inability to reduce mean lesion area with no reductions observed in March and May and only a 0.9 % reduction observed in April (Table 6.4). Single treatments of 0.24 kJm^2 showed 9.5 %, 24.8 % and no reduction in mean lesion area for March, April and May respectively, (Table 6.3).

Finally, four treatments were applied to both Amica and Temira plants with 2 DBT. Either 0.12 or 0.24 kJm^2 conventional UV-C or 7 and 14 pulse treatments were used. Plants were treated at the 6-8 true leaf stage and inoculated at 2 DPT with 4mm agar plugs of *R. solani*. Five biological and two technical replicates were used.

The pulsed treatment showing the greatest level of disease reduction for Amica was the 14-pulse treatment giving a 9.4 % reduction in mean lesion area (Table 6.2). For conventional UV-C treatments the largest reduction in lesion area was observed for the 0.12 kJm^2 treatment at 2.4 %, table 6.2. For Temira plants the 0.12 kJm^2 conventional treatments showed the greatest reduction in lesion area at 7.3 %, table 6.3. The pulsed treatments showed the greatest success with the 7-pulse treatment which showed a 1 % reduction in mean lesion area (Table 6.4).

Table 6.1: Experimental results from conventional UV-C light source treated Amica plants that have undergone multiple treatments. This includes the month and date treatment commenced, the growth stage of the plants, the number of treatments, the most successful treatment dose, days between treatment and percentage disease reduction

Month	Date	No. true leaves	No. of treatments	ST disease reduction (%)	Disease reduction		
					Treatment (kJm^2)	DBT	Reduction (%)
March	24.03.16	6-8	2	NR[1]	0.12	2	18.8
April	18.04.16	6-8	2	2.9	0.12	2	8.8
May	19.05.16	6-8	2	3.2	0.12	6	11.2
May	19.05.16	6-8	4	-	0.12	2	2.4

[1]No reduction in disease was observed. -Denotes that results were not collected.

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Table 6.2: Experimental results from high intensity pulsed polychromatic light treated Amica plants that have undergone multiple treatments. This includes the month and date treatment commenced the growth stage of the plants, the number of treatments, the most successful treatment dose, days between treatment and percentage disease reduction.

Month	Date	No. true leaves	No. of treatments	ST disease reduction (%)	Disease reduction		
					Treatment (pulses)	DBT	Reduction (%)
March	24.03.16	6-8	2	19.3	7	4	5.1
April	18.04.16	6-8	2	0.4	7	2	5.8
May	19.05.16	6-8	2	4.4	14	2	5.7
May	19.05.16	6-8	4	-	14	2	9.4

-Denotes that results were not collected

Table 6.3: Experimental results from conventional UV-C light source treated Temira plants that have undergone multiple treatments. This includes the month and date treatment commenced the growth stage of the plants, the number of treatments, the most successful treatment dose, days between treatment and percentage disease reduction.

Month	Date	No. true leaves	No. of treatments	ST disease reduction (%)	Disease reduction		
					Treatment (kJm^2)	DBT	Reduction (%)
March	24.03.16	6-8	2	9.5	0.24	4	15.8
April	18.04.16	6-8	2	24.8	0.12	6	14.7
May	19.05.16	6-8	2	NR[1]	0.24	6	5.6
May	19.05.16	6-8	4	-	0.12	2	7.3

[1]No reduction in disease was observed. -Denotes that results were not collected.

Table 6.4: Experimental results from high intensity pulsed polychromatic light treated Temira plants that have undergone multiple treatments. This includes the month and date treatment commenced the growth stage of the plants, the number of treatments, the most successful treatment dose, days between treatment and percentage disease reduction.

Month	Date	No. true leaves	No. of treatments	ST disease reduction (%)	Disease reduction		
					Treatment (pulses)	DBT	Reduction (%)
March	24.03.16	6-8	2	NR[1]	7	4	25.8
April	18.04.16	6-8	2	0.9	14	6	12.4
May	19.05.16	6-8	2	NR[1]	14	2	6.31
May	19.05.16	6-8	4	-	7	2	1.0

[1]No reduction in disease was observed. -Denotes that results were not collected.

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Summary and conclusions

Multiple LIUV and HIPPL treatments, for both Amica and Temira, either did not reduce mean lesion area, increased mean lesion area or reduced mean lesion area by only a few percent. It is also evident that the low-level repeated treatments are not as effective as some of the single, higher dose treatments that have been performed in objective five. It is therefore recommended that research be focused on investigating appropriate single treatments.

In addition, the variation observed in the treatments for reducing disease progression, as was observed in objective five, continued to persist. Variation between the most effective doses continued to show some level of cultivar dependence. The cultivar Temira, as seen in a number of experiments in objective five, continued to show reduced sensitivity to both LIUV and HIPPL treatments, thus requiring an increased dose for the induction of resistance. Furthermore, the most effective treatment regime could not be elucidated. The most successful number of DBT seemed to change from experiment to experiment. Finally, it appeared that the most successful treatment, again, increased as the months proceeded through to spring.

The large amount of variation that was been observed for all experiments throughout objective four could not be mitigated through the application of multiple low-dose treatments with the observation that environmental conditions may still play a key role in deciding the effective treatment. It is suggested that research should be moved into a controlled environment to allow for the successful determination of HIPPL and LIUV induced resistance's viability as an alternative to chemical control. It could be hypothesised that almost any change to the plants homeostasis, through environmental cues, during development would alter the treatment that induced resistance. Such a treatment would, therefore, require an extremely tightly controlled glasshouse environment to fulfil the full efficacy of any treatments applied. The full and comprehensive modelling of the effect of changing environmental conditions, through the use of a controlled environment, could allow the prediction of glasshouse treatments in real time and increase the likelihood of an effective treatment and preventing unwanted damage occurring to crops. Further research to test this hypothesis, however, is required.

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Objective 7- LIUV and HIPPL Treatments of Lettuce in a Controlled Environment

Introduction

In meeting objective five, a great deal of variation was observed when treating lettuce with HIPPL and LIUV in the glasshouse. Such variation included the damage threshold and potential treatments for disease control. Variation for both factors was seen both across the growing season and with the cultivar being treated. Furthermore, variation in the treatment dose for each of the pathogens undergoing investigation was also observed. In objective 6 we attempted to mitigate some of the variation by applying low-dose treatments, that were not damaging at any point during the growing season, multiple times with either 2, 4 or 6 days between treatment applications. Variation in the treatment dose for disease control, however, was still apparent with differing doses proving most effective dependant on the date of application and cultivar. It was, therefore, concluded that to determine whether pre-harvest LIUV and HIPPL treatments were a reliable alternative to chemical control the experiments should be performed within a controlled environment.

Aims

The aims of this study were to determine at what point pre-harvest LIUV and HIPPL treatments of lettuce cause damage to the crop. Treatments will then be assessed for their ability to control grey mould (*Botrytis cinerea*) through a leaf disc bioassay.

Materials and methods

Plant husbandry, LIUV and HIPPL treatment and inoculation

Plants of the commercial cultivars Amica and Temira were grown in a nutrient film technique (NFT) system, as stated in objective five, but with the use of a controlled environment. A lighting period of 16/8 at 21/12 ºC and a light intensity of 250 µmol/m/s-1 was delivered by 400 W HPS lights at a relative humidity of 70 to 80 %. Seedlings were grown to the 8-true leaf stage for treatment.

For LIUV treatments, plants were treated with either 0.32, 0.64, 0.98, 1.28 or 1.92 kJ/m^2 delivered at 2000 µW/cm^2. For HIPPL treatments, plants were treated from 40 cm with 12, 24, 36, 48 or 72 pulses. At 2 days post treatments (DPT) plants were inspected for the presence of damage and then inoculated with *Botrytis cinerea* as outlined in objective five.

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Experimental design

Five biological and four technical repeats were used for each of the treatments groups across two independent replicate experiments carried out in May and June 2017, n=10. One-way ANOVA with Tukey's post-hoc testing was performed. Where the homogeneity of variances assumption could not be met, Welch's robust ANOVA was performed followed by the Games-Howell post-hoc test. Statistical significance is defined as $p \leq 0.05$. All statistical analysis was performed in SPSS (IBM). Results from each individual experimental replicate are given in appendix 7.

Results and discussion

At two days following treatment, damage was observed at LIUV treatments > 0.98 kJ/m^2 for both cv. Amica and Temira (Figure 7.1A and 7.2A). In line with observations in objective five, cv. Amica showed a greater susceptibility to damage than Temira with 100 % of plants exhibiting damage at ≥ 1.28 kJ/m^2 and 1.92 kJ/m^2 for each cultivar, respectively. For the HIPPL treatments, damage was observed at 48 and 72 pulses for cv. Amica and Temira (Figure 7.1B and 7.2B). Amica showed a 20 and 100 % damage rate for the 48 and 72 pulse treatments, respectively, whereas Temira only showed a 70 % damage rate for treatments of 72 pulses.

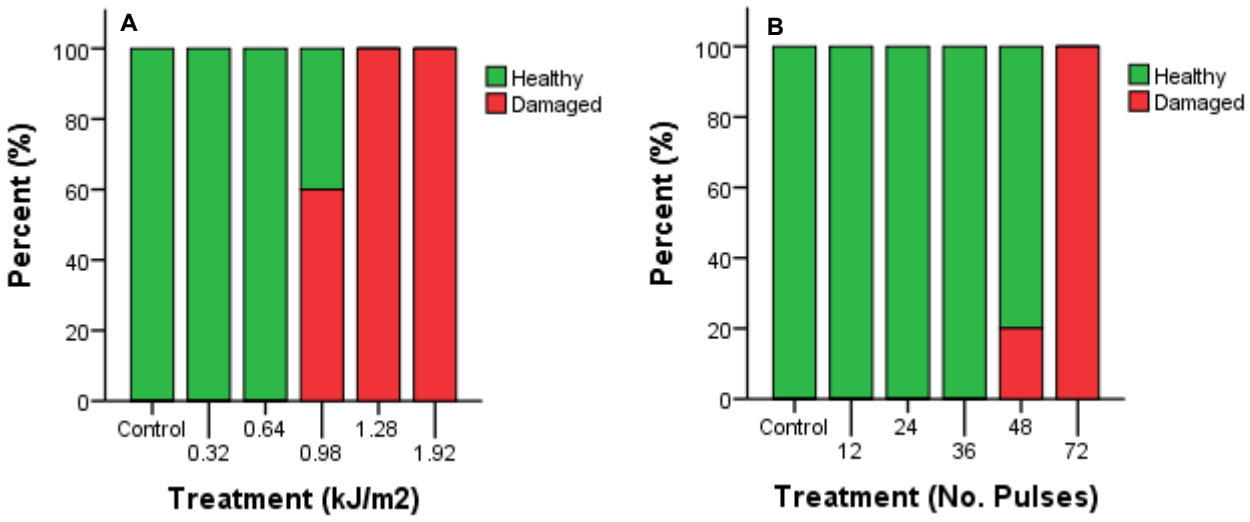

Figure 7.1: The percentage of healthy and damaged plants for the lettuce variety Amica. Plants were either treated with a conventional low-intensity mercury UV-C source (LIUV) (**A**) or a high intensity pulsed polychromatic light (HIPPL) source (**B**) from two independent replicate experiments carried out during May and June 2017 n=10.

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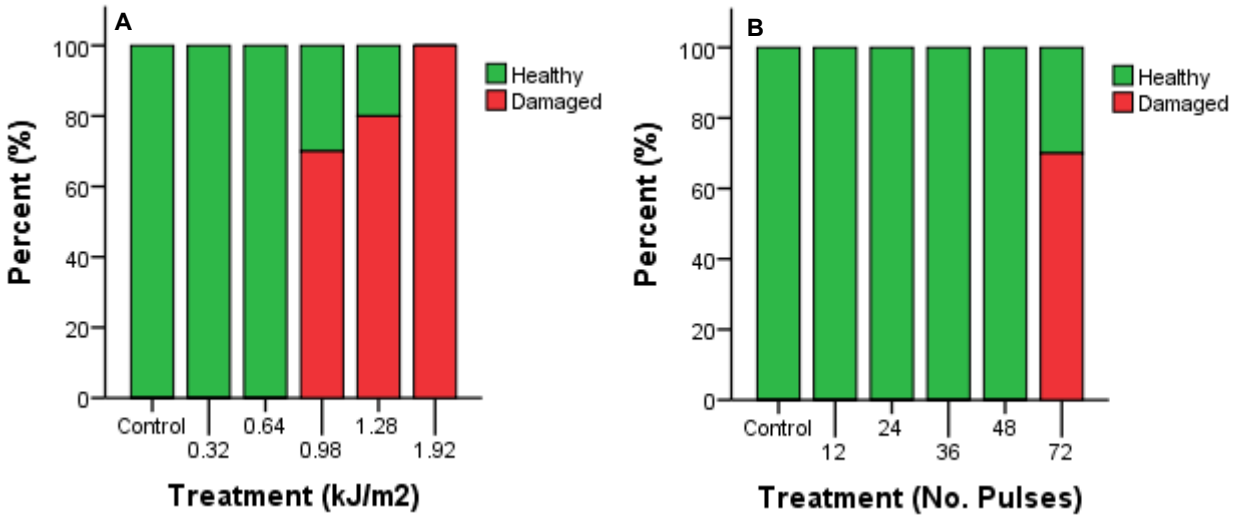

Figure 7.2: The percentage of healthy and damaged plants for the lettuce variety Temira. Plants were either treated with a conventional low-intensity UV-C source (LIUV) (**A**) or a high intensity pulsed polychromatic light (HIPPL) source (**B**) from two independent replicate experiments carried out during May and June 2017 n=10.

Following damage assessments, a leaf disc bioassay was performed to assess the ability of treatments to control disease caused by *B. cinerea.* Treatments that caused damage to the plant were not statistically analysed for their ability to reduce disease progression. The LIUV treatment that gave the greatest levels of control for cv. Amica was 0.98 kJ/m^2 which reduced disease by 24.4 % (Figure 7.3A). This treatment, however, caused damage to the crop (Figure 7.1) and is, therefore, not hormetic or suitable for commercial use. The most successful non-damaging treatment was 0.32 kJ/m^2 which reduced disease progression by 12.1 %. None of the LIUV treatments were significantly different from the control. None of the HIPPL treatments gave significant levels of disease control (Figure 7.3B). The 24-pulse treatment, however, did show some levels of disease reduction at 14.1 %. The damaging treatment of 72-pulses also showed disease control with reductions in disease progression at 11.3 %. Further replications with cv. Amica would be needed to identify any potential beneficial effects of treatment. This, however, was not possible due to time constraints.

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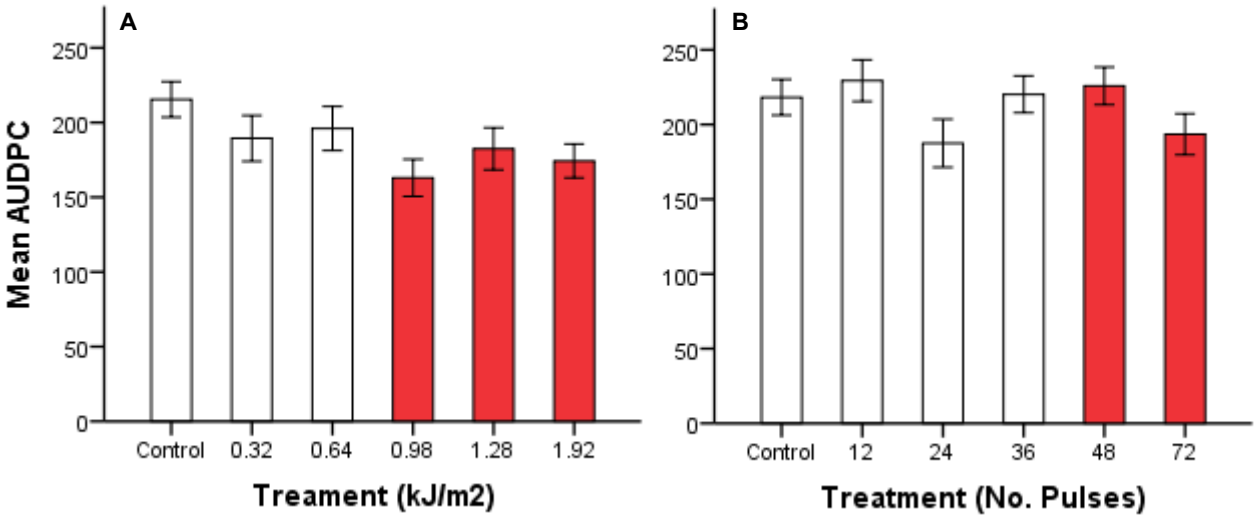

Figure 7.3: The area underneath the disease progression curve (AUDPC) from lettuce plants (Amica) treated with either low-intensity UV-C (A) or high-intensity, pulsed polychromatic light (B) and inoculated with *Botrytis cinereal* at 2 days post-inoculation. Disease progression measurements were taken at 2 and 3 days post inoculation to allow calculation of AUDPC. Red bars indicate that treatments caused damage to the crop. Damaging treatments were not statistically analysed. Graphs show the data from two independent replicate experiments performed during May and June 2017. n=10. Bars show ± 1 S.E.M. Labelling indicates statistical significance where groups sharing labels are not significantly different at $p< 0.05$.

The LIUV treatment showing the greatest levels of control for the cv. Temira was also damaging with the 1.92 kJ/m^2 treatment giving a 40.4 % reduction in disease progression (Figure 7.4A). Of the two treatments that fell below the damage threshold only the 0.64 kJ/m^2 treatment was significantly different from the control, reducing disease progression by 21.0 %. HIPPL treated cv. Temira gave statistically significantly levels of disease control (Figure 7.4B). The most successful of which, however, was also damaging (72-pulses) but reduced disease progression by 23.4 %. The 48-pulse treatment, however, was not damaging and provided a 21.4 % reduction in disease progression.

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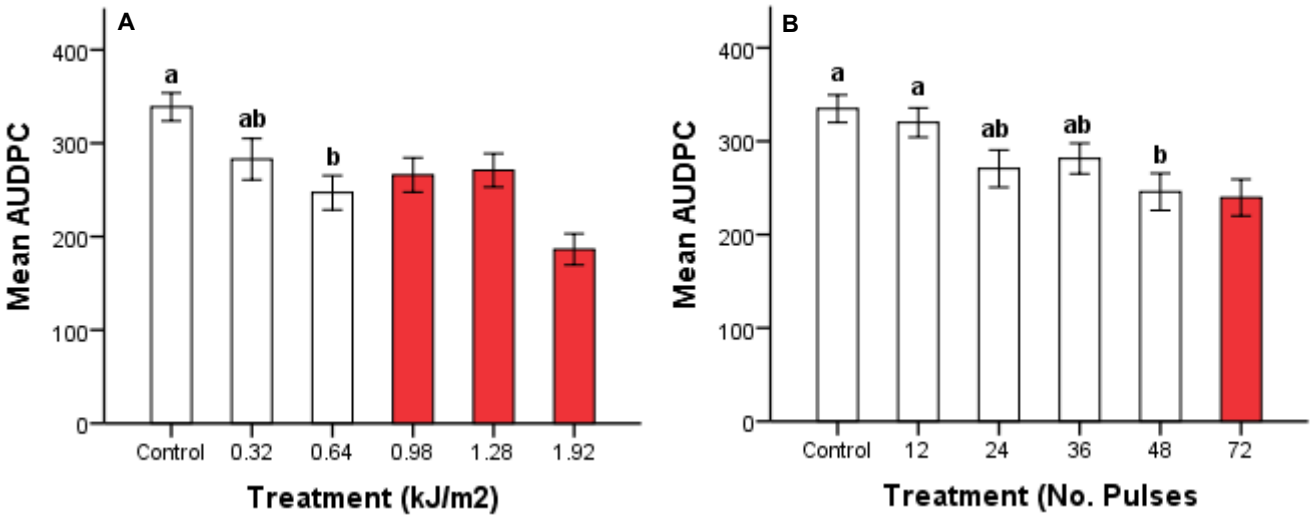

Figure 7.4: The area underneath the disease progression curve (AUDPC) from lettuce plants (Temira) treated with either low-intensity UV-C (**A**) or high-intensity, pulsed polychromatic light (**B**) and inoculated with *Botrytis cinerea* at 2 days post-inoculation. Disease progression measurements were taken at 2 and 3 days post inoculation to allow calculation of AUDPC. Red bars indicate that treatments caused damage to the crop. Damaging treatments were not statistically analysed. Graphs show the data from two independent replicate experiments performed during May and June 2017. n=10. Bars show ± 1 S.E.M. Labelling indicates statistical significance where groups sharing labels are not significantly different at $p < 0.05$.

The results obtained here yielded similar findings regarding cultivar susceptibility to LIUV or HIPPL damage and the levels of protection provided by treatments to those obtained in objectives five and six. As was previously reported in these experiments, we have observed an increased susceptibility to damage for the cv. Amica in comparison to Temira (Figures 7.1 and 7.2). Furthermore, the levels of protection provided from both LIUV and HIPPL treatments are greater for Temira. This is also observed alongside an increased susceptibility to *B. cinerea* for cv. Temira. Similar observations of elevated levels of susceptibility to disease and greater levels of disease protection following treatment were also seen while working with tomato fruit in previous preliminary studies. These results suggest that LIUV and HIPPL treatments can successfully control disease on the lettuce cultivar Temira. The mechanisms of disease control, however, are not yet known. Due to the experimental design, however, we can discount any direct effects of LIUV and HIPPL treatment on the phytopathogen. It is likely that disease control is achieved through similar mechanisms to that outlined in objective two. Further investigations, however, are required into the molecular mechanisms underpinning disease control.

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Conclusions

In meeting objectives five and six, LIUV and HIPPL treatments of lettuce showed potential for controlling disease on lettuce grown in the glasshouse. Variation in the damage threshold and the identification of treatments which controlled disease proved problematic. Using a controlled environment with fixed lighting intensity, temperature and stable humidity has mitigated the variation and allowed us to successfully replicate experiments. It can now be confirmed that both LIUV and HIPPL treatments can control disease, with statistical significance, for the cv. Temira.

Future work should now be focused on further replicating experiments, testing further cultivars and determining how changing environmental factors effect damage thresholds and treatments that may provide disease control. Investigations can then be carried out determining the mechanisms by which disease control is achieved and how treatments affect plant development, physiology, microbial ecology of the leaf surface and producer and consumer attributes including yield, plant compactness and nutritional qualities.

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Objective 8- LIUV Seed Treatments of Tomato to Control *B. cinerea*

Introduction

As was discussed in objective 5 and 6, pre-harvest LIUV and HIPPL treatments for inducing resistance on lettuce proved to be challenging. Applying light stress treatments to actively growing plants can potentially be problematic due to the plant continually changing its homeostasis in response to the changing environmental cues. For example, in times of increased light intensity and duration and UV-B/A exposure to lettuce plants will alter their production of phenolic compounds, which act as light quenchers, to protect them from light induced cellular damage. It was therefore decided that UV-C treatment of seeds would be investigated in order to remove the variation observed in the glasshouse applying foliar treatments.

The observation that seed treatments can induce resistance to disease was first published by Brown *et al.* (2001). To date, however, only a small pool of literature is available. Brown *et al.* (2001) showed that a treatment of 3.6 kJ/m^2 reduced the incidence of *Xanthomonas campestris* pv. *campestris* by 75 %, improved crop colour, increased head diameter delayed maturity and also doubled the dry mass of cabbage (*Brassica oleracea*).

Only one further investigation into UV-C seed treatments' ability to reduce pathogen burden has been performed. Siddique *et al.* (2011) treated groundnut (*Arachis hypogaea*) and mung bean (*Vigna radiata*) seeds with 0 to 60 minute exposures of UV-C; no dose or intensity data was given. Reductions of up to 88 % were observed in the incidence of disease for *Fusarium* spp., *Rhizoctonia solani* and *Macrophomina phaseolina*. The optimal treatment for disease resistance was shown to be dependent on crop and the pathogen undergoing investigation.

Aims

It was decided, with our industrial representatives, that LIUV seed treatment trials would be of a greater interest to tomato producers. Furthermore, HIPPL seed treatments were not of interest due to the large quantity of seeds that can be treated with a single LIUV source. All seed treatment studies were, therefore, carried out on tomato. The aim of this study was to explore a range of UV-C treatments and whether they could induce resistance against the necrotrophic tomato pathogens *Botrytis cinerea.*

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Materials and methods

Seeds, seed storage and UV-C treatment

Seeds of the cv. Shirley, as a previously commercial cultivar, were chosen due to their physiological similarities with current commercial tomato cultivars. Shirley F1 tomatoes, however, lack the high levels of resistance to *B. cinerea* seen in modern commercial cultivars. Seeds were purchased from the DEFRA-registered seed merchant Sow Seeds (UK). Seeds were stored at 6 °C at a relative humidity of 50 %. For treatment, seeds were placed in a 50 mm Petri dish lined with aluminium foil on the exterior. Lids were removed and seeds were treated at 20 W m^{-2} to total doses of 2, 4 and 6 kJ/m^2. Seeds were immediately stored in the dark at 21 °C for 5 days following the procedures of (Brown *et al.,* 2001).

Plant husbandry

Seeds were planted in in 1L pots of Levington™ M3 compost. Plants were grown in a temperature controlled, ventilated glasshouse at The University of Nottingham. Assimilation lighting by the means of 400W HPS SON-T lighting (Phillips) was used on a 16-hour photoperiod. Day and night temperatures were 24/18 ± 2 °C. Plants were grown to the first signs of flowering, as susceptibility to *B. cinerea* is greatest following flowering (Borges *et al.,* 2014).

Pathogen maintenance, spore preparation and inoculation

Pathogen propagation and spore preparation was performed as stated in objective 1. Inoculations were performed in a controlled environment with a 16/8 photoperiod set at 21 º C as preliminary inoculation experiments in the glasshouse proved to be highly variable. Petiole stub inoculations were performed according to Beyers *et al.* (2014) with an amended spore solution optimised in preliminary experiments. Three technical repeat inoculations were performed on each plant at true leaves 3, 4 and 5. Petioles were inoculated with 10 µl of 5 x 10^6 spores per ml amended with 40 % grape juice which was found to give the most consistent and uniformly-sized lesions in preliminary experiments (Figure 7.1 & Table 7.1). Lesion size was measures as total lesion length with Vernier callipers at 4 and 6 days post inoculation (DPI). Multiple measurements allowed the calculation of the area underneath the disease progression curve as stated in objective 1, equation 1.2.

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Experimental design and data analysis

For experiments concerning disease resistance three UV-C treatments, 2, 4 and 6 kJ/m^2 were utilised. *Botrytis cinerea* resistance assays had a completely randomised experimental design within the glasshouse. Three independent experimental replicates were performed with a total of 24 biological replicates per treatment group. The dates of each experimental replicate can be seen in table 8.1. Due to variation in susceptibility to *B. cinerea* between experiments factor correction was used to normalise the data following Ruijter *et al.,* (2006). Data was analysed in SPSS (IBM) by Kruskal Wallis (non-parametric ANOVA) with post-hoc testing by utilising the adjusted p-values for pairwise comparisons. Statistical significance is defined as p=0.05 in all experiments. All the results from individual experimental replicates in this chapter can be found in appendix 8.

Table 8.1. The dates that independent experimental replicates were performed

Experimental replicate	Date
1	5th September 2016
2	24th September 2016
3	27th January 2017

Results

Optimisation of inoculation techniques

A number of spore concentrations and amended buffers were attempted after inoculations using water to carry the spores at 1 x 10^6 proved to be ineffective. Concentrations of 1 x 10^6, 5 x 10^6 and 1 x 10^7 were attempted. The first amendment was potato dextrose broth (PDB) at 50 %; shown to be effective for *B. cinerea* inoculations in objective 5. Amendments with 40 % grape juice and also a potassium phosphate and glucose buffer were also attempted (Rossall, 2014; Beyers *et al.,* 2014). All PDB inoculations showed a fairly small inter quartile ranges (IQR) (Figure 8.1). Incidence of disease, however, was low and a number of outliers was observed (Table 8.2). The potassium phosphate buffer showed the greatest IQRs indicating that lesion progression was the most variable. The inoculations with grape juice were the most successful in terms of incidence with all concentrations giving incidences ≥ 93.3 %. Furthermore, IQRs for the 1 x 10^6 and 5 x 10^6 concentrations were small and the 5 x 10^6 concentrations showed a distribution closest to normal and had no outliers. For this reason

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the 5 x 10^6 concentration amended with 40 % grape juice was chosen for the following experiments.

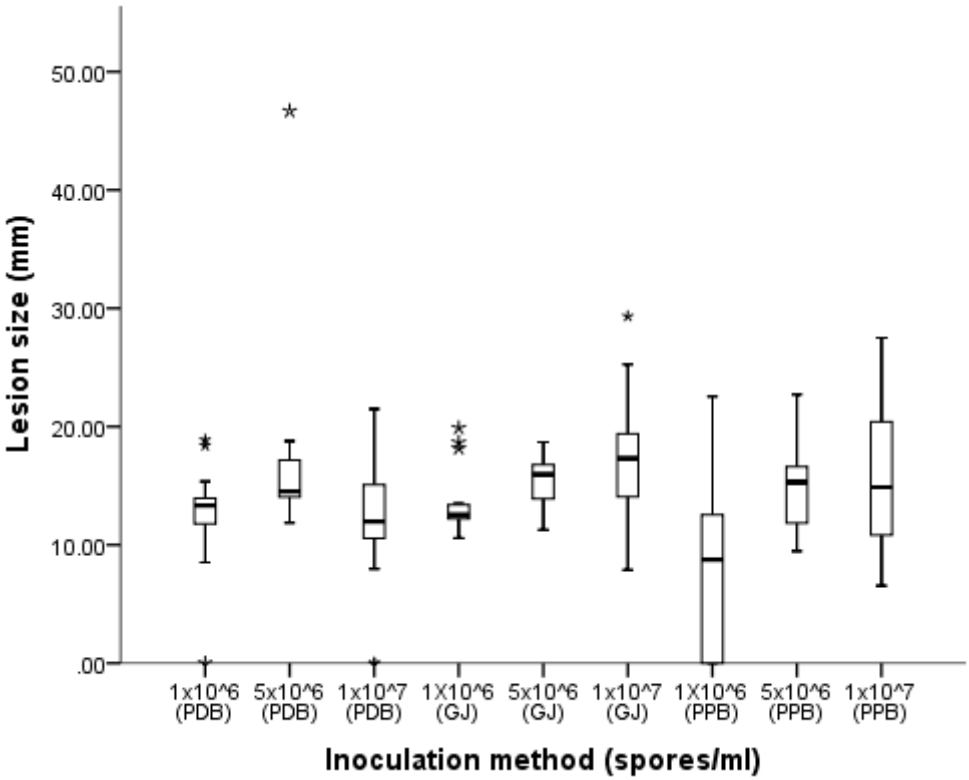

Figure 8.1. The lesion size from tomato plants inoculated with *Botrytis cinerea* at the flowering stage. Plants were inoculated at the wound site of a petiole stub with 10 µl of a calibrated spore suspension. Suspensions were amended with either 50 % potato dextrose broth (PDB), 40 % grape juice (GJ) and a potassium phosphate & glucose buffer as in Beyers *et al.* (2014).

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Table 8.2 Incidence of disease for tomato plants (cv. Shirley F1) following inoculation with *Botrytis cinerea* at the flowering stage. Plants were inoculated at the wound site of a petiole stub with 10 µl of a calibrated spore suspension. Suspensions were amended with 50 % potato dextrose broth (PDB), 40 % grape juice (GJ) and potassium phosphate & glucose buffer as in Beyers *et al.* (2014).

Inoculation technique	Incidence of disease (%)
1 x 10^6 in 50 % PDB	93.3
5 x 10^6 in 50 % PDB	100
1 x 10^7 in 50 % PDB	86
1 x 10^6 in 40 % GJ	100
5 x 10^6 in 40 % GJ	100
7 x 10^6 in 40 % GJ	93.3
1 x 10^6 in PPB	60
5 x 10^6 in PPB	100
1 x 10^7 in PPB	100

Disease control bioassays

Plants of the cv. Shirley were inoculated with *B. cinerea* at the flowering stage. Both a significant decrease in disease incidence and progression were observed for the 4 kJ/m^2 treatment. Control plants showed a disease incidence of 98.61 % with the 2 and 4 kJ/m^2 treatments showing 6.5 and 9.8 % reductions in incidence in comparison to the control (Figure 8.2). Incidence for the 6 kJ/m^2 showed no change from the control at 98.61 % and was also significantly different from the 4 kJ/m^2 treatment.

The disease progression was also recorded at 4 and 6 days following inoculation. Median disease progression was lower for all treatments in comparison to the control (Figure 8.3). The 4 kJ/m^2 showed the greatest reduction at 10.7 % while the 2 and 6 kJ/m^2 treatments showed 1.8 and 3.6 % reductions, respectively. Only the 4 kJ/m^2 was significantly different from the control.

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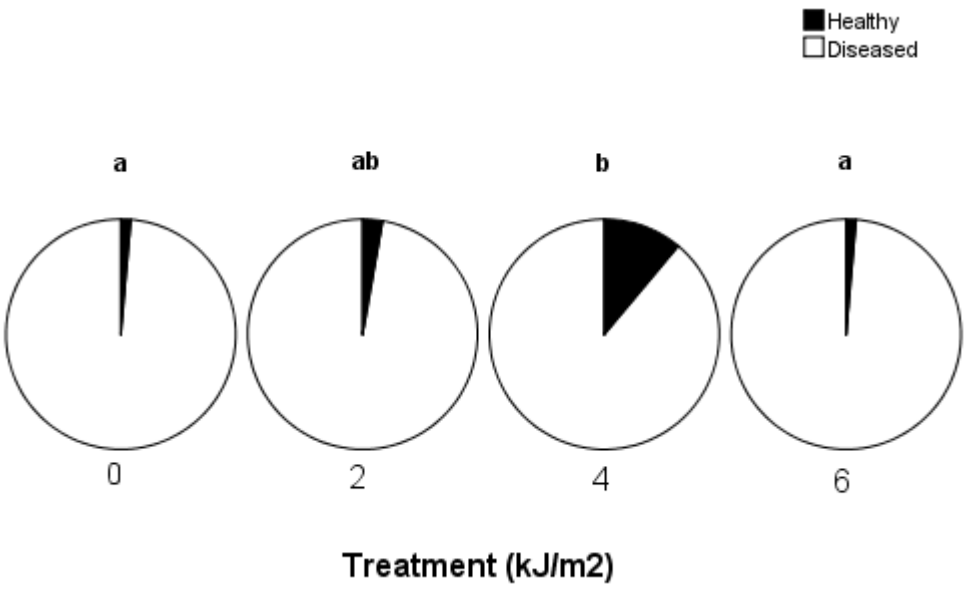

Figure 8.2. Disease incidence (%) for tomato plants of the cv. Shirley inoculated with *B. cinerea* following UV-C treatment of seeds. Labelling indicates statistical significance at $p < 0.05$. Means sharing the same label are not significantly different from each other. N=24.

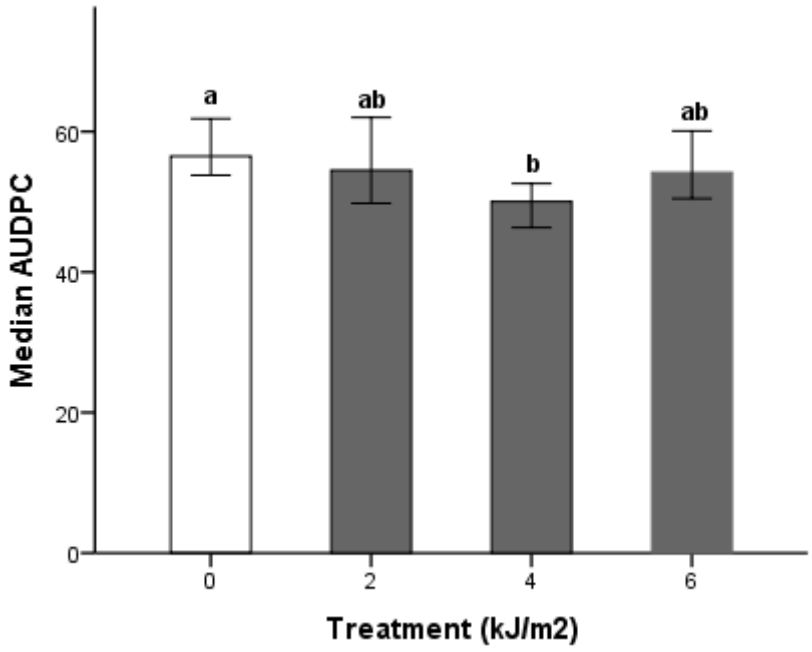

Figure 8.3. Area underneath the disease progression curve (AUDPC) for tomato plants of the cv. Shirley inoculated with *Botrytis cinerea* following UV-C treatment of seeds. Error bars show confidence intervals at 95 %. Labelling indicates statistical significance at $p < 0.05$. Means sharing the same label are not significantly different from each other. N=24.

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This is the first observation of UV-C seed treatment-induced resistance on tomato. The observation here is supported by reductions in disease incidence on both cabbage, mung bean and groundnut (Brown *et al.,* 2001; Siddique *et al.,* 2011). However, this is the first report of a reduction in disease progression alongside a reduction in incidence. Furthermore, the data may indicate good longevity for induced resistance as plants were 5 to 6 weeks old at the point of inoculation. Further investigation, however, is required to fully establish the longevity of resistance.

Conclusions and summary

For disease resistance assays, tomato seeds were treated with either, 2, 4 or 6 kJ/m^2. The 4 kJ/m^2 treatment showed significant reductions of 9.8 % and 10.7 % in both the incidence and disease progression of *B. cinerea*, respectively. Further investigation is required to determine how UV-C seed treatments reduce disease progression and incidence. It is likely to be achieved by similar means to the post-harvest induced resistance observed on tomato fruit. Further investigation, however, is required. This could be focused on the molecular changes at both early seedling development and before and after inoculation. This would allow the determination of a gene-priming response.

Seed treatments have been shown to be far more reliable and reproducible in comparison to the experiments on foliar treatments of lettuce in the glasshouse. Foliar treatments of lettuce in the glasshouse were shown to be variable in both their damage threshold to treatment and also treatment doses to reduce disease progression; depending on the timing of treatment within the year. This is to be expected as plants are continuously adapting to their environment and will, therefore, contain differing levels of light-quenching molecules, such as phenolics, which will alter the levels of stimulation achieved by doses dependant on the environmental conditions the plants are exposed to. Through utilising seed treatments, a single dose has proven effective and repeatable at 3 differing points during the year.

The finding that UV-C seed treatments can successfully induce resistance to disease is a significant one. For commercial tomato growers in the UK there is currently not a single fungicide against *B. cinerea* for which resistance has not been observed. To increase the likelihood of commercial integration, however, much more research is needed into how treatments effect crop physiology.

Furthermore, UV-C seed treatments have also been shown to reduce the impact of salt stress on two crops, lettuce and green beans (*Phaseolus vulgaris*). Ouhibi *et al.* (2014) treated lettuce with 0.85 or 3.42 kJ/m^2 of UV-C. The 0.85 kJ/m^2 treatment reduced the impact of salt

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stress on the dry weight of both roots and leaves, leaf number, total area, thickness, succulence and sclerophylly. Furthermore, the water content of roots and leaves was increased along with total phenolics. There was no change in the level of flavonoids and a reduction in the total antioxidant capacity.

Fotouh *et al.* (2014) showed reduced sensitivity to salt stress for seeds of green beans. Again, seeds were treated with 0 to 60 minutes of UV-C; no dose or intensity data was given. Treatments showed a reduction in the impact of salt stress to shoot and root dry and fresh mass. Furthermore, treatment increased proline concentrations; a marker for stress in plants. An increase in the activities of antioxidant enzymes superoxide dismutase, catalase, guaiacol peroxidase and ascorbate peroxidase was also observed. The UV-C treatments, however, were performed on pre-germinated seeds. Exploration into the impact of crop physiology following abiotic stressors could, therefore, be performed on tomato.

Objective 9-Effects of LIUV seed treatment on germination and early seedling growth

Introduction

In objective 8 it was shown that UV-C seed treatments of tomato (cv. Shirley) can reduce both disease progression and incidence of *Botrytis cinerea* by approx. 10 %. Although such a result may be extremely beneficial to commercial growers it is important to determine whether any potentially detrimental effects are occurring to the plants' growth and development. Previous literature, however, indicates quite the opposite with biostimulation and increases in yield being observed (Siddique *et al.,* 2011; Hamid & Javvaid, 2011; Shaikat *et al.,* 2013; Neelamegan & Sutha, 2015). It is unknown whether such effects will be observed on tomato.

Siddique *et al.* (2011) treated groundnut (*Arachis hypogaea*) and mung bean (*Vigna radiata*) seeds with 0 to 60 min exposures of UV-C; no dose or intensity data was given. Plants responded in a dose-responsive manner with different treatments showing the greatest increase in shoot and root weight and length, leaf area and number of nodules. Similar observations were seen on groundnut. The majority of treatments, however, showed increases for all of the measurements. Increases in total germination were also observed with the 30 min and 60 min treatments increasing germination to the greatest extent at 40 and 20 % for mung bean and groundnut, respectively.

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Hamid & Javvaid (2011), treated mung beans with UV-C and UV-A exposures of 2, 4 and 6 hours; no dose or intensity data was given. They found that specific leaf area, dry mass and length of shoots and roots were increased with the greatest doses proving to be most effective for both UV-C/A. The effects continued to become more pronounced for up to 60 days following planting- no further time points were analysed. The total number of germinated seeds increased to the greatest extent for the 6 and 2 hours treatments for UV-C and UV-A, respectively.

Neelamegan & Sutha (2015) treated groundnut for 0 to 60 min; no intensity or dose information was given. The length, fresh and dry mass of shoots and roots, number of branches and leaves, seedling vigour index and seedling tolerance index increased to the maximum treatment. Furthermore, increased yield and vegetative biomass was also observed along with both delayed maturity and pod production. An increase in the number of root nodules, leaf length, breadth, leaf area and number of flowers was also shown to increase. The measured effects, however, appeared to be dose-dependent with differing doses showing the greatest increases.

Finally, Shaikat *et al.* (2013) performed UV-B treatments on mash-bean (*Vigna mungo*). They observed increased germination velocity, reduced root and shoot growth, reduced levels of chlorophyll a and b, along with increased total phenol, anthocyanin and flavone accumulation along with an increase in PAL (phenyl ammonium lyase) and TAL (tyrosine ammonium lyase) activity; two enzymes involved in the biosynthesis of phenolic compounds.

Aims

Investigations into how UV-C seed treatment effects the germination and early seedling growth and development of tomato were performed. As identified in objective 8, a 4 kJ/m^2 treatment induced resistance against *B. cinerea.* The aims of this chapter were to assess the impact of treatment on seed germination and early seedling growth. Two higher doses of 8 and 12 kJ/m^2 were, therefore, used to determine at what point treatments become detrimental to plant growth.

Materials and methods

Monitoring seed germination and seedling development

UV-C treatments were performed as stated in objective 8. Seeds were sterilised to prevent growth of microorganisms shown to directly affect root development in preliminary studies. Seeds were washed in 70 % ethanol for 2 min and 3 % sodium hypochlorite (Sigma-Aldrich)

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for 20 min followed by 3 washes in SDW. Seeds were plated in a 120 mm square Petri dishes in a complete block randomised design. Plates were filled with 50 ml of Murashige Skoog media at 4.3g/l (pH 5.8) and 0.8 % agar technical No.3 (Oxoid). Plates were sealed with surgical tape (3MM). Plates were placed in a rack, allowing them to stand vertically, and exposed to a 16 h photoperiod at an intensity of 100 mE m^{-2} s^{-1} at 22 °C ± 1 °C and a relative humidity of 50-75 %. Germination was monitored for 7 days following plating. Primary root length and hypocotyl length was measured at 2 and 5 days post germination (DPG) with ImageJ. At 5 DPG seedlings were dissected and dried for 24 h at 50 °C. Dry mass of the roots, hypocotyl and cotyledon were then taken. To monitor germination a number of metrics were used including total germination percentage, germination index (Eq. 9.1), T_{50} (Eq. 9.2) and Z-index (Eq. 9.3) (Coolbear *et al.,* 1984; Walker-Simmons, 1987; Ranal *et al.,* 2009). For the monitoring of root growth and stem mass fraction, specific root and stem mass, root length ratio, root-shoot ratio and the seedling vigour index were calculated (Table 9.1).

$$GI = (7 \times n_1) + (6 \times \mathrm{n}_2) + \ \ldots \ + \ (1 \times \mathrm{n}_7)$$

Equation 9.1. Germination index (GI), where n_1, n_2, … , n_7 are the number of germinated seeds on the first, second and subsequent days until the 7th; 7, 6, … , 1 are the weights given to the seeds germinated on the first, second and 7th days, respectively (Walker-Simmons, 1987).

$$T_{50} = t_i + \frac{(N+1)/2 \ - \ n_i}{n_j \ - \ n_i} \ \times (t_j \ - \ t_i)$$

Equation 9.2. The time to reach 50 % germination (T_{50}) of the total number of seeds planted. *N* is the final number of seeds that have germinated, n_i and n_j are the total number of seeds germinated at adjacent time points t_i and t_j where $n_i < (N+1)/2 < n_j$ (Coolbear *et al.,* 1984).

$$Z = \sum \frac{Cn_{i,2}}{N} \quad Cn_{i,2} = \frac{n_i(n_i - 1)}{2} \quad N = \frac{\sum n_i(n_i - 1)}{2}$$

Equation 9.3. Synchrony of germination (Z-index) where n_i is the number of seeds germinated during the *i*th time (Ranal *et al.,* 2009).

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Table 9.1. Growth metric equations

Measure	Equation	Reference
Root mass fraction	Root mass ÷ total plant mass	Poorter & Ryser 2015
Stem mass fraction	Stem mass ÷ total plant mass	Poorter & Ryser 2015
Root length ratio	Root length ÷ plant mass	Poorter & Ryser 2015
Specific root length	Root length ÷ root length	Poorter & Ryser 2015
Specific stem length	Stem length ÷ stem length	Poorter & Ryser 2015
Root-shoot ratio	Root mass ÷ Shoot mass	Monk, 1966
Seedling vigour index II	Germination percentage × mean dry weight	Kharb *et al.,* 1994

Experimental design and data analysis

Three treatments of 4, 8 and 12 kJ/m^2 were used. Three independent replicate experiments were performed with 21 biological repeats per experiment n = 63. Data was analysed by one-way ANOVA with Tukey's post-hoc testing was performed. Where the homogeneity of variances assumption could not be met Welch's robust ANOVA was performed followed by the Games-Howell post-hoc test. All experimental replicates were performed during February 2017. All data for individual experimental repeats can be found in appendix 9.

Results and discussion

Effects on germination

To determine the effect of UV-C on seedling growth an extended range of treatments were performed starting with the treatment that showed the greatest promise for disease resistance against *B. cinerea* (4 kJ/m^2) and then increasing the treatment dose by two and three-fold to 8 and 12 kJ/m^2. These treatments were chosen to ascertain at what point the treatments became detrimental to plant growth and development.

When monitoring germination for 7 days following planting, no significant differences were observed. The 8 kJ/m^2 treatment, however, showed a stimulatory effect to germination with germination occurring from 2 DPP whereas all other treatments began germination at 3 DPP (Figure 9.3). Furthermore, an increase in the cumulative germination percentage was also

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observed for all DPP when compared to the control (Figure 9.3). At 3 and 4 DPP, the 4 and 12 kJ/m^2 treatments showed slightly reduced levels of germination in comparison to the control. From day 5 onwards, however, germination rate was similar for the control, 4 and 12 kJ/m^2 treatments. This can be highlighted by the total germination percentages which are all similar with small increases for all treatments apart from a slight decrease for the 4 kJ/m^2 treatment at 87.3 % (Table 9.2). The stimulatory effect on germination of the 8 kJ/m^2 can be seen by an increase in germination index to 79.0 in comparison to the control at 66.33, a reduction in the time to 50 % germination (T_{50}) from 4.23 days for the control to 3.41. Finally, the synchronicity of germination was also shown to increase from 0.21 in the control to 0.25 (scale from 0-1). The 4 kJ/m^2 treatment, which showed a significant reduction in disease incidence and disease control, showed minor non-significant decreases in germination index, T_{50}.

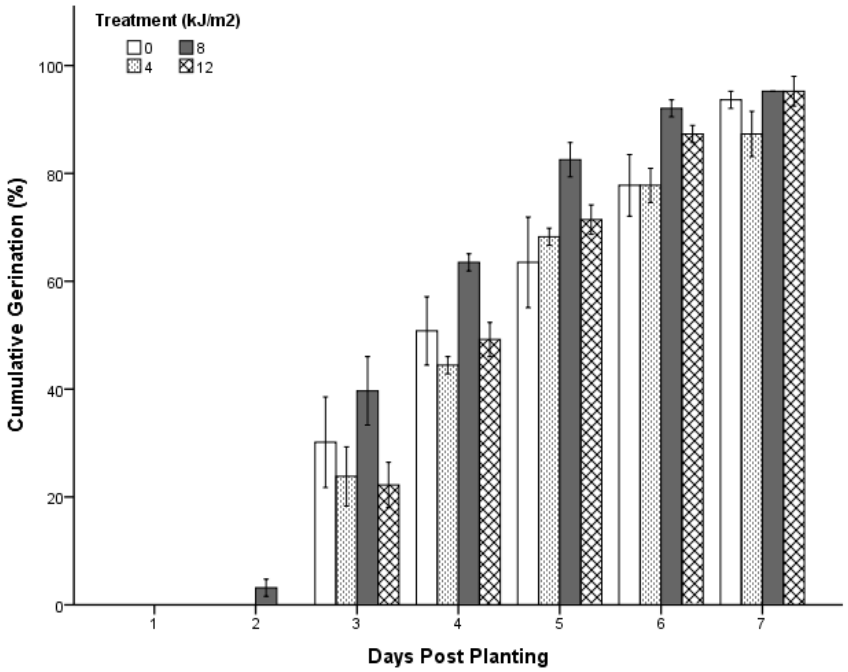

Figure 9.3: The cumulative germination percentage of tomato seeds cv. Shirley (F1) following treatment with UV-C. Error bars show ± 1 S.E.M. N=63.

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Table 9.2: Germination metrics of cv. Shirley (F1) tomato seeds following UV-C treatment

Treatment (kJ/m2)	Total germination (%)	Germination index	T_{50}	Z-index
0	93.65 ± 2.75	66.33 ± 10.97	4.23 ± 0.87	0.21 ± 0.04
4	87.30 ± 7.27	63.33 ± 1.15	4.32 ± 0.08	0.21 ± 0.03
8	95.24 ± 0.00	79.00 ± 3.00	3.41 ± 0.36	0.25 ± 0.05
12	95. 24 ± 4.76	68.33 ± 4.04	4.13 ± 0.23	0.19 ± 0.01

± 1 standard deviation

Effects on seedling growth

At 5 days after germination, seedlings were measured, dried and their total dry mass was taken. Increases in dry mass of 5.9, 11.4 and 4.3 % were observed for the 4, 8 and 12 kJ/m^2 treatments, respectively (Figure 9.4). Only the 8 kJ/m^2 treatment was significantly different from the control. The increase in dry mass was also complemented with an increase in the seedling vigour index for all treatments. The greatest increase was, again, observed for 8 kJ/m^2 at 386.5 in comparison to the control seeds which gave a SVI-II value of 335.9 (Table 9.3). No significant differences were observed for mean seedling length at 2 or 5 days post germination (Figure 9.5).

Observations of a biostimulatory effect after UV-C seed treatment of seeds has also been observed by Brown *et al.* (2001); Siddique *et al.* (2011); Hamid & Javvaid (2011) and Neelamegan & Sutha (2015). Furthermore, Hamid & Javvaid (2011) showed an increase in the biostimulatory effect of treatment on the plants up to 60 days following treatment and Neelamegan & Sutha (2015) showed an increase in yield. Further investigation into vegetative development, anthesis and fruit development is, therefore, required to elucidate the full potential of UV-C treatment on tomato.

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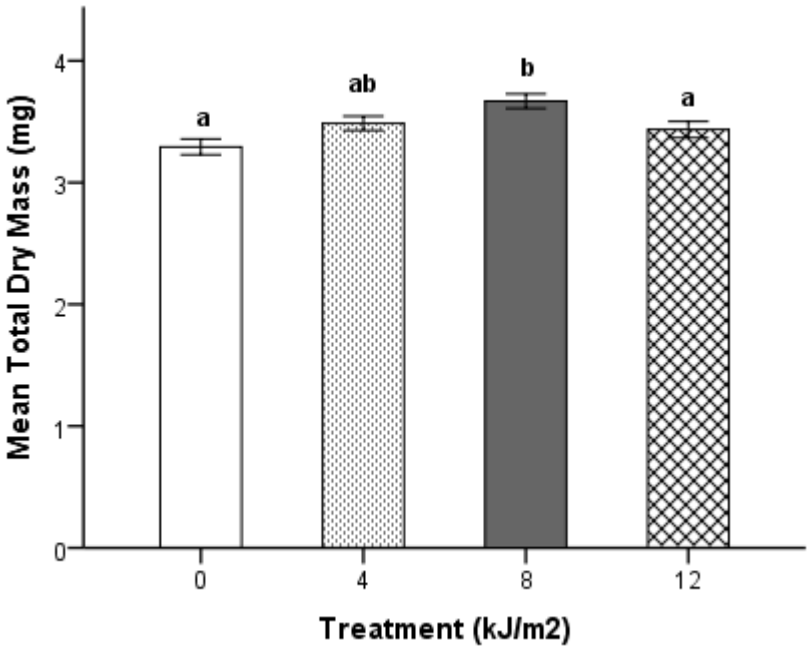

Figure 9.4: Mean total dry mass (mg) of cv. Shirley (F1) tomato seedlings at 5-days post germination following UV-C treatment of seeds. Error bars show ± 1 S.E.M. N=63.

Table 9.3: Seedling vigour index-II (SVI-II) of tomato seeds cv. Shirley (F1) following UV-C treatment

Treatment (kJ/m2)	Seedling vigour index
0	335.87 ± 39.61
4	339.07 ± 80.40
8	386.51 ± 73.71
12	377.39 ± 78.10

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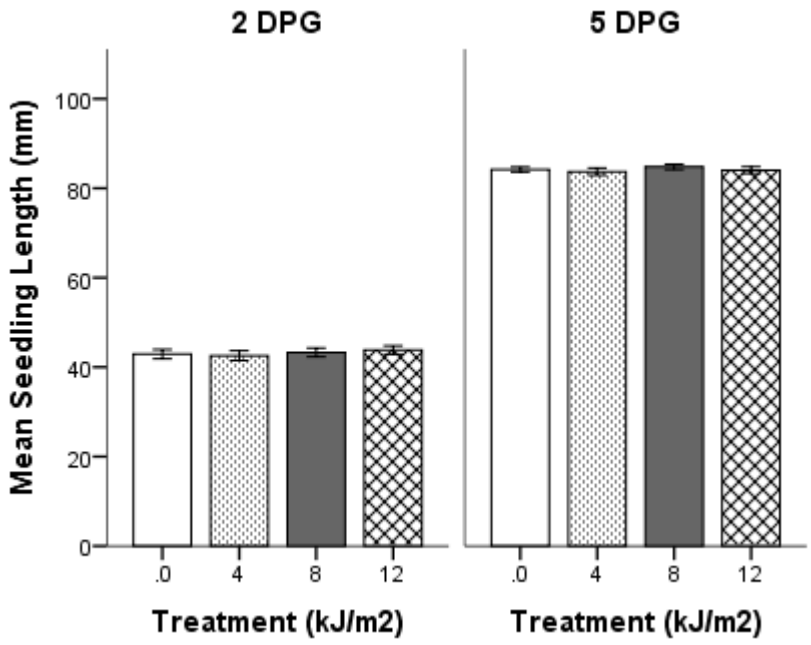

Figure 9.5: Mean seedling length of cv. Shirley (F1) tomato seedlings at 2 and 5-days post germination (DPG) following UV-C treatment of seeds. Error bars show ± 1 S.E.M. N=63.

To further elucidate the effect of UV-C seed treatments on growth, seedlings were dissected to allow the determination of the effect on growth of the major plant organs. The growth of shoots was stimulated for each of the treatments. Shoot dry mass was significantly increased for the 8 kJ/m^2 treatment with a 9.6 % increase in comparison to the control (Figure 9.6). The 4 and 12 kJ/m^2 treatments showed smaller increases at 5.9 and 3.4 %, respectively.

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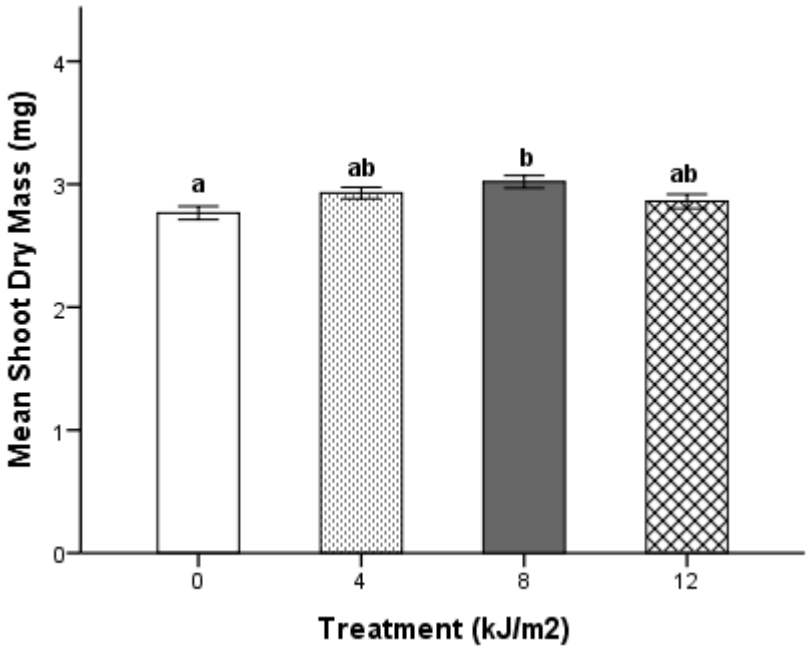

Figure 9.6: Mean shoot dry mass of cv. Shirley (F1) tomato seedlings at 5-days post germination (DPG) following UV-C treatment of seeds. Error bars show ± 1 S.E.M. N=63

The dry mass of cotyledons was increased for both the 4 and 8 kJ/m^2 treatments by 4.4 and 8.0 %, respectively. The effects of the 12 kJ/m^2 treatment, however, were similar to that of the control at 0.52 and 0.57 mg, respectively (Figure 9.7). None of the differences, however, were significant. Further investigation is required during vegetative growth to determine how the efficiency of photosynthesis is affected.

A stimulatory effect on the mass of hypocotyls was also observed (Figure 9.8). Mass was increased for all of the treatments, in comparison to the control, with 9.4, 12.0 and 7.6 % increases for 4, 8 and 12 kJ/m^2, respectively. Only the 8 kJ/m^2 treatment, however, was significantly different from the control. As was observed for the total seedling length, for all of the treatments, at both 2 and 5 days following germination no significant differences were observed (Figure 9.9).

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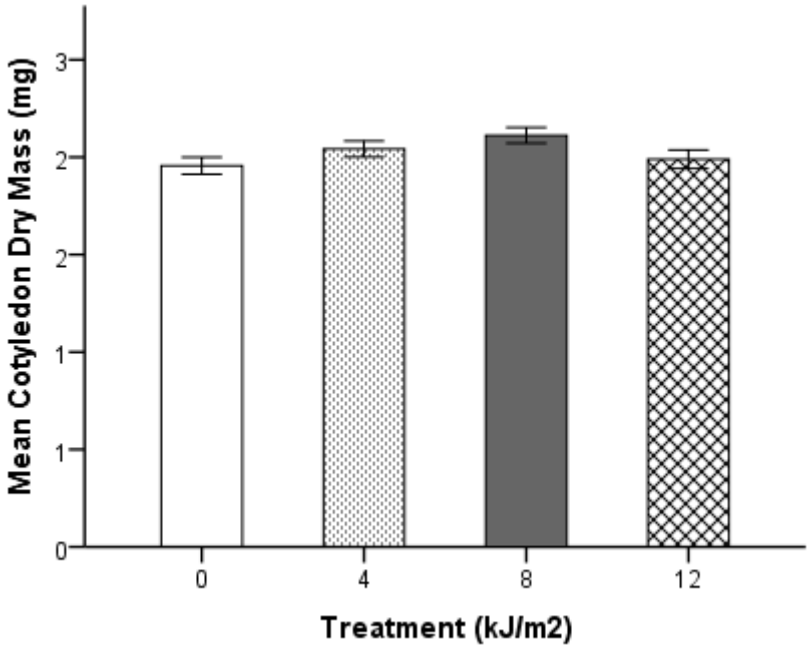

Figure 9.7: Mean cotyledon dry mass of cv. Shirley (F1) tomato seedlings at 5-days post germination (DPG) following UV-C treatment of seeds. Error bars show ± 1 S.E.M. N=63.

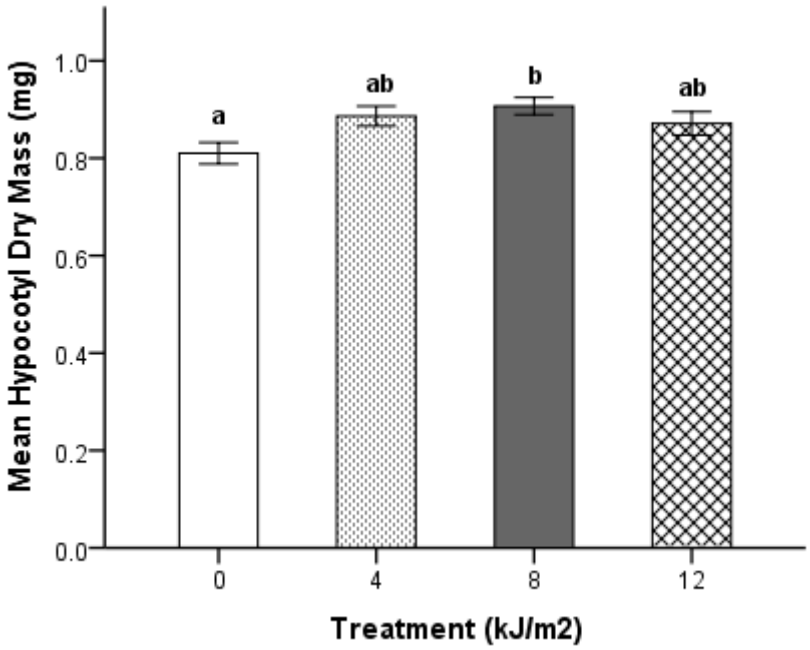

Figure 9.8: Mean hypocotyl dry mass of cv. Shirley (F1) tomato seedlings at 5-days post germination (DPG) following UV-C treatment of seeds. Error bars show ± 1 S.E.M. N=63.

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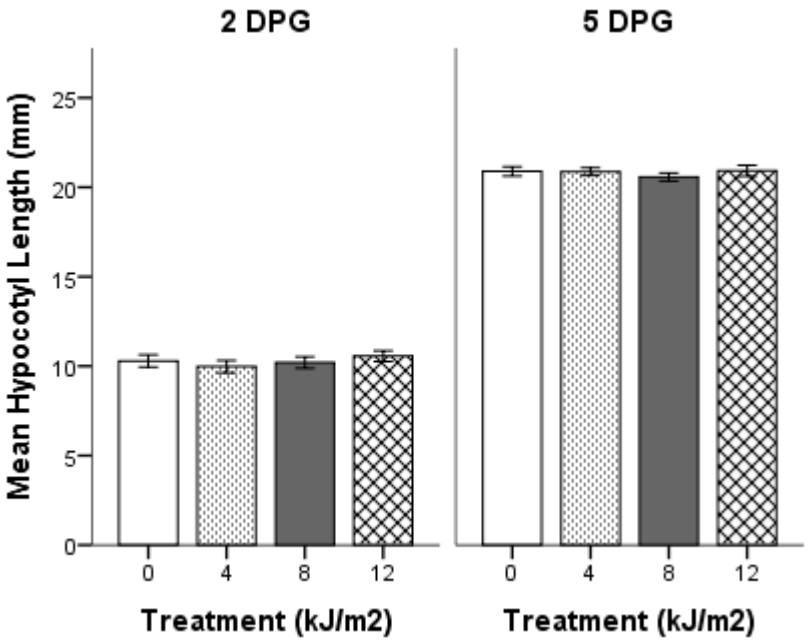

Figure 9.9: Mean hypocotyl length of cv. Shirley (F1) tomato seedlings at 2 and 5-days post germination (DPG) following UV-C treatment of seeds. Error bars show ± 1 S.E.M. N=63.

The number of lateral roots at 5 DPG was not significantly affected by any of the treatments, although small increases at 2.2, 3.2 and 2.5 % were observed for the 4, 8 and 12 kJ/m^2 treatments, respectively (Figure 9.10). Root dry mass, however, showed a significant increase at 23.1 % for 8 kJ/m^2 in comparison to the control (Figure 9.11). The 4 and 12 kJ/m^2 treatments also showed smaller increases at 5.8 and 9.1 %, respectively. Mean primary root length, for all treatments, showed little variation from the control (Figure 9.12). None of the UV-C treatments, therefore, showed any negative impact on early root growth or their basic architecture. Furthermore, an increase in root dry mass may lead to greater efficiency in nutrient and water acquisition.

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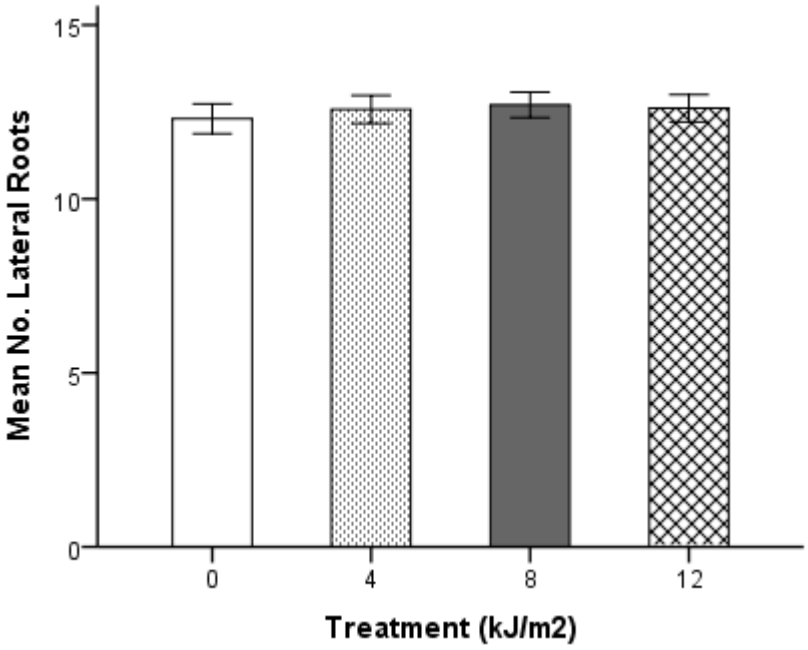

Figure 9.10: Mean number of lateral roots for the tomato seedlings of the cv. Shirley (F1) at 5-days post germination (DPG) following UV-C treatment of seeds. Error bars show ± 1 S.E.M. N=63.

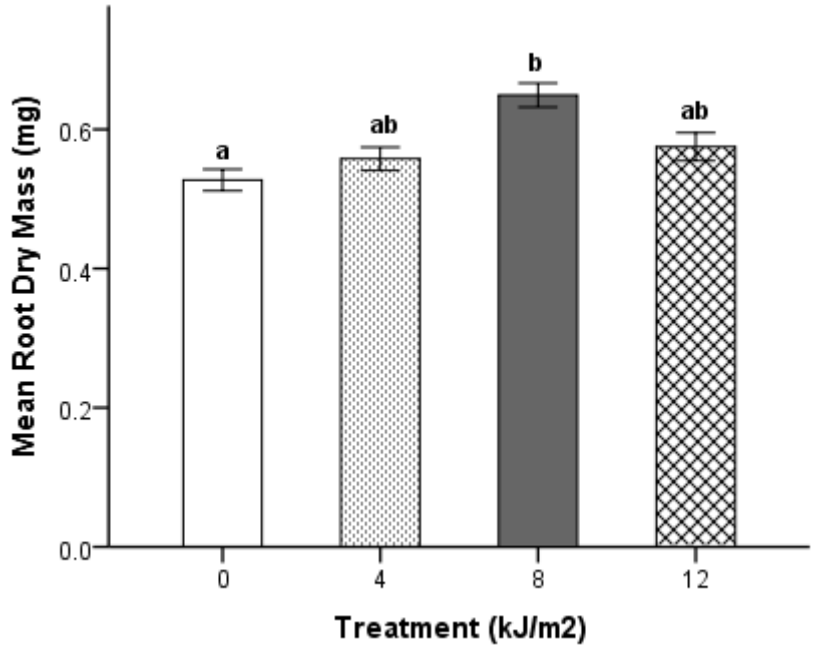

Figure 9.11: Mean root dry mass of cv. Shirley (F1) tomato seedlings at 5-days post germination (DPG) following UV-C treatment of seeds. Error bars show ± 1 S.E.M. N=63.

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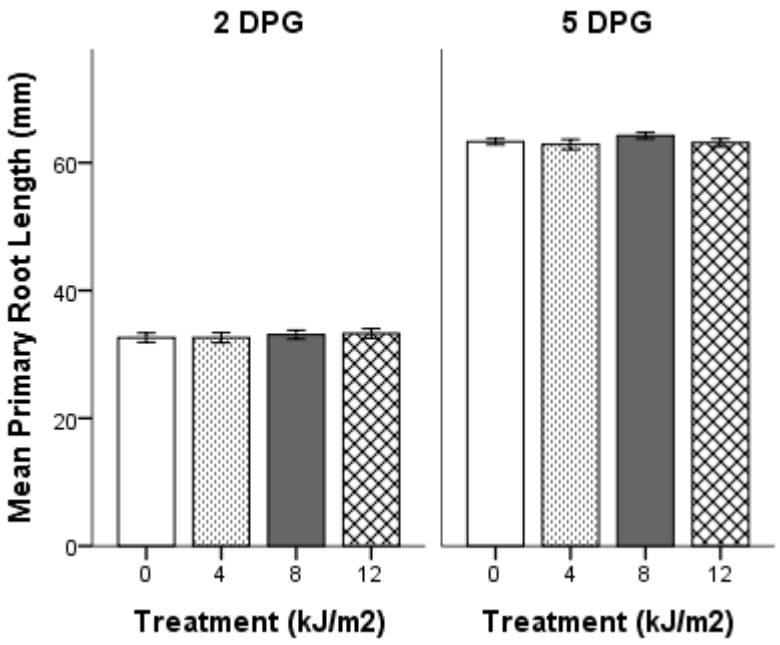

Figure 9.12: Mean primary root length of cv. Shirley (F1) tomato seedlings at 2 and 5-days post germination (DPG) grown from UV-C treated seeds. Error bars show ± 1 S.E.M. N=63.

Although significant increases in both root and shoot dry mass were observed for the 8 kJ/m^2 treatment, a significant increase in root mass fraction and decrease in stem mass fraction were also seen (Figure 9.13 A and E). This indicates a general biostimulatory effect that is weighted towards the roots; inferring photoassimilates are being directed primarily towards the root system. Increased root growth may lead to increased efficiency of both water and nutrient uptake. This may have been achieved without any negative effect to the growth or efficiency of photosynthetic organs, which is associated with increasing root mass fraction, as cotyledon mass also exhibited an increase following treatment (Figure 9.7).

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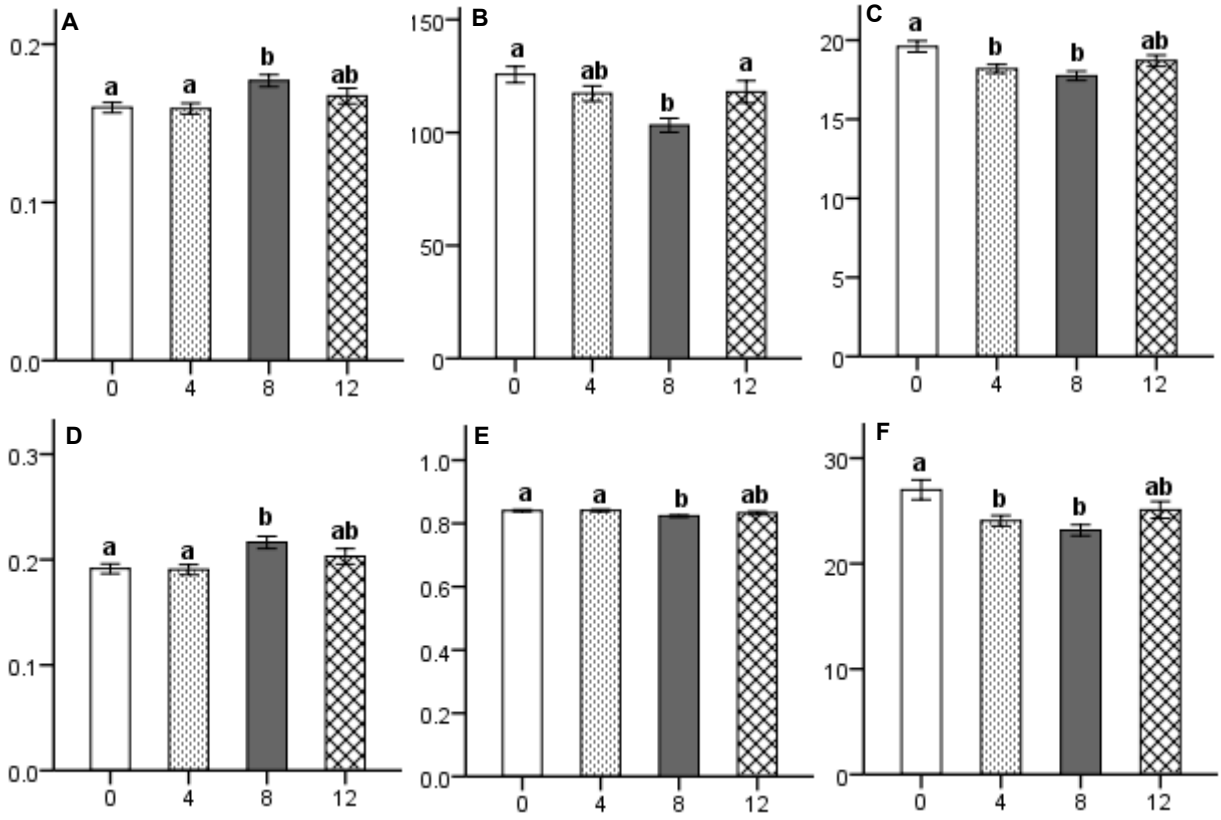

Figure 9.13: Mean root mass fraction (A), specific root length (B), root length ratio (C), root-shoot ratio (D), stem mass fraction (E) and specific stem length (F) from tomato plants grown from UV-C treated seeds of the cv. Shirley (F1). Seeds were treated with total doses of either 0, 4, 8 or 12 kJ/m^2. Error bars show ± 1 S.E.M. N=63.

A significant reduction in both specific stem and root lengths and root-length ratios were also observed for the 8 kJ/m^2 treatment (Figure 9.9, 9.12 & 9.13 B, C and F). Taken together with the absence of any changes to root or shoot length this point towards an increase in root and shoot volume. Further investigation into the changes to organ-specific cellular structure is required. For example, are changes to root volume increased by greater root hair density, increased cellular volume or potentially an increase in cell wall deposition. Increases in cell wall deposition were observed by Charles *et al.,* (2008b) following UV-C treatment of tomato fruit. Finally, a significant increase, from the control, in the root-shoot ratio was also observed for 8 kJ/m^2 signifying an increase in general plant health.

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Conclusions and summary

No detrimental effects were observed for any of the UV-C treatments performed here. Conversely, the increased 8 kJ/m^2 treatment showed significant biostimulatory effects during seedling growth. Root growth was stimulated to the greatest extent with a 23.1 % increase in dry mass. No significant differences in primary root length or lateral root number were observed. Shoot growth was also stimulated to a lesser extent (9.1 %) to that of the roots with the hypocotyl showing greater increases in comparison to that of cotyledons at 12.0 and 8.0 %, respectively. The positive effects were also observed for the 4 kJ/m^2 treatment. None, however, were significantly different from the control. Moreover, preliminary studies indicate both reduced disease progression and incidence for the 8 kJ/m^2 treatment.

Positive influences were also observed for seed germination with the 8 kJ/m^2 treatment giving an increase in total germination %, germination index and synchronicity (Z index). Furthermore, the time to 50 % germination was also reduced. No differences were observed for the 4 kJ/m^2 treatment.

Further investigation into potential changes to crop physiology from vegetative growth to fruiting is required as previous work has indicated an increase in biostimulation up to 60 days following planting and increases in yield (Hamid & Javvaid, 2011). Investigation into the molecular mechanisms leading to both the reduction in disease burden and biostimulation are also required.

UV-C seed treatment of tomatoes may have an extremely beneficial impact on commercial tomato production. As stated previously, there is not a single fungicide against *B. cinerea* for which resistance has not been observed. The ability to induce resistance prior to planting could potentially reduce costs by reducing fungicide applications and losses due to disease. Furthermore, with further investigation the positive influence on root growth may allow increased nutrient and water uptake efficiency, potentially leading to an increase in yield. UV-C seed treatments are an exciting area of research, which will be advanced by the results produced here.

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Conclusions

Post-harvest fruit treatments:

- The HIPPL source was shown to successfully induce resistance and delay ripening on cv. Mecano.
- A 16-pulse treatment gave comparative levels of disease resistance, against *B. cinerea,* and delayed ripening as did the established LIUV treatment of 3.7 kJm^2.
- High intensity pulsed polychromatic light sources can reduce treatment times by 97.3 %.
- Both LIUV and HIPPL treatments elicited local disease resistance and delayed ripening when treating fruit from the side, blossom end or calyx.
- The molecular mechanisms underpinning both LIUV and HIPPL hormesis are highly similar.
- Both salicylic acid and jasmonic acid biosynthesis markers and pathogenesis-related proteins are upregulated indicating that induced resistance may act not only against necrotrophic pathogens but also biotrophic pathogens and plant pests.
- Ethylene and polygalacturonase production is downregulated and play a role in the observed delayed ripening.
- Changes in secondary metabolism are observed though upregulations to PAL and carotene hydroxylase. Whereas downregulation of flavonol synthase is observed.

Pre-harvest foliar treatments of lettuce in the glasshouse:

- Preliminary in work the glasshouse shows: -
    - Preliminary results showed 2-54 % reductions in disease.
    - Lettuce in the glasshouse show variation in the damage threshold and treatment which reduced disease for both pulsed and conventional treatments.
    - Repeated treatments, utilising doses that are not damaging at any point of the year, do not successfully reduce disease progression.

Pre-harvest foliar treatments of lettuce in the controlled environment:

- LIUV and HIPPL treatments control the disease progression of *B. cinereal* by 21.0 and 21.4 % on the cv. Temira, respectively.
- Damage thresholds vary between the cvs. Amica and Temira, with Amica showing increased susceptibility to damage.

UV-C seed treatment of tomatoes

- Control of *Botrytis cinerea* with an approx. 10% reduction in both incidence and disease progression.

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- Biostimulation of both roots, hypocotyls and cotyledons.
- An increase in root mass fraction indicating that root growth is stimulated to a greater extent to that of shoots which may lead to increased water and nutrient uptake.
- No change to root length or No. lateral roots indicating an increase in root volume which may lead to increased nutrient and water uptake efficiency.

Future work:

- Continued research into the effectiveness of preharvest LIUV and HIPPL treatments on lettuce.
- Investigations into how environmental conditions affect the treatment dose for controlling disease for lettuce in a controlled environment.
- Elucidate the physiological changes to the tomato crop following UV-C seed treatment.
- Investigation into the molecular mechanisms controlling stimulated root growth and reduction in disease.
- Determining the potential for UV-C seed treatments to control against abiotic stress and pests.

Knowledge and Technology Transfer

Project meetings:

- Initiation meeting, Sutton Bonington, 16th March 2015
- Annual meeting, Sutton Bonington, 23rd October 2015
- Annual meeting, Sutton Bonington, October 2016

Conferences:

- Molecular Biology of Plant Pathogens; poster presentation, 9th April 2015.
- AHDB: Studentship Conference; poster presentation, 16th September 2015.
- British Tomato Conference; oral presentation, 24th September 2015.
- BCPC: Crop Diseases Are We Losing Control; industry forum, 3rd December 2015.
- KTN: Early Career Researchers; poster presentation 22nd March 2016.
- BSPP: Food Security, Biosecurity and Trade; poster presentation 12th September 2016.
- AHDB Annual Studentship Conference; oral presentation 16th November.

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Publications:

- AHDB Grower [Article in preparation], May 2017
- UV-C Treatment of Tomato Seed Induces Disease Resistance to Botrytis cinerea and Stimulates Growth. [Manuscript in preparation], May, 2017.
- A Comparison of the Molecular Mechanisms Underpinning High Intensity Pulsed Polychromatic Light and Low Intensity UV-C Hormesis in Tomato Fruit. Postharvest Biology and Technology [under review], April, 2017.
- A Comparison of Low Intensity UV-C and High Intensity Pulsed Polychromatic Sources as Elicitors of Hormesis in Tomato Fruit. Postharvest Biology and Technology, 125, pp.52-5, March 2017.
- AHDB Grower; “A Little Light Goes a Long Way”, May 2016.

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Appendix

Appendix 1

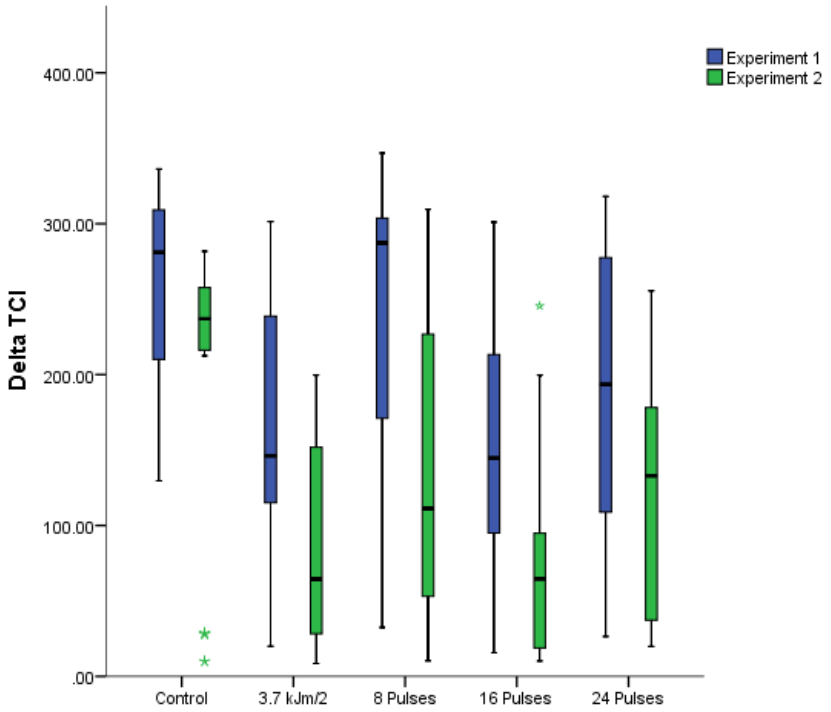

Figure A1.1. The Δ TCI (tomato colour index) from day 0 - 10 of mature green fruit from cv. Mecano. Box plots show the data from the individual independent replicate experiments (n=15). Asterisks represent observed outliers.

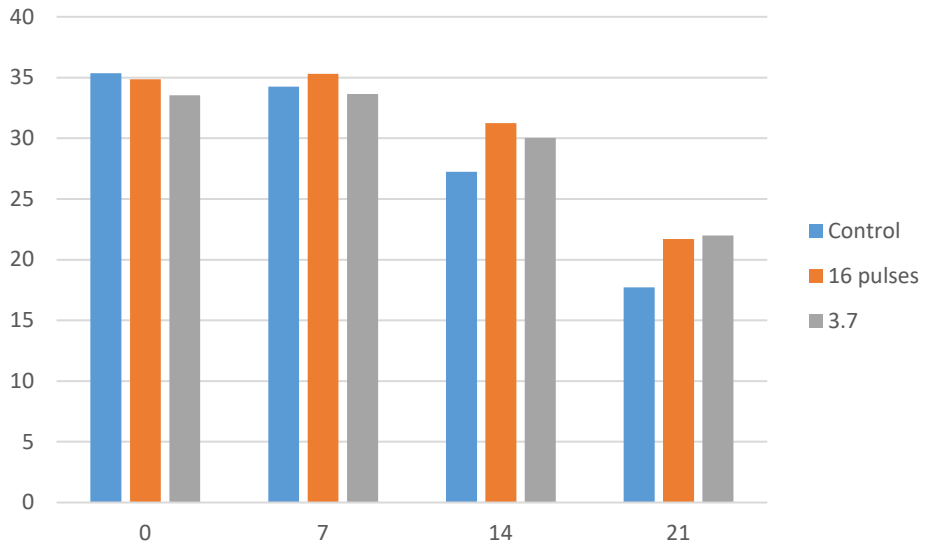

Figure A1.2A. Texture analysis performed on mature green fruit of the cv. Mecano. Fruit were treater either with 16 pulses of high intensity pulsed polychromatic light (HIPPL) or 3.7 kJ/m2 of low intensity UVC (LIUV) N= 10.

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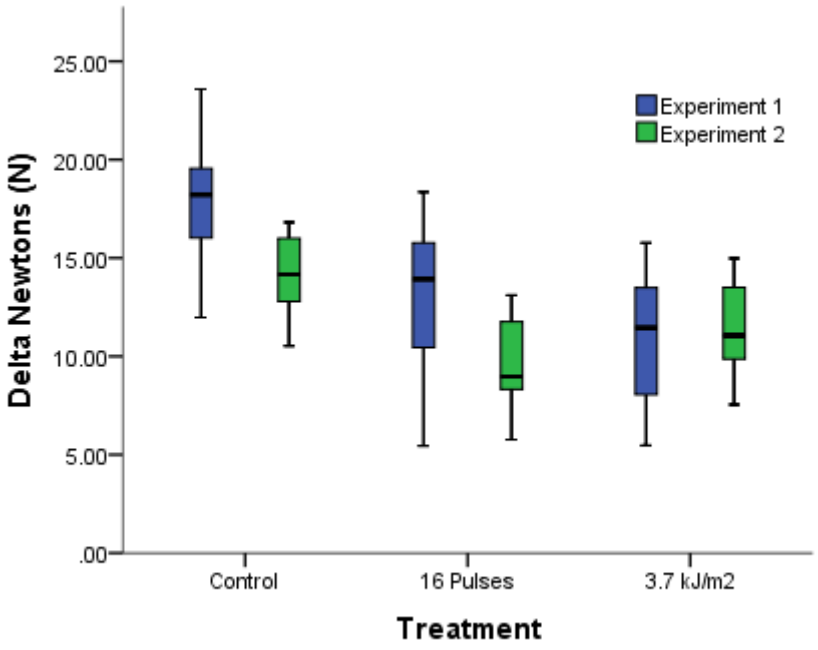

Figure A1.3 The change (delta) in firmness (newtons) from day 0 - 21 of mature green fruit from cv. Mecano treated with either a low-intesity UV-C source or high-intensity pulsed polychromatic source. Box plots show the data from the individual independent replicate experiments (n=10). Asterisks represent observed outliers.

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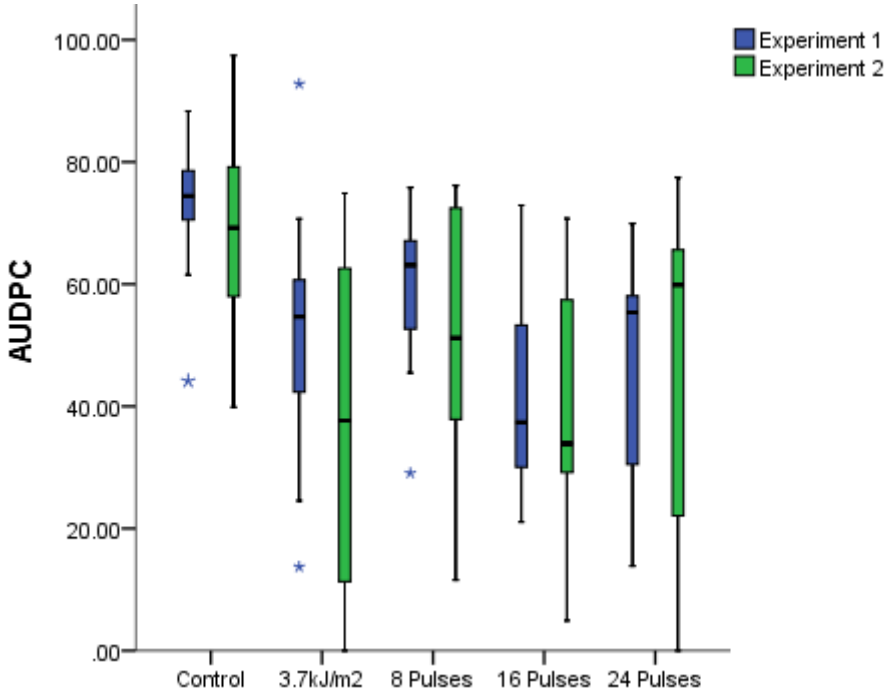

Figure A1.4 Area underneath the disease progression curve (AUDPC) from the independent experimental replicates from mature green fruit cv. Mecano treated with a conventional low intensity UV-C source and a high intensity pulsed polychromatic light source. Inoculations were performed with *B. cinerea* at 10 d post treatment; n = 15. Box plots show the distribution of data from the independent experimental replicates. Asterisks represent observed outliers.

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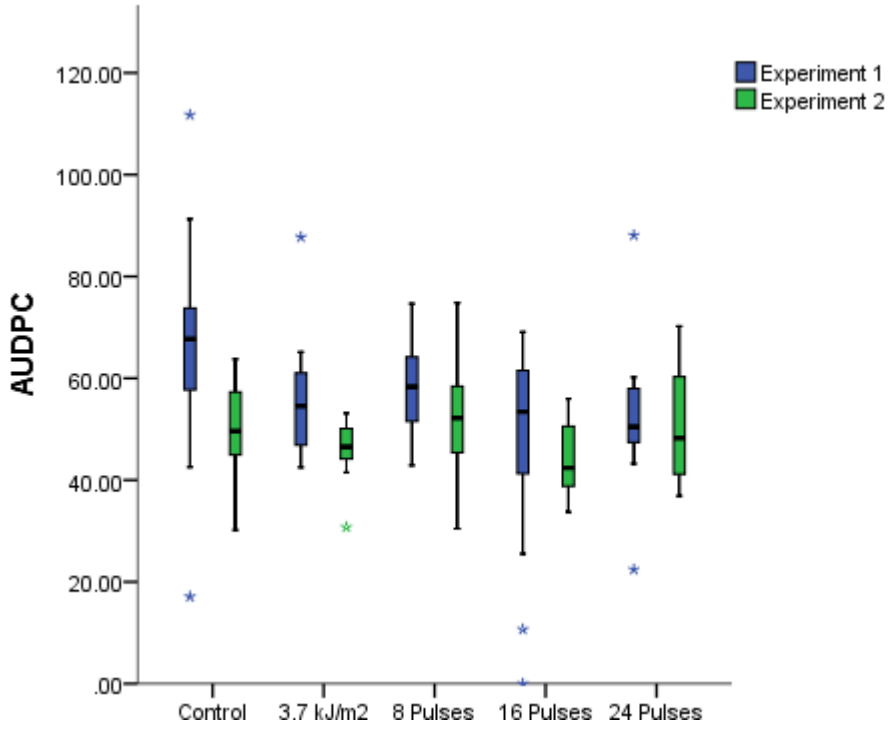

Figure A1.5 Area Underneath the Disease Progression Curve (AUDPC) for ripe fruit cv. Mecano treated with a conventional low intensity UV-C source with and a high intensity pulsed polychromatic light source, followed by inoculation with *B. cinerea* at 10 d post treatment; n = 15. Box plots show the distribution of data from the independent experimental replicates. Asterisks represent observed outliers.

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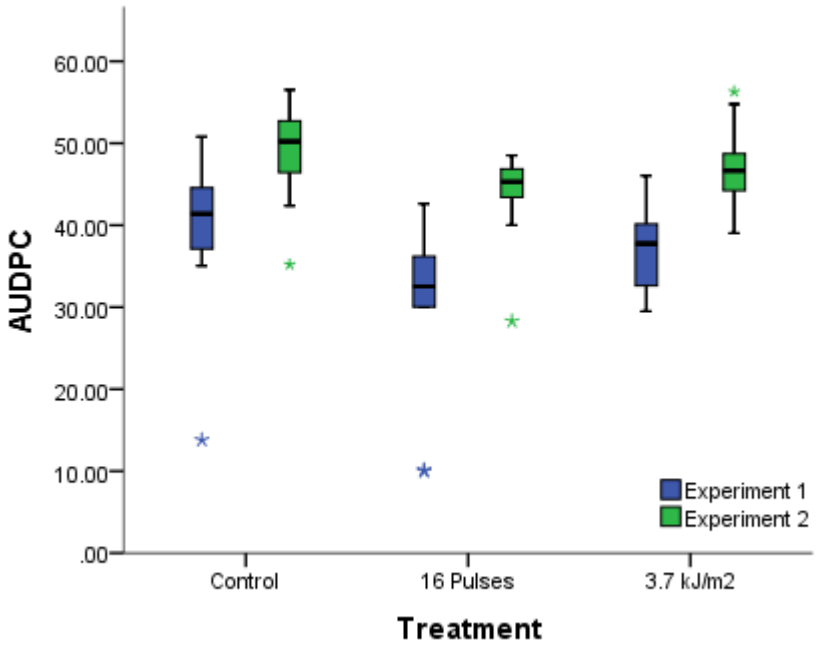

Figure A1.5 Area Underneath the Disease Progression Curve (AUDPC) for ripe fruit cv. Mecano treated with a conventional low intensity UV-C source with and a high intensity pulsed polychromatic light source, followed by inoculation with *P. expansum* at 10 d post treatment; n = 10. Box plots show the distribution of data from the independent experimental replicates. Asterisks represent observed outliers.

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Appendix 2

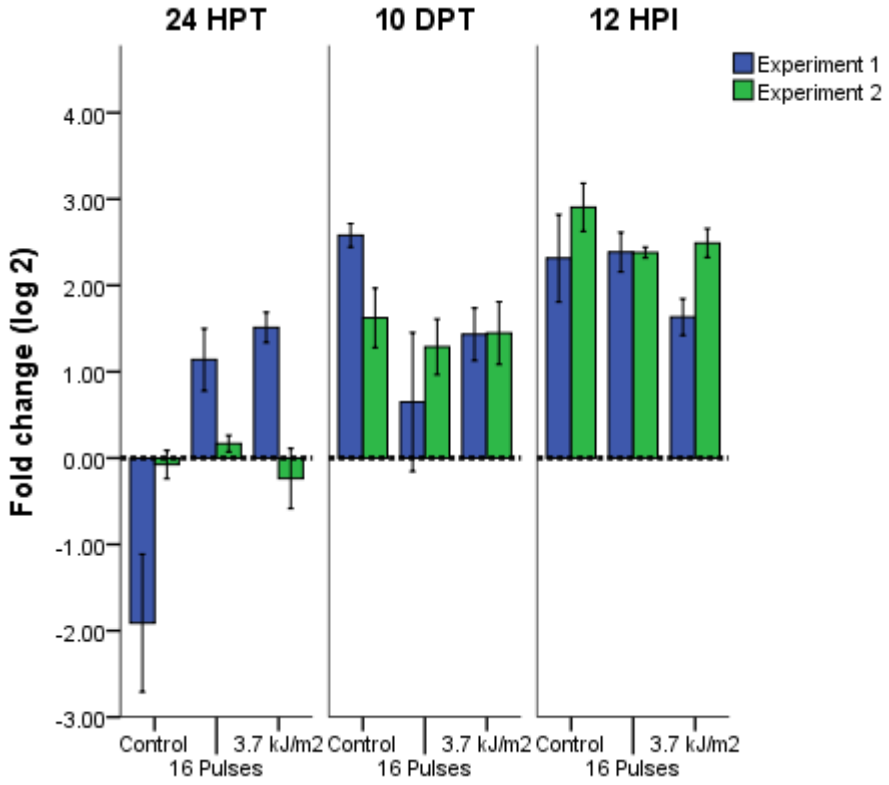

Figure A2.1. Relative expression of *ACO1* (1-aminocyclopropane-1-carboxylic acid oxidase 1), a bottleneck enzyme in ethylene biosynthesis, following treatment with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C source (LIUV). Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). N=3 and bars show ± 1S.E.M.

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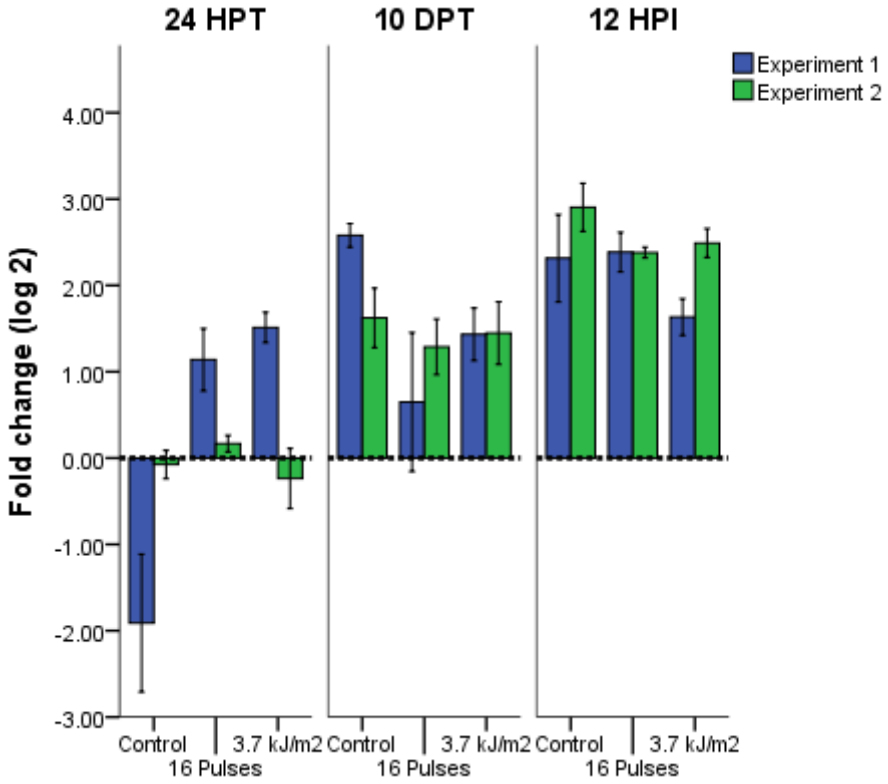

Figure A2.2 Relative expression of *CHI9* (Chitinase 9) a jasmonic acid induced pathogenesis related protein transcript following treatment with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). N=3. Bars show ± 1S.E.M.

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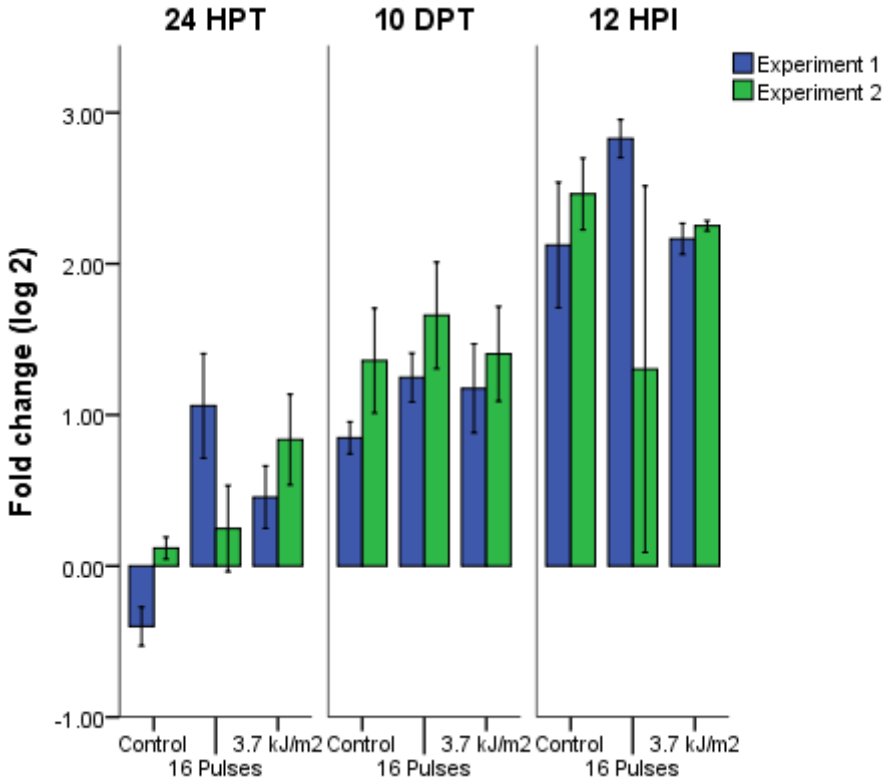

Figure A2.3: Relative expression of *CRTR-B1* (β -carotene hydroxylase) following treatment with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). N=3. Bars show ± 1S.E.M.

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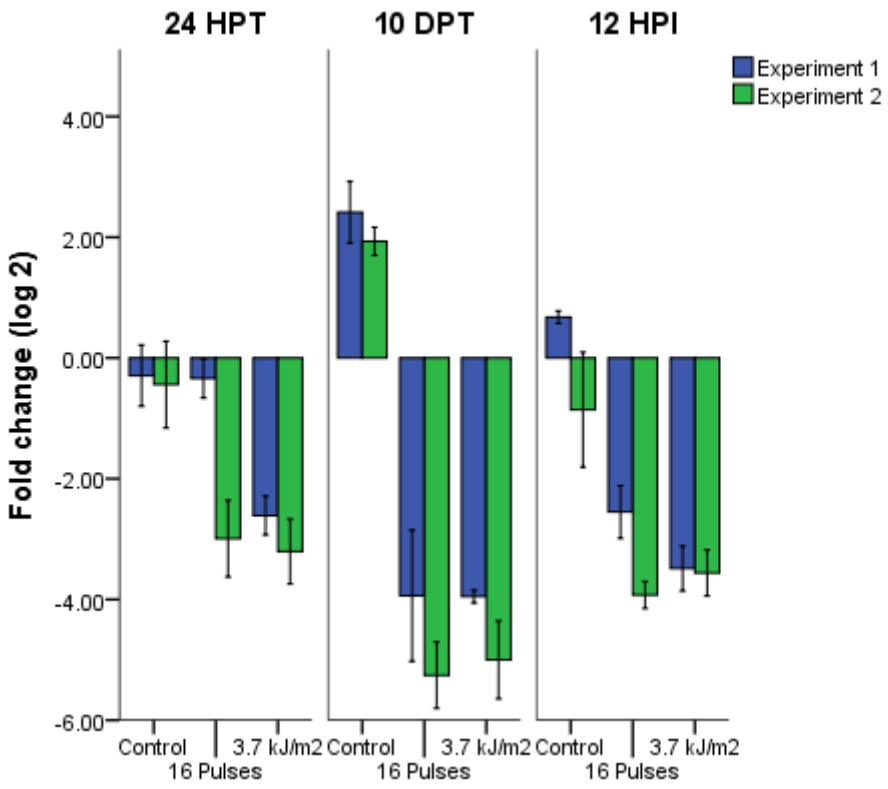

Figure A2.4 Relative expression of *FLS* (flavonol synthase) following treatment with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). N=3. Bars show ± 1S.E.M.

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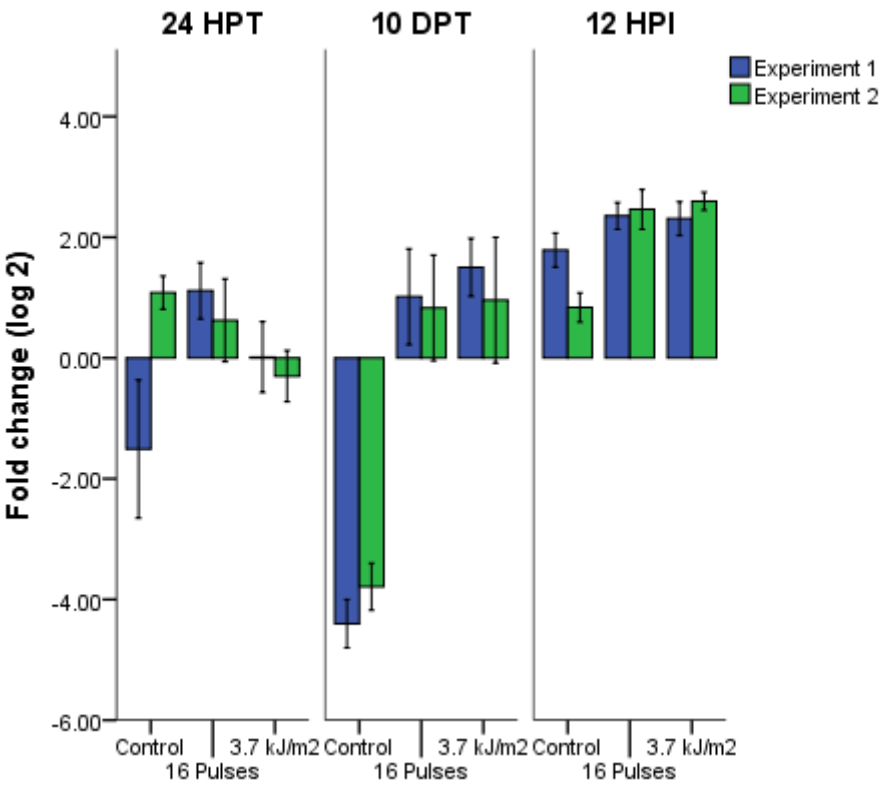

Figure A2.5: Relative expression of *GluB* (β-1,3,-Glucanase) an the ethylene inducible pathogenesis related protein transcript following treatment with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). N=3. Bars show ± 1S.E.M.

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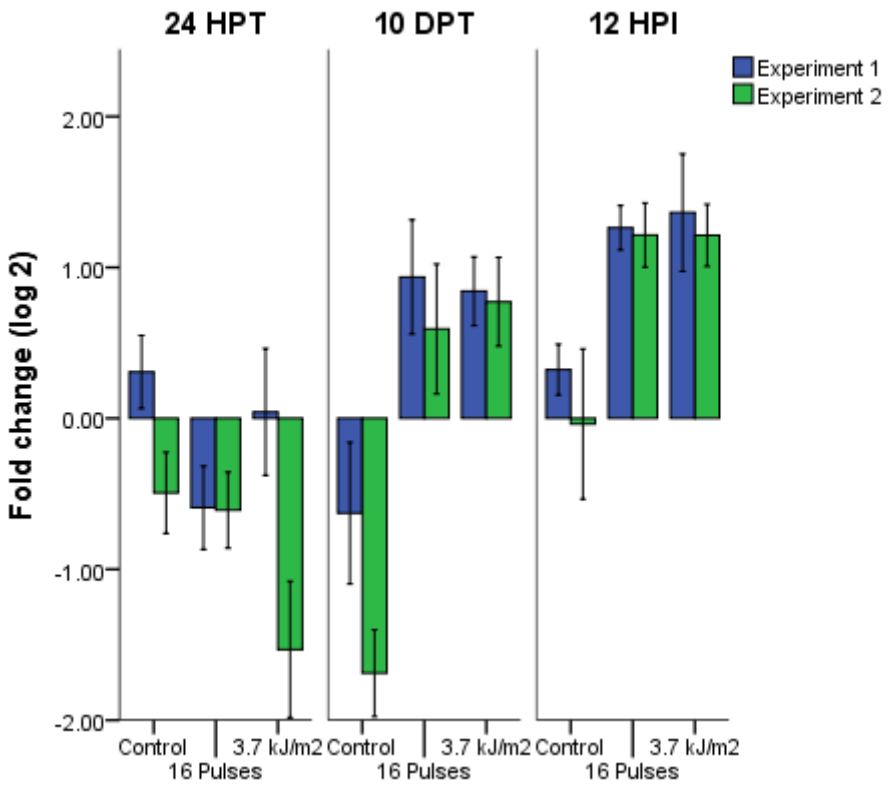

Figure A2.6: The relative expression of *OPR3* (12-Oxophytodienoate reductase 3) a jasmonate biosynthesis protein transcript following treatment with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). N=3. Bars show ± 1S.E.M.

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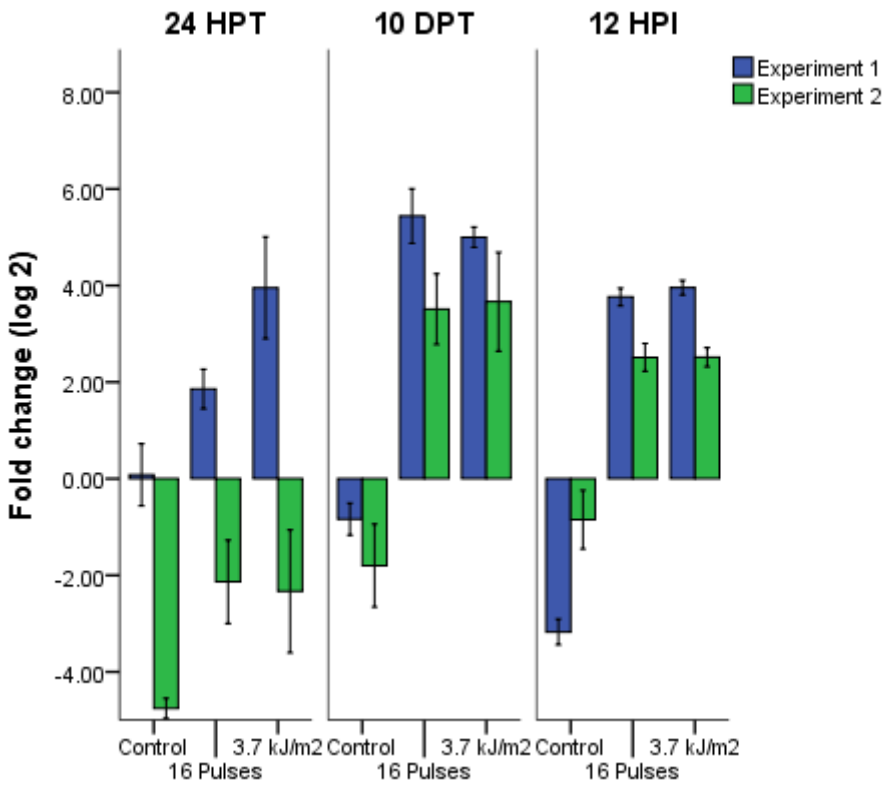

Figure A2.7: The relative expression of *P4* (*PR1a*) a salicylic acid inducible pathogenesis related protein and marker of systemic acquired resistance (SAR) following treatment with either 16 pulses from a high intensity pulsed polychromatic light source (HIPPL) or 3.7 kJ/m2 from a low intensity UV-C source (LIUV). Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). N=3 Bars show ± 1S.E.M.

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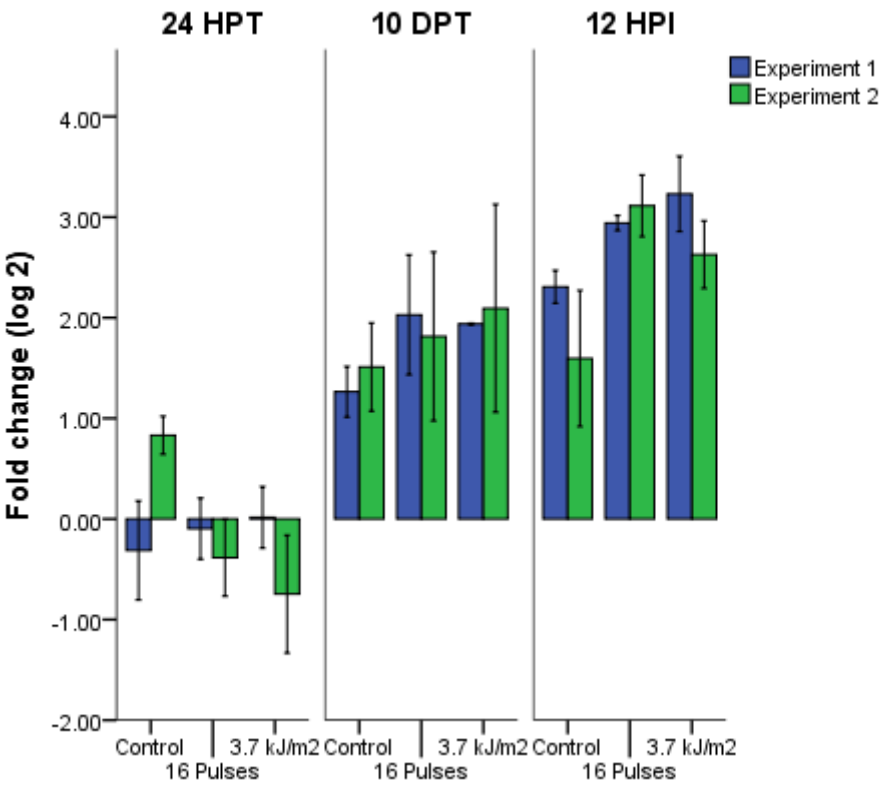

Figure A2.8: The relative expression of *PAL* (phenylalanine ammonia lyase) following treatment with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). N=3. Bars show ± 1S.E.M.

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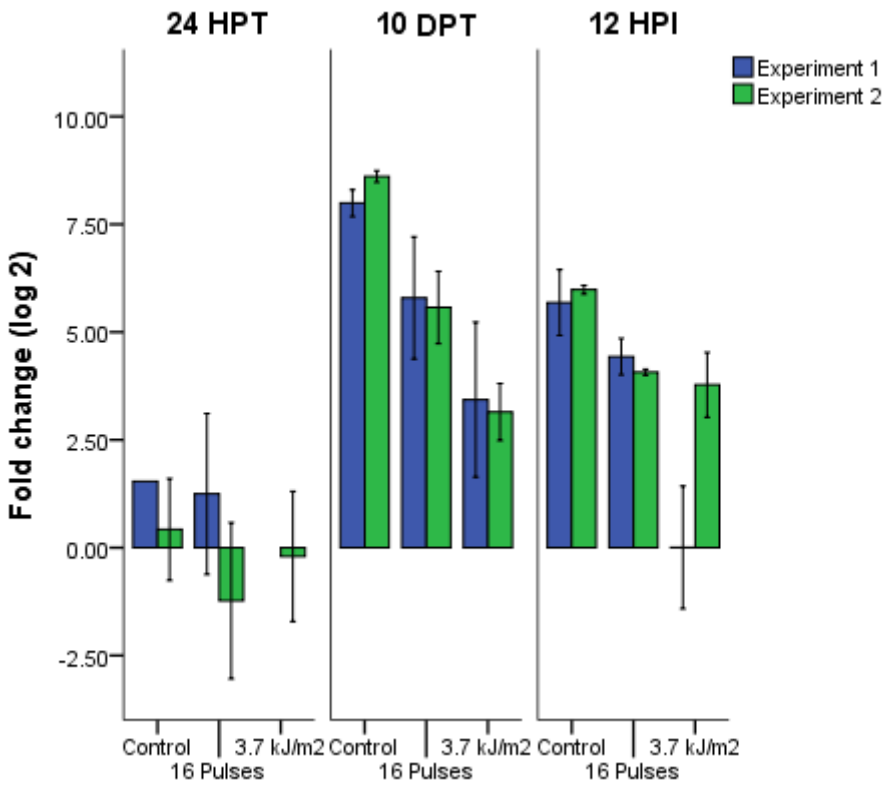

Figure A2.9: The relative expression of *PG* (polygalacturonase) following treatment with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). N=3. Bars show ± 1S.E.M.

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Appendix 3

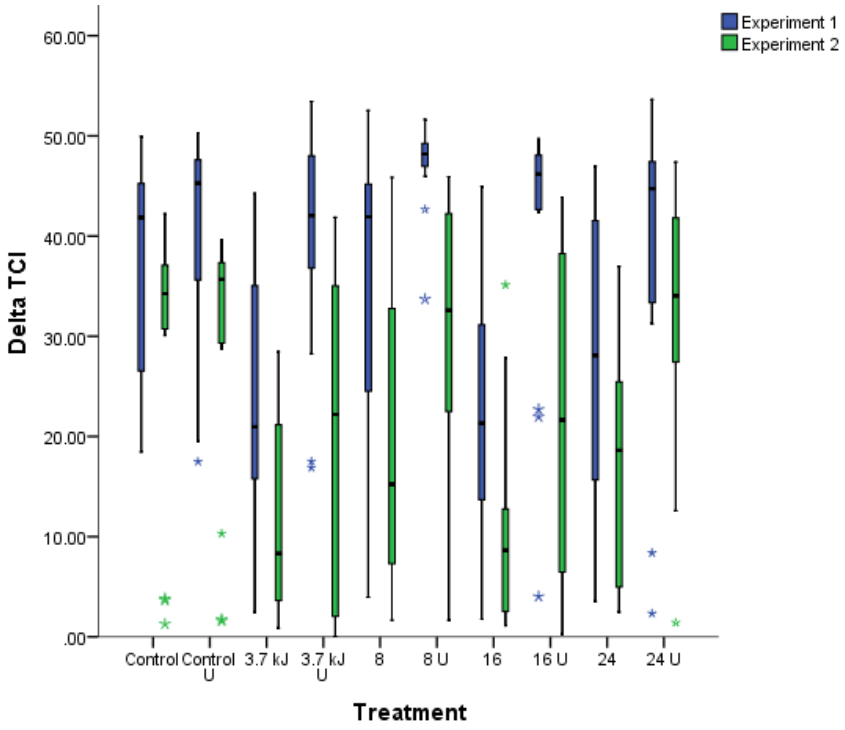

Figure A3.1. The change in tomato colour index over days 0-10 following treatment with either a low-intensity UV-C source or a high-intensity, pulsed polychromatic light source. Measurements were taken from tissue either directly facing the light sources or unexposed tissue (U) at 90 degrees from that facing the sources.

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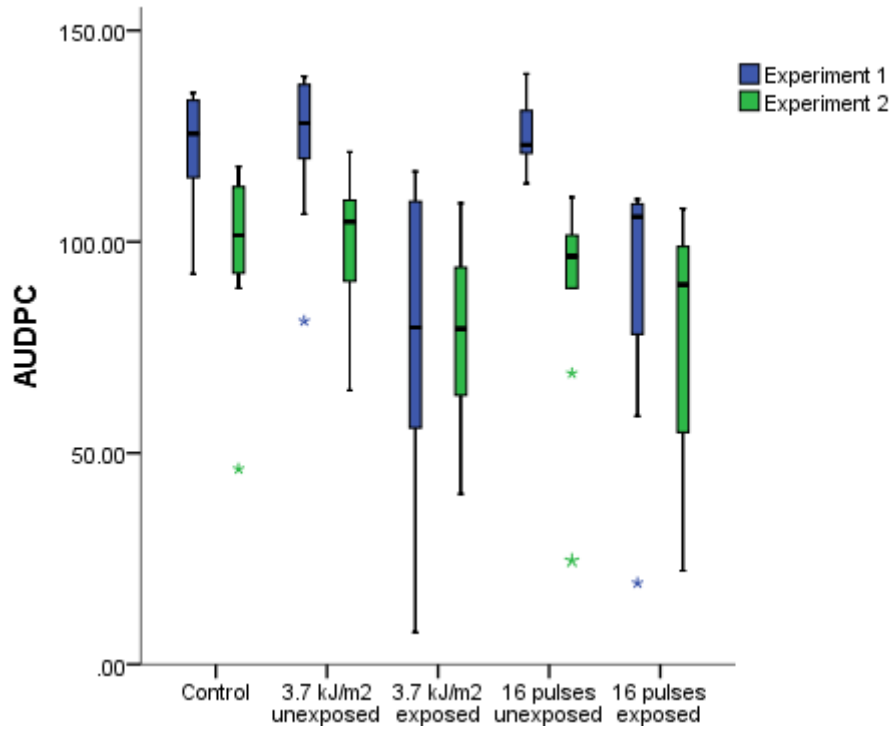

Figure A3.2. Area underneath the disease progression curve (AUDPC) of tomatoes, cv. Mecano, treated on a single side and inoculated with *B. cinerea* at 10 d post treatment (DPT). Fruit were treated with an established low intensity UV-C treatment of 3.7 kJ/m^2 and a high intensity pulsed polychromatic treatment of 16 pulses. Exposed tissue or unexposed, n = 10. Box plots show the distribution of data from the independent experimental replicates. Asterisks represent observed outliers.

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Appendix 4

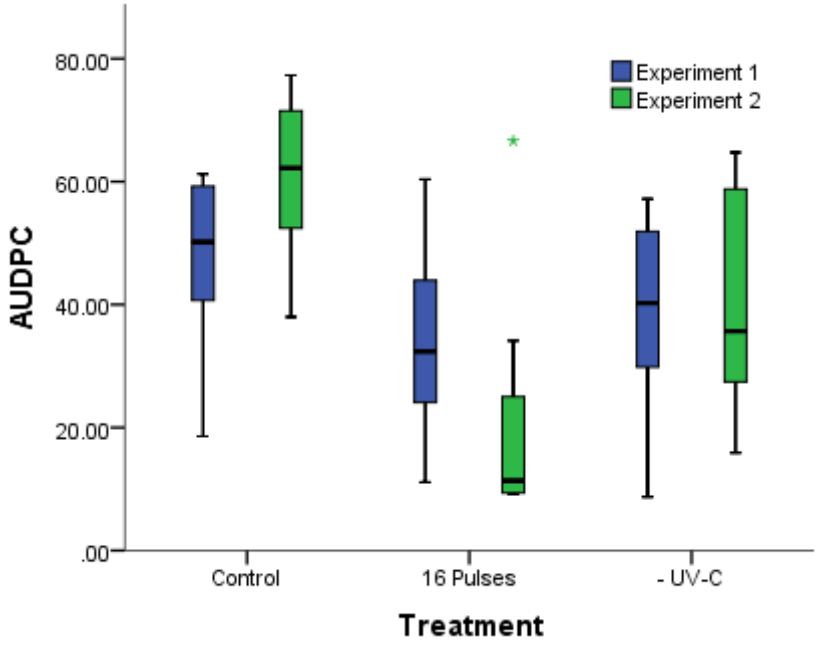

Figure A4.1. Area underneath the disease progression curve (AUDPC) of tomatoes, cv. Mecano, treated a high intensity pulsed polychromatic treatment of 16 pulses with or without UV-C filtering glass. N = 10. Box plots show the distribution of data from the independent experimental replicates. Asterisks represent observed outliers.

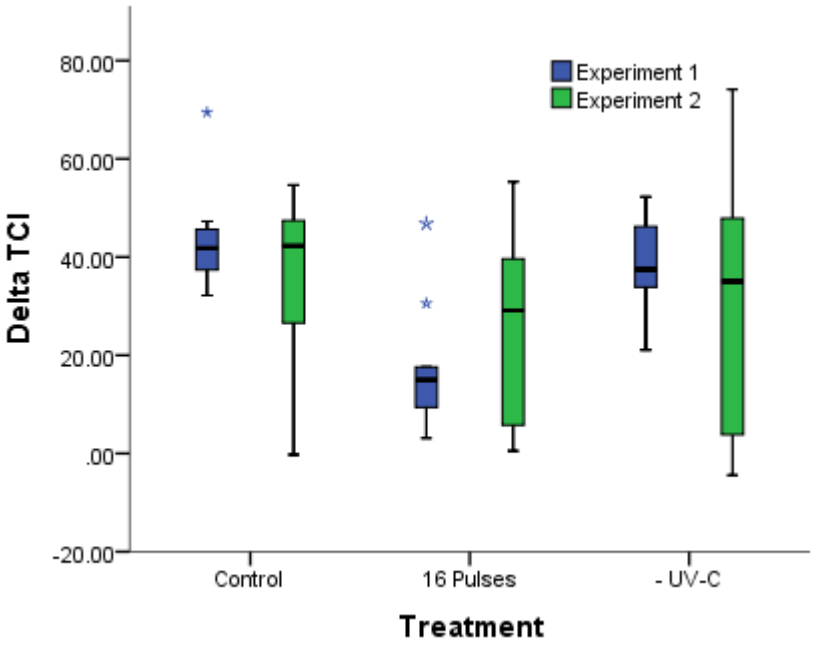

Figure A4.2. The change in tomato colour index over days 0-10 following treatment with a high-intensity, pulsed polychromatic light source without (16 pulses) or with UV-C filtering glass in place (- UV-C). Box plots show the distribution of data from the independent experimental replicates. Asterisks represent observed outliers.

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Appendix 5

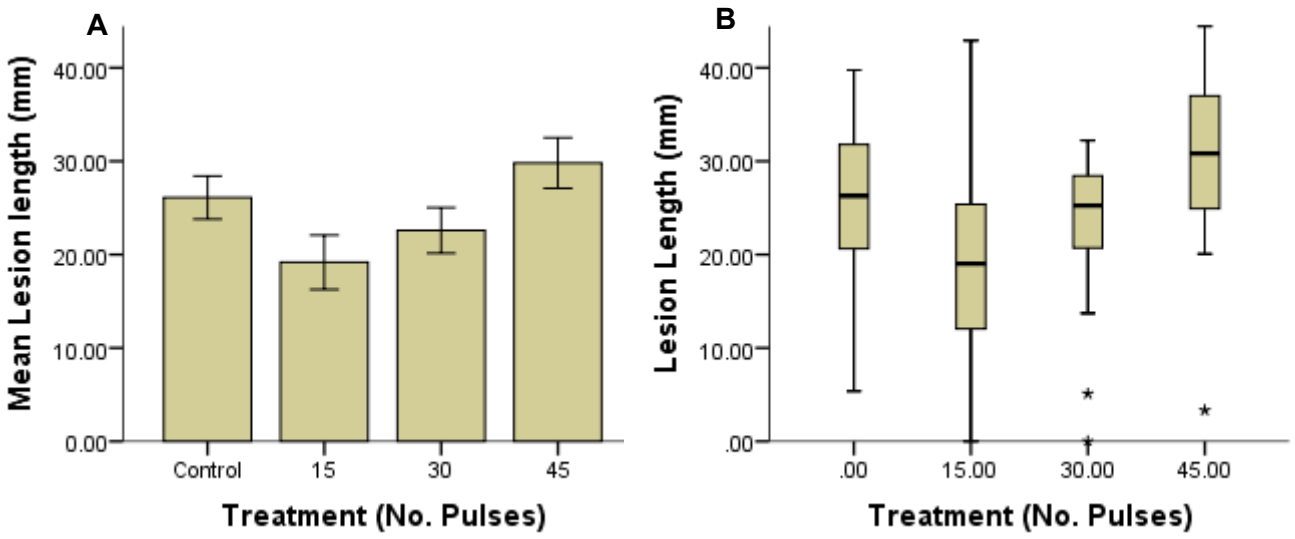

Figure A5.1. Disease resistance assays performed in April 2015 (14.04.2015) on the butterhead variety Amica. Plants were treated at early head formation and inoculated 9 days after treatment with 4mm agar plugs from 5 day old *B.cinerea* cultures. Lesion lengths were measured at 3 days after inoculation with Vernier callipers. (**A**) Shows the mean lesion length and ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 5.

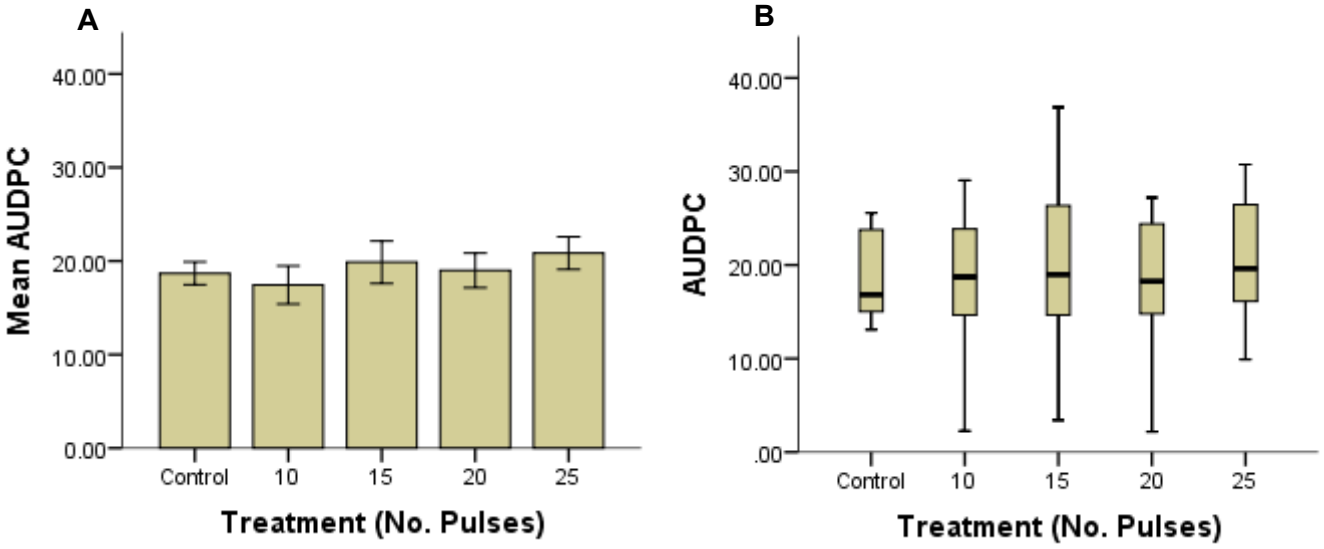

Figure A5.2. Disease resistance assays performed in May 2015 (05.05.2015) on the butterhead variety Temira. Plants were treated at early head formation and inoculated with *B. cinerea* 6 days after treatment with 10 µl of $1x10^6$ spores/ml amended with 50 % potato dextrose broth. Lesion lengths were measured at 2 and 3 days after inoculation with Vernier callipers to allow calculation of AUDPC. (**A**) Shows the mean lesion length ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 5.

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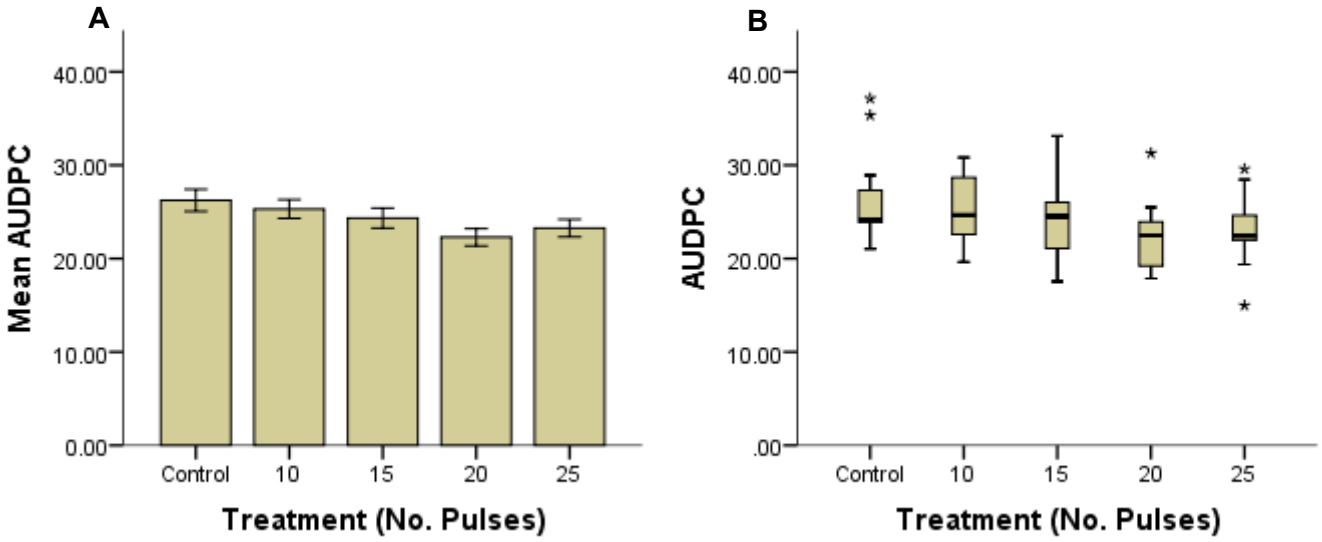

Figure A5.3. Disease resistance assays performed in May 2015 (05.05.2015) on the butterhead variety Amica. Plants were treated at early head formation and inoculated with *B. cinerea* 6 days after treatment with 10 µl of $1x10^6$ spores/ml amended with 50 % potato dextrose broth. Lesion lengths were measured at 2 and 3 days after inoculation with Vernier callipers to allow calculation of AUDPC. (**A**) Shows the mean lesion length ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 5.

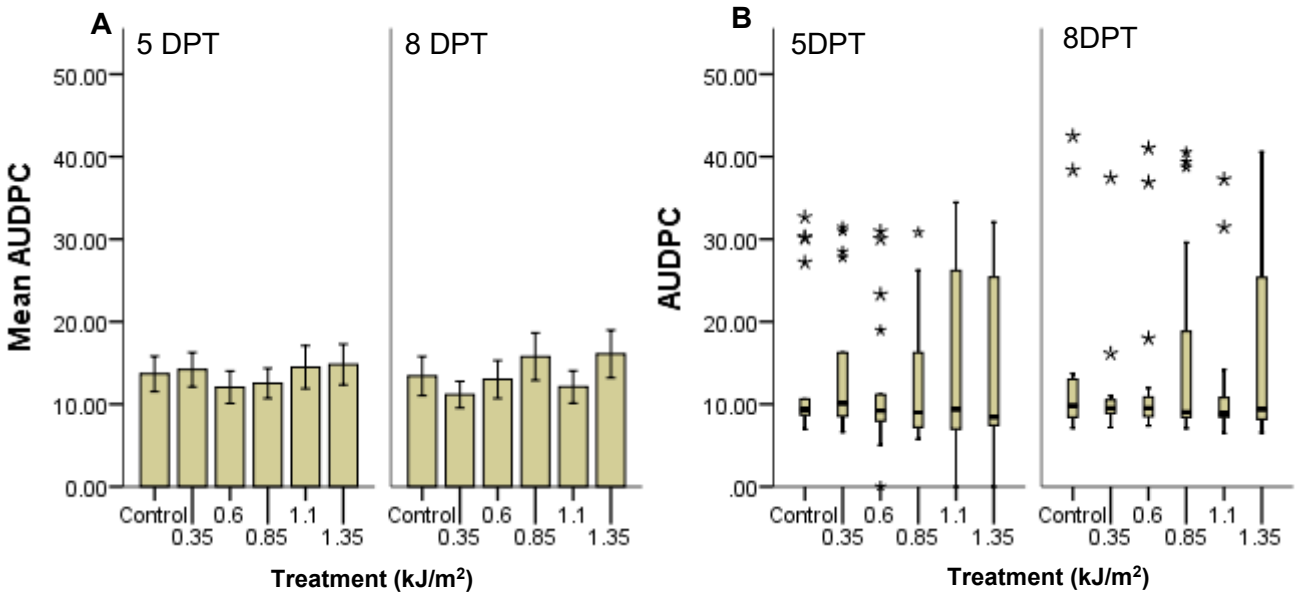

Figure A5.4. Disease resistance assays performed in September 2015 (10.09.2015) on the butterhead variety Amica treated with the conventional UV-C source. Plants were treated at 3-5 true leaves and inoculated 5 and 8 days with 10 µl of $1x10^6$ spores/ml of *B. cinerea* amended with 50 % potato dextrose broth. Lesion lengths were measured at 2 and 3 days after inoculation with Vernier callipers to allow calculation of AUDPC. (**A**) Shows the mean lesion length ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 6.

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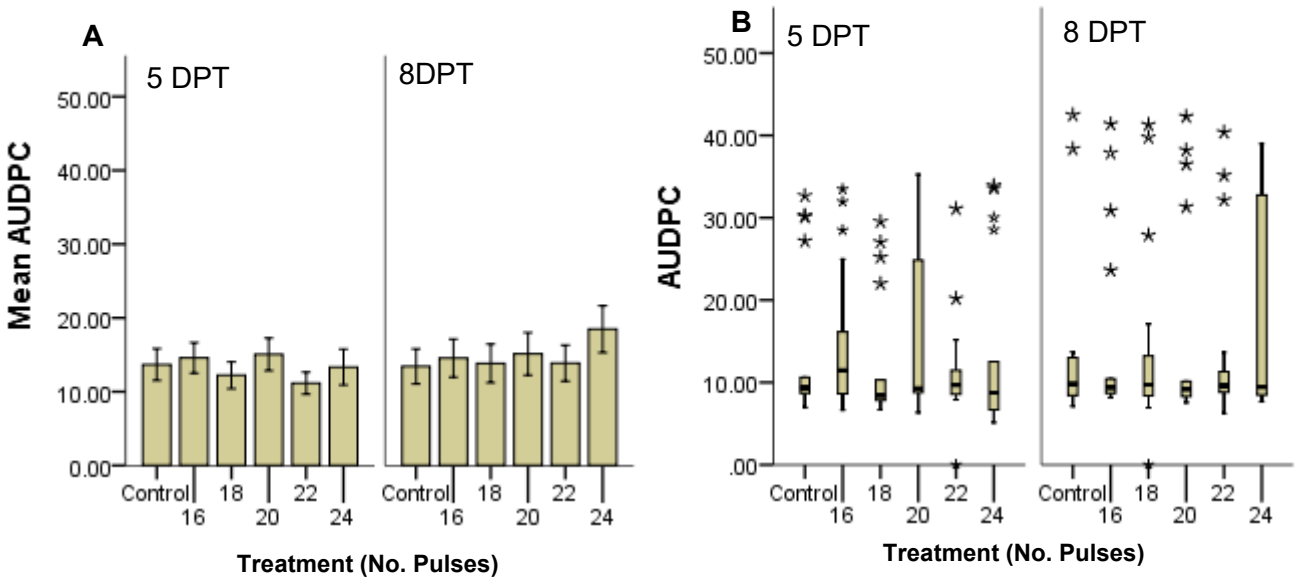

Figure A5.5. Disease resistance assays performed in September 2015 (10.09.2015) on the butterhead variety Amica treated with the high intensity pulsed polychromatic source. Plants were treated at 3-5 true leaves and inoculated 5 and 8 days after treatment with 10 µl of 1x10^6 spores/ml of *B. cinerea* amended with 50 % potato dextrose broth. Lesion lengths were measured at 2 and 3 days after inoculation with Vernier callipers to allow calculation of AUDPC. (**A**) Shows the mean lesion length ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 6.

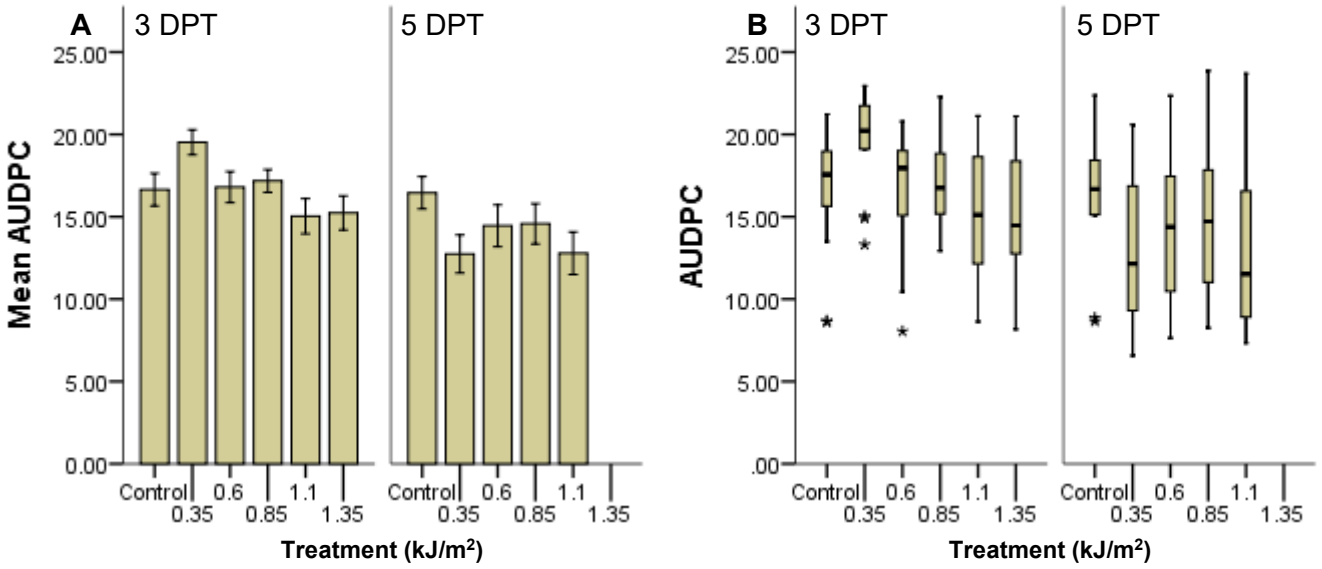

Figure A5.6. Disease resistance assays performed in September 2015 (29.09.2015) on the butterhead variety Temira treated with the conventional UV-C source. Plants were treated at 3-5 true leaves and inoculated 3 and 5 days after treatment with 10 µl of 1x10^6 spores/ml of *B. cinerea* amended with 50 % potato dextrose broth. Lesion lengths were measured at 2 and 3 days after inoculation with Vernier callipers to allow calculation of AUDPC. (**A**) Shows the mean lesion length ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 6.

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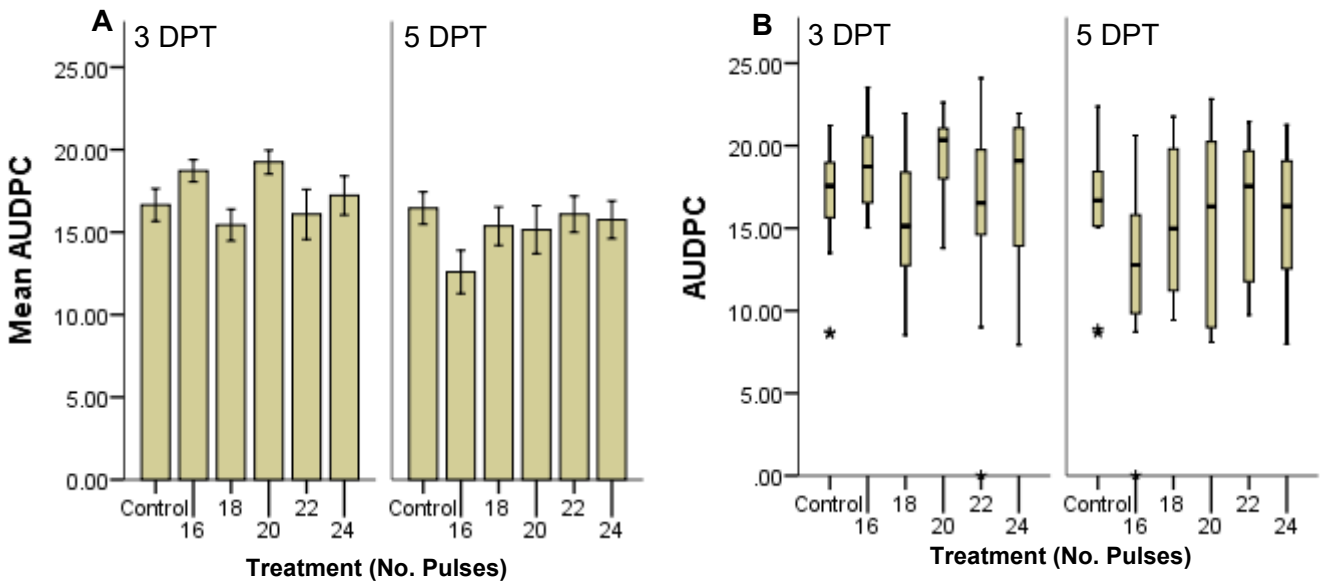

Figure A5.7. Disease resistance assays performed in September 2015 (29.09.2015) on the butterhead variety Temira treated with the high intensity pulsed polychromatic source. Plants were treated at 3-5 true leaves and inoculated 3 and 5 days after treatment with 10 µl of 1x10^6 spores/ml of *B. cinerea* amended with 50 % potato dextrose broth. Lesion lengths were measured at 2 and 3 days after inoculation with Vernier callipers to allow calculation of AUDPC. (**A**) Shows the mean lesion length ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 6.

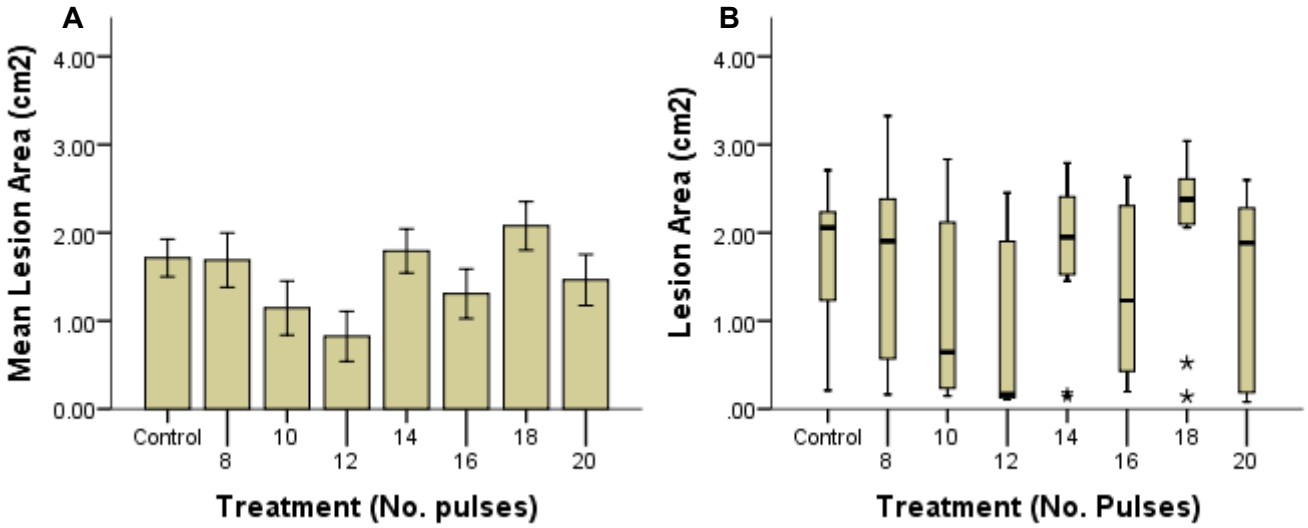

Figure A5.8. Disease resistance assays performed in October 2015 (28.10.2015) on the butterhead variety Temira treated with the high intensity pulsed polychromatic source. Plants were treated at late head formation and inoculated 2 days after treatment with 10 µl of 1x10^6 spores/ml of *B. cinerea* amended with 50 % potato dextrose broth. Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. (**A**) Shows the mean lesion length ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 5.

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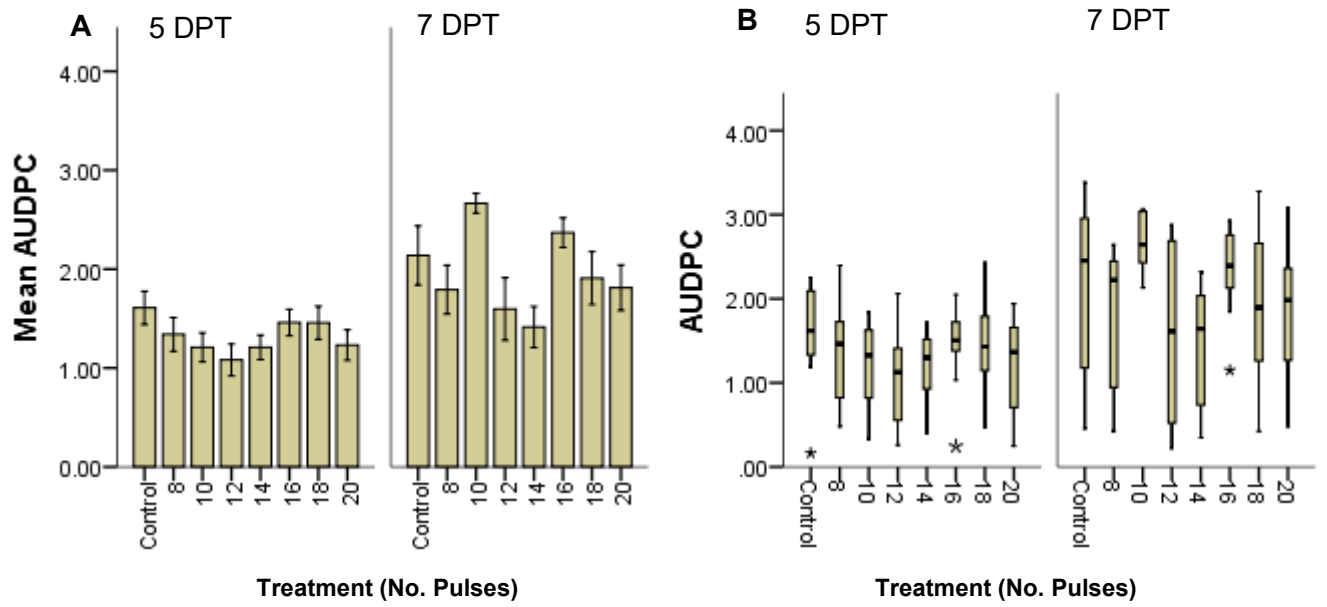

Figure A5.9. Disease resistance assays performed in October (28.10.2015) on the butterhead variety Temira treated with the high intensity pulsed polychromatic source. Plants were treated at late head formation and inoculated 5 and 7 days after treatment with 10 µl of 1x10^6 spores/ml of *B. cinerea* amended with 50 % potato dextrose broth Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. (**A**) Shows the mean lesion length ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 5.

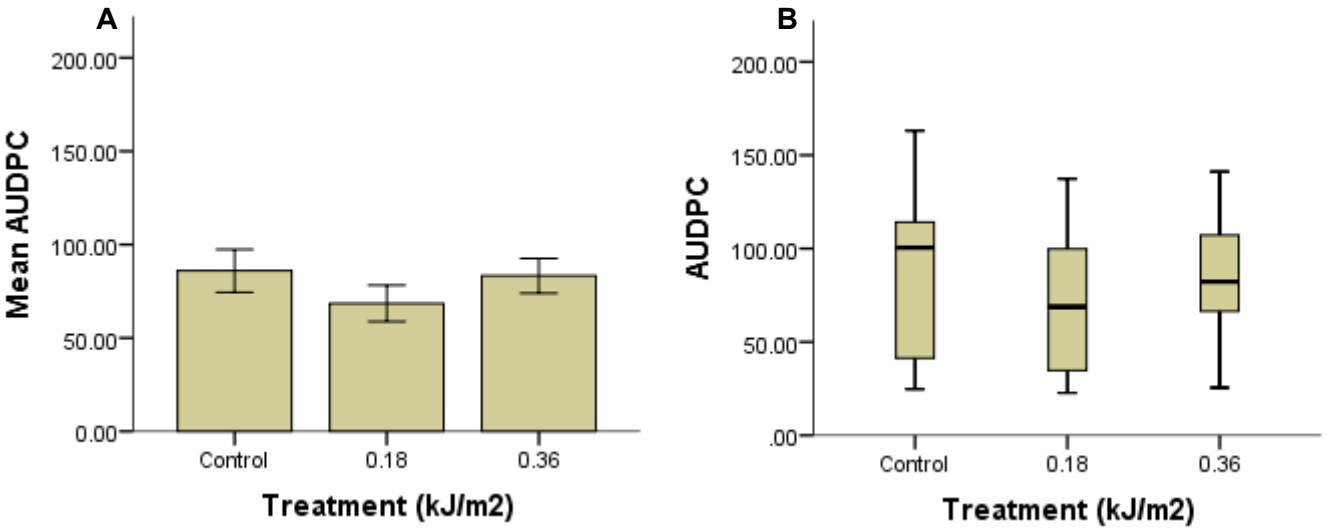

Figure A5.10. Disease resistance assays performed in November 2015 (18.11.2015) on the butterhead variety Amica treated with the conventional UV-C source. Plants were inoculated 2 days after treatment with 10 µl of 1x10^6 spores/ml of *B. cinerea* amended with 50 % potato dextrose broth. Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. (**A**) Shows the mean lesion length ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 4.

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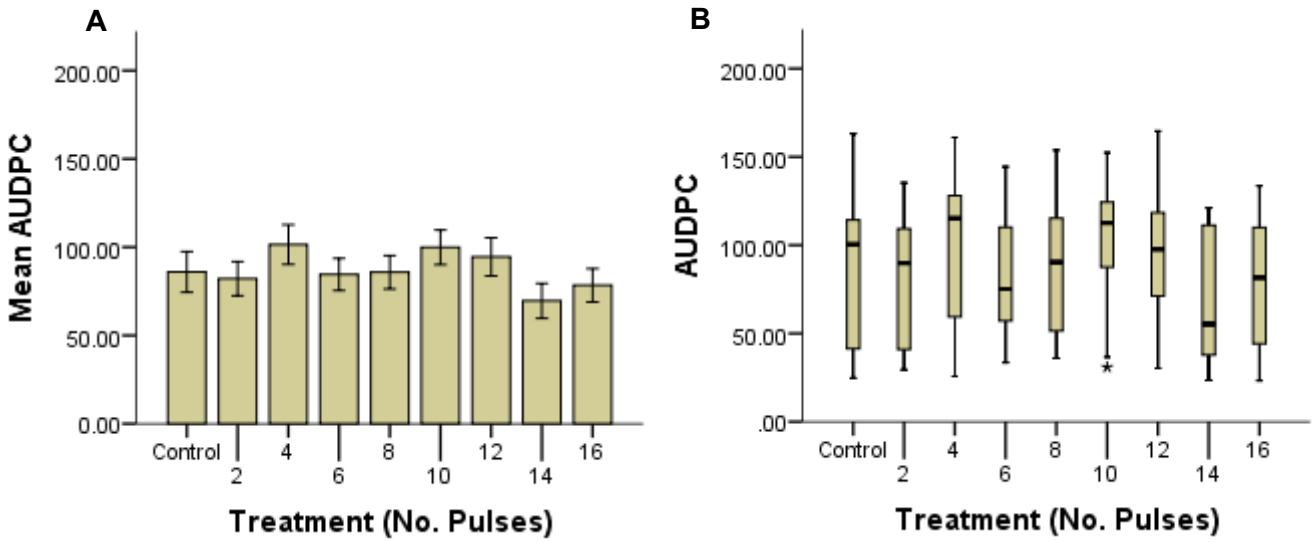

Figure A5.11. Disease resistance assays performed in November 2015 (18.11.2015) on the butterhead variety Amica treated with the pulsed polychromatic source. Plants were treated at 3-5 true leaves and inoculated 2 days after treatment with 10 µl of $1x10^6$ spores/ml of *B. cinerea* amended with 50 % potato dextrose broth. Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. (**A**) Shows the mean lesion length ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 5

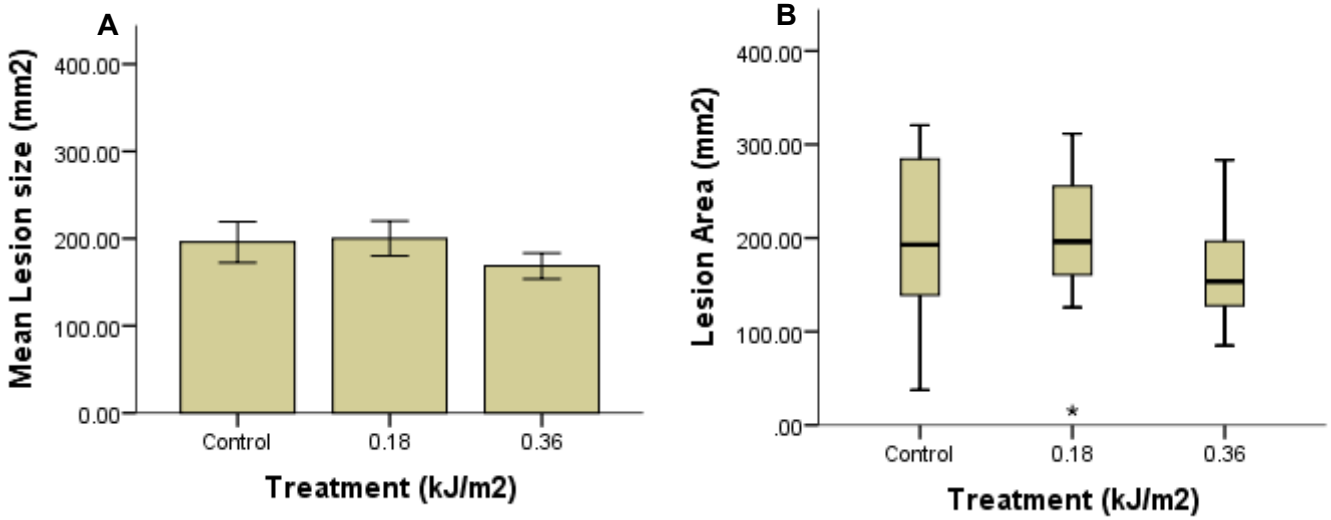

Figure A5.12. Disease resistance assays performed in November 2015 (18.11.2015) on the butterhead variety Amica treated with the conventional UV-C source. Plants were treated at 3-5 true leaves and inoculated 2 days after treatment with 4mm agar plugs from 3 day old *Rhizoctonia solani* cultures. Lesion areas were measured at 3 days after inoculation with ImageJ. (**A**) Shows the mean lesion length ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 4

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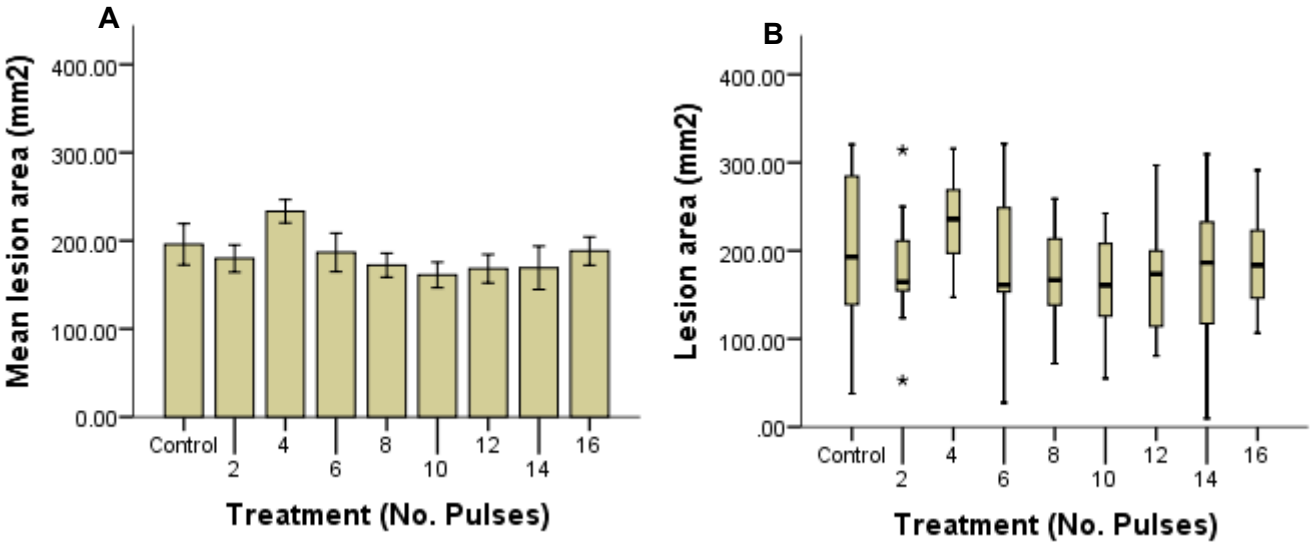

Figure A5.13. Disease resistance assays performed in November 2015 (18.11.2015) on the butterhead variety Amica treated with pulsed polychromatic source. Plants were treated at 3-5 true leaves and inoculated 2 days after treatment with 4mm agar plugs from 3 day old *Rhizoctonia solani* cultures. Lesion areas were measured at 3 days after inoculation with ImageJ. (**A**) Shows the mean lesion length ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 5

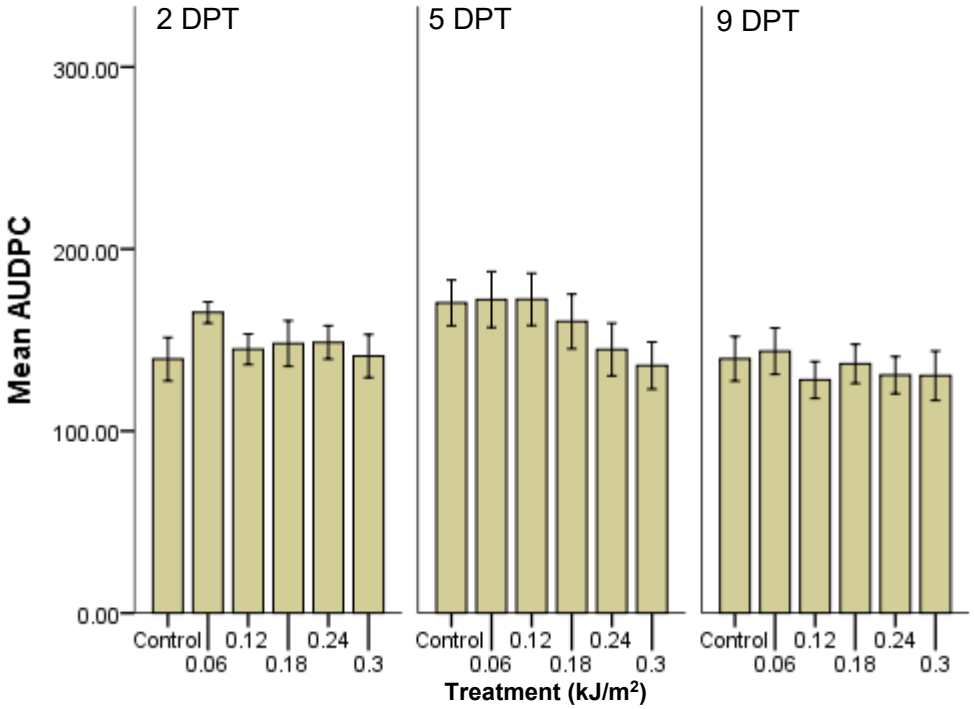

Figure A5.14. Disease resistance assays performed in December 2015 (09.12.2015) on the butterhead variety Amica treated with the conventional UV-C source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 10 µl of $1x10^6$ spores/ml of *B. cinerea* amended with 50 % potato dextrose broth. Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. Bars show mean lesion length ± 1 standard error of the mean. N= 7

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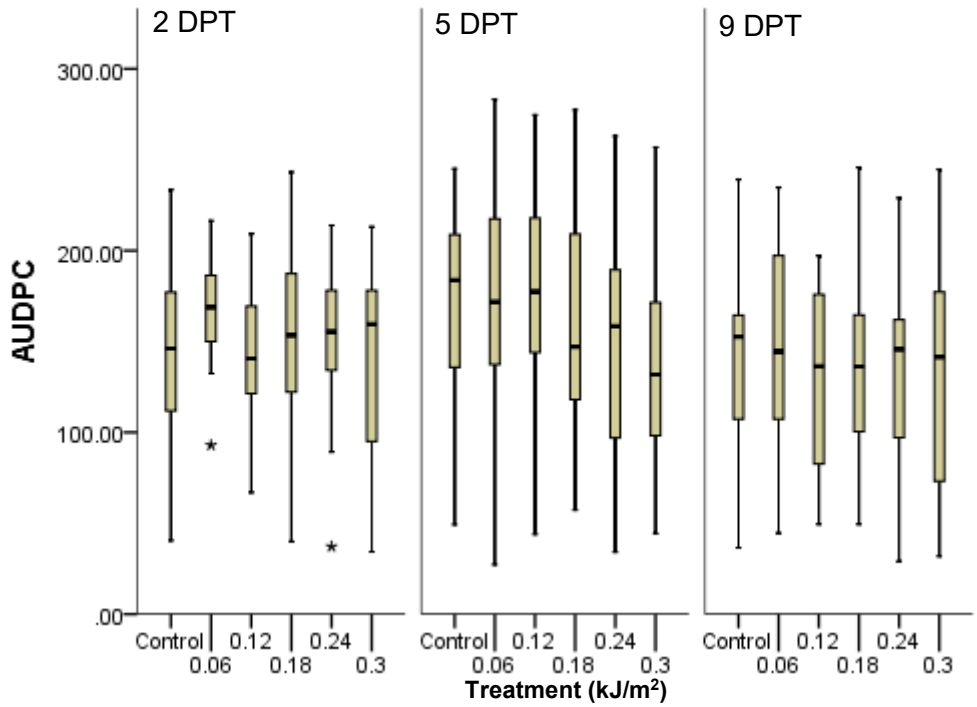

Figure A5.15. Disease resistance assays performed in December 2015 (09.12.2015) on the butterhead variety Amica treated with the conventional UV-C source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 10 µl of $1x10^6$ spores/ml of *B. cinerea* amended with 50 % potato dextrose broth. Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. Asterisks represent observed outliers. N= 7

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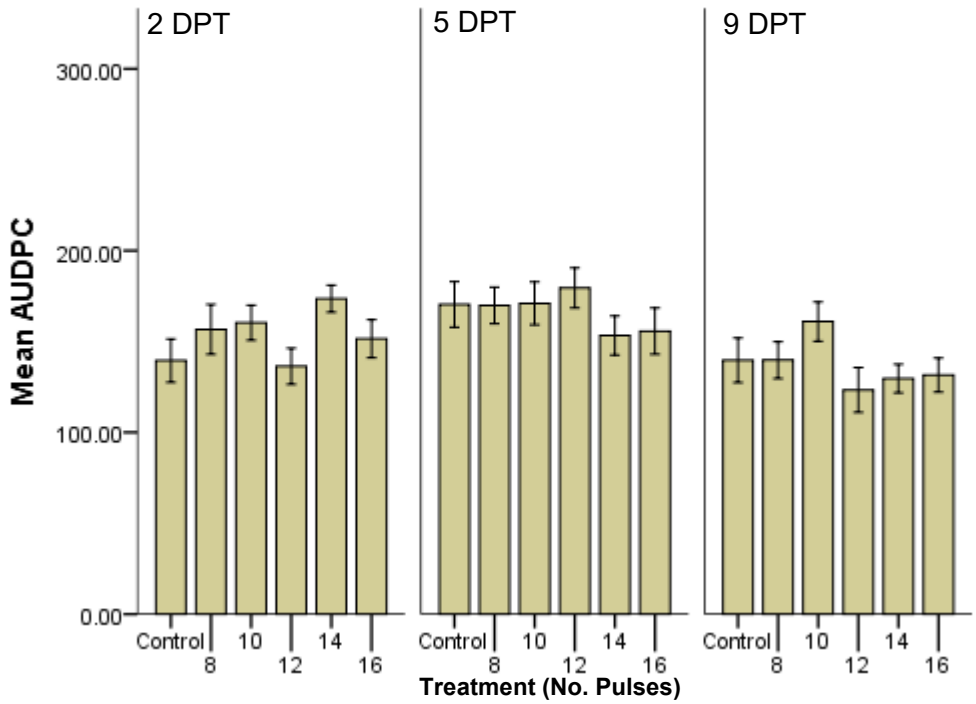

Figure A5.16. Disease resistance assays performed in December 2015 (09.12.2015) on the butterhead variety Amica treated with the pulsed polychromatic source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 10 µl of $1x10^6$ spores/ml of *B. cinerea* amended with 50 % potato dextrose broth. Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. Bars show the mean lesion length ± 1 standard error of the mean. N=

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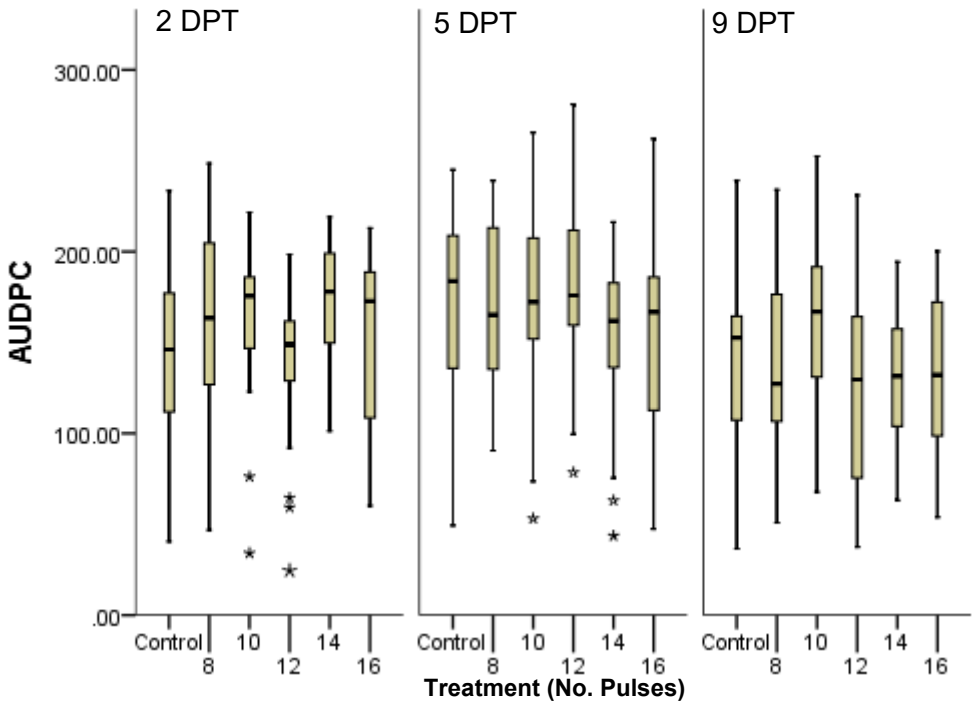

Figure A5.17. Disease resistance assays performed in December 2015 (09.12.2015) on the butterhead variety Amica treated with the pulsed polychromatic source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 10 µl of $1x10^6$ spores/ml of *B. cinerea* amended with 50 % potato dextrose broth. Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. Asterisks represent observed outliers. N= 7

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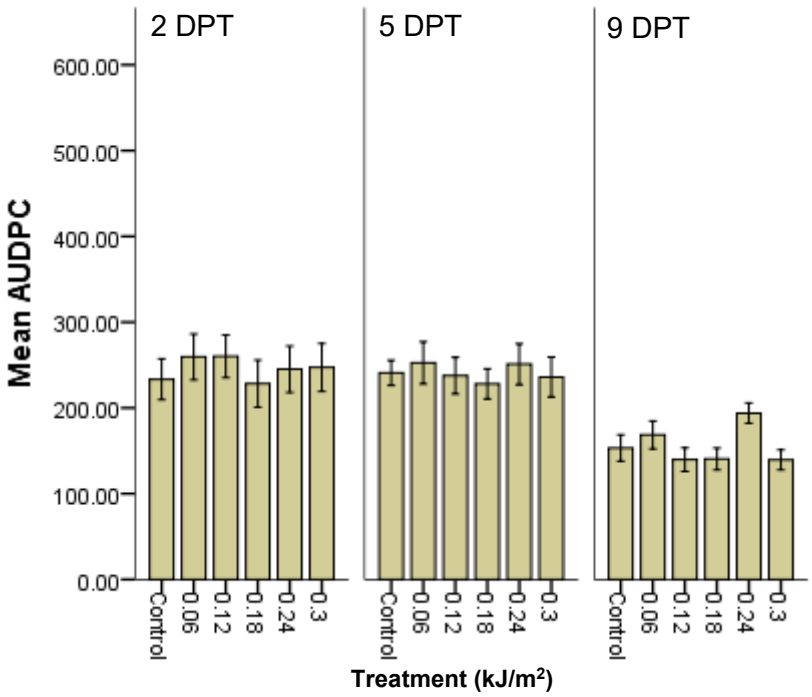

Figure A5.18. Disease resistance assays performed in December 2015 (09.12.2015) on the butterhead variety Amica treated with the conventional UV-C source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 4mm agar plugs of *R. solani*. Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. Bars shows the mean lesion length ± 1 standard error of the mean. N= 7

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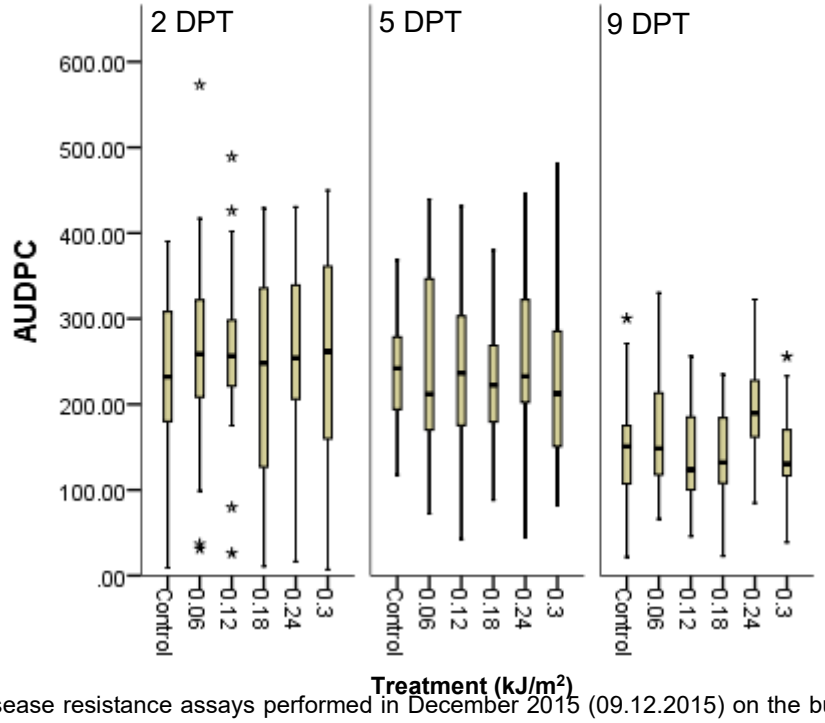

Figure A5.19. Disease resistance assays performed in December 2015 (09.12.2015) on the butterhead variety Amica treated with the conventional UV-C source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 4mm agar plugs of *R. solani*. Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. Asterisks represent observed outliers. N= 7

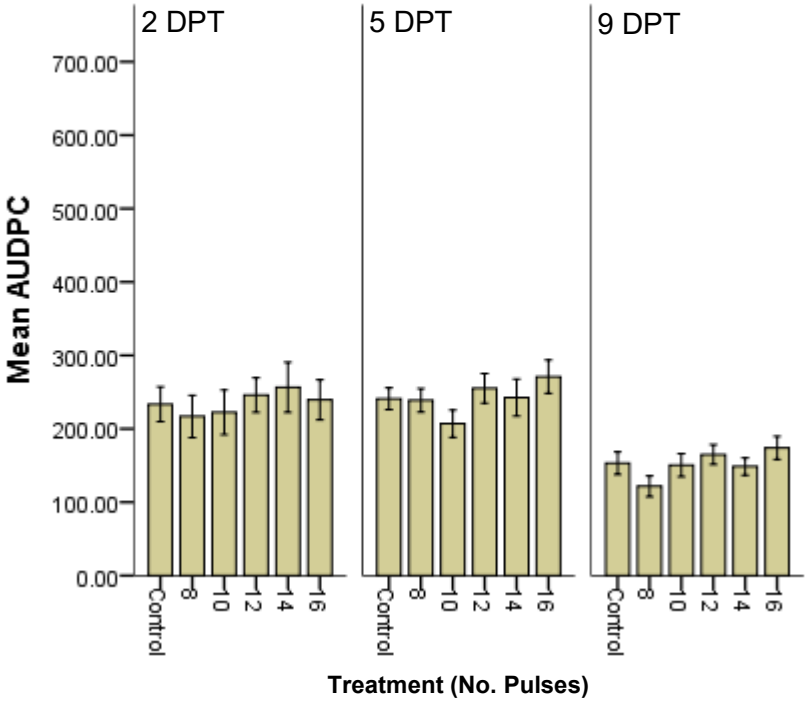

Figure A5.20. Disease resistance assays performed in December 2015 (09.12.2015) on the butterhead variety Amica treated with the pulsed polychromatic source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 4mm agar plugs of *R. solani*. Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. Bars shows the mean lesion length ± 1 standard error of the mean. N= 7

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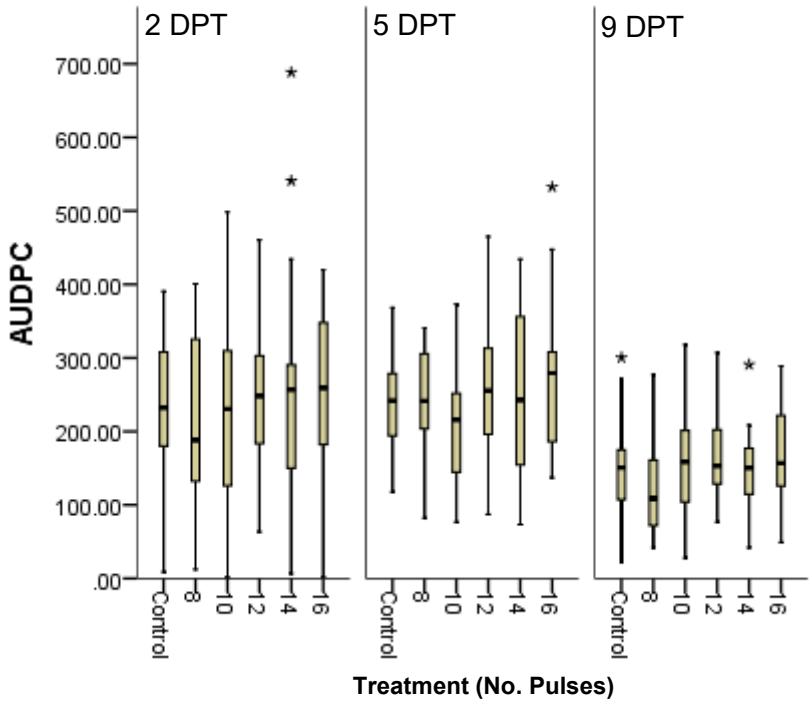

Figure A5.21. Disease resistance assays performed in December 2015 (09.12.2015) on the butterhead variety Amica treated with the pulsed polychromatic source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 4mm agar plugs of *R. solani*. Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. Asterisks represent observed outliers. N= 7

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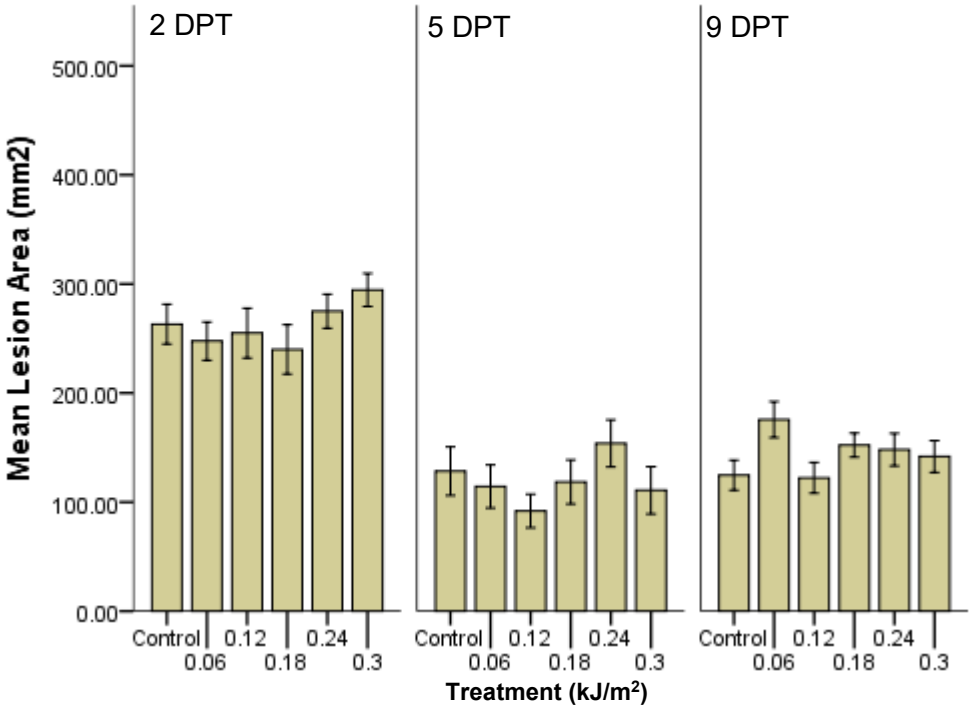

Figure A5.21. Disease resistance assays performed in December 2015 (09.12.2015) on the butterhead variety Amica treated with the conventional UV-C source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 4mm agar plugs of *S. sclerotiorum*. Lesion areas were measured at 2 days after inoculation with ImageJ. Bars shows the mean lesion length ± 1 standard error of the mean. N=

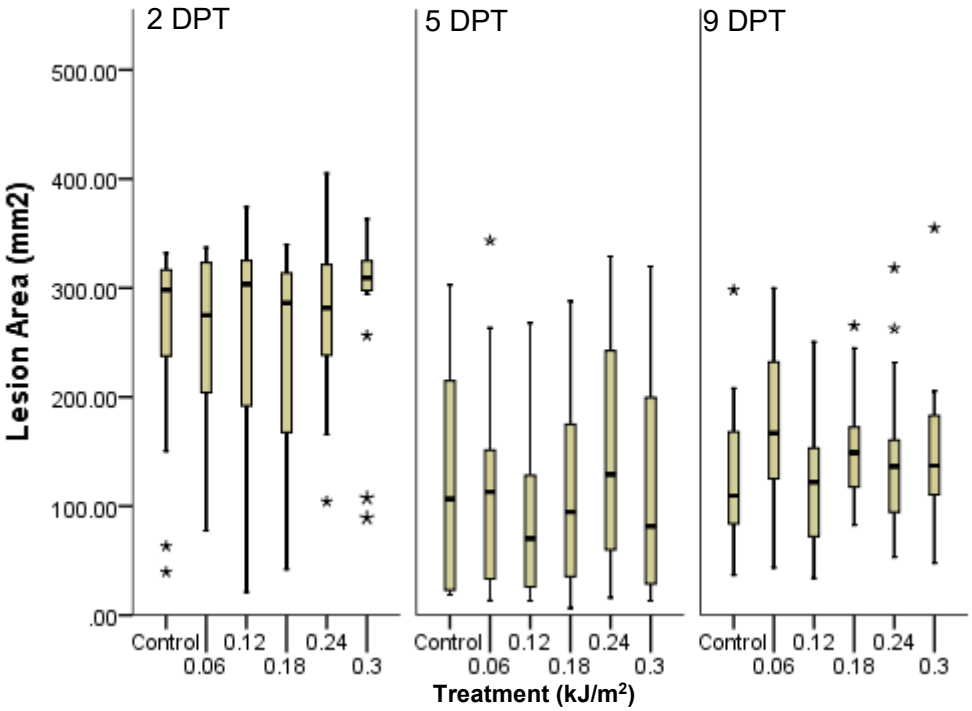

Figure A5.22. Disease resistance assays performed in December 2015 (09.12.2015) on the butterhead variety Amica treated with the conventional UV-C source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 4mm agar plugs of *S. sclerotiorum*. Lesion areas were measured at 2 days after inoculation with ImageJ. Asterisks represent observed outliers. N= 7

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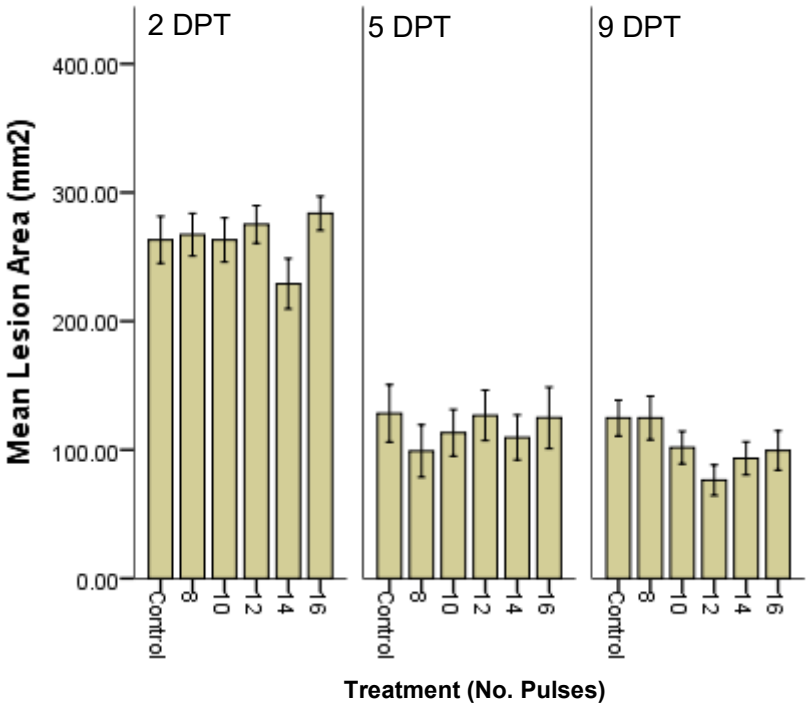

Figure A5.23. Disease resistance assays performed in December 2015 (09.12.2015) on the butterhead variety Amica treated with the pulsed polychromatic source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 4mm agar plugs of *S. sclerotiorum*. Lesion areas were measured at 2 days after inoculation with ImageJ. Bars shows the mean lesion length ± 1 standard error of the mean. N= 7

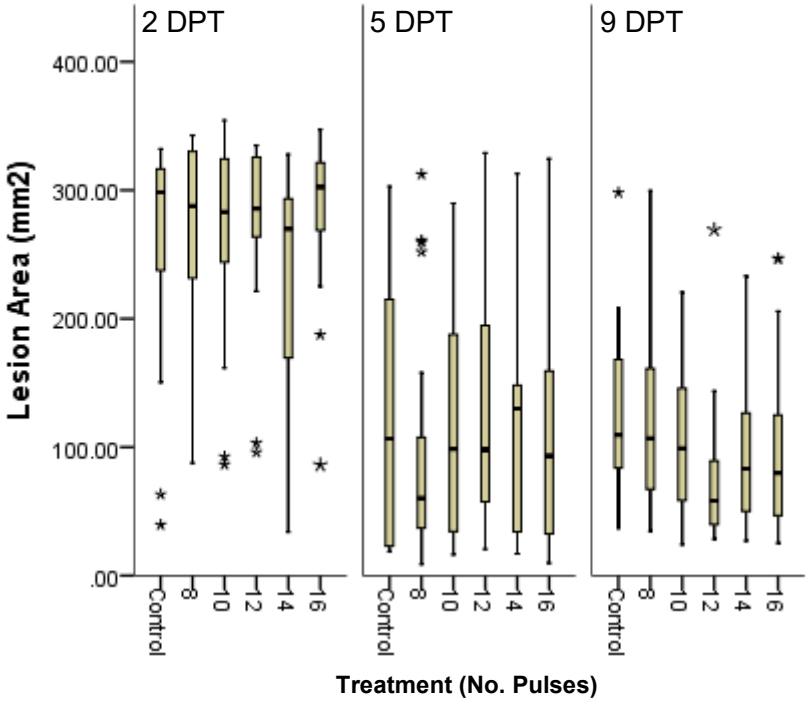

Figure A5.24. Disease resistance assays performed in December 2015 (09.12.2015) on the butterhead variety Amica treated with the pulsed polychromatic source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 4mm agar plugs of *S. sclerotiorum*. Lesion areas were measured at 2 days after inoculation with ImageJ. Asterisks represent observed outliers. N= 7

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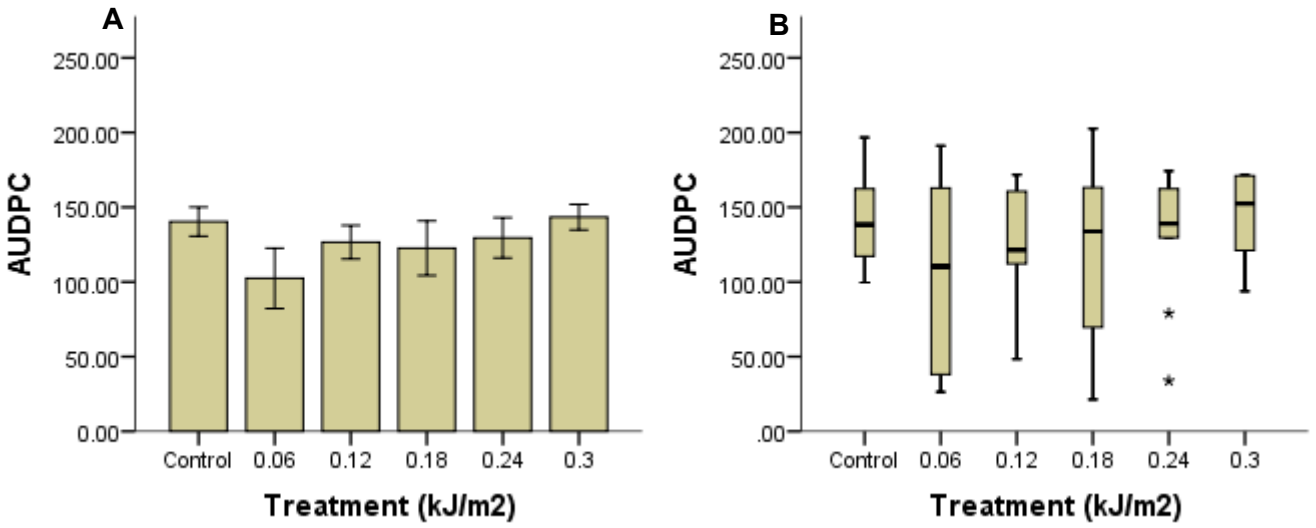

Figure A5.25. Disease resistance assays performed in February 2016 (12.02.2016) on the butterhead variety Amica treated with the conventional UV-C source. Plants were treated at 6-8 true leaves and inoculated 2 days after treatment with 10 µl of 1x10^6 spores/ml of *B. cinerea* amended with 50 % potato dextrose broth. Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. (**A**) Shows the mean lesion length ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 7

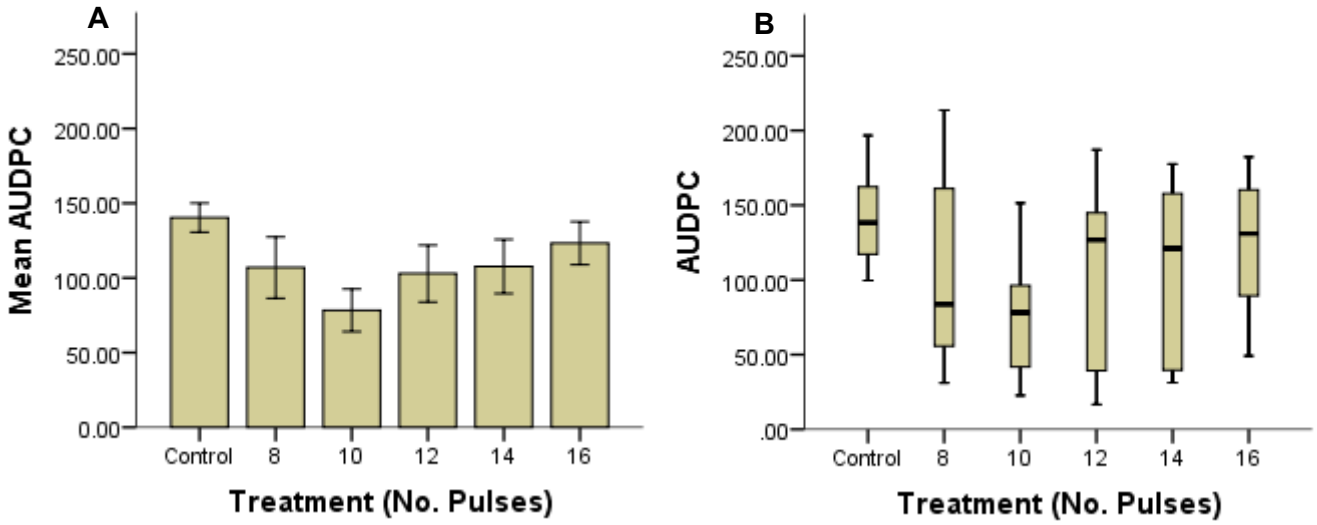

Figure A5.26. Disease resistance assays performed in February 2016 (12.02.2016) on the butterhead variety Amica treated with the conventional UV-C source. Plants were treated at 6-8 true leaves and inoculated 2 days after treatment with 10 µl of 1x10^6 spores/ml of *B. cinerea* amended with 50 % potato dextrose broth. Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. (**A**) Shows the mean lesion length ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 7

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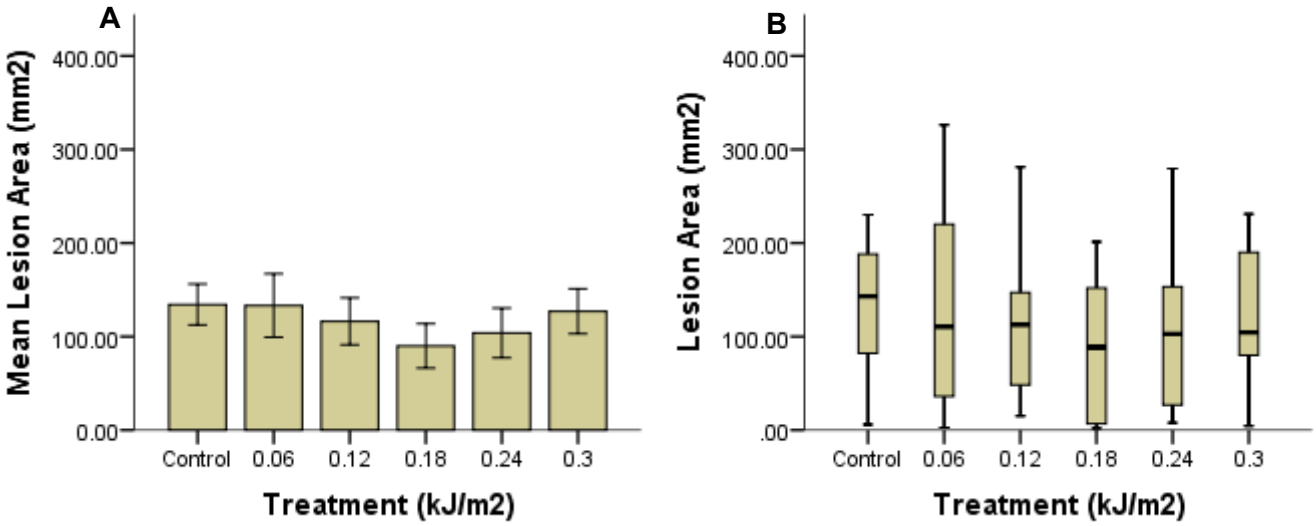

Figure A5.27. Disease resistance assays performed in February 2016 (12.02.2016) on the butterhead variety Amica treated with the conventional UV-C source. Plants were treated at 6-8 true leaves and inoculated 2 days after treatment with 4 mm agar plugs of *R. solani*. Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. (**A**) Shows the mean lesion length ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 7

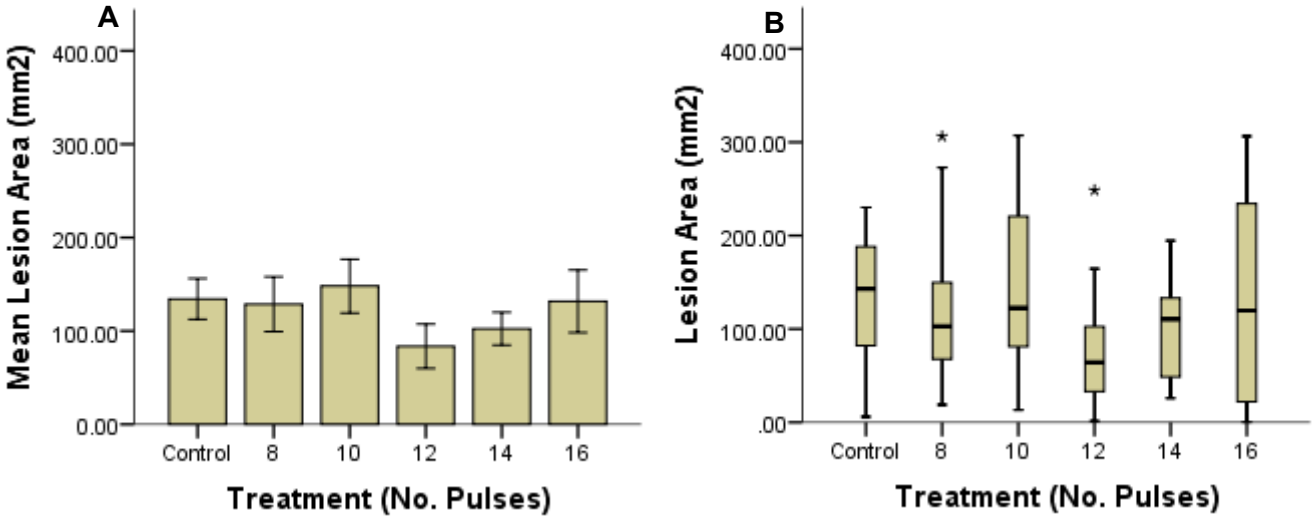

Figure A5.28. Disease resistance assays performed in February 2016 (12.02.2016) on the butterhead variety Amica treated with the pulsed polychromatic source. Plants were treated at 6-8 true leaves and inoculated 2 days after treatment with 4 mm agar plugs of *R. solani*. Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. (**A**) Shows the mean lesion length ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 7

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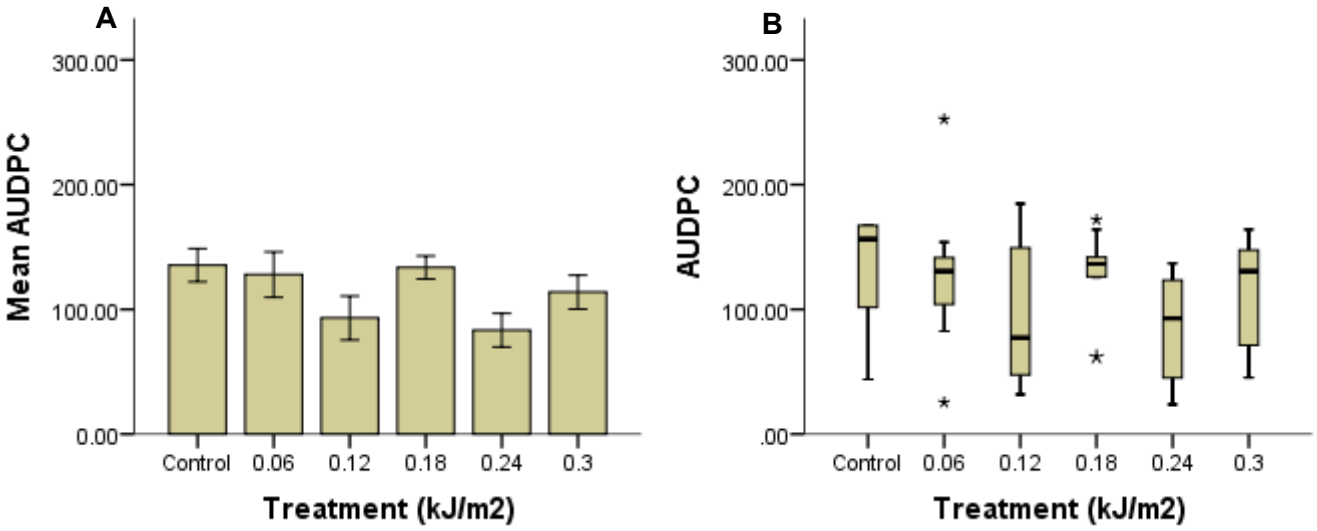

Figure A5.29. Disease resistance assays performed in February 2016 (12.02.2016) on the butterhead variety Temira treated with the conventional UV-C source. Plants were treated at 6-8 true leaves and inoculated 2 days after treatment with 10 µl of 1x10^6 spores/ml of *B. cinerea* amended with 50 % potato dextrose broth. Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. (**A**) Shows the mean lesion length ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 7

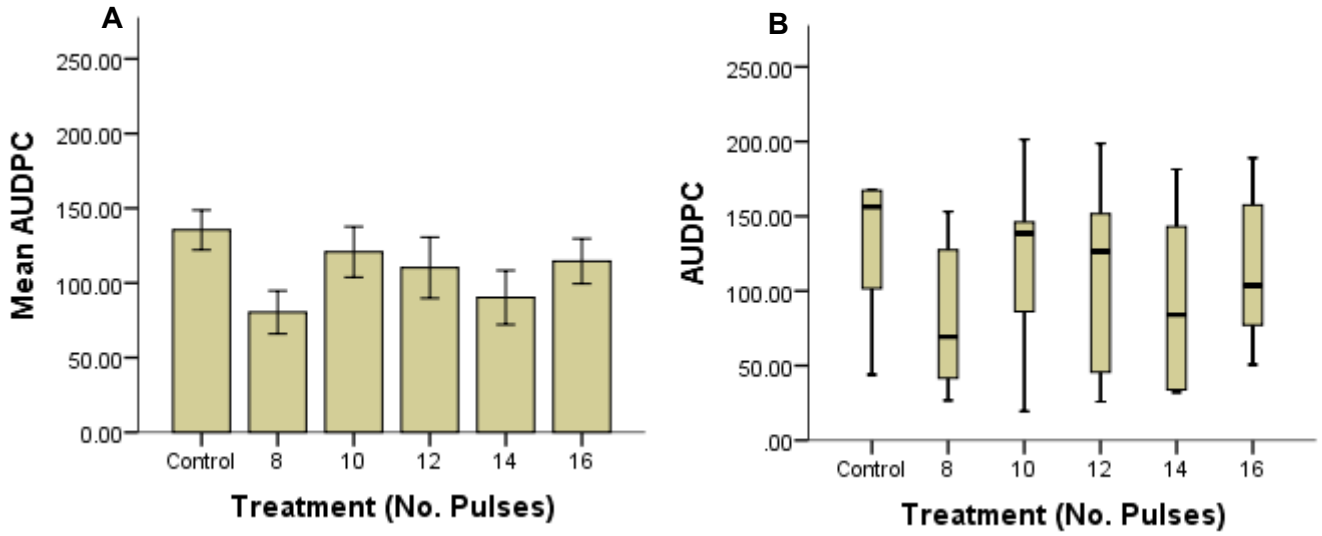

Figure A5.30. Disease resistance assays performed in February 2016 (12.02.2016) on the butterhead variety Temira treated with the conventional UV-C source. Plants were treated at 6-8 true leaves and inoculated 2 days after treatment with 10 µl of 1x10^6 spores/ml of *B. cinerea* amended with 50 % potato dextrose broth. Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. (**A**) Shows the mean lesion length ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 7

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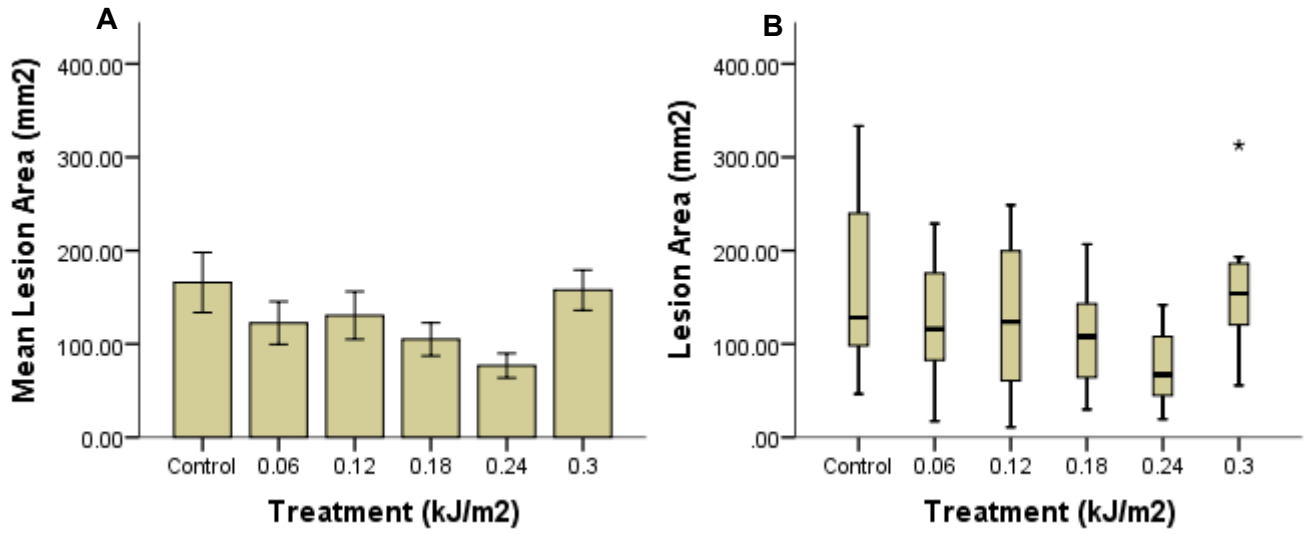

Figure A5.31. Disease resistance assays performed in February 2016 (12.02.2016) on the butterhead variety Temira treated with the conventional UV-C source. Plants were treated at 6-8 true leaves and inoculated 2 days after treatment with 4 mm agar plugs of *R. solani*. Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. (**A**) Shows the mean lesion length ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N=

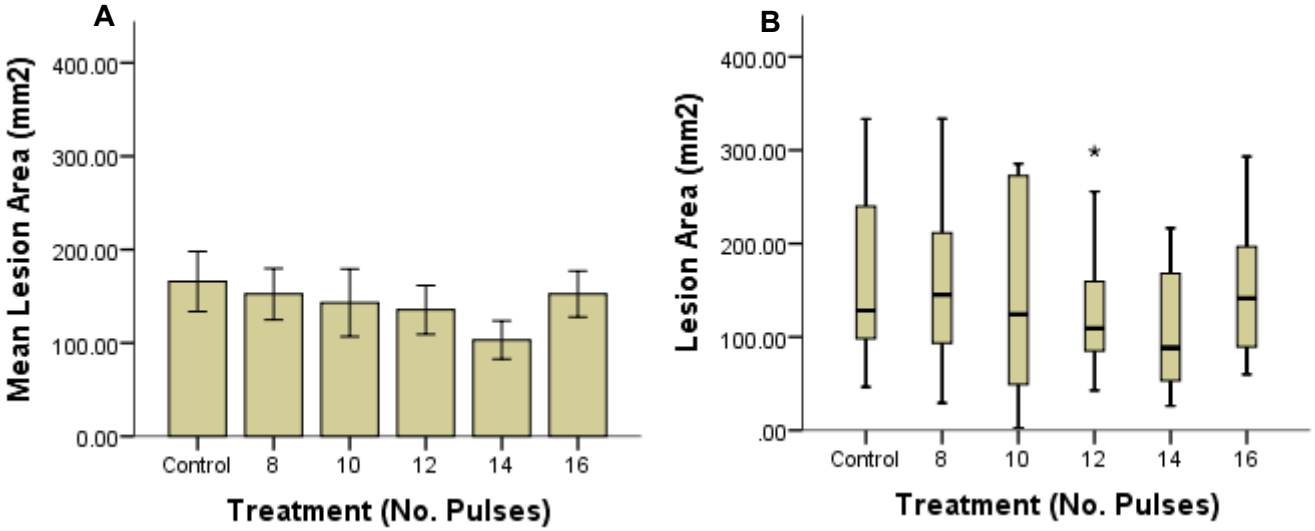

Figure A5.32. Disease resistance assays performed in February 2016 (12.02.2016) on the butterhead variety Temira treated with the pulsed polychromatic source. Plants were treated at 6-8 true leaves and inoculated 2 days after treatment with 4 mm agar plugs of *R. solani*. Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. (**A**) Shows the mean lesion length ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 7

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Appendix 6

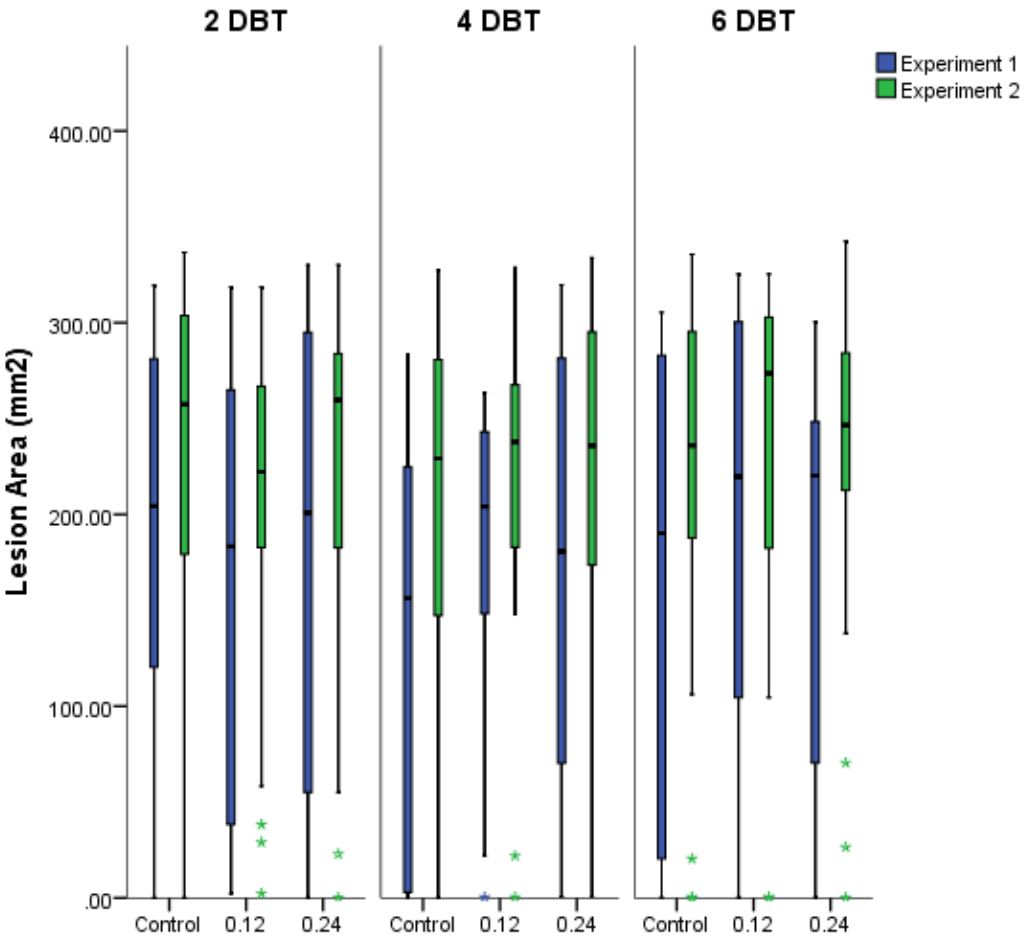

Figure A6.1. The mean lesion area from lettuce (cv. Amica) treated with multiple low-dose, low-intensity UV-C treatments (kJ/m^2) followed by inoculation with *R. solani.* Treatments were applied with either 2, 4 or 6 days between treatments (DBT). N=5 and 7 for experimental replicates 1 and 2, respectively. Box plots show the distribution of data from the independent experimental replicates. Asterisks represent observed outliers.

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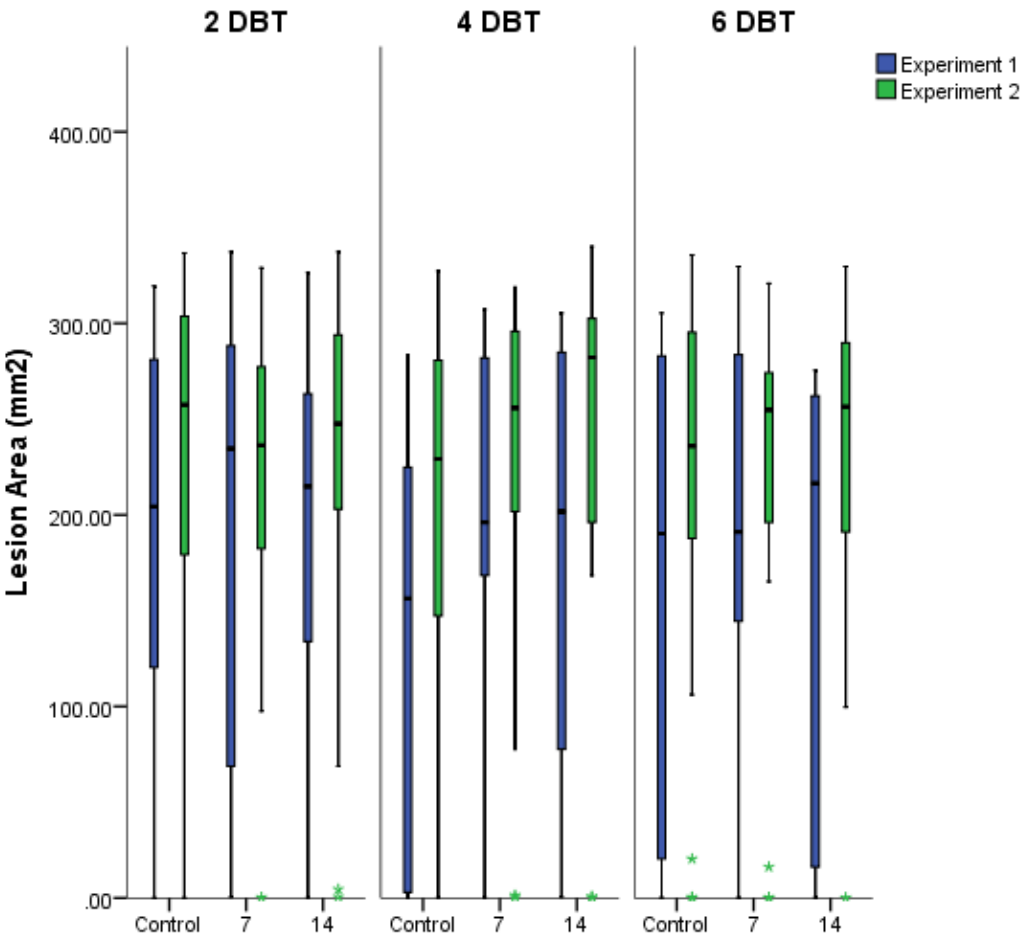

Figure A6.2. The mean lesion area from lettuce (cv. Amica) treated with multiple low-dose, high-intensity, pulsed polychromatic treatments (No. pulses) followed by inoculation with *R. solani.* Treatments were applied with either 2, 4 or 6 days between treatments (DBT). N=5 and 7 for experimental replicates 1 and 2, respectively. Box plots show the distribution of data from the independent experimental replicates. Asterisks represent observed outliers.

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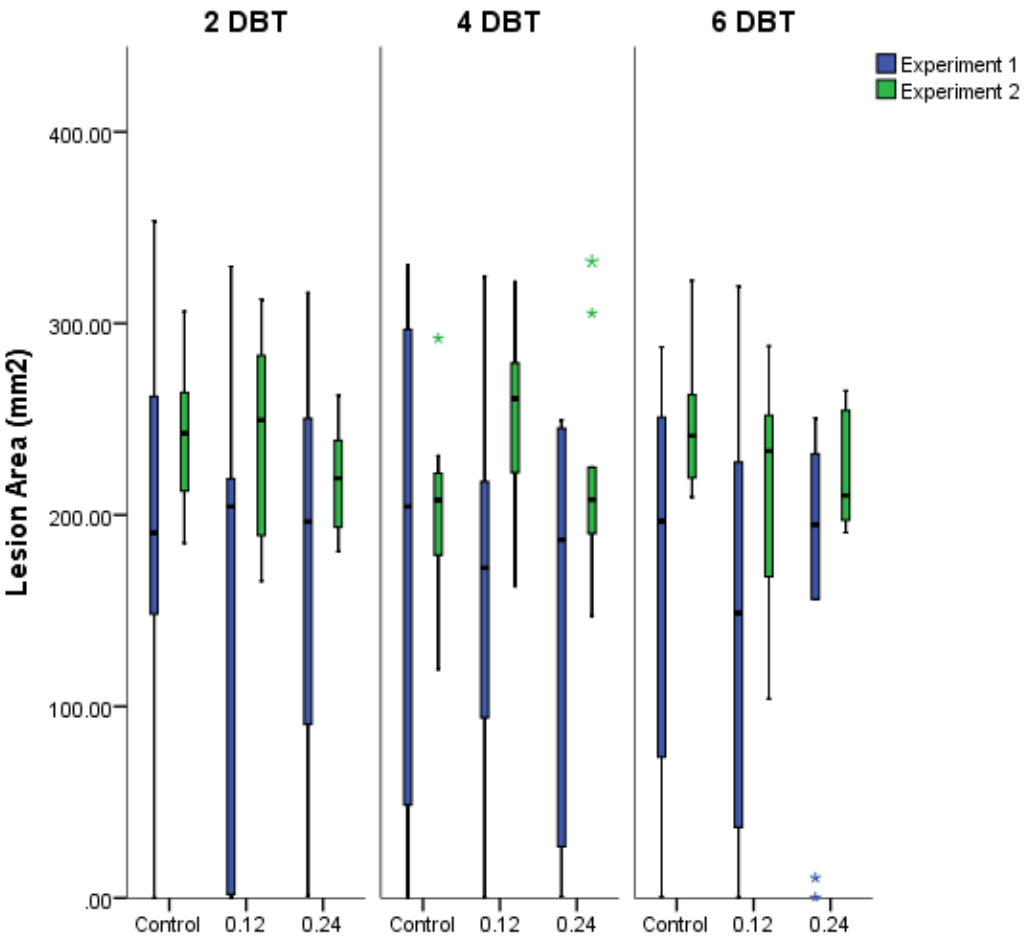

Figure A6.3. The mean lesion area from lettuce (cv. Temira) treated with multiple low-dose, low-intensity UV-C treatments (kJ/m^2) followed by inoculation with *R. solani.* Treatments were applied with either 2, 4 or 6 days between treatments (DBT). N=5 and 7 for experimental replicates 1 and 2, respectively. Box plots show the distribution of data from the independent experimental replicates. Asterisks represent observed outliers.

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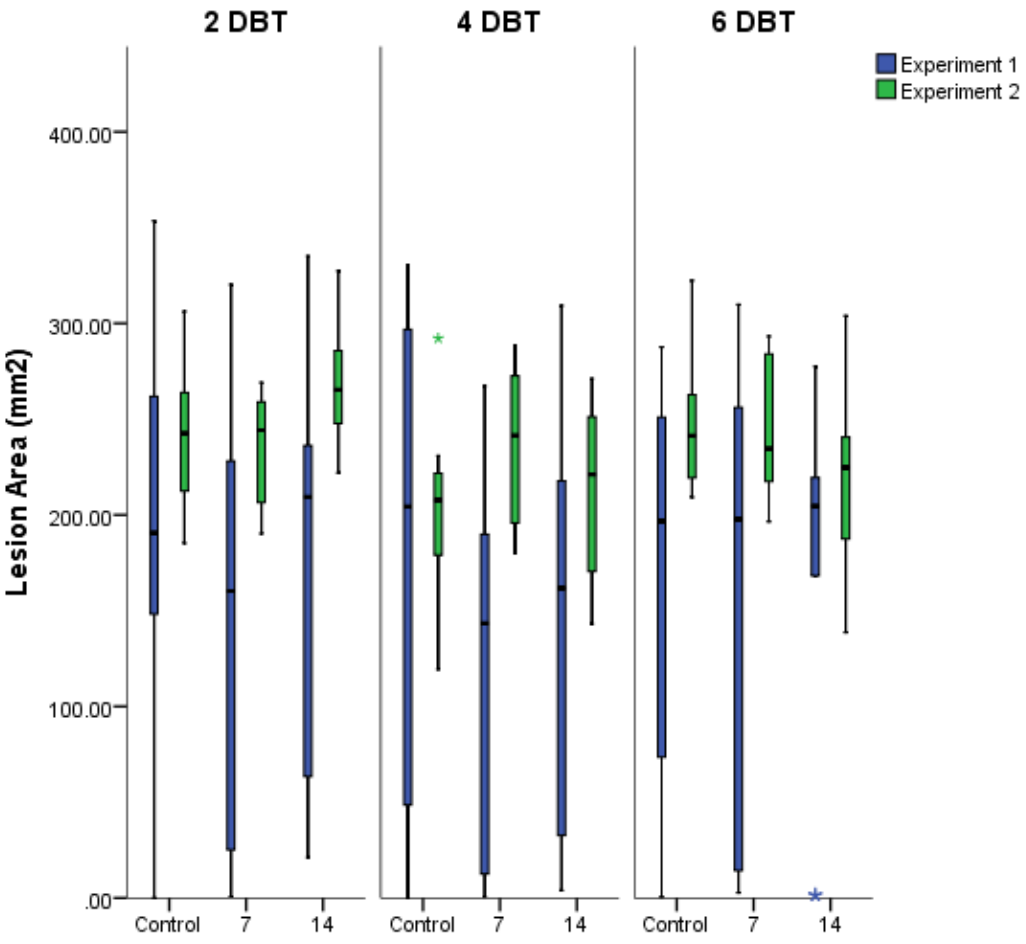

Figure A6.4. The mean lesion area from lettuce (cv. Temira) treated with multiple low-dose, high-intensity, pulsed polychromatic treatments (No. pulses) followed by inoculation with *R. solani.* Treatments were applied with either 2, 4 or 6 days between treatments (DBT). N=5 and 7 for experimental replicates 1 and 2, respectively. Box plots show the distribution of data from the independent experimental replicates. Asterisks represent observed outliers.

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Appendix 7

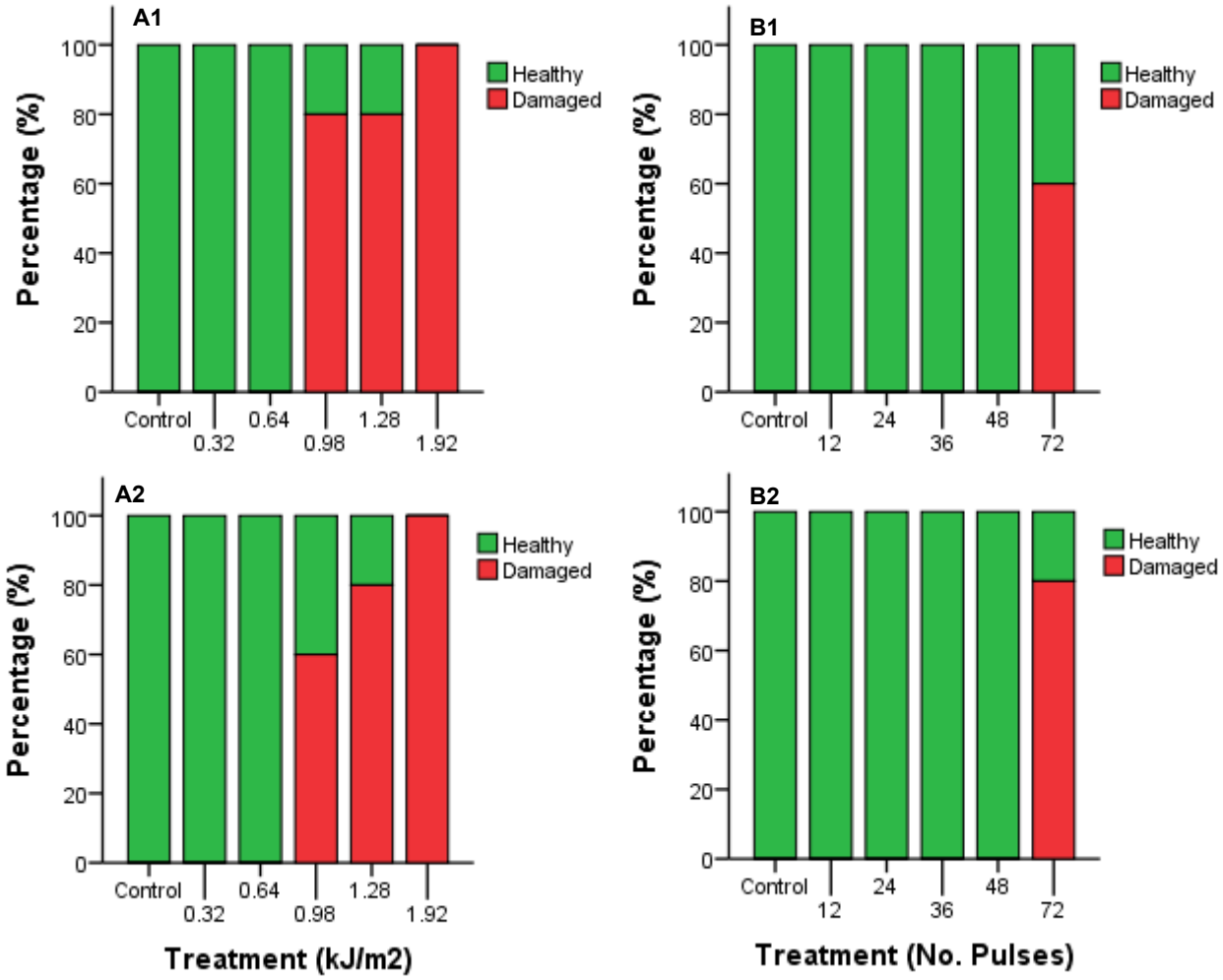

Figure A7.1. The percentage of healthy and damaged plants for the lettuce variety Temira. Plants were either treated with a conventional low pressure mercury UV-C source (**A**) or a high intensity pulsed polychromatic source (**B**) during May (**1**) and June (**2**) 2017 n=5.

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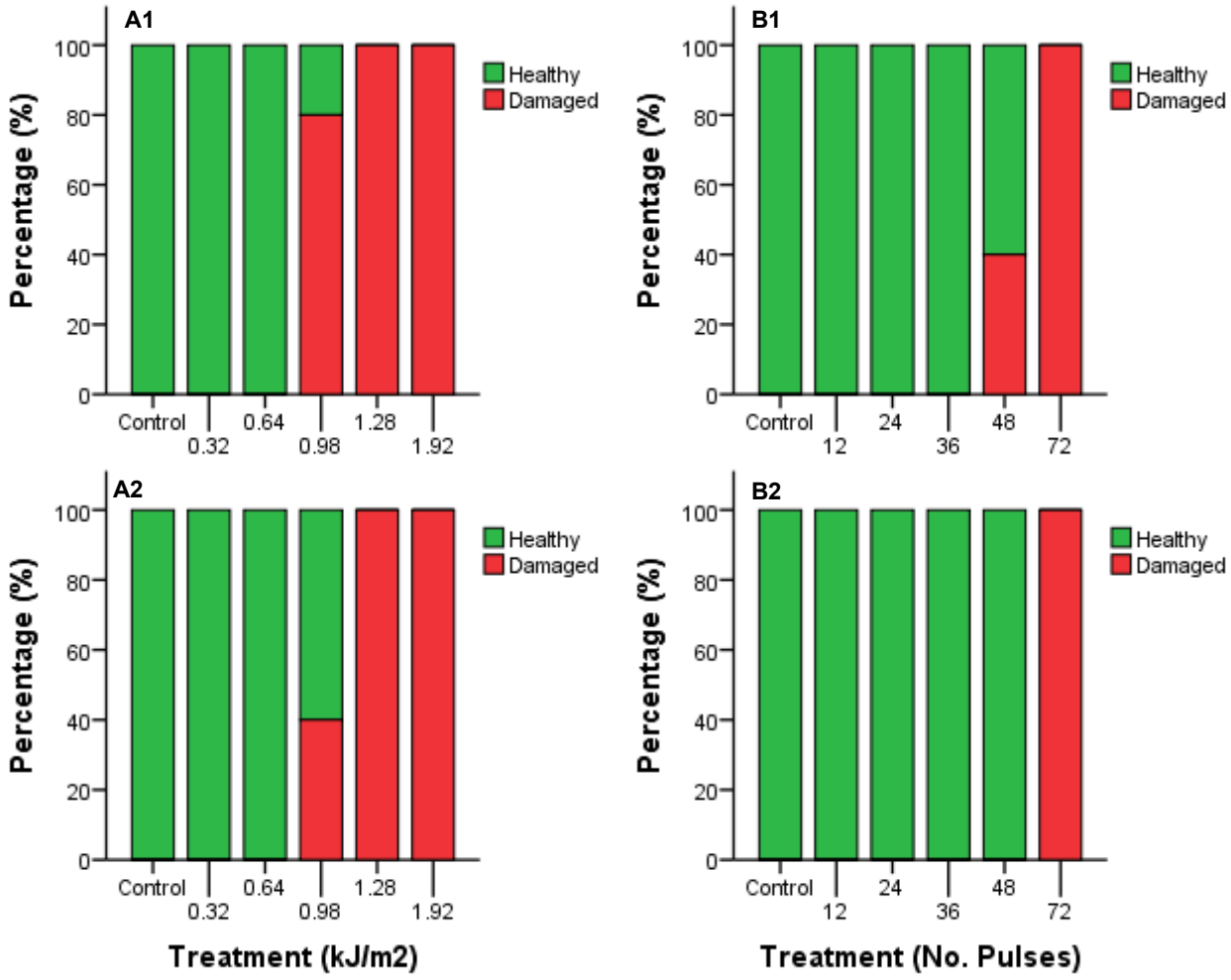

Figure A7.2. The percentage of healthy and damaged plants for the lettuce variety Amica. Plants were either treated with a conventional low pressure mercury UV-C source (**A**) or a high intensity pulsed polychromatic source (**B**) during May (**1**) and June (**2**) 2017 n=5.

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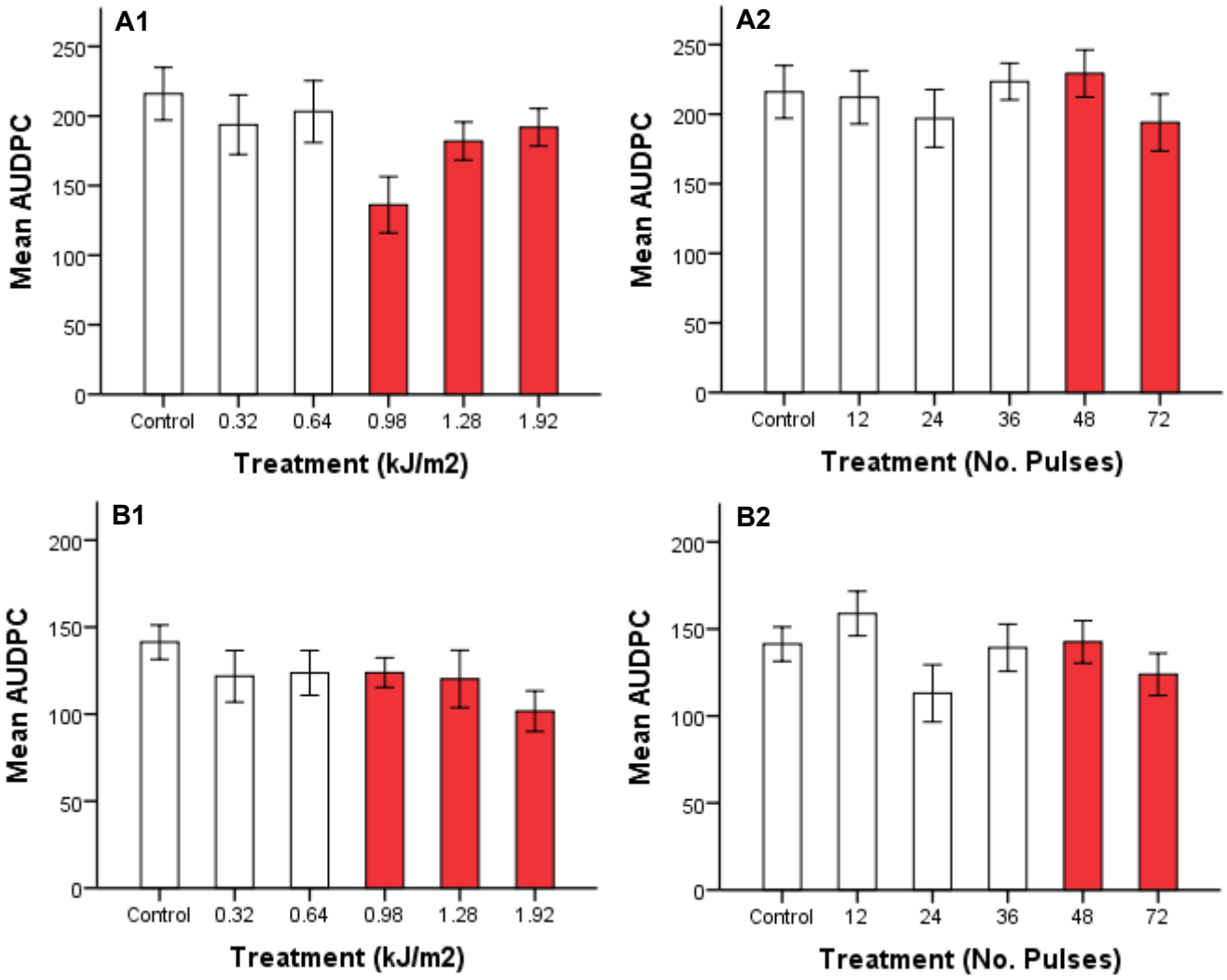

Figure A7.3: The area underneath the disease progression curve (AUDPC) from lettuce plants (Amica) treated with either low-intensity UV-C (A) or high-intensity, pulsed polychromatic light (B) and inoculated with *Botrytis cinereal* at 2 days post-inoculation. Disease progression measurements were taken at 2 and 3 days post inoculation to allow calculation of AUDPC. Graphs show the data from two independent replicate experiments one performed during May (**1**) and June (**2**) 2017 n=5.

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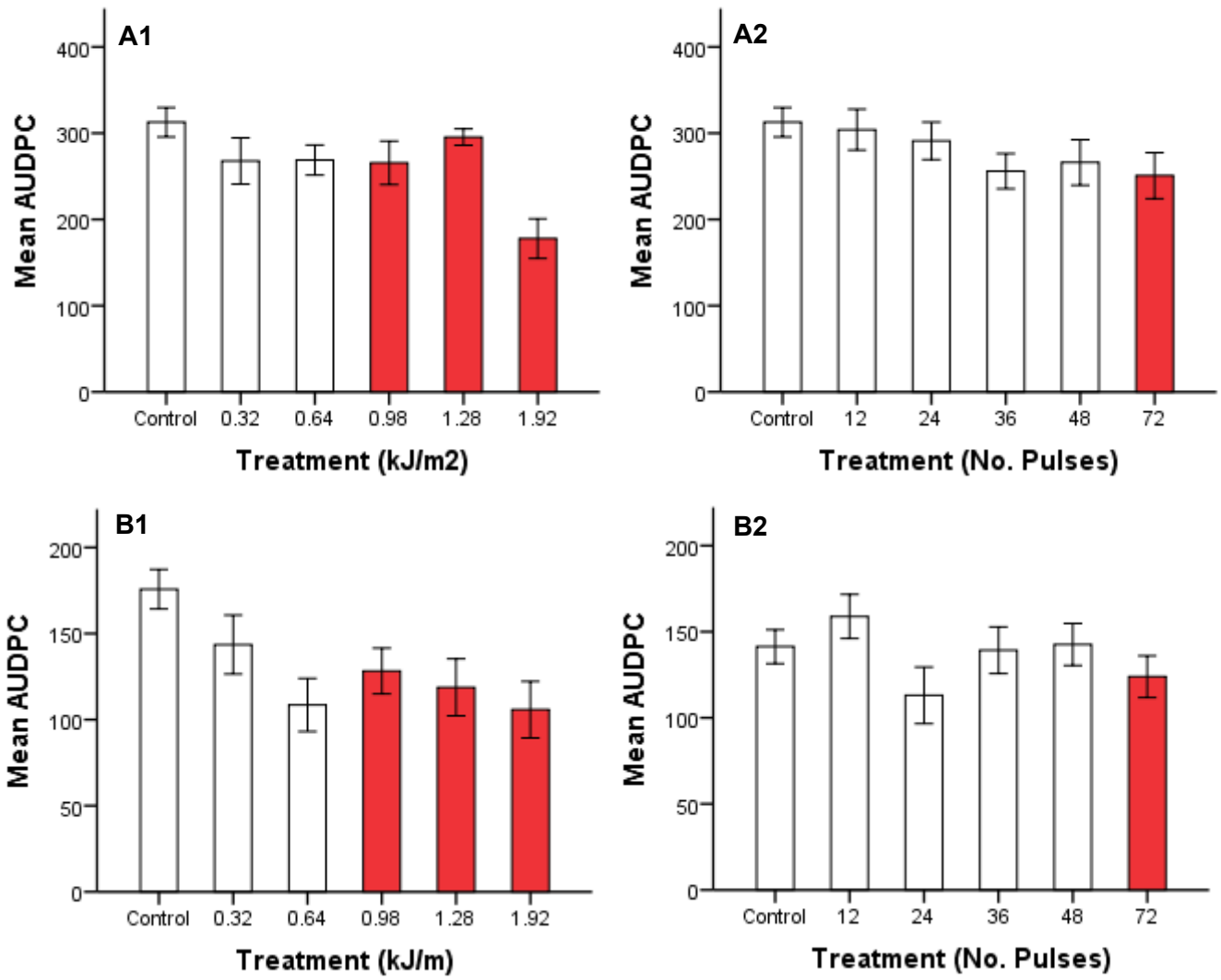

Figure A7.4: The area underneath the disease progression curve (AUDPC) from lettuce plants (Temira) treated with either low-intensity UV-C (A) or high-intensity, pulsed polychromatic light (B) and inoculated with *Botrytis cinereal* at 2 days post-inoculation. Disease progression measurements were taken at 2 and 3 days post inoculation to allow calculation of AUDPC. Graphs show the data from two independent replicate experiments one performed during May (**1**) and June (**2**) 2017 n=5.

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Appendix 8

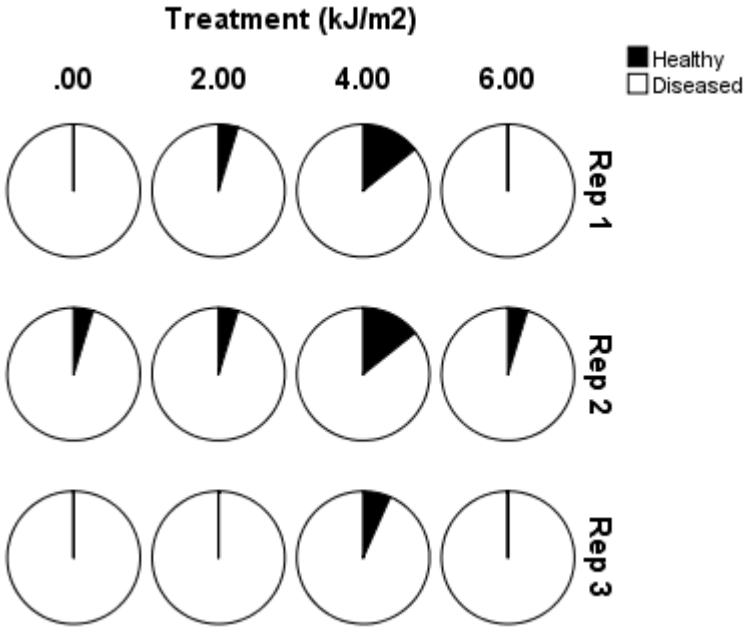

Figure A8.1: Disease incidence of tomato plants (cv. Shirley) grown from UV-C treated seeds and inoculated with *B. cinerea* at flowering. N= 7, 10 and 10 for replicates 1, 2 and 3, respectively.

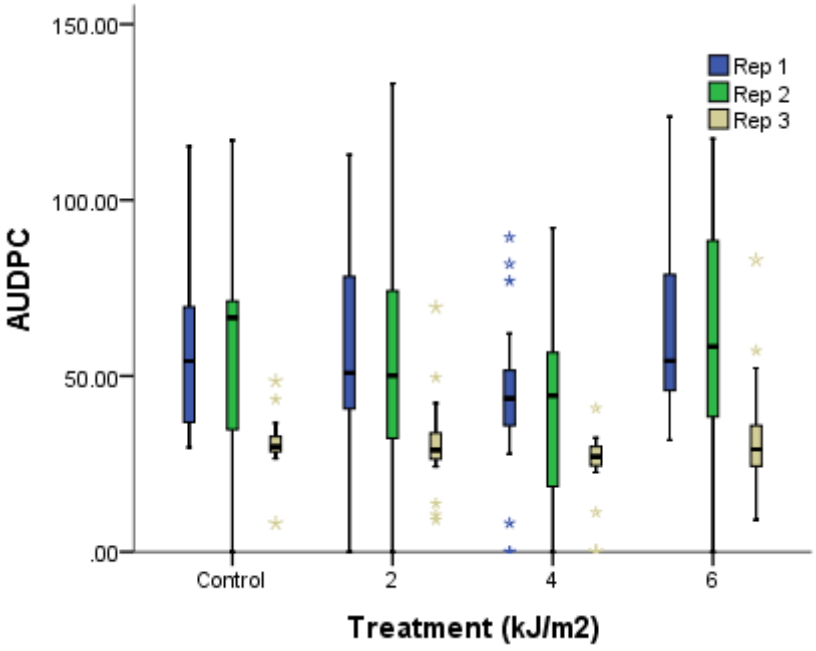

Figure A8.2: Area underneath the disease progression curve of tomato plants (cv. Shirley) grown from UV-C treated seeds and inoculated with *B. cinerea* at flowering. N= 7, 10 and 10 for replicates 1, 2 and 3, respectively.

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Appendix 9

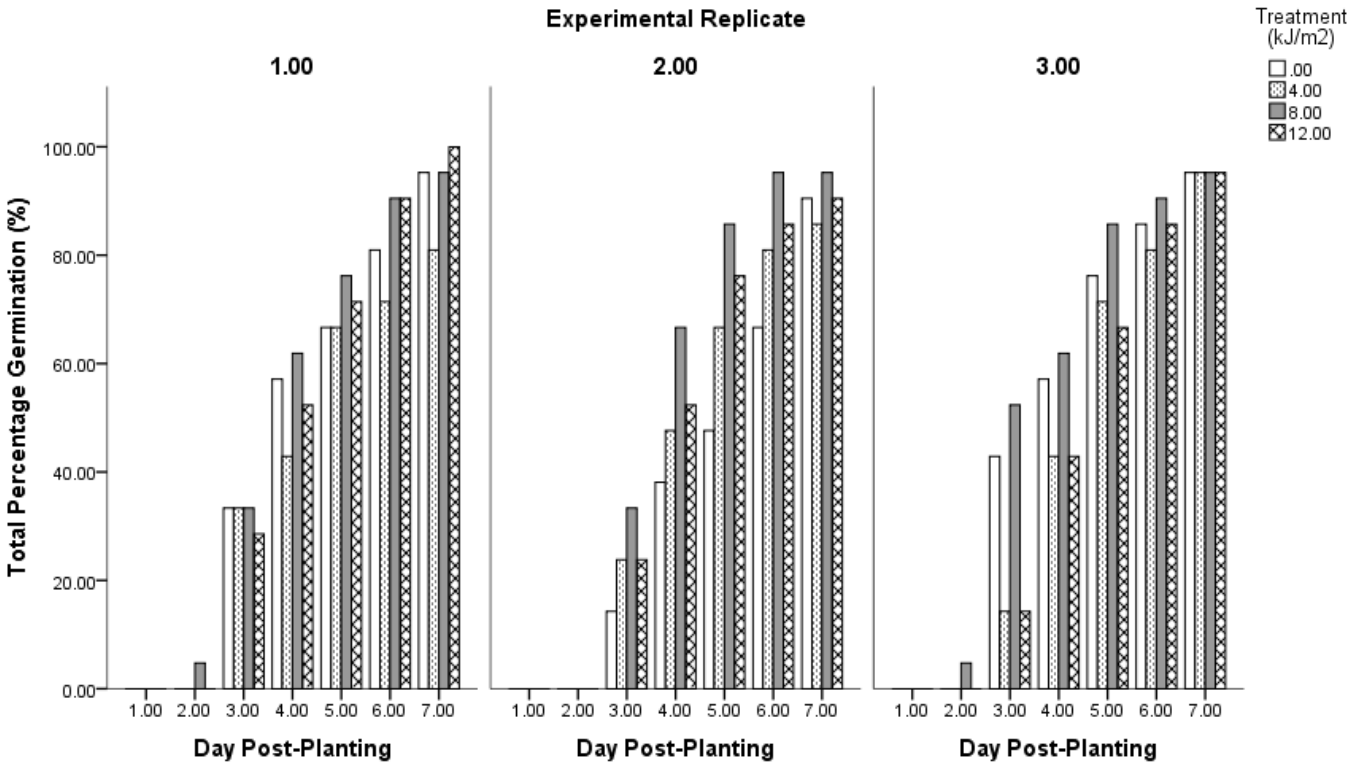

Figure A9.1: The total germination percentage of UV-C treated tomato (cv. Shirley) seeds from three experimental replicates. N= 21.

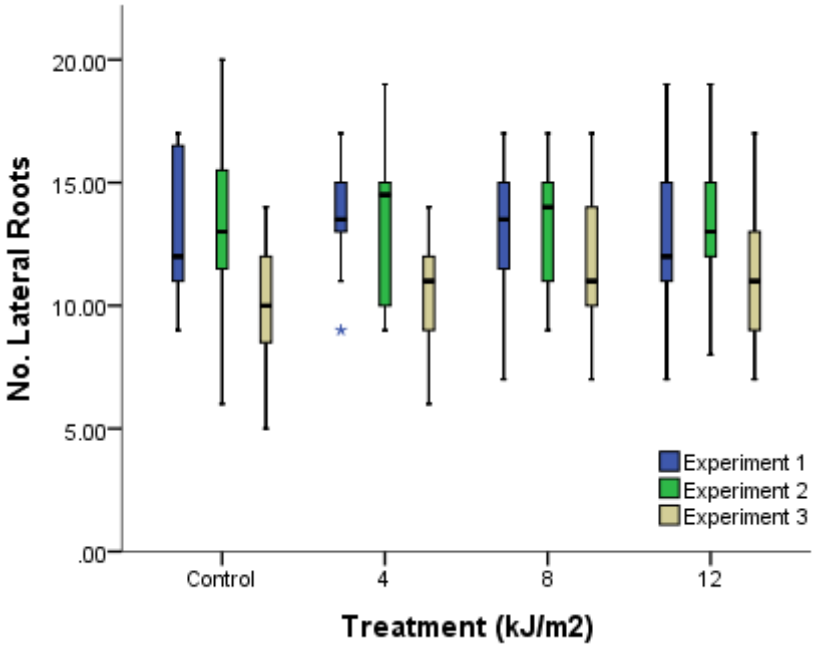

Figure A9.2: The number of lateral roots, at 5 days post germination, of UV-C treated tomato (cv. Shirley) seeds from three experimental replicates. N= 21.

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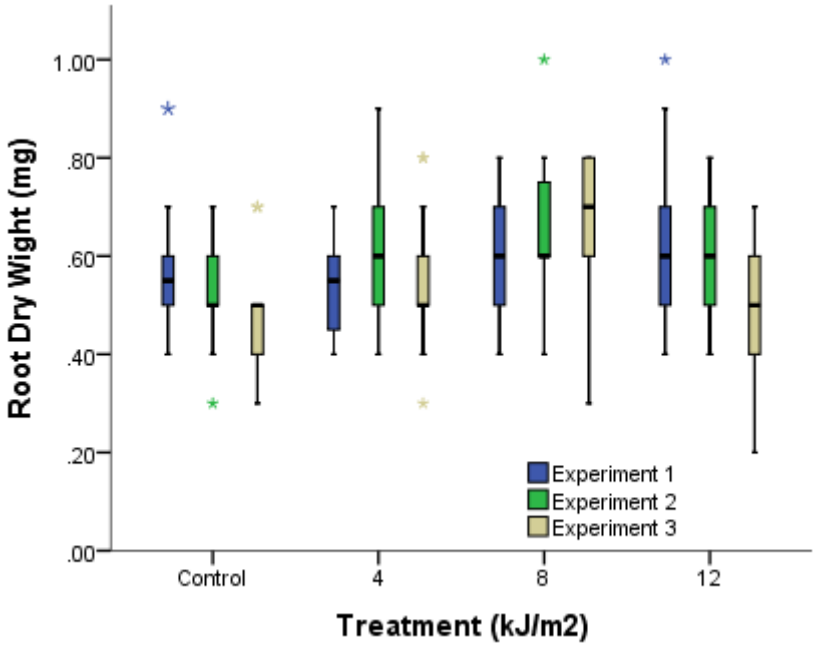

Figure A9.3: The dry root weight, at 5 days post germination, of UV-C treated tomato (cv. Shirley) seeds from three experimental replicates. N= 21.

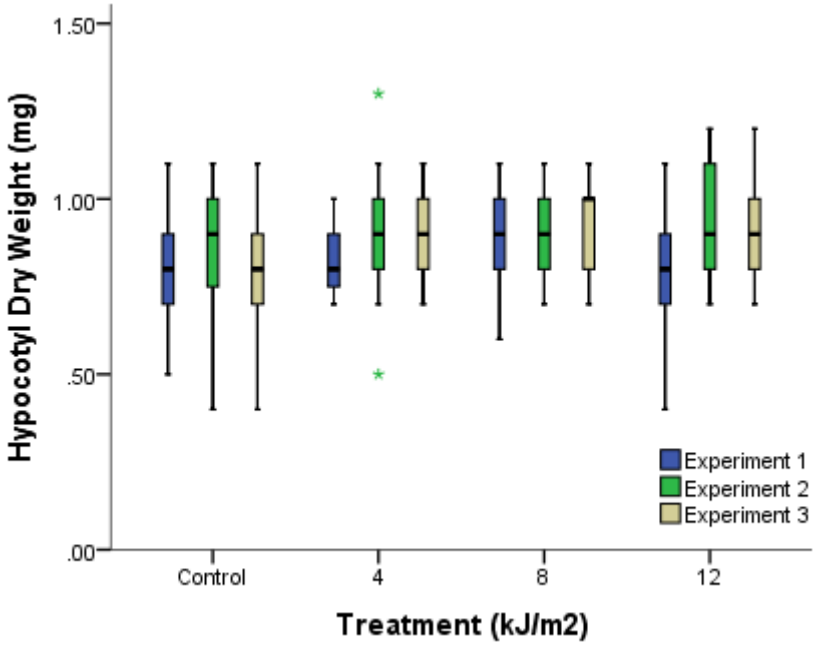

Figure A9.4: The dry hypocotyl weight, at 5 days post germination, of UV-C treated tomato (cv. Shirley) seeds from three experimental replicates. N= 21.

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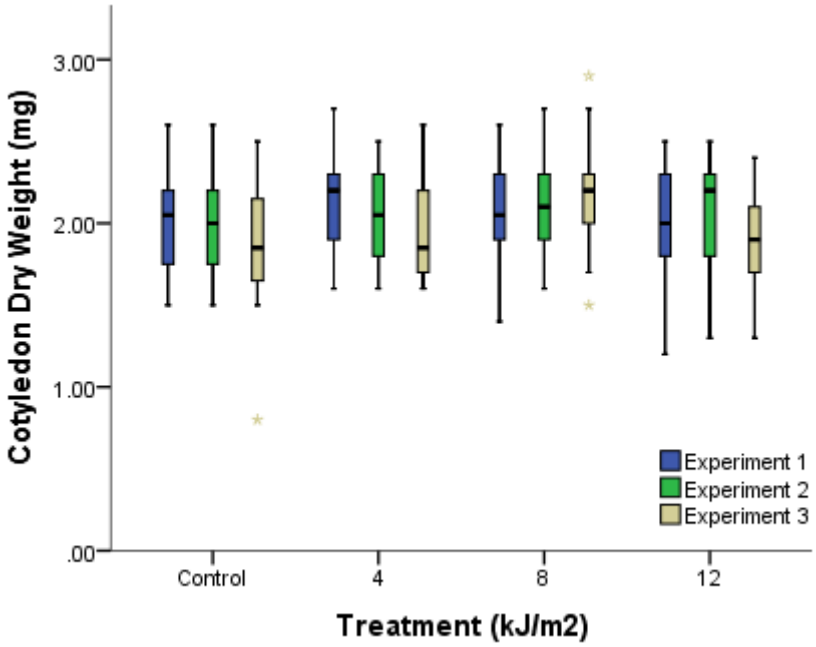

Figure A9.5: The dry cotyledon weight, at 5 days post germination, of UV-C treated tomato (cv. Shirley) seeds from three experimental replicates. N= 21.

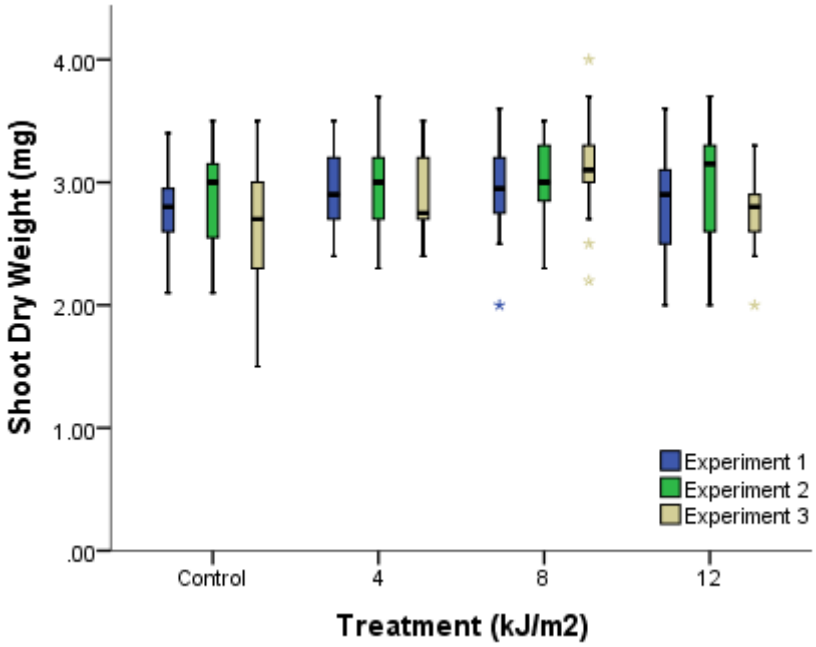

Figure A9.6: The dry shoot weight, at 5 days post germination, of UV-C treated tomato (cv. Shirley) seeds from three experimental replicates. N= 21.

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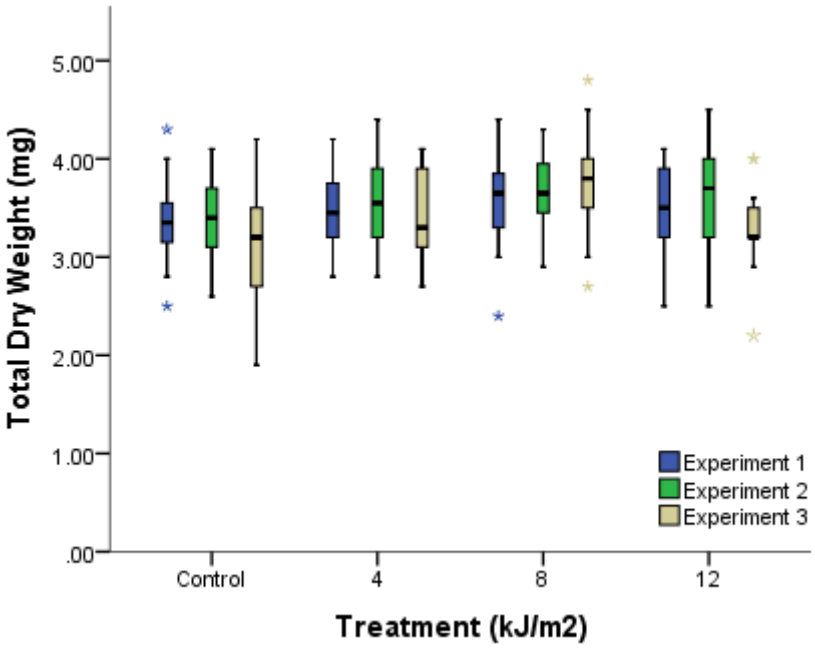

Figure A9.7: The total dry weight, at 5 days post germination, of UV-C treated tomato (cv. Shirley) seeds from three experimental replicates. N= 21.

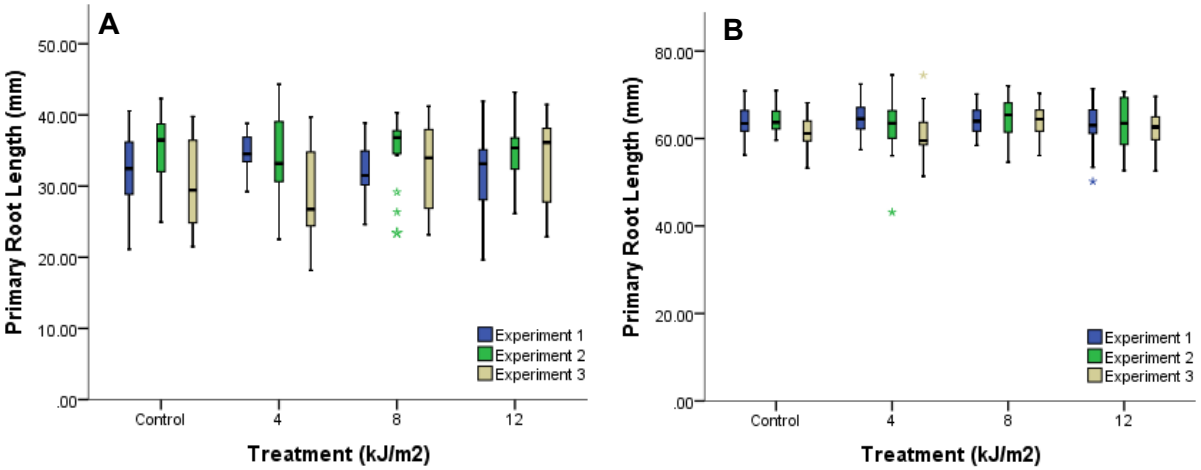

Figure A9.8: The primary root length at 2 (**A**) and 5 (**B**) days post germination of UV-C treated tomato (cv. Shirley) seeds from three experimental replicates. N= 21.

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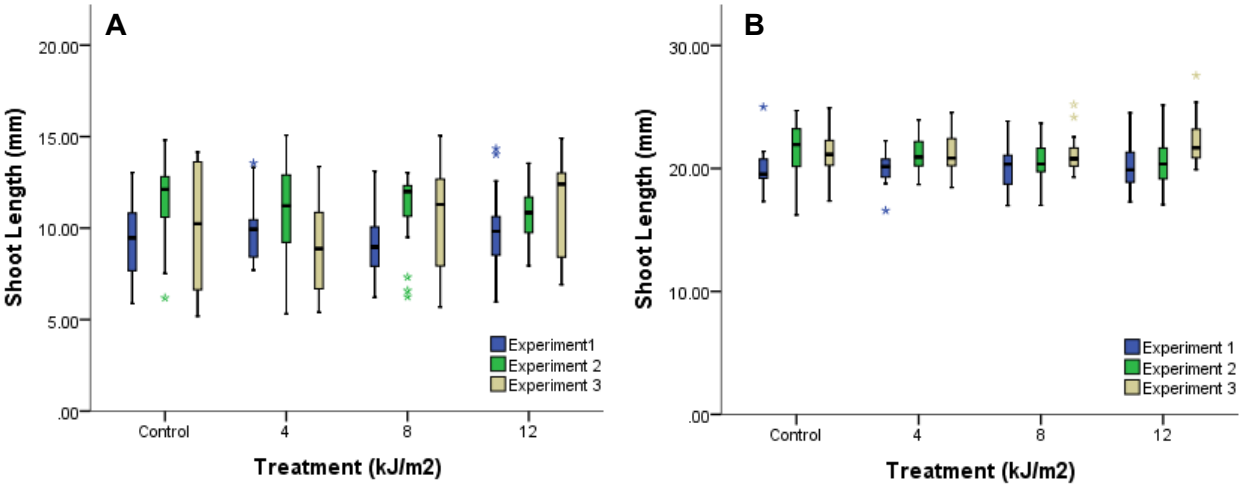

Figure A9.9: The shoot length at 2 (**A**) and 5 (**B**) days post germination of UV-C treated tomato (cv. Shirley) seeds from three experimental replicates. N= 21.

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