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Project leader:	Dr Gilbert Shama, Loughborough University
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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.

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.

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CONTENTS

GROWER SUMMARY

Headline

- Post-harvest treatments of tomato fruit with a high intensity, pulsed polychromatic source, rich in UV-C, show induced disease resistance against *Botrytis cinerea* and delayed ripening. Treatment time is reduced by 97.3 % in comparison to low intensity, conventional UV-C sources.
- Post-harvest treatments of tomato fruit from the side with high intensity, pulsed polychromatic or conventional UV-C sources elicit a local response and full surface exposure is, therefore, required.
- Post-harvest treatments of tomato fruit with a high intensity, pulsed polychromatic or conventional UV-C sources induce resistance to *B. cinerea* on both mature green and ripe tomatoes.

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 majority of studies have been performed using conventional low pressure mercury UV-C sources on post-harvest produce. Commercial application of these treatments has, in part, been prevented due to the lengthy exposure times necessitated. Conventional treatments of tomato fruit take in excess of six minutes. High intensity, pulsed polychromatic light sources, rich in UV-C, however, have been developed which hold the potential of drastically reducing treatment times and making UV-C treatments a commercial possibility. However, it is necessary to demonstrate that such sources have the ability to induce disease resistance and delay ripening on tomato fruit through post-harvest treatments (Objective 1).

Recently, exposure of foliage to UV has been shown to induce resistance against downy mildew and grey mould on *Arabidopsis thaliana*. The horticultural application of such treatments, however, have not been explored. We, therefore, aim to research pre-harvest UV treatments to induce resistance on both tomato and lettuce crops (Objectives 2 & 3). Utilisation of such UV 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

Tomato fruit of the cv. Meccano were treated at both the mature green and ripe stage. An established conventional UV-C treatment was performed alongside a number of pulsed polychromatic treatments. This was to allow a comparison of the sources' ability to induce both disease resistance against *B. cinerea* and delay ripening. Both conventional and pulsed sources successfully induced resistance, to comparable levels, against *B. cinerea* on mature green fruit following artificial inoculation. Disease progression on ripe fruit, however, was inhibited to a greater extent on ripe fruit treated by the pulsed source. Furthermore, ripening was delayed by the pulsed source to comparative levels of that observed for the conventional source. Both ripe and mature green fruit showed optimal treatment of 16 pulses giving a total treatment time of 10 seconds yielding a 97.3 % reduction in treatment time. The ability to induce resistance to *B. cinerea* at both the mature green and ripe stages shows that post-harvest UV-C treatment could be adopted by growers who harvest at differing fruit maturities. The majority of previously published research was focused only on fruit at the mature green stage.

Further investigation has highlighted that both the conventional UV-C and pulsed polychromatic sources elicit a local response in tomato fruit. Fruit would, therefore, require full surface exposure to the optimal dose to effectively induce the hormetic benefits. The local response is seen for both disease resistance and delayed ripening. To assess the need for direct tissue exposure to induce resistance, fruit were treated on a single side and then inoculated at either the directly exposed tissue or unexposed tissue. Unexposed tissue gave results homologous to those observed for the control, figure 1. An example of the local delayed ripening response to treatment can be seen in figure 2 where the uneven ripening in treated groups B, C, D and E is caused by only partial exposure to UV-C or polychromatic light.

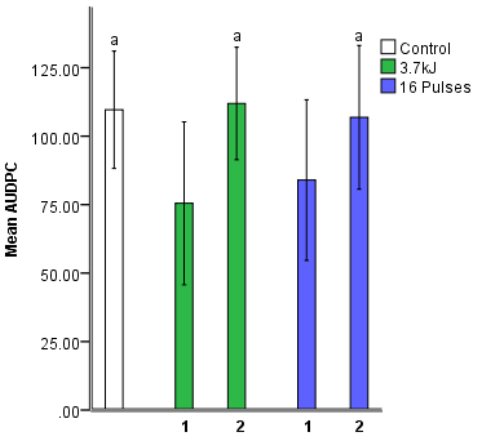

Figure 1. Area underneath the disease progression curve (AUDPC) of tomatoes, cv. Mecano, treated on a single side and inoculated with *B. cinerea* at 10 d post treatment. Fruit were treated with an established low intensity UV-C treatment of 3.7 kJ/m^{2}and a high intensity pulsed polychromatic treatment of 16 pulses. (**1**) Exposed tissue and (**2**) unexposed tissue. Error bars show ± 1 standard deviation; n = 20. Labelling denotes a statistically significant result at $p < 0.05$. Means sharing the same superscript are not significantly different from each other at $p< 0.05$. (Scott *et al.,* 2016)

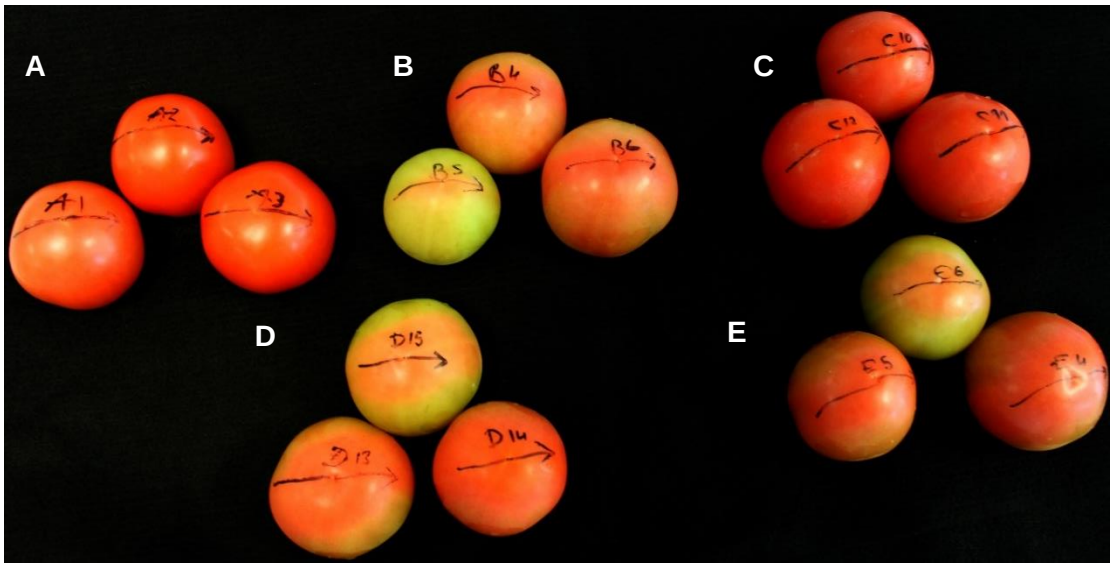

Figure 2. A representative sample from the fruits treated post-harvest showing: **A)** Control fruit. **B)** Conventional treatment with the low pressure mercury source. **C)** An 8 pulse treatment. **D)** A 16 pulse treatment and **E)** A 24 pulse treatment. Black lines on the fruit run parallel to the direction of UV source exposure which highlights the dependency of full surface exposure for delayed ripening. (Scott *et al.,* 2016)

Objective 2 - Foliar UV-C and Polychromatic Treatments of Tomato

No previous work has been carried out on the induction of resistance on tomato through exposure of the foliage to UV. The first step was, therefore, to find the point at which damage was observed on plants exposed to both conventional and pulsed UV sources. This was performed at two developmental stages; early vegetative and early flowering. Damage was observed above 0.5 kJ/m^2 for the conventional source and at 20 pulses. Hormetic treatments will, therefore, fall below these thresholds.

Objective 3 - Foliar UV-C and Polychromatic Treatments of Lettuce

Damage assessments for lettuce were carried out at the 3-5 true leaf and early, mid and late head formation developmental stages. The point at which damage was caused to the plant following UV-C and polychromatic treatments varied across the year. Within the middle of the growing season damage was caused at pulsed treatments above 60 pulses and conventional treatments above 1.5 kJm^2. At the end of the season, however, damage was observed at pulsed treatments of 16 pulses and conventional treatments of 0.35 kJm^2. In preliminary studies the treatments for which reduced levels of disease was observed also showed variation across the year. Furthermore, in preliminary work the optimal treatment also varied depending on the pathogen undergoing investigation and the cultivar being used.

Financial Benefits

Calculation of financial benefits are not possible at this time.

Action Points

There are no immediate action points.

SCIENCE SECTION

Objective 1 - Validation of 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 20 years and have shown to be effective on orange, strawberry and sweet potato to mention a few (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 investigations have relied on initiation of disease through natural inoculum or have performed inoculations pre-treatment. This may create some confusion as it may fail to truly attribute the level of disease reduction to the UV-C induced effects alone. This is because the direct effect of UV-C on the 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 *B. cinerea* through post-harvest fruit treatment with the intention of extending its application to pre-harvest, whole plant treatments.

Materials and Methods

Experimental Design and Data Analysis

All data presented here was collected from two independent replicate experiments. Fifteen fruit were used in each treatment group, per experiment, for testing of disease resistance and delayed ripening; n = 30. For experiments on the necessity for direct tissue exposure to elicit disease resistance ten fruit per group, per replicate were used; n=20. Data analysis was performed using SPSS 22 (IBM). One-way ANOVA with Tukey's post-hoc testing was performed to assess the differences in means. Where the homogeneity of variances could not be met Welch's robust ANOVA was performed followed by the Games-Howell post-hoc test. Statistical significance is here defined as $p \leq 0.05$.

Table 1. Experimental replicate dates for investigations concerning 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

Tomato Fruit Production

Tomatoes of the cv. Mecano were grown in the glasshouse at APS Salads (UK) and delivered at ambient temperature to the University of Nottingham within 24 h of harvesting. Fruit were then selected for their developmental stage, uniformity of size and absence of surface damage.

Tomato Colour Measurement

Prior to treatment, colour measurements were taken to assess the effects on ripening. Mature green fruit were measured with a calibrated CR-200 Chroma meter (Konica, Minolta) in l*a*b* mode. Readings were taken at a single point directly facing the source and at a 90° axial rotation from that point. A second colour measurement was taken using the same reference points at 10 days post treatment. This was used to calculate the change in tomato colour index (TCI) over 10 days, Equation 1.

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

Equation 1. Tomato colour index (TCI) formula where L= lightness, a= red-green and b = blue-yellow values (Hobson, 1987).

UV-C and High Intensity Pulsed Polychromatic Light Treatments

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

Pulsed treatments were carried out with the RT-847 cabinet along with RC-802 controller and LH-840 ozone-free B lamp (XENON) supplied by Lambda Photometrics (Harpenden, Herts). The source produced 505 J of energy per pulse with a pulse width of 360 µs at 3.2 pulses per second. Spectral emissions of the source were between 240 nm and 1050 nm. Fruit were placed at a distance of 10 cm from the window of the lamp housing. Through extrapolation of the manufacturer's data an estimated 4.6 kJ/m^2/pulse was delivered at fruit level.

An established conventional UV-C treatment of 3.7 kJ/m^2 delivered at 2000µW/cm^2 (Charles *et al.,* 2008) was used as a benchmark to assess the efficacy of induced disease resistance from the pulsed source. For both sources fruit received exposure on two sides through 180° axial rotation. Following treatment, fruit were immediately incubated in the dark. Fruit were then surface-sterilised in 2 % sodium hypochlorite and rinsed three times in sterile distilled water and allowed to air dry. Fruit were stored at 13 °C to prevent photoreversal. Fruit were stored in humidity boxes lined with damp paper and raised by a double layer of plastic mesh.

Inoculum Production and Artificial Inoculation

At 10 days after treatment fruit were inoculated; this was shown to be close to the optimum point of UV-C induced disease resistance by Charles *et al.,* (2008). A calibrated spore solution was made from 10-14 day old cultures of *B. cinerea.* Fruit were then wounded with a sterile hypodermic needle to the depth of 3 mm. Ripe fruits were inoculated with 5 µl of 1x10^5 spores. Mature green fruits, however, were inoculated with 5 µl of 1x10^6 spores due to reduced levels of susceptibility. Fruit were then stored at 21 °C.

Total lesion diameter was measured with digital Vernier callipers at 3 and 4 days post inoculation. Lesion sizes 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, Equation 2, (Simko & Piepho, 2011).

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

Equation 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).

Results

Tomatoes were treated with an established 3.7 kJ/m^2 treatment from a conventional low intensity UV-C source and either 8, 16 or 24 pulses from a high intensity pulsed polychromatic light source. Analysis of the colour change of tomatoes revealed that the conventional, 16 and 24 pulse treatments, showed significantly slower ripening progression (Δ TCI) in comparison to the control fruit, Figure 3 (1). The 8 pulses treatment, however, did not delay ripening to a significant level. The 16 pulse treatment gave a delay in colour change similar to that of the conventional treatment. The results from the independent experimental replicates can be seen in Figure 4.

Figure 4 shows the same pattern emerges between the two experimental replicates with the greatest Δ TCI being observed in the control group for both instances. The conventional 3.7 kJ/m^2 treatment shows a reduction in Δ TCI but not to the extent observed for the 16 pulse treatments. The 8 and 24 pulse treatments also show the same pattern between the experimental replicates both giving greater Δ TCIs than the 3.7 kJ/m^2 treatment and the 16 pulses treatments. There is, however, an observable difference between the two replicates with the first experiment showing a greater change in the Δ TCIs for all of the treatment groups. This may be due to the point of harvest during the season variation in the developmental stage of the tomatoes being treated.

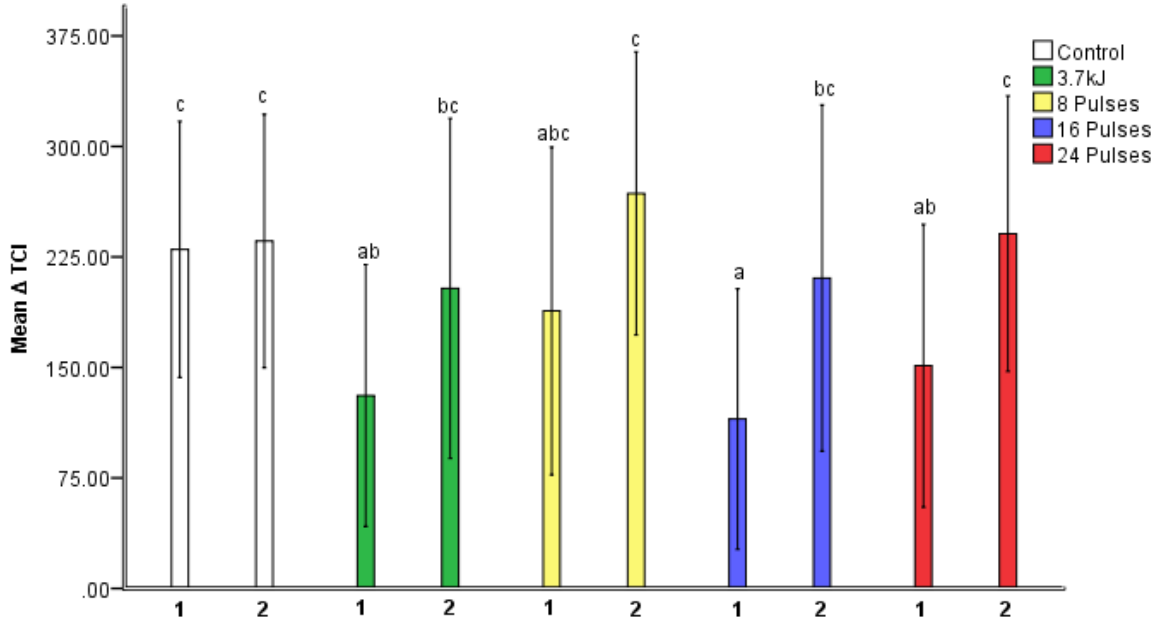

Figure 3. The Δ TCI (tomato colour index) from day 0 - 10 of mature green fruit from cv. Mecano. Fruit were treated with a hormetic conventional treatment of 3.7 kJ/m2 from a low intensity UV-C source and three high intensity pulsed treatments of 8, 16 and 24 pulses. TCI measurements were taken from tissue directly facing the light source (1) and at 90 ^{0} from the source (2). Error bars show ± 1 standard deviation; n = 30. Labelling indicates statistical significance. Means sharing the same superscript are not significantly different from each other at p< 0.05. (Scott *et al.,* 2016)

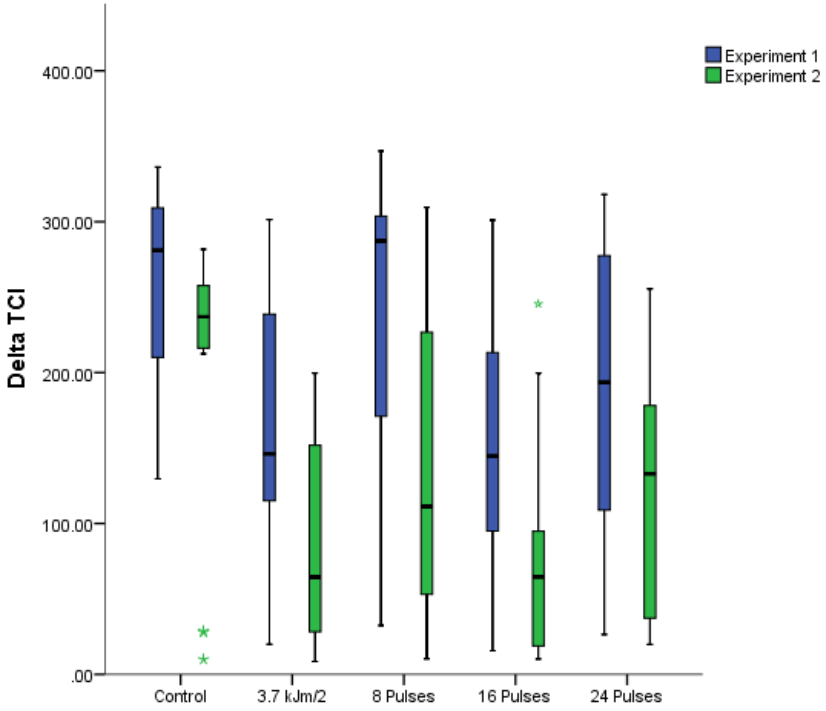

Figure 4. The Δ TCI (tomato colour index) from day 0 - 10 of mature green fruit from cv. Mecano. Box plots show the data from the individual independent replicate experiments (n=15). Asterisks represent observed outliers.

Colour measurements were also taken at 90° from tissue directly exposed to the light sources to assess if direct exposure to the light sources is necessary for the successful induction of delayed ripening, Figure 3 (2). This showed that no significant changes and only minor increases or decreases in colour change were observed for tissue not directly exposed to the light sources. This indicated that UV-C and polychromatic light induced delayed ripening is a local response in tomato fruit treated from the side following harvest. Obande *et al.,* (2011), however, showed a UV-C induced systemic delay in ripening for fruits treated on the truss prior to harvesting. Examples of induced delayed ripening can be seen in Figure 5.

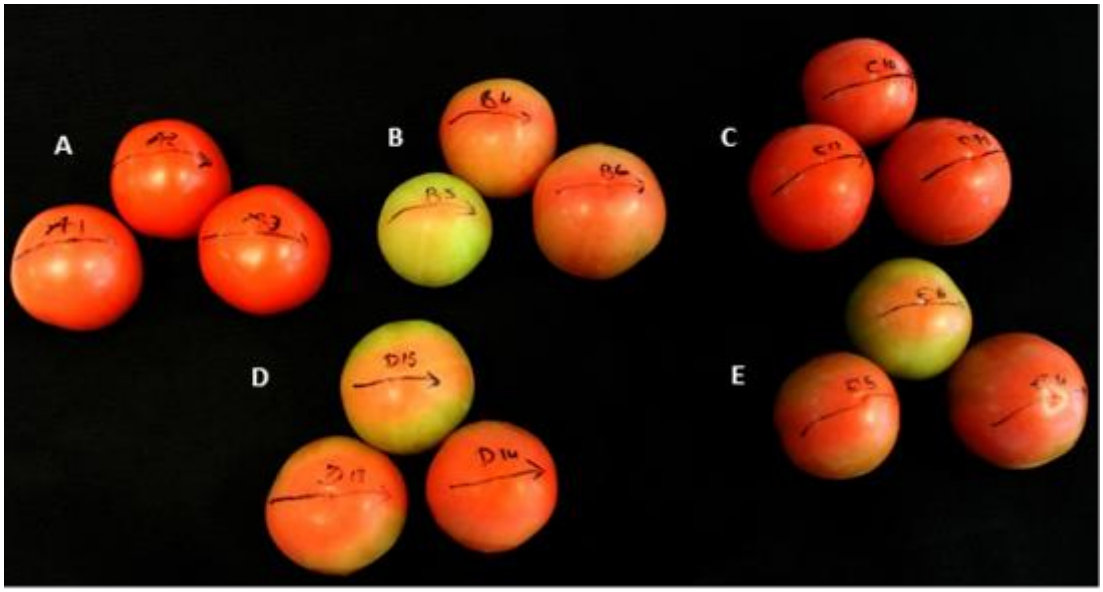

Figure 5. Representative samples of tomato fruit of the cultivar Mecano at 10 d post treatment. Groups show the control fruit (**A**), the 3.7 kJ/m^2 conventional treatment (**B**) and fruit treated with the high intensity pulsed polychromatic source with 8 (**C**), 16 (**D**) and 24 (**E**) pulses. (Scott *et al.,* 2016)

It has been hypothesised that changing fruit orientation may allow the propagation of a systemic signal utilising the fruits vasculature system (Stevens *et al.,* 2005). To test this hypothesis alternative treatment orientations were performed focusing the sources at either the blossom end or calyx. All treatments performed, however, produced uneven ripening analogous to that observed in the previous experiments, Figure 6.

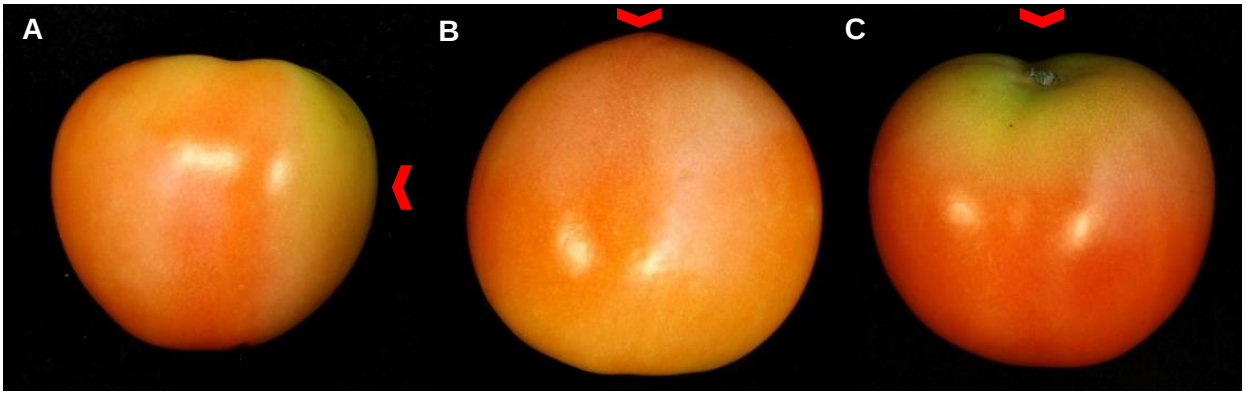

Figure 6. Representative samples of tomato fruit exposed to 16 pulses of the high intensity polychromatic light source from different orientations. Red arrows indicate the positioning of the source. **A**) Treatment from the side. **B**) Treatment from the blossom end. **C**) Treatment from the calyx. (Scott *et al.,* 2016)

To further validate the pulsed source, an additional hormetic benefit elicited by conventional UV-C sources was also observed; induced resistance against *Botrytis cinerea*, the causal agent of Grey Mould. This was initially performed on mature green cv. Mecano as mature green fruit have been the focus for the majority of previous research papers.

All treatments for both the conventional and pulsed sources significantly reduced the progression of *B. cinerea*, Table 2. No significant differences, however, were observed between pulsed and conventional treatments. The 16 pulse treatments, however, were the most effective and gave similar levels of resistance to the conventional treatments, as was observed for the analysis of colour change following treatment. The 8 pulse treatment was the least effective treatment tested and showed significantly faster disease progression to that of the 16 pulse treatment. No differences in the treatment effects were observed between the independent experimental repeats, Figure 6.

Table 2. Area underneath the disease progression curve (AUDPC) 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 = 30. (Scott *et al.,* 2016)

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

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

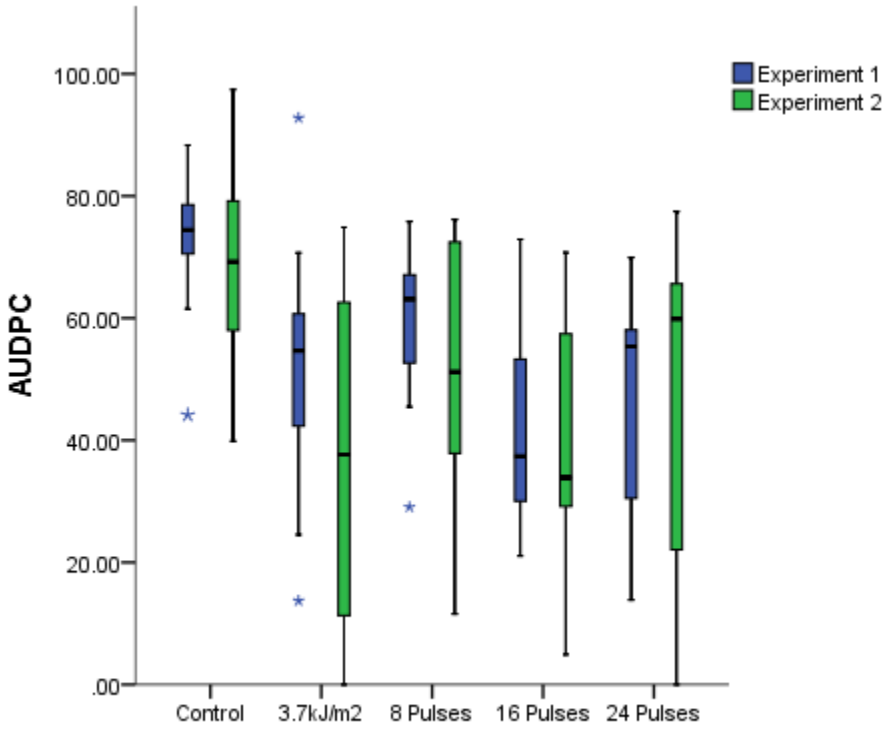

Figure 6. Area underneath the disease progression curve (AUDPC) from the independent experimental replicates from mature green fruit cv. Mecano treated with a conventional low intensity UV-C source and a high intensity pulsed polychromatic light source. Inoculations were performed with *B. cinerea* at 10 d post treatment; n = 15. Box plots show the distribution of data from the independent experimental replicates. Asterisks represent observed outliers.

To build upon the data showing that the induced delay in ripening following treatment was a local response, Figure 3, fruit were treated from a single side and then inoculated either at the directly exposed or unexposed side with *B. cinerea.* This was performed to assess whether induced resistance was also a local response. Inoculations were performed following the methods as described for the previous investigation.

Inoculations performed on unexposed tissue showed no reduction in disease progression and AUDPC levels similar to that of the control, Figure 7. Inoculations performed at directly exposed tissue, however, were significantly smaller than that of both the control and unexposed tissue. Again, no differences were observed for the effects of treatment between the independent replicate experiments, Figure 8. The second experimental replicate, however, showed slightly lower levels of disease progression for all treatment groups.

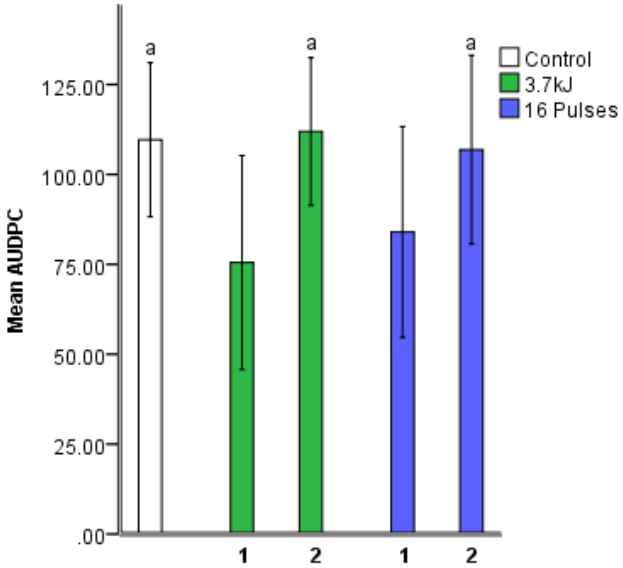

Figure 7. Area underneath the disease progression curve (AUDPC) of tomatoes, cv. Mecano, treated on a single side and inoculated with *B. cinerea* at 10 d post treatment (DPT). Fruit were treated with an established low intensity UV-C treatment of 3.7 kJ/m^2 and a high intensity pulsed polychromatic treatment of 16 pulses. Exposed tissue (1) or unexposed (2). Error bars show ± 1 standard deviation; n = 20. Labelling indicates statistical significance. Means sharing the same superscript are not significantly different from each other at p< 0.05. (Scott *et al.,* 2016)

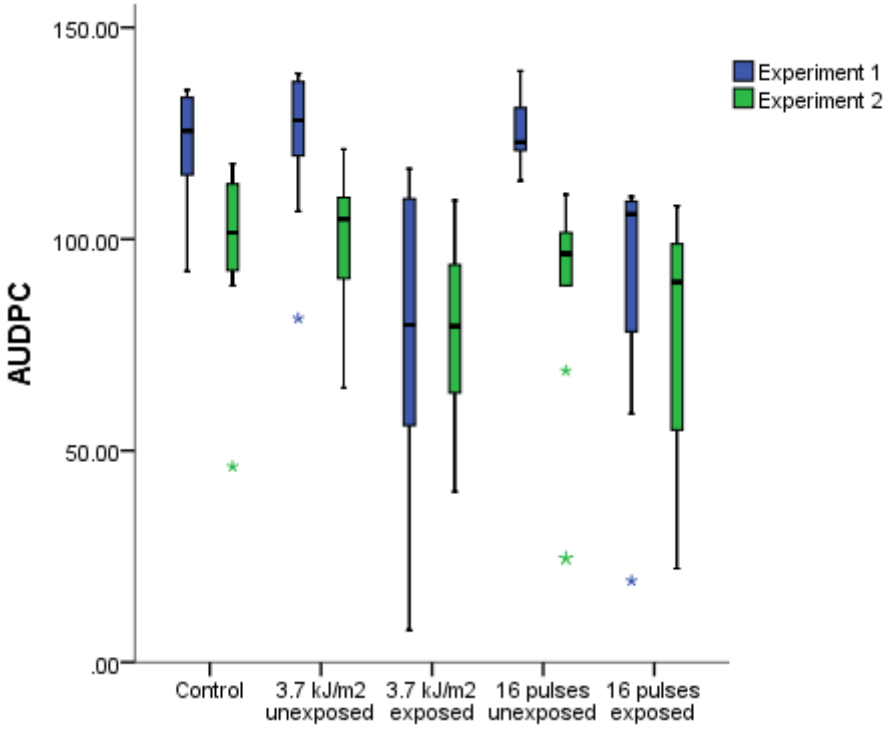

Figure 8. Area underneath the disease progression curve (AUDPC) of tomatoes, cv. Mecano, treated on a single side and inoculated with *B. cinerea* at 10 d post treatment (DPT). Fruit were treated with an established low intensity UV-C treatment of 3.7 kJ/m^2 and a high intensity pulsed polychromatic 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.

As previously discussed the majority of postharvest research on tomato fruit has been carried out on mature green fruit (Stevens *et al.,* 1998, Charles *et al.,* 2008 & Charles *et al.,* 2009). This, however, is not relevant for the UK tomato industry who almost solely harvest their fruit at the ripe stage due to improved consumer qualities. We, therefore, investigated the effect of conventional and pulsed treatments on ripe fruit and their ability to induce resistance against *B. cinerea*.

Fruit treated with both the established 3.7 kJ/m^2 conventional treatment and 8 pulses showed a slight decreases in disease progression, table 3. They, however, were not significant. Fruit treated with 16 and 24 pulses, however, reduced disease progression by 28.54 and 28.15 %, respectively, both of which were significant decreases. Independent experimental replicates showed no variation in the observed effect of treatment, Figure 9. The AUDPC, however, from the second replicate showed slightly lower values for all of the treatment groups tested.

Table 3. 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 = 30. (Scott *et al.,* 2016)

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

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

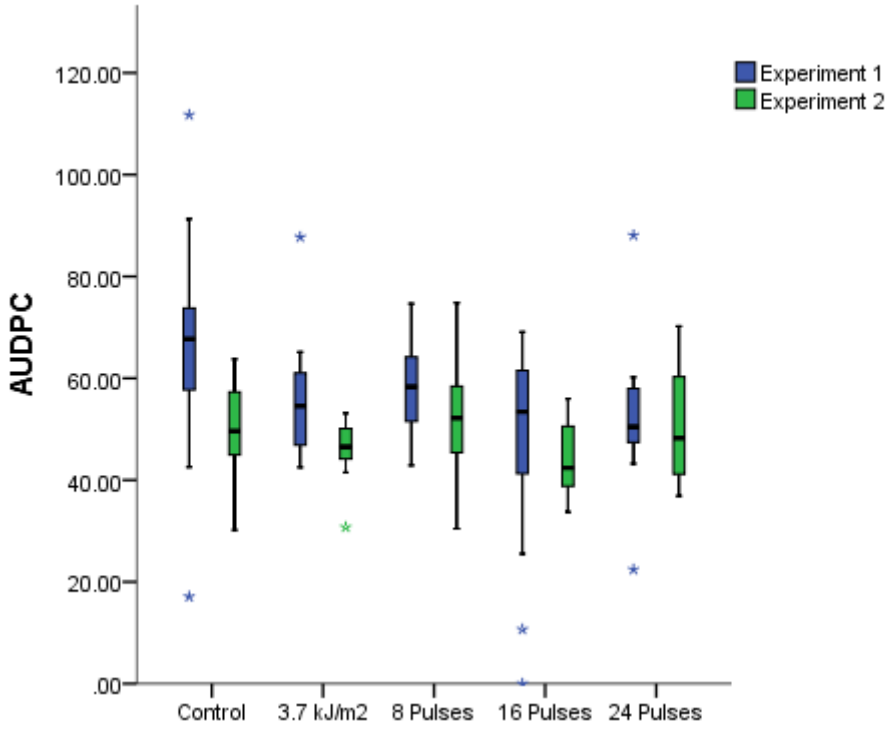

Figure 9. Area Underneath the Disease Progression Curve (AUDPC) for ripe fruit cv. Mecano treated with a conventional low intensity UV-C source with and a high intensity pulsed polychromatic light source, followed by inoculation with *B. cinerea* at 10 d post treatment; n = 15. Box plots show the distribution of data from the independent experimental replicates. Asterisks represent observed outliers.

Discussion

The commercial integration of hormetic UV-C treatments has been unfeasible, in part, due to the lengthy exposure times necessitated to successfully induce hormesis. The established UV-C treatment of 3.7 kJ/m^2 takes over six minutes. We have, shown here that a 16 pulse treatment of high intensity pulsed polychromatic light can induce both delayed ripening and disease resistance against *B. cinerea* to similar levels as that observed for conventional UV-C treatment. A 50.1% reduction in the progression of ripening was observed for the 16 pulse treatment while the 3.7 kJ/m^2 delayed ripening by 43.1 %. Moreover, *B. cinerea* disease progression was reduced by 41.7 % with the pulsed source similar to that of the conventional source at 38.1 %. The 16 pulse treatment can be achieved in a total of 10 seconds, a 97.3 % reduction in treatment time. Furthermore, we have shown here that delayed ripening and disease resistance are local responses for both the pulsed and conventional sources and that changing the orientation of fruit treatment dose not elicit a systemic response for delayed ripening. Finally, we have shown that a 16 pulse treatment successfully induces resistance against *B. cinerea* on ripe fruit, with a 28.54 % reduction, whereas the 3.7 kJ/m^2 conventional treatment only slightly reduces disease progression at 13.43 %.

Objective 2 - Pre-harvest Foliar UV-C and Pulsed Polychromatic Treatment Treatments of Tomato

Introduction

To date the majority of laboratory experiments on the induction of UV-C hormesis have been focused on preventing post-harvest spoilage of fruit. Post-harvest UV-C hormesis has shown vast potential applications for fruits with beneficial effects from reduced chilling injury and chlorophyll degradation to delayed ripening and disease resistance. The commercial application of such treatments have, however, been prevented due to the long exposure times - typically of the order of several minutes. In Objective 1 the use of a high intensity pulsed polychromatic source was validated for use in the induction of hormesis which, for delayed ripening and disease resistance on tomato, can reduce treatment time by 97.3 %.

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

For *Diplodia pinea,* the causative agent of dieback on *Pinus radiata* incidence and susceptibility was also shown to be reduced following UV-C treatment at a dose of 1.2 kJ/m^2 (Reglinski *et al.,* 2013). Single treatments were performed either 1, 3 or 6 weeks before inoculation with treatment 1 week before inoculation showing the greatest resistance. Multiple treatments at 6, 3 and 1 week before inoculation showed the greatest reduction in disease incidence and severity. The application of pre-harvest UV hormesis through foliar treatments has, however, with the exception of Reglinski *et al.,* 2013 not been explored with horticultural relevance. Pre-harvest UV-C induced resistance has, however, been shown to induce disease resistance and systemic delayed ripening through the treatment of fruit on the truss (Obande *et al.*, 2011).

The aim here is to explore the use of conventional and pulsed UV-C sources as inducers of disease resistance through the foliar treatments of tomato. Initially the point of visible damage will be determined. Where visible damage is not evident, treatments will be assessed for disease control against a number of diseases including the pathogens *Oidium neolycopersici* and tomato spotted wilt virus. Fungal pathogens will be used for initial resistance assays.

Following initial observations of resistance the length and periods of optimal resistance will be explored. This will then be used to determine optimal treatment plans and the applicability of the respective sources within a horticultural setting.

Materials and Methods

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

Results

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

Damage was manifested in the form of generalised wilting of the foliage for larger doses, see Figure 10A, and slight curling of the leaves for treatments of 1 and 0.5 kJ/m^2, Figure 10B. Mature leaves tended to be more prone to damage. The younger leaves, however, showed hyperplasia-like symptoms and a glossy appearance to their surface. Furthermore, the stem also showed a glossy-like appearance and damage to the trichromes, Figure 10C. All damage appeared to be of a highly directional nature with the most severe damage developing closest to the source and diminishing drastically across the plant, Figure 10A.

Table 4. Summary of the observed damage on tomato plants of the cv. Shirley at both 4 – 5 and 7 - 10 leaf stage at 2 days post treatment with the low pressure conventional mercury UVC source.

Treatment (kJ/m^2)	0.1	0.5	1	2	3	4	5
4-5 leaf	N/T	N/T	+	+	+	+	+
7-10 leaf	-	+	+	+	+	+	+

N/T denotes treatments were not tested. + Indicates damage was observed. – Indicates no damage was observed.

Table 5. Summary of the observed damage on tomato plants of the cv. Shirley at 7 -10 leaf stage at 2 DPT with the high intensity pulsed UV source.

No. pulses	5	10	15	20	25	30	35	40	45
Damage	-	-	-	+	+	+	+	+	+

+ Indicates damage was observed. – Indicates no damage was observed.

Figure 10. Damage induced by over exposure to UV sources on tomato plants cv. Shirley. **A)** The influence of source positioning on damage elicited to the plant and example of heavy damage. Red arrow indicates the side of the plant closest to the source. **B)** The mild leaf curling symptoms that develop at the lower exposure treatments that cause damage. **C)** Two sides of a treated plants stem. The top side was facing the source and shows distortion of trichromes on the stem and "shiny" appearance. The bottom stem was facing away from the source.

Initial resistance assays for *Oidium neolycopersici* the causative agent of powdery mildew indicate that in the initial stages of infection that the percentage of leaf covered by sporulating fungi is reduced following treatment. The disease severity index used, however, was not appropriate for determining disease burden at the latter stages of disease, Figure 11. It can be seen that at 11 days post inoculation that both pulsed and conventionally treated plants showed a decreased disease score. At 14 days post inoculation, however, little difference

was seen. This may be as the severity index used in the preliminary investigations did not take the spore density into account. From visual inspection the untreated plants appeared to have a higher density of spores on the leaf surface.

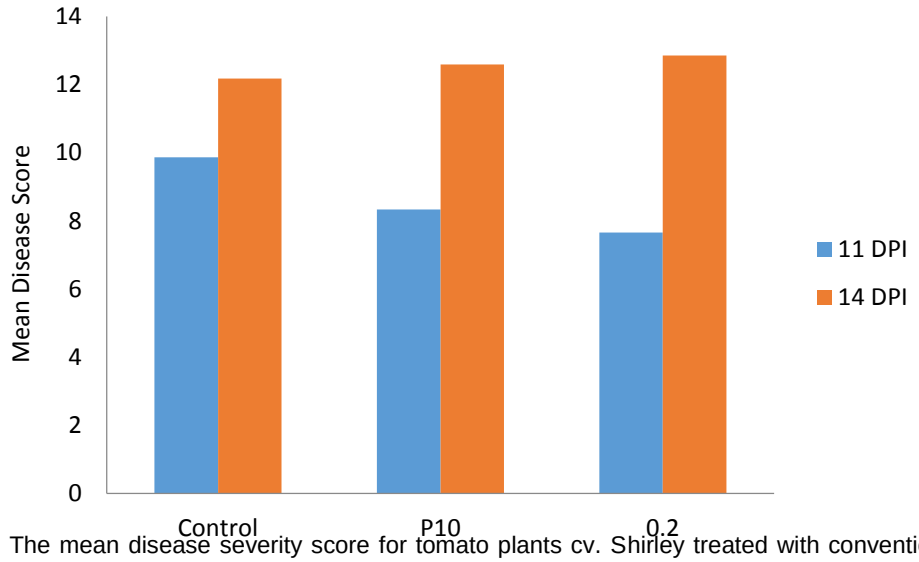

Figure 11. The mean disease severity score for tomato plants cv. Shirley treated with conventional and pulsed polychromatic UV-C sources. Plant scores were taken at 11 and 14 days post inoculation.

Discussion

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

Initial resistance assays for the pathogen *Oidium neolycopersici* have shown good promise. Further disease severity indexes, however, need to be tested and potentially a Q-PCR assay should be undertaken. The study will be extended by looking at resistance against the viral pathogen Tomato Spotted Wilt Virus and replicated on a second cultivar, moneymaker. Moneymaker was chosen due to its previous use as a commercial cultivar and similar physiology to those cvs currently being used without the broad range of pathogen resistance observed for modern commercial cultivars

Objective 3 – Pre-harvest Foliar UV-C and Pulsed Polychromatic Treatment of Lettuce

Introduction

Until recently the focus of UV-C research on lettuce has been twofold. Postharvest applications for extension of shelf life and surface decontamination of minimally processed lettuce and pre-harvest research into the effects of restoring natural UV levels through the use of UV-permeable housing for crops grown under protection (Allende & Artes, 2003, Allende *et al.,* 2006, Tsormpatsidis *et al.,* 2008). The former was mainly concerned with Enterobacteria associated with human pathology but did show a reduction in *Erwinia carotovora* a soft rot causing phytopathogen (Allende *et al.,* 2006). The results, however, do not mitigate the direct germicidal effects of 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 biomass, changes in colouration and a reduced incidence of diseases caused by *Bremia lactucae* and *B. cinerea* (Paul *et al.,* 2012, Wargent *et al.,* 2005). Park *et al.,* 2007 treated lettuce with 1.65 kJ/m^2 of UV-B per day for 10 days and observed that an increase in red colouration correlated with accumulation of anthocyanins.

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 (days post inoculation), respectively. One would expect the application doses shown to be similar for both pre and post-harvest treatments as it is the foliage undergoing treatment for both.

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

Materials and Methods

Experimental Design and Statistical Analysis

All data presented in this chapter, apart from those concerning multiple treatments, was collected from single experiments and can therefore be considered preliminary. Between 3 and 7 biological replicates were used per treatment group with 2-4 technical replicates for experiments assessing disease resistance. Detailed experimental design can be found with each individual experiment.

Data analysis was performed using SPSS 22 (IBM). One-way ANOVA with Tukey's post-hoc testing was performed to assess the differences in means. Where the homogeneity of variances could not be met Welch's robust ANOVA was performed followed by the Games-Howell post-hoc testing. Statistical significance is here defined as $p \leq 0.05$.

Lettuce propagation

Lettuce were germinated in rockwool propagation cubes until emergence of roots from the propagation cubes and then transferred to an NFT system under natural light conditions. Day and night temperatures were 12 - 14 °C and 2 - 6 °C, respectively. Vents were opened above 10 °C in the day and 4 °C during the evening. During the winter months LED assimilation lighting was used to extend the growing period to 16 hours.

UV-C and Polychromatic Light Treatments

Lettuce of the commercial butterhead varieties Amica and Temira (Enza Zaden) were subjected to treatment with both pulsed polychromatic light and conventional low-pressure mercury UV-C sources. Pulsed treatments were delivered from 40 cm and conventional treatments were delivered at either 2000 $\mu W/cm^2$ or 1000 $\mu W/cm^2$ from directly above the lettuce plant. Treatments were performed at both 3-5 and 6-8 true leaf stage and early, mid and late head formation. Further information on the pulsed source can be found in the materials and methods section of Objective 1.

Damage Assessments Following Light Treatment

Damage to the lettuce plants was visually inspected at both/either 2 DPT and 5 DPT and recorded qualitatively as simply the presence or absence of damage, examples of which can be seen in Figures 12 and 13.

Figure 12: A lettuce, cv. Amica, at early head formation treated with 75 pulses of high intensity pulsed polychromatic light and exhibiting severe damage to its mature leaves which is manifested as dry brown lesions.

Figure 13: A lettuce from a plant at early head formation treated with 45 pulses showing veins with a yellow/brown hue as a symptom of mild damage caused after UV treatment with the high intensity pulsed source.

Disease Resistance Assay

Following treatment, a leaf disc bioassay based on the method of Laboh, 2009, was used. Briefly, 20 mm leaf discs were cut with a cork borer and placed into 120 mm square petri dishes with up to a maximum of 16 leaf discs per plate. Prior to this the plates were filled with 25 ml of molecular grade agar to prevent leaf drying and humidity for pathogen growth. Leaf discs were then inoculated with either *B. cinerea*, *Rhizoctonia solani* or *Sclerotinia sclerotiorum*. At 2 and 3 DPI photos of the leaf discs were taken and analysed in Image J allowing calculation of the diseased area in mm^2 and the area under the disease progression curve as discussed in Objective 1. Example images taken at 2 and 3 DPI for all of the pathogens can be seen in Figure 14.

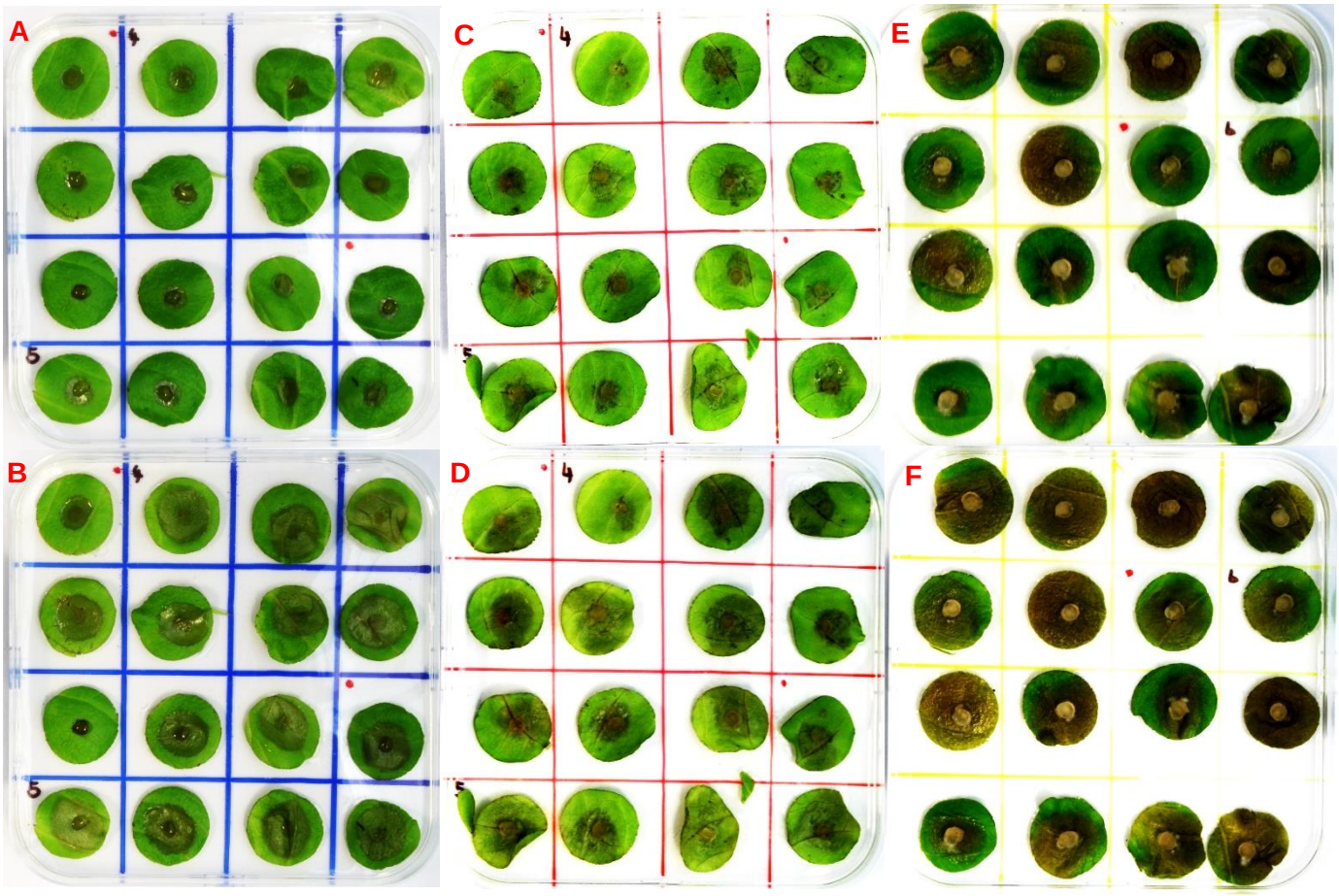

Figure 14. Examples of the leaf disc bioassay adapted from Laboh, (2009). Images show *Botrytis cinerea* spore solution inoculations at 2 days post inoculation (DPI) (**A**) and 3 DPI (**B**). *Rhizoctonia solani* inoculations with 4mm agar plugs at 2 DPI (**C**) and 3 DPI (**D**). *Sclerotinia sclerotiorum* inoculations with 4mm agar plugs at 2 DPI (**E**) and 2 DPI (**F**).

Pathogen Propagation and Inoculum Preparation

For *B. cinerea* inoculations 10 µl of a calibrated spore solution at $1x10^6$, made from 10-14 day old cultures and amended with 50 % PDB, was pipetted into the centre of the leaf disc. For *R. solani* and *S. sclerotiorum* inoculations were performed with 4 mm agar plugs from 3 day old cultures and placed on the underside and top of the leaf, respectively. All cultures were grown at room temperature.

Results

Damage Across the Growing Season

Damage on lettuce following both UV-C and high intensity pulsed polychromatic treatments was observed as dry brown lesions and vascular discolouration, see Figures 12 and 13. Damage susceptibility towards both the conventional UV-C source and high intensity pulsed polychromatic sources varied throughout the year on the cv. Amica. It is unclear what influences the damage susceptibility but it may be the hours and intensity of daylight or other environmental factors, Figures 15, 16, 17, and 18.

For example, damage was caused above 60 pulses in March which reduced to 45 pulses in April, Figures 15 and 16. Damage susceptibility increased dramatically from April, the beginning of the commercial growing season towards October; the latter stages of the season. For pulsed treatments this dropped from a threshold of 60 to 16 pulses, respectively. For conventional UV-C treatments damage was observed at and above treatments of 2.25 kJ/m^2 in April which dropped to below 0.35 kJ/m^2 in October, Figures 15 and 17.

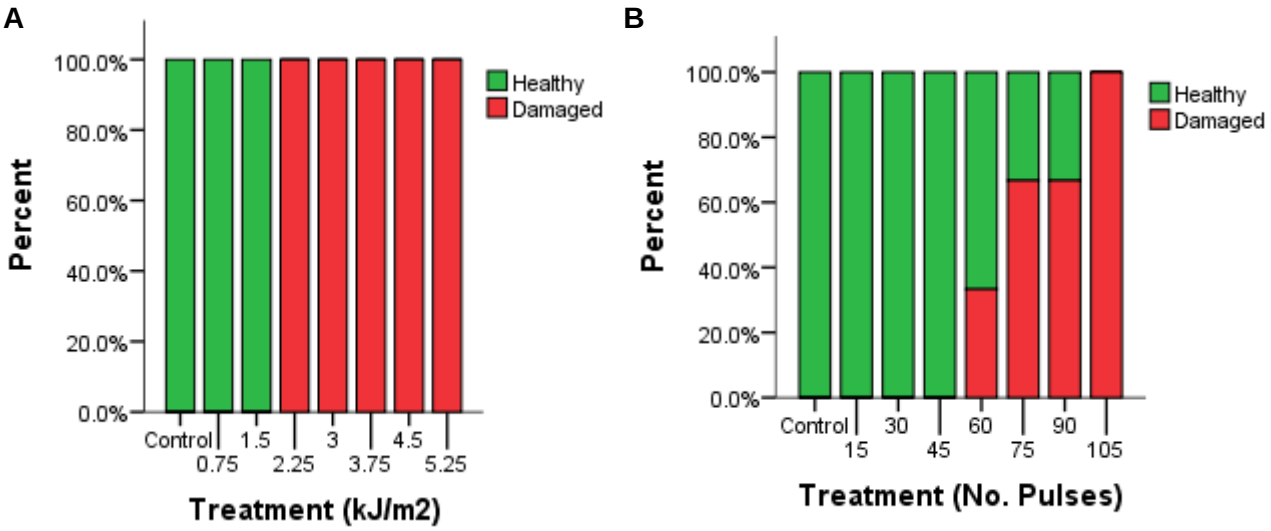

Figure 15. The percentage of healthy and damaged plants per treatment group for the lettuce variety Amica treated during March 2015. Plants were either treated with a conventional low pressure mercury UV-C source (**A**) or a high intensity pulsed polychromatic source (**B**), n=3.

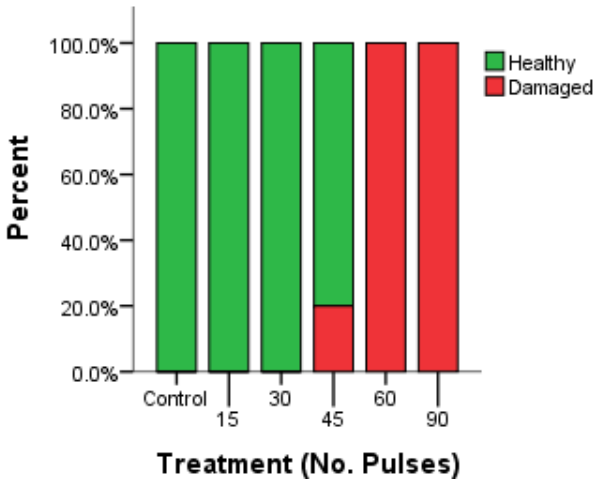

Figure 16. The percentage of healthy and damaged plants per treatment group for the lettuce variety Amica treated during April 2015. Plants were either treated with a high intensity pulsed polychromatic source (**B**), n=5.

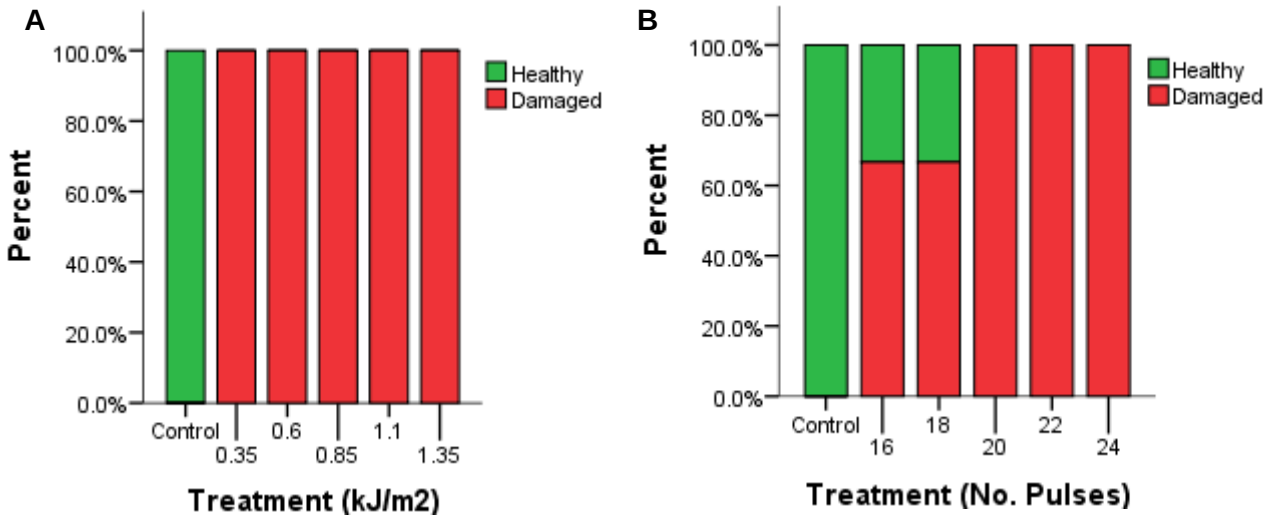

Figure 17. The percentage of healthy and damaged plants per treatment group for the lettuce variety Amica treated during October 2015. Plants were either treated with a conventional low pressure mercury UV-C source (**A**) or a high intensity pulsed polychromatic source (**B**), n=3.

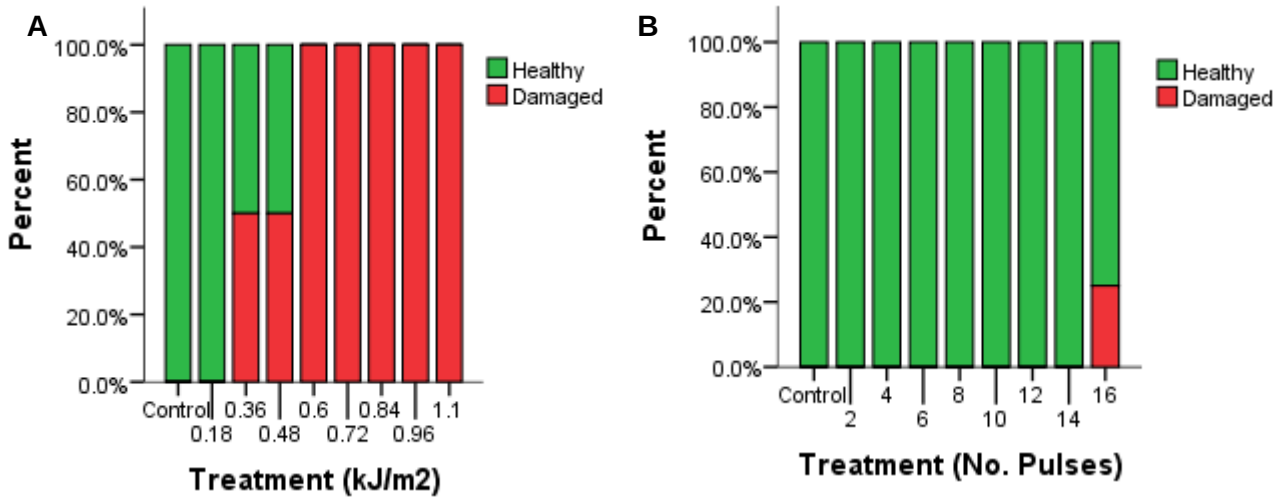

Figure 18. The percentage of healthy and damaged plants per treatment group for the lettuce variety Amica treated during November 2015. Plants were either treated with a conventional low pressure mercury UV-C source (**A**) or a high intensity pulsed polychromatic source (**B**), n=4.

Disease Resistance Assays

During the periods that damage was being assessed preliminary disease resistance assays were also being carried out. The first trials began in April 2015 and were utilising the butterhead variety Amica, plants were treated at early head formation with the high intensity pulsed polychromatic source only; n = 5. The treatment range was 15-45 pulses in 15 pulse increments. At 9 DPI plants were removed from the glasshouse and placed into a growth chamber at 21 °C. Agar plugs from 5 day old *B. cinerea* plates were placed on 3 leaves at either the top, middle or bottom of the plant. Lesions were measured at 3 DPI with Vernier callipers.

A 15 pulse treatment showed the smallest mean lesion length at 19.17 (Figure 19A) The largest lesions from plants treated with 15 pulses were, however, greater than those of the control (Figure 19B). Measuring lesions accurately with this method, however, was difficult due to leaf topology. Due to the problems with measuring lesion length accurately a leaf disc based bioassay was adapted from Laboh (2009). Furthermore, a spore solution based inoculation technique was adopted due to inter-plate variation observed when inoculating with agar plugs.

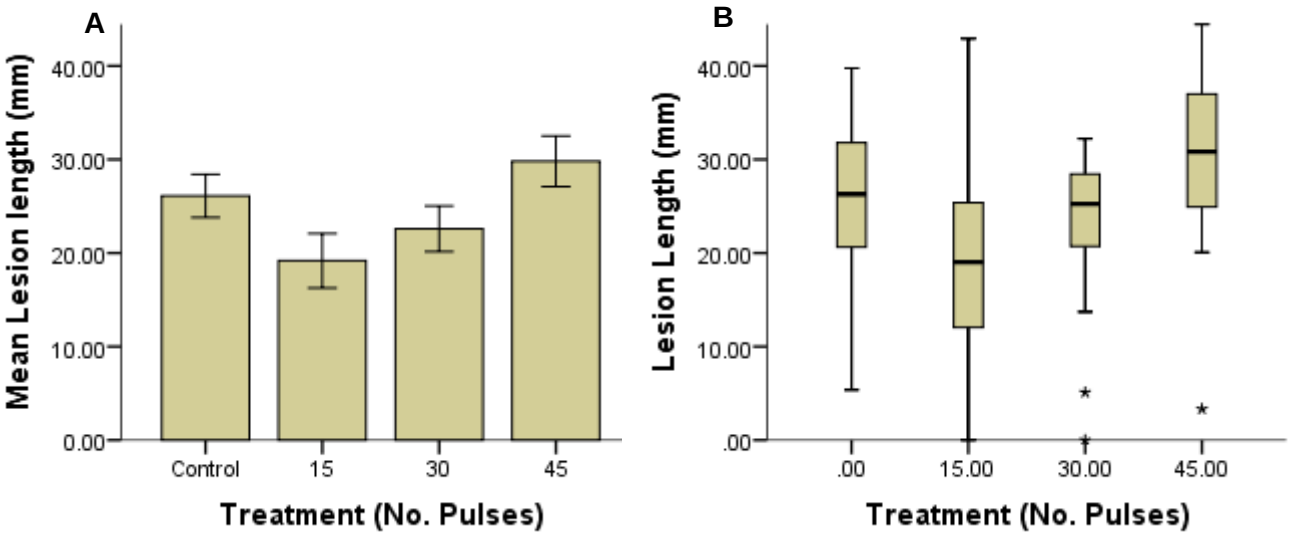

Figure 19. Disease resistance assays performed in April 2015 (14.04.2015) on the butterhead variety Amica. Plants were treated at early head formation and inoculated 9 days after treatment with 4mm agar plugs from 5 day old *B.cinerea* cultures. Lesion lengths were measured at 3 days after inoculation 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.

The next round of resistance assays were performed in May 2015. These assays utilised the leaf disc bioassay mentioned previously. Only the high intensity pulsed polychromatic source was used for this trial. Treatments from 10 to 25 pulses in 5 pulse increments were used. The maximum lesion diameter, here, was measured with Vernier callipers at 2 DPI and 3 DPI to allow the calculation of AUDPC. Measurements were not recorded after 3 DPI as the whole leaf disc was destroyed after this point. These trials were performed for both the varieties Amica and Temira at early head formation. Leaf discs were inoculated at 6 DPT with 10 µl of *B. cinerea* spores at a concentration of $1x10^6$ spores/ml amended with 50 % potato dextrose broth (Sigma-Aldrich). Five biological replicates were used with 3 technical replicates from each leaf.

For Temira the treatment showing the lowest AUDPC was the 10 pulse treatment with a mean of 17.4 and an 11 % reduction in disease progression, Figure 20A. All of the treatment groups, however, showed maximum lesion diameters greater than that of the control. This was also the case with the minimum AUDPCs as all treatment groups showed smaller minimum values to that of the control group, Figure 20B.

For the cv. Amica all treatment groups showed a lower mean AUDPC when compared to the control. The treatment showing the lowest AUDPC was the 20 pulse treatment giving an 8 % decrease in mean AUDPC, Figure 21A. Again, the maximum lesion sizes for all but the 20 pulse treatment were greater than that of the control, Figure 21B. Minimum lesion sizes were also smaller for each of the treatment groups than for the control.

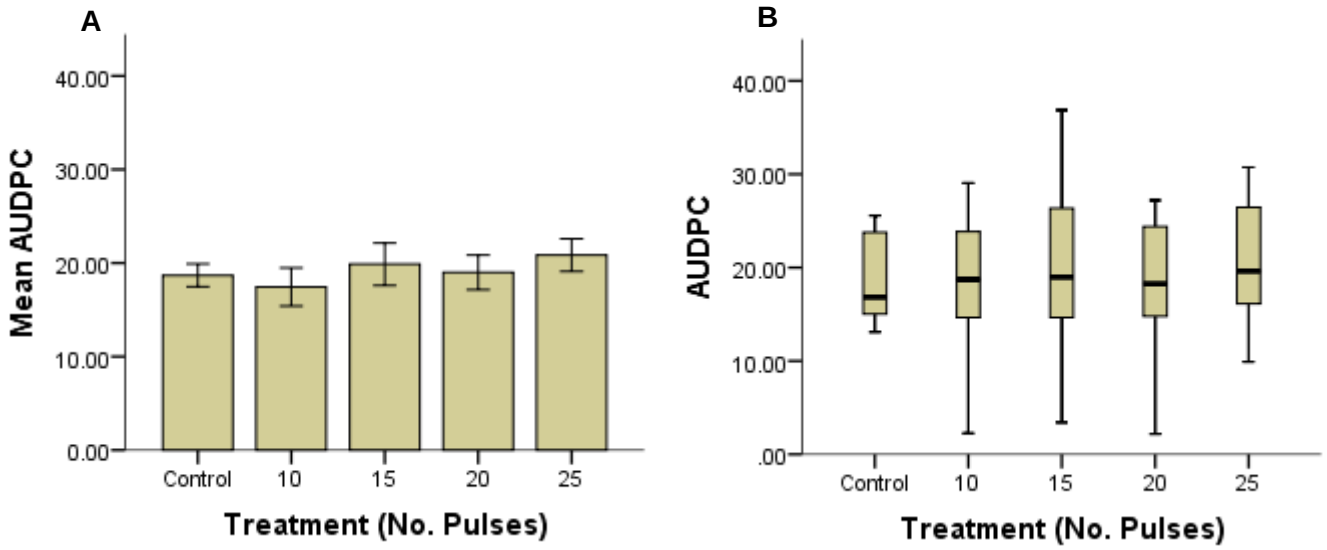

Figure 20. Disease resistance assays performed in May 2015 (05.05.2015) on the butterhead variety Temira. Plants were treated at early head formation and inoculated with *B. cinerea* 6 days after treatment with 10 µl of $1x10^6$ spores/ml amended with 50 % potato dextrose broth. Lesion lengths were measured 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.

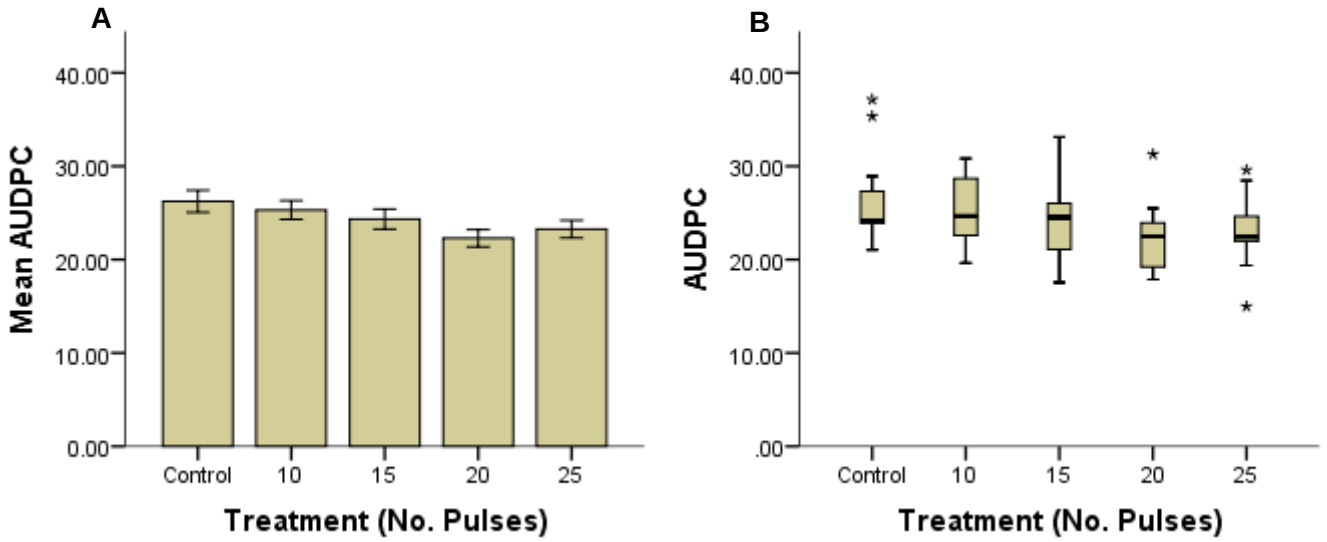

Figure 21. Disease resistance assays performed in May 2015 (05.05.2015) on the butterhead variety Amica. Plants were treated at early head formation and inoculated with *B. cinerea* 6 days after treatment with 10 µl of $1x10^6$ spores/ml amended with 50 % potato dextrose broth. Lesion lengths were measured 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.

The following resistance assay trials were performed in September 2015 with *B.* cinerea on the variety Amica and Temira and utilised 6 biological replicates and 3 technical replicates. For Amica plants inoculations were performed as above and at either 5 or 8 DPT. For plants treated with the UV-C source the treatment showing the lowest mean AUDPC was 0.6 kJ/m^2 giving an 11.9 % decrease in disease progression, Figure 22A. The treatment showing the greatest decrease in AUDPC at 8 DPT, however, was 0.35 kJ/m^2 with a 16.71 % reduction. The 0.6 kJ/m^2 treatment was, however, the only treatment to show a reduction in mean AUDPC across both time points with a 3.1 % reduction at 8 DPT. If we look at the distribution of the AUDPC data we can see that a large number of outliers were observed and the data should therefore be interpreted with caution, Figure 22B. Furthermore, as observed with the previous experiments the maximum lesions size tended to increase for all treatment groups.

For Amica plants treated with the high intensity pulsed polychromatic source the treatment showing the lowest mean AUDPC was 22 pulses with a 18.5 % reduction in disease progression, Figure 23A. No treatments at 8 DPT showed any reduction in AUDPC in comparison to the control, Figure 23A. Furthermore, all showed an increase in disease progression with the 24 pulse treatment showing a 37.7 % increase in disease progression. Again the box plots show a large number of outliers within the data set for both 5 DPT and 8 DPT inoculated plants, Figure 23 B.

Plants from the variety Temira utilised the same treatments, inoculation techniques and lesion measurement as those described for Amica. The plants, however, were inoculated at 3 and 5 DPT. For conventional UV-C treatments with inoculations at 3 DPT the treatment showing the lowest mean AUDPC was 1.1 kJ/m^2 with a 9.7 % reduction in AUDPC, Figure 24A. At 5 DPT, however the treatment with the lowest AUDPC was 0.35 kJ/m^2 with a 22.5 % reduction in AUDPC. The 1.1 kJ/m^2 treatment, however, was similar with a 22.3 % reduction in mean AUDPC, Figure 24A. The distribution of the data here still shows a large range but contains fewer outliers. Moreover, the maximum lesion sizes at 3 DPT are similar for the control with exception of the 0.35 and 0.85 kJ/m^2 treatments whose maximums are slightly higher. The medians of treatments above 0.85 kJ/m^2 at 3 DPT are lower than the control as are the smallest lesion sizes, Figure 24B. At 5 DPT all of the treatment groups medians fall below the minimum lesion size for the control.

Temira plants treated with the high intensity pulsed polychromatic source showed the smallest mean AUDPC with inoculations at 3 DPT. The 18 pulse treatment, here, showed a 7.3 % reduction in disease progression in comparison to the control, Figure 25A. For inoculations at 5 DPT the 16 pulse treatments showed the smallest mean AUDPC with a 23.5 % reduction. The 18 pulse treatment was the most successful over the two time points also showing a 6 % reduction at 5 DPT. The distribution of the data can be seen in Figure 25B.

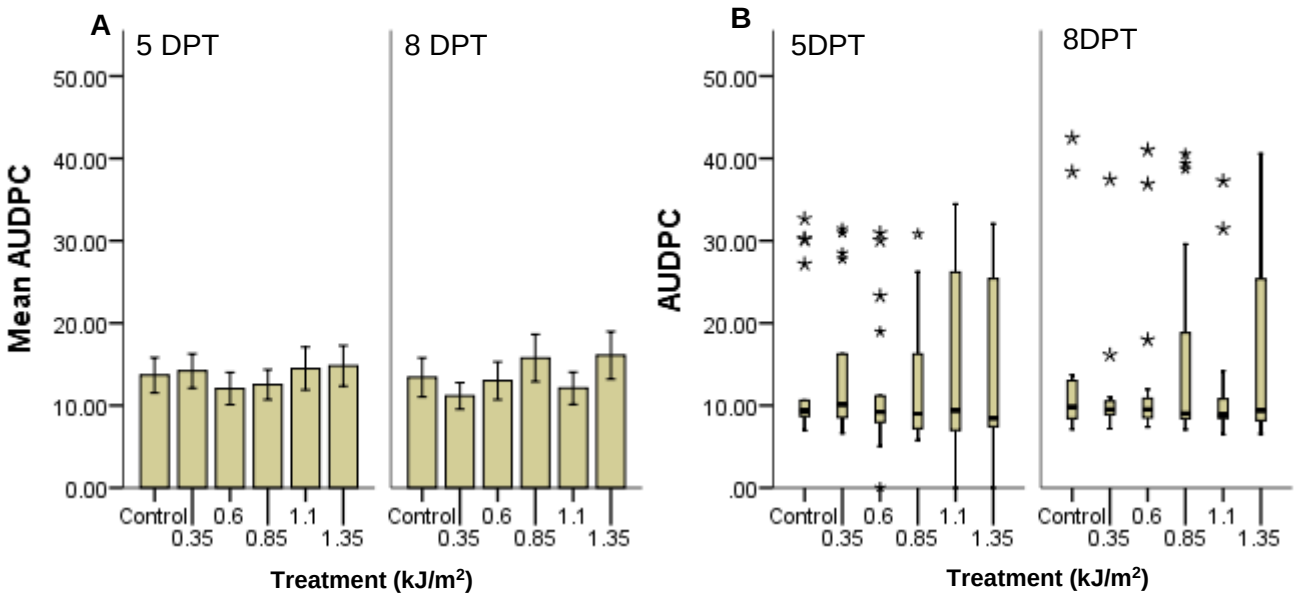

Figure 22. Disease resistance assays performed in September 2015 (10.09.2015) on the butterhead variety Amica treated with the conventional UV-C source. Plants were treated at 3-5 true leaves and inoculated 5 and 8 days with 10 µl of 1x10^6 spores/ml of *B. cinerea* amended with 50 % potato dextrose 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.

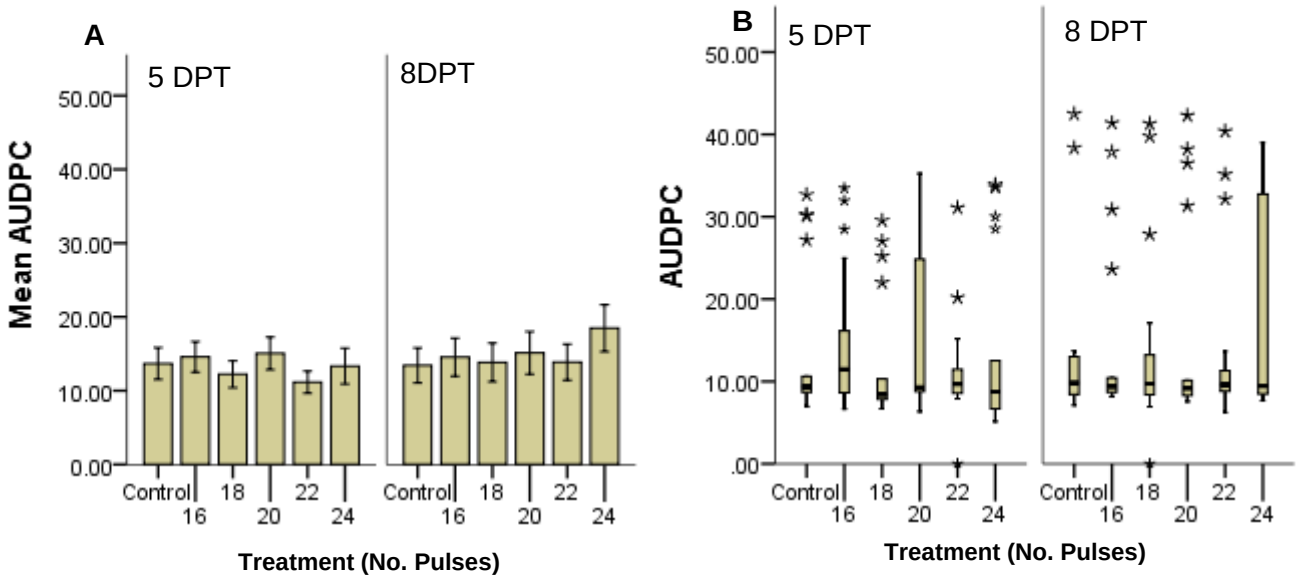

Figure 23. Disease resistance assays performed in September 2015 (10.09.2015) on the butterhead variety Amica treated with the high intensity pulsed polychromatic source. Plants were treated at 3-5 true leaves and inoculated 5 and 8 days after treatment with 10 µl of 1x10^6 spores/ml of *B. cinerea* 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.

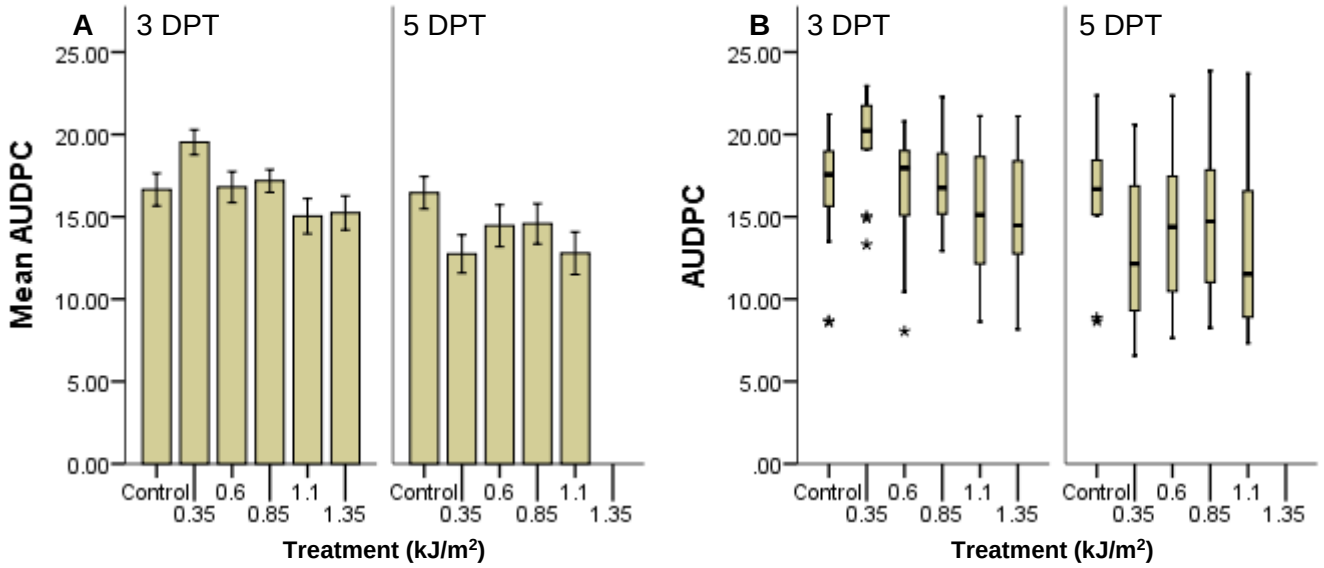

Figure 24. Disease resistance assays performed in September 2015 (29.09.2015) on the butterhead variety Temira treated with the conventional UV-C source. Plants were treated at 3-5 true leaves and inoculated 3 and 5 days after treatment with 10 µl of 1x10^6 spores/ml of *B. cinerea* amended with 50 % 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.

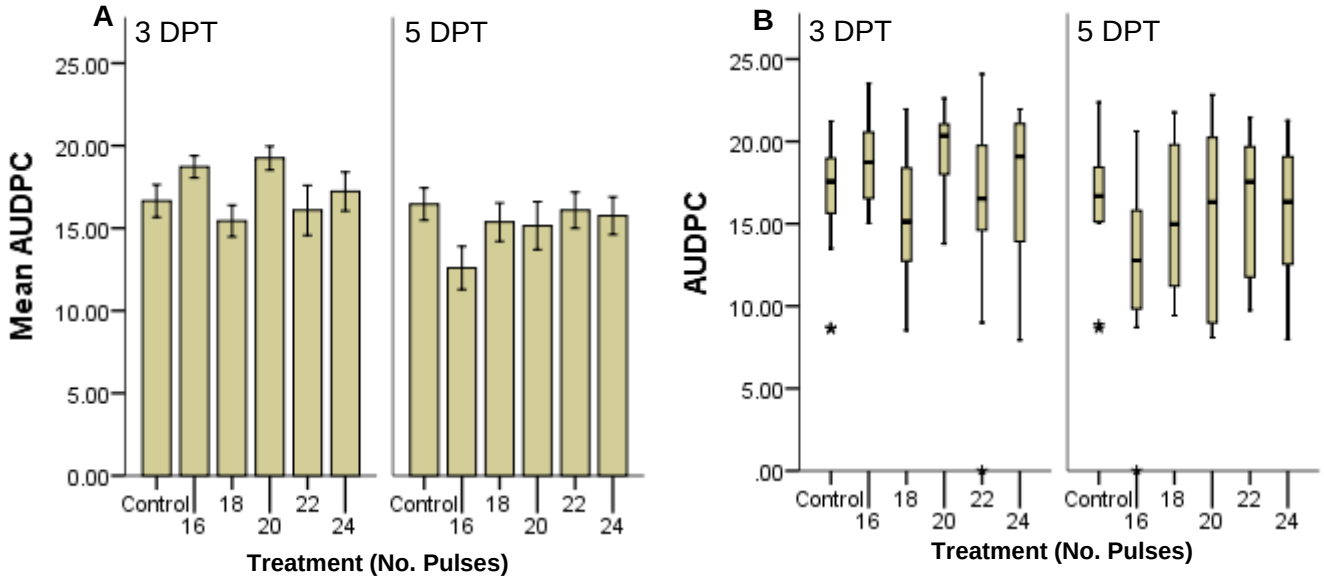

Figure 25. Disease resistance assays performed in September 2015 (29.09.2015) on the butterhead variety Temira treated with the high intensity pulsed polychromatic source. Plants were treated at 3-5 true leaves and inoculated 3 and 5 days after treatment with 10 µl of 1x10^6 spores/ml of *B. cinerea* 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.

It was noted while measuring maximum lesion diameters that the disease did not always progress equally creating lesions that were not always circular in shape. This could lead to the generation of inaccurate data and it was therefore decided that to increase accuracy and precision the image processing software ImageJ would be used to measure lesion area.

The next round of disease resistance trials were performed in October 2015 on both of the selected varieties. Inoculations were performed as in September with *B. cinerea.* For Temira only pulsed treatments were performed with inoculations at 2, 5 and 7 DPT. For inoculations at 2 DPT the treatment showing the lowest mean lesion area was 12 pulses giving a 51.9 % reduction in disease progression, Figure 26A. Inoculations performed at 5 DPT also showed the 12 pulse treatment gave the lowest level of disease progression with a 36 % reduction, Figure 27A. At 7 DPT, however, the treatments showing the lowest disease progression was the 14 pulse treatment with 26.0 % reduction. The 12 pulse treatment, however, did still show a good level of disease progression reduction in comparison to the control at 25.9 %, 26 A. At 2 DPT the 8 and 10 pulse treatments showed maximum lesions greater than that of the control, Figure 26B. For 5 and 7 DPT, however, the majority of maximum lesion sizes were either similar or lower than that of the control with medians generally lower than the control and the lower 50 % of the data positioned below that of the control, Figures 26 and 27B.

For Amica plants treated with both the conventional UV-C and high intensity pulsed polychromatic sources damage was observed for all of the treatments both pulsed polychromatic and conventional UV-C. The treatment ranges for the pulsed source were 16-24 pulses and 0.35-1.35 kJ/m^2 for the conventional UV-C treatments. The lowest two of the treatment ranges were still inoculated. These treatments however cannot be considered hormetic due to the fact that damage was caused to the plant. The 0.35 kJ/m^2 treatment showed a 9 % reduction in disease progression in comparison to the control and the 16 pulse treatment showed a 2.8 % reduction in AUDPC. Due to the observed damage on all of the treatments undergoing investigation the treatment range was lowered.

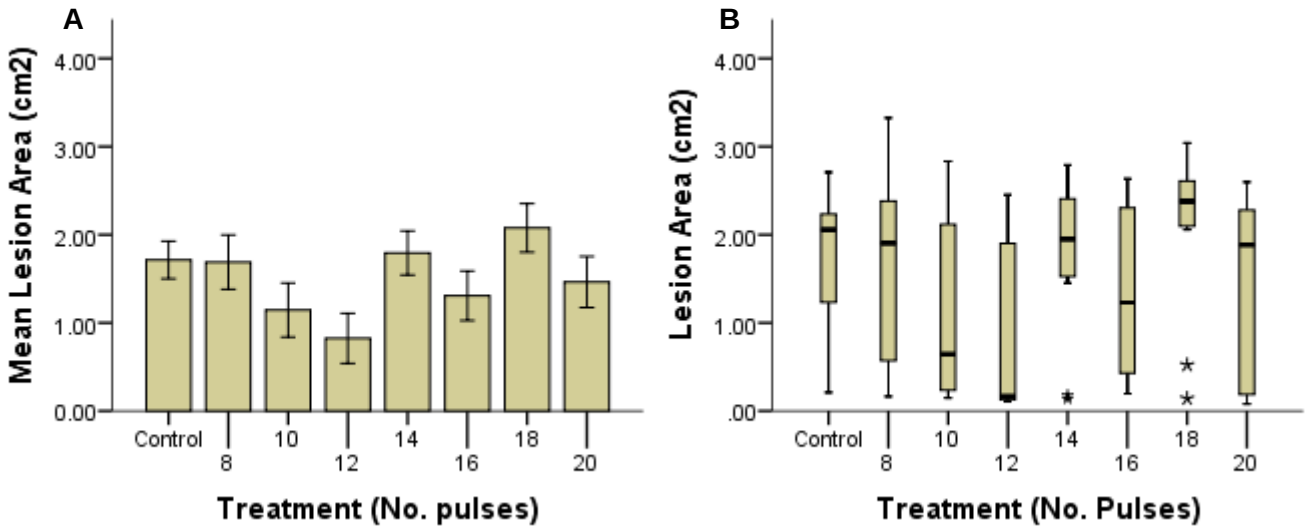

Figure 26. Disease resistance assays performed in October 2015 (28.10.2015) on the butterhead variety Temira treated with the high intensity pulsed polychromatic source. Plants were treated at late head formation and inoculated 2 days after treatment with 10 µl of $1x10^6$ spores/ml of *B. cinerea* amended 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.

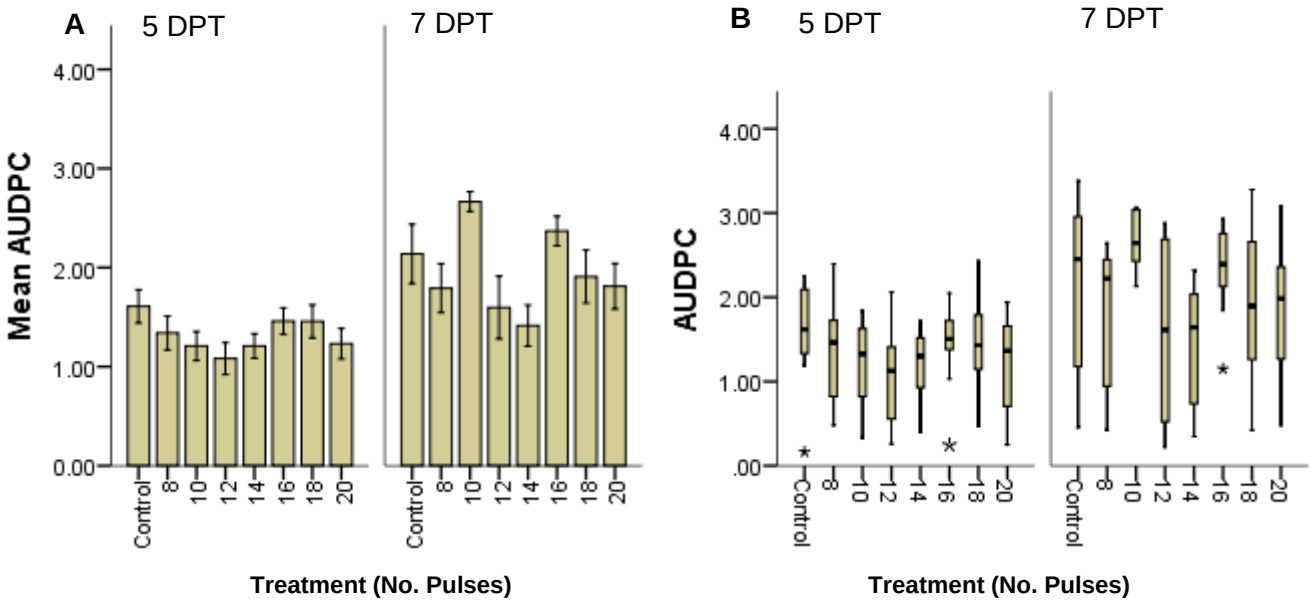

Figure 27. Disease resistance assays performed in October (28.10.2015) on the butterhead variety Temira treated with the high intensity pulsed polychromatic source. Plants were treated at late head formation and inoculated 5 and 7 days after treatment with 10 µl of $1x10^6$ spores/ml of *B. cinerea* 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.

The next trials to be carried out were during November 2015 and utilised only the variety Amica. The treatment ranges were 2-16 pulses and 0.18-1.1 kJ/m^2 for the pulsed polychromatic and conventional UV-C sources, respectively. Plants were treated at 3-5 true leaves and inoculated at 2 DPT with *B. cinerea* or 4mm agar plugs from 3 day old cultures of *Rhizoctonia solani*. Four biological repeats and four technical repeats per plant were used. Plants treated with the conventional UV-C source showed that treatments of 0.36 kJ/m^2 and above experienced damage. Plants treated with 0.18 kJ/m^2, however, showed no damage and a 21.8 % reduction in mean disease progression, Figures 28A. Maximum and median AUDPCs for the 0.18 kJ/m^2 were below that of the control (Figure 28B).Plants treated with 0.36 kJ/m^2 showed a 14.0 % reduction in disease progression for *R. solani,* Figure 30A. This treatment, however, cannot be considered as hormetic as damage was observed on 50 % of the plants treated, Figure 18.

Resistance assays utilising the pulsed polychromatic source used 5 biological repeats and 3 technical repeats. The treatment showing the greatest reduction in mean AUDPC was the 14 pulse treatment with a 21.7 % reduction in AUDPC for *B. cinerea*, Figure 29A. For resistance assays with *R. solani*, however, the 10 pulse treatment was most effective with a 17 % reduction in disease progression, Figure 31A.

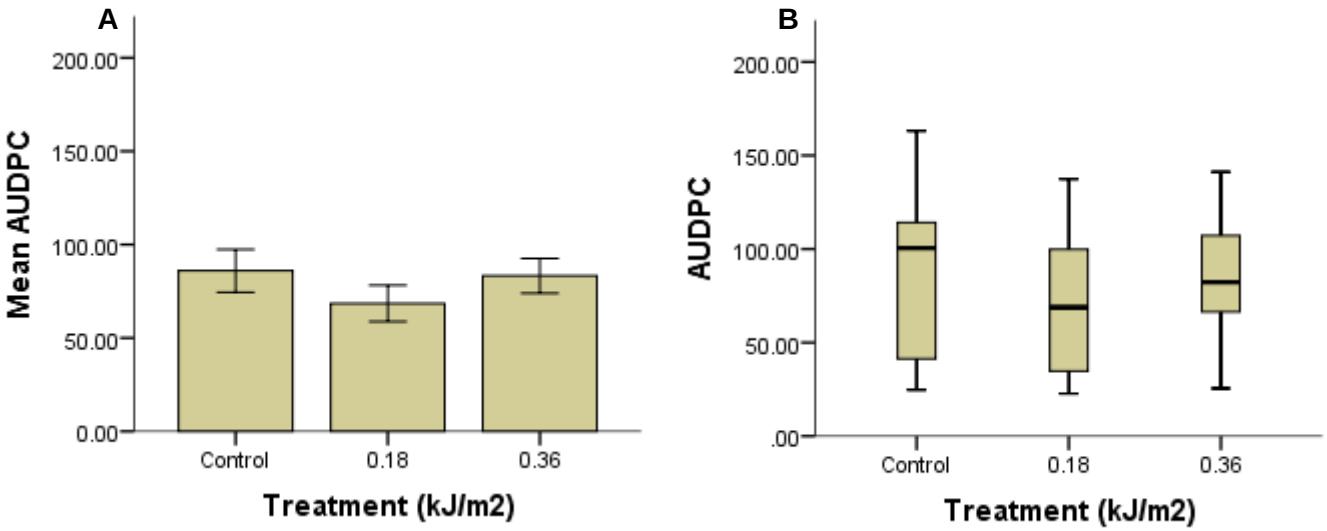

Figure 28. Disease resistance assays performed in November 2015 (18.11.2015) on the butterhead variety Amica treated with the conventional UV-C source. Plants were inoculated 2 days after treatment with 10 µl of 1x10^6 spores/ml of *B. cinerea* amended with 50 % potato dextrose broth. Lesion areas were 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.

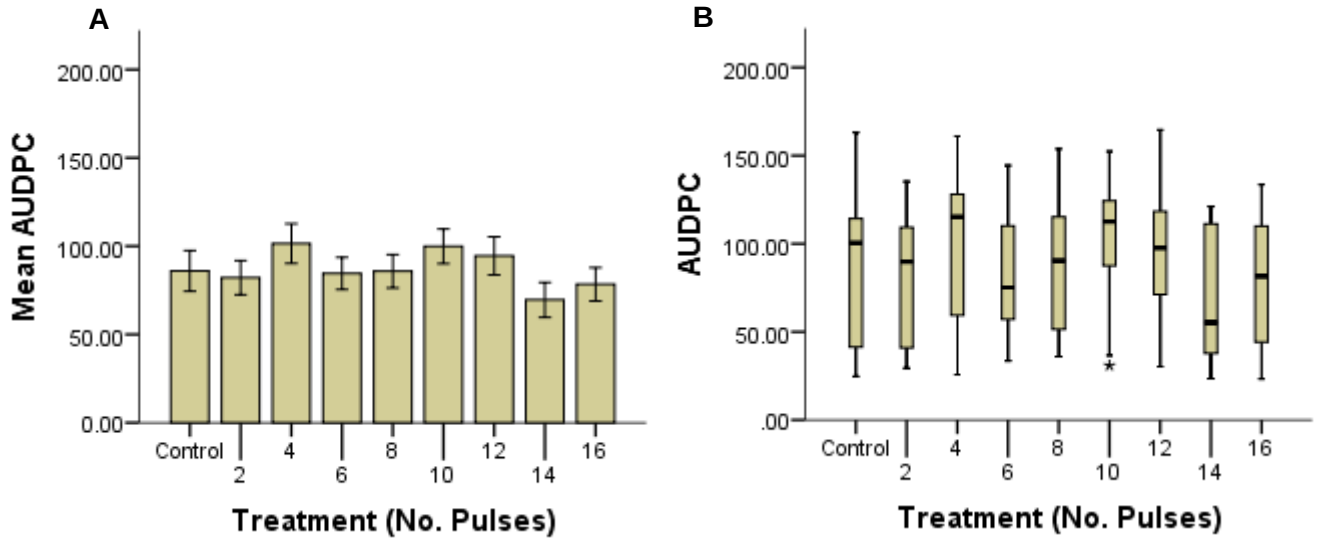

Figure 29. Disease resistance assays performed in November 2015 (18.11.2015) on the butterhead variety Amica treated with the pulsed polychromatic source. Plants were treated at 3-5 true leaves and inoculated 2 days after treatment with 10 µl of $1x10^6$ spores/ml of *B. cinerea* amended with 50 % potato 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

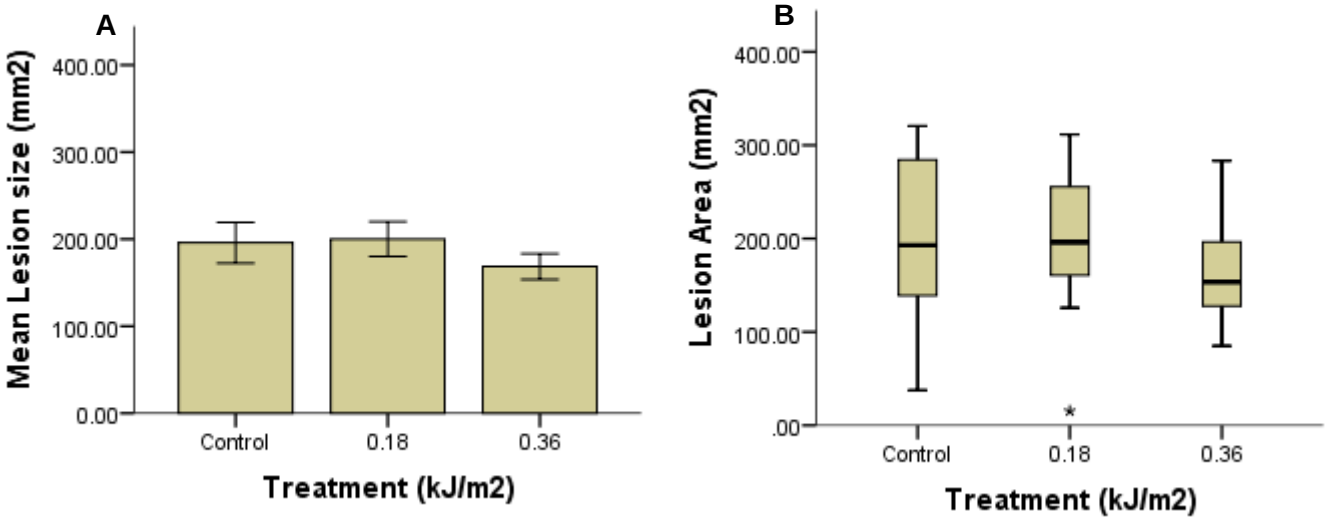

Figure 30. Disease resistance assays performed in November 2015 (18.11.2015) on the butterhead variety Amica treated with the conventional UV-C source. Plants were treated at 3-5 true leaves and inoculated 2 days after treatment with 4mm agar plugs from 3 day old *Rhizoctonia solani* cultures. Lesion 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

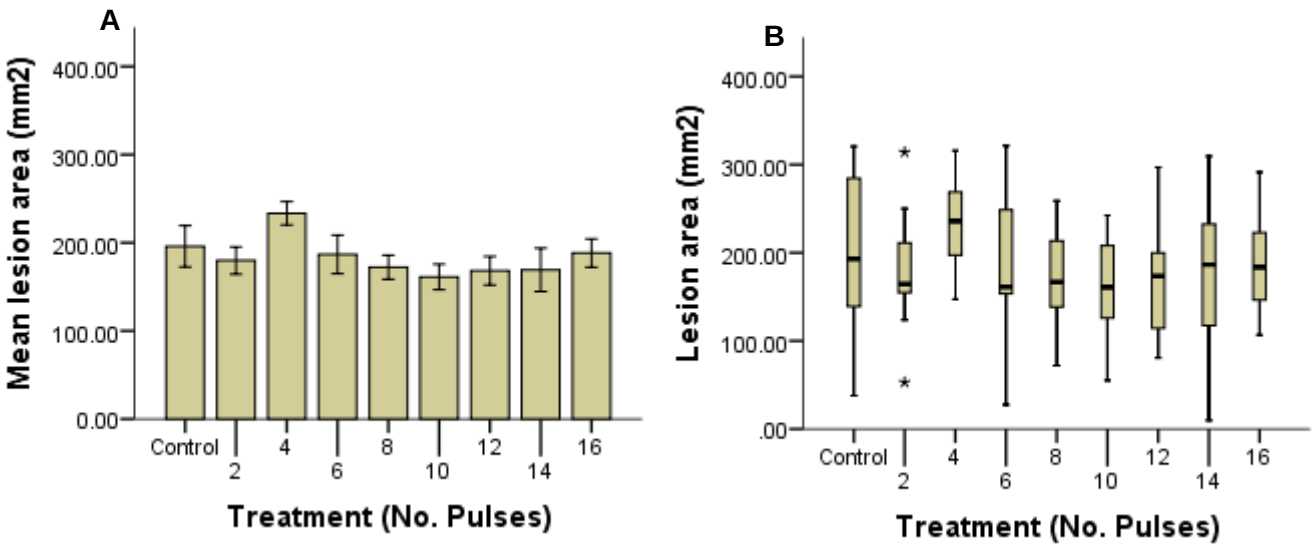

Figure 31. Disease resistance assays performed in November 2015 (18.11.2015) on the butterhead variety Amica treated with pulsed polychromatic source. Plants were treated at 3-5 true leaves and inoculated 2 days after treatment with 4mm agar plugs from 3 day old *Rhizoctonia solani* cultures. Lesion 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

The following round of resistance assay trials were carried out in December 2015 and utilised Amica plants only. Plants were treated with either the pulsed polychromatic source or the conventional UV-C source with ranges of 8-16 pulses and 0.06-0.24 kJ/m^2, respectively. Seven biological repeats were used with three technical repeats. Plants were either inoculated with *B. cinerea, R. solani* or *S. sclerotiorum* at 2, 5 and 9 DPT.

For conventional UV-C treated plants inoculated with *B. cinerea* no reductions in disease progression were observed at 2 DPT, Figure 32. At 5 DPT, however, the 0.3 kJ/m^2 treatment showed a 20.2 % reduction in mean AUDPC with the 0.18 and 0.24 kJ/m^2 treatments also showing reductions in mean AUDPC. The plants inoculated at 9 DPT showed lower reductions in mean AUDPC the largest of which was the 0.12 kJ/m^2 treatments with an 8.3 % reduction. As has been observed previously the greatest maximum AUDPCs are observed for groups that have received treatment, Figure 33.

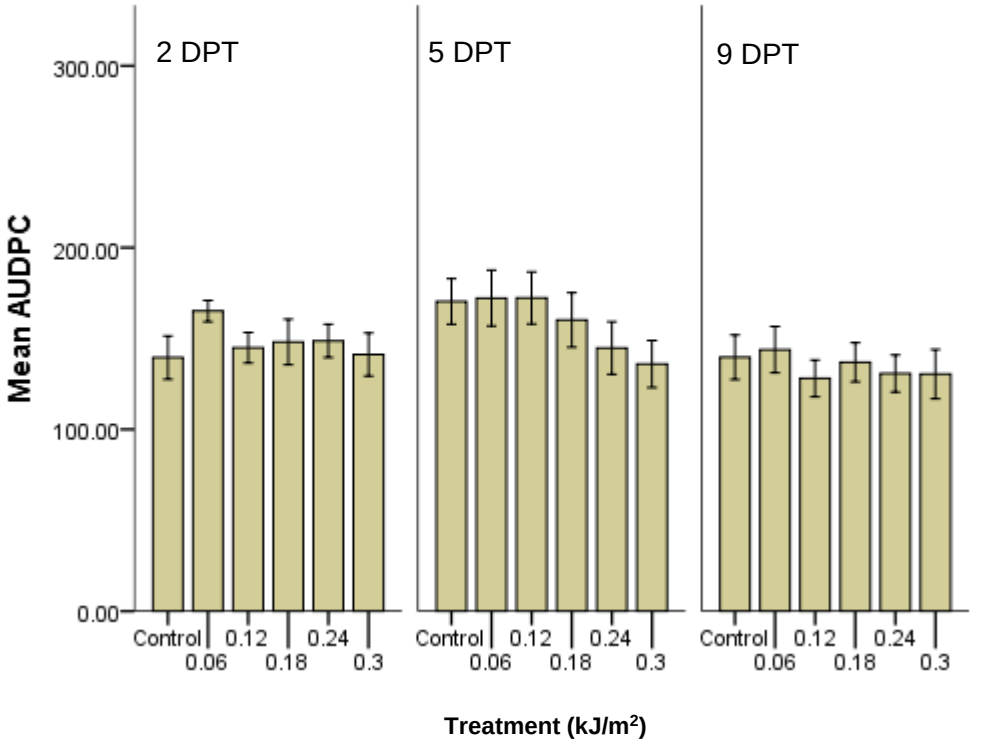

Figure 32. Disease resistance assays performed in December 2015 (09.12.2015) on the butterhead variety Amica treated with the conventional UV-C source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 10 µl of 1x10^6 spores/ml of *B. cinerea* amended with 50 % 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

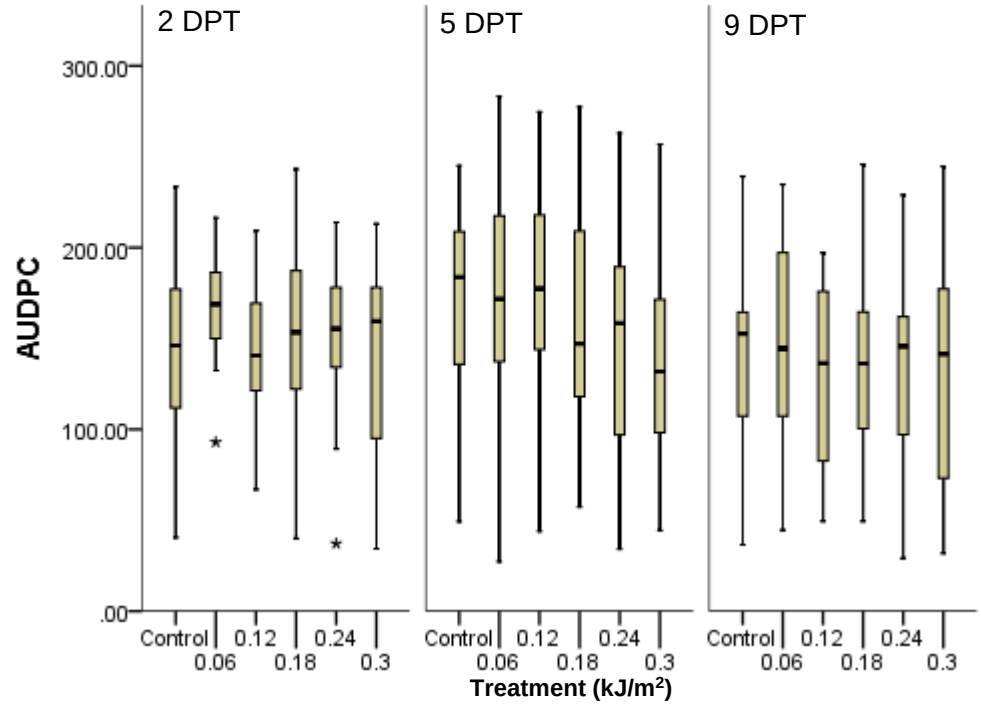

Figure 33. Disease resistance assays performed in December 2015 (09.12.2015) on the butterhead variety Amica treated with the conventional UV-C source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 10 µl of 1x10^6 spores/ml of *B. cinerea* amended with 50 % 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

For plants receiving pulsed polychromatic treatments prior to inoculation with *B. cinerea* only a single group, 12 pulses, showed a reduction in mean AUDPC at 2 DPT of 2.9 %, Figure 34. At 5 DPT, as with the conventional treatments, the higher treatments showed a reduction in mean AUDPC the greatest being observed for the 14 pulse treatments with a 10.3 % reduction in disease progression. At 9 DPT, again, only the higher doses showed reduction in mean AUDPC with the largest being that for the 12 pulse treatment.

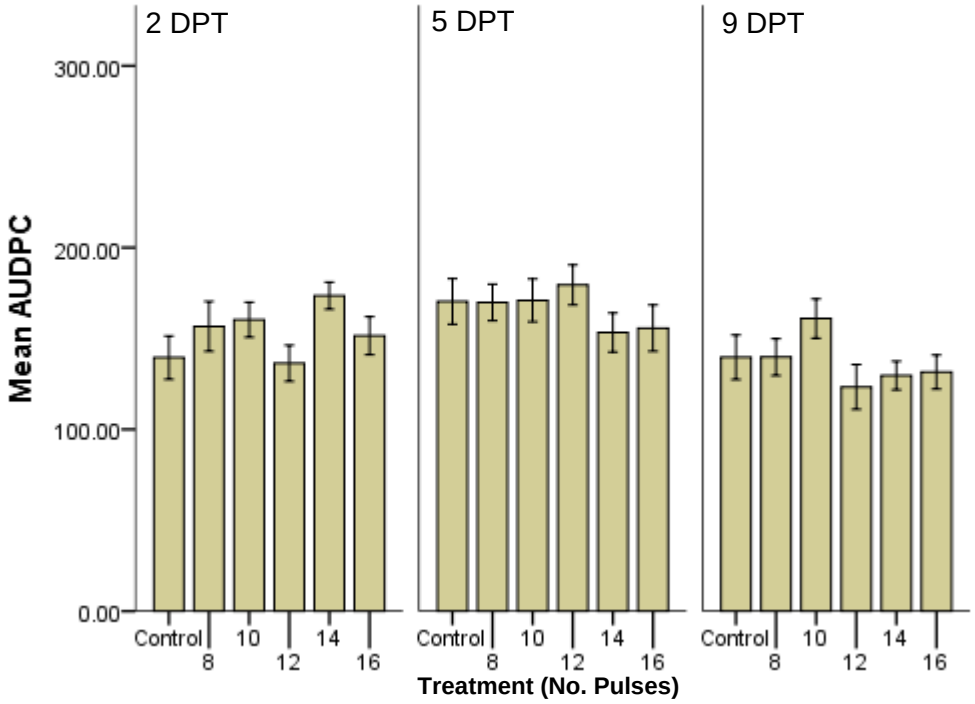

Figure 34. Disease resistance assays performed in December 2015 (09.12.2015) on the butterhead variety Amica treated with the pulsed polychromatic source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 10 µl of $1x10^6$ spores/ml of *B. cinerea* amended with 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= 7

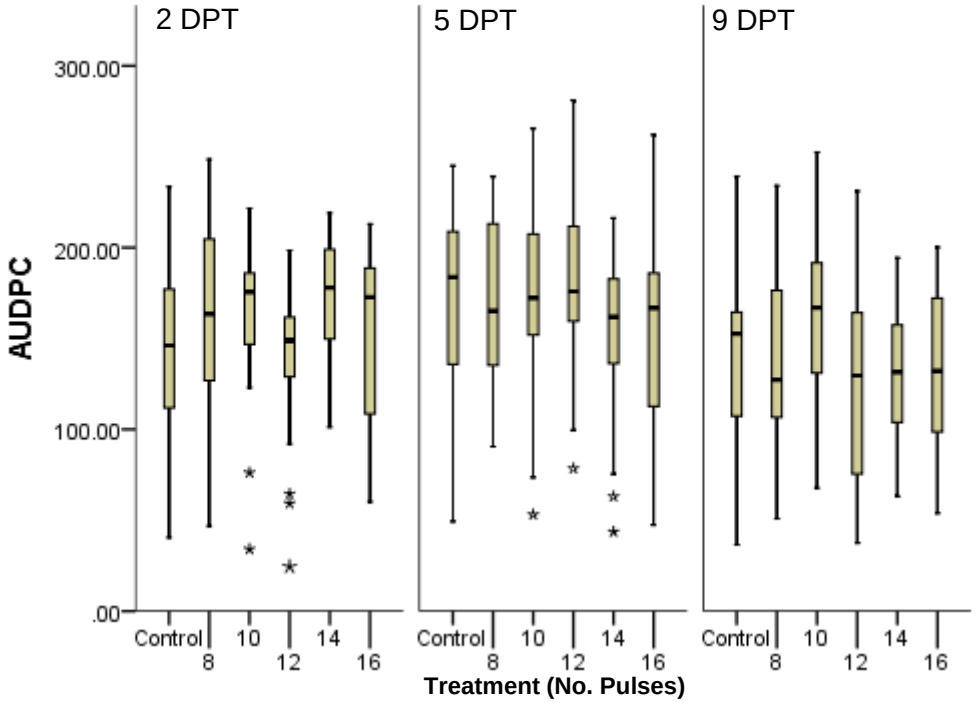

Figure 35. Disease resistance assays performed in December 2015 (09.12.2015) on the butterhead variety Amica treated with the pulsed polychromatic source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 10 µl of $1x10^6$ spores/ml of *B. cinerea* amended with 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

Plants treated with the conventional UV-C source and then inoculated with *R. solani* showed only the 0.18 kJ/m^2 treatment gave a reduction in mean AUDPC of 2.2 % at 2 DPT, Figure 36. For plants inoculated at 5 DPT the treatment showing the largest reduction in mean AUDPC was also 0.18 kJ/m^2 with a 5.3 % reduction, Figure 35. At 9 DPT the largest reduction in mean AUDPC was observed for the 0.12 kJ/m^2 treatment at 8.8 %. The 0.18 kJ/m^2 treatment, however, showed a similar reduction at 8.3 %. At both 2 DPT and 5 DPT treatment groups showed greater maximum AUDPCs than the control and similar medians, figure 36. At 9 DPT the 0.12, 0.18 and 0.3 kJ/m^2 treatments showed lower maximum and median AUDPCs than the control.

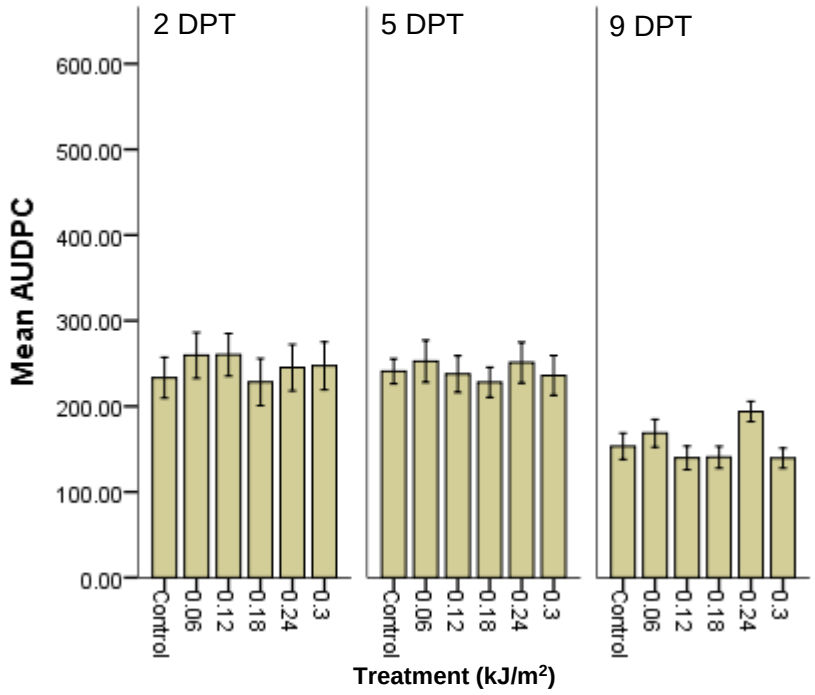

Figure 36. Disease resistance assays performed in December 2015 (09.12.2015) on the butterhead variety Amica treated with the conventional UV-C source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 4mm agar plugs of *R. solani*. Lesion areas were measured 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

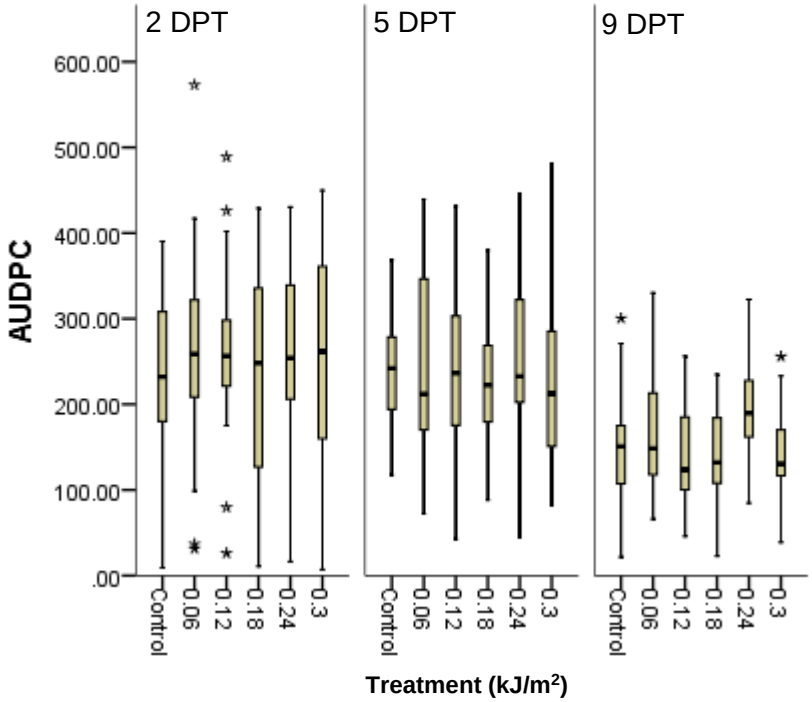

Figure 37. Disease resistance assays performed in December 2015 (09.12.2015) on the butterhead variety Amica treated with the conventional UV-C source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 4mm agar plugs of *R. solani*. Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. Asterisks represent observed outliers. N= 7

For plants treated with the pulsed polychromatic source and then inoculated with *R. solani* the treatments showing the largest mean reductions in AUDPC were the 8, 10 and 8 pulse treatments with reductions of 7.2, 14.1 and 20.6 % at 2, 5 and 9DPT, respectively, Figure 38. At 2 DPT maximum AUDPCs for treated plants were larger than that of the control, figure 38. Minimum AUDPC values were similar to that of the control. At 9 DPT maximum AUDPCs for the 8 and 10 pulse treatments were similar to that of the control. Minimum values, however, were lower than that of the control. At 9 DPT the maximum values for the 8 pulse treatment were similar to that of the control with the interquartile range showing reduced values.

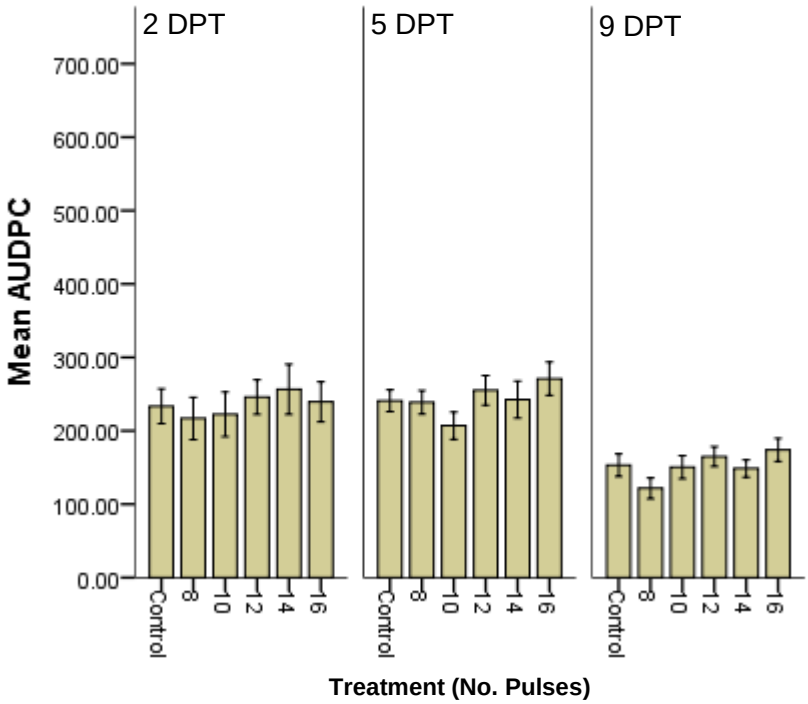

Figure 38. Disease resistance assays performed in December 2015 (09.12.2015) on the butterhead variety Amica treated with the pulsed polychromatic source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 4mm agar plugs of *R. solani*. Lesion areas were measured 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

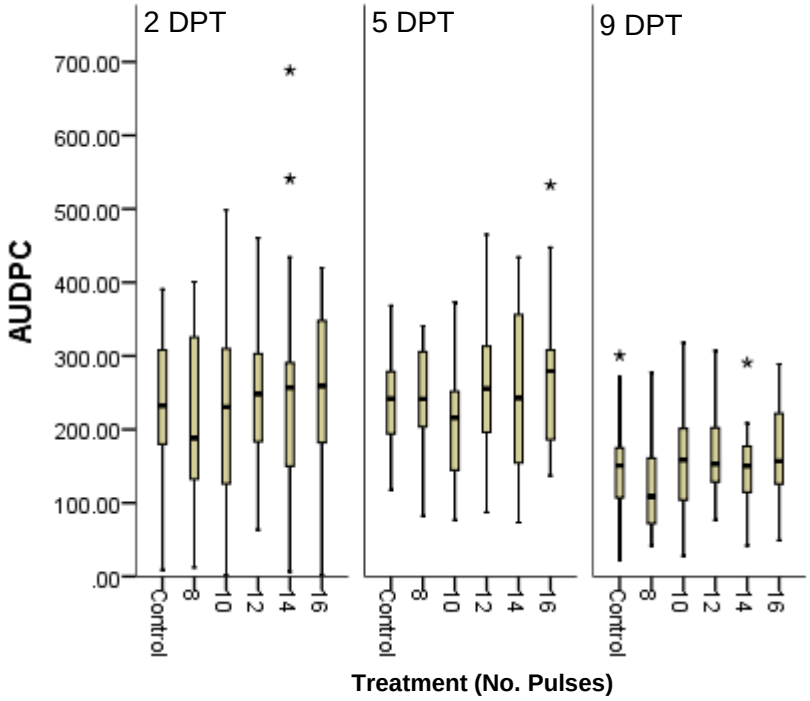

Figure 39. Disease resistance assays performed in December 2015 (09.12.2015) on the butterhead variety Amica treated with the pulsed polychromatic source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 4mm agar plugs of *R. solani*. Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. Asterisks represent observed outliers. N= 7

For *S. sclerotiorum* inoculated plants conventional UV-C treatment showed reductions in mean AUPDC at all DPT. The greatest reductions were observed for the 0.18, 0.12 and 0.12 kJ/m^2 treatments at 8.8, 16.8 and 1.96 % at 2, 5 and 9 DPT, respectively, Figure 40. All other treatments showed an increase mean AUPDC at 9 DPT. At 2 DPT maximum AUDPC values were higher for the 0.12 and 0.24 kJ/m^2 treatments, Figure 41. The maximums for 0.06 and 0.18 kJ/m^2 were similar to that of the control with reduced minimum values and inter quartile ranges. At 9DPT all treatments showed increased maximum and median AUDPCs.

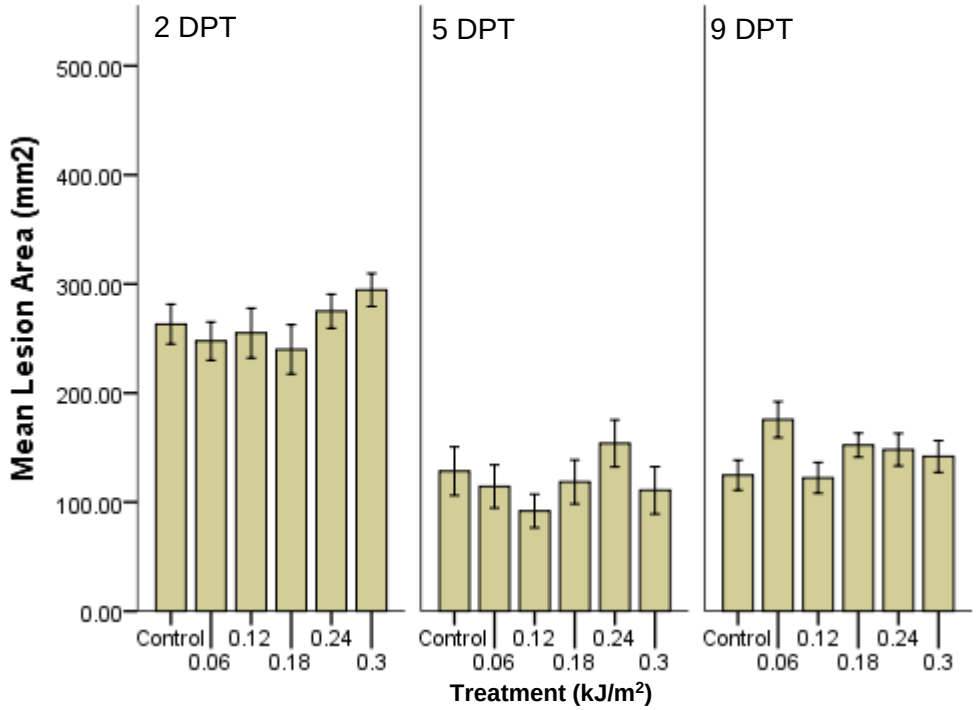

Figure 40. Disease resistance assays performed in December 2015 (09.12.2015) on the butterhead variety Amica treated with the conventional UV-C source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 4mm agar plugs of *S. sclerotiorum*. Lesion areas were measured at 2 days after inoculation with ImageJ. Bars shows the mean lesion length ± 1 standard error of the mean. N= 7

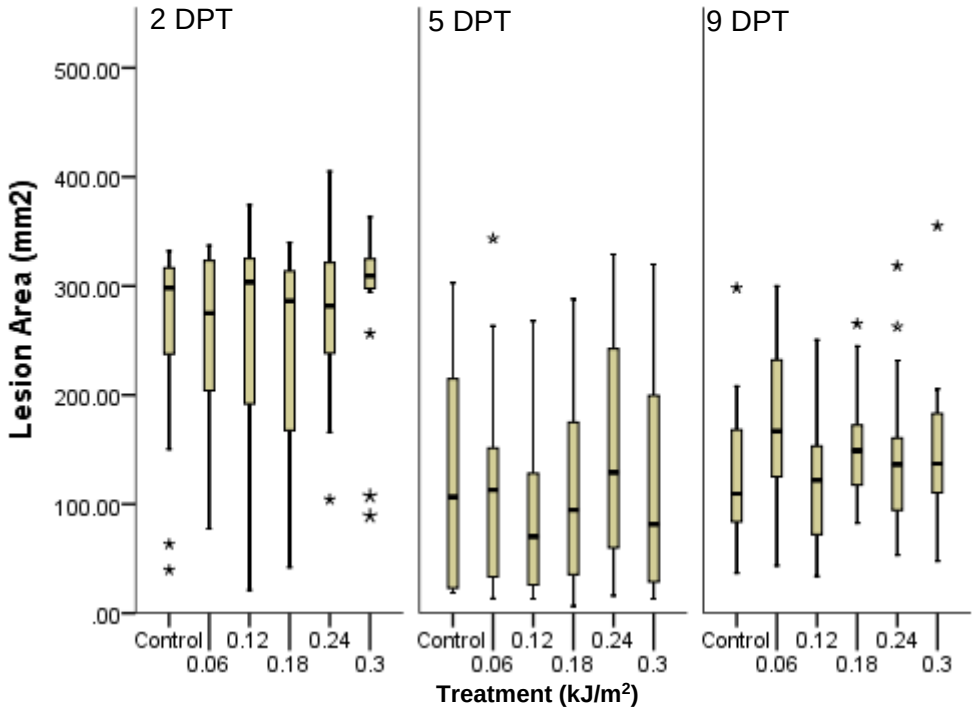

Figure 41. Disease resistance assays performed in December 2015 (09.12.2015) on the butterhead variety Amica treated with the conventional UV-C source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 4mm agar plugs of *S. sclerotiorum*. Lesion areas were measured at 2 days after inoculation with ImageJ. Asterisks represent observed outliers. N= 7

For plants treated with the pulsed polychromatic source prior to inoculation with *S. sclerotiorum* the treatments showing the greatest mean reduction in AUDPC were 14, 8 and 12 pulses at 2, 5 and 9 DPT with reductions of 12.9, 17.8 and 38.65 %, respectively, Figure 42. AT 2 DPT all treatments apart from the 14 pulse treatment showed increases in mean AUDPC. At 5 and 9 DPT, however, treatments either showed similar levels to that of the control or reductions in mean AUDPC. At 2 DPT maximum AUDPC values of treatment groups were either slightly larger than that of the control or at similar levels, Figure 43. Medians were also similar or lower than that of the control with the interquartile range of the 14 pulse treatment much lower than that of the control. At 5 DPT all maximum and minimum values for the treated groups were similar to that of the control with all the treatments showing lower interquartile ranges. At 9 DPT the data showed a similar pattern, however, the 8 pulse treatment showed a much greater maximum value.

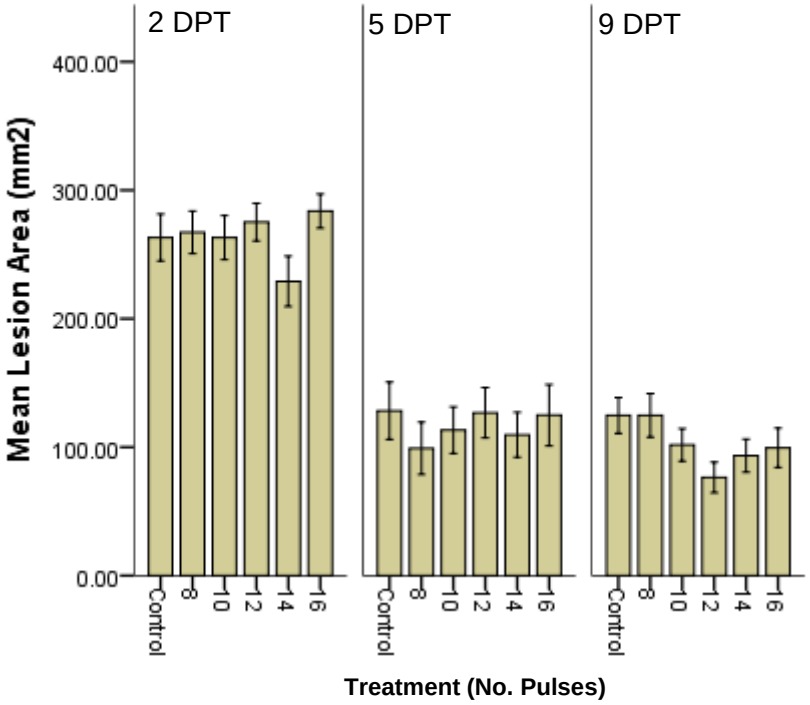

Figure 42. Disease resistance assays performed in December 2015 (09.12.2015) on the butterhead variety Amica treated with the pulsed polychromatic source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 4mm agar plugs of *S. sclerotiorum*. Lesion areas were measured at 2 days after inoculation with ImageJ. Bars shows the mean lesion length ± 1 standard error of the mean. N= 7

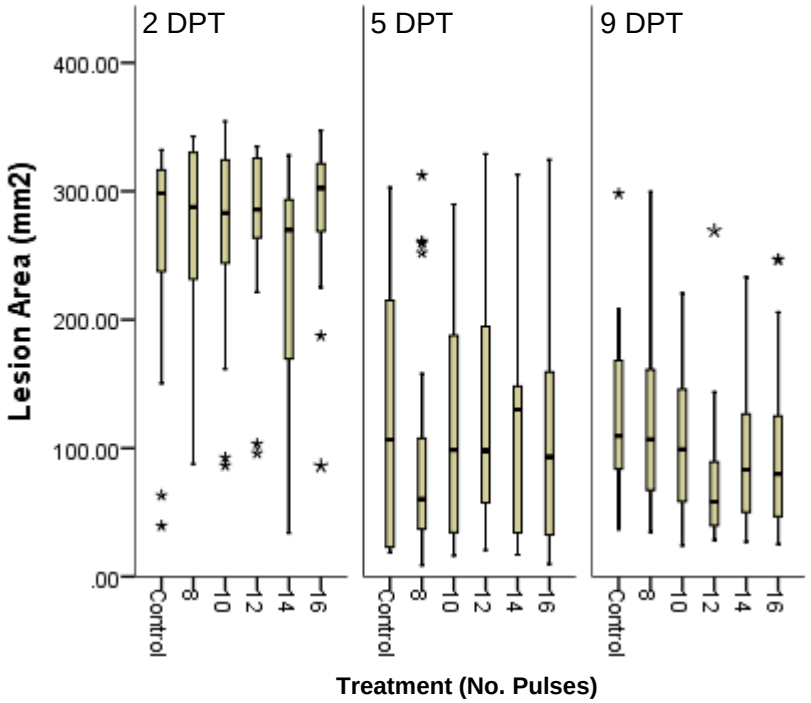

Figure 43. Disease resistance assays performed in December 2015 (09.12.2015) on the butterhead variety Amica treated with the pulsed polychromatic source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 4mm agar plugs of *S. sclerotiorum*. Lesion areas were measured at 2 days after inoculation with ImageJ. Asterisks represent observed outliers. N= 7

The next round of resistance assays were performed in February 2016 and used both Amica and Temira plants at 6-8 true leaves. Plants were inoculated with both *B. cinerea* and *R. solani*. Seven biological replicates and three technical replicates were used. For plants treated with the conventional UV-C source the treatment range was 0.06-0.3 kJ/m^2. Pulsed treatments were between 8-16 pulses. Plants were inoculated at 2 DPT. During this experiment *R. solani* lesions were only measures at 3 DPI due to the slow progression of disease.

For UV-C treated Amica plants inoculated with *B. cinerea* the largest reductions in mean AUDPC were observed for the 0.06 kJ/m^2 treatment with a 27 % reduction, Figure 44A. All other treatments apart from the 0.3 kJ/m^2 showed reductions in mean AUDPC. Maximum AUDPC values for the 0.06 kJ/m^2 group were similar to that of the control with a negative shift in interquartile range, median and minimum values, Figure 44B. Plants treated with the polychromatic source also all showed reductions in mean AUDPC in comparison to the control with the 10 pulse treatment showing the greatest reduction of 44.1 %, Figure 45A. Maximum, minimum and interquartile range AUDPC values for the 10 pulse treatment were all lower than that of the control, Figure 45B. All other treatments showing a similar pattern apart from that of the 8 pulse treatment that showed an increase in the maximum AUDPC.

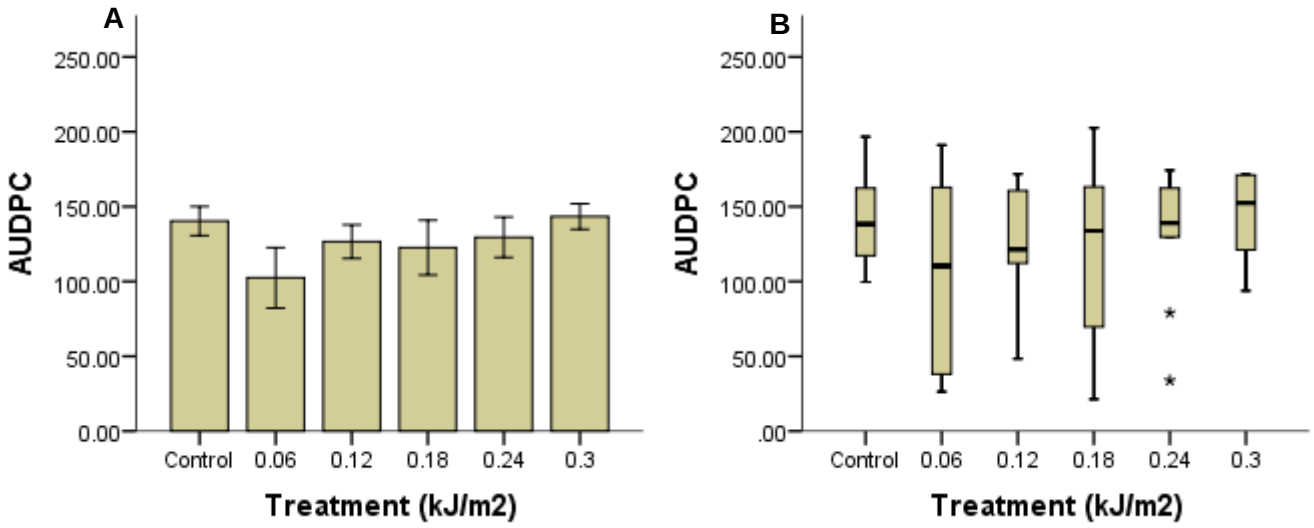

Figure 44. Disease resistance assays performed in February 2016 (12.02.2016) on the butterhead variety Amica treated with the conventional UV-C source. Plants were treated at 6-8 true leaves and inoculated 2 days after treatment with 10 µl of $1x10^6$ spores/ml of *B. cinerea* amended with 50 % potato 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

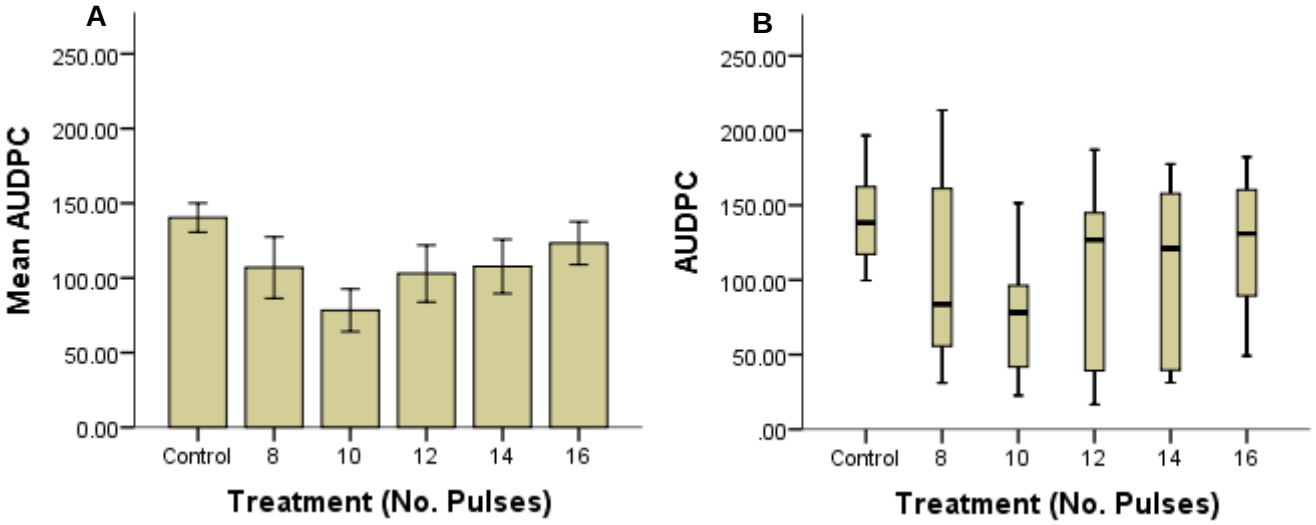

Figure 45. Disease resistance assays performed in February 2016 (12.02.2016) on the butterhead variety Amica treated with the conventional UV-C source. Plants were treated at 6-8 true leaves and inoculated 2 days after treatment with 10 µl of $1x10^6$ spores/ml of *B. cinerea* amended with 50 % potato 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

Amica plants treated with the conventional UV-C source and inoculated with *R. solani* showed reductions in mean lesion area for the 0.12m 0.18 and 0.24 kJ/m^2 treatments with the greatest reduction observed for the 0.18 kJ/m^2 treatment at 32.9 %, Figure 46. The 0.06 and 0.3 kJ/m^2 showed similar levels to that of the control. Maximum values for the 0.06, 0.12 and 0.24 treatments were greater than that of the control, Figure 46B. For the 0.12, 0.18 and 0.24 treatments inter quartile ranges showed smaller lesion areas.

Polychromatic treated plants inoculated with *R. solani* showed only two treatments, 12 and 14 pulses, with mean lesion areas reduced in comparison to the control with the 12 pulse treatment showing the greatest reduction of 37.7 %, Figure 47A. Maximum lesion areas were increased for all treatments apart from the 12 and 14 pulse treatments who showed reduced inter quartile ranges but similar minimum lesion sizes, Figure 47B.

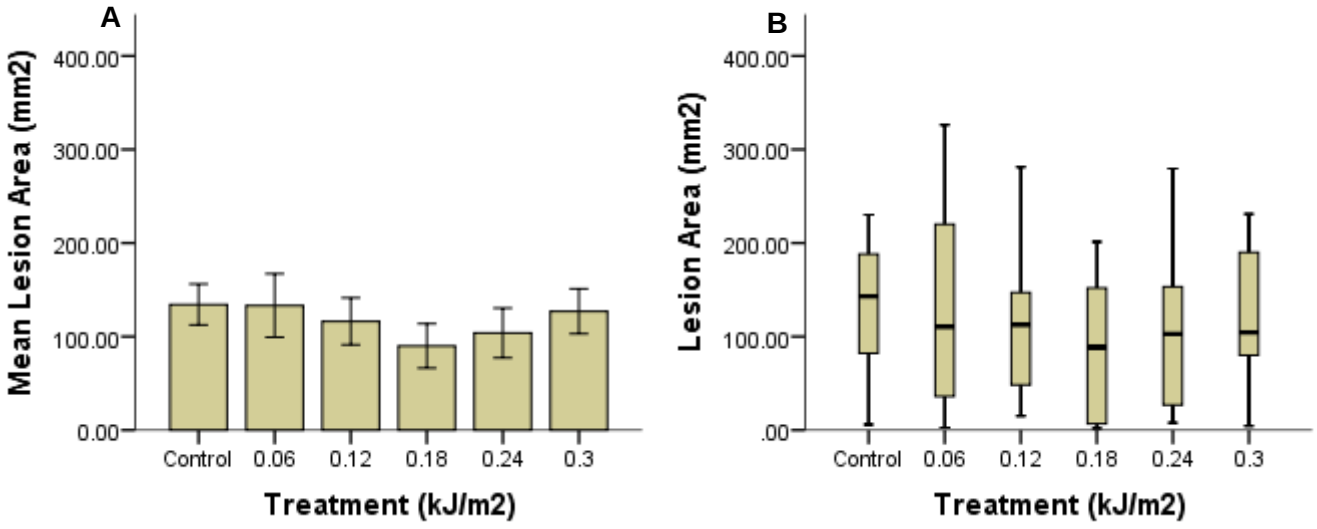

Figure 46. Disease resistance assays performed in February 2016 (12.02.2016) on the butterhead variety Amica treated with the conventional UV-C source. Plants were treated at 6-8 true leaves and inoculated 2 days after treatment with 4 mm agar plugs of *R. solani*. Lesion areas were measured at 2 and 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

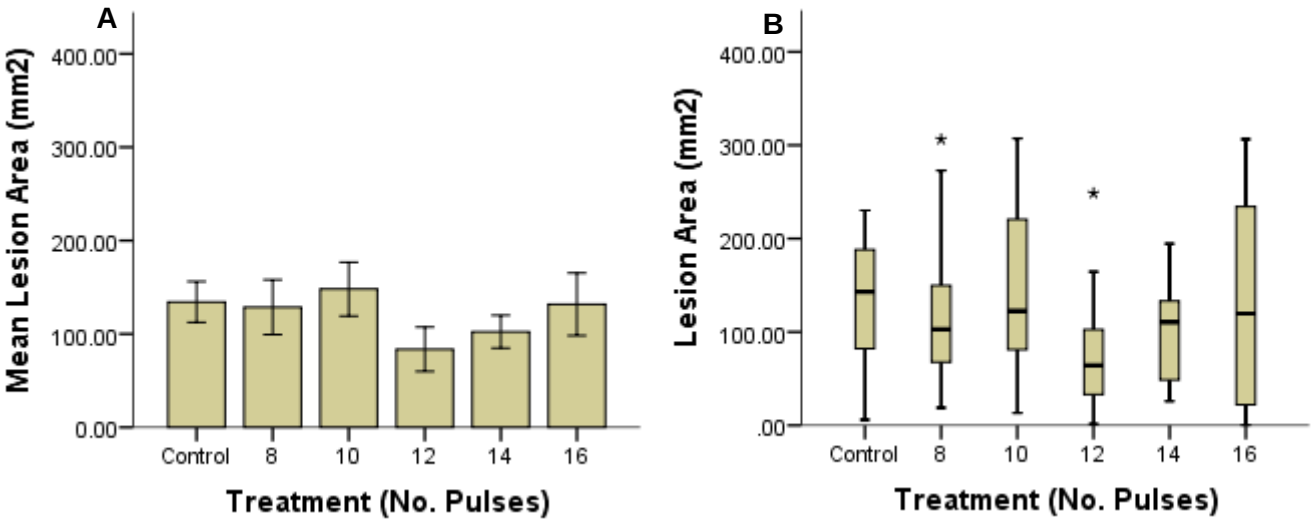

Figure 47. Disease resistance assays performed in February 2016 (12.02.2016) on the butterhead variety Amica treated with the pulsed polychromatic source. Plants were treated at 6-8 true leaves and inoculated 2 days after treatment with 4 mm agar plugs of *R. solani*. Lesion areas were measured at 2 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

Temira plants treated with the conventional UV-C source and inoculated with *B. cinerea* showed reductions for the 0.06, 0.12, 0.24 and 0.3 kJ/m^2 treatments with the 0.24 kJ/m^2 treatment showing the greatest reduction in mean AUDPC at 31.3 %. Maximum AUDPC values were similar for all of the treatment groups including the control, Figure 48B. Interquartile ranges for the 0.12, 0.24 and 0.3 kJ/m^2 treatments showed lower AUDPCS than that of the control. All minimum AUDPCs, however, were similar to that of the control.

Polychromatic treated plants also showed reductions in mean AUDPC for all of the treatments tested with the 8 and 14 pulse treatments showing similar reductions at 40.6 % and 33.3 %, respectively, Figure 49A. All treatment groups, however, showed greater maximum AUDPC values in comparison to that of the control, Figure 49B. All treatment interquartile ranges showed a negative shift in comparison to that of the control.

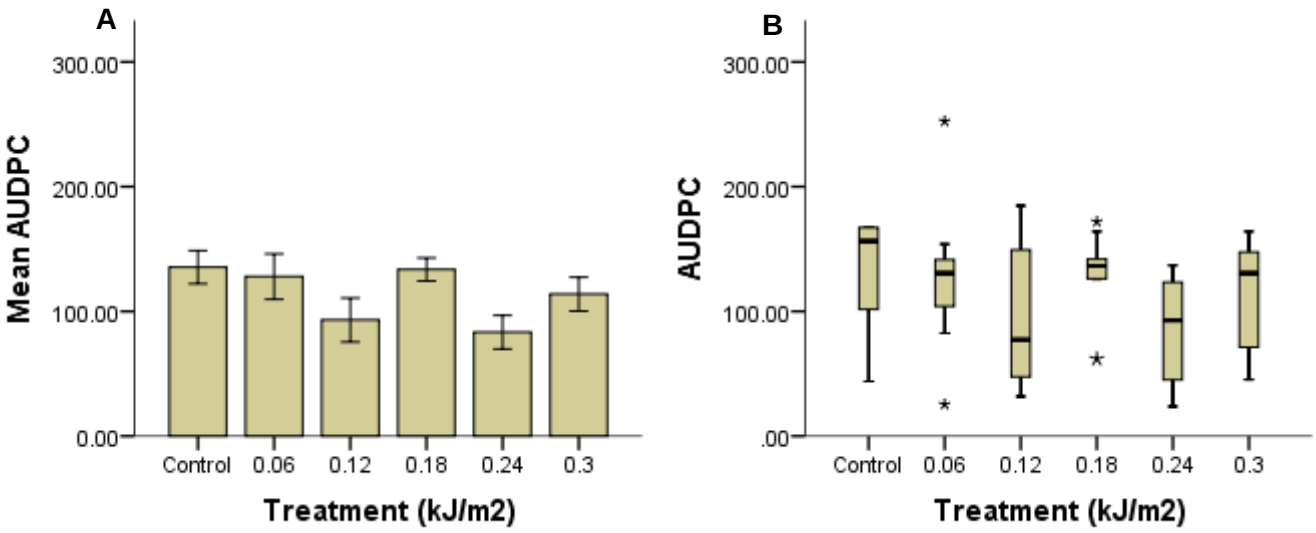

Figure 48. Disease resistance assays performed in February 2016 (12.02.2016) on the butterhead variety Temira treated with the conventional UV-C source. Plants were treated at 6-8 true leaves and inoculated 2 days after treatment with 10 µl of $1x10^6$ spores/ml of *B. cinerea* amended with 50 % potato 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

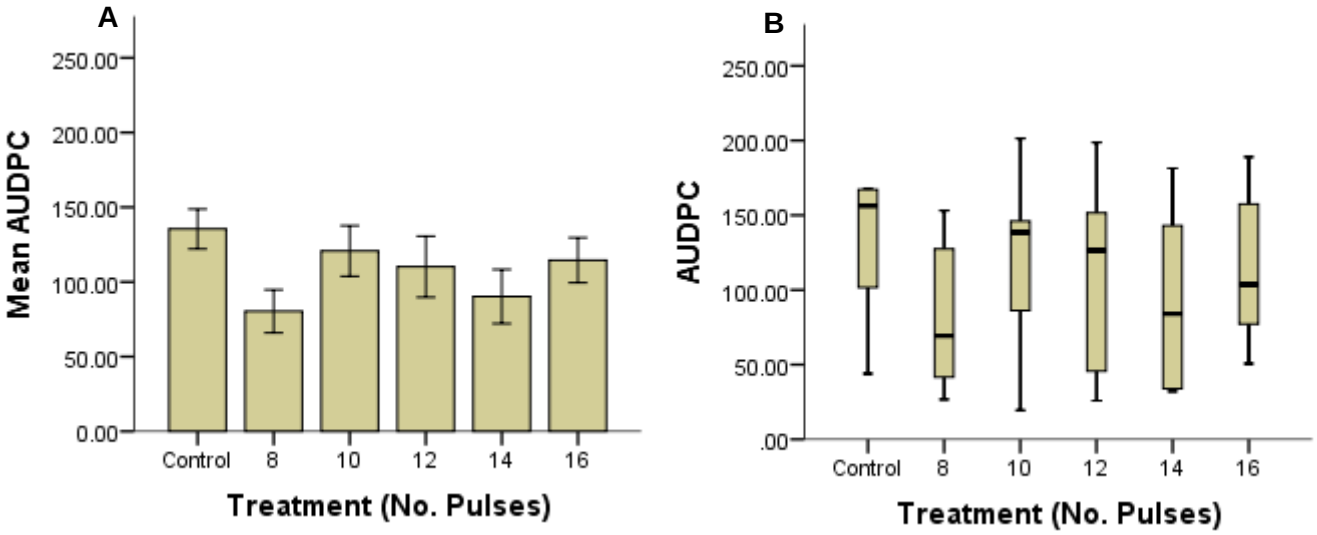

Figure 49. Disease resistance assays performed in February 2016 (12.02.2016) on the butterhead variety Temira treated with the conventional UV-C source. Plants were treated at 6-8 true leaves and inoculated 2 days after treatment with 10 µl of $1x10^6$ spores/ml of *B. cinerea* amended with 50 % potato 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

Temira plants treated with the conventional UV-C source and inoculated with *R. solani* showed reductions in mean lesion area for all groups apart from the 0.3 kJ/m^2 treatment who showed a similar level to the control, Figure 50A. The largest reduction in mean lesion area was observed for the 0.24 kJ/m^2 treatment at 53.8 %. All maximum lesion areas and interquartile ranges were reduced in comparison to the control, Figure 50B. Minimum values and medians were similar to that of the control apart from the 0.24 kJ/m^2 treatment that showed a reduced median.

Plants treated with the pulsed source showed a similar pattern in mean lesion area with all but the highest treatment showing a reduction the greatest of which being for the 14 pulse treatment at 37.8 %, Figure 51A. All treatments apart from the 8 pulse showed a reduction in maximum lesion area, Figure 51B. Interquartile ranges for the 8, 12, 14 and 16 pulse treatments were also reduced.

Summaries of all the resistance assay data collected to date can be found in Tables 6, 7, 8 and 9.

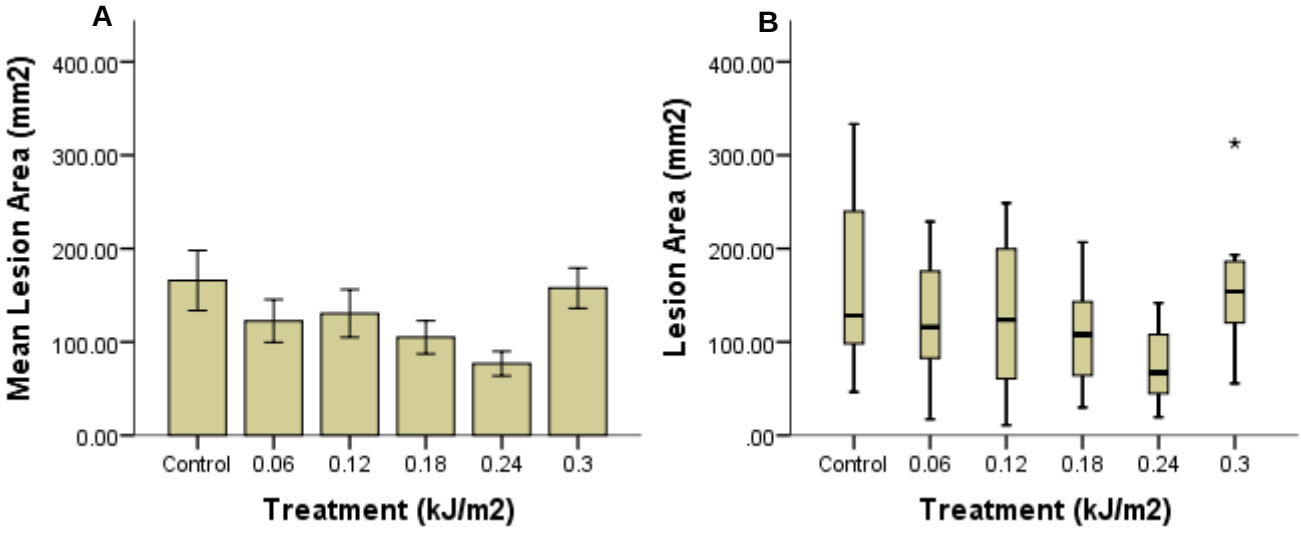

Figure 50. Disease resistance assays performed in February 2016 (12.02.2016) on the butterhead variety Temira treated with the conventional UV-C source. Plants were treated at 6-8 true leaves and inoculated 2 days after treatment with 4 mm agar plugs of *R. solani*. Lesion areas were measured at 2 and 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=

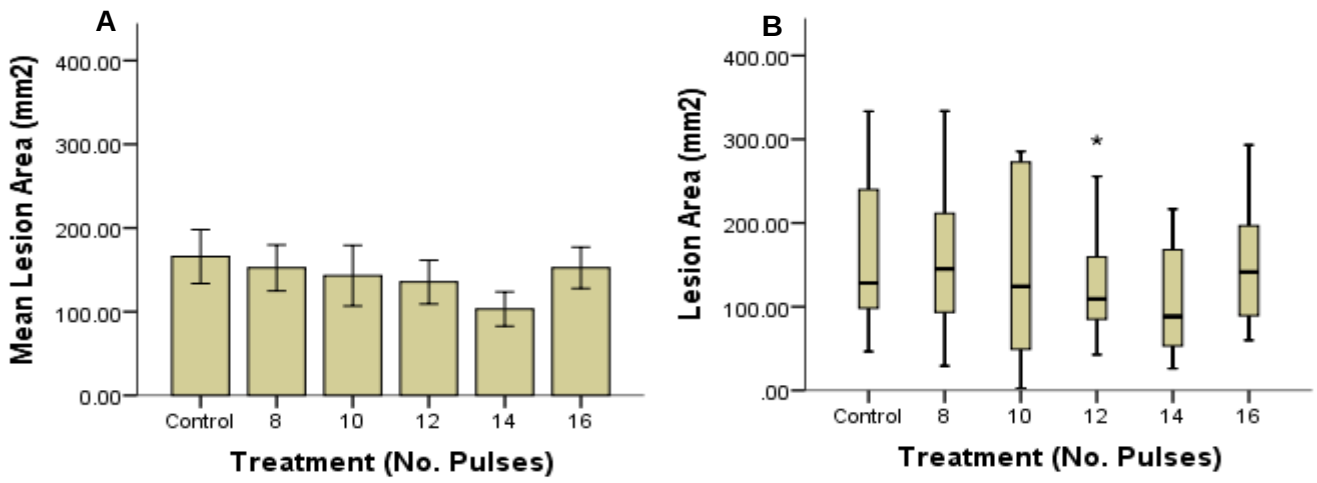

Figure 51. Disease resistance assays performed in February 2016 (12.02.2016) on the butterhead variety Temira treated with the pulsed polychromatic source. Plants were treated at 6-8 true leaves and inoculated 2 days after treatment with 4 mm agar plugs of *R. solani*. Lesion areas were measured at 2 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

Table 6. 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 optimum treatments and percentage reduction in disease for *Botrytis cinerea, Rhizoctonia solani* and *Sclerotinia sclerotiorum.*

Month	Date	No. true leaves	Treatment range (pulses)	Damage threshold	Inoculation day (DPT)	Optimal 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.

Table 7. 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 optimum treatments and percentage reduction in disease for *Botrytis cinerea, Rhizoctonia solani* and *Sclerotinia sclerotiorum.*

Month	Date	No. true leaves	Treatment range (kJm^2)	Damage threshold	Inoculation day (DPT)	Optimal 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.

Table 8. 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 optimum treatments and percentage reduction in disease for *Botrytis cinerea, Rhizoctonia solani* and *Sclerotinia sclerotiorum.*

Month	Date	No. true leaves	Treatment range (pulses)	Damage threshold	Inoculation day (DPT)	Optimal 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 9. 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 optimum treatments and percentage reduction in disease for *Botrytis cinerea, Rhizoctonia solani* and *Sclerotinia sclerotiorum.*

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

Month	Date	No. true leaves	Treatment range (kJm^2)	Damage threshold	Inoculation day (DPT)	Optimal 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	0.12	31.3	0.24	53.8	-	-

To summarise the findings of these preliminary experiments the treatments for which the greatest reductions in disease progression were observed appears across the year as does the plants susceptibility to damage. The optimal pulsed treatment for cv. Amica dropped from 22 pulses in September to 16 in October, Tables 6, 7, 8 and 9. The most effective treatments also differed between cultivar. For example in September the optimal treatments against *B. cinerea* was 22 pulses for cv. Amica but was 16 pulses for cv. Temira. Optimal conventional UV-C treatments also showed between cultivar variation at 0.6 kJm^2 for Amica and 1.1 for Temira during September. This was further complicated by differences in the optimal treatments for the pathogens under investigation. For cv. Amica the optimal treatments against *B. cinerea, R. solani* and *S. sclerotiorum* were 12, 8 and 14 pulses in December, respectively. This was also observed for the conventional source with optimal treatments at 0.3, 0.18 and 0.12 kJm^2, respectively. Moreover, 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. This was also observed for conventional treatments with 14.41 % and 31.3 % average reductions for Amica and Temira, respectively.

Due to the observed fluctuating damage thresholds the treatment ranges undergoing investigation for disease resistance were changed. Apart from during initial damage susceptibility trials for the spring and summer months no damage was observed. The doses that gave the lowest levels of disease progression across the year, however, also appeared to fluctuate, Figure 53. Figure 53 shows the most complete set of resistance assays performed on the variety Amica utilising the pathogen *B. cinerea*. The treatments showing the greatest reduction in disease were plotted against the month that the experiment was conducted in. Pulsed treatments show an increase in optimum treatment from 15 to 22 pulses between April and September and a drop to 10 in February. The conventional UV-C treated plants also show a homologous drop in dose from September where the most effective treatment was 0.6 kJm^2 to 0.06 in February.

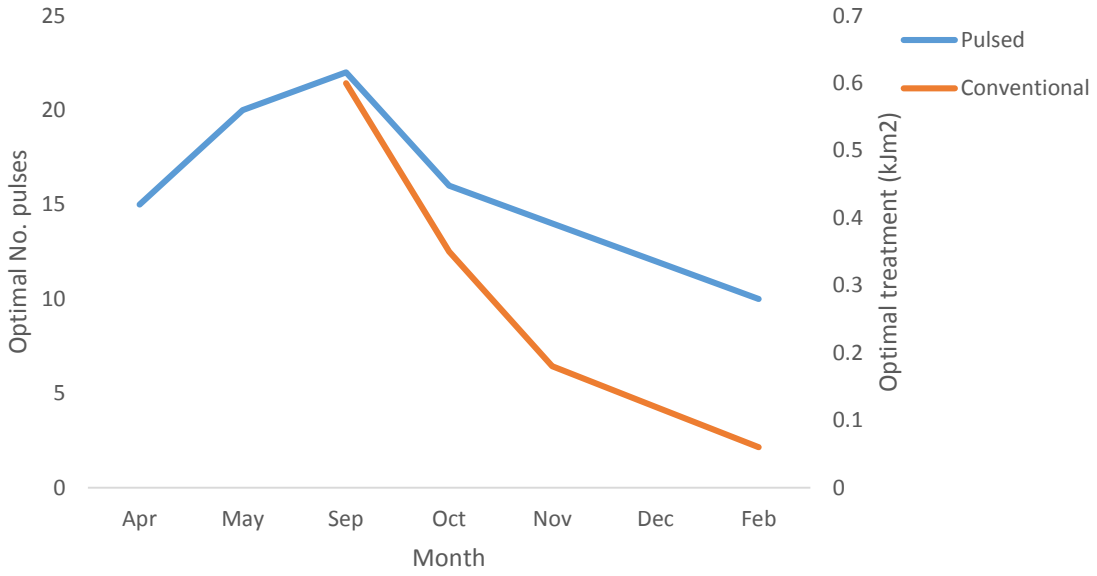

Figure 53: The optimal pulsed and conventional treatments for reducing the disease progression of against *Botrytis cinerea* at differing months of the year for the cv. Amica

This may indicate a complex situation where not only the environmental conditions could affect the necessary treatment to induce resistance but also the cultivar and the pathogen undergoing investigation. It was therefore hypothesised whether utilising a low treatment and repeating its application could induce resistance without damaging the plant. The chosen treatments were 0.12 and 0.24 kJm^2 for the conventional treatments and 7 and 14 pulses for the pulsed source. Each of the higher treatments, for the conventional and pulsed sources, produced no damage at any point during the year and showed some level of induced resistance against each of the pathogens undergoing investigation. Treatments were applied with either 2, 4 or 6 days between treatment (DBT) and applied twice. *R. solani* was chosen as the pathogen of interest as it had shown the highest level of responsiveness to treatment in previous studies. Plants were treated at the 6-8 leaf stage and inoculated 2 DPT. Five biological replicates and two technical replicates were used. Lesion area was measures at 3 DPI only. Both Amica and Temira varieties were used during the trials.

Three replicate experiments were carried out between March and May 2016. A summary of the results for each experimental replicate can be seen in Tables 10, 11, 12 and 13. 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. The aforementioned data, however, can be found in the Tables 10, 11, 12 and 13.

All conventional UV-C treatments showed slight reductions in mean lesion size at 2 DBT for the variety Amica. All treatments, however, showed an increase in mean lesion area for

treatments with 6 DBT. The conventional UV-C treatment showing the greatest reduction was the 0.12 kJm^2 giving a 13.24 % reduction at 2 DBT, Figure 54A. This pattern was mirrored by a small drop in maximum lesion size, median and negative shift in the interquartile range, Figure 54B. None of the conventional treatments showed a significant difference from the control with a large p value 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. 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 %, Figure 53C. The maximum lesion sizes, medians and interquartile ranges were similar for all of the treatments including the control, Figure 54D. Again statistical testing showed large p values of 0.896 and 0.672 for the 2 DBT and 6 DBT treatments respectively.

For the variety Temira multiple low level conventional UV-C treatments showed small reductions with both 2 and 6 DBT. The treatment showing the largest decrease in mean lesion area was that of the 0.24 kJm^2 treatment with 2 DBT showing a 7.5 % decrease, figure 55A. With 6 DBT the treatment showing the greatest reduction in mean lesion size was the 0.12 kJm^2 at 9.5 %. Slight reduction in the medians for all treatments at both 2 and 6 DBT were observed, Figure 55B.

For plants that had undergone pulsed polychromatic treatment only the 14 pulse treatment applied with 2 DBT showed a reduction in mean lesion size at 9.9 %, Figure 55C. Maximum, minimum, median and interquartile ranges for the 14 pulse 2 DBT showed a negative shift in comparison to that of the control, Figure 55D. Statistical analysis highlighted no significant differences and again showed particularly large p values. Conventional UV-C treatments gave p values of 0.722 and 0.587 for the 2 and 6 DBT trials, respectively. Pulsed treatments similarly showed high p values at 0.374 and 0.929 for the 2 and 6 DBT trials.

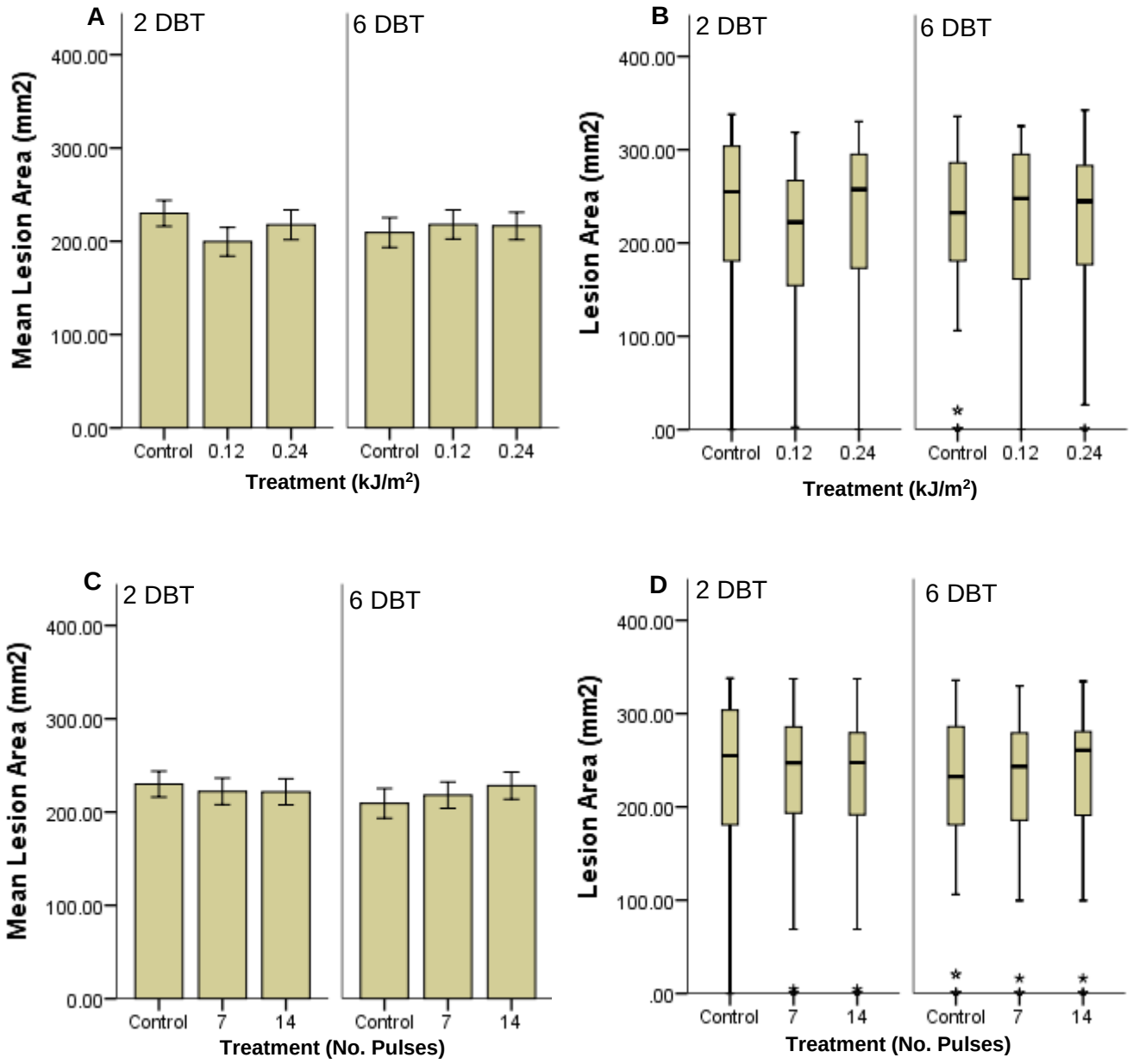

Figure 54. The combined experimental data from three replicate experiments utilising multiple low level treatments of the variety Amica. Experimental replicates were performed during March, April and May 2016. Plants were inoculated at 2 days post treatment with 4mm *R. solani* agar plugs and lesions 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.

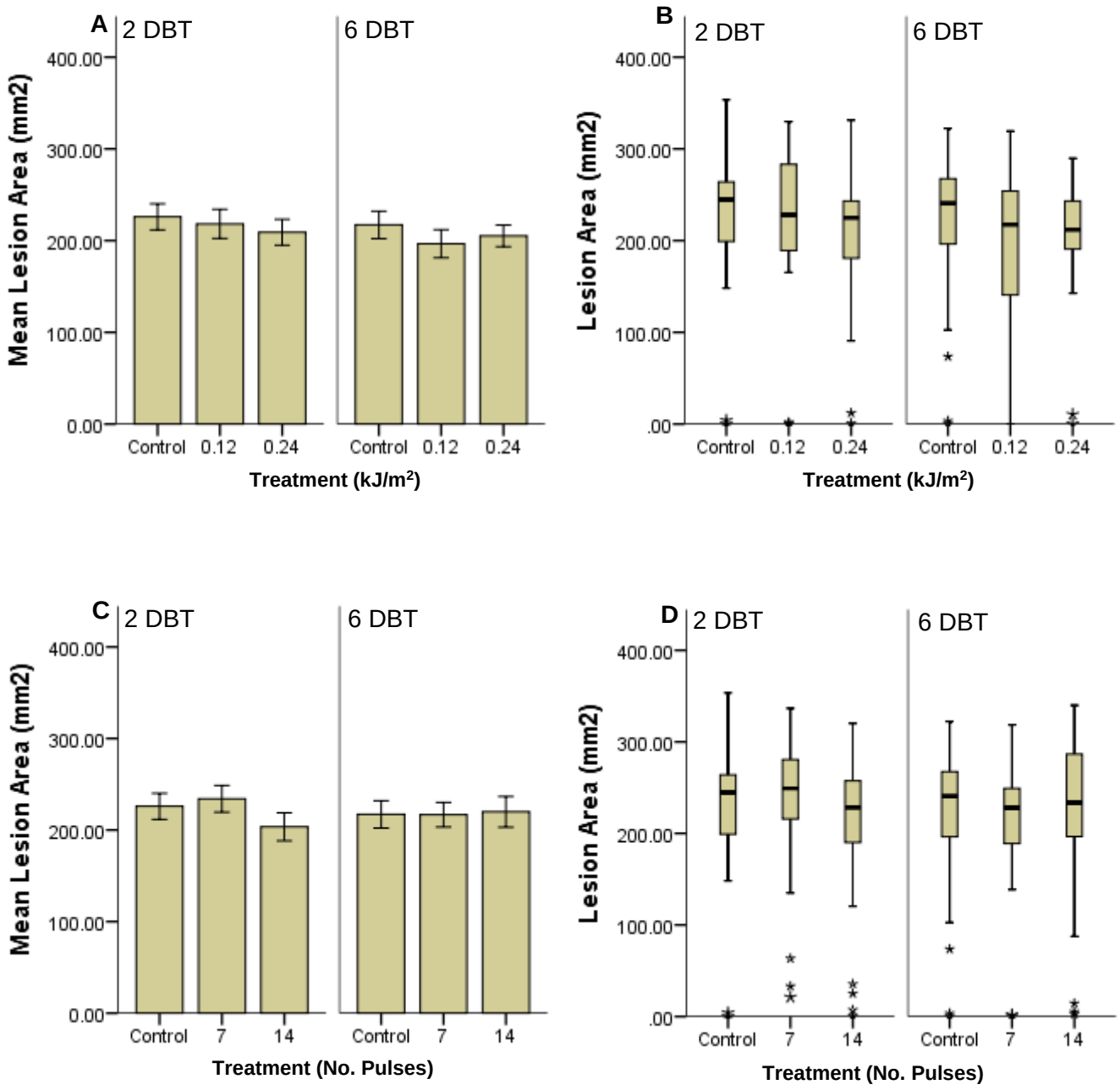

Figure 55. The combined experimental data from three replicate experiments utilising multiple low level treatments of the variety Temira. Experimental replicates were performed during March, April and May 2016. Plants were inoculated at 2 days post treatment with 4mm *R. solani* agar plugs and lesions 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.

The failure to significantly reduce disease here is not unexpected. If we look at the data from the experimental replicates the most effective treatment regime, be that 2, 4 or 6 DBT, showed variation between each replicate experiment. For example, Temira plants treated with the conventional source showed the most successful DBTs were 4, 6 and 2 for the replicates in March, April and May respectively, Table 12. Moreover, 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 10.

Interestingly it is also clear that single treatments performed with high doses that weren't damaging at any point of the year also showed little promise to reliably reduce 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 10. For plants treated with the conventional UV-C 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 11.

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 12. 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 13.

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 6-8 true leaves and inoculated at 2 DPT with 4 mm 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 10. For conventional UV-C treatments the largest reduction in lesion area was observed for the 0.12 kJm^2 treatment at 2.4 %, Table 11. For Temira plants the 0.12 kJm^2 conventional treatments showed the greatest reduction in lesion area at 7.3 %, Table 13. The pulsed treatments showed the greatest success with the 7 pulsed treatment which showed a 1 % reduction in mean lesion area, Table 12

Table 10. 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 optimal treatment dose, days between treatment and percentage disease reduction

Month	Date	No. true leaves	No. of treatments	Single treatment disease reduction (%)	Optimal disease reduction		
					Treatment (kJm^2)	Days between treatments	Disease 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.

Table 11. 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 optimal treatment dose, days between treatment and percentage disease reduction.

-Denotes that results were not collected.

Month	Date	No. true leaves	No. of treatments	Single treatment disease reduction (%)	Optimal disease reduction		
					Treatment (pulses)	Days between treatments	Disease 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

Table 12. 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 optimal treatment dose, days between treatment and percentage disease reduction.

Month	Date	No. true leaves	No. of treatments	Single treatment disease reduction (%)	Optimal disease reduction		
					Treatment (kJm^2)	Days between treatments	Disease 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 13. 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 optimal treatment dose, days between treatment and percentage disease reduction.

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

Month	Date	No. true leaves	No. of treatments	Single treatment disease reduction (%)	Optimal disease resistance		
					Treatment (pulses)	Days between treatments	Disease 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

Discussion

Pre-harvest conventional UV-C and pulsed polychromatic treatments have shown a mixed ability to reduce disease levels in preliminary trials. Reduced levels of disease progression have been observed for a number of pathogens including *B. cinerea*, *R. solani* and *S. sclerotinia*. Levels of disease reduction have varied from 2-54 % and have shown to last approximately 8 days. Choosing the correct treatment, however, may not be without its complications. Firstly, preliminary studies show there may be a difference in the levels of disease reduction which can be achieved for specific cultivars. It may, therefore, not be a viable choice for all cultivars and some in fact may show increased susceptibility to disease. Further investigation, however, is required. Furthermore, the use of UV-C and polychromatic treatments should be optimised for each cultivar across an entire growing season to avoid any damage to crops. This is due to large variation in the point at which damage is caused by treatments across the growing season with a drastic drop from October onwards.

Preliminary work also appears to show that the specific treatments that elicit reductions in disease also appear to change across the growing season. This appears to be in line with an increase in the number of hours of daylight. Data to support this hypothesis, however, was not collected in the glasshouse. Treatments appear to be further complicated as optimal treatments also appear to change with the pathogen undergoing investigation. Successful treatment ranges for specific pathogens, however, tend to overlap and it could, therefore, be possible to choose a treatment that would allow work against a broad range of pathogens.

If the variation that has been identified in this preliminary work is supported by further investigations then choosing the correct treatment will ultimately be determined by the point during the year, the cultivar being grown and potentially the pathogens that pose the greatest risk. It should also be noted that further investigation into how environmental conditions may change the plants response to treatment is required. A plants response to both biotic and abiotic factors are highly linked and it could therefore be hypothesised that changes in the plants homoeostatic processes due to, for example, high salinity could lead to an adverse response to treatment and potentially an increase in susceptibility of disease.

Finally, multiple 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 can therefore be stated that the low-level repeated treatments tested here are not as effective as some of the single, higher dose treatments that have been performed in preliminary trials. It is therefore recommended that research is focused on investigating appropriate single treatments.

Conclusions

Post-harvest fruit treatments:

- The high intensity, pulsed polychromatic light 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 conventional UV-C treatment of 3.7 kJm^2.
- High intensity pulsed polychromatic light sources can reduce treatment times by 97.3 %.
- Both conventional UV-C and pulsed polychromatic treatments elicited local disease resistance and delayed ripening when treating fruit from the side.

Pre-harvest foliar treatments of lettuce:

- Preliminary work shows:-
- Single treatments showed 2-54 % reductions in disease.
- Reductions are observed for approximately 8 days following treatment
- Both pulsed and conventional treatments cause damage to crops at differing levels throughout the year.
- Optimal treatments may change depending on the time point during the year.
- Optimal treatments may change depending on cultivar undergoing investigation.
- Optimal treatments may change depending on the pathogen of interest.
- Repeated treatments utilising doses that are not damaging at any point of the year do not successfully reduce disease progression.

Future work:

- Completion of the investigation into optimal treatments for lettuce across the year.
- Completion of molecular comparison of post-harvest pulsed and conventional treatment induced changes to gene expression of tomato fruit.
- Further investigation into post-harvest treatment of ripe fruit, including high sugar varieties, and its induced resistance against spoilage pathogens.
- Investigation into the necessity for UV-C in the high intensity pulsed polychromatic light source.
- Conventional UV-C seed treatments for the induction of resistance.

Knowledge and Technology Transfer

Project meetings:

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

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.

Publications:

- AHDB Grower; "A Little Light Goes a Long Way", May 2016
- Postharvest Biology and Technology; "A Comparison of Low Intensity UV-C and High Intensity Pulsed Polychromatic Sources as Elicitors of Hormesis in Tomato Fruit, *Solanum lycopersicum*", May 2016 [under review].

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