Hormetic UV treatments for control of plant diseases on protected edible crops

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Abstract

Hormesis is a dose response phenomenon where low doses of a stress bring about a positive response in the organism undergoing treatment. UV-C hormesis has been known for over three decades and has a broad range of benefits on postharvest produce. Benefits include increased nutritional content, delayed chlorophyll degradation and disease resistance. The beneficial effects have been observed on many varieties of fresh produce including climacteric and non-climacteric fruit, tubers, salads and brassicas. The majority of previous studies have used low-intensity (LIUV) UV-C sources. LIUV sources require lengthy treatment times, which are in the region of 6 minutes for tomato fruit. This has, in part, prevented the commercial application of this technique. High-intensity, pulsed polychromatic light (HIPPL) sources, however, have recently been developed. HIPPL sources may have the potential to drastically reduce treatment times and increase their commercial viability.

It was shown, here, that the use of HIPPL can control disease (reduce disease progression) caused by *Botrytis cinerea* and *Penicillium expansum* and also delay ripening on tomato fruit. Both disease control and delayed ripening were at similar levels for LIUV and HIPPL treatments on mature green fruit. The HIPPL treatments used in these studies can reduce treatment times for tomato fruit by 97.3%. Both HIPPL and LIUV treatments elicit local responses irrespective of the treatment orientation and tomato fruit, therefore, require full surface irradiation. Furthermore, UV-C in the HIPPL source is not required for disease control or delayed ripening. It does, however, contribute approximately 50% towards the total observed effects.

Investigations into the mechanisms underpinning postharvest HIPPL and LIUV hormesis, on tomato fruit, identified that the expression of genes involved in plant hormone biosynthesis, defence, secondary metabolism and ripening were affected. This indicates that disease control is achieved through induced resistance. Changes to expression, following treatment, were highly similar for both HIPPL and LIUV treatments and were mediated by salicylic acid, jasmonic acid and ethylene. This may lead to broad range resistance against necrotrophic and biotrophic pathogens as well as abiotic stresses and herbivorous pests.

Recently, the exposure of foliage to UV-C has been shown to induce resistance against *B. cinerea* on *Arabidopsis thaliana*. The horticultural applications of such treatments, however, have not been explored. Pre-harvest treatments of lettuce in the glasshouse showed variation in damage threshold and optimal treatment to control disease following LIUV and HIPPL treatment. Further sources of variation included the cultivar, pathogen of interest and the point that treatment was applied during the year. Using a controlled environment allowed seasonal variation to be mitigated and both HIPPL and LIUV treatments controlled disease against *B. cinerea*. For pre-harvest treatments to be a success in the glasshouse, further studies into how both biotic and abiotic factors influence treatment is required.

To circumvent the problems associated with pre-harvest treatments and environmental variation in the glasshouse, LIUV seed treatments were performed on tomato. Control of *B. cinerea* was established with an approximately 10% reduction in incidence and disease progression with a 4 kJ/m^2 treatment. When monitoring the effect of treatment on germination and early seedling development it was also identified that an 8 kJ/m^2 treatment led to biostimulation of germination and root and shoot growth.

Key Words: Hormesis; induced resistance; high-intensity, pulsed polychromatic light; UV-C; disease control; biocontrol.

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List of Abbreviations

ABA	Abscisic acid
AUX	Auxin
AzA	Azelaic acid
BS*	Brassinosteroids
CHP	Combined heat and power
CYK*	Cytokinins
DAMP	Damage associated molecular pattern
DFT	Deep flow technique
DPI	Days post inoculation
DPG	Days post germination
DPP	Days post planting
DPT	Days post treatment
EF-Tu	Elongation factor thermo unstable
ET	Ethylene
ETI	Effector triggered immunity
FAO	Food and Agriculture Organisation of the United Nations
FAOSTAT	Food and Agriculture Organisation of the United Nations Statistics Division
G3P	Glycerol-3-phosphate
GA	Gibberellic acid
HPI	Hours post inoculation
HPT	Hours post treatment
HR	Hypersensitive response
ISR	Induced systemic resistance
IRE	Independent replicate experiment
JA	Jasmonic acid
JA-Ile	Jasmonate-isoleucine
MAPK	Mitogen-activated protein kinase
MeJA	Methyl-jasmonate
MeSA	Methyl-salicylate
NFT	Nutrient film technique
PA	Polyamines
PAMP	Pathogen associated molecular pattern
PCD	Programmed cell death
PCR	Polymerase chain reaction
PGRR	Plant growth regulating rhizobacteria
PPB	Potassium phosphate buffer
PR protein	Pathogenesis related protein
PRR	Pathogen recognition receptor
PTI	PAMP triggered immunity
qPCR	Quantitative-polymerase chain reaction
QTL	Quantitative trait loci
RNAi	RNA interference
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SA	Salicylic acid
SAR	Systemic acquired resistance
SDW	Sterile distilled water

*Cytokinins and brassinosteroids are commonly abbreviated to CK and BR, respectively.

Chapter 1: Introduction

The global population is set to reach 9 billion in 2050. It is estimated that currently 805 million people are undernourished (FAO, 2014). The situation, however, is improving with 205 million more people living with proper nourishment in comparison to 1991, (FAO, 2014). Every year, however, it is estimated that 30% or 1.3 billion tonnes of the food produced is discarded; this figure includes both fresh and processed foods (FAO, 2014; Winkworth-Smith & Morgan, 2014).

In the UK alone 45% of all purchased salad and 26% of fruit are wasted (WRAP, 2012). The Courtauld commitment, a government run scheme to reduce household food waste, has failed to achieve its goal of reducing household food waste by 5% between 2012 and 2015. Instead, a 4.4% rise in household food waste has been observed (WRAP, 2015). In the developed world, these losses can be attributed to a number of factors including a general practice of discarding fresh produce in line with the use before dates, impulse buying of more produce than is required, poor planning of meals and the decay of fresh produce. In the UK, household food waste accounts for 70% post-farm-gate food waste (WRAP, 2017). Sixty percent of this waste has been identified as avoidable and costs the average household £720 per annum (WRAP 2017). Loss of produce, however, can occur both pre- and post-harvest.

Pre-harvest losses are caused by two key factors. The first of which are poor horticultural techniques including mechanical damage to fruit and inappropriate supply of nutrients causing physiological disorders such as blossom-end rot of tomato**.** The second factor is that of plant pathogens. Plant pathogens can not only directly affect yield through fruit rots such as grey mould of tomato caused by *Botrytis cinerea* but can also indirectly reduce yield due to a reduction in plant vigour. As a whole, plant pathogenic organisms can account for crop-dependent pre and postharvest losses of 8 to 15% per annum (Oerke, 2006). This thesis will be focused on the pre- and post-harvest losses caused by plant pathogens with specific application to tomato and lettuce production within the protected edibles sector of the UK's horticultural industry. The following research will focus on the use of low-intensity UV-C (LIUV) and high-intensity, pulsed polychromatic light (HIPPL) to control disease both pre- and postharvest. Disease control, here, is defined as a reduction in disease progression following a successful inoculation or a reduction in the incidence of disease (the number of successful inoculations).

1.1 Tomatoes

Tomato (*Solanum lycopersicum*) is a solanaceous perennial plant that originates in the Andes of South America (Sims, 1980). Wild varieties of tomato are observed growing in a variety of conditions from 1 m to 3200 m above sea level and are comprised of a number of morphologically distinct species (Peralta & Spooner, 2006). It is perhaps the tomato's ability to survive in a plethora of conditions that has led to its domestic success. Until recently the domestic origin of tomato was under debate with its origins of domestication believed to be in either in Mexico or Peru (Peralta & Spooner, 2006). Domestication is now believed to have been a two-step process which began in Ecuador and Northern Peru and then migrated to Mesoamerica where domestication was completed (Liang *et al.,* 2017).

The wild ancestor of modern domestic cultivars is believed to be *S. lycopersicum* var. *cerasiforme,* or the wild cherry tomato (Peralta & Spooner, 2006). The first domesticated species, however, would have been different as tomatoes were initially cultivated as an ornamental as it was feared that the fruit was toxic (Costa & Heuvenlink, 2005). Tomatoes were first introduced into Europe in the 16th century but it was not until the 18th century that tomato consumption became widespread across Europe (Peralta & Spooner, 2006).

The economic importance of the humble tomato has risen dramatically from its ornamental beginnings to the culinary staple it is today. In 1961 tomato had a global gross production value of 12.7 billion US dollars in contrast to that of 62.2 in 2013 (FAOSTAT, 2015). This has been seen alongside an increase in annual per capita production increasing from 9.0 kg in 1961 to 23.0 in 2013 setting them as the 10th most economically important non-meat commodity (FAOSTAT, 2015).

1.1.1 Tomato production in the UK

The UK is the 78th largest producer of tomatoes; producing 98,500 tonnes in 2014 (FAOSTAT, 2017). This is in contrast to the top three producers China, India and the USA who produced 53, 19 and 15 million tonnes respectively (FAOSTAT, 2017). In 2014 the UK, however, produced the third greatest yield at 468 tonnes/Ha only behind that of the Netherlands and Belgium at 557 and 549 tonnes/Ha, respectively (FAOSTAT, 2017).

The outdoor growing season for tomatoes runs between July and October due to short hours of daylight and adverse weather conditions (The British Tomato Growers Association, 2013). Commercial tomato production in the UK is, therefore, undertaken under protection which allows year-round production. The high start-up costs of commercial greenhouses

has led to the adaptation of growing techniques that are more sustainable from both a business and environmental perspective. The first of these is the utilisation of soilless cultivation (Raviv & Leith, 2008). Soilless cultivation is achieved by the use of a substrate on which the plant is rooted and supplied nutrients to via the application of liquid nutrient solution. For fruiting vegetable production nutrients are supplied via drip irrigation which allows tight regulation of nutrient supply (Fig. 1.1A). The adaptation of soilless systems not only allowed the regulation of nutrient supply but also reduced the high incidence of soil-borne pathogens observed when intensively growing a single crop in soil year after year.

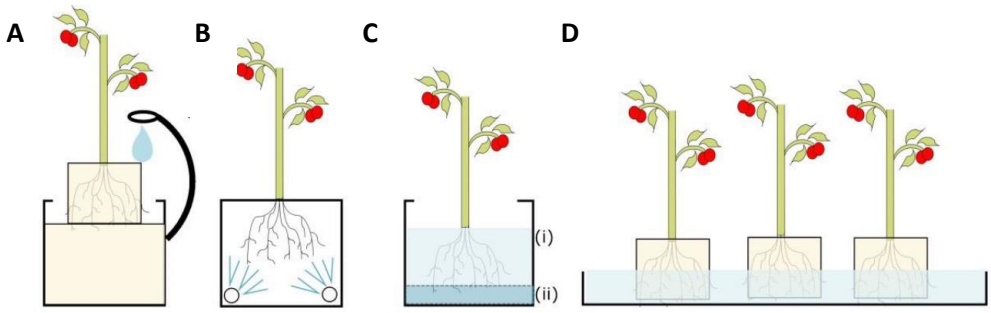

Figure 1.1: Representation of irrigation techniques utilised within soilless growing systems. **A)** Drip irrigation of plant placed on a rockwool cube (top) and rockwool slab (bottom). **B)** Aeroponic irrigation; plants are suspended above a box within which the roots are housed and constantly fed with a fine mist of nutrient solution. **C)** Deep Flow Technique (i) and Nutrient Film Technique (ii) where plants are suspended on a buoyant material such as polystyrene and their roots are fully immersed in nutrient solution or nutrients are supplied as a film of water along the base of the gutter housing, respectively. **D)** Ebb and Flood system in which plants are grown on a potted substrate within an area that is flooded and subsequently drained.

The second major advance in greenhouse tomato production was the integration of combined heat and power (CHP) systems. CHP is achieved through the running of an on-site combustion generator often powered by the burning of household waste. CHP has been shown to improve yield by 22 to 55% (Compernolle *et al.*, 2011). The generator is first used to power supplementary lighting during times of sub-optimal light. Heat produced during combustion is stored in water pipes and used during cold periods. Finally, CO_2 is extracted from the exhaust fumes, collected and pumped into the greenhouse which has led to a 30% decrease in CO_2 emissions (Raviv & Leith, 2008). It is the combination of soilless cultivation systems grown under protection with advanced CHP systems along with high yield tomato cultivars that has led to the much-improved yield levels seen in the UK. Furthermore, commercial tomato producers now predominantly use grafted plants with vigorously

growing and hardy rootstocks that ensures good nutrient retrieval and pathogen protection (O'Neil, 2014).

1.1.2 Diseases of tomato prevalent in the UK

There are a number of diseases affecting tomato crops in the UK. The majority of diseases, however, do not commonly affect the foliage or fruit of plants grown under protection. Of the fungal and oomycete pathogens (Table 1.1), only *Botrytis cinerea*, *Oidium neolycopersici* and *Passalora fulva* commonly cause problems to fruit and foliage in the UK. The remainder of the pathogens that occur under protection such as *Colletotrichum coccodes, Didymella lycopersici, Rhizoctonia solani* and *Sclerotinia sclerotiorum* are polyphagous and more commonly observed as stem and root rots. Bacterial diseases are much less common than their fungal and viral counterparts. Only three bacterial diseases occur in the UK and all are uncommon (Table 1.2).

Table 1.1: Fungal and oomycete diseases affecting tomato crops in the UK. Information adapted from Blancard, (2012)

Disease	Causative agent	Feature effected	Distribution	Disease severity	Prevalence under protection
Anthracnose / Black Dot	*Colletotrichum coccodes*	Fruit and Roots	Worldwide	Severe	Common
Canker and Fruit Rot	*Didymella lycopersici*	Stem, Fruit and Foliage	Europe	Highly variable	Common
Damping off/ Fruit Rot	*Rhizoctonia solani*	Fruit and Roots	Worldwide	-	Common
Early Blight	*Alternaria tomatophila*	Stem, Fruit and Foliage	Worldwide	Severe	Uncommon
Grey Mould	*Botrytis cinerea*	Stem, Fruit and Foliage	Worldwide	Severe	Common
Late Blight	*Phytophthora infestans*	Stem, Fruit and Foliage	Worldwide	Severe	Uncommon
Powdery Mildew	*Oidium neolycopersici*	Fruit and Foliage	Worldwide	Considerable	Common
Sclerotinia Drop	*Sclerotinia sclerotiorum*	Stem, Fruit and Foliage	Worldwide	-	Common
Tomato Leaf Mould	*Passalora fulva*	Fruit and Foliage	Worldwide	Considerable	Common

Table 1.2: Bacterial diseases affecting tomato crops in the UK. Information adapted from Blancard, (2012).

Disease	Causative agent	Feature effected	Distribution	Disease severity	Prevalence under protection
Bacterial Speck	*Pseudomonas syrinage*	Fruit and Foliage	Worldwide	Severe	Uncommon
Soft Rot	*Erwinia caratovora*	Fruit and Stem	Worldwide	-	Uncommon
Tomato Pith Necrosis	*Pseudomonas corrugata*	Fruit and Stem	Europe, USA, New Zealand	-	Absent

A number of viral pathogens exist within the UK (Table 1.3). The majority are, however, uncommon for plants grown under protection due to a number of factors, the first of which is the use of resistant cultivars. Nearly all modern cultivars have been bred for resistance against the two closely related viruses, tobacco and tomato mosaic virus, which can be easily spread by physical contact. Resistance against pepino mosaic virus, however, has not been achieved and this remains one of the largest problems facing the UK tomato industry today (Pearson, 2015). The remaining viral pathogens, with the exception of tomato spotted wilt virus, are all uncommon as the greenhouse not only provides a physical barrier to the vectors that carry viruses but also creates a geographical separation from any sources of inoculum from wild or outdoor grown crops.

Table 1.3: Viral diseases affecting tomato crops in the UK. Information adapted from Blancard, (2012).

Viral Agent	Vector	Distribution	Disease severity	Prevalence under protection
Alphalpha Mosaic Virus	Aphid	Worldwide	-	Uncommon
Cucumber Mosaic Virus	Aphid	Worldwide	Severe	Uncommon
Pepino Mosaic Virus	Physical contact	Worldwide	Severe	Common
Potato Leaf Roll Virus	Aphid	Potato Growing Regions	Severe	Absent
Potato Virus X	Physical contact	Potato Growing Regions	Severe	Uncommon
Potato Virus Y	Aphid	Potato Growing Regions	Severe	Uncommon
Tomato Aspermy Virus	Aphid	Chrysanthemum growing regions	Minor	Absent
Tomato Blackening Virus	Nematode	Europe	-	Absent
Tobacco Mosaic Virus	Physical contact	Worldwide	Severe	Uncommon
Tomato Mosaic Virus	Physical contact	Worldwide	Severe	Uncommon
Tomato Spotted Wilt Virus	Thrips	Worldwide	Severe	Common
Tobacco Streak Virus	Thrips	Worldwide	-	Absent

1.2 Lettuce

Lettuce (*Lactuca sativa*) is the most important of the leafy vegetables and a member of the family Asteracea, the largest of the dicotyledonous families (Judd *et al.*, 1999; Davies,

2009). Its origins are believed to be in the Middle-East and the first evidence of cultivation was observed in ancient Egyptian tomb paintings of stem lettuce dated to 2500 BC (Harlan, 1986; Ryder, 1999; Ryder, 2002). By the 5th century the consumption of stem lettuce had moved to China but it was not until the 15th century that it reached Europe (Ryder, 2002).

There are six edible forms of lettuce; Butterhead, Crisphead, Latin, Leaf, Romaine and Stem, alongside oilseed lettuce which is used in oil production (Ryder, 1986; Ryder, 2002). Until the 1970s the production of different lettuce forms was isolated to specific regions, with Northern Europe growing mainly Butterhead and Crisphead, the Mediterranean regions growing Romaine and Asia the Stem form (Ryder, 2002). With increased accessibility of international travel in the 1970s the popularity of all forms of lettuce expanded and it is now observed as a staple in our everyday diets from the ever-familiar Iceberg to the more extravagant Lollo Rossa. Global lettuce production has seen nearly a 3-fold increase in production between 1961 and 2013 with production at 24.8 million tonnes. Per capita production, however, has only seen an increase from 2.1 to 3.5 kg in 1961 and 2013, respectively (FAOSTAT, 2015). All FAOSTAT data for lettuce also includes the data for chicory.

1.2.1 Lettuce production in the UK

The UK was the 14th largest producer of lettuce in 2014 producing 135,500 tonnes at a yield of 23.4 tonnes/Ha- the 38th greatest yield. The largest three yields were produced by Belgium, Democratic Republic of the Congo and the Congo at 44.7, 49.4 and 43.3 tonnes/Ha in 2014, respectively (FAOSTAT, 2017). Lettuce production in the UK, in contrast to that of tomato, is a very different story. Approximately 90% of all lettuce is field-grown utilising the hardier Iceberg and Romaine varieties; the remaining crops are mostly grown under protection in soil with only a small percentage grown on soilless systems (Bean, 2015). This explains the contrast observed in the UK total production and yield positions as although the UK is one of the top producers it is investing large areas to return relatively small yields.

The soilless systems utilised also differ from tomato with mainly the Deep Flow Technique (DFT) being utilised in the UK and Nutrient Film Technique (NFT) being utilised throughout Europe (Bean, 2015). Examples of soilless systems can be seen in Figure 1.1C. DFT systems are favoured in the UK due to the more erratic weather patterns. The immersion of the

plants roots in DFT systems buffers the roots from any sudden changes in the root environment (Bean, 2015).

Crops grown under protection in the UK are mostly comprised of Butterhead and loose-leaf varieties (British Leafy Salad Association, 2015). Moreover, unlike tomato no supplementary lighting is used and only small levels of heat are applied to the greenhouses (Bean, 2015). The cultivars grown, however, are season-dependent and respond to the length of light exposure with certain cultivars growing more vigorously in the colder winter months than others. Furthermore, cultivar dependent light exposure can lead to bolting; the initiation of the flowering stage, which leads to a bitter tasting product. The correct winter and summer varieties, therefore, have to be grown during their respective seasons.

1.2.2 Diseases of lettuce prevalent in the UK

Lettuce crops in the UK have four main fungal and oomycete pathogens that cause both severe disease and economic impact (Table 1.4). These include *B. cinerea, Bremia lactucae, Rhizoctonia solani* and *Sclerotinia sclerotiorum*, all of which affect both field-grown and protected crops with the exception of *R. solani* which predominantly affects crops grown under protection (McPherson, 2013). As *S. sclerotiorum* is soil-borne, reduced losses on soilless systems may be observed due to the removal of their natural niche. Soilless systems, however, as stated in section 1.2.1 comprise only a small percentage of the crops grown in the UK making the diseases worthy of study in this project. The first outbreak of lettuce wilt and root rot (*Fusarium oxysporum* f.sp. *lactucae* race 4) has recently been recorded in the UK (AHDB, 2017). As there are no known treatments or varietal resistance that provide protection against the aggressive race 4 pathogen this may be a major concern for growers (AHDB, 2017).

Bacterial pathogens of lettuce appear to be more diverse than those of tomato with the crop being vulnerable to five potential diseases in the UK (Table 1.5). Bacterial pathogens, however, predominantly affect crops in warmer climates that allow more rapid proliferation of the bacterial populations and for this reason outbreaks are rare in the UK. Bacterial diseases may become more prevalent within the UK as global climate change continues to progress.

Viral pathogens of lettuce, as with tomato, although generally causing severe disease and are both highly diverse and have a wide geographical spread, seem to be mainly restricted to crops grown in the field (Table 1.5). Tomato spotted wilt and lettuce big vein viruses,

however, both cause severe disease and can be observed on protected lettuce crops within the UK.

Table 1.4: Fungal and oomycetes diseases of lettuce crops in the UK. Information adapted from Blancard, (2006)

Disease	Causative agent	Feature effected	Distribution	Disease severity	Prevalence under protection
Anthracnose	*Microdochium panattonianum*	Leaves	USA/Europe/Australia	Minor	Uncommon
Damping Off/Bottom Rot	*Rhizoctoia solani*	Leaves and crown	Worldwide	Severe	Common
Downy Mildew	*Bremia lactucae*	Leaves	Worldwide	Severe	Common
Grey Mould	*Botrytis cinerea*	Leaves and crown	Worldwide	Severe	Common-increased severity
Mycocentraspora leaf spot	*Mycocentrospora acerina*	Leaves	Worldwide-Rare in the UK	Minor	Uncommon
Powdery Mildew	Erysiphe cichoracearum	Leaves	USA/Europe/ E. Asia	Minor	Absent
Sclerotinia Drop	*Sclerotinia sclerotiorum*	Leaves and crown	Worldwide	Severe	Yes
Septoria Leaf Spot	*Septoria lactucae*	Leaves	Worldwide	Minor	Uncommon

Table 1.5: Bacterial diseases of lettuce crops grown in the UK. Information adapted from Blancard , (2006)

Disease	Causative agent	Feature effected	Distribution	Disease severity	Prevalence under protection
Bacterial Leaf Spot/Head Rot	*Xanthomonas campestris* pv. *vitians*	Leaves	Worldwide	Severe	Yes
Corky Root	*Rhizomonas suberfaciens*	Stem or Roots	USA/Australia/New Zealand/ Europe	Minor	Yes
Marginal Leaf Spot	*Pseudomonas marginalis*	Leaves	Europe/India/USA	Minor	Yes
Soft Rot	*Erwinia caratovora*	Stem or Roots	Worldwide	Severe	Yes
Swingle	*Pseudomonas cichorii*	Leaves	Worldwide	-	Yes

Table 1.6: Viral diseases of lettuce crops grown in the UK. Information adapted from Blancard, (2006)

Viral Agent	Vector	Distribution	Disease severity	Prevalence under protection
Lettuce Mosaic	Aphid	Worldwide	Severe	Uncommon
Cucumber Mosaic	Aphid	Worldwide	Severe	Uncommon
Beet Western Yellow	Aphid	USA/Europe	Severe	Uncommon
Alfalfa Mosaic	Aphid	Worldwide	Severe	Uncommon
Broad Bean Mosaic	Aphid	Japan/Australia/UAS/N.Europe/Turkey	Minor	Uncommon
Dandelion Mosaic	Aphid	N. Europe/China	-	Uncommon
Turnip Mosaic	Aphid	Worldwide	Minor	Uncommon
Endive Necrotic	Aphid	Europe	Minor	Uncommon
Tomato Spotted Wilt	Thrip	Worldwide	Severe	Common
Lettuce Big Vein	Fungi	Europe and Oceania	Severe	Common

1.3 Plant disease

Plant disease can be caused by both biotic and abiotic factors, the latter of which are outside the scope of the study described here. Biotic agents of disease come from at least two domains of life; the Bacteria and Eucaryota. Within the Eukarya there are three distinct causes of disease the first of which are predatory nematodes. Members of the *Meloidogyne* species of nematodes, for example, cause Root-knot disease; a galling of the roots which drains nutrients and reduced plant vigour (Mitkowski & Abawi, 2003). An example of Root-knot symptoms can be seen in Figure 1.2A. The second and third agents of disease are members of the fungal kingdom and class Oomycota who can cause a plethora of diseases from necrotrophic moulds such as grey mould to yield reducing biotrophic diseases such as the powdery and downy mildews (Figures 1.2B, C and D, respectively). Prokaryotes cause many diseases from soft rots caused by *Pectobacterium carotovora* to crown gall caused by *Agrobacterium tumefaciens* (Figures 1.2E and F, respectively). Bacterial diseases, however, are less common than fungal or viral diseases and tend to cause less damage and economic losses (Kennedy & Alcorn, 1980). The third domain of life the Archaea harbours no known true pathogens of either plants or mammals (Gill & Brinkman, 2011). Further to the aforementioned pathogens, there are also viral and sub-viral viroid pathogens that shall be classified, here, as biotic factors.

Viral pathogens are by definition obligate parasites and require their hosts for replication, and often an intermediate host or "vector" for transmission between plants due to a highly-restricted genome that only encodes a few proteins. Tobbaco mosaic virus, was the first virus to be described in 1892 and there are now over 4,000 recognised viruses, approximately 1,000 of which are associated with plants (Gergerich & Dolja, 2006). This number is believed to be a gross underestimate (Roossinck, 2012).

Viroids do not code for any proteins and consist only of a covalently closed RNA molecule. There are currently only 29 known viroid species most of which have a narrow host range affecting only 1 to 6 host species (Hammond & Owens, 2006). The potato spindle tuber viroid, however shows a broad host range of at least 138 species (Matsushita *et al.,* 2016).

There are a plethora of diseases that can affect plants. The diseases affecting specific crops, however, will vary between geographic regions and also the horticultural methods employed during cultivation. Here the focus will be on the foliar and fruit pathogens of tomato and lettuce.

Figure 1.2: Visual symptomology of a diverse range of plant pathogens. **A**) Gall-like symptoms of root-knot disease caused by nematode species *Meloidogyne incognita* on the roots of tomato (*Solanum lycopersicum*). **B**) The characteristic symptoms of grey mould caused by the necrotrophic fungal pathogen *Botrytis cinerea.* Image depicts *B. cinerea* during sporulation on fig (*Ficus carica*). **C**) A heavily sporulating powdery mildew infection on *Acacia magnium* **D**) Sporulating downy mildew (*Peronospora parasitica*) of collard (*Brassica oleracea*) with chlorotic and necrotic lesions. **E**) Soft rot on napa cabbage (*Brassica rapa*) caused by the bacterium *Pectobacterium carotovorum*. **F**) The distinctive galling caused by the bacteria *Agrobacterium tumefaciens,* the causative agent of crown gall disease on a white trumpet tree (*Tabebuia roseoalba*). All images are from www.flickr.com/photos/scotnelson and are held under a creative commons licence (CC BY 2.0). Image URLs and a written copy of permission can be found in Appendix 11.

1.4 Plant innate immunity

Unlike higher vertebrates, plants do not have an adaptive immune system whereby exposure of a naive host to a pathogen leads to the generation of antigen specific antibodies (Gust *et al.,* 2017). Plants, however, can successfully identify pathogens and produce an immune response that, like the mammalian system, can spread systemically and give resistance over a plant's lifetime and also between generations. This is achieved through innate immunity which recognises non-specific pathogen molecular patterns and is present in both plants and higher vertebrates and is a prerequisite to the adaptive immune system (Gust *et al.,* 2017). It is believed that every plant cell can initiate an innate immune response which is achieved through a multi-layered system that gives plants broad range resistance in a phenomenon called non-host resistance (Spoel & Dong, 2012; Bigeard *et al.,* 2015).

Plants are protected by two physical barriers, the cuticle and the cell wall, alongside constitutive production of antimicrobial compounds (Bigeard *et al.,* 2015). When these physical and chemical defences are breached, however, the plants immune system is activated. The first line of defence exhibited by plants is pathogen associated molecular pattern (PAMP) triggered immunity (PTI). PTI is triggered by the recognition of conserved PAMPs such as bacterial flagellin or fungal chitin at the plant cell wall. Recognition is achieved by transmembrane proteins that act as pattern recognition receptors (PRR) such as the flagellin-sensitive 2 PRR in *Arabidopsis thaliana* (Bigeard *et al.,* 2015). Pathogens, however, have evolved mechanisms to avoid recognition via the PTI system through the production of effector molecules that can interfere with the plants defence recognition, signalling and gene transcription. Similarly plants can also detect damage-associated molecular patterns (DAMPs). DAMPs are plant cellular debris that are produced following pathogen attack, their detection results in a response analogous to PTI (Boller & Felix, 2009; Yamaguchi & Huffaker, 2011; Gust *et al.,* 2017).

Through co-evolution plants have developed effector triggered immunity (ETI) and resistance genes (R-genes) to overcome the actions of effectors. R-genes can encode enzymes or receptors that either breakdown phytotoxins or directly and indirectly detect effector molecules (Mukhtar *et al.,* 2011). Unlike the majority of PTI induced defences, ETI often precedes the hypersensitive response (HR) which involves the production of a number of antimicrobial compounds including reactive oxygen species (ROS) followed by plant cell degradation and programmed cell death to prevent pathogen spread.

Following the activation of PTI or ETI, with the exception of HR and specific antimicrobial compounds, the plant's response is broad range and generic (Spoel & Dong, 2012). The response observed from ETI, however, is often stronger and longer-lasting (Tsuda & Katagiri, 2010). PTI and ETI are initially localised at the site of infection and lead to the upregulation of defence responses. These include pathogenesis related (PR) proteins such as β-1,3-glucanases which function to break down the fungal cell wall, secondary metabolites called phytoalexins that act as broad spectrum antimicrobial compounds, and reinforcement of cell walls via callose, lignin and suberin. Overlap between the observed effects of PTI and ETI have led to the distinction between them becoming increasingly vague, and it has been suggested that PTI and ETI should be viewed as early-extracellular and late-intracellular recognition of pathogens, respectively (Abramovitch *et al.,* 2006). In many instances the elicitation of local PTI and ETI leads to acquisition of resistance in systemic non-infected tissue.

1.4.1 Activation of local and systemic resistance

Following PAMP, effector or DAMP recognition there is a calcium influx into the cytosol that causes production of reactive oxygen and nitrogen species (ROS & RNS) and mitogen-activated protein kinase (MAPK) phosphorylation cascades that end in activation and nuclear localisation of transcription factors and the upregulated biosynthesis of hormones involved in the defence response (Tsuda & Katagiri, 2010; Bigeard et al. 2015) (Figure 1.3). There are three plant hormones, salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) which are considered to be the "backbone" of signalling within the plant's innate immune response (Vidhyasekaran, 2015). Recently, a group plant peptide hormones have been identified as secondary endogenous danger signalling molecules which have been named "phytocytokines" due to their similar function to cytokines (Gust *et al.,* 2017). SA, JA and ET have all been shown to play roles in the acquisition of both local and systemic resistance (Vidhyasekaran, 2015).

Signalling from SA, JA, and ET is likely to be a highly integrated response that relies on cross-talk between each of the hormones to produce a response tailored to the specific stress; antagonistic and synergistic responses between the hormones have both been reported (van Wees *et al.,* 2000; Kunkel *et al.,* 2002; Rojo *et al.,* 2003). It is considered that in general SA plays important roles in signalling against predominantly biotrophic pathogens, insect pests and abiotic stresses, and JA and ET act in responses against insect pests and necrotrophic pathogens (van Loon *et al.,* 2006; Spoel & Dong 2012). Their roles in

these responses, however, may not be as clearly defined as reported. Following recognition of the pathogen and biosynthesis of plant hormones, transcriptional activation of defence genes occurs giving rise to a local cellular defence response.

Plants then utilise their vascular system to transport immune signals from the site of local infection to uninfected tissue to initiate systemic resistance. This is achieved through the propagation of mobile signalling molecules azelaic acid (AzA), ET, glycerol-3-phosphate (G3P), jasmonate-isoleucin (JA-Ile) methyl-salicylate (MeSA) and methyl-jasmonate (MeJA) (Spoel & Dong, 2012; Vidhyasekaran, 2015). This signal is spread systemically whereupon reaching systemic tissue it induces a defence response mediated by SA, JA or ET. Furthermore, SA and JA along with ET elicit two different systemic responses known as systemic acquired resistance (SAR) and induced systemic resistance (ISR), respectively (Vidhyasekaran, 2015). ISR and SAR are involved in the priming of defence-related genes and production of pathogenicity-related proteins, respectively (van Loon *et al.,* 2006). AzA, G3P, DIR1 and MeSA are involved in the mobile signalling of SAR and ET, MeJA and JA-Ile signal for ISR (Spoel & Dong, 2012; Vidhyasekaran, 2015; Cameron *et al.,* 2017).

Furthermore, studies have shown a novel signalling pathway in plants (Miller *et al.,* 2009; Zimmermann *et al.,* 2009; Mittler *et al.,* 2011; Oyarce & Gurovich, 2011; Suzuki & Mittler, 2012). The pathway is induced by wounding and a number of abiotic stressors including light, salinity and temperature. The pathway relies on the production of ROS, specifically H_2O_2, in the apoplast. Signal transduction is self-propagating and spreads systemically through the apoplast dependent on the functioning of NADPH-oxidase homologue RbohD. The ROS-mediated signal can travel at 8.4 cm/min and 20.9 cm/s in *A. thaliana* and avocado, respectively (Miller *et al.,* 2009; Oyarce & Gurovich, 2011). RbohD, however, has also been shown to be directly mediated by PRRs EFR and FLS2 which recognise prokaryotic EF-Tu (elongation factor thermo unstable) and flagellin (Kadota *et* al., 2014). This indicates that the apoplastic production of ROS, stimulated by invading pathogens, may also spread systemically. Recent studies have shown that nitric oxide (NO) may also play a role in plant innate immunity and the response to pathogens. Yun *et al.,* (2016) reported that endogenous NO accumulation led to the loss of R-gene mediated resistance, basal resistance and defence against non-adapted pathogens in *Arabidopsis thaliana*. They also reported a suppression of SA signalling and SA-dependant gene expression.

There are a number of key plant hormones which interact to regulate the defence response in a manner which may relay specific information about the pathogen encountered and

mediate the appropriate systemic response (Spoel & Dong, 2012). The biosynthesis, signalling pathways and induced gene transcription mediated by the SA, JA and ET signalling pathways can be further regulated by a group of 5 hormones intimately involved in plant growth and development. These include abscisic acid (ABA), auxin (AUX), cytokinins (CYK) brassinosteroids (BS) and gibberellic acid (GA) which act in both antagonistic and synergistic manners and can enhance resistance or increase susceptibility (Vidhyasekaran, 2015). A summary of the interactions between SA, JA and ET and their regulators can be seen in Figure 1.4.

To conclude, defence signalling in plants is a highly-integrated process which is mediated by rapid ROS/RNS production in both the apoplast and cytosol, calcium-dependent MAPK phosphorylation cascades and hormone signalling that can lead to both local and systemic responses. The highly-integrated response to pathogens also shows overlap with the pathways through which plants manage their defences against abiotic factors, discussed further in section 1.5.4.

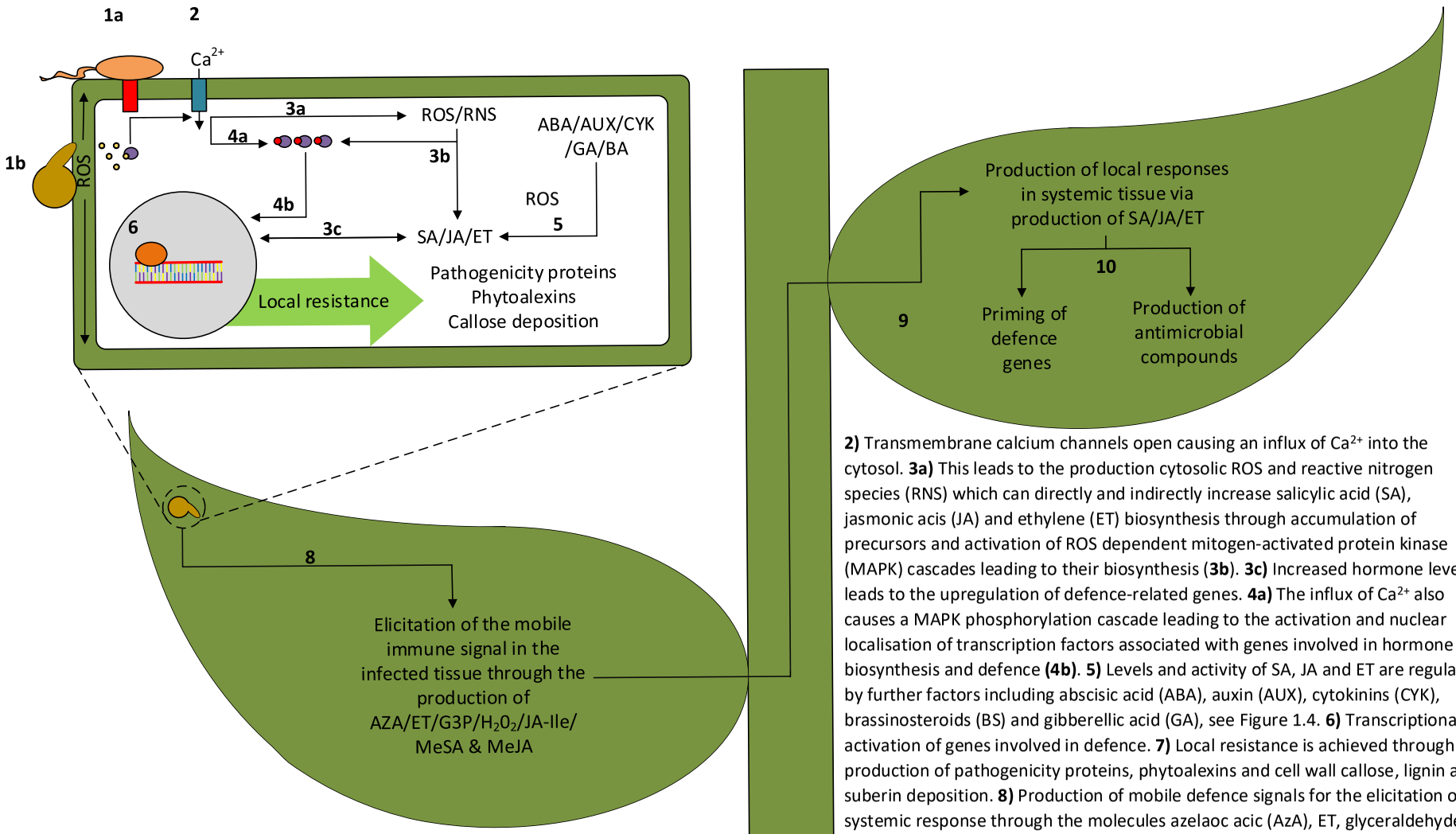

Figure 1.3: A simplified model of local and systemic induction of resistance. **1a)** A pathogen is recognised externally by a pattern recognition receptor (PRR) to elicit pathogen-associated molecular pattern triggered immunity (PTI). **1b)** Effector molecules secreted by a pathogen are recognised internally by a Resistance-gene (R-gene) encoded protein. Wounding of the plant also stimulates apoplastic systemic reactive oxygen species (ROS) signalling. **2)** Transmembrane calcium channels open causing an influx of Ca^{2+} into the cytosol. **3a)** This leads to the production cytosolic ROS and reactive nitrogen species (RNS) which can directly and indirectly increase salicylic acid (SA), jasmonic acis (JA) and ethylene (ET) biosynthesis through accumulation of precursors and activation of ROS dependent mitogen-activated protein kinase (MAPK) cascades leading to their biosynthesis (**3b**). **3c)** Increased hormone levels leads to the upregulation of defence-related genes. **4a)** The influx of Ca^{2+} also causes a MAPK phosphorylation cascade leading to the activation and nuclear localisation of transcription factors associated with genes involved in hormone biosynthesis and defence **(4b)**. **5)** Levels and activity of SA, JA and ET are regulated by further factors including abscisic acid (ABA), auxin (AUX), cytokinins (CYK), brassinosteroids (BS) and gibberellic acid (GA), see Figure 1.4. **6)** Transcriptional activation of genes involved in defence. **7)** Local resistance is achieved through production of pathogenicity proteins, phytoalexins and cell wall callose, lignin and suberin deposition. **8)** Production of mobile defence signals for the elicitation of a systemic response through the molecules azelaoc acic (AzA), ET, glyceraldehyde 3-phosphate (G3P), H_2O_2, jasmonic acid-isoleucine (JA-Ile), methyl-salicylate (MeSA) and methyl-jasmonate (MeJA). **9)** Mobile signals elicit SA, JA and ET responses in systemic tissue. **10)** Production of antimicrobial products and the priming of defence genes lead to systemic immunity. Information taken from Bigeard *et al.*, 2015; Spoel & Dong, 2012; Vidhyasekaran, 2015.

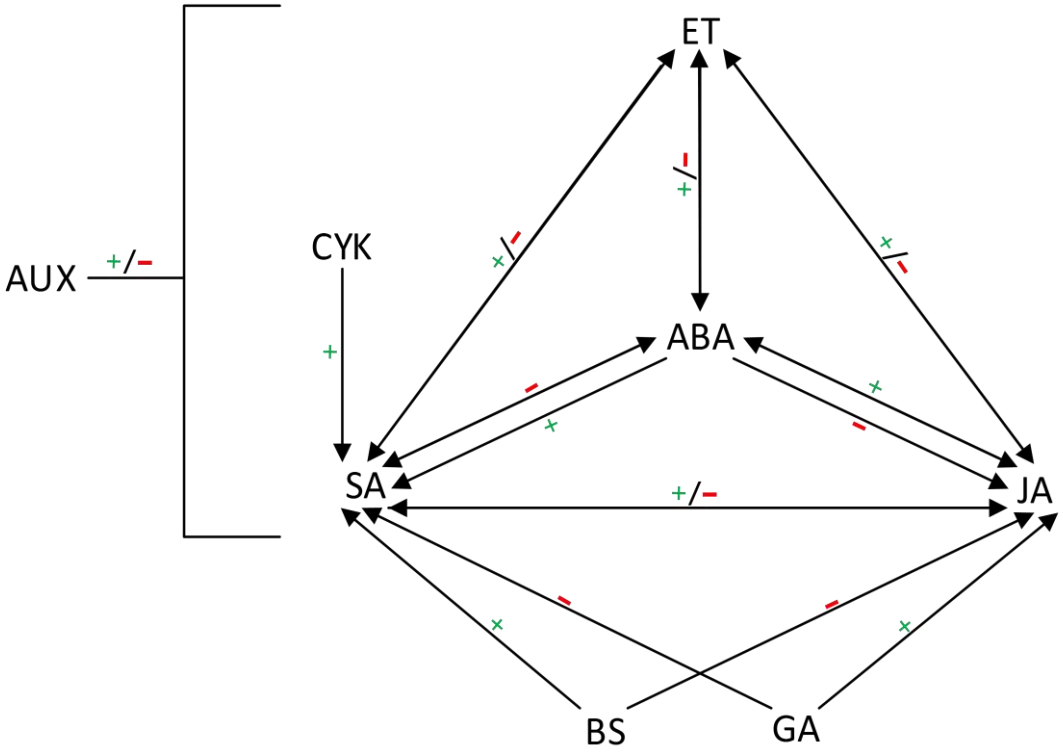

Figure 1.4: The regulation of the salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) plant-defence, hormone signalling backbone. Auxin (AUX) has shown to be correlated with every major factor involved in the plant immune response, excluding brassinosteroids (BS) and gibberellic acid (GA). SA, JA and ET can all regulate each other in either positive or negative directions. Abscisic acid (ABA) and ET can interact with each other in either a positive or negative fashion. ABA and JA can cooperate to achieve increased levels of JA induced resistance. ABA, however, can also negatively regulate the JA pathway to repress certain JA induced genes. ABA and SA can regulate each other in a negative fashion. ABA, however, can positively enhance SA mediated resistance. Cytokinins (CYK) have only been shown to enhance SA facilitated responses. BS and GA can mediate both SA and JA resistance. BS can enhance SA responses and restrict the JA. GA is capable of performing the opposite. All information was obtained from Vidhyasekaran, 2015; Spoel & Dong, 2012; van Loon *et al*., 2006.

1.5 Disease control

Plant disease control has traditionally been dependent on the breeding of resistance genes into commercial cultivars and application of chemical-based pesticides. In 2009, however, the European Commission set legislation to achieve the sustainable use of pesticides and set maximum residue levels for each of the commercially available control methods (European Commission, 2009). Their overall aim was to reduce chemical-based pesticides' impact on public health and the surrounding environment. The main actions of the legislation included the prohibition of aerial application of pesticides, the minimisation and banning of pesticide use in "critical" areas and the promotion of low-pesticide input and non-chemical management of disease (European Commission, 2009). This legislation saw an increase of funding into alternate methods of control which explored areas such as biocontrol and inducing disease resistance, discussed in sections 1.5.3 and 1.5.4 respectively. Such techniques may provide new solutions to the problems of crop

protection. Their success, however, requires more biological knowledge, they show more experimental variation and are more complicated to deploy (Bruce *et al.,* 2017).

1.5.1 Resistance breeding

Breeding for disease resistance and yield have been two of the main aims for commercial breeders with other consumer properties such as taste, colour and texture being only of secondary importance. Much of the observed resistance in current commercial cultivars has been achieved from either the introgression of resistance genes or loci from wild relatives and closely related species, utilisation of spontaneously occurring mutations in commercial cultivars or via mutagenesis treatment (Causse *et al.*, 2007). The wild relative of the tomato, *Solanum hirsutum,* has provided resistance to five pathogens including TMV and *O. neolycopersici*-the causative agent of powdery mildew (Blancard, 2015).

Breeding of diseases resistance, however, is time-consuming and often only provides monogenic resistance that pathogens can easily overcome. Breakdown of resistance is more likely to occur in intensive farming strategies that create an environment that is selective towards the breakdown of resistance. Durable resistance, however, has been achieved is some circumstances; this is far harder to achieve and in many instances relies on the introgression of numerous genes or quantitative trait loci (QTL) that cumulatively result in the resistant phenotype. The advances in molecular biology and genetics in recent years have allowed the plant breeding process to become more efficient allowing breeders to directly view the successful introgression of genes and QTL.

1.5.2 Chemical-based pesticides

Chemical-based pesticides vary in their modes of action from blocking metabolic pathways to the disturbance of cell walls. All pesticides, however, can be characterised by their movement through the plant and are either contact or systemic; remaining on the plant surface or spreading systemically though the plant, respectively. Furthermore, classification of fungicides also relies on their capacity to control disease either as a preventative treatment or an eradicant. Few eradicants, however, exist and most pesticides are applied as preventative treatments requiring regular applications of pesticides at differing intervals depending on their half-life.

Between 1940 and 1960 the heavy use of chemical pesticides coupled with high intensity farming led to key pathogens gaining pesticide resistance (van Lentern, 2000). The development of novel pesticides has been limited, primarily due to both the costly and

lengthy development process (van Lentern, 2000). Techniques, however, have been employed to reduce the number and frequency of pesticide applications. Disease forecasting better allows growers to apply the correct pesticides when certain diseases pose a greater risk and decide when an application may not be necessary. Disease forecasting, however, tends to be limited to diseases effecting crops that aren't grown under protection such as oilseed rape and wheat.

1.5.3 Biocontrol

The use of biocontrol to control disease is relatively new. The first biocontrol agent, *Agrobacterium radiobacter*, was released in 1979 to control crown gall disease (Junid *et al.,* 2013). The biocontrol agent, however, was closely related to the pathogen *A. tumefaciens*, the causative agent of crown gall and acquired the necessary pathogenicity plasmid that allowed it to cause disease. Such interactions require intense scrutiny to prevent the production of new pathogenic species.

Biocontrol agents act on the premise that a non-pathogenic or weakly pathogenic microorganisms can compete for a niche, predate, antagonise or form a mutualistic relationship with the plant and thus prevent disease occurring on the host plant (Pal & Mc Spadden-Gardner, 2006). A wide variety of biocontrol agents against plant diseases have been established in the laboratory, however, their success in the field has been variable (Blancard, 2012). It may be this observed variation between laboratory and field success that has resulted in only a handful of biocontrol agents making it to the commercial market (Asaka & Shoda, 1996).

To increase the success of biocontrol agents a more integrated approach should be taken which encompasses breeding microorganism-optimised plants, plant-optimised microorganisms and the use of biofertilisers and biopesticides (Farhana *et al.,* 2018). Interactions within the microbiome, the plants genotype, environmental factors and management practices must all also be considered for biological control to be a success (Busby *et al.,* 2017).

A number of biological control agents such as rhizosphere-colonising fungi, mycorrhiza-forming fungi and plant growth-promoting rhizobacteria (PGPR) have been shown to not only antagonise pathogens but also induce disease resistance through the upregulation of defence responses in the plant (Walters *et al.,* 2013).

1.5.4 Induced disease resistance

Induced disease resistance can be achieved through the treatment of plants with both abiotic and biotic factors that either constitutively upregulate or prime the plants defences both locally or systemically through induction of SAR- or ISR-like responses (Goellner & Conrath, 2008; Walters & Fountian, 2009; Walters *et al.,* 2013). Resistance can be elicited by avirulent pathogens, non-pathogens, plant metabolites and extracts, pathogen extracts, synthetic chemicals and abiotic stressors such as water, heat and light (Walters *et al.,* 2005; Miwa *et al.,* 2017). Induced resistance rarely leads to complete control of pathogens, however, it is commonly effective against a broad range of pathogens through the production of a non-specific defence response.

ISR is largely induced as a result of PGPR colonising the root and is mediated by the JA- and ET-sensitive pathways (Spoel & Dong, 2012). The PGPR *Azospirillum brasilense* and rhizosphere-colonising fungus *Fusarium equiseti* have been shown to induce disease resistance against *Colletotricum acutatum* and *Rhizoctonia solani* on strawberry and cucumber, respectively (Saldajeno & Hyakumachi, 2011; Tortora *et al.,* 2012). Furthermore, *Bacillus amyloliquefaciens* (F2B42) JA-dependant ISR is initiated by the suppression of microRNA miR846 *Arabidopsis thaliana* (Xie *et al.,* 2017).

Mycorrhizal fungi have been shown to confer resistance against both root and foliar pathogens. Resistance seemed to be determined by the pathogens lifestyle with necrotrophic pathogens being supressed and biotrophic pathogens showing increased susceptibility (Fritz *et al.,* 2007; Pozo & Azcon-Aguilar, 2007).

SAR can be induced by biotrophic and necrotising pathogens along with chemical treatment and environmental factors that are mediated by SA-dependent pathways (Spoel & Dong, 2012). SAR is induced by a number of chemicals including probenzole, acibenzolar-s-methyl and phosphate salts (Walters *et al.,* 2005). Potassium salts such as di and tripotassium phosphates K_2HPO_4 and K_3HPO_4 have proved to be effective against a variety of diseases on a number of crops including anthracnose, powdery mildew and neck blast on cucumber, barley and rice, respectively (Gottstein & Kuc, 1989; Mandahar *et al.,* 1998; Mitchell & Walters, 2004). Phosphate salts results in accumulation of SA and production of superoxide and hydrogen peroxide leading to localised cell death on the introduction of a pathogen (Oostendrop *et al.,* 2001). Fungal cell wall and insect exoskeleton component chitosan has also proven successful in the induction of SAR against *Fusarium oxysporum* f.sp. *lycopersici* crown and root rot on tomato and is now commercially available as a foliar spray (Benhamou *et al.,* 1998; Agostini *et al.,* 2003).

Recent work by Chen *et al,* (2017), has identified a group of 75 genes whose expression is modified by more than 4-fold following the induction of SAR by exogenous application of 3-acetonyl-3-hydroxyoxindole. The majority of differently expressed genes showed an increase in expression and were predominantly involved in metabolic pathways including the phenylpropanoid pathway, secondary metabolism and sesquiterpenoid and triterpenoid biosynthesis (involved in the production in the protective cuticle and wax).

Abiotic factors, heat, light and water can also induce disease resistance through overlap in the signalling pathways of abiotic and biotic stress (Rejeb *et al.,* 2014). Abiotic stressors elicit their defence response through the production of ROS and RNS which, in turn, can directly or indirectly lead to the production or suppression of SA, JA and ET through interactions with ABA which plays a key role in the plants adaptation to abiotic stress (Vlot *et al.,* 2009). Drought and osmotic stress can induce resistance to *B. cinerea* and *O. neolycopersici* on tomato and *Blumeria graminis* f.sp. *hordei* on barley, respectively (Wiese *et al.,* 2005; Achou *et al.,* 2006). Drought stress on tomato induced a two-fold increase in the levels of ABA (Achou *et al.,* 2006). Furthermore, heat-shock induced resistance against *B. cinerea* and induced SAR on melon and *A. thaliana*, respectively (Widiastuti *et al.,* 2011; Kusajima *et al.,* 2012).

Although numerous inducers of disease resistance have been identified, a number of problems can arise from their commercial application. Biotic factors that rely on the introduction of a non-virulent pathogen or non-pathogenic microorganisms into the ecosystem can be problematic on two fronts. As seen before with the use of *A. radiobacter* this can lead to the formation of new pathogenic species. Moreover, the introduction of new species onto the plant surface can be difficult as the microbial ecology of the plant surface is highly dynamic and will vary on the genotype, throughout the course of the plant's life and between geographical locations. The exact microbial communities present on a plant at the time of introduction may play a role in determining the efficacy of defence response induction.

There has been a great deal of laboratory success with the development of plant and pathogen extracts and synthetic chemicals for the induction of resistance. These still, however, require exogenous application as either seed or foliar dressings which may leave residues on produce. Furthermore, heat and water stress may be commercially applicable for postharvest treatments but not for pre-harvest ones as the high temperatures and wilting regimes required may be unfeasible for large scale commercial greenhouses. Light

stress induced resistance such as UV treatment, however, may mitigate some of the problems such as residues that are encountered for the previously discussed methods.

1.6 UV and hormesis

UV is genotoxic electromagnetic radiation with wavelengths that fall between 100 and 400 nm. UV exhibits its genotoxicity through the direct absorption of energy from UVB (320 - 290 nm) and UVC (290 – 100 nm) by DNA and the production of cellular ROS, from UVA, UVB and UVC, that lead to strand breaks and base lesions (Hollósy, 2002). Furthermore, UV can cause photooxidation of amino acids such as phenylalanine, photolysis of proteins and peroxidation of lipids (Hollósy, 2002). UV hormesis, however, is a dose response phenomenon where small doses of UV bring about a positive reaction in the target organism. The positive effects of UV on fresh produce have been known for over 30 years and have shown to be effective on orange, strawberry and sweet potato to mention just 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). Here the focus is on the induction of disease resistance; a comprehensive list of induced responses can be found in Appendix 1.

1.6.1 UV-induced disease resistance

To date, induction of disease resistance has been focused primarily on post-harvest treatment with most experiments aimed at monitoring disease progression. Caution needs to be exercised in reviewing the literature, however, as a number investigations have relied on initiation of disease through natural inoculations or have performed inoculations pre-treatment. This may create some confusion as it fails to truly attribute the level of disease reduction to the induced resistance alone the direct effect of UV on the inoculum cannot be account for. The results, however, highlight critical factors that support the induction of defence responses (Table 1.7), which shall be discussed in more detail in section 1.6.2.

There are a number of studies whose experimental design allows the quantification of resistance induced by UV hormesis (Table 1.8). As with other elicitors of induced resistance, UV does not provide complete control of disease with reductions in severity and incidence ranging from 10 to 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 (DPT) that a fruit is inoculated but also by the day post inoculation (DPI) 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 treatments to date have been focused primarily on the use of UV-C in the induction of disease resistance; few experiments have utilised UV-B/A as a post-harvest or UV-C as a pre-harvest treatment.

Table 1.7: Postharvest reduced decay exhibited by a combination of direct germicidal action and UVC induced resistance.

Fruit	Inoculation	Effective Dose (kJ/m^2)	Intensity ($\mu W/cm^2$)	Results	Reference
Bell pepper cv. Zafiro	Natural	7	-	No *Alternaria*- and *Botrytis*-based decay observed 12 DPT compared to 40% on controls. UV treated fruit also showed reduced chilling injury. Increase in antioxidants 18 DPT treatment.	Vicente *et al.,* 2005
Blueberries cv. Collins and Bluecrop	Natural	1.00 -4.00	2100	Decay by *Colletotrichum acutatum* was reduced by 10% at 1-4 kJ. Increased antioxidants at 1kJ. Increased anthocyanin content at 2-4 kJ	Perkins-Veazie *et al.,* 2008
Grapefruit cv. Ruby	Natural	0.5	1923	Delayed and reduced *Penicillium* based rot 2-12% dependent on harvest date. Increased phytoalexins scarpone and scopoletin which accumulated in flavedo tissue.	D'Hallewin. *et al.,* 2000
Mango cv. Haden	Natural	4.93	822	Reduced fungal decay from 100 to 40% 18 DPT. Increased phenylalanine ammonia-lyase levels in treated fruit. Increased total phenol content.	Gonzalez-Aguilar *et al.,* 2007
Mango cv. Tommy Atkins	Natural	4.93	822	Reduced microbial decay from 22 – 8% 14 DPT. Fruit showed increased levels of spermidine and putrescine.	Gonzalez-Aguilar *et al.,* 2001
Orange	Natural	0.5- 3	-	0.5 kJ effectively reduced decay development. 1.5 kJ, however, was more effective in early harvested fruit. In late harvest 3.0 kJ improve decay control further. Phytoalexins scaparone and scopoletin accumulated in flavedo tissue in a cultivar, fruit age and UV treatment dependent manner.	D'Hallewin *et al*., 1999
Persimmon cv. Karaj	Natural	1.5-3.0	630	Reduction disease incidence from 100 to 60% 96 DPT. No detrimental effects on firmness, ethylene production or skin colour.	Khademi *et al.,* 2012
Pineapple	Natural	13.2-39.6	2202	Treatments of 13.2, 26.4 and 39.6 kJ/m^2 all successfully reduced disease incidence at 14, 21 and 28 days post treatment. Although there were no significant differences between the treatments, the 26.4 kJ/m^2 showed the greatest reduction at each of the time points.	Sari *et al.,* 2016
Potato cv. superior	Pre-treatment	15	1400	Completely supressed the development of both dry rot and soft rot in potato dependent on incubation period. No effect on texture, firmness or colour of potato.	Ranganna *et al.,* 1997
Strawberry	Natural	0.43-4.30	716	0.43, 2.15 and 4.30 kJ promoted antioxidant capacity, enzyme activities and phenolic content to reduce decay. Anthocyanin content also increased for all treatments. A dose of 4.3 kJ/m^2 gave the best decay inhibition reducing disease incidence by 62% 20 DPT	Erkan *et al.*, 2007

Table 1.8: Postharvest hormetic UV-C treatments showing induced disease resistance supported by direct inoculation of fruit.

Fruit	Resistance against	Effective Dose (kJ/m^2)	Intensity ($\mu W/cm^2$)	Results	Reference
Apple cv. Red Delicious	*Penicillium expansum*	7.5	-	Fruit inoculated 1, 2 and 3 DPT. UVC was more effective than harpin, chitosan and antagonistic yeasts. Fresh fruit were more responsive to treatment than stored fruit. Treatments 3DPT were the most successful giving a 60% reduction in disease progression. UVC alone showed similar efficacy to combined treatments.	De Capdeville *et al.,* 2002
Bell pepper cv. Delphine	*Botrytis cinerea*	0.88	-	Fresh fruit inoculated 1 DPT showed increased resistance over fruit stored for 7 days before treatment. Disease progression was delayed for 10 days in treated fruit and gave a 22.8% reduction in lesion diameter at 22 DPI. Resistance was induced in both green and red fruit. Disease progressed fastest on red fruit but percentage decrease in lesion size was similar at all developmental levels. Two successive doses of 0.44 showed additive effect equal to a single dose of 0.88. Two doses of 0.88, however, were less successful than one.	Mercier *et al.,* 2001
Carrot cv. Caropak	*Botrytis cinerea*	0.88	-	UV showed reduced systemic effect in comparison to *B. cinerea*- induced resistance. UV induced strong local accumulation of 6-methoxymellein and an 82% reduction in lesion size when inoculated 50 DPT. A 25% reduction of lesion size was seen on treated but non-exposed tissues. A 24kDa chitinase was also upregulated after UV treatment. No β-1,3-glucanase activity was observed.	Mercier *et al.*, 2000
Grape cv. Autumn Black and B36-55	*Botrytis cinerea*	3.6	600	Cultivar-specific levels of induced resistance were shown with 38 and 28% reductions in disease incidence for Autumn Black and B36-55, respectively, when inoculated 2 DPT and inspected 7DPI. Treatment induced trans-resveratrol in both cultivars and catechin only in Autumn Black.	Romanazzi *et al.*, 2006
Grape cv. Italia	*Botrytis cinerea*	0.125 -0.5	-	A 10 – 15% and 28 – 38% lower disease incidence and lesion size was observed on berries inoculated from 1-4 DPT compared to controls. Fruit inoculates at 5 DPT showed increasing levels of incidence and severity. Lower level of disease on berries inoculated 1-2 DPT to 10-15 minutes after treatment. The population of epiphytic yeasts and bacteria on the fruit surface was also significantly higher after UV treatment.	Nigro *et al.,* 1998
Lemon cv. Eureka	*Penicillium digitatum*	5	-	Time of inoculation greatly affected disease susceptibility. No reduction in disease was observed when inoculations were performed 1 day prior to immediately before treatment. Resistance was greatest on fruit inoculated 2 DPT with a 44% reduction in incidence reducing to 24% for fruit inoculated 37 DPT. Increased levels of scoparone were observed after UV treatment.	Ben-Yehoshua *et al.,* 1992

Table 1.8: continued.

Fruit	Resistance against	Effective Dose (kJ/m^2)	Intensity ($\mu W/cm^2$)	Results	Reference
Melon	*Fusarium oxyspoum* and *Alternaria alternata*	4.0/6.0	555	The 4 and 6 kJ/m^2 both effectively reduced both the incidence and lesion diameter of both pathogens. Levels of incidence and progression for both treatments were similar. Fruit quality was not effected by any of the treatments.	Huang *et al.,* 2015
Mango 'Tommy Atkins'	Botryosphaeria berengeriana	2.5	-	The 2.5 kJ/m^2 treatment controlled disease by 70%. This treatment produce d damage on the fruit surface. Damage, however, was very slight and did not appear on all treated fruits. The pH, firmness and acidity of fruit was not changed by any of the treatments at 15 days post treatment.	Terao *et al.,* 2015
Orange	*Penicillium digitatum*	2.0	370	UV-C treatment reduced the disease progression by 70%. The 2 kJ/m^2 also inhibited 100% of spore germination when its direct germicidal abilities were assessed.	Terao *et al.,* 2017
Peach cv. Elberta and Loring	*Monilinia fructicola*	7.5	1260	Incidence of disease was reduced by 44 and 78% and lesion size was reduced by 84 and 89% on Elberta and Loring cultivars, respectively. Peaches were inoculated 2 DPI and lesions measured at 15 and 17 DPI for Elberta and Loring, respectively.	Stevens *et al.,* 1997
Sweet potato cv. Georgia-Jet Tuskegee-155	*Rhizopus stolonifer*	3.6	1260	Incidence of disease was reduced in both cultivars for inoculations at both 7 and 14 DPI when inoculated at 3 DPT. Gerorgia-Jet showed decreases in incidence of 61 and 60% at 7 and 14 DPI, respectively. Tuskegee-155 showed a 48 and 55% reduction in disease incidence at 7 and 14 DPI.	Stevens *et al.,* 1997
Strawberry	*Botrytis cinerea*			UV-C treatment reduced lesion diameter by 36 and 24% on 9 and 12 days following inoculation. Chitinase activity increased on the 9^{th} day following inoculation and then decreased. β-1,3-glucanase activity, however, increased gradually during storage. Six defence-related genes (were upregulated following treatment (*CCR-1, CAT, CHI2, PPO* and *PAL6*)	Jin *et al.,* 2017
Tangerine cv. Dancy	*Penicillium digitatum*	1.3	1260	Fruit were inoculated 2 DPT and incidence of disease was measured at 2 DPI showing a reduction in incidence of 45 % and lesion size was reduced by 59%.	Stevens *et al.,* 1997

Table 1.8: continued.

Fruit	Resistance against	Effective Dose (kJ/m^2)	Intensity (µW/cm^2)	Results	Reference
Tomato cv. Tuskege- 80-130 and Floradade	*Rhizopus stolonifer*	3.6	1260	Incidence of disease was reduced by 50 and 78% and lesion size was reduced by 33 and 51% on cultivars Tuskegee 80-130 and Floadade, respectively. Tomatoes were inoculated 2 DPT and disease assessed at 1 DPI.	Stevens *et al.,* 1997
Tomato cv. Trust	*Botrytis cinerea*	3.7	2055	Treated fruit were immediately more susceptible to disease and gradually became resistant which lasted up to 35 days post treatment. Optimum disease resistance was observed at 10 -12 DPT with a 91% reduction in lesion size. Production of phytoalexin rishitin was induced and a hypersensitive-like response was induced around the point of inoculation. The capacity to accumulate rishitin declined with ripening.	Charles *et al.,* 2008
Pineapple	*Chalara paradoxa*	4.0	555	The 4, 6 and 8 kJ/m^2 treatments showed the greatest ability to reduce the incidence and progression of disease. UV-C treatment also reduced tissue softening from 4 to 10 days of storage.	Ou *et al.,* 2016

1.6.2 Induction of defence responses

There are at least three potential mechanisms that play a role in the initiation of UV induced defence responses. The first of which may function through the production of ROS from chloroplasts, mitochondria and peroxisomes following UV exposure. Overexposure of UV can lead to the initiation of programmed cell death (PCD) mediated through ROS production (Gao *et al.,* 2008). Hormetic UV doses, however, may allow the accumulation of ROS without triggering PCD. Cytosolic ROS can lead to the upregulated biosynthesis of ABA, JA, SA and ET (Vidhyasekaran, 2015) which may lead to both the induction of biotic and abiotic stress defences and priming of defence genes.

ROS signalling is now recognised as playing a key role in stress signalling and acclimatisation in plants. Furthermore, antioxidants may also play a key role as ROS processing and signalling mediators (Noctor *et al.,* 2017). One such antioxidant is glutathione which may be key in the transmission of ROS signals by acting as an interfacing molecule (Noctor *et al.,* 2017). This is supported by its role as a modulator of plant stress (SA, JA, & ET) and development (AUX & ABA) hormones (Noctor *et al.,* 2017). For more information on induction of defence responses see section 1.4.

ABA, JA, SA and ET have all been shown to play roles in the signalling of UV hormesis. In apple seedlings treated with UVC a transient peak in ABA and prolonged upregulation of JA biosynthesis occurred at 3 DPT and 6-10 DPT, respectively (Kondo *et al.,* 2011). UV-C treated *A. thaliana* exhibits a two fold increase in the production of SA that is initially localised to the chloroplast (Fragnire *et al.,* 2011). Tomato fruit show a transient increase in ethylene production shortly after treatment with a lag in ethylene production and lower maximum ethylene levels from 7 days following treatment (Maharaj *et al.,* 1999). Changes to the levels of ABA, SA and gibberellic acid have all been shown to change in a dose responsive manner following the UV-C treatment of strawberry plants (Xu *et al.,* 2017). Furthermore, Kondo *et al.* (2011) showed that UV-C treated apple seedlings' levels of putrescine and spermidine peaked at 1-5 DPT and 3-10 DPT, respectively. Both showed a three-fold increase in comparison to controls.

Spermine and putrescince are both ubiquitous polyamines (PA) that can function to modulate the plants response against abiotic and biotic stress through direct interactions with DNA (Gill & Tuteja, 2010). Moreover, polyamines can act in a number of ways to directly strengthen defence responses, including but not limited to, binding to the cell wall and membranes to prevent degrading enzymes, scavenging of ROS and inhibition of RNAses and proteases (Balestreri *et al.,* 1987; Charnay *et al.,* 1992; Ha *et al.,* 1998; Altman, 2006).

PAs are therefore likely to play a role in the induction of UV hormesis downstream of ROS production.

Secondly, a UV-B specific photoreceptor, UVR8 encoded by *UV resistance locus 8* in *A. thaliana* acts to protect plants from UV stress through the production of flavonoids and sinapates (Demkura & Ballaré, 2012); their function is discussed in section 1.6.3. UVR8 induction of sinapates led to resistance against *B. cinerea* in *A. thaliana. UVR8-1* mutants exhibit a reduction in UV-B induced flavonoid biosynthesis and an increase in PR1 and PR5, two SA-dependent PR proteins (Ward *et al.,* 1991; Kliebenstein *et al.,* 2002). This suggests the induction of SA-mediated response in the absence of UVR8.

Finally, in a study by Kucera *et al*. (2003) it was shown that the UV-B treated bean (*Phaseolus vulgaris*) leaves exhibited upregulation of PR protein β-1,3-glucanase via a separate pathway to flavonoid synthesis. β-1,3-glucanase was induced via shortwave UV-B, 310 – 275 nm, and flavonoid synthesis was elicited by longwave UV-B; 360-310 nm. Specific wavelengths of UV has also been shown to elicit differing responses by Conconi *et al.,* (1996).

Conconi *et al.* (1996) showed that in tomato leaves treated with 2 kJ/m^2 of UV-C transcription of *Proteinase Inhibitor I* and *II,* associated with JA signalling, was induced to a degree similar to that observed after wounding. UV-B treatments, however, only induced a 50% accumulation of mRNA, in comparison to that of wounding, through an optimal 35 kJ/m^2 treatment. UV-A did not induce any changes. It is now known that the production of flavonols, as speculated by Kucera *et al.* (2003), can be mediated by the UVR8 mediated response to UV-B although UV-C treatments also stimulate flavonoid biosynthesis (Demkura & Ballaré, 2012). The differential defence responses for varying wavelengths of UV indicate a further pathway that may initiate the hormetic response.

Kucera *et al*. (2003) additionally showed that levels of β-1,3-glucanase correlated with the number of photoreversible DNA lesions, and were localised to directly exposed tissue. Moreover, during conditions that allow photorepair of DNA lesions β-1,3-glucanase production was prevented. The localisation of the β-1,3-glucanase expression, however, could be attributed to the failure to elicit a systemic response. Recently Yan *et al.* (2013) and Song & Bent (2014) have shown that SA and NPR1, a transcription factor integral to the induction of SA defence responses, can mediate DNA damage repair machinery. This is supported by previous work that genotoxic substances induce PR gene expression and that DNA damage repair proteins RAD51, BRCA2 and SSN2 are intimately involved in the

regulation of the plants SA-mediated immune response (Choi *et al.,* 2001; Kunz *et al.,* 2006; Durrant *et al.,* 2007; Wang *et al.,* 2010; Song *et al.,* 2011). The correlation between the number of DNA lesions and localised β-1,3-glucanase expression, its independence from flavonol biosynthesis and the intimate relationship between DNA damage repair proteins and SA point towards the potential for DNA damage detection and repair, mediated through SA, to induce defence related genes.

With at least three potential mechanisms for the induction of UV-induced disease resistance it can therefore be speculated that the use of multiple wavelengths of UV could induce multiple defence responses in plants and, therefore, increase the spectrum of disease control. Moreover, with elicitation of defence responses upstream of JA, ABA, PA, SA and ET, through the production of ROS, the simultaneous induction of both SA and JA/ET mediated responses could be hypothesised.

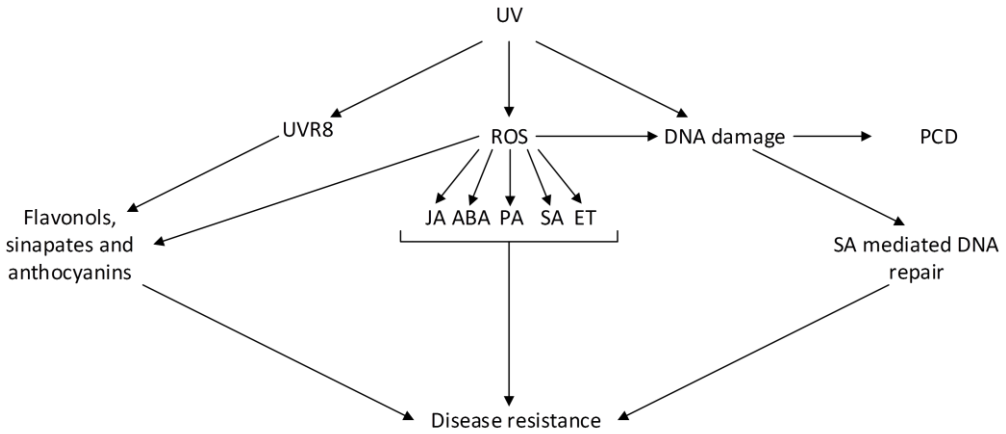

Figure 1.5: The potential pathways leading to the induction of defence responses through hormetic UV treatment of plants. Long wavelength UV-B can be detected by photoreceptor UVR8 leading to the upregulated biosynthesis of secondary metabolites such as flavonoids and sinapates, which can act as antimicrobials. Short wavelength UV-B and UV-C lead to reactive oxygen species (ROS) production from the chloroplasts, mitochondria and peroxisomes and can lead to the biosynthesis of jasmonic acid (JA), abscisic acid (ABA), phosphatidic acid (PA), salicylic acid (SA) and ethylene (ET) leading to increased levels of biotic and abiotic defence responses. Moreover, it can be envisaged that ROS may play a direct role in the signalling of flavonoid and sinapate biosynthesis due to their antioxidant activities. Short wavelength UV-B and UV-C can directly and indirectly damage DNA. High levels of damage can lead to programmed cell death (PCD). Hormetic doses may, however, induce an SA-mediated DNA damage response and the recruitment of transcription factors whose actions function in both DNA repair and biotic defence.

1.6.3 Changes in secondary metabolism

Secondary metabolites are produced from the end- and by-products of the primary metabolic pathways and are not essential for photosynthesis, reproduction or respiration (Cetin, 2014). They are chemically and functionally diverse with roles in attracting

pollinators and stress defence modulation to mention a few (Cetin, 2014). UV treatment of plants can cause drastic changes to a plant's secondary metabolism leading to biosynthesis of an array of products that protect the plant from both UV and ROS as well as abiotic stress and plant pathogens through their antimicrobial activities (Schreiner *et al.,* 2014).

The largest studied group of UV-induced secondary metabolites are the phenolic compounds derived from the phenylpropanoid pathway. An array of phenolic compounds have been observed to be upregulated. These including the flavonoids scoparone and scopoletin which accumulate in the flavedo tissue of both grapefruit and orange (D'Hallewin, 1999; D'Hallewin *et al.,* 2000). The former has also been observed at increased levels in lemon (Ben-Yehoshua *et al.,* 1992). Increased anthocyanin content occurs in blueberry, apple and bean leaves. Conversely, strawberries showed no change in anthocyanin levels (Vincente *et al.,* 2005; Erkan *et al.,* 2007). See Tables, A1.1 for a list of changes in secondary metabolism.

Phenolic compounds including the flavonoids and anthocyanins not only act as chromophores, absorbing damaging wavelengths of light, but also as antioxidants, preventing ROS-mediated cellular damage such as lipid peroxidation, in a dual role to protect plants against further light and ROS stress (Pietta, 2000; Sourivong *et al.,* 2007; Lev-Yadun & Gould, 2009). Many phenolic compounds, including all of the aforementioned, also act as phytoalexins showing direct antimicrobial properties (Pietta, 2000; Lev-Yadun & Gould, 2009). These properties are also shared by a group of esters, the sinapates, produced by *A. thaliana* after UV exposure (Demurka & Ballare, 2012).

A further group of upregulated secondary metabolites are formed from the terpenoid pathways, members of which have been observed to be upregulated in melon and tomato. A group of three terpenoids; β-ionone, geranylacetone and terpinyl acetate, were observed at increased levels in melon treated with UV-C (Widiastuti *et al.,* 2011). Rishitin biosynthesis was induced in tomato fruit and led to *B. cinerea* resistance (Charles *et al.,* 2008). Terpenoids are the largest and most diverse group of natural compounds in nature and vary in their function from plant defence to communication (Lopez *et al.,* 2015). β-ionone, geranylacetone, terpinyl acetate and rishitin have all been shown to act as phytoalexins. Moreover, β-ionone is an oxidation product of β-carotene and can induce the transcription of H_2O_2 mediated genes including an oxidoreductase (Ramel *et al.,* 2012). β-ionone may, therefore, also play a role in signalling during UV hormesis. To date the most extensive research on modelling the physiological basis of UV-C hormesis has been performed post-

harvest on tomato fruit which shall now be summarised for a broad analysis of the changes observed during UV-C hormesis.

1.6.4 UV-C induced resistance in tomato fruit

Charles *et al.* published a series of five papers between 2008 and 2009 outlining the physiological basis of UV-C induced resistance against *B. cinerea* in tomato fruit. To summarise their findings; UV-C treatment of tomato fruit causes increased biosynthesis of a broad range of phenolic compounds, phytoalexin rishitin and three PR proteins comprised of a 33.1 kDa β-1,3-glucanase and two chitinases of 37.1 and 20.6 kDa in size (Charles *et al.,* 2008a, 2008d). Moreover, physical modifications were observed including modifications to the fruit surface and pericarp (Charles *et al.,* 2008b, 2008c, 2008d). After UV-C treatment the fruit surface exhibited flattening of cellular mounds, surface wrinkling, failure to fully form opercula over broken trichomes and the quasi-absence of epicuticular wax which may additively result in the reduced level of spore colonisation and production of appressoria (Charles *et al.,* 2008b). The fruit pericarp exhibited plasmolysis in the epicarp and mesocarp leading to cell collapse and formation of cell wall stacking zones, similar to those observed in the hypersensitive response (Charles *et al.,* 2008c). Cell wall stacking zones underwent reinforcement through the deposition of phenolic compounds lignin and suberin, a processes known to be involved with pathogen defence and wound healing (Dean & Kolattukudy, 1976; Charles *et al.,* 2009; Bhuiyan *et al.,* 2009). Charles *et al.* gave a good grounding for the continuation of research in to the complexities of UV-C induced disease resistance. It is now known, however, that UV-C hormesis has a significant effect on the fruit.

High-throughput transcriptional analyses were carried out by Liu *et al*. (2011) through the use of the Tomato GeneChip microarray (Affymetrix) which monitors 9254 genes. This is approximately one quarter of the genes in the tomato genome (van der Hoven *et al.,* 2002). A total of 677 genes were differentially expressed after treatment with 4 kJ/m^2 of UV-C; the actual level of gene transcription affected is assumed to be larger. Out of the 677 genes 274 were upregulated whose functions were mainly in signal transduction, defence responses and metabolism. Out of the 24 signal transduction genes that were upregulated 12 were involved with either ethylene biosynthesis, signalling or perception. The remaining genes were downregulated and involved in cell wall disassembly, photosynthesis and lipid metabolism.

To conclude, UV-C treatment has complex effects on its target that are active on the structure, transcriptome, proteome, metabolome and hormones; all of which, excluding the former, are intimately linked through the overlap of biochemical and signalling pathways. The cumulative results of these studies point towards, at least in tomato fruit, a major role for ethylene in the induction of UV-C induced defence responses. This is further supported by work showing increased synthesis of ethylene and upregulation of 14 ethylene responsive factors in tomato after treatment with UV-C (Servo *et al.,* 2015).

1.7 Aims

To date the research into low intensity UV-C (LIUV) hormesis has been limited to postharvest treatments for extending shelf life and adding nutritional value to produce. The length of exposure times are one of the limiting factors preventing adoption of the technology by commercial growers. The aims of this project, therefore, are two-fold. The first is to validate the use of a high intensity pulsed polychromatic light (HIPPL) source as an inducer of hormesis; for more information on HIPPL see Chapter 3. Such new sources may allow reductions in the lengthy exposure times required to induce LIUV hormesis. Experiments will be performed postharvest on tomato fruit as a large collection of literature for LIUV hormesis on tomato fruit is available. Following validation of the HIPPL source as an inducer or hormesis, a comparative gene expression study will be conducted to identify the molecular mechanisms underpinning LIUV and HIPPL hormesis in postharvest produce.

Secondly, preharvest LIUV and HIPPL treatments will be evaluated for their ability to control disease against a number of fungal pathogens. Treatments will be applied to the foliage or as seed treatments. These investigations will be performed on protected lettuce and tomato crops, respectively. Disease assays will be performed for a number of fungal pathogens including *B. cinerea, R. solani* and *S. sclerotiorum* on lettuce and *B. cinerea* on tomato. Following the identification of successful treatments the effects of treatment on plant physiology will be monitored.

2.0 General materials and methods

2.1 Culturing, growth and storage of microorganisms

A number of organisms were chosen to study as pathogenic agents of tomato and lettuce. See sections 1.1.2 and 1.2.2 for information on tomato and lettuce pathogens, respectively. *B. cinerea*, *Rhizoctonia solani,* and *Sclerotinia sclerotiorum* were supplied by The University of Nottingham, FERA and The University of Warwick, respectively. Cultures were maintained on Potato Dextrose Agar (Sigma-Aldrich) at 39 g/l amended with Penicillin G sodium salt (Sigma-Aldrich) at 33 mg/l and Streptomycin sulphate salt (Sigma-Aldrich) at 133mg/l. Cultures were grown on 90 mm petri dishes and stored at 21°C. For long term storage all organisms were grown on agar slants in 30 ml universal tubes, stored at 21°C to allow complete coverage of slant and then stored at 4°C for preservation.

For long term storage of *B. cinerea,* spores were collected from cultures following instructions in section 2.3, suspended in 20% glycerol and stored at -80°C. *R. solani* cultures were preserved on barley seeds. Briefly, seeds were soaked in water for 24 hours and autoclaved three times, allowing 24 hours for the seeds to cool between each replicate. Barley seeds were then placed on 2 - 3 day old cultures of *R. solani* and left for 1 week to allow the seeds to be colonised. Seeds were then transferred to a sterile universal tube and stored at -20°C. For the long term storage of *S. sclerotiorum* cultures were allowed to grow until the maturation of sclerotia; fungal resting bodies. Sclerotia were then removed from the petri dish and stored at 4°C in a container along with the silicone gel as a desiccant.

2.2 Identification of microorganisms

Cultures were identified via colony PCR. A sterile pipette tip was used to transfer spores or hyphal fragments directly into the PCR mixture. The PCR mixture was comprised of 12.5 µl of MangoMix™ (Bioline), 10.5 µl of HyClone™ molecular grade water (GE Healthcare) and 0.5 µl of both forward and reverse universal fungal primers, table 2.1. PCRs were performed on a BioRad® S1000™ thermal cycler programme using the following reaction cycle. Initial denaturation 94°C for 2 minutes followed by 35 cycles of; 94°C for 30 seconds, 53°C for 1 minute and 72°C for 1 minute; with a final extension of 72°C for 5 minutes.

Table 2.1: The universal fungal and oomycete primers, designed from the internal transcribed spacer (ITS) region of the ribosomal-DNA, used for the identification of microorganisms in this study (White *et al.,* 1990).

Primers	Sequence	Target
$2234C^{F}$	GTTTCCGTAGGTGAACCTGC	3' of 18S
$3126T^{R}$	ATATGCTTAAGTTCAGCGGGT	5' of 26/28S

Key: F= Forward primer, R= Reverse primer.

Products were then visualised via gel electrophoresis on a 0.7% TAE (Tris base-Acetic acid-EDTA) gel amended with 50 µl/l ethidium bromide run at 100 V for 60 minutes using a PowerPac Baisic™ (BioRad). The gel was viewed by UV camera InGenius3 on software platform GeneSys manufactured by Syngene, UK. PCR products were then purified using the GenElute ™ kit (Sigma-Aldrich) following the manufactures protocol and sent to Eurofins (Germany) for sequencing. Returned sequences were analysed on the software platform Molecular Evolutionary Genetics Analysis (MEGA 6.1) and identified via the "megablast" algorithm of the Basic Local Alignment Search Tool (BLAST) software produced by The National Center for Biotechnology Information (NCBI).

2.3 Calibrating spore solutions

Petri dishes were flooded with 15 ml of sterile distilled water and 0.03% Tween 20. Spores were released via agitation and filtered through a double layer of muslin cloth. An additional 15 ml was added to plates followed by agitation and filtration through muslin. Samples were vortexed vigorously, centrifuged at 1000 rpm for 10 minutes (184 g) in a Centaur 2 (MSE). The supernatant was discarded and the pellet was re-suspended in 30 ml of water, vortexed and centrifuged as stated before. The supernatant was discarded and the pellet was re-suspended in 5ml of sterile distilled water. A haemocytometer was used to calculate the spore concentration and the inoculum concentration was adjusted accordingly.

2.4 Low-intensity UV-C and high-intensity, pulsed polychromatic light sources and treatments

All treatments were carried out in an enclosed gantry to protect users from the genotoxic effects of UV light (Figure 2.1A). A UV protective face shield was worn at all times and along with LaserShield (NoIR Laser Company) glasses while using the pulsed source due to the damaging effects of intense white (visible) light. LIUV treatments were carried out using a U-shaped amalgam UV-C source (UVI 12OU2G11 CP15/469) obtained from Dr Hőnle AG,

Gräfelfing, Germany. Its peak emission were at 254 nm and the source was housed within an anodised aluminium parabolic reflector (Figure 2.1B). The LIUV source was switched on at least 30 minutes before treatment and not terminated until the end of the experiment to allow constant emission. Intensity was measured with a portable radiometer (Model UVX, UVP Instruments, Cambridge) fitted with a 254 nm sensor.

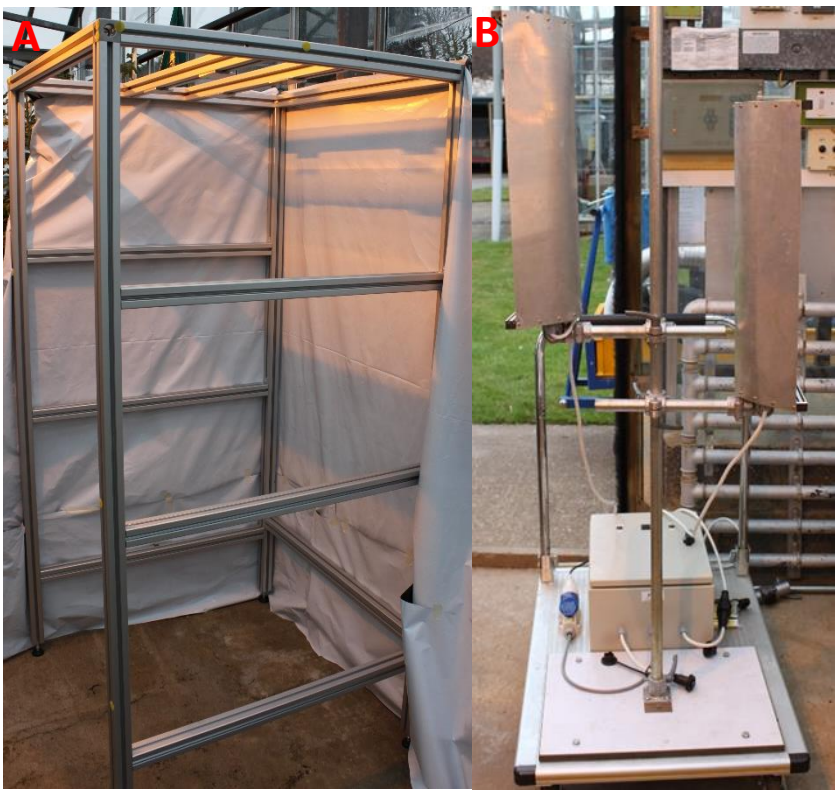

Figure 2.1: The enclosed gantry in which treatments were performed (**A**) and the low-intensity UV-C source with two separate sources mounted in parabolic reflectors (**B**).

HIPPL treatments were carried out with a XENON LH-840 16" ozone free B lamp powered and controlled by RT-847 cabinet and RC-802 controller, supplied by Lambda Photometrics (Harpenden, Herts) (Figure 2.2). 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 polychromatic source were between 240 nm and 1050 nm. It was not possible to measure total dose energies or spectral irradiance delivered per pulse during this study as an appropriate polychromatic radiometer could not be obtained.

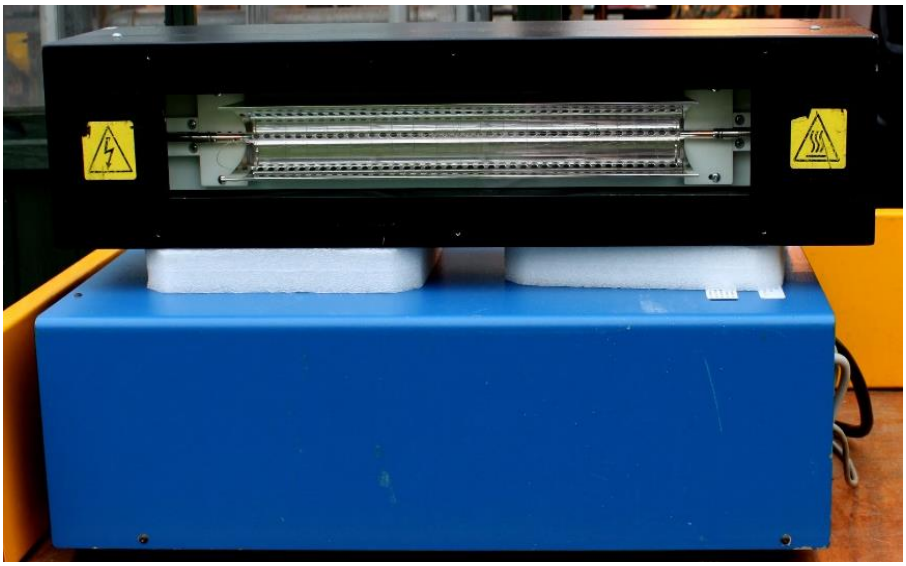

Figure 2.2: The high-intensity, pulsed polychromatic light source with light source LH-840 and RT-847 cabinet with inbuilt RC-802 controller (XENON).

2.5 Tomato fruit production

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

2.6 Lettuce husbandry in an NFT system

Commercial butterhead lettuce varieties Amica and Temira (Enza Zaden) were germinated in 25 mm rockwool propagation cubes (Grodan). Upon the emergence of roots from the cubes (approximatley 14 days) seedling were then placed into pre-wetted 3” Delta Cubes (Grodan) and then transferred to an NFT system (Figure 2.3). The NFT system was set to a gradient of 1:50 and filled with 1g/l HortiMix Standard (Hortifeed) water soluble nutrient fertiliser. Plants were then grown to the desired developmental stage before treatment.

Figure 2.3: An example of a nutrient film technique (NFT) soilless system. Lettuce seedlings are in 25 mm rockwool cubes (Grodan) and are 14 days old.

Chapter 3: Validation of high-intensity, pulsed polychromatic light as an inducer of disease resistance and delayed ripening on tomato fruit

The post-harvest hormetic low-intensity UV-C (LIUV) treatments of fresh produce are well-established and have been shown to induce a number of beneficial effects including increased nutritional content and disease resistance through modifications in the plant's secondary metabolism and upregulation or priming of defence genes, see section 1.6. Yet, to date the commercial application of LIUV induced hormesis has not occurred. This is attributed to a number of factors including long exposure times which require a treatment time of 100 seconds to achieve an exposure of 1 kJ/m^2 at 10 W m^{-2}. Using LIUV sources in line with a common published intensity of 20 W m^{-2} would require an exposure time of approximately six min per fruit to induce hormesis. Moreover, the necessity to expose the entire fruit surface to the full hormetic dose, due to the localised responses observed in some produce, further complicates treatment (Mercier *et al.,* 2000). This may not be the case however, for all produce as Obande *et al.,* (2011), who treated fruit pre-harvest, observed delayed ripening on fruit covered with a UV impermeable film on trusses distinct from those receiving treatment.

With the recent advent of high-intensity pulsed polychromatic light (HIPPL) sources which have considerable emission in the UV-C region a substantial reduction in treatment times, from minutes to seconds, could be achieved. There have been a number of studies utilising HIPPL to induce beneficial properties in fresh produce. There has been only one study on the modification of disease responses using HIPPL by Marquenie et al., (2003) who reported no positive or negative effect on *Botrytis cinerea* inoculated strawberries.

Treatment of fresh produce with HIPPL, however, has been shown to increase the concentration of anthocyanins and total phenolics along with improving colour in nethouse grown fig, *Fiscus carica* (Rodov *et al.,* 2012). Both LIUV and HIPPL treatments have been shown to significantly increase the total lycopene, carotenoid and phenolic content as well as antioxidant activities of tomato fruit (Liu *et al.,* 2009; Liu *et al.,* 2012; Pataro *et al.,* 2015). HIPPL has also been shown to increase anthocyanin and Vitamin D_2 levels in mushrooms, *Agaricus bisporus* (Oms-Oliu *et al.,* 2010; Koyyalamudi *et al.,* 2011).

There are a number of differences between the delivery of UV from a HIPPL source and LIUV sources. These include the dose being delivered in pulses, the spectrum of light emitted and its intensity. LIUV sources have a relatively short band of emissions from approximately 160 to 480 nm with a varying peak, either UVA, B or C (Heering, 2004). For the purposes of UV-C treatments a peak at 254 nm is usually employed. HIPPL sources, with reference to the source used in this study, can deliver energy across a broader spectrum from 240 to 1,050 nm including UV, visible light and infra-red light at a maximum of 40 kJ/m^2 per pulse, each lasting 320 µs (Middleton, 2015). Approximately 2% of the total energy output is germicidal i.e. between 200 - 300 nm and includes UV-C and shortwave UV-B (Middleton, 2015). The effect of such a high intensity treatment and broad spectral emission on the induction of defence responses is unknown. The pulsing of treatments, however, has been shown to have an additive effect on the induction of resistance (Mercier *et al.,* 2001).

3.1 Aims

The aim of this study was to investigate whether HIPPL sources were able to delay colour and texture changes during ripening and induce resistance against *B. cinerea* on mature green tomato. Treatments were also conducted with a LIUV source as a basis for comparison. Additionally, treatments using both types of source, HIPPL and LIUV, were conducted to assess their ability to induce disease resistance against *B. cinerea* and *Penicillium expansum* on ripe fruit, as an increasing number of tomato growers are harvesting at this stage due high consumer demand.

3.2 Materials and methods

3.2.1 Colour and texture analysis

Mature green fruit were measured with a calibrated CR-200 Chroma meter (Konica Minolta, UK) in l*a*b* mode. Readings were taken at a single point directly facing the source and at a 90° axial rotation from that point. A second colour measurement was taken using the same reference points at 10 DPT. This was used to calculate the change in tomato colour index (TCI) over 10 days. Fruit firmness was measures with a TA.XT plus texture analyser (Stable Micro Systems, UK). Four measurements were taken with a 40 mm diameter non-destructive probe which measured the force required to compress the tomato by 4 mm at a speed of 1.5 mm/s. Of the four measurements, three were taken from the equatorial region

and one from the blossom end. Measurements for each fruit were averaged and taken from the same position at every time point monitored.

3.2.2 Low-intensity UV-C and high-intensity, pulsed polychromatic light treatments

Fruit were produced as stated in section 2.5 and light treatments were performed as stated in sections 2.4. An established conventional LIUV treatment of 3.7 kJ/m^2 delivered at 20 W m^{-2} was used as a benchmark to assess the efficacy of induced disease resistance from the pulsed source (Charles *et al.,* 2008a). Fruit were positioned 10 cm from the pulsed source and treated with a range of pulses. Through extrapolation of the manufacturer's data, approximately 4.6 kJ/m^2/pulse was delivered at fruit level. This was estimated by fitting a logarithmic curve to polychromatic energy data for distances of 2.5, 5.0 and 7.5 cm from the source.

For both sources fruit received exposure on two sides through 180° axial rotation. Following treatment fruit were immediately stored in the dark until sterilisation. For sterilisation tomatoes were immersed in 2% Sodium hypochlorite (Sigma-Aldrich, UK) for approximately 5 – 10 seconds; to prevent growth of naturally occurring microorganisms during the incubation period. Fruit were then rinsed three times in sterile distilled water (SDW), dried and immediately incubated in the dark at 13°C to prevent photoreversal. Fruit were stored in humidity boxes lined with damp paper and raised by a double layer of plastic mesh at≥ 98% RH (Figure 3.1). At 10 days after treatment fruit were inoculated to allow for the induction of defence responses; this was shown to be the optimum point of UV-C induced disease resistance by Charles *et al.,* 2008a. For more information on the UV-C and HIPPL equipment used in this study can be found in Chapter 2.

Figure 3.1: An example tomato fruit (cv. Mecano) during storage following treatment by either high-intensity, pulsed polychromatic light or low-intensity UV-C. Fruit were stored in a humidity box with a relative humidity > 98%. Boxes were lined with moist tissue and two layers of plastic mesh to raise tomatoes from direct contact with the tissue paper.

3.2.3 Pathogen maintenance, spore preparation and inoculation

A *Botrytis cinerea* culture, originally isolated from a plant of the genus *Rosa*, was supplied from The University of Nottingham's collection. Cultures were grown and stored according to sections 2.1. A calibrated spore solution was made from 10 day old cultures of *B. cinerea* following the protocol in section 2.3. Fruit were then wounded with a sterile hypodermic needle to the depth of 3mm. Ripe fruits were inoculated with 5 µl of $1x10^5$/ml spores. Green fruits, however, were inoculated with 5 µl of $1x10^6$ spores due to decreased levels of susceptibility observed in preliminary work. Total lesion diameter including all sunken lesions, splitting and tissue maceration was measured with digital Vernier callipers at 3 and 4 DPI. For *Penicillium expansum* inoculations on ripe fruit a culture was also obtained from The University of Nottingham and cultured as stated for *B. cinerea.* Spores were isolated from 7 day old cultures and fruit were inoculated with 5 µl of spores at a concentration of $1x10^6$/ml.

3.2.4 Experimental design and data analysis

All data presented here was collected from two independent replicate experiments. For

experiments concerned with tomato colour change and *B. cinerea* disease resistance fifteen fruit per treatment group per replicate experiment were used. For experiments concerned with *Penicillium expansum* resistance and texture analysis 10 fruit per treatment group per experiment were used.

Tomato colour measurements were transformed into the tomato colour index and the first reading was subtracted from the second to calculate change in TCI (Lopez Camelo & Gomez, 2004; Corcuff, *et al.,* 2012; Hobson, 1987). The change in TCI was used to allow statistical analysis of ripening progression as opposed to analysis of the start and end values. No significant differences were observed between the starting TCI values of any of the treatment groups (graphs available in Appendix 2). The formulae for calculating TCI can be found in equation 3.1. Lesion size measurements were used for the calculation of the area under the disease progression curve (AUDPC); a method used in both epidemiology and resistance breeding for the calculation of disease progression (Eq. 3.2).

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

Equation 3.1: Tomato colour index (TCI) formula where L= lightness, a= red-green and b = blue-yellow values (Hobson, 1987).

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

Equation 3.2: Area Underneath the Disease Progression Curve formula where n= total number of observations, i= observation, y= disease score and t= time (Jeger and Viljanen-Rollinson, 2001).

Statistical analysis was performed using statistical software package SPSS 22 (IBM). One-way ANOVA with Tukey's post-hoc testing was performed. Where the homogeneity of variances assumption could not be met Welch's robust ANOVA was performed followed by the Games-Howell post-hoc test. Statistical significance is here defined as $p \leq 0.05$. Data from independent experimental replicates can be found in Appendix 2.

3.3 Results and discussion

3.3.1 Delayed ripening

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

The 3.7 kJ/m^2 UV-C, 16 and 24 HIPPL treatments showed significantly lower ripening progression, Δ TCI, in comparison to the control (Figure 3.2). The 3.7 kJ/m^2 and 16 pulse HIPPL treatments reduced change in TCI over 10 days by 43.1 and 50.1%, respectively. This data supports the successful induction of delayed ripening with either HIPPL or LIUV. Fruit treated with 8 pulses, however, did not ripen at a rate significantly different from the control. Representative samples of tomato fruit are shown in Figure 3.3.

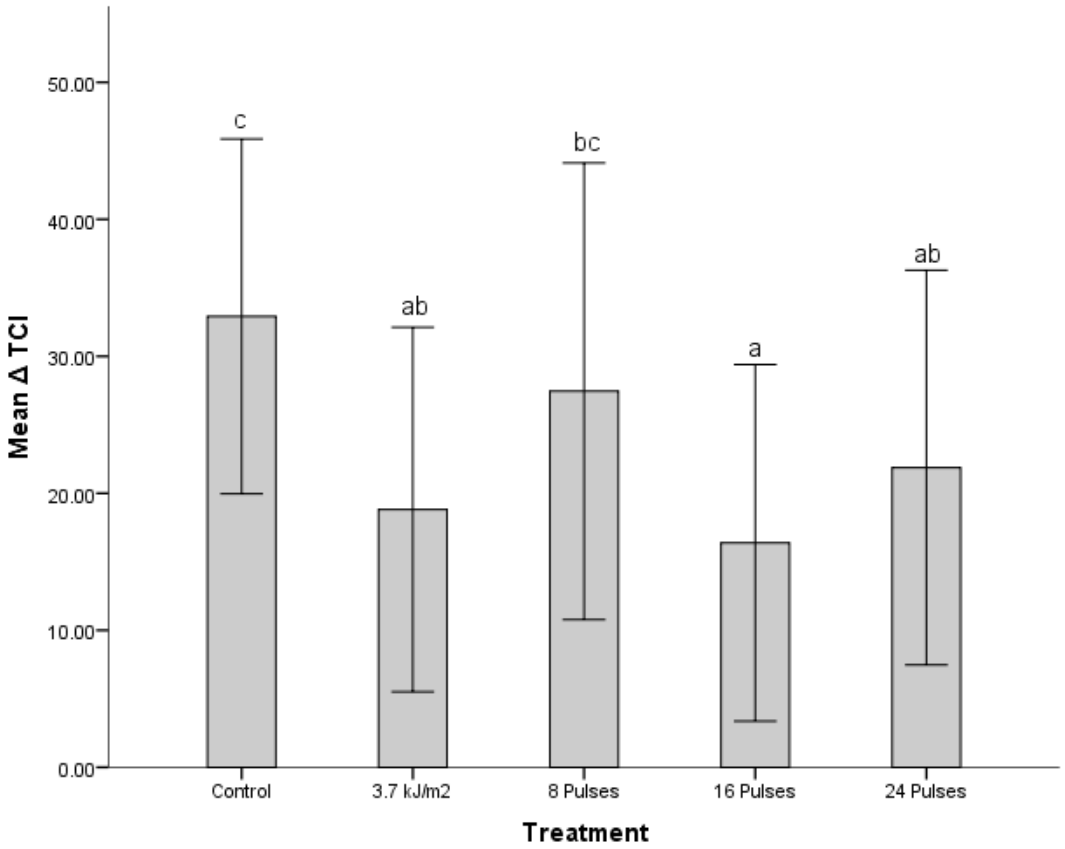

Figure 3.2 The change (Δ) in TCI (tomato colour index) of mature green fruit (cv. Mecano) during storage in the dark from day 0 – 10. Fruit were treated with a low-intensity UV-C treatment of 3.7 kJ/m^2 (peak emissions at 254 nm) and three high-intensity, pulsed polychromatic light (HIPPL) treatments of 8, 16 and 24 pulses. Error bars show ± 1 standard deviation; n = 30. Labelling indicates statistical significance. Means sharing the same label are not significantly different from each other at $p < 0.05$.

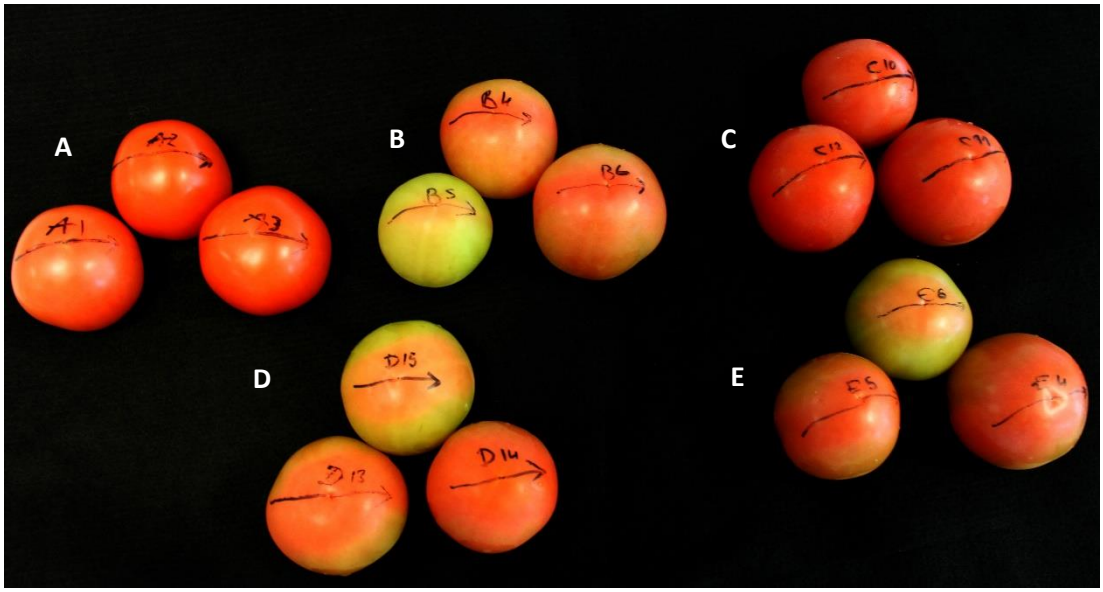

Figure 3.3: Representative samples of tomato fruit (cv. Mecano) at 10 days post treatment. Fruit were treated at the mature green stage and stored in the dark. Groups show the control fruit (**A**), the 3.7 kJ/m^2 low-intensity UV-C treatment with peak emissions at 254 nm (**B**), and fruit treated with the high-intensity, pulsed polychromatic light source at 8 (**C**), 16 (**D**) and 24 (**E**) pulses (Scott *et al.,* 2017a).

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

To further investigate the effects of HIPPL treatment on fruit ripening, fruit texture was monitored following treatment. During preliminary work fruit texture was measured at 7 day intervals and it was found that there was a difference in fruit firmness that became apparent in HIPPL and LIUV treated fruit at 21 days following treatment (Appendix 2). Unfortunately due to the industry partners discontinuing the production of the cv. Mecano it was not possible to repeat the initial experiments in full. Evidence, however, is avaiable to show a significant difference in the texture change of tomato fruit at 21 DPT.

Both the LIUV and HIPPL treatments (16 pulses) show a significant reduction in the softening of tomato fruit over the 21 days of storage when compared to the control. The control exhibited a mean change in texture of 15.83 N whereas the HIPPL and LIUV treated fruit were approximately 5 Newtons firmer at 11.27 and 11.42 N, respectively (Figure 3.4).

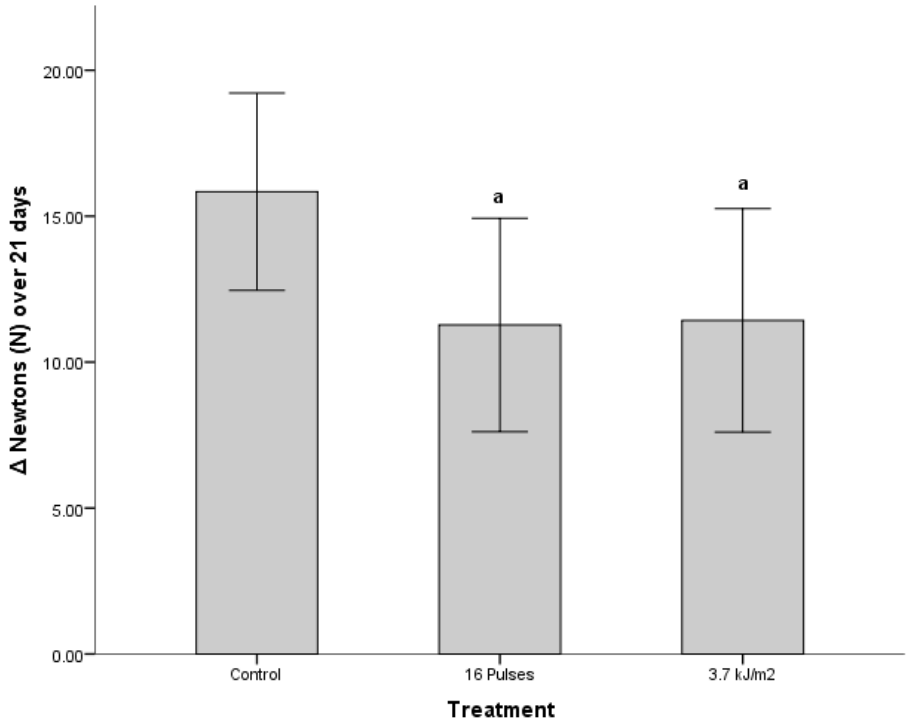

Figure 3.4: The change (Δ) in firmness (Newtons) of tomatoes (cv. Mecano) over 21 days of storage. Fruit were treated with 3.7 kJ/m^2 of low-intensity UV-C (with peak emissions at 254 nm) or 16 pulses from a high-intensity, pulsed polychromatic light source. Error bars show ± 1 standard deviation; n = 30. Labelling indicates statistical significance. Means sharing the same label are not significantly different from each other at $p < 0.05$.

3.3.2 Disease control

LIUV has previously been shown to induce disease resistance against *B. cinerea* on tomato fruit (Charles et al., 2008a). The possibility of inducing resistance with HIPPL was, therefore, investigated. HIPPL- and LIUV-treated fruit showed reductions in mean AUDPCs indicating reduced disease progression (Table 3.1). Welch's ANOVA showed that disease progression for all treated groups was significantly lower than the control. No significant differences were observed between HIPPL treatments and the LIUV treatment. However, a significant difference between the AUDPCs of the 8 and 16 pulse treatments was observed showing increased disease resistance for the 16 pulse treatment. These results show that HIPPL can induce resistance to *B. cinerea* on mature green tomatoes to similar levels to that of LIUV

treatment. This is in contrast to the results obtained by Marquenie *et al.* (2003) who reported no effect of pulsed light on the disease progression of *B. cinerea* on strawberries, *Fragaria ananassa*. This could be due to the employment of a different plant species or to differences in the spectral emission of the HIPPL sources. The HIPPL source used by Marquenie *et al.* (2003) produced 30 µs pulses at 15 pulses per second (15 Hz). The source in this study, however, produces 360 µs pulses at 3.2 pulses per second. Furthermore, the authors reported that the percentage of light falling within the UV region was 50% of a 7 J pulse in contrast to the output obtained here (1% of a 505 J pulse).

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

Table 3.1: Area underneath the disease progression curve (AUDPC) from mature green fruit (cv. Mecano) treated with 3.7 kJ/m^2 of low-intensity UV-C (peak emissions at 254 nm) and a range of high-intensity, pulsed polychromatic light treatments (8, 16 and 24 pulses). Inoculations were performed with *Botrytis cinerea* at 10 d post treatment and measured at 3 and 4 days post-inoculation. N=30, IRE=2 (Scott *et al,* 2017a).

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

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

The majority of studies on LIUV-induced disease resistance have been carried out postharvest on mature green tomatoes. Treatment at this stage is not entirely relevant for the UK tomato industry where tomatoes are picked when at the red ripe stage to meet

consumer preferences. Induced resistance against *B. cinerea* and *P. expansum* on red ripe tomatoes was, therefore, investigated.

LIUV treated fruit did not show significantly reduced disease progression against *B. cinerea* (Table 3.2). Moreover, an 8 pulse treatment did result in a slight reduction of disease progression but it was not statistically significant. Both 16 and 24 pulse HIPPL treatments, however, did significantly reduce the AUDPC in comparison to the control. Variation in the induction of hormetic responses for the HIPPL and LIUV sources is not unexpected due to the differences in spectral emission, the intensity of dose delivery and fractionation of the dose with HIPPL sources.

Similar results were observed for *P. expansum* as those for *B. cinerea* resistance assays on ripe fruit. The 16 pulse HIPPL treatment showed a greater reduction in AUDPC when compared to the 3.7 kJ/m^2 with reductions in disease progression of 18.2 and 13.5%, respectively, Figure 3.5.

Table 3.2: Area underneath the disease progression curve (AUDPC) from ripe fruit (cv. Mecano) treated with 3.7 kJ/m2 of low-intensity UV-C (peak emissions at 254 nm) and a range of high-intensity, pulsed polychromatic light treatments (8, 16 and 24 pulses). Inoculations were performed with Botrytis cinerea at 10 d post treatment and measured at 3 and 4 days post-inoculation. N=30, IRE=2 (Scott et al, 2017a).

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

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

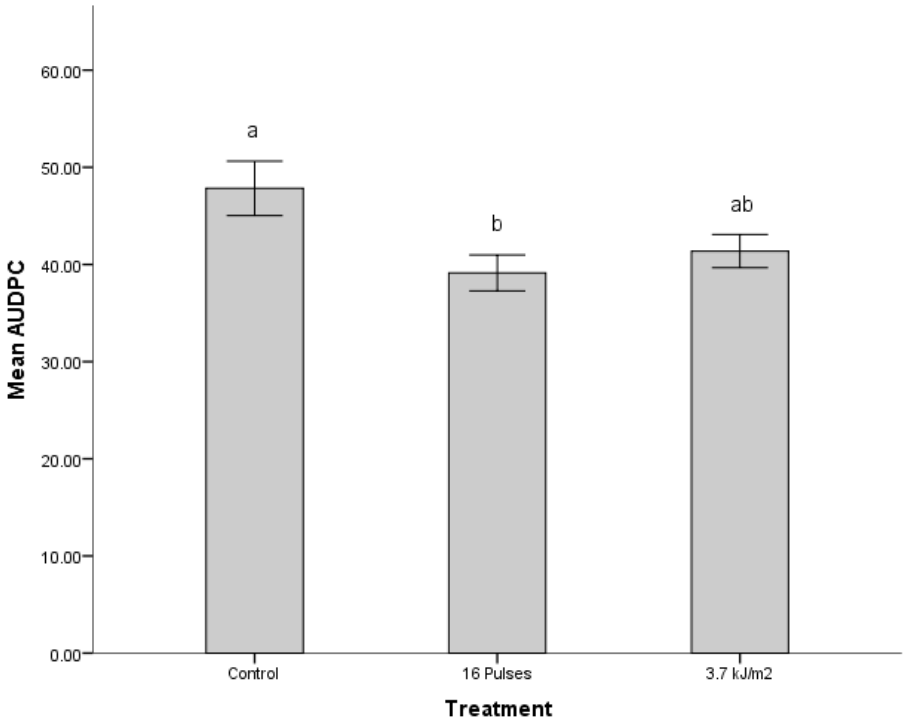

Figure 3.5: Area underneath the disease progression curve (AUDPC) from ripe fruit (cv. Mecano) treated with 3.7 kJ/m2 of low-intensity UV-C (peak emissions at 254 nm) or 16 pulses of high-intensity, pulsed polychromatic light. Inoculations were performed with *Penicillium expansum* at 10 d post treatment and measured at 3 and 4 days post-inoculation. Error bars show ± 1 S.E.M. Labelling indicates statistical significance. Means sharing the same label are not significantly different from each other at $p < 0.05$. N=30, IRE=3.

3.4 Summary and conclusions

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

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

In addition to the investigations on mature green fruit, induced disease resistance was also monitored on ripe fruit. Fruit, generally, becomes more susceptible to disease as it ripens as its nutrients become more easily available to pathogens. Furthermore, it is this developmental state that is of the greatest interest to commercial growers in the UK as fruit is harvested at the ripe stage due to improved consumer qualities. The majority of pathogen-related problems are observed in storage before shipment to the supermarkets.

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

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

Chapter 4: Comparative gene expression analysis following hormetic low-intensity UV-C and high-intensity, pulsed polychromatic light treatments on tomato fruit

In our previous study (Chapter 3) we found that a 16-pulse treatment at 4.6 kJ/m^2/pulse of HIPPL induced both delayed ripening and disease resistance on tomato fruit at comparable levels to a 3.7 kJ/m^2 treatment with LIUV (Scott *et al.,* 2017a). The use of HIPPL reduced treatment times from 350 s to 10 s when LIUV treatments were delivered at 20 W m^{-2}.

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

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

A further benefit of hormesis in tomato fruit is that of increased nutritional content through changes in secondary metabolism. Changes to secondary metabolism have been observed on a wide range of LIUV-treated fruit including blueberries (*Vaccinium*

corymbosum), grapefruit (*Citrus paradisi*) and mango (*Mangifera indica*) -to mention but a few (D'Hallewin. *et al.,* 2000; Gonzalez-Aguilar *et al.,* 2007; Perkins-Veazie *et al.,* 2008). Both HIPPL and LIUV treatments significantly increase total carotenoid and phenolic content as well as the antioxidant activities of tomato fruit (Liu *et al.,* 2009; Liu *et al.,* 2012; Pataro *et al.,* 2015). To date, however, little is known of the molecular mechanisms underpinning HIPPL hormesis in tomato fruit.

4.1 Aims

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

4.2. Materials and methods

4.2.1 Fruit production, low-intensity UV-C and high-intensity, pulsed polychromatic light treatment and inoculation

Fruit production, treatment and inoculation was performed as stated in Chapter 3.

4.2.2 Sampling, RNA extraction and reverse transcription

A No.2 cork borer (6.25 mm outer diameter) was used to take a 50-75 mg sample of pericarp from tissue directly facing the light sources. Samples were placed into microcentrifuge tubes and immediately frozen in liquid nitrogen. Samples were stored at -80°C until use. Twenty-four hours before tissue homogenisation a single 4 mm steel bead (Qiagen) was cooled in liquid nitrogen and added to each microcentrifugetube. Samples were placed into a Tissuelyser II (Qiagen) block and stored at -80°C overnight. Samples were homogenised using two runs of a Tissuelyser II (Qiagen) at 30Hz for 1 minute. Homogenised samples were stored at -80°C until RNA extraction.

RNA was extracted with the RNeasy Plant Mini Kit (Qiagen) following the manufacturers guidelines. An on-column DNase treatment was performed with the RNASE free DNASE kit (Qiagen). A further off column DNase step was performed with the TURBO^{TM} DNase kit (Ambion) following the manufacturers guidelines. RNA purity and yield was assessed via

NanoDrop (Thermo Scientific). All samples were then diluted to a concentration of ≤ 50 ng/µl.

The integrity of RNA samples was then assessed. Approximately 250 ng (5 µl) of RNA was combined with 1 µl of 6X orange/green loading dye (Promega). Samples were heated to 70 ^{o} C for 1 minute and then placed on ice. A 5 µl aliquot was loaded into a 1.2% (w/v) Agarose, TAE (tris base, acetic acid & EDTA) gel and run in a 1 x TAE buffer for 30 minutes at 60 v (Appendix A3.1). Products were checked for the presence of clear 28S and 18S rRNA bands. A 20 µl Reverse transcription reaction was then performed using the High-Capacity RNA-to-cDNA kit (Applied Biosystems) following the manufacturers protocol. The resulting cDNA was stored at -20°C until use.

4.2.3 qPCR

Two technical replicates were performed for each sample. Each 10 µl reaction contained 5 µl of 2x Fast SYBR® Green master mix (Applied Biosystems) and 2 µl of template cDNA. Primer concentrations and annealing temperatures were as stated in Table 4.1. Reactions were run on a LightCycler 480 ® (Roche) with a two-step amplification cycle. The cycle was as follows; a pre-incubation of 10 minutes at 95°C followed by 40 cycles of 95°C for 5 s and the anneal for 45 s. Ct values were calculated utilising the second derivative maximum method. A melting curve was run between 90°C and 60°C following the amplification to allow for checking of product specificity. Primers were optimised utilising a pooled sample and a 5-point 5-fold dilution series from which efficiency was calculated (Eq. 4.1). Specificity of products from each primer pair were confirmed by sequencing and NCIB basic local alignment search tool (BLAST) analysis.

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

Equation 4.1: Amplification efficiency of qPCR, showing amplification efficiency (AE), fold dilution (D) used in serial dilutions and gradient of the logarithmically plotted dilution curve (∇) (Pfaffl, 2004).

Table 4.1: Information on the primers used in qPCR

Target gene	Reference	Accession	Product Tm (°C)	Conc. (nm)	Anneal (°C)	Efficiency (%)	Sequence 5'-3'
ACT	Aime *et al.*, 2008	Solyc03g078400.2.1	75.4	100	60	81.0	F: AGGCACACAGGTGTTATGGT R: AGCAACTCGAAGCTCATTGT
ACO1	Van de Poel *et al.*, 2012	Solyc07g049530	76.4	500	60	85.8	F: ACAAACAGACGGGACACGAA R: CCTCTGCCTCTTTTTCAACC
CHI9	Aime *et al.*, 2008	Solyc10g055810.1	78.5	50	58	80.0	F: GAAATTGCTGCTTTCCTTGC R: CTCCAATGGCTCTTCCACAT
CRTR-B1	Tiecher *et al.*, 2013	Solyc06g036260.2	77.8	500	60	101.4	F: TTGGGCGAGATGGGCACAC R: TGGCGAAAACGTCGTTCAGC
FLS	Tiecher *et al.*, 2013	Solyc11g013110.1	71.7	250	60	97.3	F: ATGGAGGCAGCTGGTGGTGAA R: CAGGCCTTGGACATGGTGGATA
GLUB	Aime *et al.*, 2008	Solyc01g059980.2	75.8	100	60	79.3	F: TCTTGCCCCATTTCAAGTTC R: TGCACGTGTATCCCTCAAAA
OPR3	Blanco-Ulate *et al.*, 2013	Solyc07g007870	76.8	300	60	86.0	F: TGGGTTTCCTCATGTGCCAG R: GCAGCTCCAGCAGGTTGATA
PAL	Bovy *et al.*, 2002	Solyc09g007920	74.0	500	60	96.3	F: ATTGGGAAATGGCTGCTGATT R: TCAACATTTGCAATGGATGCA
PG	Xie *et al.*, 2014	Solyc10g080210.1	74.6	250	58	78.5	F: ATACAACAGTTTTCAGCAGTTCAAGT R: GGTTTTCCACTTTCCCCTACTAA
PR1a	Aime *et al.*, 2008	Solyc09g007010.1	80.9	250	58	78.9	F: TCTTGTGAGGCCCAAAATTC R: ATAGTCTGGCCTCTCGGACA

4.2.4 Experimental design and data analysis

Data was collected from two independent replicate experiments. For each experiment three fruit per treatment group per time point were analysed; n=6. Fruit were sampled before treatment (baseline expression), at 24 HPT, 10 DPT and 12 HPI. Each gene of interest was run on its own 384 well plate (Roche) along with a 5-point, 5-fold dilution series that was used to calculate the efficiency of amplification (Eq. 4.1). Following qPCR samples were checked for non-specific products (melt curve analysis), Ct values ≥ 35 and technical replicate standard deviations > 0.5. Samples exhibiting these characteristics were considered unsuitable for further analysis and the data was re-collected. Inter-plate calibration was performed with a pooled sample to correct for interpolate bias (Eq. 4.2). Amplification efficiency was then used to correct Ct values following Eq. 4.3. Technical replicates were then averaged before further analysis.

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

Equation 4.2: Interplate calibration equation for qPCR. The cycle threshold for any given sample is Ct. The Ct value of the interplate calibrator is Ct^{IPC} and N is equal to the number of plates that are being calibrated between (TATAABiocenter, 2012).

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

Equation 4.3: Efficiency correction of qPCR cycle threshold (Ct) values. CtE is the efficiency corrected Ct value and AE is the efficiency of amplification (Kubista & Sindelka, 2007).

Actin was used as reference gene as in previous UV-C studies and *B. cinerea* inoculation studies (Liu *et al.,* 2011; Virk *et al.,* 2012; Blanco-Ulate *et al.,* 2013; Tiecher *et al.,* 2013). Following efficiency correction actin was used to normalise the data giving ΔCt (Eq. 4.4). Data was normalised to baseline gene expression and fold change between treatment groups was calculated following Eq. 4.5. For experiments utilising theoretical copy number a copy number of 100 was assigned to the baseline (pre-treatment) gene expression levels and the further data was adjusted accordingly.

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

Equation 4.4: Normalisation of qPCR gene of interest with reference gene. CtE(goi) is the efficiency corrected cycle threshold (Ct) value for the gene of interest and CtE(ref) is the efficiency corrected Ct value for the reference gene (Pfaffl, 2004).

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

Equation 4.5: Calculation of fold change for qPCR. ΔCtET is the normalised and efficiency corrected mean cycle threshold (Ct) value for the treatment group and ΔCtEC is the normalised and efficiency corrected mean Ct value of the control group (Livak & Schmittgen, 2001).

Statistical analysis was performed on the normalised Ct values (ΔCt) using statistical software package SPSS 22 (IBM). One-way ANOVA with Tukey's post-hoc testing was performed. Where the homogeneity of variances assumption could not be met Welch's robust ANOVA was performed followed by the Games-Howell post-hoc test. Statistical significance is defined as $p \leq 0.05$. Results from the independent experimental replicates can be found in Appendix 3.

4.3 Results and discussion

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

4.3.1 Phytohormones and disease resistance

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

Expression of *ACO1* in treated fruit was shown to be significantly different from that of the control at 24 HPT. Expression levels for HIPPL- and LIUV-treated fruit were both 3.1-fold higher than that of the control. At 10 DPT and 12 HPI, however, the levels of *ACO1* were not significantly different across groups. Expression in control fruit however, was approximately 1.2 to 2.2-fold higher than treated fruit, Figure 4.1.

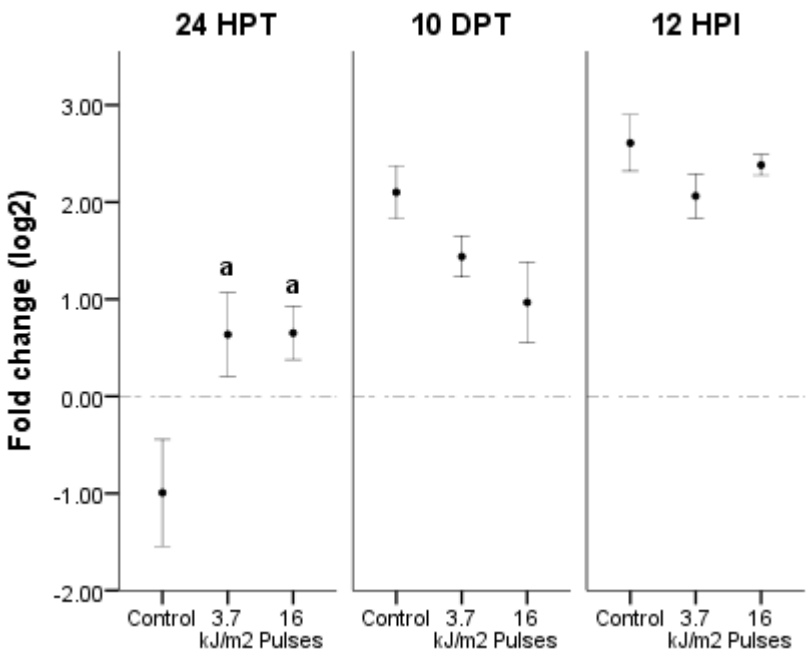

Figure 4.1: Relative expression of *ACO1* (1-aminocyclopropane-1-carboxylic acid oxidase 1), an enzyme in ethylene biosynthesis, following treatment with either 16 pulses from a high-intensity, pulsed polychromatic light (HIPPL) source or 3.7 kJ/m^2 from a low-intensity UV-C source (LIUV). Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). Labelling indicates statistical significance, within a given time point, where groups sharing labels are not significantly different at $p< 0.05$. N=6, IRE=2. Bars show ± 1 S.E.M. (Scott *et al.,* 2017b).

The downregulation of *ACO1* at 10 DPT and 12 HPI may contribute towards the delayed ripening phenotype observed following HIPPL and LIUV treatment of tomato fruit (Liu *et al.,* 1993; Scott *et al.,* 2017a). This is supported by two studies. Firstly, Zhefeng *et al.,* (2008) showed that virus induced gene silencing of an *ACO1* transcription factor (LeHB1) in tomato led to a reduction in *ACO1* mRNA and delayed ripening (colour change). Secondly, RNA interference inhibition of *ACO1* has been shown to lead to a reduction in ethylene biosynthesis and a prolonged shelf life (Behboodian *et al.,* 2012)

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

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

In HIPPL treated fruit we detected a slight downregulation of *OPR3* (<2-fold) at 24 HPT, Figure 4.2. Expression in control fruit remained at the baseline levels. After 10 days of storage (10 DPT) a significant increase in *OPR3* expression was observed at 3.8 and 3.9-fold for HIPPL and LIUV treatments in comparison to the control. Following inoculation (12 HPI) *OPR3* expression increased in all groups. Expression, however, was still significantly higher in treated fruit at 2.1 and 2.2-fold greater in HIPPL and LIUV treated fruit. OPR3 upregulation following LIUV and HIPPL treatments may result in increased JA levels and activation of JA inducible plant defences involved in resistance against necrotrophic pathogens (Glazebrook, 2005).

This is supported by Scalschi *et al.,* (2015) who showed, through RNAi silencing of OPR3, that its expression determines the availability of 12-oxo phytodienoic acid (ODPA) and expression of major genes involved in JA synthesis (Scalschi *et al.,* 2015). Furthermore, silencing of OPR3 also increased susceptibility to *B. cinerea* and reduced callose deposition in tomato; a defence response against the invading pathogen (Scalschi *et al.,* 2015).

Upregulation of OPR3 may, therefore, contribute towards disease control of *B. cinerea* following HIPPL and LIUV treatment (Liu *et al.,* 1993; Scott *et al.,* 2017a). The initial reduction in *OPR3* expression was analogous to the results observed by Liu *et al.,* (2011) who showed a 3.9-fold reduction in *OPR2* at 24 HPT following LIUV treatment; no further time points were monitored.

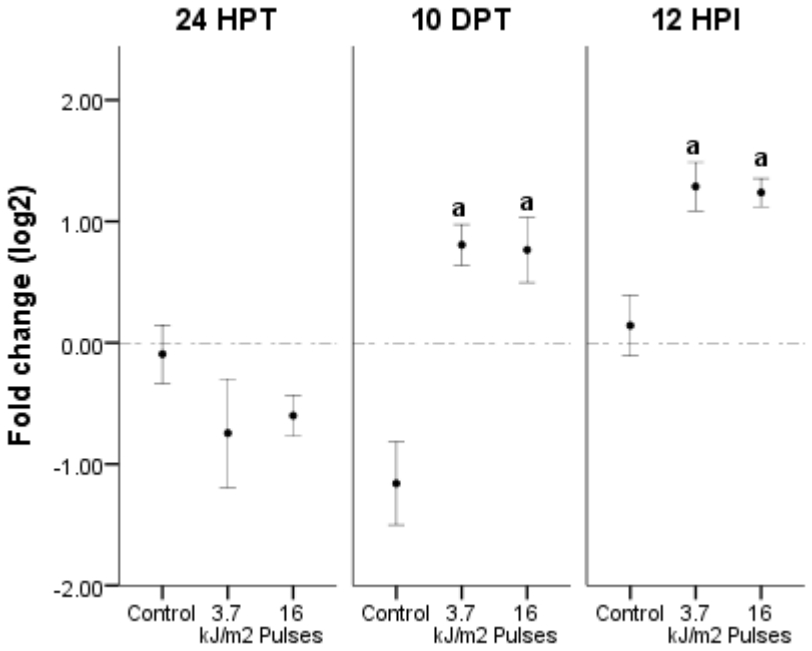

Figure 4.2: The relative expression of *OPR3* (12-Oxophytodienoate reductase 3) a jasmonate biosynthesis protein transcript, following treatment with either 16 pulses from a high-intensity, pulsed polychromatic light (HIPPL) source or 3.7 kJ/m^2 from a low-intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). Labelling indicates statistical significance, within a given time point, where groups sharing labels are not significantly different at $p< 0.05$. N=6, IRE=2. Bars show ± 1 S.E.M. (Scott *et al.,* 2017b).

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

P4 expression was increased in comparison to the control at each of the time-points, Figure 4.3. The differences, however, were only significant at 10 DPT and 12 HPI. *P4* levels in LIUV and HIPPL treated fruit were 50.3 and 55.5-fold and 38.0 and 35.5-fold higher than that of the control at 10 DPT and 12 HPI, respectively. The results indicate that HIPPL and LIUV treatments induce SA signalling upon treatment.

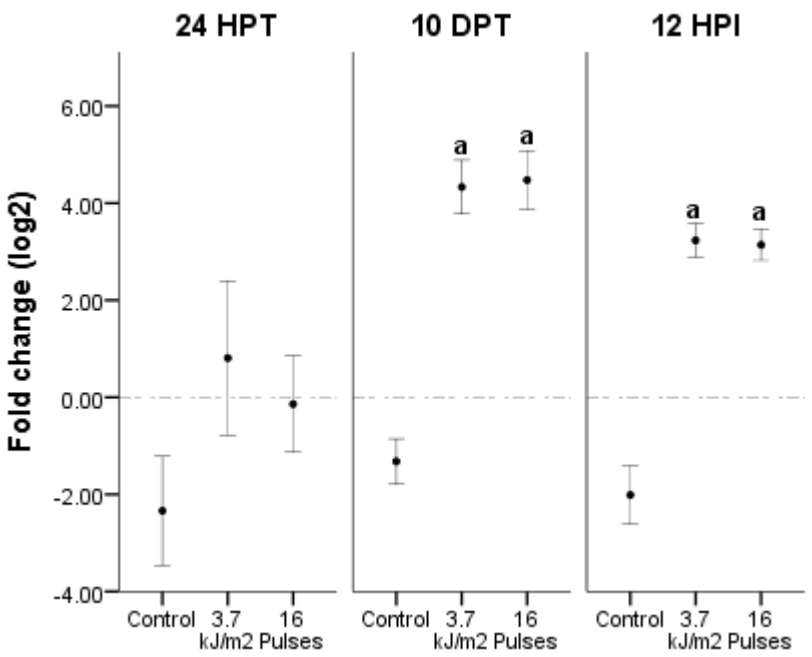

Figure 4.3: The relative expression of *P4* (*PR1a*) a salicylic acid-inducible pathogenesis-related protein and marker of systemic acquired resistance (SAR) following treatment with either 16 pulses from a high-intensity, pulsed polychromatic light source (HIPPL) or 3.7 kJ/m^2 from a low-intensity UV-C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

SA, however, has been shown to only play a small part in resistance against *B. cinerea.* Tomato plants expressing the bacterial gene *nahG* cannot accumulate SA and were shown to be slightly more susceptible to *B. cinerea* (Asselbergh *et al.,* 2007). SA and P4, however, play a greater role in protecting the plants against biotrophic pathogens (Glazebrook, 2005). The results may, therefore, indicate that HIPPL and LIUV hormesis could potentially be used as a means to protect against a broad range of pathogens

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

Levels of *GluB* were similar in all groups at 24HPT, Figure 4.4. At 10 DPT, however, expression of *GluB* was increased 32.4 and 40.1 –fold in HIPPL- and LIUV-treated tomato

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

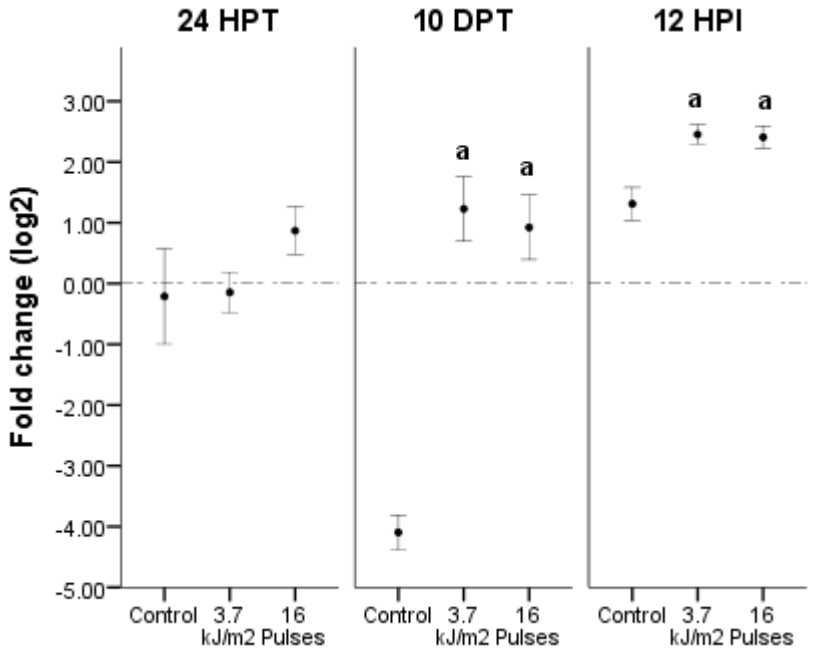

Figure 4.4: Relative expression of *GluB* (β-1,3,-Glucanase) an the ethylene inducible pathogenesis-related protein transcript, following treatment with either 16 pulses from a high-intensity, pulsed polychromatic light (HIPPL) source or 3.7 kJ/m^2 from a low-intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). Labelling indicates statistical significance, within a given time point, where groups sharing labels are not significantly different at $p< 0.05$. N=6 IRE=2. Bars show ± 1 S.E.M. (Scott *et al.,* 2017b).

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

Expression profiles observed for *CHI9* were similar to profiles of *GluB*. At 24 HPT, a slight increase in *CHI9* expression was detected in HIPPL- and LIUV-treated fruit while expression in control decreased below baseline, Figure 4.5. At 10 DPT a statistically significant increase in expression can be seen with 10.0 and 7.3-fold differences between the control and LIUV and HIPPL treatments, respectively. This was approximately 2-fold above baseline.

Following inoculation (12 HPI) expression of *CHI9* only increased in the control fruit. The expression in treated samples, however, was still significantly greater than the control at 2.9 and 3.8-fold for the HIPPL and LIUV groups. Our results indicate that disease resistance due to increased chitinase expression is a mechanism shared by both light treatments. Two chitinases observed by Charles *et al.,* (2008b) also showed a similar pattern of expression to those observed here with little change in expression at 3 DPT but intensified expression at 10 DPT and also induced following inoculation as seen for the control fruit which showed approximately a 2-fold increase in expression following inoculation.

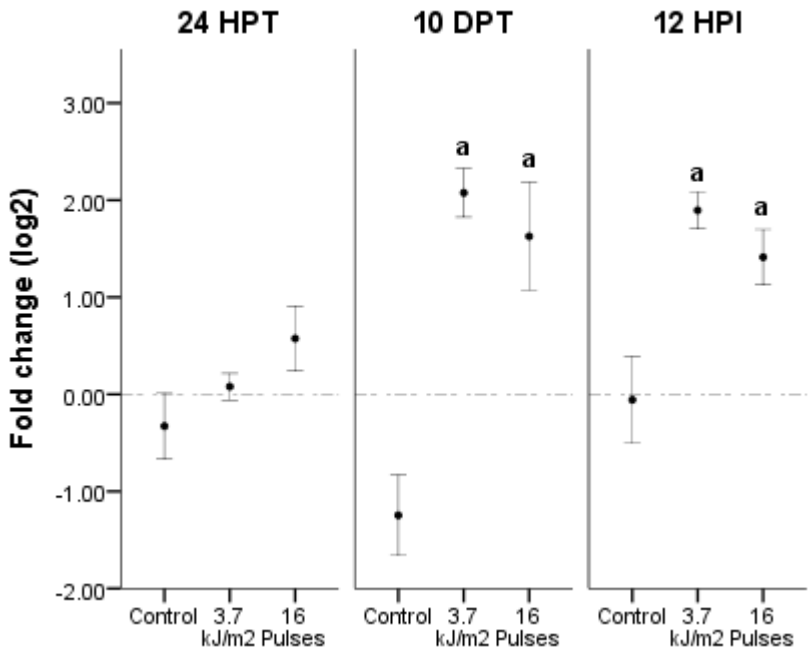

Figure 4.5: Relative expression of *CHI9* (Chitinase 9) a jasmonic acid induced pathogenesis-related protein transcript, following treatment with either 16 pulses from a high-intensity, pulsed polychromatic light (HIPPL) source or 3.7 kJ/m^2 from a low-intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). Labelling indicates statistical significance, within a given time point, where groups sharing labels are not significantly different at $p< 0.05$. N=6. Bars show ± 1 S.E.M. (Scott *et al.,* 2017b).

The upregulation PR proteins P4, GLUB and CHI9 following HIPPL and LIUV treatment supports the hypothesis that the disease control of *B. cinerea* is achieved through induced resistance (Liu *et al.,* 1993; Scott *et al.,* 2017a). Furthermore, the postulated broad-range resistance is further supported as all three PR proteins are also upregulated in tomatoes' defence responses against both the greenhouse and silverleaf whitefly (*Bemisia tabaci* and *Trialeurodes vaporariorum*) and *Fusarium oxysporum* f.sp. *lycopersici* (Puthoff *et al.,* 2010; Aime' *et al.,* 2008). HIPPL and LIUV hormesis may, therefore, be an effective pre-harvest alternative to chemical control against both pathogens and pests

4.3.2 Ripening and secondary metabolism

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

At 24 HPT *PG* expression was at baseline levels in all groups, Figure 4.6. The expression in all groups increased at 10 DPT. In HIPPL- and LIUV-treated fruit however, levels of *PG* were significantly lower than the control with 6.1 and 32.2-fold decreases, respectively. *PG* levels decreased in response to inoculation (12 HPI) with *B. cinerea* in all groups. Fruit from both treated groups however, still showed significantly lower expression than control fruit with 15.4 and 3.0-fold less *PG* in LIUV and HIPPL treated fruit, respectively. Expression of *PG* in HIPPL treated fruit supports our observations that control fruit were 14.6 and 22.4% softer than HIPPL treated fruit at 14 and 21 days post treatment (Appendix 4). Furthermore, Barka *et al.,* (2000) showed a reduction in PG activity following LIUV treatment.

The reductions in *PG* expression are, therefore, likely to play a role in the delayed tissue softening observed following LIUV and HIPPL treatments (Liu *et al.,* 1993; Appendix 4). This is supported by Langley *et al.,* (1994) who showed that antisense RNA gene silencing of *PG* reduced the tissue softening of tomato fruit

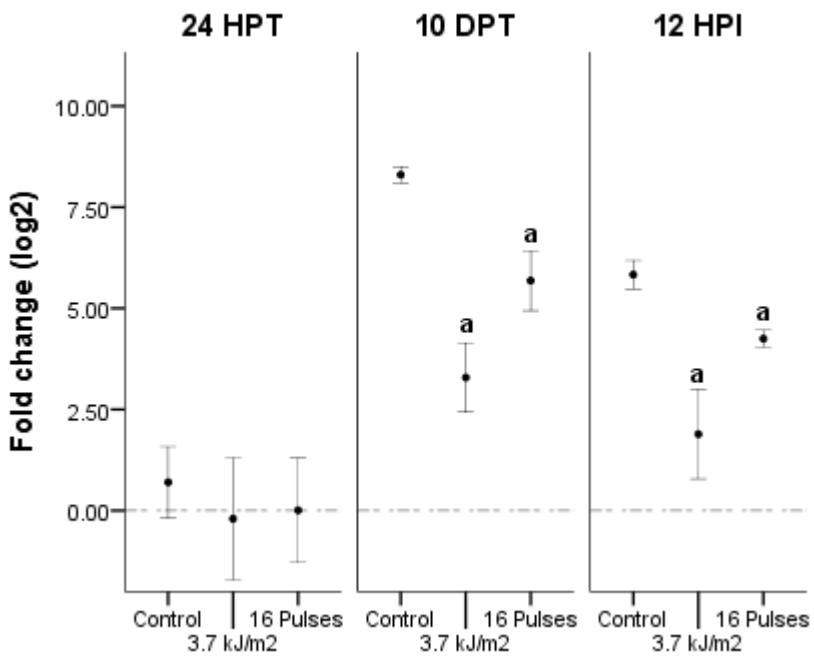

Figure 4.6: The relative expression of *PG* (polygalacturonase) following treatment with either 16 pulses from a high-intensity, pulsed polychromatic light (HIPPL) source or 3.7 kJ/m^2 from a low-intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). Labelling indicates statistical significance, within a given time point, where groups sharing labels are not significantly different at $p< 0.05$. N=6, IRE=2. Bars show ± 1 S.E.M. (Scott *et al.,* 2017b).

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

We have shown a significant 1.7-fold increase in *CRTR-B1* expression in HIPPL and LIUV treated fruit 24 HPT, Figure 4.7. At 10 DPT and 12 HPI, however, expression of *CRTR-B1* was not significantly different from that of the control. Analogous patterns of expression of CRTR-B1 along with zeaxanthin and lutein concentrations were observed by Teicher et al.,

(2013) who observed increases in both at 1 DPT following LIUV treatment and similar levels to the control at 7 DPT.

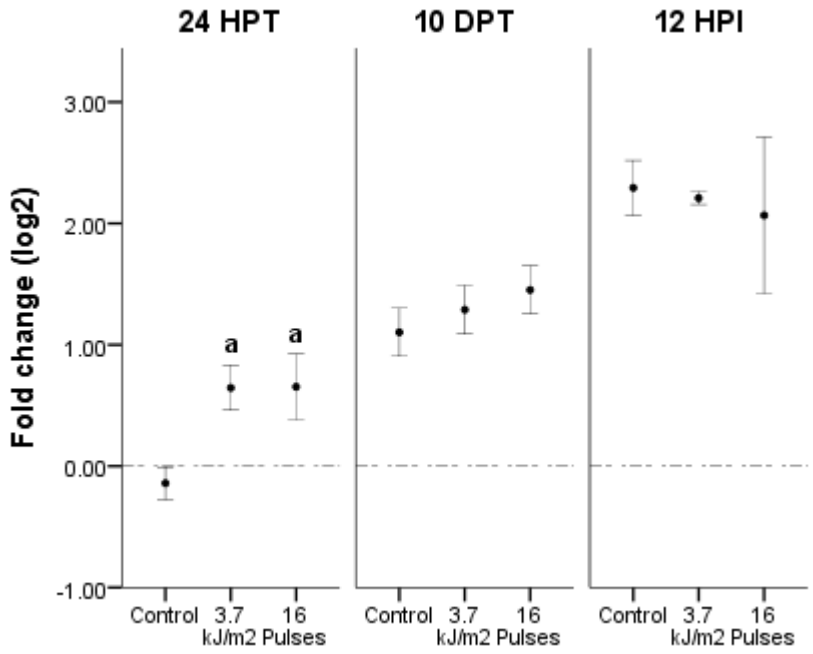

Figure 4.7: Relative expression of *CRTR-B1* (β -carotene hydroxylase) following treatment with either 16 pulses from a high-intensity, pulsed polychromatic light (HIPPL) source or 3.7 kJ/m^2 from a low-intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). Labelling indicates statistical significance, within a given time point, where groups sharing labels are not significantly different at $p< 0.05$. N=6, IRE=2. Bars show ± 1 S.E.M. (Scott *et al.,* 2017b).

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

Following treatment expression of *PAL* was approximately at baseline levels in all groups at 24 HPT, Figure 4.8. Following 10 days of storage and immediately before inoculation (10 DPT) a slight increase in expression of *PAL*, in comparison to the control, was observed for the treated fruit with a 1.4 and 1.5-fold increases for HIPPL and LIUV treatments, respectively. The differences, however, were not significant. Following inoculation (12 HPT) *PAL* expression was significantly greater for both HIPPL and LIUV with a 2.0 and 2.1-fold increase in comparison to the control, respectively. The results are in agreement with Teicher et al., (2013) showed an approximately 2 to 3-fold increase in *PAL* following LIUV treatment in the mesocarp of tomato fruit at both 1 and 7 DPT. The exocarp, however, showed no increase in *PAL* at either 1 or 7 DPT. Expression of *PAL*, however, was not monitored following inoculation.

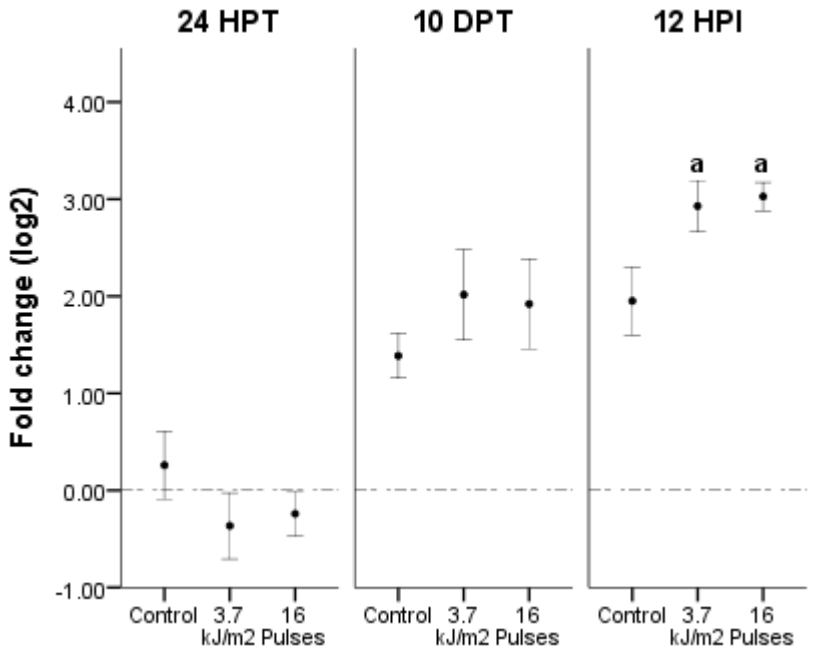

Figure 4.8: The relative expression of *PAL* (phenylalanine ammonia lyase) following treatment with either 16 pulses from a high-intensity, pulsed polychromatic light (HIPPL) source or 3.7 kJ/m^2 from a low-intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). Labelling indicates statistical significance, within a given time point, where groups sharing labels are not significantly different at $p< 0.05$. N=6, IRE=2. Bars show ± 1 S.E.M. (Scott *et al.,* 2017b).

An increase in the expression of *PAL* following inoculation indicates upregulation of the phenylpropanoid pathway as PAL catalyses its first step converting phenylalanine to cinnamic acid. With products including SA, flavonols and anthocyanins, upregulation of the phenylpropanoid pathway following inoculation may allow treated fruit to respond to pathogens faster than the control fruit resulting in resistance to *B. cinerea* as observed in Liu *et al.,* (1993) and Scott *et al.,* (2017a).

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

FLS expression was decreased at 24 HPT with 5.8 and 2.5- fold higher concentration in the control fruit when compared to the LIUV and HIPPL sources, respectively, Figure 4.9. Only the LIUV treatment was significantly different from the control. At 10 DPT *FLS* expression further decreased with the HIPPL and LIUV treated fruit showing 100.3 and 109.1-fold differences when compared to the control. At 12 HPI *FLS* expression in the control fruit decreased by approx. 4-fold to baseline levels. Expression for both treatments increased to 8.9-fold and 10.8 below the control for HIPPL and LIUV treated fruit, respectively. This was still significantly lower than the control.

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

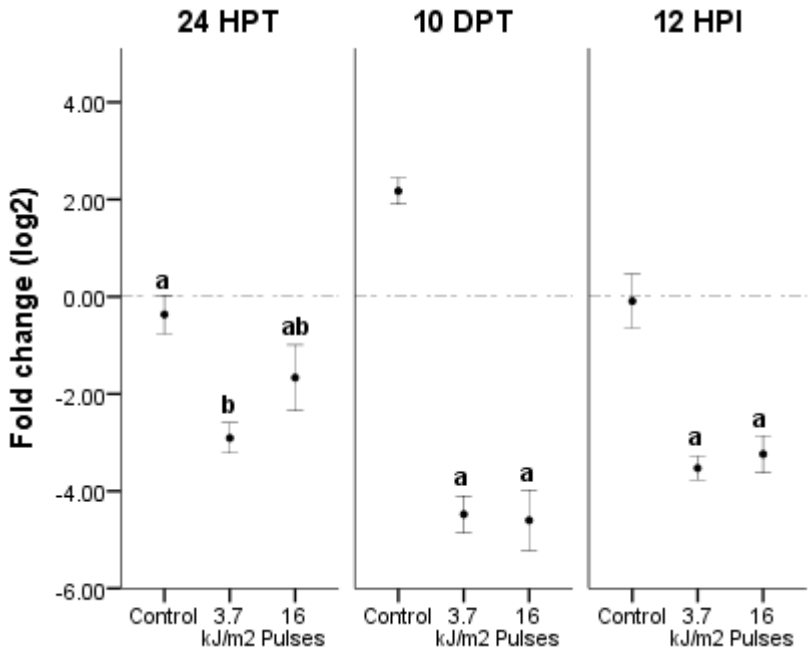

Figure 4.9: Relative expression of *FLS* (flavonol synthase) following treatment with either 16 pulses from a high-intensity, pulsed polychromatic light (HIPPL) source or 3.7 kJ/m^2 from a low-intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). Labelling indicates statistical significance, within a given time point, where groups sharing labels are not significantly different at $p< 0.05$. N=6. Bars show ± 1 S.E.M. (Scott *et al.,* 2017b)

4.3.3 Gene priming

It has been shown that biotic and abiotic inducers of disease resistance can prime plant defences; reducing the impact of the phytopathogens (Mur *et al.,* 1996; Latunde-Dada & Lucas, 2001; Cools & Ishii, 2002; Yang *et al.,* 2015). Defence priming is postulated to be an adaptive, low-cost defensive measure activated by a given priming stimulus, in our case HIPPL and LIUV treatments. In primed plants, transcriptional responses are deployed in a faster, stronger or more sustained manner following the perception by the plant of a secondary stress (Martinez-Medina *et al.,* 2016).

In Martinez-Medina *et al.,* (2016) a number of priming-related expression profile identifiers were defined. Firstly, a small or transient change in expression following the initial priming stimulus should be present. To identify this change we have monitored gene expression at 24 HPT. To assess whether changes were transient, samples were taken at 10 DPT, where

genes exhibiting priming should show similar levels of expression to the control. Secondly, following exposure to a secondary (trigger) stimulus a faster, stronger or more sustained response should be observed. The trigger stimulus, used here, was inoculation with *B. cinerea*. Samples were taken at 12 HPI to assess whether a stronger response was observed. Ct values were transformed into theoretical copy number allowing the change in theoretical copy number from 10 DPT to 12 HPI to be calculated.

All of the genes, in this study, showed small changes in gene expression at 24 HPT; following the priming stimulus (Figures 4.1-4.9). Excluding *ACO1*, *CRTR-B1* and *PAL*, all of the genes from LIUV and HIPPL treated samples, however, showed increased changes in expression at 10 DPT. This indicates that the changes were not transient and may have an increased fitness cost indicative of direct induction (van Hulten *et al.,* 2006). Following the triggering stimulus only P4 and PAL (from HIPPL and LIUV treated samples) showed a stronger response in gene expression associated with gene priming (Figure 4.10). P4, however, also exhibited an increase in expression at 10 DPT, indicating direct induction (Figure 4.3). Expression levels of *PAL* at 10 DPT, from LIUV and HIPPL treated fruit, is similar to that of the control and, therefore, meets the identifiers of a gene priming-associated expression profile outlined by Martinez-Medina *et al.,* (2016) (Figure 4.8).

The majority of the genes investigated in this study appear to be directly induced and fail to meet the expression profile of gene priming; a summary of the results is available in Table 4.2. Further investigations, however, are required to provide conclusive evidence that gene priming is involved in LIUV and HIPPL hormesis. Such investigations may include analyses of histone modifications, DNA methylation and changes to the expression of transcription factors (WRKYs and MYC2) and mitogen-activated protein kinases MPK3 and MPK6 that are associated with priming (Conrath *et al.,* 2015). An involvement for priming in LIUV and HIPPL hormesis is also supported by further criteria outlined in Martinez-Medina *et al.,* (2016). This includes a more robust defence response and broad-spectrum activity. LIUV hormesis has been shown to induce resistance against a number of pathogens on tomato fruit including *B. cinerea, Rhizopus stolonifera, Penicillium expansum* and Colletotrichum gloeosporioides (Liu *et al.,* 1993; Stevens *et al.,* 1997; Fletcher, unpublished data). Furthermore, HIPPL hormesis can induce resistance against *B. cinerea, C. gloeosporioides and P. expansum* on tomato fruit (Scott *et al.,* 2017a; Fletcher*, unpublished data).

* Alex Fletcher is a doctoral researcher in Prof. Matthew Dickinson's Laboratory in Plant Science (the University of Nottingham). We collaborated to performed postharvest inoculations with *C. gloeosporioides* following high-intensity, pulsed polychromatic light and low-intensity UV-C treatments of tomato (cv. Mecano).

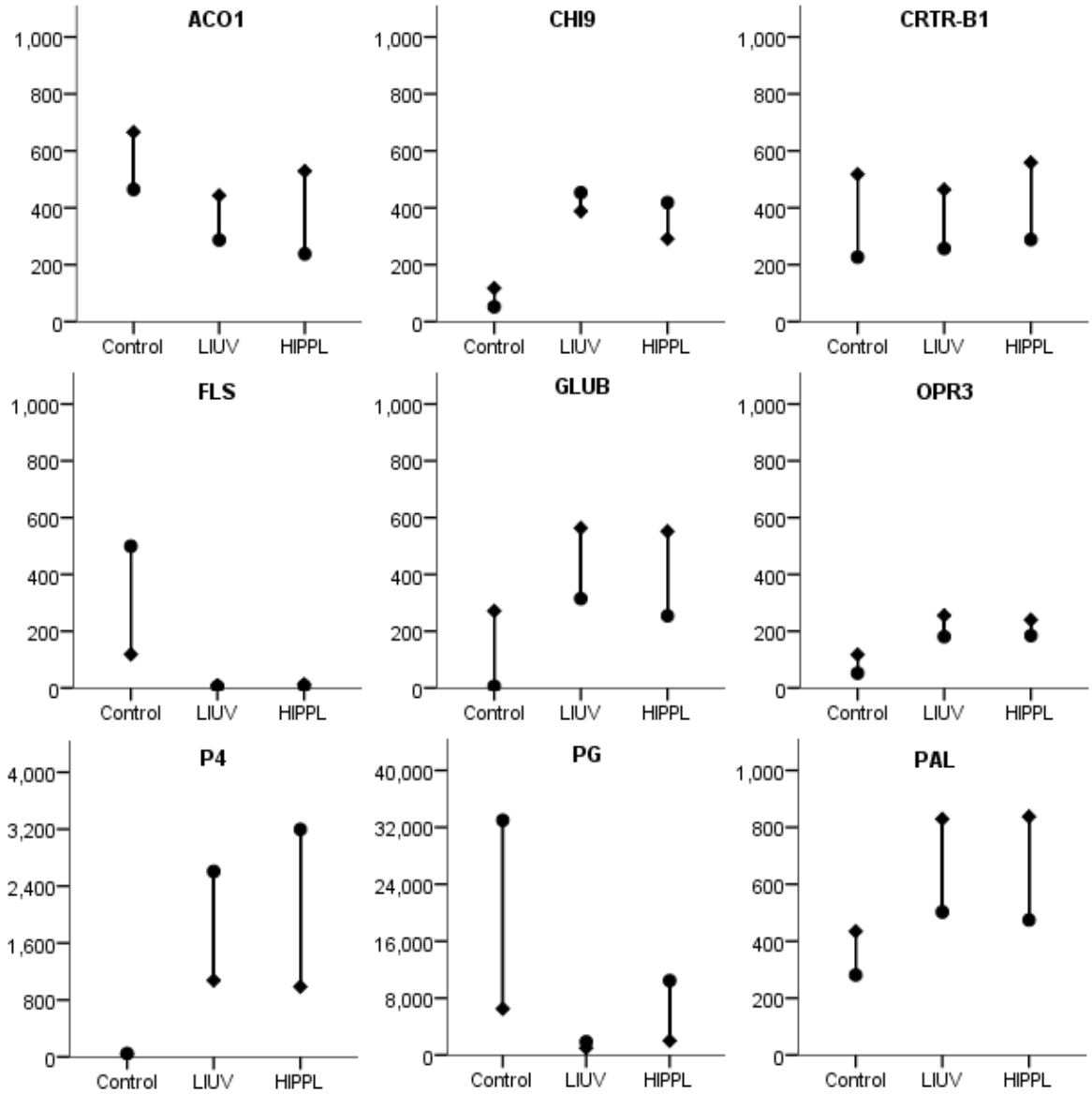

Figure 4.10: Gene expression levels shown as theoretical copy number (TCN) between samples taken at 10-days post treatment (●) and 12-hours post inoculation with *Botrytis cinerea* (◆). The vertical line denotes the magnitude of change. Fruit were treated with either 16 pulses from a high-intensity, pulsed polychromatic light (HIPPL) source or 3.7 kJ/m^2 from a low-intensity UV-C (LIUV) source and compared to untreated control Graphs show the following genes; ***ACO1*** (1-aminocyclopropane-1-carboxylic acid oxidase; an enzyme in ethylene biosynthesis), ***GLUB*** (β-1,3,-Glucanase an ethylene inducible pathogenesis related protein) , ***CHI9*** (chitinase 9 a jasmonic acid pathogenesis related protein) ***CRTR-B1*** (β -carotene hydroxylase), ***FLS*** (flavonol synthase), ***OPR3*** (12-Oxophytodienoate reductase 3, a jasmonate acid biosynthesis protein), ***PAL*** (phenylalanine ammonia lyase), ***PG*** (polygalacturonase), ***P4*** (a salicylic acid inducible pathogenesis related protein). N=6, IRE=2. (Scott *et al.,* 2017b)

Table 4.2: Gene priming expression profile identifier summary as stated in Martinez-Medina *et al.,* (2016) (Scott *et al.,* 2017b).

Gene	Small change following priming stimulus	Transient change following priming stimulus	Stronger response following triggering stimulus	Potential priming response
ACO1	1	1	0	0
CHI9	1	0	0	0
CRTR-B1	1	1	0	0
FLS	1	0	0	0
GluB	1	0	0	0
OPR3	1	0	0	0
P4	1	0	1	0
PAL	1	1	1	1
PG	1	0	0	0

0 = No and 1 = Yes

The observed HIPPL- and LIUV-induced resistance may, therefore, be mainly due to increased expression and/or accumulation of transcripts between treatment and the day of inoculation (10 DPT). This could result in a gradual increase in resistance following light treatment, like that observed by *Charles* et al. (2008a) following LIUV treatment of tomatoes. Priming, however, may also play a role in the induction of resistance as an expression profile analogous to that of the priming response seen for *PAL.* It is also possible that the priming response for other genes monitored here may have occurred before or after 12 HPI and went unnoticed in our experimental design as such responses have shown greater levels of protein activity and gene expression > 3 hours following inoculation (Mur *et al.,* 1996, Latunde-Dada & Lucas, 2001, Cools & Ishii, 2002, Yang *et al.,* 2015). Further investigation is required to elucidate the full extent to which priming may play a role in LIUV an HIPPL induced resistance.

4.4 Summary and conclusions

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

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

Importantly we can infer that HIPPL induced resistance, similarly to that of LIUV, is due to the production of PR proteins including P4, B-1,3-Glucanase and Chitinase 9. Moreover, a reduction in PG and ACO1 expression may contribute towards delayed ripening and reduced susceptibility to B. cinerea in HIPPL and LIUV-treated tomato fruit (Barka *et al.,* 2000; Scott *et al.,* 2017a)

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

Chapter 5: Assessing the importance of direct tissue exposure and fruit orientation during low-intensity UV-C and high-intensity, pulsed polychromatic light treatment

During the work carried out and described in Chapter 3, it was noted that following treatment the delay in colour change appeared to be most intense at the points of the fruit directly facing either the low-intensity UV-C (LIUV) or high-intensity, pulsed polychromatic light (HIPPL) light source. There is limited information on whether LIUV hormesis induces a systemic or local effect. What limited information is available seems to be dependent on the produce undergoing treatment. No information to date is available on the nature of induced resistance for the HIPPL source.

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

In addition to the variation in responsiveness from both the developmental stage and organ treated, Stevens *et al.,* (2005) showed that alterations in treatment orientation may influence the propagation of a systemic signal and induction of systemic disease resistance.

Stevens *et al.,* (2005) showed that treatment at the calyx followed by inoculation at the blossom end resulted in systemic disease resistance on apples (*Malus domestica*) against *Collectotrichum gloesporioides*, peaches (*Prunus persica*) against *Monilinia fructicola* and tangerines (*Citrus reticulate*) against *Penicillum digitatum*.

5.1 Aims

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

5.2 Materials and methods

All experimental protocols were as stated in Chapter 3, section 3.2. Fruit, however, were treated from various orientations including treatments directed at the side, calyx and blossom end.

5.2.1 Experimental design and data analysis

For the investigations concerned with the necessity for direct tissue exposure for delayed ripening, when fruit are treated from the side, 15 fruit per group were used in each experimental replicate (n=30). For disease control assays 10 fruit per group per independent replicate experiment were used (n=20). For investigations into blossom end- and calyx-focused treatments 10 fruit per treatment group per experiment were used. For experiments concerning colour change, with blossom end- and calyx-directed treatments, two experimental replicates were performed (n=20). Only a single experimental replicate was performed for the experiments concerning disease resistance (n=10). Analysis was performed using statistical software package SPSS 22 (IBM). One-way ANOVA with Tukey's post-hoc testing was performed. Where the homogeneity of variances assumption could not be met Welch's robust ANOVA was performed followed by the Games-Howell post-hoc test. Statistical significance is here defined as $p \leq 0.05$. Results from the independent experimental replicates can be found in Appendix 4.

5.3 Results and discussion

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

To establish whether LIUV and HIPPL delayed ripening is a local response, Δ TCI was calculated for tissue directly facing the light sources and at 90 º from that directly exposed to the sources. For all groups, the tissue at 90 º from the source showed no significant difference in ripening progression. When compared with directly exposed tissue, however, tissue at 90 º from the 16 and 24 pulse treatments showed a significantly greater progression in ripening to that of the directly exposed tissue (Figure 5.1). Tissue at 90 º for the LIUV treatment showed no statistically significant difference from directly exposed LIUV tissue or the control. The data presented here indicates that direct exposure to both LIUV and HIPPL is required for the induction of delayed ripening. Disease resistance was also monitored to determine whether direct tissue exposure is required for induction of hormesis. Unexposed tissue inoculations showed no reduction in AUDPC and similar levels of disease progression to that of the control (Figure 4.2). The directly exposed tissue, however, showed significant reductions following both HIPPL and LIUV treatment. It can therefore be stated that treatments employing HIPPL or LIUV sources require direct tissue exposure to successfully induce resistance to *B. cinerea*. This is in agreement with previous findings (Stevens *et al.,* 1998a; Charles *et al.,* 2008; Liu *et al.,* 2011) who routinely rotated the fruit during LIUV treatment to ensure that the entire surface area of the fruit was irradiated, although they but did not specifically set out to show that failure to do so would not result in systemic resistance.

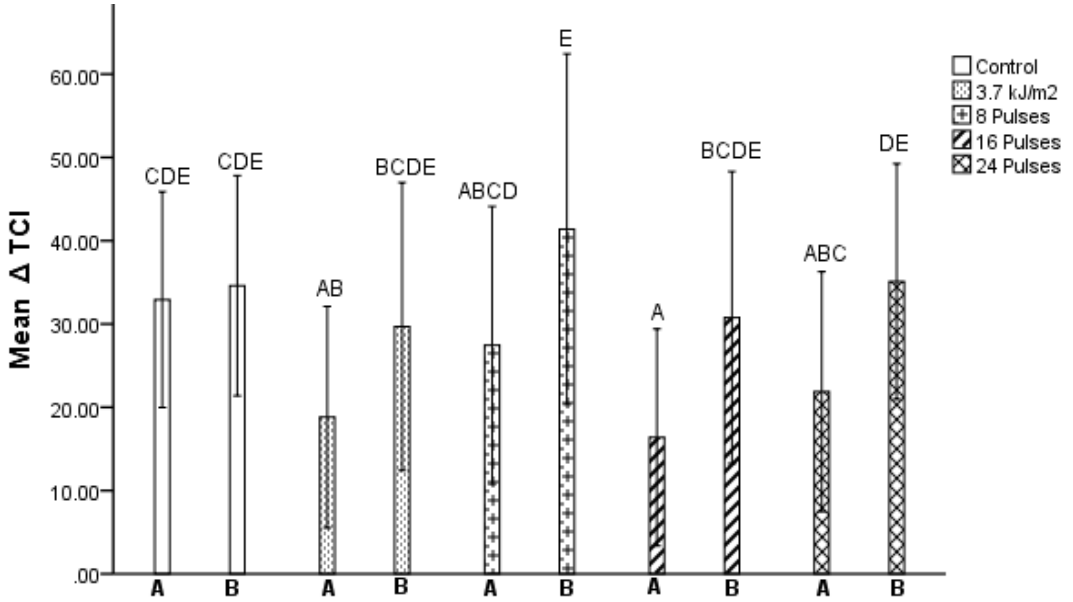

Figure 5.1: The change in (Δ) TCI (tomato colour index) from day 0 - 10 of mature green fruit from cv. Mecano. Fruit were treated with a hormetic low-intensity UV-C treatment of 3.7 kJ/m^2 from a source with peak emissions at 254nm and three high-intensity, pulsed polychromatic light treatments of 8, 16 and 24 pulses. TCI measurements were taken from tissue directly facing the light source (A) and at 90^o from the source (B). Error bars show a single standard deviation; n = 30, IRE=2. Means sharing the same label are not significantly different from each other at $p < 0.05$. (Scott *et al.,* 2017a).

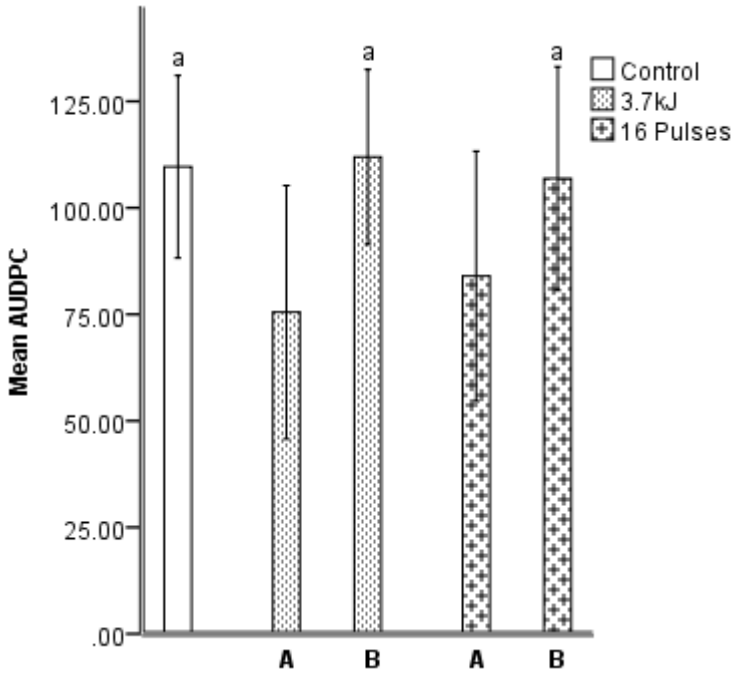

Figure 5.2: Area underneath the disease progression curve (AUDPC) of tomatoes, cv. Mecano, treated on a single side and inoculated with *B. cinerea* at 10 d post treatment (DPT). Fruit were treated with an established low-intensity UV-C treatment of 3.7 kJ/m^2, peak emissions at 254 nm, and a high-intensity, pulsed polychromatic light treatment of 16 pulses. Exposed tissue (**A**) or unexposed tissue (**B**). Error bars show ± 1 standard deviation; n = 20, IRE=2. Labelling indicates statistical significance. Means sharing the same label are not significantly different from each other at $p < 0.05$. (Scott *et al.,* 2017a).

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

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

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

Both the fruit receiving direct exposure to a 3.7 kJ/m^2 LIUV treatment and monitoring of colour at the calyx and blossom end exhibited a significant reduction in colour change Figures 5.3 A and C, respectively. The change in TCI was 59.8 and 59.9% less for the calyx- and blossom end-treated fruit, respectively. For fruit receiving indirect treatment the TCI changed to a greater extent to that of their respective controls. These differences, however, were not significant (Figure 5.3 B and D). This indicates that direct tissue exposure is required for the induction of delayed ripening LIUV on fruit treated from both the blossom end and calyx.

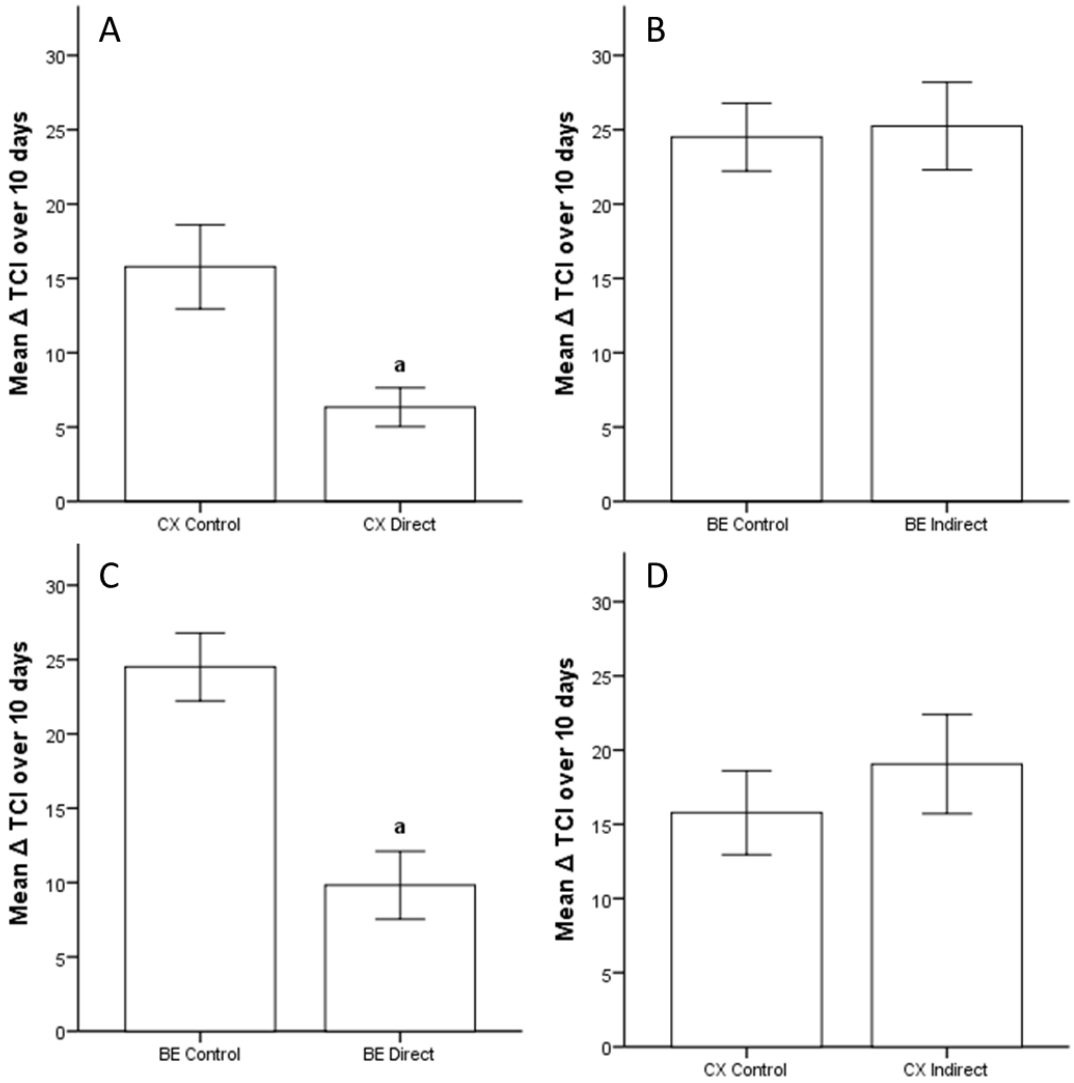

Figure 5.3: The change in tomato colour index (TCI) over ten days of storage following a 3.7 kJ/m^2 treatment with a low-intensity UV-C source. **A)** Measurements taken from the calyx (CX) of control fruit and fruit treated at the CX. **B)** Measurements taken from the blossom end (BE) of control fruit and of fruit treated at the CX **C)** Measurements taken from the BE of control fruit and fruit treated at the BE. **D)** Measurements taken from the CX of control fruit and the CX of fruit treated at the BE. N=20, IRE=2. Bars show ± 1 S.E.M. Means sharing the same label are not significantly different from each other at $p < 0.05$.

For the monitoring of induced disease resistance an identical pattern was observed with both direct treatments which showed a reduction is disease progression and indirect treatments resulting in similar or increased levels of disease in comparison to the control (Figure 5.4). Fruit inoculated and treated from the calyx showed a significant reduction in disease progression with a 47.0% reduction in comparison to the control (Figure 5.4 A). Similarly, the fruit treated and inoculated at the blossom end showed a 33.7% reduction in disease progression (Figure 5.4 C). This reduction was not significant but further experimental replicates, however, may possibly increase confidence in the mean and show

a significant difference. The results from directly exposed tissue are in contrast to fruit receiving treatment from the calyx and inoculated at the blossom end, and treatments at the blossom end and inoculation at the calyx. Both of the treatments showed similar or slightly increased levels of disease progression in comparison with the control (Figures 5.4 B and D).

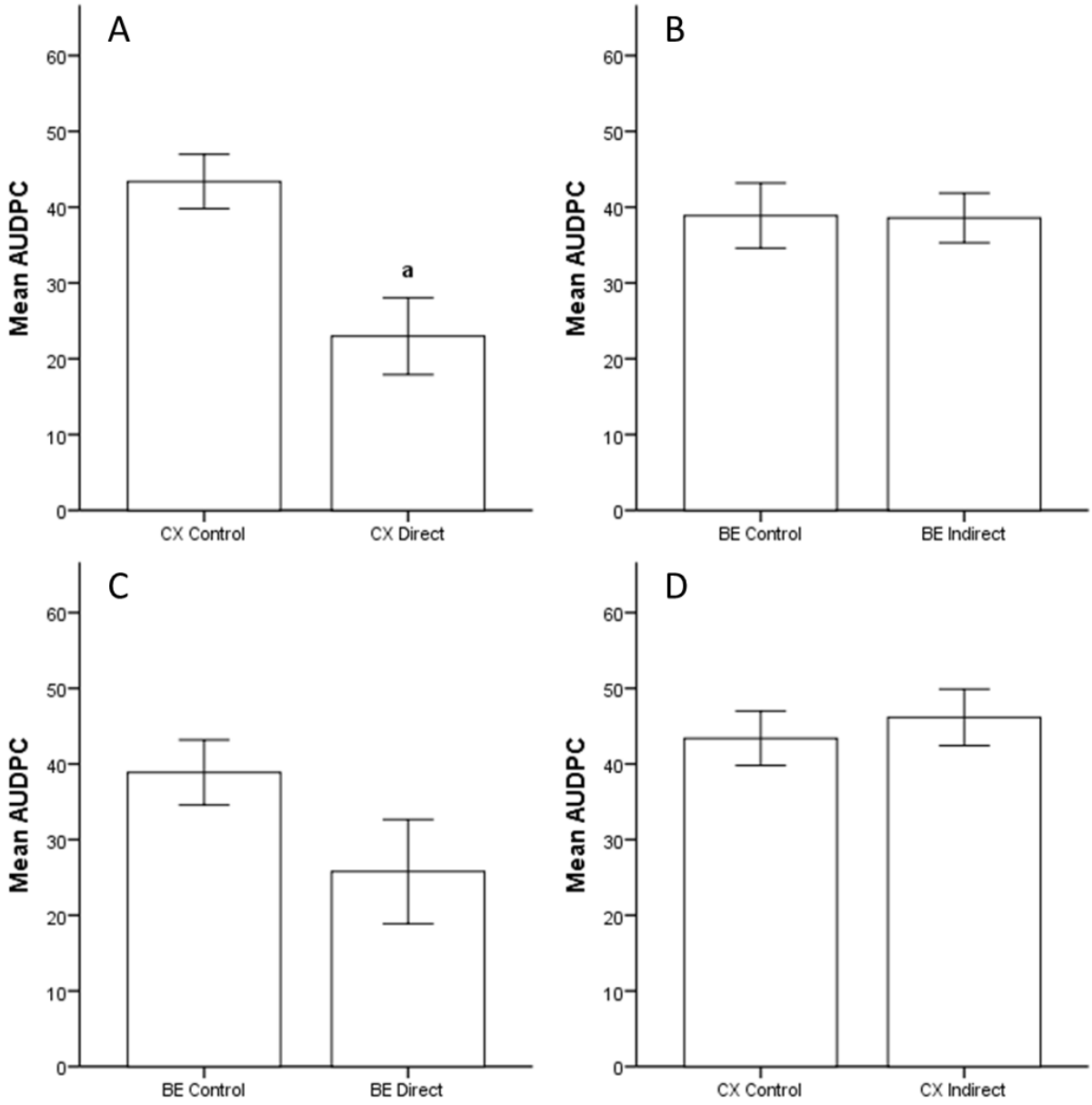

Figure 5.4: Area underneath the disease progression curve (AUDPC) from tomato fruit of the cv. Mecano following a 3.7 kJ/m^2 treatment with a low-intensity UV-C source and inoculation with *Botrytis cinerea*. **A)** Measurements taken from the calyx (CX) of control fruit and fruit treated at the CX. **B)** Measurements taken from the blossom end (BE) of control fruit and of fruit treated at the CX **C)** Measurements taken from the BE of control fruit and fruit treated at the BE. **D)** Measurements taken from the CX of control fruit and the CX of fruit treated at the BE. N=10, IRE=1. Bars show ± 1 S.E.M. Means sharing the same label are not significantly different from each other at $p < 0.05$.

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

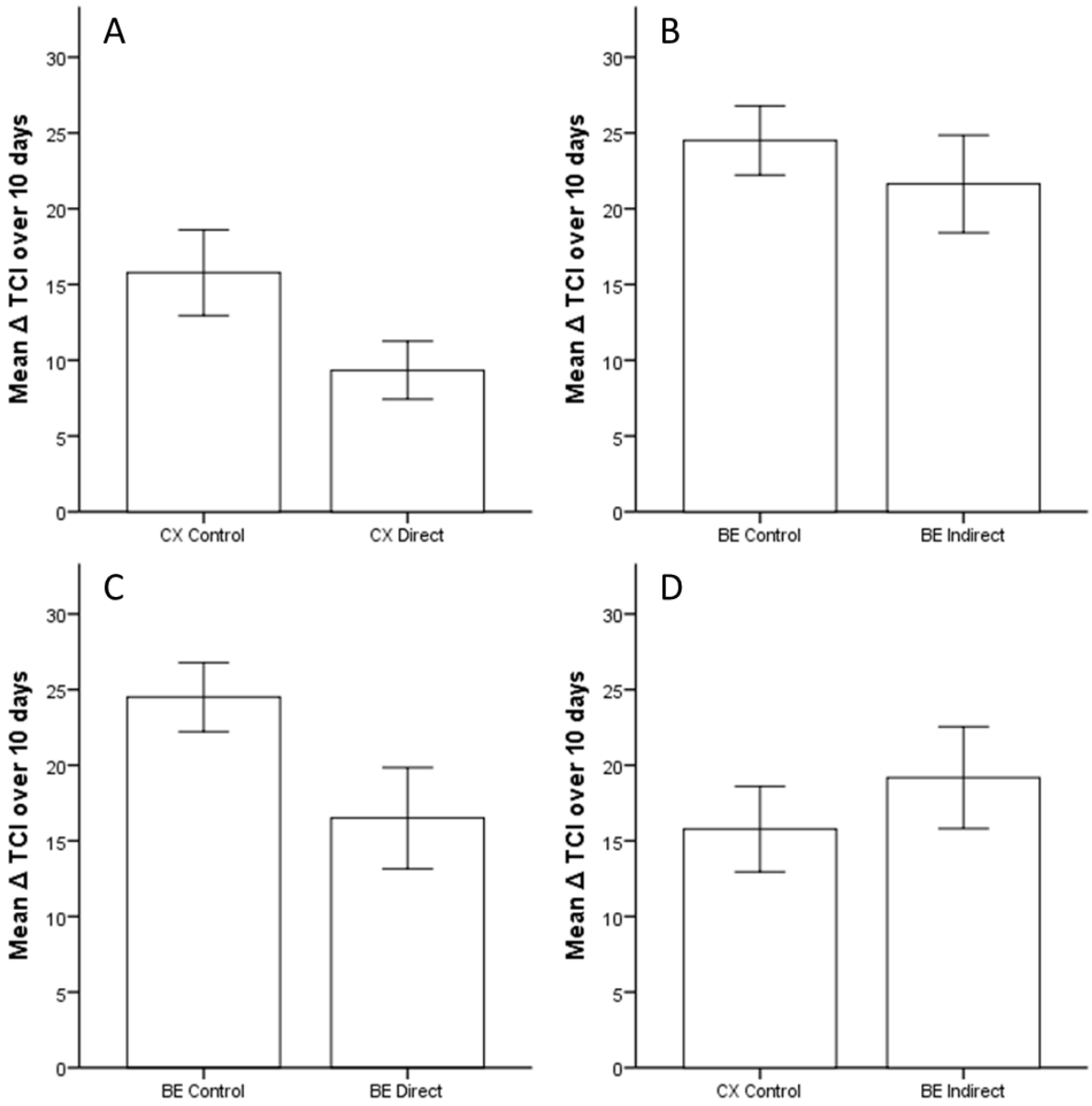

Figure 5.5: The change in tomato colour index (TCI) over ten days of storage following a 16 pulse treatment with a high-intensity, pulsed polychromatic light source. **A)** Measurements taken from the calyx (CX) of control fruit and fruit treated at the CX. **B)** Measurements taken from the blossom end (BE) of control fruit and of fruit treated at the CX **C)** Measurements taken from the BE of control fruit and fruit treated at the BE. **D)** Measurements taken from the CX of control fruit and the CX of fruit treated at the BE. N=20, IRE=2. Bars show ± 1 S.E.M. Means sharing the same label are not significantly different from each other at $p < 0.05$.

Unlike the LIUV source, a slight decrease in colour change was observed for the fruit treated at the calyx with colour monitored at the blossom end (Figure 5.5 B). This was not statistically significant and further testing would be required to determine this definitively. It is, however, likely to be due experimental variation as treatment from the blossom end while monitoring the calyx gave an increase in ripening in comparison to the control, Figure

5.5 D, as has been seen for all LIUV treated fruit for both colour change and disease resistance, (Figures 5.4 and 5.3).

Treatment orientation also showed no difference in the acquisition of a systemic response for HIPPL-treated fruit when monitoring disease resistance. Again, both tissue directly treated at the blossom end and calyx showed reductions in disease progression when compared to the control. Reductions were 53.8 and 23.6% for the calyx and blossom end respectively (Figures 5.6 A and C). The former was significantly different from the control. As was seen for LIUV-treated fruit, the reduction in disease progression when fruit are treated from the blossom end was not as great as that from the calyx. With reductions of 53.8 and 23.6% for the HIPPL-treated fruit from the blossom end and calyx, respectively, and 47.0 and 33.7% for LIUV-treated fruit. This may indicate that not only do specific plant organs vary in their sensitivity and reactivity to UV-C, as shown by Petit *et al.,* (2009), but there may also be a differential response in spatially separated tissues of those organs. This is supported by evidence from Stevens *et al.,* (2005) who also showed that levels of induced resistance varied depending on the orientation of treatment.

One could hypothesise that the tissue of the blossom end of fruit would contain fewer photoreceptors to those at the calyx, as its natural positioning is towards the ground. Alternatively, its cellular homeostasis may be geared away from any severe adaptation to changes in lighting conditions and may therefore respond to a lesser extent to treatment. The reduction in the level of delayed ripening when treated from the blossom end can be seen in Figure 5.7 where treatments from the calyx (C) and side (A) produce islands of chlorophyll-rich tissue whereas the treatment from the blossom end (B) the production of carotenoids has become evident, but to a lesser extent to that of the untreated tissue at the calyx.

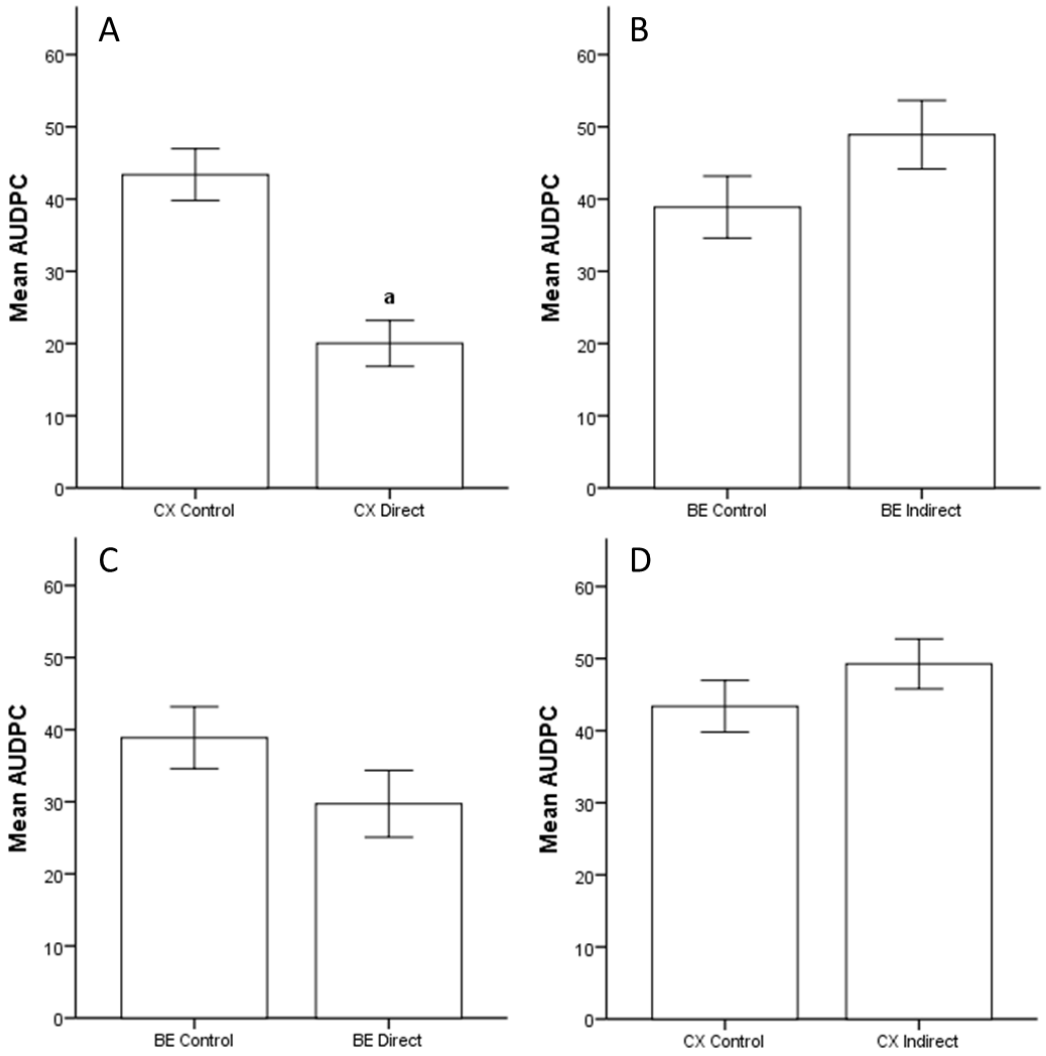

Figure 5.6: Area underneath the disease progression curve (AUDPC) from tomato fruit of the cv. Mecano following a 16-pulse treatment with a high-intensity, pulsed polychromatic light source and inoculation with *Botrytis cinerea*. **A)** Measurements taken from the calyx (CX) of control fruit and fruit treated at the CX. **B)** Measurements taken from the blossom end (BE) of control fruit and of fruit treated at the CX **C)** Measurements taken from the BE of control fruit and fruit treated at the BE. **D)** Measurements taken from the CX of control fruit and the CX of fruit treated at the BE. N=10, IRE=1. Bars show ± 1 S.E.M. Means sharing the same label are not significantly different from each other at $p < 0.05$.

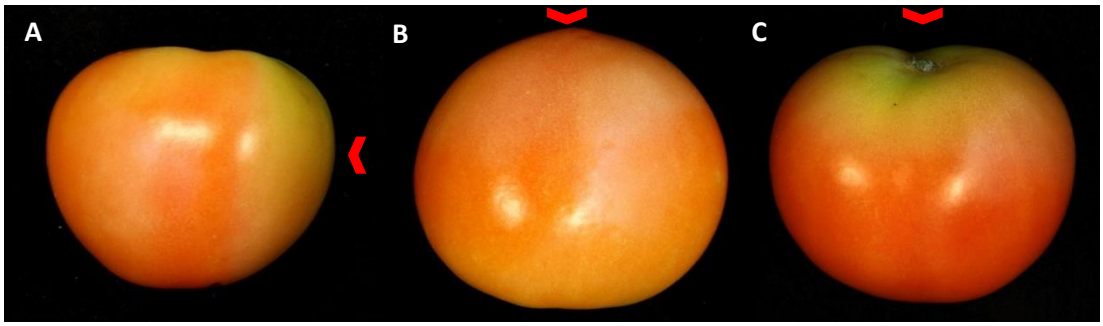

Figure 5.7: Representative samples of tomato fruit exposed to high-intensity, polychromatic light from different orientations. Mature green fruit (cv. Mecano) were treated with 16 pulses and photographed at 10 days post treatment. Red arrows indicate the positioning of the source. **A**) Treatment from the side. **B**) Treatment from the blossom end. **C**) Treatment from the calyx (Scott *et al.,* 2017a).

5.4 Summary and conclusions

In this study preliminary observations from Chapter 3, in which uneven ripening progression was observed, were built upon. It was shown by Stevens *et al.,* (2005) that treatments of apples, peaches and tangerines from the calyx resulted in the induction of disease resistance in tissues not directly exposed to the source both the side of the fruit and at the blossom end.

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

The data collected here directly contradicts that of Stevens *et al.,* (2005), however, a different fruit is being used which highlights the fact that different species, genera and cultivar may respond to LIUV and HIPPL treatments differently. Furthermore, the spatial

location of tissues within plant organs may also respond differently to treatment. This is supported by work done by both Stevens *et al.,* (2005) and Petite *et al.,* (2009) who showed differing levels of resistance depending on the spatial local of the tissue undergoing treatment and also differing level of reactivity to UVC depending on the plant organ undergoing treatment, respectively. In depth analysis of the variation of response to spatially distinct tissues require much greater investigation.

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

Chapter 6: Assessing the importance of UV-C, B and A and visible light within the high-intensity, pulsed polychromatic light source, for inducing the hormetic effects observed on tomato fruit

In Chapter 3 it was established that high-intensity, pulsed polychromatic light (HIPPL) treatments can induce both disease resistance and delayed ripening on tomato fruit to similar levels to that obtained with LIUV treatment. Unlike the low-intensity UV-C (LIUV) source, the HIPPL source emits broad spectrum (polychromatic) light. It is unclear to what extent wavelengths longer than UV-C contribute to HIPPL-induced resistance.

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

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

conditions. For example, post-treatment exposure to visible light leads to photoreversal and the absence of any hormetic benefits.

It is well established that storage of tomato fruits under visible light leads to increased ripening when measured in terms of colour change (Boe & Salunkhe, 1967). The effects of high intensity visible light and its ability to induce hormesis, however, have not been explored to a great extent. It is known that high intensity visible light can lead to the loss of viability of microrganisms such as *Escherchia coli, Porphyromonas gingivalis, Staphylococcus aureus* and *Fusobacterium nucleatum,* human pathogenic bacteria, and cells of the human retina (Lipovsky *et al.,* 2008). By definition hormesis is a phenomenon where low doses of a stressor bring about a positive change in the organism undergoing treatment. As high intensity visible light can cause damage to a broad range of cells and organisms, including plants, the ability for visible light to induce hormesis remains viable and a possibility which requires investigation.

Further evidence supports high intensity visible light-induced hormesis in plants. Firstly, exposure of plants to high intensity visible light can lead to the production of ROS (Schmitt *et al.,* 2015). ROS production and signalling can play an important role in plant's adaptation to stress (Vidhyasekaran, 2015) and is one of the key potential components that may lead to UV-C-induced hormesis (see section 1.6.2). ROS production is also noted as a potential mechanism for the bacteriocidal effect of visible light (Lubart *et al.,* 2011). Secondly, the hormetic or photobiomodulatory effects of visible light on wound healing have been observed in a number of studies; as reviewd by Tchanque-Fossuo *et al.,* (2016). The bacteriocidal effects of visible light, however, cannot be discounted. Finally, it has also been observed that growing plants under high intensity visible light, for intermittant periods, can induce disease resistance (Al-Jafar, 2016).

6.1 Aims

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

6.2 Materials and methods

Fruit were obtained as stated in section 3.2. Light treatments were performed as stated in sections 3.2.2 with the addition of 5 mm Borofloat ® 33 UV-C filtering glass (Schott, UK). The glass was cut to 500 x 300 (+/-1.0) mm and placed into a bespoke frame. The frame was mounted onto the front of the HIPPL source. The optical transmission of the glass can be seen in Table 6.1 and Figure 6.1. Inoculum preparation and colour and firmness measurements were performed as stated in sections 3.2.3 and 3.2.1, respectively.

Table 6.1: Average optical transmittance of Borofloat ® 33 UV-C filtering glass (Schott, UK) for the major electromagnetic radiation groups emitted by the high-intensity, pulsed polychromatic light source.

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

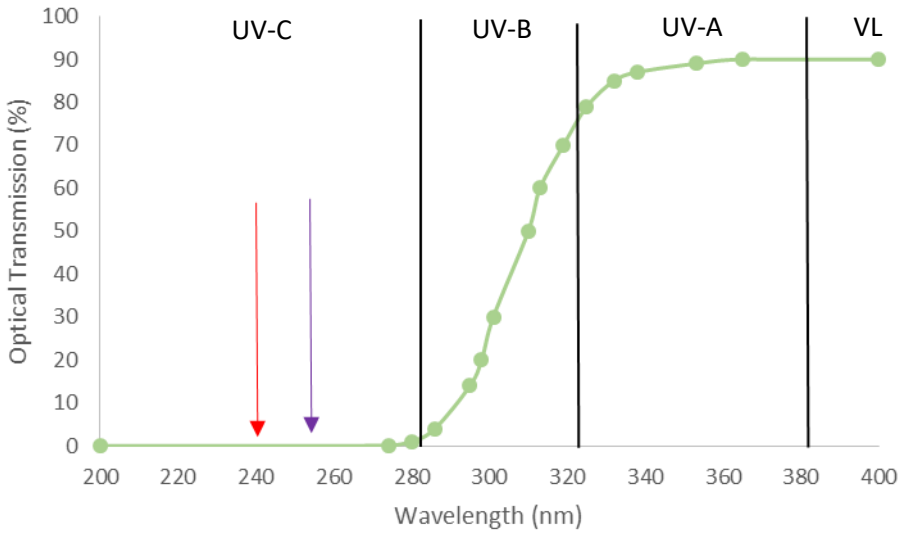

Figure 6.1: Optical transmission for the Borofloat ® 33 UV-C filtering glass (Schott). The red arrow indicates the optical cut off point (minimum wavelength) emmited by the high-intensity, pulsed polychromatic light source. The purple arrow indicates the standard peak emission for low-intensity UV-C sources (254 nm). Vertical lines indicate the groups of electromagnetic radiation UV, groups C, B and A along with VL (visible light).

6.2.1 Experimental design data analysis

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

6.3 Results and discussion

HIPPL treatments, either with or without UV-C, significantly reduced disease progression of *B. cinerea* in comparison to the control (Figure 6.2). Full spectrum HIPPL treatments reduced disease progression by 50.5% whereas treatments without UV-C only gave a 21.8% reduction. This indicates that UV-C in the HIPPL source accounts for approximately 56.8% of the disease control. It should, however, also be noted that as UV-B, UV-A and visible light transmittance is reduced, and some of the disease control attributed towards UV-C may be elicited by the aforementioned wavelengths. Future work to rectify this may be achieved by increasing the number of pulses. This, however, may be difficult to achieve as the transmittance of specific wavelengths is not reduced equally by the filtering glass.

HIPPL treatments with the UV-C filtered out also led to a delay in the ripening process following treatment (Figure 6.3). HIPPL and UV-C treatments led to a 44.1 and 16.6% reduction in ripening progression, respectively. A similar contribution was also seen for the involvement of UV-C in the response to delayed ripening where UV-C in the pulsed source accounted for 62.4% of the observed delayed ripening. For delayed ripening, however, the UV-C treatment was not significantly different to the control. Further replications of the experiment may lead to increased confidence in the mean leading to a significantly different result. Unfortunately, further replications were not possible due to our commercial partners ceasing the production of cv. Mecano. Results from the independent experimental replicates can be found in Appendix 5.

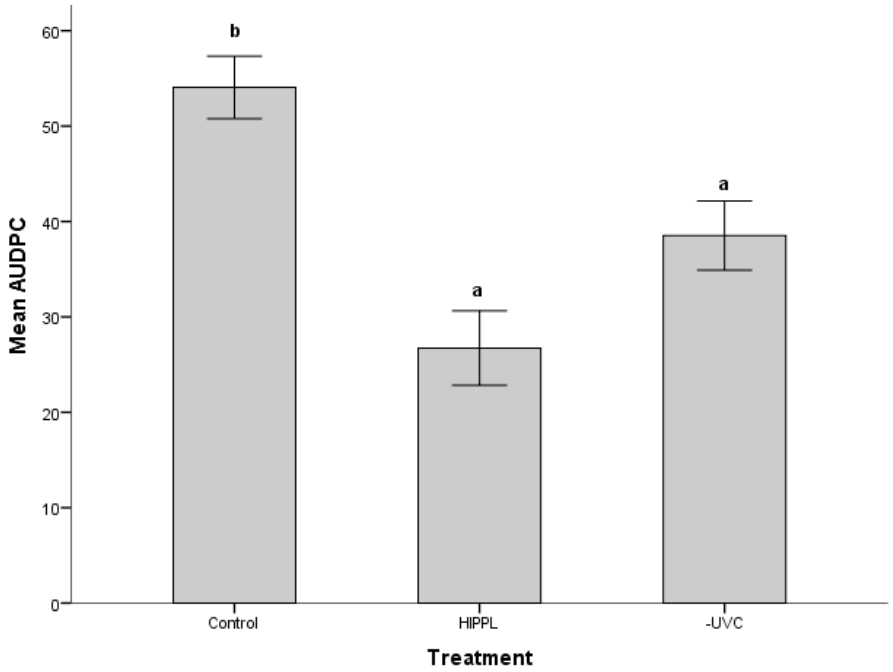

Figure 6.2: Area underneath the disease progression curve (AUDPC) from tomatoes of the cv. Mecano inoculated with *Botrytis cinerea* at 10 d post treatment. Inoculations were performed following treatment with 16 pulses of high- intensity, pulsed polychromatic light and a 16-pulse treatment utilising a Borofloat ® 33 UV-C filter (Schott) which removes wavelengths below 280 nm. N=20 and IRE=2. Error bars show ± 1 S.E.M. Means sharing the same label are significantly different at $p<0.05$.

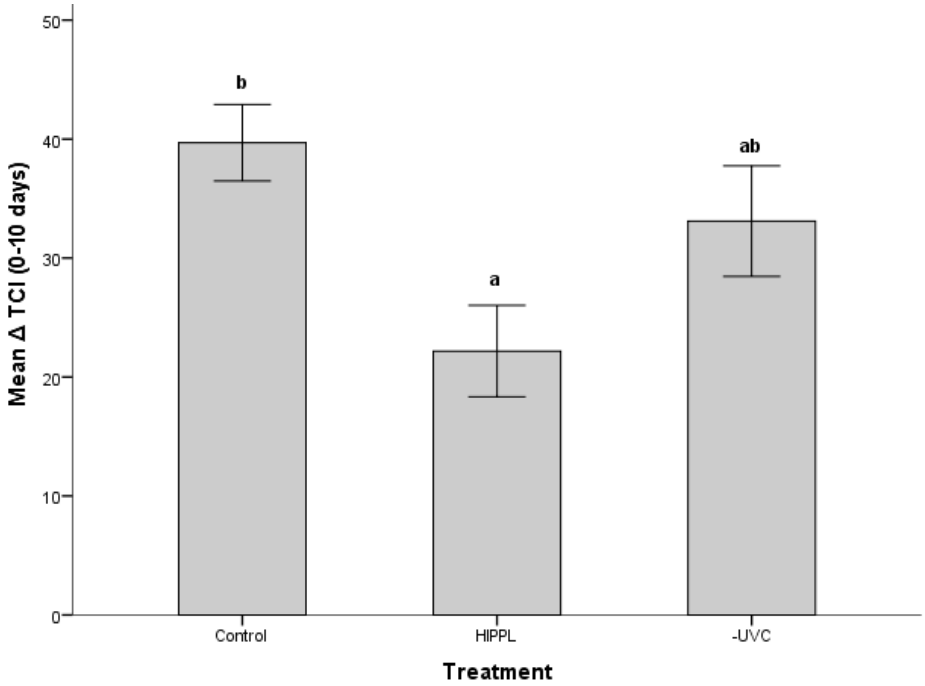

Figure 6.3: The change (Δ) in tomato colour index (TCI) over 10 days from tomatoes of the cv. Mecano following treatment with 16 pulses of high-intensity, pulsed polychromatic light and a 16-pulse treatment utilising a Borofloat ® 33 UV-C filter (Schott) which removes wavelengths below 280 nm. N=20 and IRE=2. Error bars show ± 1 S.E.M. Means sharing the same label are significantly different at $p<0.05$.

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

It is difficult to determine the factors leading to such a high reliance on UV-C to induce the full effects of HIPPL treatments. It may be due to a number of factors including a peak in spectral irradiance in the germicidal UV region (Middleton, 2015). Furthermore, UV-C is the most biologically active region of the UV spectra. Smaller doses, therefore, would be required to stimulate a beneficial response. This can be highlighted by the much greater UV-B treatment that is required for successful induction of delayed ripening when treating light from this portion of the spectrum (20 kJ/m^2). This is in comparison to the much smaller (3.7 kJ/m^2) dose required for successful induction of UV-C hormesis (Charles *et al.,* 2008; Liu *et al.,* 2011).

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

It could therefore be hypothesised that high-intensity visible light, alone, could lead to the induction of hormesis. Further investigation is required to asses this hypothesis.

6.4 Summary and conclusions

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

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

Chapter 7: Investigation into low-intensity UV-C and high-intensity, pulsed polychromatic light treatments for the control of disease through pre-Harvest foliar treatments of lettuce in the glasshouse environment

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

Research on the use of UV-permeable sheeting and supplementary UV-B lighting for protected lettuce crops has shown a number of induced effects such as the production of a more compact plant, reduction in mass, changes in colouration and a reduced incidence of diseases caused by *Bremia lactucae* and *Botrytis cinerea* (Paul *et al.,* 2012; Wargent *et al.,* 2005). Park *et al.* (2007) treated lettuce with 1.65 kJ/m^2 of UV-B per day for 10 days and observed that an increase in red colouration correlated with accumulation of anthocyanins. Phenolic compounds including anthocyanins and flavonoids have been shown to have direct antimicrobial effects, see section 1.6.3. Furthermore, they observed upregulation of putative genes involved in signal transduction, disease, defence and secondary metabolism amongst others.

Recently, UV-C induced disease resistance has been shown on lettuce by Ouhibi *et al.* (2014a). A treatment of 0.85 kJ/m^2 gave post-harvest resistance against *B. cinerea* and *Sclerotinia minor* with 20 and 34% reductions in lesion size at 4 DPI, respectively. Moreover, treated plants showed accumulation and significant increases in the levels of the ROS H_2O_2 at 4 DPI which may be playing a role in defence, aside from its role in cellular signalling, as the greatest difference in disease was observed at 4 DPI. One would expect the application

of doses employed here to be similar for both pre and post-harvest treatments and could infer from this report that UV-C can successfully provide pre-harvest protection.

7.1 Aims

Post-harvest LIUV treatments of lettuce were previously shown by Ouhibi *et al.* (2014a) to have the potential to provide pre-harvest disease resistance. In Chapter 3, it was observed that new high-intensity pulsed polychromatic light (HIPPL) sources can induce resistance on tomato fruit (cv. Mecano) following postharvest treatment. Here, it is intended to extend these findings and build upon this data to show the scope and longevity of the protection of the two contrasting light sources HIPPL and LIUV; see section 2.4 for further information on the light sources. Resistance assays against *B. cinerea, Rhizoctonia solani, B. lactucae* and *Sclerotinia sclerotiorum* will be performed. This data will be used to determine a treatment regime suiTable for commercial glasshouses.

7.2 Materials and methods

7.2.1. Plant husbandry

Plants were grown as stated in section 2.6. Venting of the glasshouse was set to open above 20°C in the day and 16°C during the night. During the winter months LED assimilation lighting was used to extend the photoperiod to 16 hours.

7.2.2 Low-intensity UV-C and high-intensity, pulsed polychromatic light treatments

Treatments were performed as stated in section 2.4. Lettuce plants were subjected to treatment with both high-intensity pulsed polychromatic light (HIPPL) and conventional low-intensity UV-C sources (LIUV). Pulsed treatments were delivered from 40 cm distance from the source and conventional treatments were delivered at either 2000 $\mu W/cm^2$ or 1000 $\mu W/cm^2$ from the apical leaf. Treatments were performed at the 3-5 and 6-8 true leaf stages along with plants at early, mid and late head formation.

7.2.3 Damage assessments following treatment

Damage to the lettuce plants was visually inspected at 2 DPT or 5 DPT and recorded qualitatively as simply the presence or absence of damage. Damage manifested itself as

dry, brown necrotic lesions and brown vascular discolouration; examples of which can be seen in Figures 7.1 and 7.2.

Figure 7.1: A lettuce plant (cv. Amica) at early head formation treated with 75-pulses of high-intensity, pulsed polychromatic light from 40 cm. The plant is exhibiting severe damage to its mature leaves which is manifested as dry brown, necrotic lesions (indicated by red arrows).

Figure 7.2: A lettuce leaf from a plant (cv. Amica) treated at early head formation treated with 45-pulses of high-intensity, pulsed polychromatic light. The picture shows vascular discolouration (yellow/brown) which intensifies towards the petiole.

7.2.4 Pathogen propagation, inoculum preparation and disease resistance assays

For calibrated spore solution inoculations spores were prepared as stated in section 2.3. A 10 µl aliquot of spore solution was pipetted into the centre of the leaf disc. Inoculations utilising agar plugs were performed with a 3 mm cork-borer. Agar plugs were taken from 3-5 day old cultures, dependent on species, and placed into the centre of leaf disks. Cultures were treated as stated in section 2.1 and spore suspensions were made as described for section 2.3.

A leaf disc bioassay based on the method of Laboh (2009), was used to assess disease resistance following treatment. Briefly, 20 mm leaf discs were cut with a cork borer and placed into 120 mm square Petri dishes with a maximum of 16 leaf discs per plate. Prior to this the plates were filled with 25 ml of 0.8% molecular grade agar (Oxoid) to prevent leaf desiccation and to provide humidity for pathogen growth.

Leaf discs were then inoculated with either *B. cinerea*, *R. solani* or *S. sclerotiorum*. At 2 and 3 days post inoculation (DPI) photos of the leaf discs were taken and analysed in Image-J. This allowed the calculation of the diseased area in mm^2. Taking measurements at multiple time points allowed the monitoring of disease progression over time. Data from the two time points was used to calculate the area under the disease progression curve; equation 3.2.

7.2.5 Experimental design and statistical analysis

The plants for each experiment were grown in a single NFT system with four gutters, thus maintaining equal nutrient supply to all plants in a single experiment. Plants were grown to their required size in the NFT system and a completely random design was used where treatments were randomly assigned to each plant in the experiment. Statistical analysis was performed either by ANOVA or Kruskal Wallis (non-parametric ANOVA) where the assumptions of normality and homogeneity of variance could not be met. Statistical significance is here defined as p=0.05. Results from the independent experimental replicates can be found in Appendix 6.

7.3 Results and discussion

7.3.1 Damage thresholds

Damage to lettuce following both LIUV and HIPPL treatments was observed as dry brown lesions and vascular discolouration (Figures 7.1 and 7.2). Damage susceptibility for both LIUV- and HIPPL-treated lettuce varied throughout the year on the cv. Amica. During March, damage was seen above 60 pulses and 2.25 kJ/m^2 for the HIPPL and LIUV sources, respectively (Figure 7.3). For April, the beginning of the commercial growing season, 45 pulses was observed to cause damage to the plants; LIUV treatments were not tested (Figure 7.4). Between April and October no damage was observed for any of the treatments. Treatments, however, were all >45 pulses.

From October onwards a dramatic reduction in the damage threshold for both HIPPL and LIUV treatments was seen. HIPPL-treated plants exhibited damage for treatments ≥ 16 pulses (Figure 7.5). Furthermore, LIUV treatments showed damage above 0.36 kJ/m^2. Up to this point in the LIUV trials doses of UV-C were applied at 2000 µW/cm^2 which gave a total treatment time of 18 seconds for a 0.36 kJ/m^2 treatment. As treatment times now had to be further reduced it was decided that treatments would be delivered at an intensity of 1000 µW/cm^2 to provide greater control of the dose application.

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

No statistical analysis was performed on the data due to the small number of biological replicates performed (3 to 5). Such a low number of biological replicates may artificially increase the differences in the percentage of plants showing damage with a single plant contributing 30% in some circumstances. Future work should be performed to replicate the preliminary results observed here, with an aim to increase resolution on the differences observed between treatments.

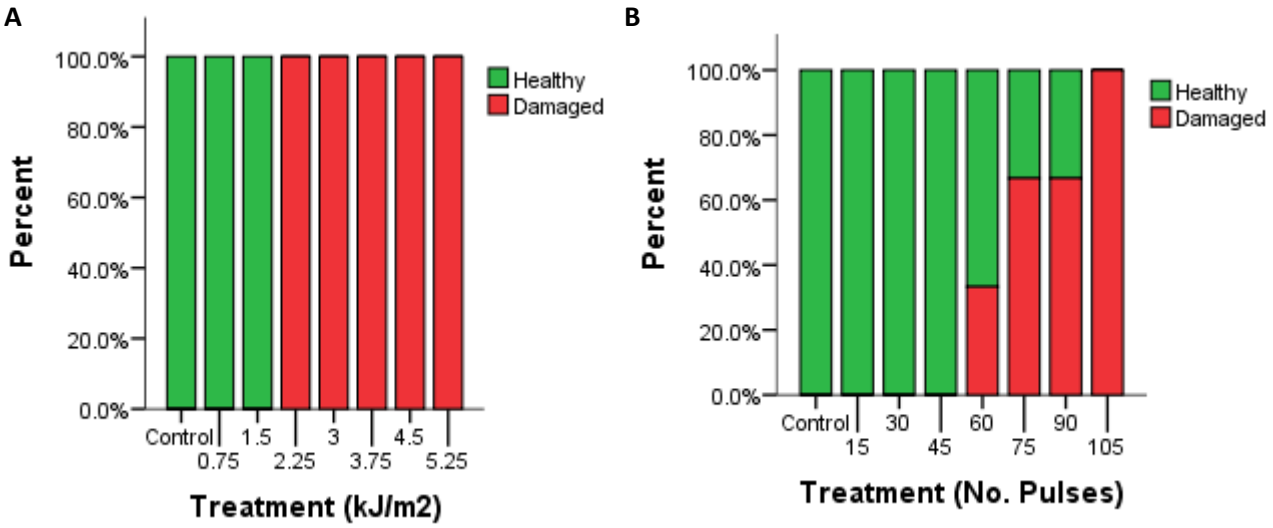

Figure 7.3: The percentage of healthy and damaged plants for the lettuce variety Amica treated during March 2015. Plants were either treated at 3 to 5 true leaves with a conventional low-intensity mercury UV-C source (**A**) or a high-intensity, pulsed polychromatic source (**B**). N=3.

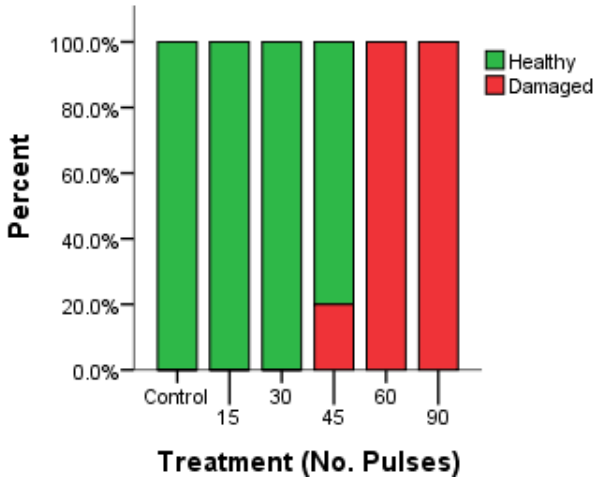

Figure 7.4: The percentage of healthy and damaged plants for the lettuce variety Amica treated during April 2015. Plants were treated at early head formation with a high-intensity, pulsed polychromatic source. N=5.

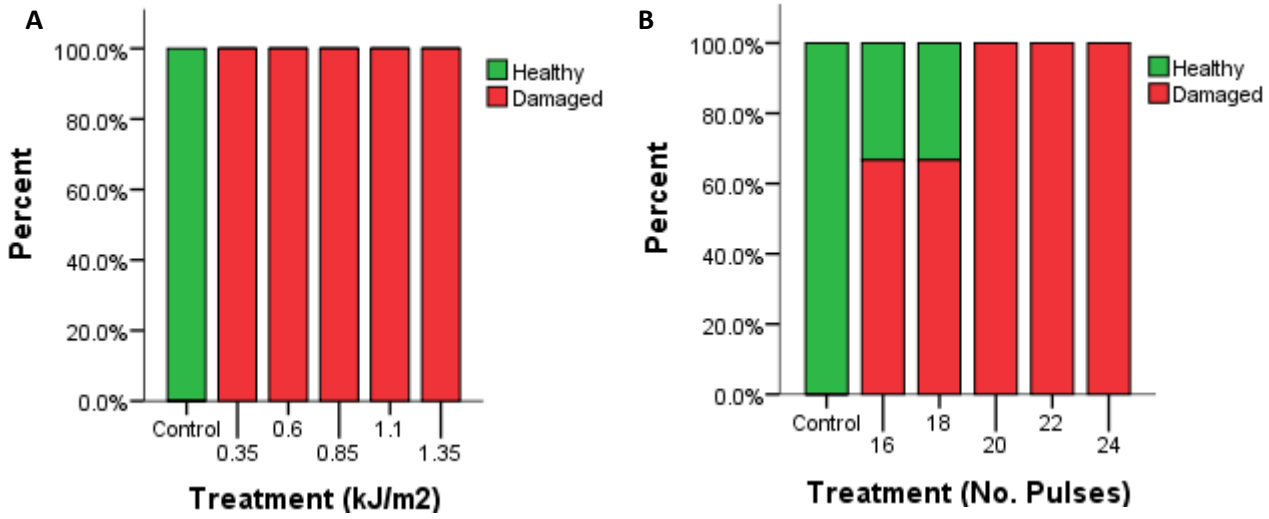

Figure 7.5: The percentage of healthy and damaged plants for the lettuce variety Amica treated during October 2015. Plants were treated at late head formation with a conventional low-intensity, UV-C source (**A**) or a high-intensity, pulsed polychromatic source (**B**). N=3.

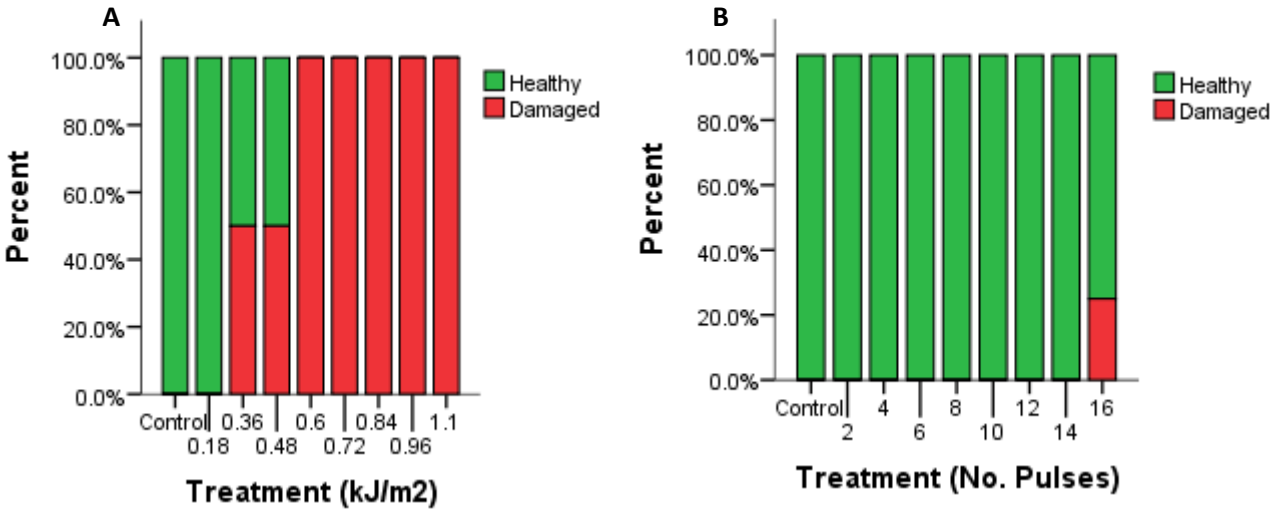

Figure 7.6: The percentage of healthy and damaged plants for the lettuce variety Amica treated during November 2015. Plants were treated at 3 to 5 true leaves with a conventional low-intensity, UV-C source (**A**) or a high-intensity, pulsed polychromatic source (**B**). N=4.

A change in the damage threshold to HIPPL and LIUV treatment at differing points of the year can be expected. With changing light conditions across the year plants have to adapt to survive. Such adaptations occur systemically and include changes to leaf orientation, chlorophyll content, modulation of photosystem light-harvesting antenna size and photosystem II efficiency (Ruban 2009). Such adaptations will lead to the plant being in either a greater or reduced state of susceptibility to light damage and, therefore, alter the treatment that is required to cause damage.

7.4 Development of disease control bioassays

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

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

For the next round of preliminary experiments plants (cv. Amica) were grown to early head formation and treated with 5 to 30 pulses of HIPPL, incubated in the dark for 12 hours, and at 6 DPT leaf discs were then inoculated with 3mm agar plugs from 5 day old *B. cinerea* cultures. Measurements were taken at 2 and 3 DPI. All treatments showed a decrease in lesion size with a statistically significant reduction of 15% for the 20 pulses treatment.

There were, however, a number of problems with the experimental procedure. Firstly, lesion development was still uneven due to the need to use multiple agar plates across the experiment (Figure 7.7 A). The use of agar plugs is inherently variable due to its lack of

calibration and one cannot produce constant hyphal densities. Furthermore, addition of 6-benzylaminopurine to the agar may have additional bias on the data as it has previously been demonstrated to be an inducer of disease resistance (Mills *et al.,* 1986). The bioassay was, therefore, further adapted with the removal of 6-benzylaminopurine and the use of a calibrated spore solution following the procedures in section 2.3.

Inoculations with 10 µl of spore solution were then tested at concentrations of $1x10^5$ and $1x10^6$ with or without the addition of 50% potato dextrose broth (Sigma-Aldrich) and measured at 2 DPI. The use of a calibrated spore solution utilising 1×10^6 spores per ml amended with 50% potato dextrose broth provided much greater homogeneity to the development of disease (Figure 7.7 B). Although inoculating leaves with spore solutions improved the within-group variation, there was still a degree of uneven lesion development.

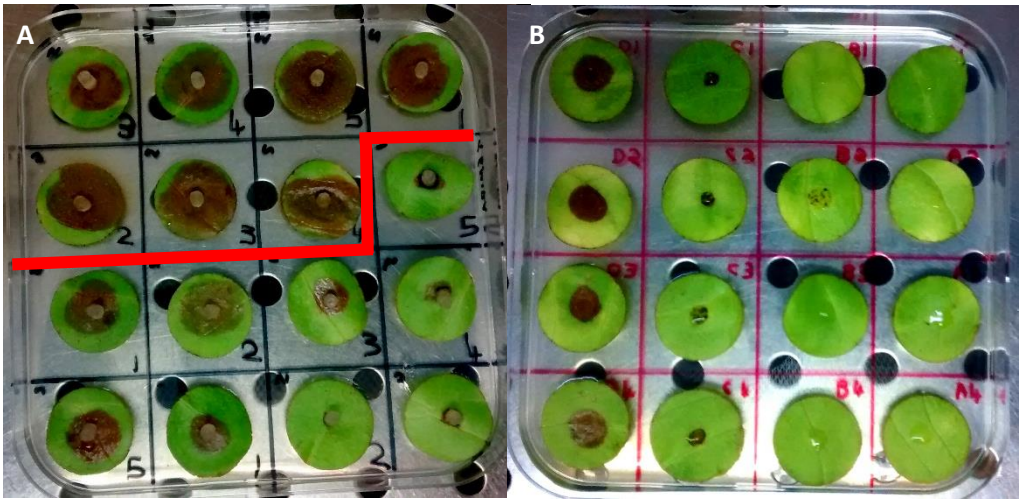

Figure 7.7: The Lettuce leaf bioassay adjusted from Laboh (2009). Lettuce plants were harvest, the leaves were removed and 20 mm leaf discs were cut. Leaf discs were placed onto 0.8% agar and inoculated with either **A)** 3 mm plugs from 5 day old plates of *Botrytis cinerea.* Red line indicates where agar plugs were taken from differing cultures. **B)** Inoculation with a 10 µl of calibrated spore solutions of *B. cinerea* from 14 day old cultures. Columns from left to right show inoculation with calibrated spore solutions of $1x10^6$ and $1x10^5$ spores per ml with 50% potato dextrose broth and $1x10^6$ and $1x10^5$ spores per ml in sterile distilled water.

To improve the accuracy of lesion measurements in further experiments, photographs of the lesions were taken. Measurements of lesion size was then performed using the software package ImageJ (Image Processing and Analysis in Java). Image colour channels were split and the green channel was kept for image analysis; this allowed the greatest contrast between healthy and diseased tissues. Lesion measurement was performed

utilising the wand tool set to 8-conntected and a threshold of 20. This allowed accurate determination of lesion area in mm^2.

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

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

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

For *R. solani* inoculations a method published by Fiddaman *et al.* (2000) was adapted. Agar plugs were placed in the centre of the underside of the leaf. It was observed that when placing agar plugs on the upper surface of leaves that disease did not progress well and often stalled. Observation of the initiation of disease suggested that hyphae entered through natural openings, such as stomata, which are present at greater levels on the underside of leaves. Inoculations with *S. sclerotiorum* were performed on the upper side of leaves. Example photographs of the final inoculation procedures and lesion development at 2 and 3 DPI can be seen in Figure 7.8.

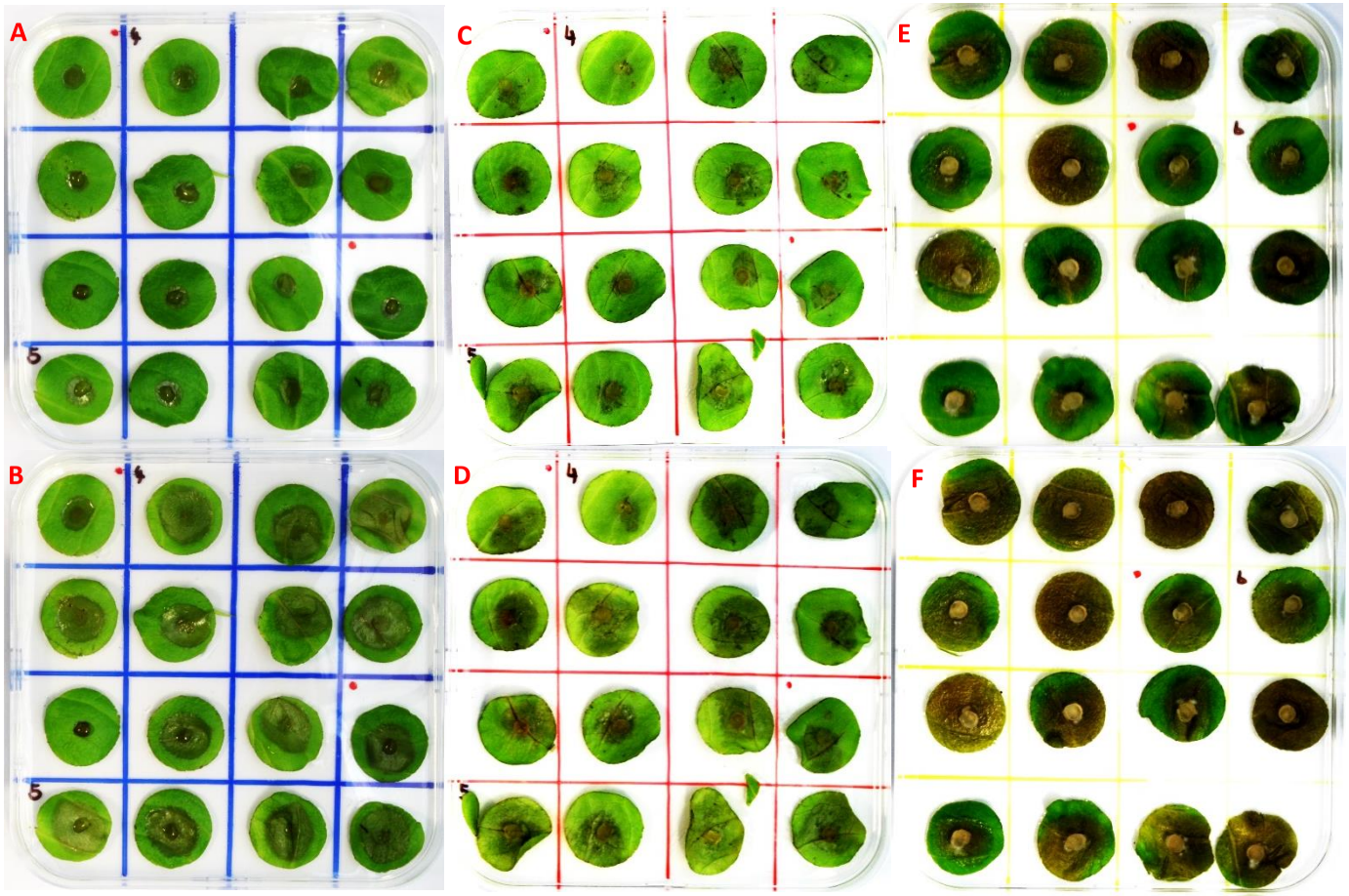

Figure 7.8: Examples of the lettuce leaf disc bioassay adapted from Laboh (2009). Images show *Botrytis cinerea* spore solution inoculations with 10 µl of 1 x 10^6 spores amended with 50% potato dextrose broth at 2 days post inoculation (DPI) (**A**) and 3 DPI (**B**). *Rhizoctonia solani* inoculations adapted from Fiddaman *et al.,* (2000) with 4 mm agar plugs taken from 3 d old cultures at 2 DPI (**C**) and 3 DPI (**D**). *Sclerotinia sclerotiorum* inoculations with 4mm agar plugs from 3 d old cultures at 2 DPI (**E**) and 3 DPI (**F**).

7.5 Disease control bioassays

Disease control assays were performed with two main objectives in mind. The first of these was to determine the optimal LIUV and HIPPL treatment for inducing resistance on the cultivars undergoing investigation. Secondly, to investigate the longevity of resistance, i.e. at what point in induced resistance at its peak level and how long does resistance last for. A large amount of variation was observed for both of the major objectives. None of the experiments utilising the adapted inoculation and lesion measurement techniques were significantly different from the control for both the HIPPL and LIUV source.

To summarise the findings of these experiments, the treatments for which the greatest reductions in disease progression were observed appeared to change across the year, as does the plant's susceptibility to damage. The optimal HIPPL treatment for *B. cinerea* resistance (cv. Amica) dropped from 22 pulses in September to 16 pulses in October 2015 (Table 7.1). This variation was also observed for LIUV treatments 0.6 to 0.35 and 0.18 kJ/m^2 in September, October and November, respectively (Table 7.2). A similar pattern was observed for cv. Temira with optimal HIPPL and LIUV treatments dropping from 18 pulses and 1.1 kJ/m^2 to 8 pulses and 0.12 kJ/m^2 in February (Tables 7.3 and 7.4). Complete data sets can be found in Appendix 8.

The most effective treatments against *B. cinerea* also differed between cultivar. For example in September the optimal treatment was 22 pulses for cv. Amica but 16 pulses for cv. Temira (Tables 7.1 and 7.3). Optimal LIUV treatments also showed between cultivar variation at 0.6 kJ/m^2 for cv. Amica and 1.1 0.6 kJ/m^2 for cv. Temira during September (Tables 7.2 and 7.4). This was further complicated by differences in the optimal treatments for the various pathogens under investigation. For cv. Amica the optimal HIPPL treatments against *B. cinerea, R. solani* and *S. sclerotiorum* were 12, 8 and 14 pulses in December, respectively (Table 7.1). This was also observed for the LIUV treatments with optimal treatments at 0.3, 0.18 and 0.12 kJm^2, respectively. Furthermore, the responsiveness to treatment also varies between cultivars with Amica showing an average reduction in *B. cinerea* disease progression of 14.47% whereas Temira showed 21.1% following pulsed treatments. Similarly, LIUV treatments showed 14.41% and 31.3% mean reductions for cv. Amica and Temira, respectively.

Table7.1: Experimental results from high-intensity, pulsed polychromatic light treated Amica plants. This includes the month and date treatment commenced, the growth stage of the plants, the treatment range under investigation, the point at which damage was observed and the day of inoculation post treatments (DPT). The results from inoculation studies are also included showing the optimum treatments and percentage reduction in disease for *Botrytis cinerea, Rhizoctonia solani* and *Sclerotinia sclerotiorum.* Complete data sets can be found in Appendix 8.

Month	Date	No. true leaves	Range (pulses)	Damage threshold	Inoculation day (DPT)	Optimal disease control					
						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 7.2: Experimental results from low-intensity 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.* Complete data sets can be found in Appendix 8.

Month	Date	No. true leaves	Range (kJm^2)	Damage threshold	Inoculation day (DPT)	Optimal disease control					
						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 7.3: Experimental results from high-intensity, pulsed polychromatic light treated Temira plants. This includes the month and date treatment commenced, the growth stage of the plants, the treatment range under investigation, the point at which damage was observed, the day of inoculation post treatments (DPT). The results from inoculation studies are also included showing the optimum treatments and percentage reduction in disease for *Botrytis cinerea, Rhizoctonia solani* and *Sclerotinia sclerotiorum.* Complete data sets can be found in Appendix 8.

Month	Date	No. true leaves	Range (pulses)	Damage threshold	Inoculation day (DPT)	Optimal disease control					
						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 7.4: Experimental results from low-intensity 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.* Complete data sets can be found in Appendix 8.

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

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

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

Pulsed treatments show an increase in optimum treatment from 15 to 22 pulses between April and September and a drop to 10 in February (Figure 7.9). LIUV treated plants also show a homologous drop in dose from September, where the most effective treatment was 0.6 kJ/m^2, to 0.06 kJ/m^2 in February

Figure 7.9: The optimal high-intensity, pulsed polychromatic light and low-intensity UV-C treatments for reducing the disease progression of *Botrytis cinerea* at differing months of the year for the lettuce cv. Amica

Such wide variation may indicate a complex situation where not only the environmental conditions effect the optimum treatment to induce resistance but also so do the cultivar and pathogen undergoing investigation. Postharvest LIUV treatments of lettuce, however, were shown to be successful by Ouhibi *et al.* (2014a) with a treatment of 0.85 kJ/m^2. After in depth review of their research, however, little in the way of experimental design is discussed. Firstly, only a single treatment was performed and compared with the control group; with no reference as to why the dose employed had been chosen. We should, therefore, interpret the data with caution. Secondly, it is stated that the disease assay data is supplied from a sample size of 20. The number of independent replicate experiments, however, is not specified. This brings into question the reliability of their work and fails to answer the question whether their experiment was, or can be successfully replicated.

To attempt to remove a degree of the variation observed here, it was decided that the most successful trials from 2015/2016 would be repeated during 2017. This was to attempt to mitigate any variation that may have been caused by day length and seasonal environmental fluctuations. It was, therefore, decided to repeat the experiments that were performed during February 2016 as all treatments on both cultivars showed a reduction in disease progression >27.0% for both *B. cinerea* and *R. solani*.

7.6 Treatments from February 2016 and February 2017

To allow for the potential seasonal variation observed in section 7.4, treatments from a single month were replicated using both LIUV and HIPPL sources. This was to ascertain the extent to which the optimal treatment ranges may differ between growing seasons and whether treatments would have to be adjusted between seasons. To summarise the experimental protocols; both varieties Amica and Temira were grown to 6-8 true leaves. They were then inoculated with at 2DPT with *B. cinerea* due to the ease of inoculation and homogeneity of inoculation procedures. Lesion measurements were taken at 2 and 3 DPI with ImageJ and used to calculate the area underneath the disease progression curve.

No significant differences were found when analysing the combined experimental data (data not shown). When analysing the experimental replicates separately, variation in the treatment giving the optimum reduction in disease progression, again, showed variation for both varieties and light sources undergoing investigation. For Amica, during 2016 the LIUV treatment showing the greatest reduction in disease was 0.06 kJ/m^2 at 27.0% (Figure 7.10A). During 2017, however, although all treatments showed a small reduction in disease progression, they were very small with the most successful treatment of 0.30 kJ/m^2 only reducing disease by 19.7% (Figure 7.10B). Similarly, HIPPL treatments showed a large degree of disease reduction in 2016 with an optimal treatment of 10 pulses giving a 44.1% reduction (Figure 7.11A). In 2017, all treatments showed a reduction in disease progression. These were, however, very small with the optimal treatment of 14 pulses only giving a 17.7% reduction in disease progression (Figure 7.11B).

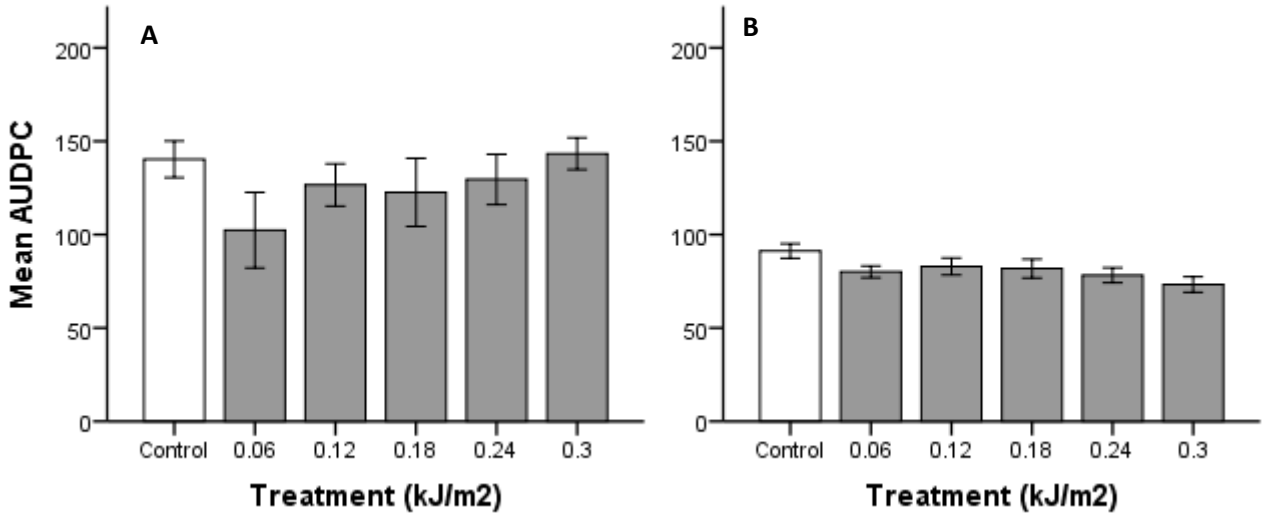

Figure 7.10: Area underneath the disease progression curve (AUDPC) for lettuce plants of the cv. Amica treated with a low-intensity UV-C source at the 6-8 true leaf stage. Plants were inoculated at 2 days post treatment with *Botrytis cinerea* and disease was measured with ImageJ at 2 and 3 days post inoculation. Replicate experiments were performed in February 2016 (**A**) and February 2017 (**B**). Two technical replicates were performed for each biological replicate. N=5 and 7 for A and B, respectively. Error bars show ± 1 S.E.M.

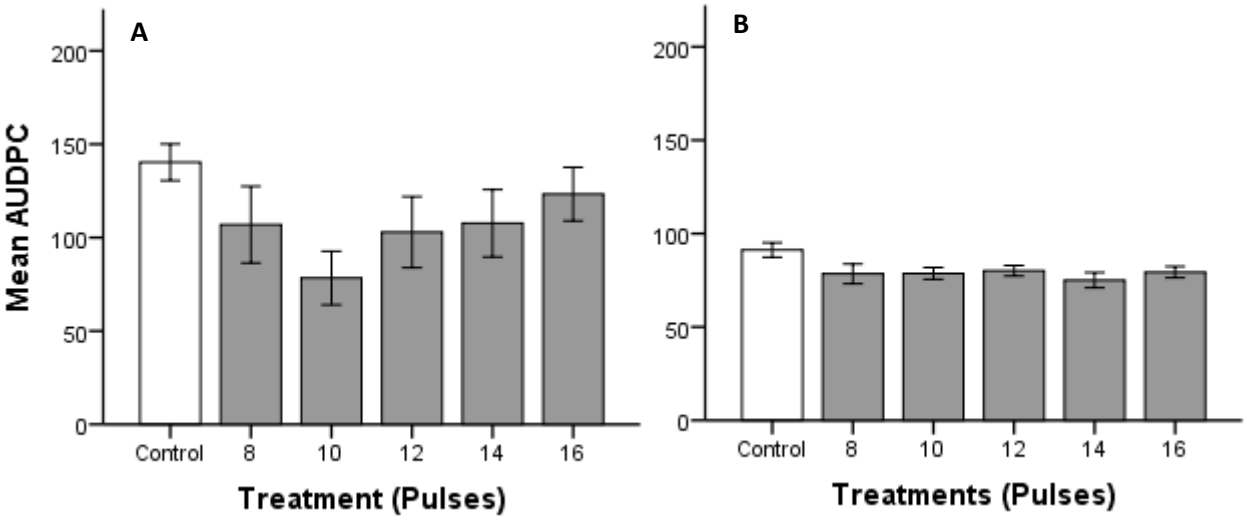

Figure 7.11: Area underneath the disease progression curve (AUDPC) for lettuce plants of the cv. Amica treated with a high-intensity, pulsed polychromatic light source at the 6-8 true leaf stage. Plants were inoculated at 2 days post treatment with *Botrytis cinerea* and disease was measured with ImageJ at 2 and 3 days post inoculation. Replicate experiments were performed in February 2016 (**A**) and February 2017 (**B**). Two technical replicates were performed for each biological replicate. N=5 and 7 for A and B, respectively. Error bars show ± 1 S.E.M.

For the cultivar Temira, a similar set of observations was made. The levels of disease reduction for LIUV treated plants were high in 2016. The optimal treatment, 0.24 kJ/m^2 showed a 31.3% reduction in disease progression (Figure 7.12A). For the experimental replicate performed in 2017, however, the 0.06 kJ/m^2 showed the greatest level of disease reduction (Figure 7.12B). Again, the levels of disease reduction during 2017 were much smaller than that observed for 2016 with only a 12.7% reduction for the optimal treatment. Experiments with HIPPL treated lettuce (cv. Temira) produced similar findings. In 2016 the greatest reduction in disease were seen for the 8 pulse treatment at 40.6% (Figure7.13A). In 2017, however, the 16 pulse treatment showed the highest level of reduction at 13.8% (Figure 7.13B).

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

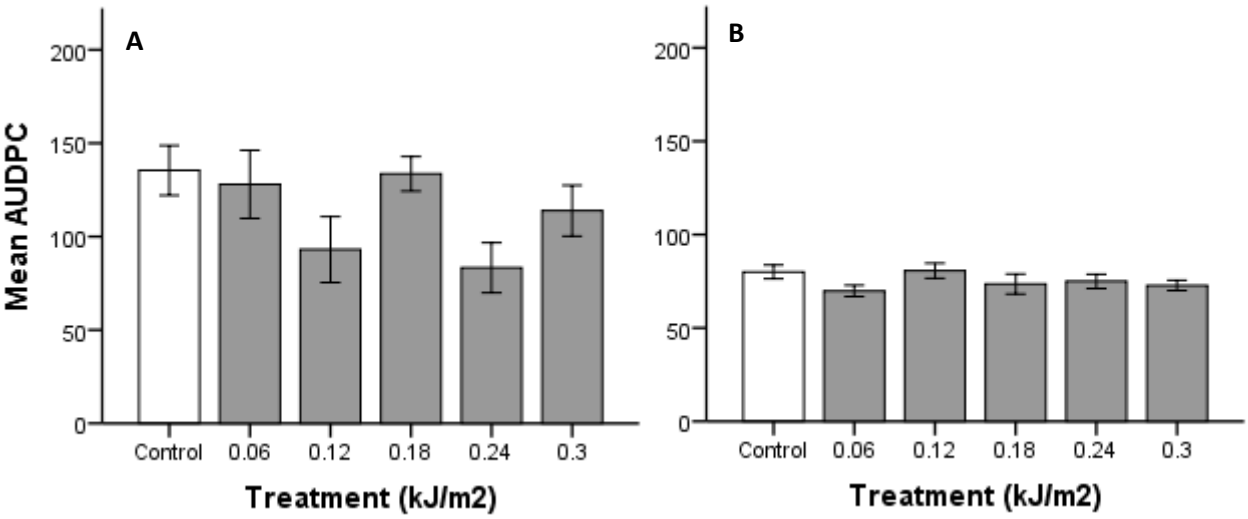

Figure 7.12: Area underneath the disease progression curve (AUDPC) for lettuce plants of the cv. Temira treated with a lowintensity UV-C source at the 6-8 true leaf stage. Plants were inoculated at 2 days post treatment with *Botrytis cinerea* and disease was measured with ImageJ at 2 and 3 days post inoculation. Replicate experiments were performed in February 2016 (**A**) and February 2017 (**B**). Two technical replicates were performed for each biological replicate. N=5 and 7 for A and B, respectively. Error bars show ± 1 S.E.M.

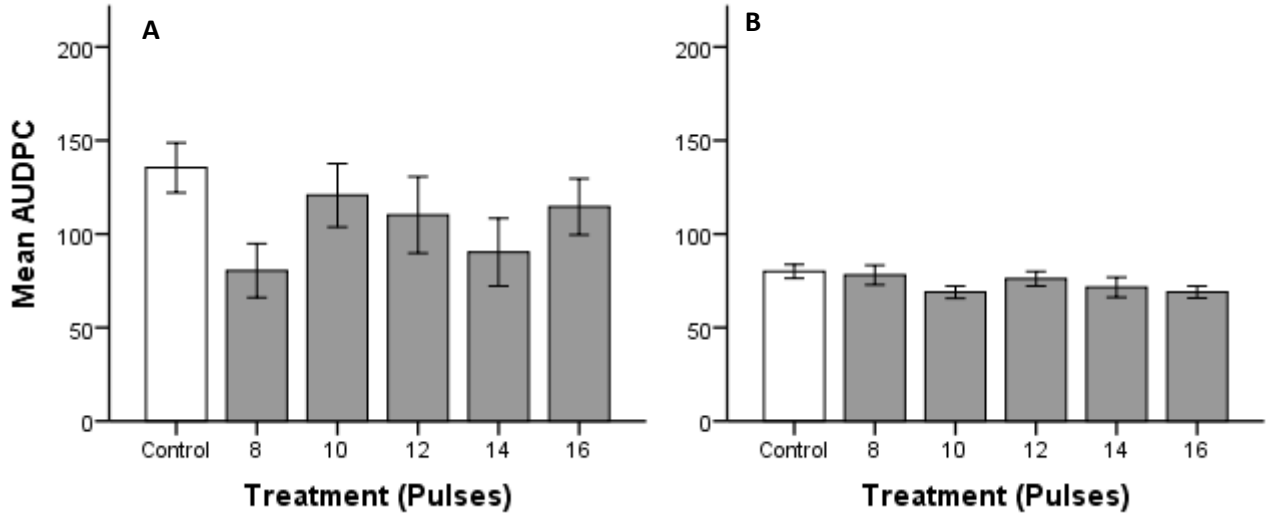

Figure 7.13: Area underneath the disease progression curve (AUDPC) for lettuce plants of the cv. Temira treated with a high-intensity, pulsed polychromatic light source at the 6-8 true leaf stage. Plants were inoculated at 2 days post treatment with *Botrytis cinerea* and disease was measured with ImageJ at 2 and 3 days post inoculation. Replicate experiments were performed in February 2016 (**A**) and February 2017 (**B**). Two technical replicates were performed for each biological replicate. N=5 and 7 for A and B, respectively. Error bars show ± 1 S.E.M.

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

7.7 Summary and conclusions

Two commercial cultivars of butterhead lettuce, Amica and Temira, were grown in a temperature-controlled glasshouse. The first aim of the study was to ascertain the damage thresholds for LIUV and HIPPL treatments. Damage is induced by both HIPPL and LIUV sources and manifests as dry brown lesions and vascular discolouration, see Figure 7.1 and 7.2. The damage thresholds of the cv. Amica showed variation across the year decreasing from 60 to 16 pulses and 2.25 kJ/m^2 to 0.36 kJ/m^2 between April and October 2015 for the HIPPL and LIUV sources, respectively.

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

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

Chapter 8: Multiple low-dose preharvest low-intensity UV-C and high-intensity, pulsed polychromatic light treatments for the control of disease on lettuce in the glasshouse

In Chapter 7, it was found that a large amount of variation was found in both the damage threshold to both LIUV and HIPPL treatments and the optimal treatment to reduce disease progression. The primary factor affecting damage to the crop appeared to be linked to environmental conditions. As they were not being monitored in real time, however, conclusions on what was causing the shift in the point of damage cannot be drawn. It is however, likely that light intensity and hours of daylight play a role in determining the outcome. Furthermore, the optimal treatment for reducing disease progression also appeared to be influenced by both the cultivar and the pathogen being studied. It was decided at this point not to conduct experiments in a controlled environment as the main objective of the study was to produce a treatment regime that could be applied directly to a commercial glasshouse environment.

8.1 Aims

The aims of this investigation were to test the hypothesis that can either single or multiple low-level treatments induce disease resistance against *R. solani* on lettuce. Treatments were chosen based on the previous data (section 7.3.1) and were shown not to be damaging at any point during the growing season.

8.2 Materials and methods

8.2.1 Plant husbandry, low-intensity UV-C and high-intensity, pulsed polychromatic light treatments and inoculation

Lettuce of the cv. Amica and Temira were grown to the 6-8 leaf stage as stated in section 7.2.2. The chosen low-dose treatments were 0.12 and 0.24 kJm^2 for the LIUV source and 7 and 14 pulses for the HIPPL source. Each of the higher treatments, for the LIUV and HIPPL sources, showed a level of reduced disease progression against each of the pathogens under investigation in Chapter 7. Treatments were applied twice with either 2, 4 or 6 days

between treatments (DBT). Treatments were performed as stated in section 7.2.2. Plants were treated and inoculated with *R. solani* utilising the detached leaf bioassay as outlined in section 7.4. Inoculations were performed at 2 DPT as described by Ouhibi *et al.,* (2014a). *R. solani* was chosen as the pathogen to undergo study as it had shown the greatest level of responsiveness to treatment in previous studies. Lesion area was measured at 3 DPI due the nature of early disease progression.

8.2.2 Experimental design

Five biological and two technical repeats were used for each experimental replicate. Three replicate experiments were carried out one in each of the months March, April and May 2016. A completely randomised design was used as stated in section 7.2.5. The data from the three replicates was combined and statistically analysed in SPSS via One-Way ANOVA where significance was measured at $p < 0.05$. 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. Results from the independent experimental replicates can be found in Appendix 7.

8.3 Results and discussion

Three replicate experiments were carried out one in the month March, April and May 2016. All LIUV treatments showed slight reductions in mean lesion size at 2 DBT for the variety Amica (Figure 8.1A). All treatments, however, showed an increase in mean lesion area for treatments with 6 DBT. The LIUV treatment showing the greatest reduction was 0.12 kJ/m^2 giving a 13.24% reduction at 2 DBT. None of the conventional treatments showed a significant difference from the control with 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 (Figure 8.1B). The greatest reduction in lesion size was observed for the 14-pulse treatment at 3.6%. The 7-pulse treatment also showed a similar reduction at 3.6%. Again, statistical testing showed large p values of 0.896 and 0.672 for the 2 DBT and 6 DBT treatments respectively.

For the variety Temira multiple LIUV treatments showed small reductions at both 2 and 6 DBT. The treatment showing the largest decrease in mean lesion area was the 0.24 kJ/m^2 treatment at 2 DBT showing a 7.5% decrease (Figure 8.1C). With 6 DBT the treatment showing the greatest reduction in mean lesion size was 0.12 kJ/m^2 at 9.5%.

For HIPPL treatments only 14 pulses applied with 2 DBT showed a reduction in mean lesion size at 9.9% (Figure 8.1D). Statistical analysis, however, highlighted no significant differences and again showed particularly large p values. LIUV treatments gave p values of 0.722 and 0.587 for the 2 and 6 DBT trials, respectively. HIPPL treatments similarly showed high p values at 0.374 and 0.929 for the 2 and 6 DBT trials. A summary of the optimal treatments for each experimental replicate is given in Tables 8.1, 8.2, 8.3 and 8.4.

The failure to significantly reduce disease here is not unexpected. The data from the experimental replicates shows the most effective treatment regime, be that 2, 4 or 6 DBT, showed variation between each replicate experiment for both cultivars and light sources (Tables 8.1, 8.2, 8.3 and 8.4). For example, Temira plants treated with LIUV showed the most effective application regimes (DBT) were 4, 6 and 2 for the replicates in March, April and May respectively (Table 8.3). Furthermore, the most successful treatment also changed. For example, for Amica plants the most successful pulsed treatments were 7, 7 and 14 at 4, 2 and 2 DBT, respectively, Table 8.2.

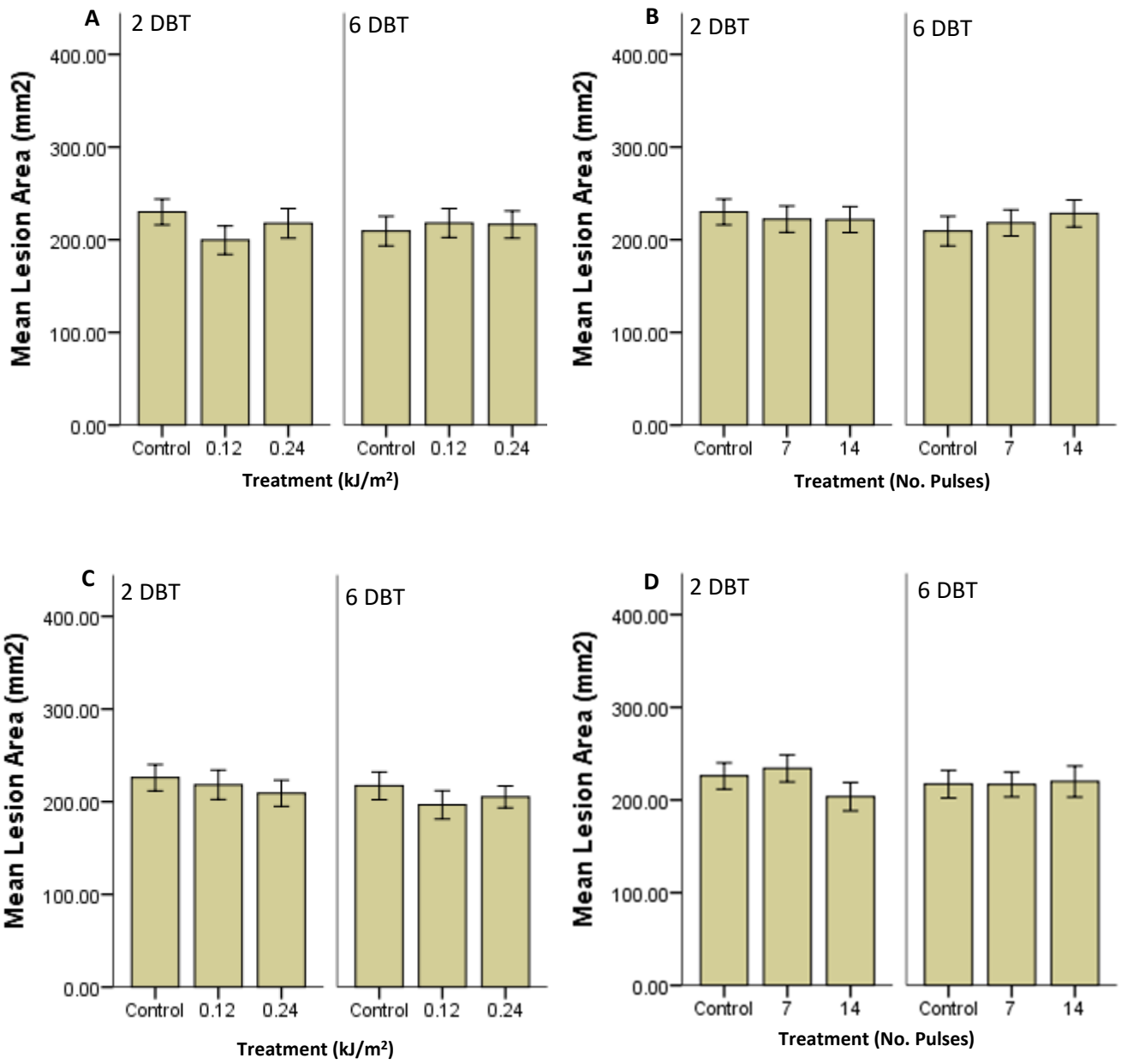

Figure 8.1. The combined experimental data from three replicate experiments utilising multiple low-level treatments. Experimental replicates were performed during March, April and May 2016. Plants were inoculated at 2 days post treatment with 4mm *Rhizoctonia solani* agar plugs and lesions were measured 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 low-intensity UV-C (LIUV) treated cv. Amica plants. (**B**) The Lesion areas of high-intensity, pulsed polychromatic light (HIPPL) treated cv. Amica plants. (**C**) The mean lesion area of LIUV treated cv. Temira plants. (**D**) The Lesion areas of HIPPL treated cv. Temira plants. Error bars show ± 1 standard error of the mean. N= 15, IRE=3.

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 effectiveness in reliably reducing disease. For Amica plants treated with the pulsed polychromatic source a single 14 pulse treatment showed a 19.3%, 0.4% and 4.4% reduction in mean lesion area for the trials during March, April and May, respectively, Table 8.2. For plants treated with the LIUV source a 0.24 kJm^2 dose showed no reduction in disease, 2.9% and 3.2% reductions in lesion area for March, April and May, respectively, Table 8.1.

Temira plants treated with a single 14 pulse treatment showed a similar inability to reduce mean lesion area with no reductions observed in March and May and only a 0.9% reduction observed in April, Table 8.4. Single treatments of 0.24 kJ/m^2 showed 9.5%, 24.8% and no reduction in mean lesion area for March, April and May respectively, Table 8.3.

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

The pulsed treatment showing the greatest level of disease reduction for Amica was the 14-pulse treatment giving a 9.4% reduction in mean lesion area, Table 8.2. For conventional UV-C treatments the largest reduction in lesion area was observed for the 0.12 kJ/m^2 treatment at 2.4%, Table 8.2. For Temira plants the 0.12 kJ/m^2 conventional treatments showed the greatest reduction in lesion area at 7.3%, Table 8.3. The pulsed treatments showed the greatest success with the 7-pulse treatment which showed a 1% reduction in mean lesion area (Table 8.4).

Table 8.1: Experimental results from low-intensity 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	Disease control (%)	Optimal disease reduction		
					Treatment (kJm^2)	DBT	Reduction (%)
March	24.03.16	6-8	2	NR[1]	0.12	2	18.8
April	18.04.16	6-8	2	2.9	0.12	2	8.8
May	19.05.16	6-8	2	3.2	0.12	6	11.2
May	19.05.16	6-8	4	-	0.12	2	2.4

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

Table 8.2: Experimental results from high-intensity, pulsed polychromatic light treated Amica plants that have undergone multiple treatments. This includes the month and date treatment commenced, the growth stage of the plants, the number of treatments, the optimal treatment dose, days between treatment and percentage disease reduction.

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

-Denotes that results were not collected

Table 8.3: Experimental results from low-intensity 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	Disease control (%)	Optimal disease reduction		
					Treatment (kJm^2)	DBT	Reduction (%)
March	24.03.16	6-8	2	9.5	0.24	4	15.8
April	18.04.16	6-8	2	24.8	0.12	6	14.7
May	19.05.16	6-8	2	NR[1]	0.24	6	5.6
May	19.05.16	6-8	4	-	0.12	2	7.3

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

Table 8.4: Experimental results from high-intensity, pulsed polychromatic light treated Temira plants that have undergone multiple treatments. This includes the month and date treatment commenced, the growth stage of the plants, the number of treatments, the optimal treatment dose, days between treatment and percentage disease reduction.

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

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

8.4 Summary and conclusions

Multiple LIUV and HIPPL treatments, for both Amica and Temira, either did not reduce mean lesion area, increased mean lesion area or reduced mean lesion area by only a few percent. It is also evident that the low-level repeated treatments are not as effective as some of the single, higher dose treatments that have been performed in Chapter 7. It is therefore recommended that research is focused on investigating appropriate single treatments.

In addition, the variation observed in the optimal treatment for reducing disease progression, as was observed in Chapter 7, continued to persist. Variation between the most effective doses continued to show some level of cultivar dependence. The cultivar Temira, as seen in several experiments in Chapter 7, continued to show reduced sensitivity to both LIUV and HIPPL treatments, thus requiring an increased dose for the induction of resistance. Furthermore, the most effective treatment regime could not be elucidated. The most successful number of DBT seemed to change from experiment to experiment. Finally, it appeared that the most successful treatment, again, increased as the months proceeded through to spring.

The large amount of variation that was been observed for all experiments throughout Chapter 7 could not be mitigated through the application of multiple low-dose treatments with the observation that environmental conditions may still play a key role in deciding the effective treatment. It is suggested that research should be moved into a controlled environment to allow for the successful determination of HIPPL and LIUV induced resistance's viability as an alternative to chemical control. It could be hypothesised that almost any change to the plants homeostasis, through environmental cues, during development would alter the optimal treatment to induce resistance. Such a treatment would, therefore, require an extremely tightly controlled glasshouse environment to fulfil the full efficacy of any treatments applied. The full and comprehensive modelling of the effect of changing environmental conditions on the optimal treatment, using a controlled environment, could allow the prediction of glasshouse treatments in real time and increase the likelihood of an effective treatment and preventing unwanted damage occurring to crops. Further research to test this hypothesis, however, is required.

Chapter 9: Foliar low-intensity UV-C and high-intensity, pulsed polychromatic light treatments of lettuce in a controlled environment

In Chapter 7 a great deal of variation was observed when treating lettuce with HIPPL and LIUV in the glasshouse. Such variation included the damage threshold and optimal treatment for disease control. Variation for both factors was seen both across the growing season and with the cultivar being treated. Furthermore, variation in the optimal treatments for each of the pathogens undergoing investigation was also observed. In Chapter 8 we attempted to mitigate some of the variation by applying low-dose treatments, that were not damaging at any point during the growing season, multiple times with either 2, 4 or 6 days between treatment applications. Variation in the optimal treatment for disease control, however, was still apparent with differing doses proving most effective dependent on the date of application and cultivar. It was, therefore, concluded that to determine whether pre-harvest LIUV and HIPPL treatments were a reliable alternative to chemical control the experiments should be performed within a controlled environment.

9.1 Aims

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

9.2 Materials and methods

9.2.1 Plant husbandry, treatment and inoculation

Plants of the commercial cultivars Amica and Temira were grown in a nutrient film technique (NFT) system as stated in section 2.6. A lighting period of 16/8 at 21/12^{o}C and a light intensity of approx. 250 µmol/m/s delivered by 400 W HPS lights at a relative humidity of 70 to 80%. Seedlings were grown to the 8 true leaf stage for treatment.

For LIUV treatments plants were treated with either 0.32, 0.64, 0.98, 1.28 or 1.92 kJ/m^2 delivered at 2000 µW/cm^2. For HIPPL treatments plants were treated from 40 cm with 12,

24, 36, 48 or 72 pulses. At 2 days post treatments (DPT) plants were inspected for the presence of damage and then inoculated with *Botrytis cinerea* and disease was measured at 2 and 3 days post inoculation (DPI), both as stated in section 7.4.

9.2.2 Experimental design and statistical analysis

Five biological repeats and 4 technical repeats were used for each of the treatments groups across two independent replicate experiments, n=10. One-way ANOVA with Tukey's post-hoc testing was performed. Where the homogeneity of variances assumption could not be met Welch's robust ANOVA was performed followed by the Games-Howell post-hoc test. Statistical significance is defined as $p \leq 0.05$. All statistical analysis was performed in SPSS (IBM). Results from each individual experimental replicate is given in Appendix 10.

9.3 Results and discussion

At two days following treatment damage was observed at LIUV treatments > 0.98 kJ/m^2 for both cv. Amica and Temira (Figure 9.1A and 9.2A). In line with observations in objective 5 cv. Amica showed a greater susceptibility to damage than Temira with 100% of plants exhibiting damage at ≥ 1.28 kJ/m^2 and 1.92 kJ/m^2 for each cultivar, respectively. For the HIPPL treatments damage was observed at 48 and 72 pulses for cv. Amica and Temira (Figure 9.1B and 9.2B). Amica showed a 20 and 100% damage rate for the 48 and 72 pulse treatments, respectively, whereas Temira only showed a 70% damage rate for treatments of 72 pulses.

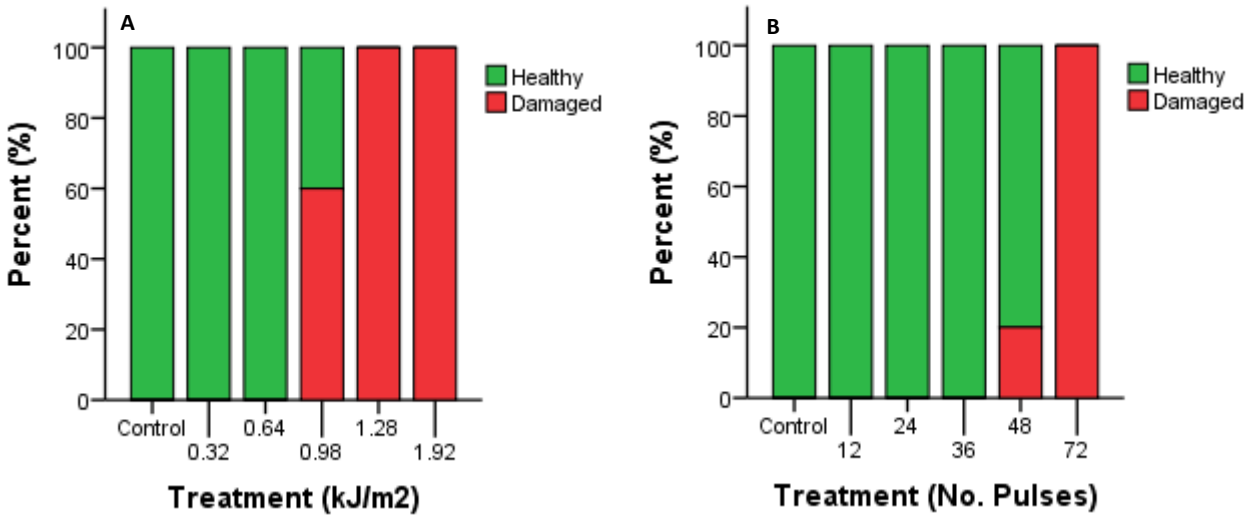

Figure 9.1: The percentage of healthy and damaged plants for the lettuce variety Amica. Plants were either treated with low-intensity UV-C source (LIUV) (**A**) or a high-intensity, pulsed polychromatic light (HIPPL) source (**B**) from two independent replicate experiments carried out during May and June 2017 n=10.

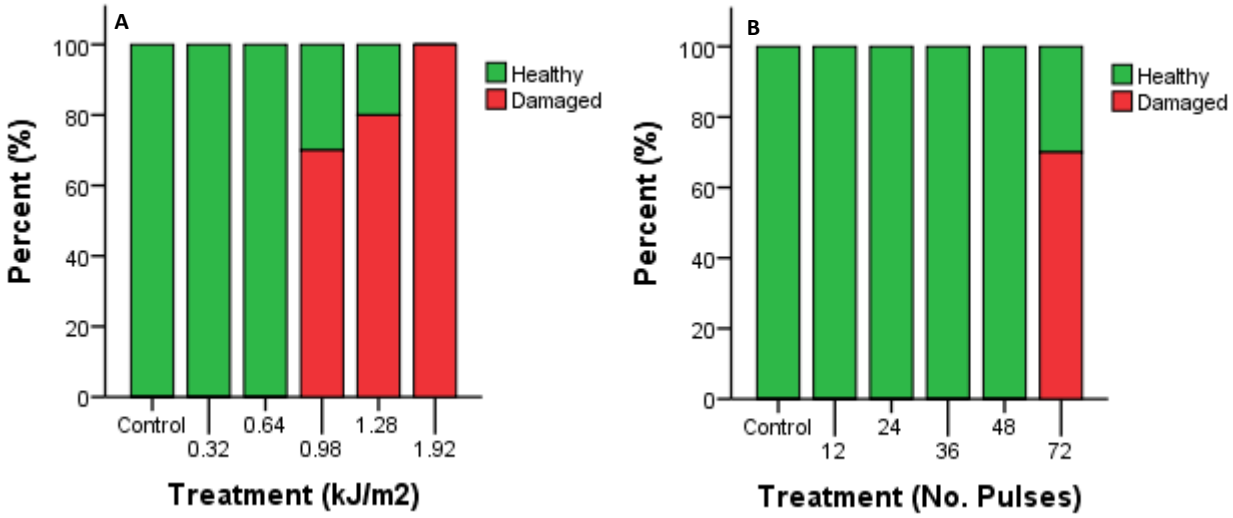

Figure 9.2: The percentage of healthy and damaged plants for the lettuce variety Temira. Plants were either treated with a low-intensity UV-C source (LIUV) (**A**) or a high-intensity, pulsed polychromatic light (HIPPL) source (**B**) from two independent replicate experiments carried out during May and June 2017 n=10.

Following damage assessments, a leaf disc bioassay was performed to assess the ability of treatments to control disease caused by *B. cinerea.* Treatments that caused damage to the plant were not statistically analysed for their ability to reduce disease progression. The LIUV treatment that gave the greatest levels of control for cv. Amica was 0.98 kJ/m^2 which reduced disease by 24.4% (Figure 9.3A). This treatment, however, caused damage to the crop (Figure 9.1) and is, therefore, not hormetic or suitable for commercial use. The most successful non-damaging treatment was 0.32 kJ/m^2 which reduced disease progression by 12.1%. None of the LIUV treatments were significantly different from the control. None of the HIPPL treatments gave significant levels of disease control (Figure 9.3B). The 24-pulse treatment, however, did show some levels of disease reduction at 14.1%. The damaging treatment of 72-pulses also showed disease control with reductions in disease progression at 11.3%. Further replications with cv. Amica would be needed to help to identify any potential beneficial effects of treatment. This, however, was not possible due to time constraints.

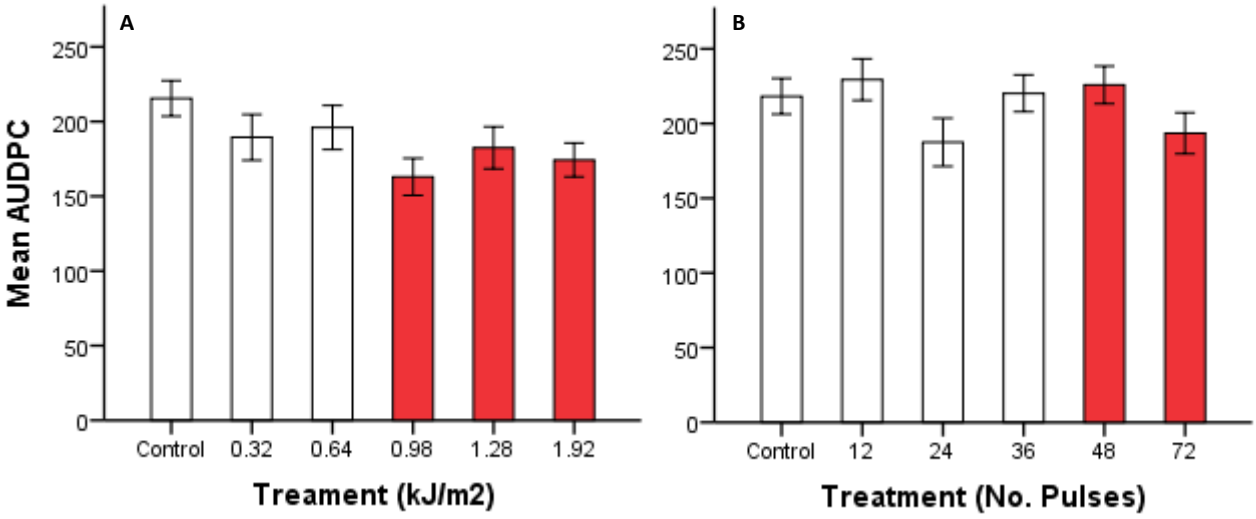

Figure 9.3: The area underneath the disease progression curve (AUDPC) from lettuce plants (Amica) treated with either low-intensity UV-C (**A**) or high-intensity, pulsed polychromatic light (**B**) and inoculated with *Botrytis cinereal* at 2 days post-inoculation. Disease progression measurements were taken at 2 and 3 days post inoculation to allow calculation of AUDPC. Red bars indicate that treatments caused damage to the crop. Damaging treatments were not statistically analysed. Graphs show the data from two independent replicate experiments performed during May and June 2017. n=10. Bars show ± 1 S.E.M. Labelling indicates statistical significance where groups sharing labels are not significantly different at $p < 0.05$.

The LIUV treatment showing the greatest levels of control for the cv. Temira was also damaging with the 1.92 kJ/m^2 treatment giving a 40.4% reduction in disease progression (Figure 9.4A). Of the two treatments that fell below the damage threshold only the 0.64 kJ/m^2 treatment was significantly different from the control, reducing disease progression by 21.0%. HIPPL treated cv. Temira gave statistically significantly levels of disease control (Figure 7.4B). The most successful of which, however, was also damaging (72-pulses) but reduced disease progression by 23.4%. The 48-pulse treatment, however, was not damaging and provided a 21.4% reduction in disease progression.

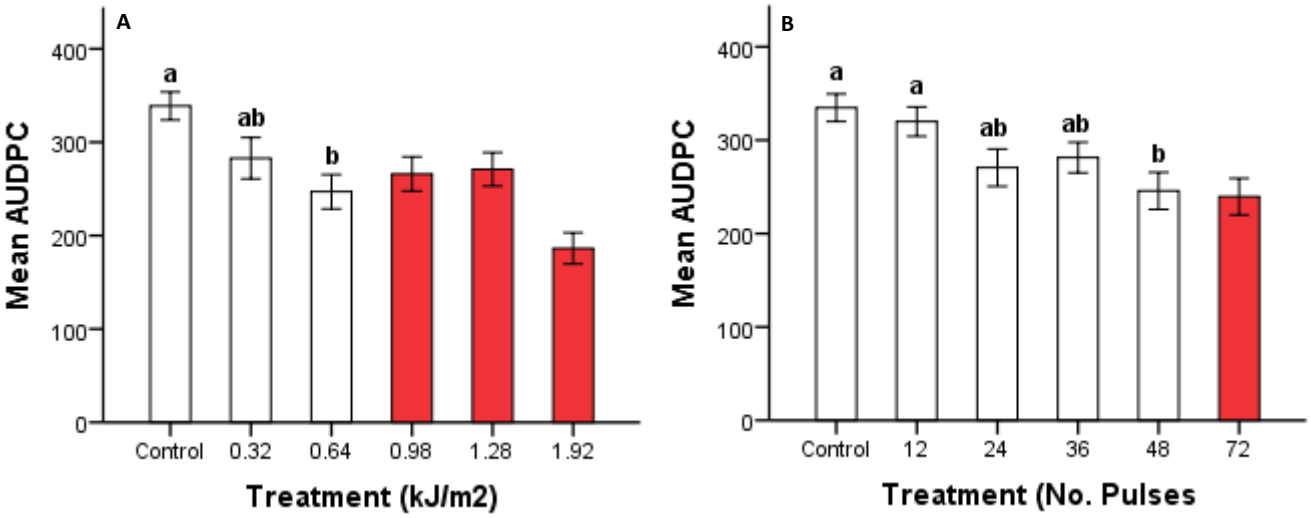

Figure 9.4: The area underneath the disease progression curve (AUDPC) from lettuce plants (Temira) treated with either low-intensity UV-C (**A**) or high-intensity, pulsed polychromatic light (**B**) and inoculated with *Botrytis cinereal* at 2 days post-inoculation. Disease progression measurements were taken at 2 and 3 days post inoculation to allow calculation of AUDPC. Red bars indicate that treatments caused damage to the crop. Damaging treatments were not statistically analysed. Graphs show the data from two independent replicate experiments performed during May and June 2017. n=10. Bars show ± 1 S.E.M. Labelling indicates statistical significance where groups sharing labels are not significantly different at $p < 0.05$.

The results obtained here yielded similar findings regarding cultivar susceptibility to LIUV or HIPPL damage and the levels of protection provided by treatments to those obtained in objectives 5 and 6. As was previously reported in these experiments, we have observed an increase susceptibility to damage for the cv. Amica in comparison to Temira (Figures 9.1 and 9.2). Furthermore, the levels of protection provided from both LIUV and HIPPL treatments are greater for Temira. This is also observed alongside an increased susceptibility to *B. cinereal* for cv. Temira. Similar observations of high levels of

susceptibility to disease and greater levels of disease protection following treatment were also seen while working with tomato fruit in previous preliminary studies.

These results suggest that pre-harvest LIUV and HIPPL treatments can successfully control disease on the lettuce cultivar Temira when grown in a controlled environment. This is similar to the findings in Ouhibi *et al.* (2014a) which showed that a post-harvest LIUV treatment of 0.85 kJ/m^2 can control both *B. cinerea* and *Sclerotinia minor* on lettuce reducing lesion area by 20.0 and 33.3% at four days following inoculation, respectively. The study, here, gave similar levels of disease control. Furthermore, Vàsquez *et al.,* (2017) have recently published work showing that a single pre-harvest LIUV treatment of 0.85 kJ/m^2 reduced mean lesion area by 10 to 20% and four successive treatments, delivered with 48 hours between treatments, reduced lesion area by 26.0% at three days post-inoculation. It is noted that the dose deliver time for the 0.85 kJ/m^2 treatment was 60 seconds indicating that the intensity of dose delivery was 1433 µW/cm^2. The HIPPL treatment in this study delivered similar levels of disease control against *B. cinereal* on the cv. Temira at 21.4% with a single treatment. The HIPPL treatment, however, can be delivered in 15 seconds in comparison to the 0.85 kJ/m^2 treatment by Vàsquez *et al.,* (2017) with a duration of 60 seconds and the 0.64 kJ/m^2 treatment, here, which gave a 21.4% reduction in disease progression with a duration of 32 seconds.

In this study we have, therefore, provided two alternative treatments. Firstly, the LIUV treatment which was delivered at a higher intensity of 2000 µW/cm^2, when compared to Vàsquez *et al.,* (2017), can reduced treatment times by 46%. Secondly, the HIPPL treatment showing the greatest potential to control disease was achieved in a mere 15 seconds a 75% and 53% reduction in treatment times in comparison to the LIUV treatment in Vàsquez *et al.,* (2017) and here, respectively.

The mechanisms of disease control, however, are not yet know. Due to the experimental design, however, we can discount any direct effects of LIUV and HIPPL treatment on the phytopathogen. It is likely, therefore, that disease control is achieved through similar mechanisms to that outlined in Chapter 4. Further investigations, however, into the molecular mechanisms underpinning disease control, following LIUV and HIPPL treatment, are required.

9.4 Summary and conclusions

To conclude, during Chapters 7 and 8, LIUV and HIPPL treatments of lettuce showed potential for controlling disease on lettuce grown in the glasshouse. Variation in the damage threshold and identification of the treatments which effectively controlled disease, proved to be problematic. Using a controlled environment with fixed lighting intensity, temperature and stable humidity has mitigated the variation and allowed us to successfully replicate experiments. It can now be confirmed that both LIUV and HIPPL treatments can control disease, with statistical significance, for the cv. Temira. Furthermore, using HIPPL treatments can drastically reduce treatment times by up to 75%.

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

Chapter 10: Investigating low-intensity UV-C seed treatments for the control of disease on tomato crops

As was discussed in Chapters 7 and 8 preharvest LIUV and HIPPL treatments for inducing resistance on lettuce proved to be challenging. Applying light stress treatments to actively growing plants can potentially be problematic due to the plant continually changing its homeostasis in response to the changing environmental cues. For example, in times of increased light intensity and duration and UV-B/A exposure lettuce plants will alter their production of phenolic compounds, which act as light quenchers, to protect them from light-induced cellular damage. It was therefore decided that UV-C treatment of seeds would be investigated in order to remove the variation observed in the glasshouse applying foliar treatments.

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

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

10.1 Aims

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

10.2 Materials and methods

10.2.1 Seeds, seed storage and UV-C treatment

Seeds of the cv. Shirley, as a previously commercial cultivar, were chosen due to their physiological similarities with current commercial tomato cultivars. Shirley F1 tomatoes, however, lack the high levels of resistance to *B. cinerea* seen in modern commercial cultivars. Seeds were purchased in bulk from the DEFRA-registered seed merchant Sow Seeds (UK) and may, therefore, fail to identify any batch-to-batch variation. Seeds were stored at 6°C at a relative humidity of 50%. For treatment, seeds were placed in a 50 mm Petri dish lined with aluminium foil on the exterior. Lids were removed and seeds were treated at 20 W m^{-2} to total doses of 2, 4 and 6 kJ/m^2 as stated in section 2.4. Seeds were immediately stored in the dark at 21°C for 5 days following the procedures of (Brown *et al.,* 2001).

10.2.2 Plant husbandry

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

10.2.3 Pathogen maintenance, spore preparation and inoculation

Pathogen propagation and spore preparation was performed as stated in section 3.2.3. Inoculations were performed in a controlled environment with a 16/8 photoperiod set at 21°C as preliminary inoculation experiments in the glasshouse proved to be highly variable. Petiole stub inoculations were performed according to Beyers *et al.* (2014) with an amended spore solution optimised in preliminary experiments. Three technical repeat inoculations were performed on each plant at true leaves 3, 4 and 5. Petioles were inoculated with 10 µl of 5 x 10^6 spores per ml amended with 40% grape juice which was found to give the most consistent and equally sized lesions in preliminary experiments (Figure 10.1 & Table 10.1). Lesion size was measured as total lesion length with Vernier callipers at 4 and 6 days post inoculation (DPI). Multiple measurements allowed the

calculation of the area underneath the disease progression curve as stated in section 3.2.4, equation 3.2.

10.2.5 Experimental design and statistical analysis

For experiments concerning disease resistance three UV-C treatments, 2, 4 and 6 kJ/m^2 were utilised. *Botrytis cinerea* resistance assays had a completely randomised experimental design within the glasshouse. Three independent experimental replicates were performed with a total of 24 biological replicates per treatment group. The dates of each experimental replicate can be seen in Table 10.1. Due to variation in susceptibility to *B. cinerea* between experiments factor correction was used to normalise the data following Ruijter *et al.,* (2006). Data was analysed in SPSS (IBM) by Kruskal Wallis (non-parametric ANOVA) with post-hoc testing utilising the adjusted p-values for pairwise comparisons. Statistical significance is defined as p=0.05 in all experiments. All the results from individual experimental replicates in this Chapter can be found in Appendix 8.

Table 10.1: The dates that independent experimental replicates were performed

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

10.3 Results and discussion

10.3.1 Optimisation of inoculation techniques

A number of spore concentrations and amended buffers were attempted after inoculations using water to carry the spores at 1 x 10^6 proved to be ineffective. Concentrations of 1 x 10^6, 5 x 10^6 and 1 x 10^7 were attempted. The first amendment was potato dextrose broth (PDB) at 50%; shown to be effective for *B. cinerea* inoculations in Chapter 7. Amendments with 40% grape juice and also a potassium phosphate and glucose buffer were also attempted (Rossall, 2014; Beyers *et al.,* 2014). All PDB inoculations showed a fairly small inter quartile ranges (IQR) (Figure 10.1). Incidence of disease, however, was low and a number of outliers was observed (Table 10.2). The potassium phosphate buffer showed the greatest IQRs indicating that lesion progression was the most variable. The inoculations with grape juice were the most successful in terms of incidence with all concentrations

giving incidences ≥ 93.3%. Furthermore, IQRs for the 1 x 10^6 and 5 x 10^6 concentrations were small and the 5 x 10^6 concentrations showed a distribution closest to normal and had no outliers. For this reason the 5 x 10^6 concentration amended with 40% grape juice was chosen for the following experiments.

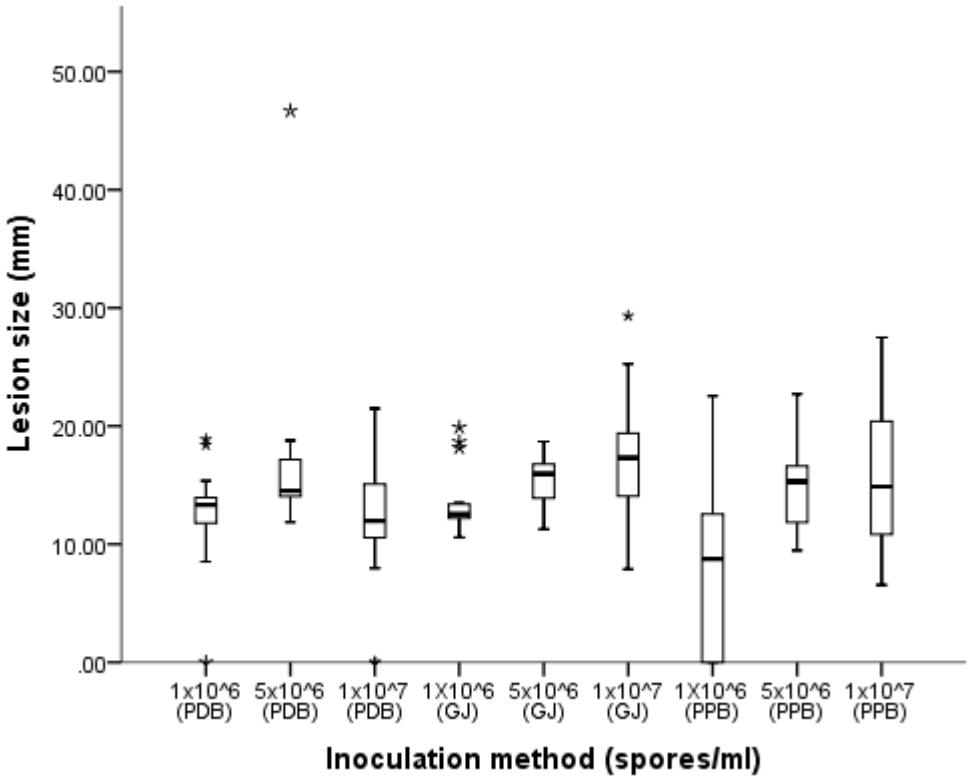

Figure 10.1: The lesion size from tomato plants (cv. Shirley) inoculated with *Botrytis cinerea* at the flowering stage. Plants were inoculated at the wound site of a petiole stub with 10 µl of a calibrated spore suspension. Suspensions were amended with either 50% potato dextrose broth (PDB), 40% grape juice (GJ) and a potassium phosphate & glucose buffer (PPB) as in Beyers *et al.* (2014).

Table 10.2: Incidence of disease for tomato plants (cv. Shirley) following inoculation with *Botrytis cinerea* at the flowering stage. Plants were inoculated at the wound site of a petiole stub with 10 µl of a calibrated spore suspension. Suspensions were amended with 50% potato dextrose broth (PDB), 40% grape juice (GJ) and potassium phosphate & glucose buffer as in Beyers *et al.* (2014).

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

10.3.2 Disease control bioassays

Plants of the cv. Shirley were inoculated with *B. cinerea* at the flowering stage. Both a significant decrease in disease incidence and progression were observed for the 4 kJ/m^2 treatment. Control plants showed a disease incidence of 98.61% with the 2 and 4 kJ/m^2 treatments showing 6.5 and 9.8% reductions in incidence in comparison to the control (Figure 10.2). Incidence for the 6 kJ/m^2 showed no change from the control at 98.61% and was also significantly different from the 4 kJ/m^2 treatment.

The disease progression was also recorded at 4 and 6 days following inoculation. Median disease progression was lower for all treatments in comparison to the control (Figure 10.3). The 4 kJ/m^2 showed the greatest reduction at 10.7% while the 2 and 6 kJ/m^2 treatments showed 1.8 and 3.6% reductions, respectively. Only the 4 kJ/m^2 was significantly different from the control.

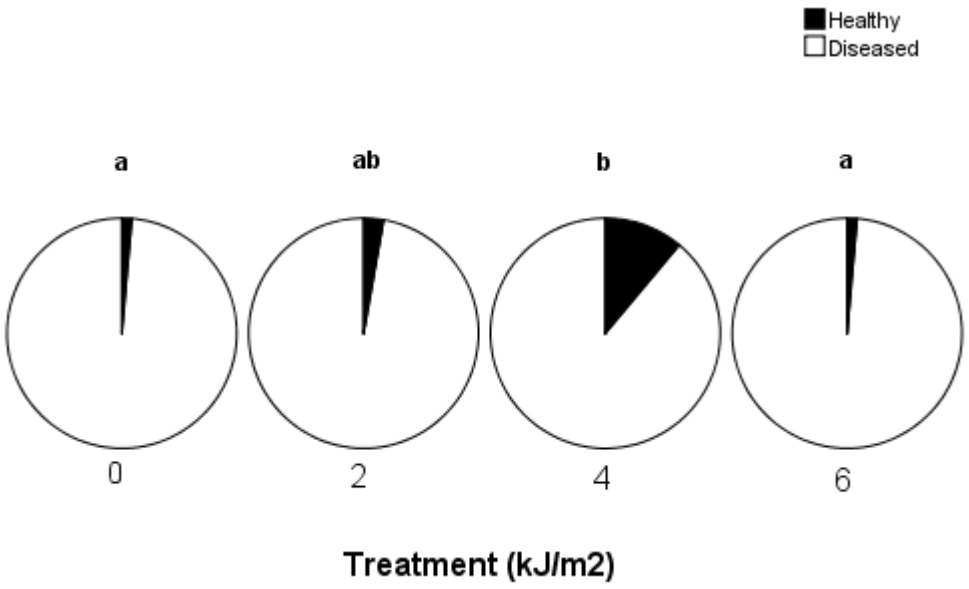

Figure 10.2: Disease incidence (%) for tomato plants (cv. Shirley) grown from UV-C treated seed and inoculated with *Botrytis cinerea* at flowering. Labelling indicates statistical significance at $p < 0.05$. Means sharing the same label are not significantly different from each other. N=24, IRE=3.

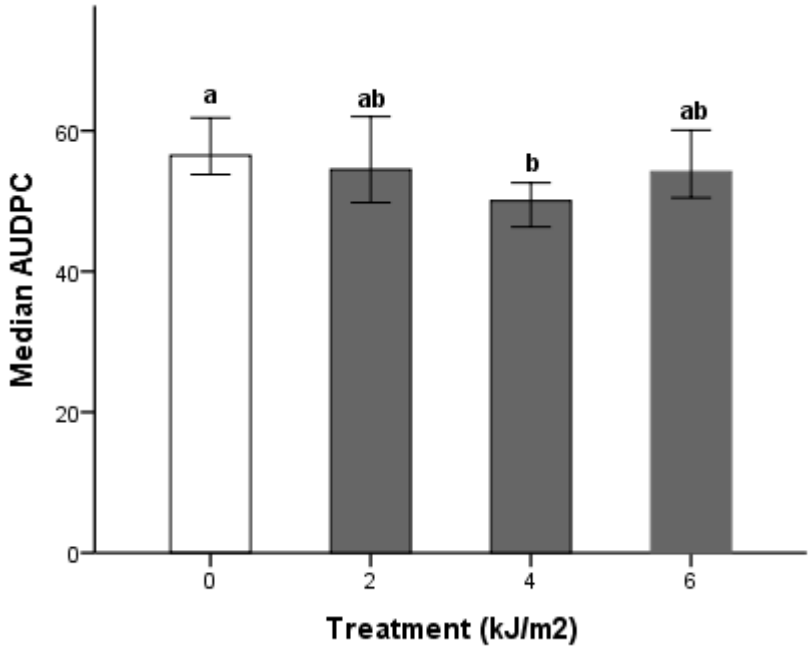

Figure 10.3: Area underneath the disease progression curve (AUDPC) for tomato plants (cv. Shirley) grown from UV-C treated seed and inoculated with *Botrytis cinerea* at flowering. Error bars show confidence intervals at 95%. Labelling indicates statistical significance at $p < 0.05$. Means sharing the same label are not significantly different from each other at $p<0.05$. N=24, IRE=3.

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

10.4 Summary and conclusions

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

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

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

Furthermore, UV-C seed treatments have also been shown to reduce the impact of salt stress on two crops, lettuce and green beans (*Phaseolus vulgaris*). Ouhibi *et al.* (2014b) treated lettuce seeds with 0.85 or 3.42 kJ/m^2 of UV-C. The 0.85 kJ/m^2 treatment reduced the impact of salt stress on the dry weight of both roots and leaves, leaf number, total area, thickness, succulence and sclerophylly. Furthermore, the water content of roots and leaves was increased along with total phenolics. There was no change in the level of flavonoids and a reduction in the total antioxidant capacity.

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

Chapter 11: Effect of low-intensity UV-C seed treatments on early seedling development

In Chapter 10, it was shown that UV-C seed treatments of tomato (cv. Shirley) can reduce both disease progression and incidence of *Botrytis cinerea* by approx. 10%. Although such a result may be extremely beneficial to commercial growers it is important to determine whether any potentially detrimental effects are occurring to the plants' growth and development. Previous literature, however, indicates quite the opposite with biostimulation and increases in yield being observed (Hamid & Javvaid, 2011; Siddique *et al.,* 2011; Shaukat *et al.,* 2013; Neelamegan & Sutha, 2015). It is not yet known whether such effects will be observed on tomato.

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

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

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

increase. The measured effects, however, appeared to be dose-dependent with differing doses showing the greatest increases.

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

11.1 Aims

Investigations into how UV-C seed treatment effects the germination and early seedling growth and development of tomato were performed. As identified in Chapter 10, a 4 kJ/m^2 treatment induced resistance against *B. cinerea.* The aims of this Chapter were to assess the impact of treatment on seed germination and early seedling growth. Two higher doses of 8 and 12 kJ/m^2 were, therefore, used to determine at what point treatments become detrimental to plant growth.

11.2 Materials and methods

11.2.1 Monitoring seed germination and seedling development

UV-C treatments were performed as stated in section 9.2.2. Seeds were sterilised to prevent growth of microorganisms shown to directly affect root development in preliminary studies. Seeds were washed in 70% ethanol for 2 min and 3% sodium hypochlorite (Sigma-Aldrich) for 20 min followed by 3 washes in SDW. Seeds were plated in a 120 mm square Petri dishes in a complete block randomised design. Plates were filled with 50 ml of Murashige Skoog media at 4.3 g/l (pH 5.8) and 0.8% agar technical No.3 (Oxoid). Plates were sealed with surgical tape (3MM). Plates were placed in a rack, allowing them to stand vertically, and exposed to a 16 h photoperiod at an intensity of 100 mE m^{-2} s^{-1} at 22°C ± 1°C and a relative humidity of 50-75%. Germination was monitored for 7 days following plating. Primary root length and hypocotyl length was measured at 2 and 5 days post germination (DPG) with ImageJ. At 5 DPG seedlings were dissected and dried for 24 h at 50°C. Dry mass of the roots, hypocotyl and cotyledon were then taken. To monitor germination a number of metrics were used including total germination percentage,

germination index (Equation 11.1), T_{50} (Equation 11.2) and Z-index (Equation 11.3) (Coolbear *et al.,* 1984; Walker-Simmons, 1987; Ranal *et al.,* 2009). For the monitoring of root growth and stem mass fraction, specific root and stem mass, root length ratio, root-shoot ratio and the seedling vigour index were calculated (Table 11.1).

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

Equation 11.1: Germination index (GI), where n_1, n_2, … , n_7 are the number of germinated seeds on the first, second and subsequent days until the 7th; 7, 6, … , 1 are the weights given to the seeds germinated on the first, second and 7th days, respectively (Walker-Simmons, 1987).

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

Equation 11.2: The time to reach 50% germination (T_{50}) of the total number of seeds planted. *N* is the final number of seeds that have germinated, n_i and n_j are the total number of seeds germinated at adjacent time points t_i and t_j where $n_i < (N+1)/2 < n_j$ (Coolbear *et al.,* 1984).

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

Equation 11.3: Synchrony of germination (Z-index) where n_i is the number of seeds germinated during the *i*th time (Ranal *et al.,* 2009).

Table 11.1: Growth metric equations

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

11.2.2 Experimental design and data Analysis

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

11.3 Results and discussion

11.3.1 Effects on germination

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

When monitoring germination for 7 days following planting no significant differences were observed. The 8 kJ/m^2 treatment, however, showed a stimulatory effect to germination with germination occurring from 2 DPP whereas all other treatments began germination at 3 DPP (Figure 11.1). Furthermore, an increase in the cumulative germination percentage was also observed for all DPP when compared to the control (Figure 11.3). At 3 and 4 DPP the 4 and 12 kJ/m^2 treatments showed slightly reduced levels of germination in comparison to the control. From day 5 onwards, however, germination rate was similar for the control, 4 and 12 kJ/m^2 treatments. This can be highlighted by the total germination percentages

which are all similar with small increases for all treatments apart from a slight decrease for the 4 kJ/m^2 treatment at 87.3% (Table 11.2). The stimulatory effect on germination of the 8 kJ/m^2 can be seen by an increase in germination index to 79.0 in comparison to the control at 66.33, a reduction in the time to 50% germination (T_{50}) from 4.23 days for the control to 3.41. Finally, the synchronicity of germination was also shown to increase from 0.21 in the control to 0.25 (scale from 0-1). The 4 kJ/m^2 treatment, which showed a significant reduction in disease incidence and disease control, showed minor non-significant decreases in germination index, T_{50}.

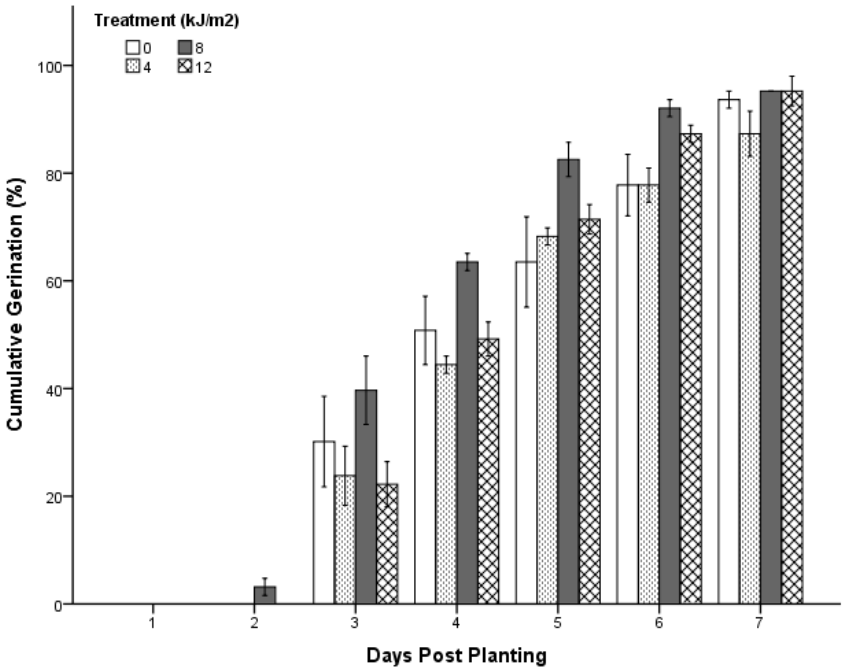

Figure 11.1: The cumulative germination percentage of tomato seeds (cv. Shirley) following treatment with UV-C and grown in controlled environment on Murashige Skoog media. Error bars show ± 1 S.E.M. N=63, IRE=3.

Table 11.2: Germination metrics of tomato (cv. Shirley) following treatment with UV-C and grown in controlled environment on Murashige Skoog media. Error bars show ± 1 S.E.M. N=63, IRE=3.

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

± 1 standard deviation

11.3.2 Effects on seedling growth

At 5 days after germination seedlings were measured, dried and their total dry mass was taken. Increases in dry mass of 5.9, 11.4 and 4.3% were observed for the 4, 8 and 12 kJ/m^2 treatments, respectively (Figure 11.2). Only the 8 kJ/m^2 treatment was significantly different from the control. The increase in dry mass was also complemented with an increase in the seedling vigour index for all treatments. The greatest increase was, again, observed for 8 kJ/m^2 at 386.5 in comparison to the control seeds which gave a SVI-II value of 335.9 (Table 11.3). No significant differences were observed for mean seedling length at 2 or 5 days post germination (Figure 11.3). Observations of a biostimulatory effect after UV-C seed treatment of seeds has also been observed by Brown *et al.* (2001), Siddique *et al.* (2011), Hamid & Javvaid (2011) and Neelamegan & Sutha (2015). Furthermore, Hamid & Javvaid (2011) showed an increase in the biostimulatory effect of treatment on the plants up to 60 days following treatment and Neelamegan & Sutha (2015) showed an increase in yield. Further investigation into vegetative development, anthesis and fruit development is, therefore, required to elucidate the full potential of UV-C treatment on tomato.

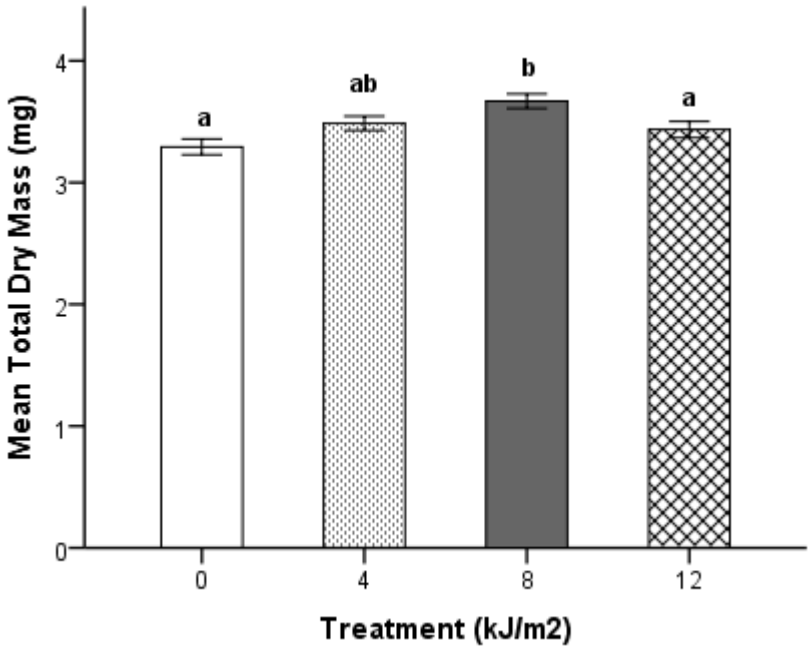

Figure 11.2: Mean total dry mass (mg) of tomato (cv. Shirley) seedlings grown from UV-C treated seed at 5-days post germination. Seedlings were grown in controlled environment on Murashige Skoog media. Error bars show ± 1 S.E.M. N=63, IRE=3. Means sharing the same label are not significantly different at p<0.05.

Table 11.3: Seedling vigour index-II (SVI-II) of tomato seeds (cv. Shirley) following UV-C treatment **a**nd grown in controlled environment in Murashige Skoog media. Error bars show ± 1 S.E.M. N=63, IRE=3.

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

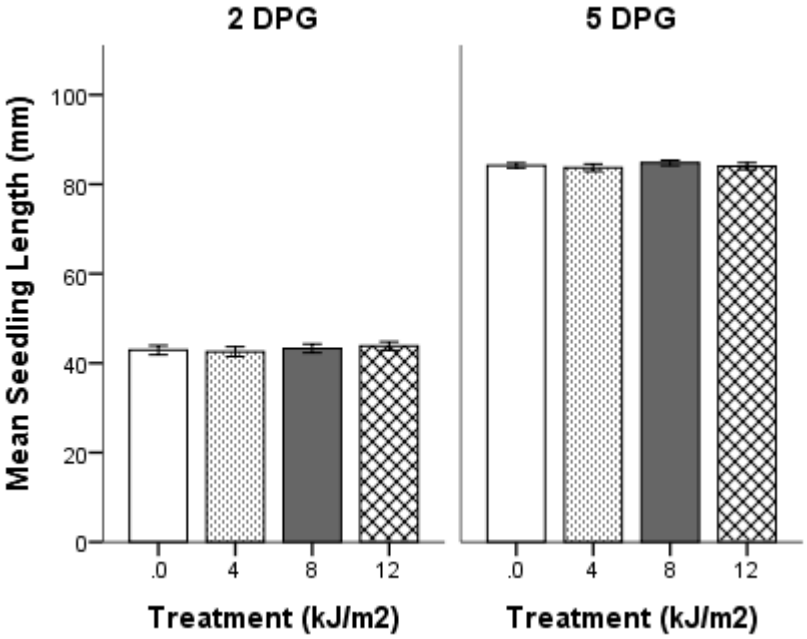

Figure 11.3: Mean seedling length of tomato (cv. Shirley) seedlings grown from UV-C treated seed at 2 and 5-days post germination (DPG). Seedlings were grown in controlled environment on Murashige Skoog media. Error bars show ± 1 S.E.M. N=63, IRE=3. Means sharing the same label are not significantly different at $p<0.05$.

To further elucidate the effect of UV-C seed treatments on growth, seedlings were dissected to allow the determination of the effect on growth of the major plant organs. The growth of shoots was stimulated for each of the treatments. Shoot dry mass was significantly increased for the 8 kJ/m^2 treatment with a 9.6% increase in comparison to the control (Figure 11.4). The 4 and 12 kJ/m^2 treatments showed smaller increases at 5.9 and 3.4%, respectively.

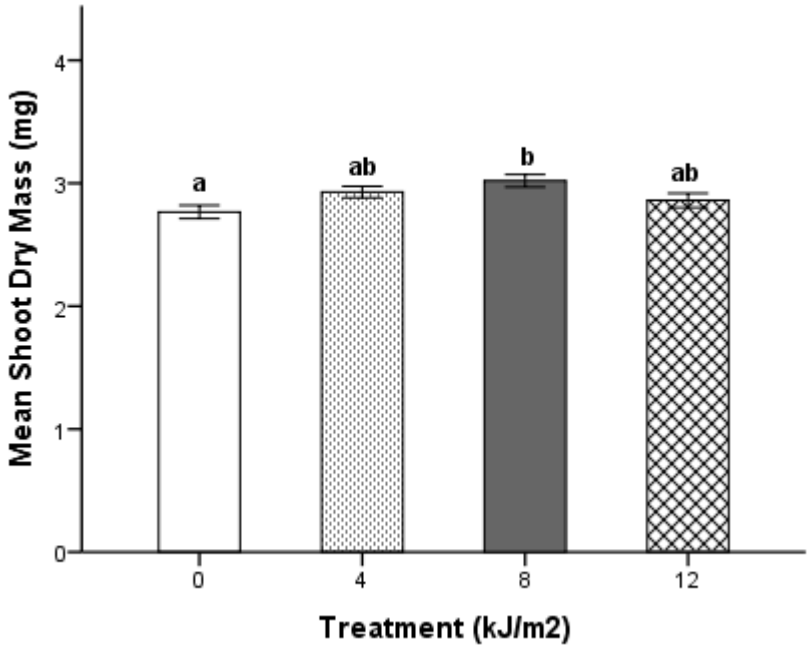

Figure 11.4: Mean shoot dry mass of tomato (cv. Shirley) seedlings grown from UV-C treated seed at 5-days post germination. Seedlings were grown in controlled environment on Murashige Skoog media. Error bars show ± 1 S.E.M. N=63, IRE=3. Means sharing the same label are not significantly different at $p<0.05$.

The dry mass of cotyledons was increased for both the 4 and 8 kJ/m^2 treatments by 4.4 and 8.0%, respectively. The effects of the 12 kJ/m^2 treatment, however, were similar to that of the control at 0.52 and 0.57 mg, respectively (Figure 11.5). None of the differences, however, were significant. Further investigation is required during vegetative growth to determine how the efficiency of photosynthesis is affected.

A stimulatory effect on the mass of hypocotyls was also observed (Figure 11.6). Mass was increased for all of the treatments, in comparison to the control, with 9.4, 12.0 and 7.6% increases for 4, 8 and 12 kJ/m^2, respectively. Only the 8 kJ/m^2 treatment, however, was significantly different from the control. As was observed for the total seedling length, for all of the treatments, at both 2 and 5 days following germination no significant differences were observed (Figure 11.7).

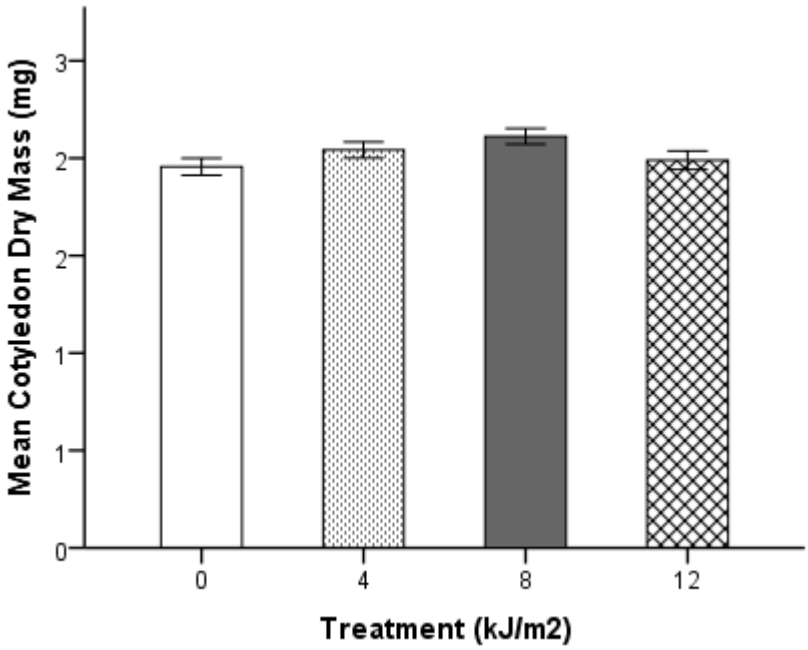

Figure 11.5: Mean cotyledon dry mass of tomato (cv. Shirley) seedlings grown from UV-C treated seed at 5-days post germination. Seedlings were grown in controlled environment on Murashige Skoog media. Error bars show ± 1 S.E.M. N=63, IRE=3. Means sharing the same label are not significantly different at p<0.05.

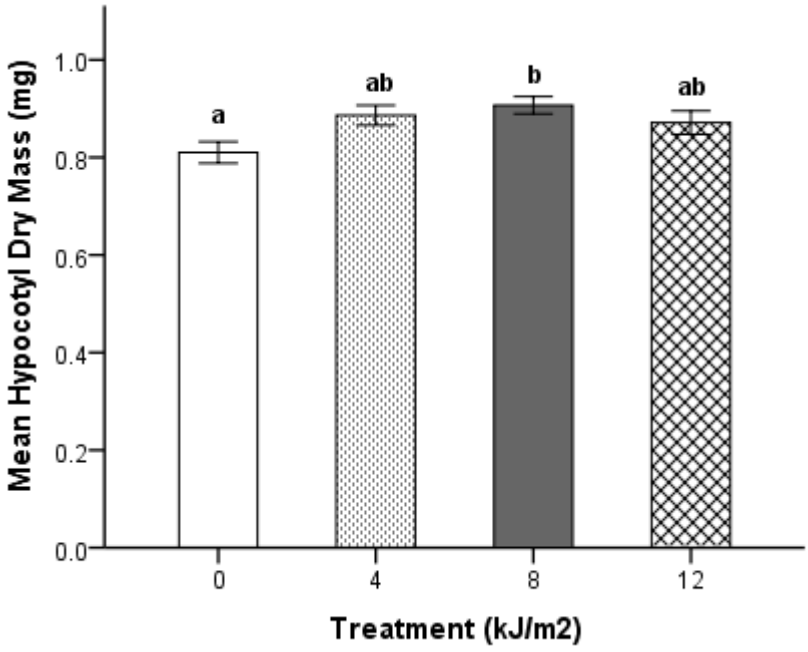

Figure 11.6: Mean hypocotyl dry mass of tomato (cv. Shirley) seedlings grown from UV-C treated seed at 5-days post germination. Seedlings were grown in controlled environment on Murashige Skoog media. Error bars show ± 1 S.E.M. N=63, IRE=3. Means sharing the same label are not significantly different at p<0.05.

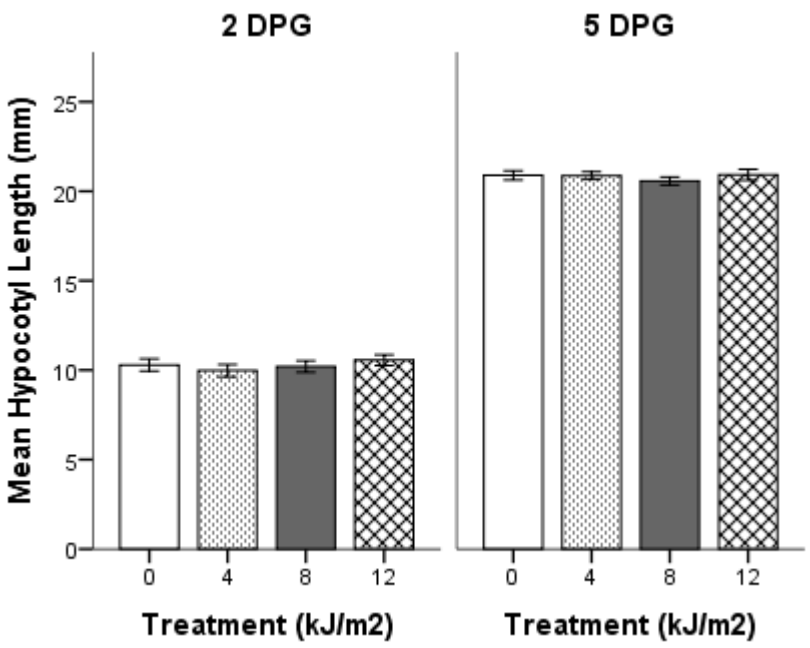

Figure 11.7: Mean hypocotyl length of tomato (cv. Shirley) seedlings grown from UV-C treated seed at 2 and 5-days post germination (DPG). Seedlings were grown in controlled environment on Murashige Skoog media. Error bars show ± 1 S.E.M. N=63, IRE=3. Means sharing the same label are not significantly different at $p<0.05$.

The number of lateral roots at 5 DPG was not significantly affected by any of the treatments, although small increases at 2.2, 3.2 and 2.5% were observed for the 4, 8 and 12 kJ/m^2 treatments, respectively (Figure 11.8). Root dry mass, however, showed a significant increase at 23.1% for 8 kJ/m^2 in comparison to the control (Figure 11.9). The 4 and 12 kJ/m^2 treatments also showed smaller increases at 5.8 and 9.1%, respectively. Mean primary root length, for all treatments, showed little variation from the control (Figure 11.10). None of the UV-C treatments, therefore, showed any negative impact on early root growth or their basic architecture. Furthermore, an increase in root dry mass may lead to greater efficiency in nutrient and water acquisition.

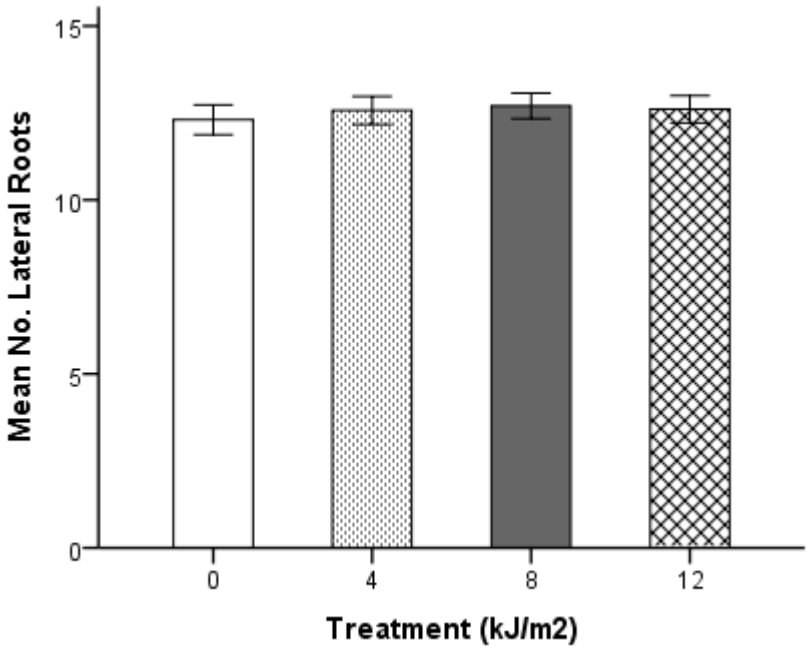

Figure 11.8: Mean number of lateral roots of tomato (cv. Shirley) seedlings grown from UV-C treated seed at 5-days post germination. Seedlings were grown in controlled environment on Murashige Skoog media. Error bars show ± 1 S.E.M. N=63, IRE=3. Means sharing the same label are not significantly different at p<0.05.

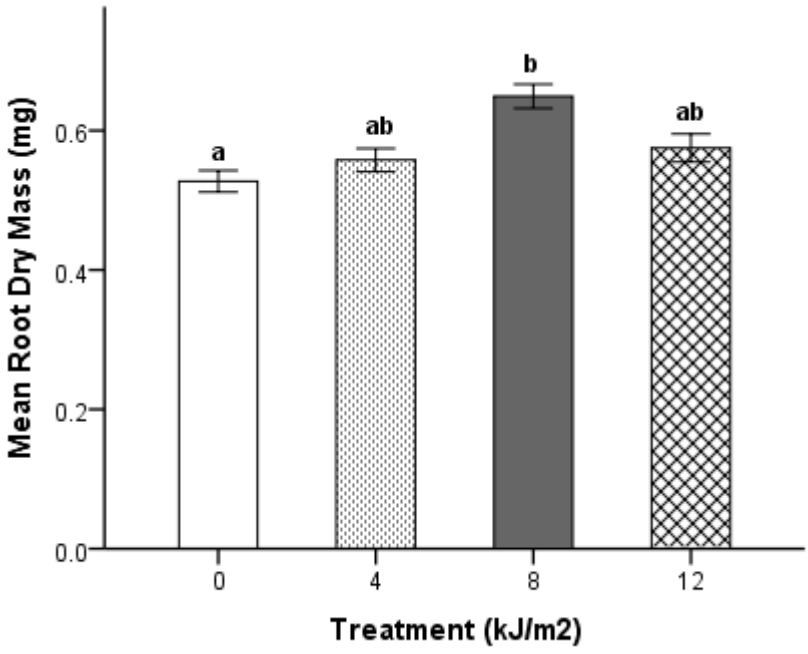

Figure 11.9: Mean root dry mass of tomato (cv. Shirley) seedlings grown from UV-C treated seed at 5-days post germination. Seedlings were grown in controlled environment on Murashige Skoog media. Error bars show ± 1 S.E.M. N=63, IRE=3. Means sharing the same label are not significantly different at p<0.05.

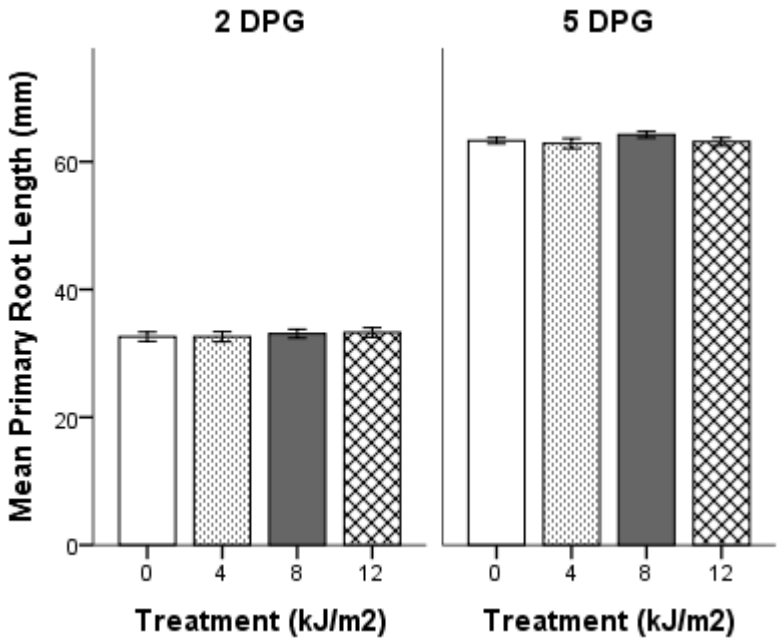

Figure 11.10: Mean primary root length of tomato (cv. Shirley) seedlings grown from UV-C treated seed at 2 and 5-days post germination (DPG). Seedlings were grown in controlled environment on Murashige Skoog media. Error bars show ± 1 S.E.M. N=63, IRE=3. Means sharing the same label are not significantly different at $p<0.05$.

Although significant increases in both root and shoot dry mass were observed for the 8 kJ/m^2 treatment, a significant increase in root mass fraction and decrease in stem mass fraction were also seen (Figure 11.11 A and E). This indicates a general biostimulatory effect that is weighted towards the roots; inferring photoassimilates are being directed primarily towards the root system. Increased root growth may lead to increased efficiency of both water and nutrient uptake. This may have been achieved without any negative effect to the growth or efficiency of photosynthetic organs, which is associated with increasing root mass fraction, as cotyledon mass also exhibited an increase following treatment (Figure 11.5). Increases in the mass of plants without changes to plant organ length may be achieved through an increase in plant cell volume or also an increase in cell wall deposition as seen by Charles *et al.,* (2008b) following UV-C treatment of tomato fruit.

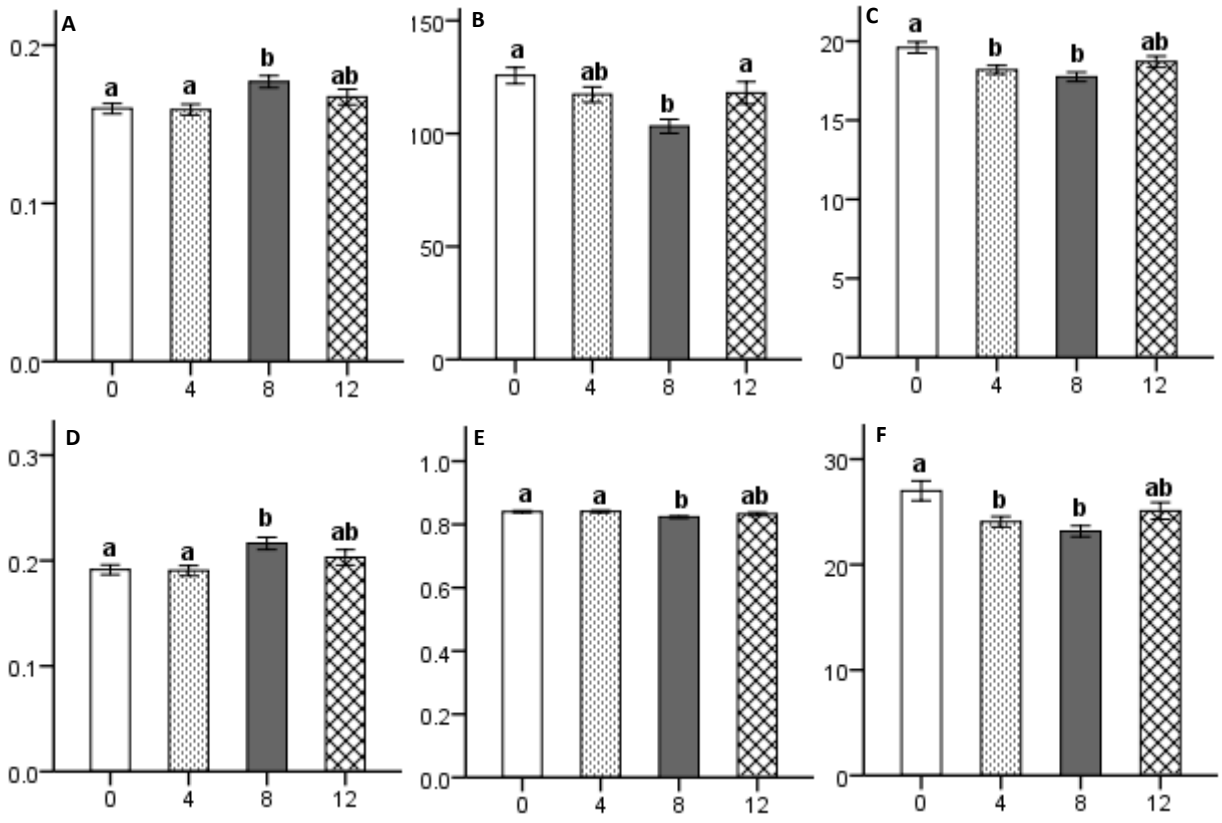

Figure 11.11: Mean root mass fraction, specific root length (B), root length ratio (C), root-shoot ratio (D), stem mass fraction (E) and specific stem length (F) of tomato (cv. Shirley) seedlings grown from UV-C treated seed at 5-days post germination. Seedlings were grown in controlled environment on Murashige Skoog media. Error bars show ± 1 S.E.M. N=63, IRE=3. Means sharing the same label are not significantly different at $p<0.05$.

A significant reduction in both specific stem and root lengths and root-length ratios were also observed for the 8 kJ/m^2 treatment (Figures 11.7, 11.10 & 11.11 B, C and F). Taken together with the absence of any changes to root or shoot length this point towards an increase in root and shoot volume. Further investigation into the changes to organ-specific cellular structure is required. For example, are changes to root volume increased by greater root hair density, increased cellular volume or potentially an increase in cell wall deposition. Increased cell wall deposition has previously been observed by Charles *et al.,* 2008b following UV-C treatment of tomato fruit. Finally, a significant increase, from the control, in the root-shoot ratio was also observed for 8 kJ/m^2 signifying an increase in general plant health.

11.4 Summary and conclusions

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

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

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

UV-C seed treatment of tomatoes may have an extremely beneficial impact on commercial tomato production. As stated previously in Chapter 9 there is not a single fungicide against *B. cinerea* for which resistance has not been observed. The ability to potentially induce resistance prior to planting could potentially reduce costs by reducing fungicide applications and losses due to disease. Furthermore, with further investigation the positive influence on root growth may allow increased nutrient and water uptake efficiency, potentially leading to an increase in yield. UV-C seed treatments are therefore, an exciting area of research which will hopefully be stimulated from the results produced here.

Chapter 12: Summary, conclusions and future research

The benefits of UV-C hormesis have been known for over 30 years. A broad range of benefits are observed from increased nutritional content to disease resistance. To date, most studies have been performed using conventional LIUV sources on postharvest produce. Commercial application of these treatments has, in part, been prevented due to the lengthy exposure times that are required; conventional treatment of tomato fruit can take in excess of six minutes. High-intensity, pulsed polychromatic light (HIPPL) sources have been developed which may hold the potential to drastically reduce treatment times, making such treatments a commercial possibility. It was, however, first necessary to demonstrate that such sources can induce disease resistance and delayed ripening on tomato fruit through postharvest treatments.

During this project, it has been shown that HIPPL sources can successfully induce resistance and delay ripening on tomato fruit (cv. Mecano). A 16-pulse treatment gave comparative levels of disease resistance, against *B. cinerea,* and delayed ripening to the established LIUV treatment of 3.7 kJ/m^2. The HIPPL sources can, therefore, reduce treatment times by 97.3% to 10 seconds per fruit. Both LIUV and HIPPL treatments elicited local disease resistance and delayed ripening responses when treating fruit from the side, blossom end or calyx.

The molecular mechanisms underpinning both LIUV and HIPPL hormesis are highly similar. Both salicylic acid and jasmonic acid biosynthesis markers and pathogenesis-related proteins are upregulated. This indicates that induced resistance may act not only against necrotrophic pathogens but also biotrophic pathogens and plant pests. ACO1, an ethylene biosynthesis enzyme and polygalacturonase production is downregulated leading to the observed delayed ripening. Changes in secondary metabolism are observed though upregulations of PAL and carotene hydroxylase and downregulation of flavonol synthase. PAL exhibits an expression profile associated with gene priming.

Pre-harvest foliar treatments of lettuce in the glasshouse showed that single treatments gave 2 to 54% reductions in the disease progression of *B. cinerea, R. solani* and *S. sclerotiorum*. Reductions in disease progression were observed for approximately 8 days following treatment. Both HIPPL and LIUV treatments show variation in the damage threshold and the optimal treatments that give protection against phytopathogens throughout the year. Furthermore, variation in optimal treatments may change depending

on the cultivar undergoing investigation and pathogen of interest. Low-dose, repeated treatments do not successfully reduce disease progression in the glasshouse.

Pre-harvest foliar treatments of lettuce were moved to a controlled environment in an attempt to control the variation observed in the glasshouse. Moving the study to a controlled environment allowed experiments to be successfully replicated. LIUV and HIPPL treatments were showed to significantly control the disease progression of *B. cinerea* by 21.0 and 21.4%, respectively, on the cv. Temira. Damage thresholds were shown to vary between the cvs. Amica and Temira. The cv. Amica showed increased susceptibility to LIUV and HIPPL damage. HIPPL and LIUV treatments showed greater levels of disease control on the cv. Temira in comparison to Amica. All of these between cultivar variations were also observed in the glasshouse.

The treatment of plants in a controlled environment was able to mitigate the variation observed while treating actively growing plants in the glasshouse. Such treatments, however, are not commercially relevant. It was, therefore, decided to attempt alternative treatment methods to mitigate variation while maintaining commercial relevance. The LIUV treatment of seeds was, therefore, attempted. The study was performed on tomato after consultation with our industry representatives.

A LIUV seed treatments of 4 kJ/m^2 gave control of *Botrytis cinerea* with an approx. 10% reduction in both incidence and disease progression on the cv. Shirley. It was then identified that LIUV treatments of 8 kJ/m^2 showed biostimulation of germination including; increased germination index, increased synchronicity of germination, increased seedling vigour and a reduced time to 50% germination (T_{50}). Biostimulation of roots, hypocotyls and cotyledons was also observed along with an increase in root mass fraction indicating that root growth is stimulated to a greater extent. This may lead to increased water and nutrient uptake and, therefore, yield. Further investigation, however, is required. No change to root length or to the No. lateral roots was observed.

Future work should focus on two principal areas. Initially, investigations into how environmental conditions affect the optimal treatments for controlling disease on lettuce in a controlled environment. This may allow the modelling and determination of treatment regimes that should be applied, in real-time, in the glasshouse. Monitoring of the physiological changes to the crop following treatment should then be performed. Secondly, the impact of seed treatments should be further investigated with an emphasis on physiological changes to the crop; including yield. Examination of the molecular

mechanisms controlling stimulated root growth and disease control should then be performed. Finally, investigations into the further potential benefits of UV-C seed treatment could then be performed with an emphasis on protection against abiotic stress and pests.

Knowledge and technology transfer

Project meetings:

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

Conferences:

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

Publications:

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

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Appendices

Appendix 1

Appendix 1 contains a summary of the observed hormetic benefits of postharvest UV-C, B and A treatments on fresh produce. The tables show the fruit undergoing treatment, the observed benefit, the successful treatment and its intensity (where available) a summary of the results and a reference.

TableA1.1 A summary of the benefits induced by UV hormesis

Fruit	Benefit	Treatment	Dose kJ/m^2	Intensity μWcm^2	Results	Reference
Bananna	Reduce oxidative stress and chilling injury	UVC	0.02-0.04	200	Reduced incidence and severity chilling injury. Higher levels of phenylalanine and phenolics. Reduced oxidiative stress and higher activity of antioxidants including superoxide dismutase. Heat shock protein 70 expression increased.	Pongprasert *et al.*, 2011
Apple	Increasing dietary value and colour.	UVB	0.002	20	Apple flesh showed no response to any of the treatments. Increased levels of flavonoids/antioxidants/phenols/anthocyanins. Improved skin colour and no change to important taste related factors.	Hagen *et al.*, 2007
Broccoli	Delaying senescence	UVA and UVB	8.8	-	UVB more effective at retaining colour. Delayed floret yellowing via supressed chlorophyll degradation.	Aiamla, *et al.*, 2009
Broccoli	Delaying senescence and increasing dietary value	UVC	10	-	Delayed yellowing via supressed chlorophyll a and b degradation. Activity of chlorophyll peroxidase and chlorophyllase were lower in treated plants. Treated broccoli showed lower respiration rate, total phenols and flavonoids but a higher antioxidant capacity. Increase in pheophytins observed.	Costa *et al.*, 2005
Melon cv. Cantelope	Reducing decay	UVC	-	-	Performed on processed fruit. Reduced aliphatic esters and produces phytoalexins to reduce decay.	Lamikanra *et al.*, 2002
Blueberries	Increasing dietary value	UVC	0.43-6.45	-	Increased flavonoids including resveratrol and many more. Significant increase in antioxidant capacity at 2.15, 4.20 and 6.45 kJ. 0.23 kJ increased phenol content and anthocyanins. Optimal results immediately after treatment.	Wang *et al.*, 2009
Cress	Delaying senescence	UVC	-	-	Chlorophyll content was increased with treatment. High electrolyte leakage in all treated samples. Reduced yellowing.	Kasim & Kasim, 2012
Pineapple	Increasing dietary value	UVC	1.6	888	Performed on processed fruit. Increased Vitamin C, β-carotene and α-carotene content.	Freitas *et al.*, 2015

Table 1.1: Continued.

Fruit	Benefit	Treatment	Dose kJm^2	Intensity μWcm^2	Results	Reference
Grapes	Improving dietary value	UVC	8.8	-	Produced stilbene enriched grapes & wine. Increase of 26 fold and 3.2 fold of piceantannol and trans-resveratol in wine, respectively.	Guerro *et al.*, 2010
Chinese kale	Delaying senescence and increasing dietary value	UVC	3.6-5.4	-	Treatments delayed leaf yellowing showed by increased chlorophyll content and lower activity of chlorophyll degrading enzymes. Prolonged levels of antioxidants including peroxidase and superoxide dismutase. Reduced ethylene production and respiration rates.	Chairat *et al.*, 2013
Oyster Mushroom	Reducing microbial load	UVC	-	-	Applied alongside ethanol, hydrogen peroxide and sodium hypochlorite. Combined UV and sanitiser produced greater reductions in microbial load that either treatment alone.	Ha *et al.*, 2011
Pear	Reducing microbial load	UVC	0-8.7	-	Performed on processed pear. Gave control against *Listeria innocua, L. monocytogenes, L. welshimeri E.coli* and *Zygosaccharomyces bailli*. Increased time (dose) led to increased microbial deactivation for all species. Best results were observed in pear without peel.	Schenk *et al.*, 2008
Strawberry	Delaying scenescence	UVC	4.1		Delayed fruit softening. Treated fruit showed increased firmness 96h after treatment. Treatment changed expression of genes and activity of enzymes. Delay in ripening possibly related to decreased transcription of cell wall degrading genes.	Pombo *et al.*, 2009
Watermel-on	Reducing microbial load	UVC	4.1		Treatment caused a 1 log reduction in the microbial population by the end of the productions shelf life. No detrimental effect to juice leakage, colour or visual quality. Lower doses of 1.4 decreased load to a lesser extent. Higher doses did not result in any difference in microbial load or quality	Fonesca *et al.*, 2006

Appendix 2

The following appendix contains preliminary results and data from each independent replicate experiment from Chapter 3 “Validation of high-intensity, pulsed polychromatic light as an inducer of disease control and delayed ripening on tomato fruit”. Such treatments were used to ascertain whether high-intensity, pulsed polychromatic light control disease and delay ripening (postharvest) on tomato fruit (cv. Mecano).

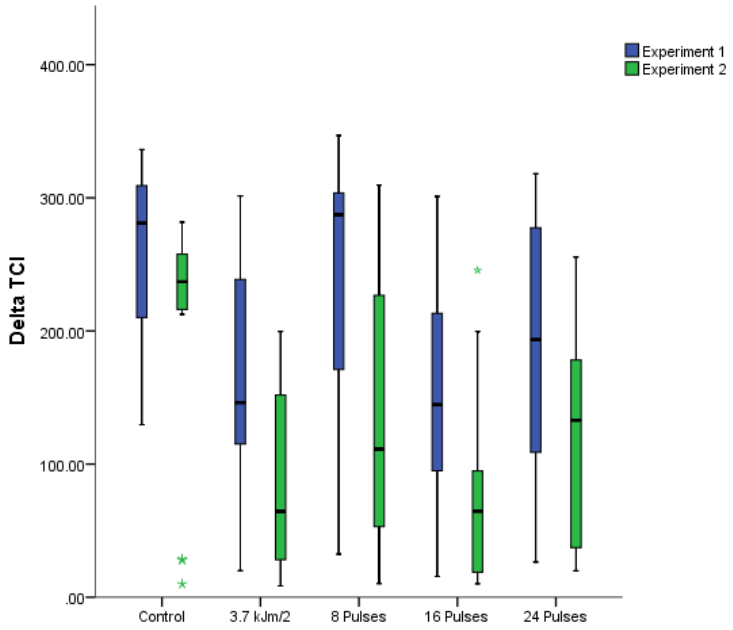

Figure A2.1: The change (Δ) in TCI (tomato colour index) from day 0 - 10 d of storage. Mature green tomato fruit (cv. Mecano) were treated with either low-intensity UV-C (3.7 kJ/m^2) or a range of high-intensity, pulsed polychromatic treatments and stored for 10 days. Box plots show the data from the individual independent replicate experiments (N=15). Asterisks represent observed outliers.

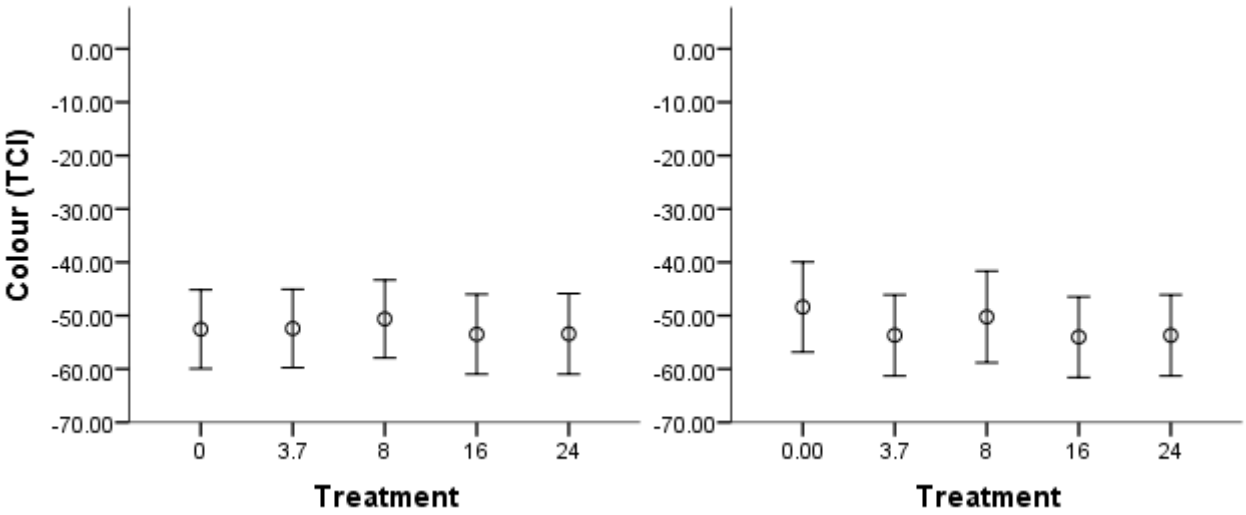

Figure A2.2: The starting tomato colour index (TCI) values for two independent replicate experiments where mature green tomatoes (cv.Mecano) were treated with either low-intensity UV-C or high-intensity, pulsed polychromatic light source postharvest. Error bars show ± 1 S.E.M, N=15.

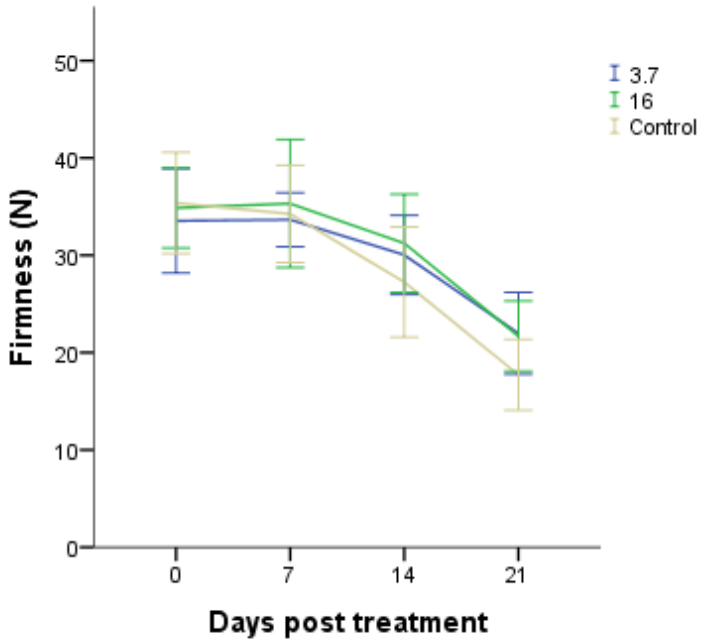

Figure A2.3: Preliminary texture analysis data performed on mature green fruit (cv. Mecano) following treatment wither with 16 pulses of high-intensity, pulsed polychromatic light or 3.7 kJ/m^2 of low-intensity UV-C. Texture measurements were taken either before (0) or 7, 14 or 21 days post treatment. Error bars show ± 1 standard deviation. N= 10.

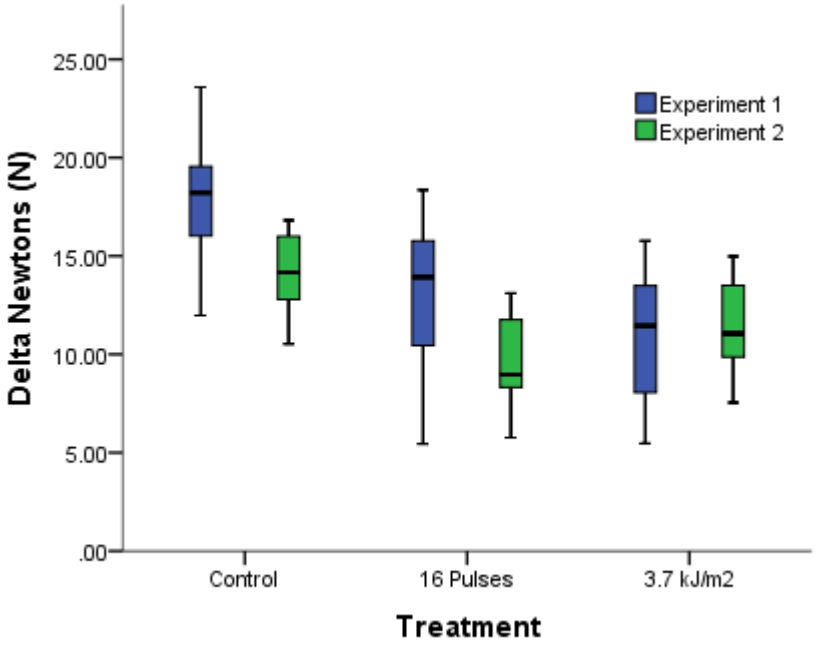

Figure A2.4: The change (delta) in firmness (Newtons) of mature green tomato fruit (cv. Mecano) treated with either a low-intesity UV-C source or high-intensity pulsed polychromatic source. Measurements are taken from day 0 - 21 following treatment. Box plots show the data from the individual independent replicate experiments (N=10). Asterisks represent observed outliers.

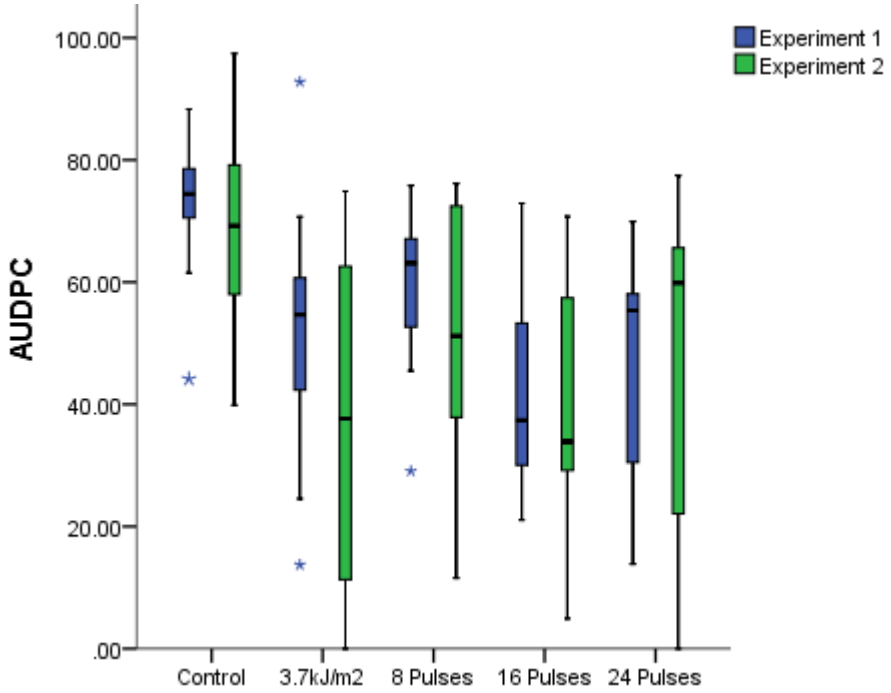

Figure A2.5: Area underneath the disease progression curve (AUDPC) from the independent experimental replicates from mature green fruit (cv. Mecano) treated with a low-intensity UV-C source and a high-intensity pulsed polychromatic light source. Inoculations were performed with *B. cinerea* at 10 d post treatment; n = 15. Box plots show the distribution of data from the independent experimental replicates. Asterisks represent observed outliers.

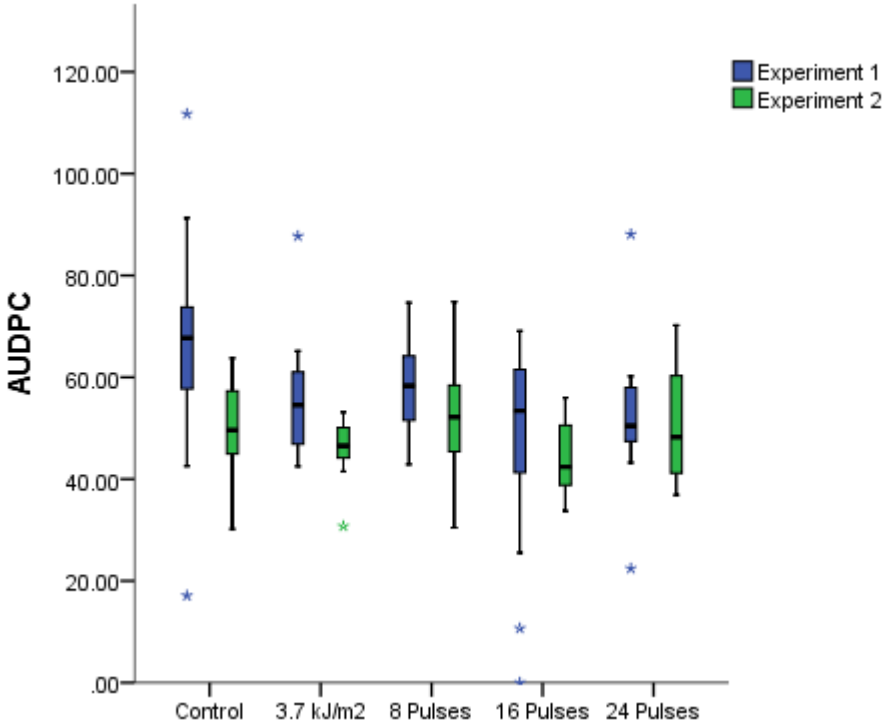

Figure A2.6: Area Underneath the Disease Progression Curve (AUDPC) for ripe fruit (cv. Mecano_ treated with a low-intensity UV-C source with and a high-intensity, pulsed polychromatic light source, followed by inoculation with *B. cinerea* at 10 d post treatment; n = 15. Box plots show the distribution of data from the independent experimental replicates. Asterisks represent observed outliers.

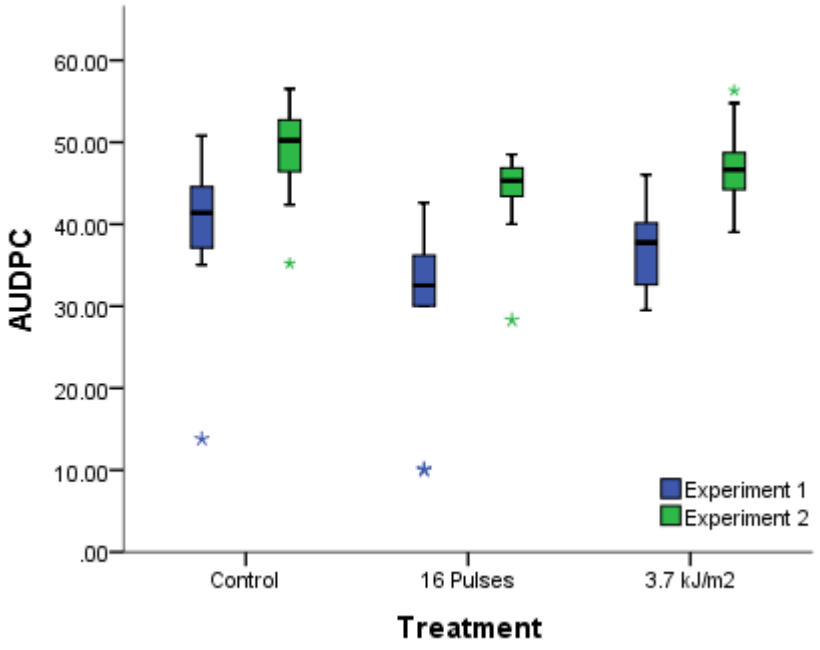

Figure A2.7: Area Underneath the Disease Progression Curve (AUDPC) for ripe fruit cv. Mecano treated with a conventional low intensity UV-C source with and a high intensity pulsed polychromatic light source, followed by inoculation with *P. expansum* at 10 d post treatment; n = 10. Box plots show the distribution of data from the independent experimental replicates. Asterisks represent observed outliers.

Appendix 3

Appendix 3 contains the supplementary data and results from each independent experimental replicate from Chapter 4 “Comparative gene expression analysis following hormetic UV-C and high intensity pulsed polychromatic light treatments on tomato fruit”. Mature green tomato fruit (cv. Mecano) were treated postharvest with either low-intensity UV-C or high-intensity, pulsed polychromatic light sources and samples taken for gene expression analysis with qPCR.

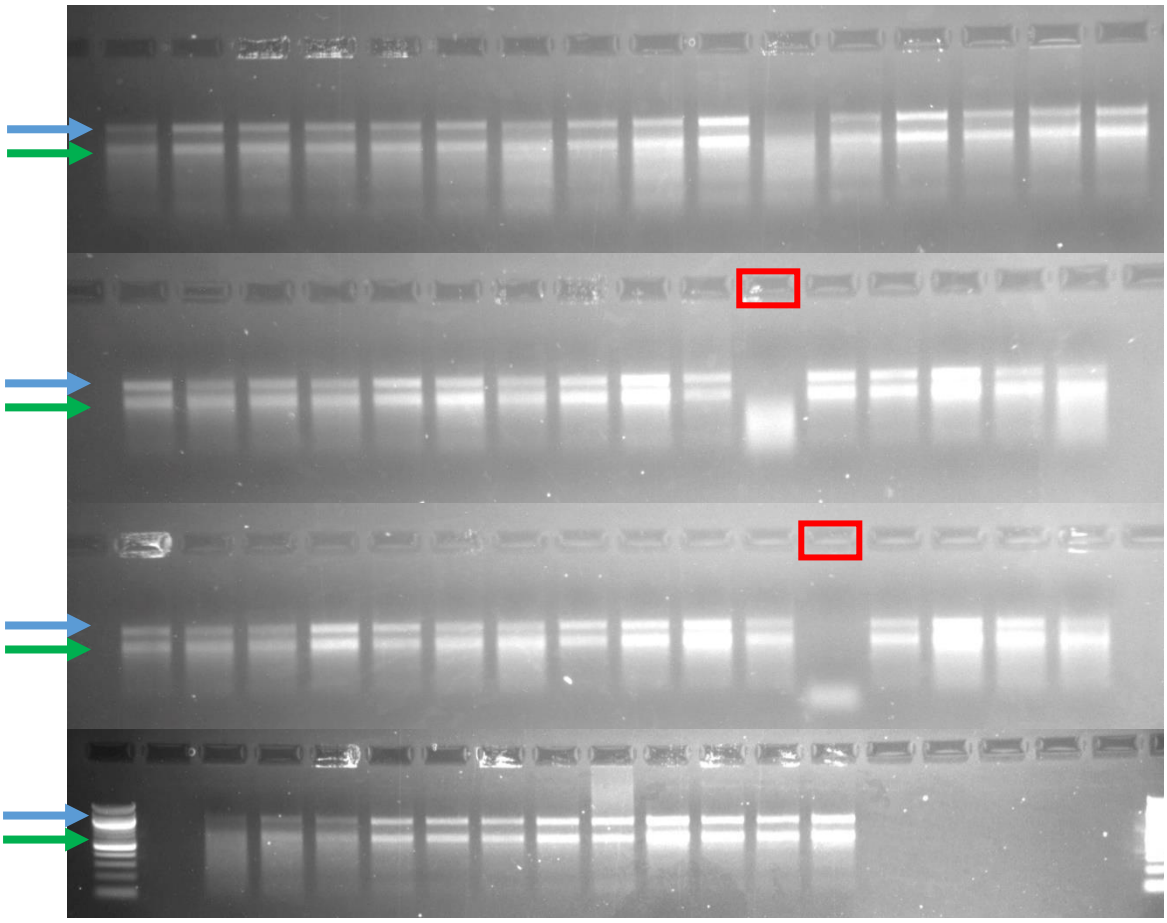

Figure A3.1: Image of TAE gel electrophoresis used to assess the integrity of RNA samples used in qPCR. Approximately 250 ng (5 µl) of RNA was combined with 1 µl of 6X orange/green loading dye (Promega). Samples were heated to 70 ^{o} C for 1 minute and placed on ice. A 5 µl aliquot was loaded into a 1.2 % (w/v) Agarose, TAE (Tris base, acetic acid & EDTA) gel and run in a TAE buffer for 30 minutes at 60 v. The upper band (red arrow) represents the 28S and the lower (blue arrow) the 18S ribosomal RNA, respectively. RNA samples which may have degraded (highlighted in red) will show dull 18S and 28S bands and lower molecular weight products. Degradation of the ribosomal RNA, however, does not indicate degradation of mRNA but is used as a general identification of RNA quality.

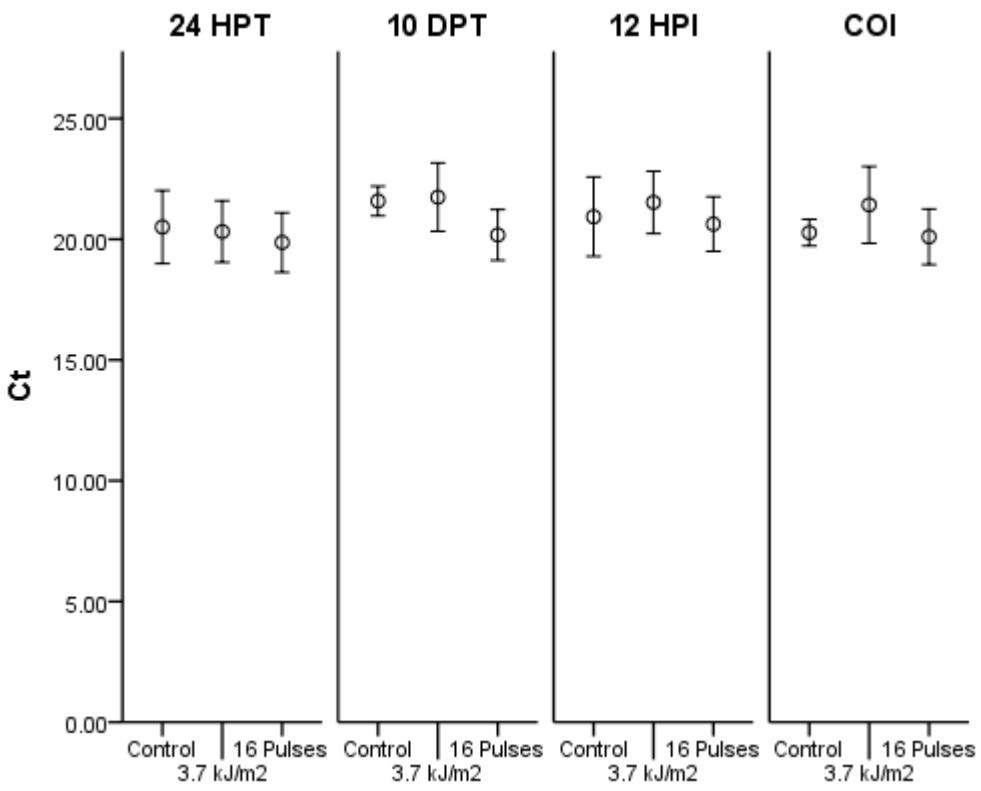

Figure A3.2: Assessment of reference gene (Actin) expression following treatment, storage and inoculation giving mean cycle threshold (Ct) values. Samples were taken from mature green tomato fruit (cv. Mecano) following treatment with either low-intensity UV-C (3.7 kJ/m^2) or a high-intensity, pulsed polychromatic light source (16 pulses). Samples were taken at 24 hours post treatment (HPT), 10 days post treatment (DPT), 12 hours post inoculation with *Botrytis cinerea* (HPI), and control of inoculation/mock inoculated fruit (COI). Error bars show confidence intervals and 95 %.

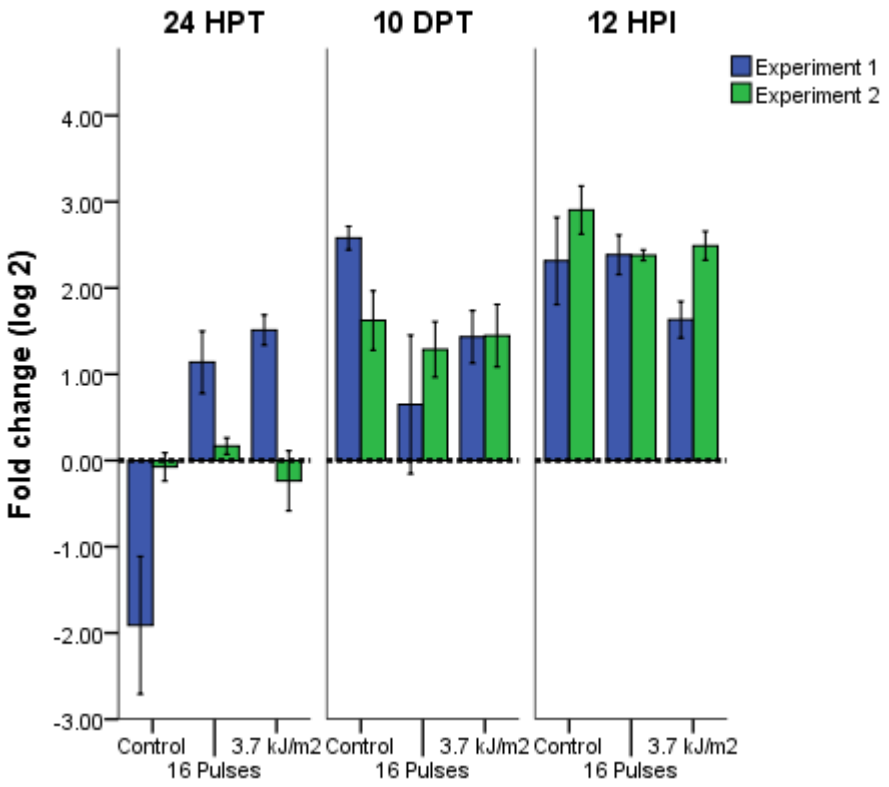

Figure A3.3: Data from the independent replicate experiments monitoring gene expression (qPCR) showing the relative expression of *ACO1* (1-aminocyclopropane-1-carboxylic acid oxidase 1), a bottleneck enzyme in ethylene biosynthesis, following treatment with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C source (LIUV). Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). N=3 and bars show ± 1S.E.M.

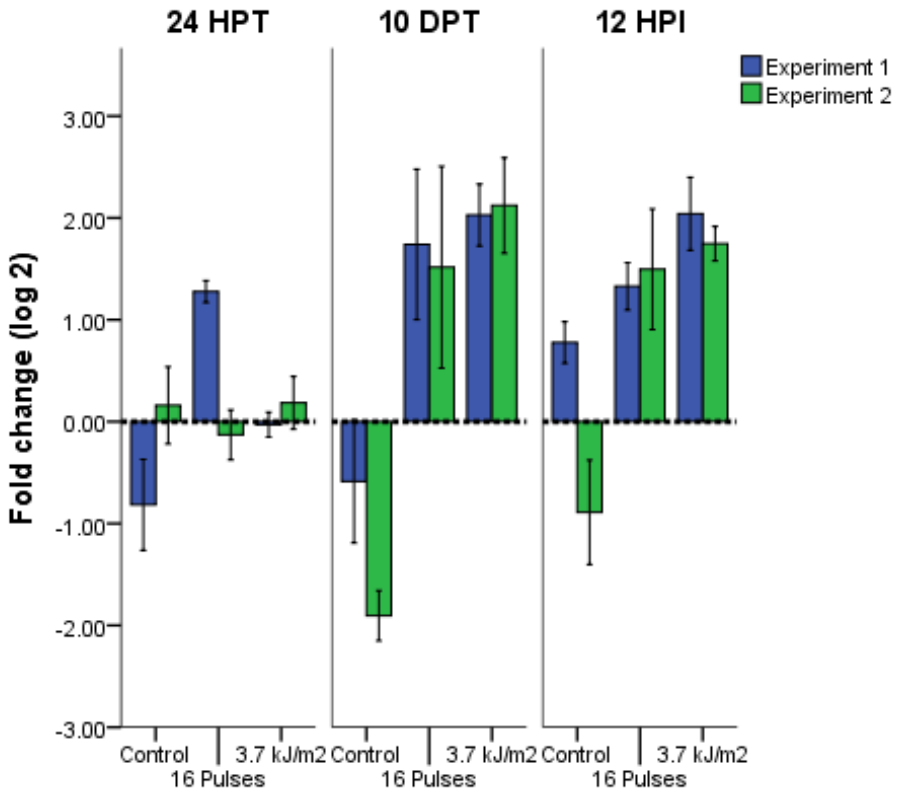

Figure A3.4: Data from the independent replicate experiments monitoring gene expression (qPCR) showing the relative expression of *CHI9* (Chitinase 9) a jasmonic acid induced pathogenesis related protein transcript following treatment with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). N=3. Bars show ± 1S.E.M.

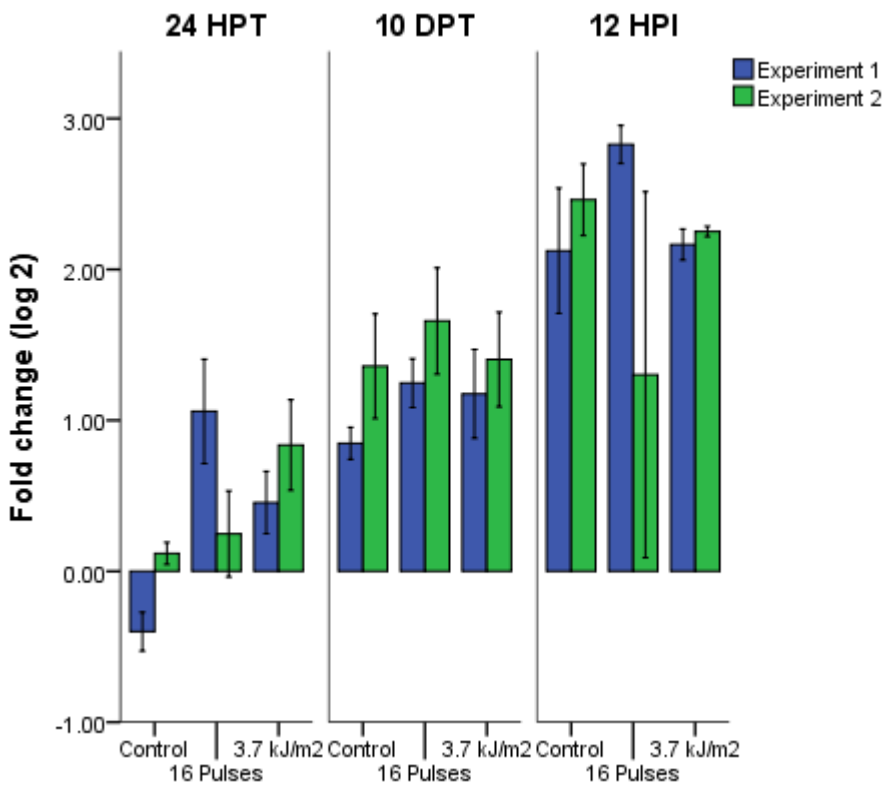

Figure A3.5: Data from the independent replicate experiments monitoring gene expression (qPCR) showing the relative expression of *CRTR-B1* (β -carotene hydroxylase) following treatment with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). N=3. Bars show ± 1S.E.M.

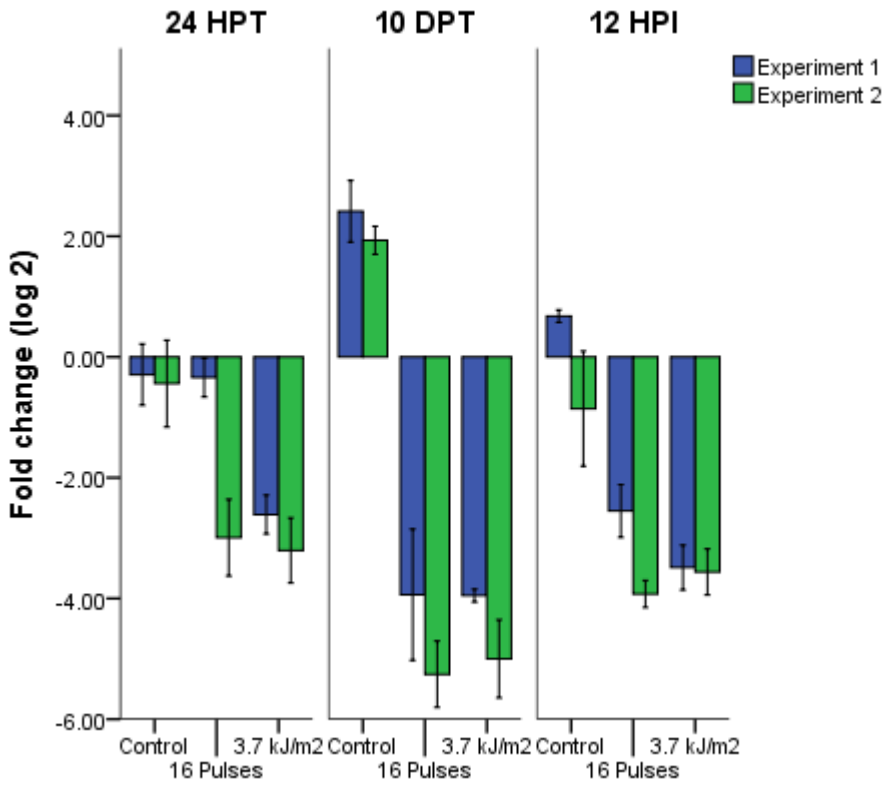

Figure A3.6: Data from the independent replicate experiments monitoring gene expression (qPCR) showing the relative expression of *FLS* (flavonol synthase) following treatment with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). N=3. Bars show ± 1S.E.M.

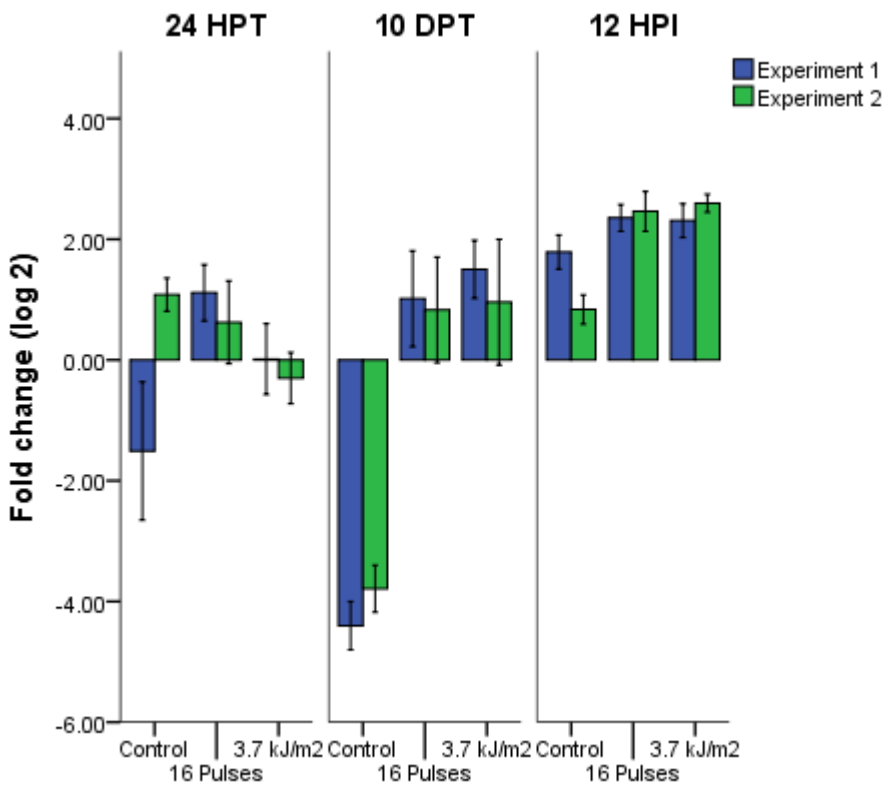

Figure A3.7: Data from the independent replicate experiments monitoring gene expression (qPCR) showing the relative expression of *GluB* (β-1,3,-Glucanase) an the ethylene inducible pathogenesis related protein transcript following treatment with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). N=3. Bars show ± 1S.E.M.

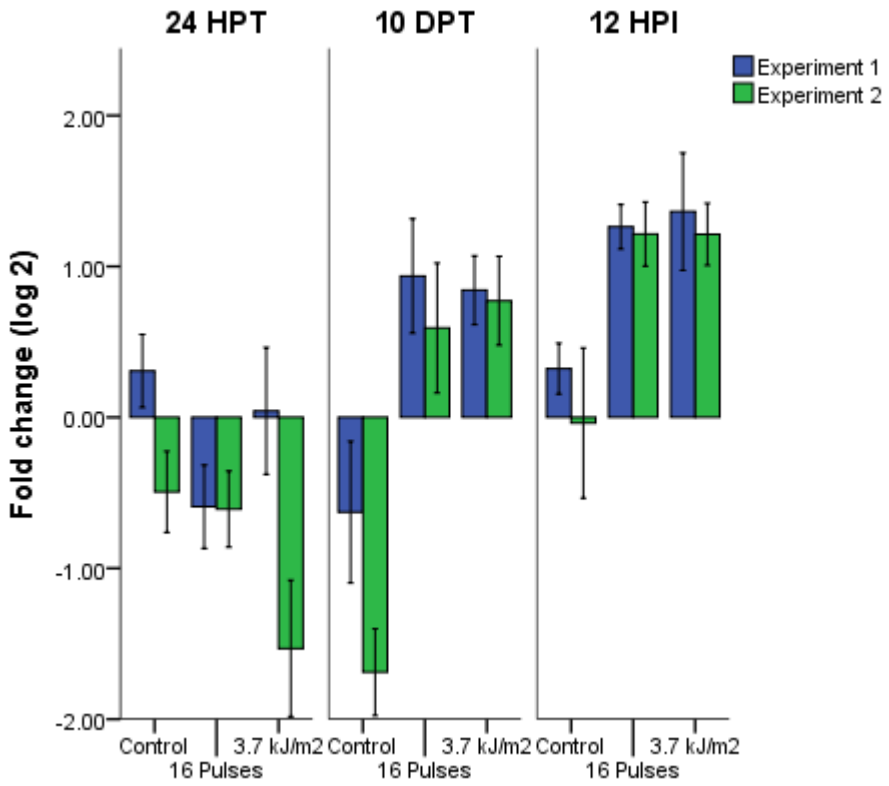

Figure A3.8: Data from the independent replicate experiments monitoring gene expression (qPCR) showing the relative expression of *OPR3* (12-Oxophytodienoate reductase 3) a jasmonate biosynthesis protein transcript following treatment with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). N=3. Bars show ± 1S.E.M.

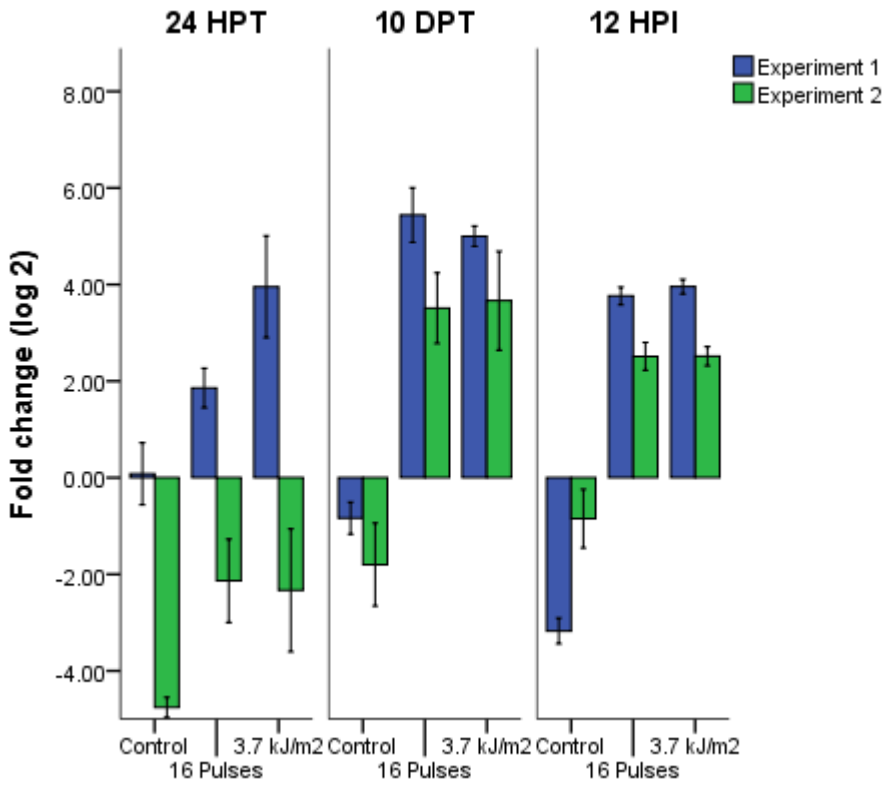

Figure A3.9: Data from the independent replicate experiments monitoring gene expression (qPCR) showing the relative expression of *P4* (*PR1a*) a salicylic acid inducible pathogenesis related protein and marker of systemic acquired resistance (SAR) following treatment with either 16 pulses from a high intensity pulsed polychromatic light source (HIPPL) or 3.7 kJ/m2 from a low intensity UV-C source (LIUV). Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). N=3 Bars show ± 1S.E.M.

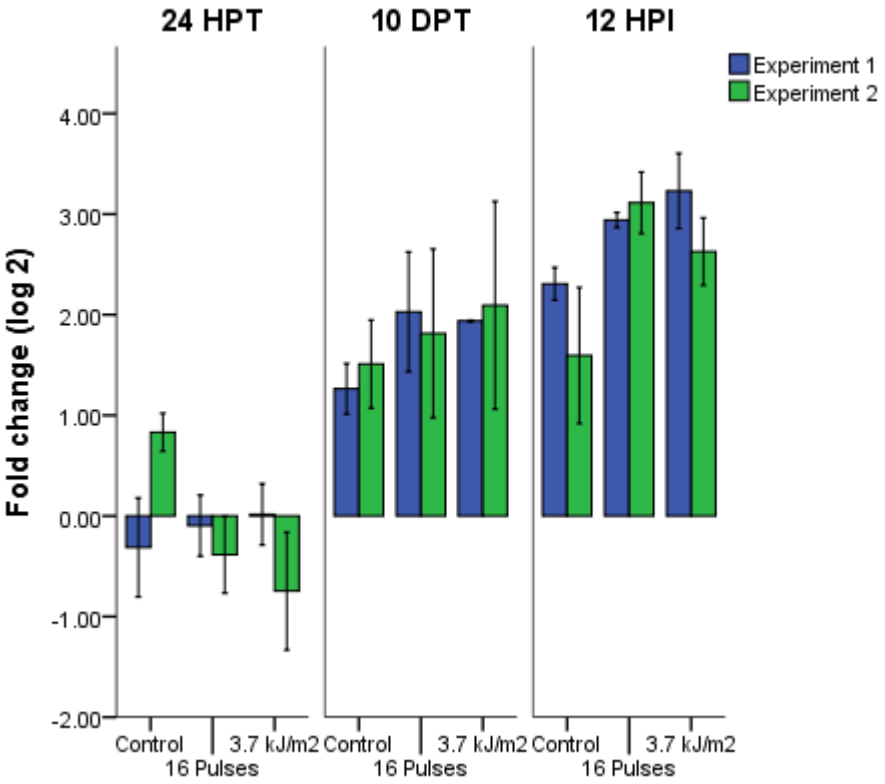

Figure A3.10: Data from the independent replicate experiments monitoring gene expression (qPCR) showing the relative expression of *PAL* (phenylalanine ammonia lyase) following treatment with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). N=3. Bars show ± 1S.E.M.

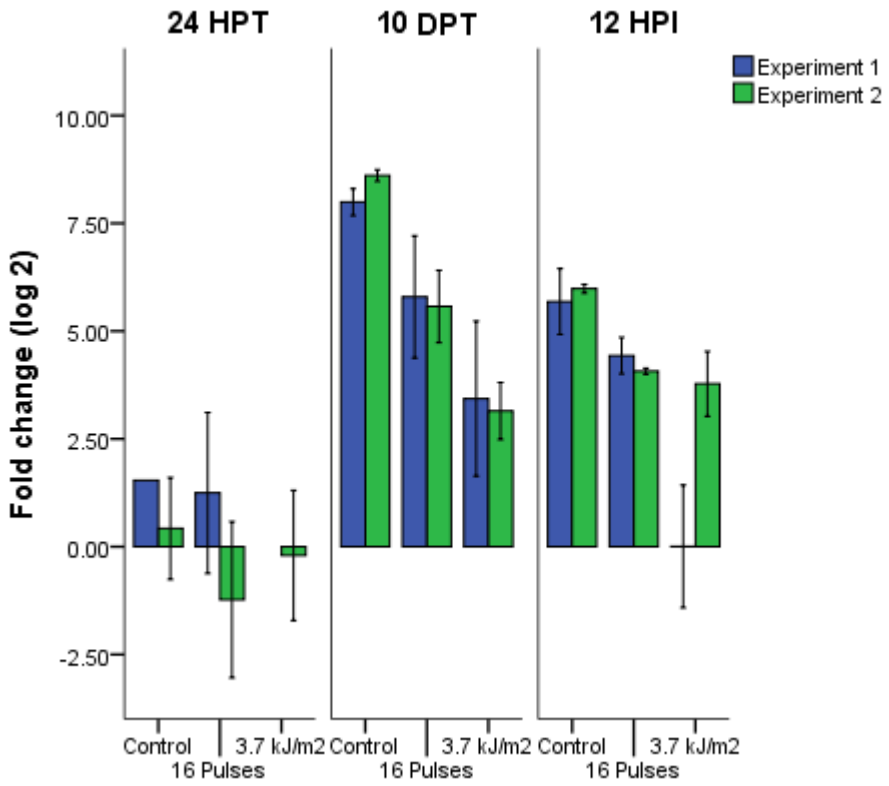

Figure A3.11 Data from the independent replicate experiments monitoring gene expression (qPCR) showing the relative expression of *PG* (polygalacturonase) following treatment with either 16 pulses from a high intensity pulsed polychromatic light (HIPPL) source or 3.7 kJ/m2 from a low intensity UV-C (LIUV) source. Samples were taken before treatment, 24-hours post treatment (HPT), 10-days post treatment (DPT), immediately before inoculation, and 12-hours post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). N=3. Bars show ± 1S.E.M.

Appendix 4

Appendix 4 gives the data from the independent replicate experiments in Chapter 5 "Assessing the importance of direct tissue exposure and fruit orientation during low-intensity UV-C and high-intensity, pulsed polychromatic light treatment". Mature green tomato fruit (cv. Mecano) were treated at differing orientations with low-intensity UV-C or high-intensity, pulsed polychromatic light (postharvest) to determine whether they stimulate a local or systemic response.

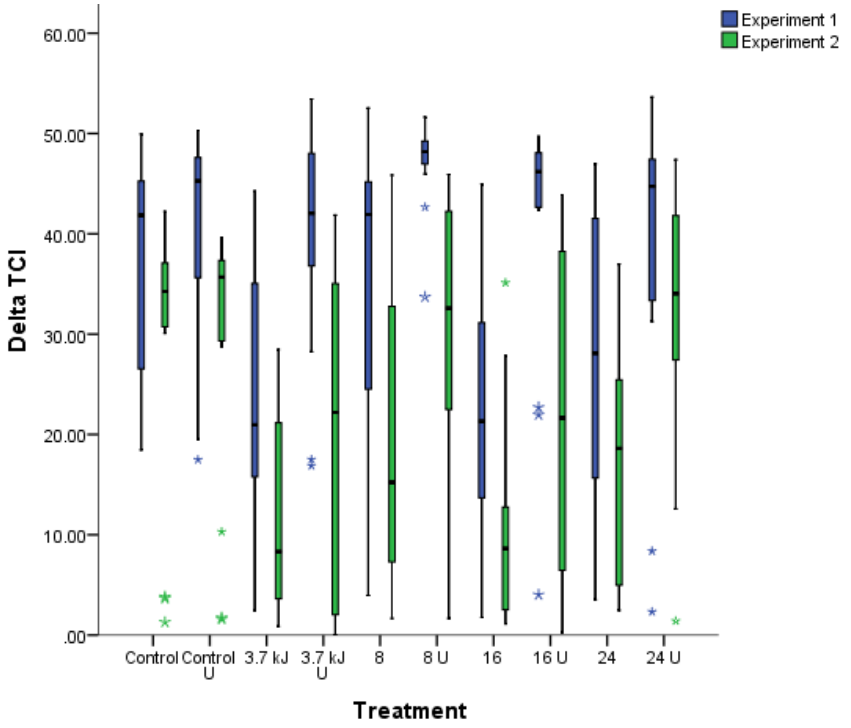

Figure A4.1: The change (delta) in tomato colour index over days 0-10 following treatment with either a low-intensity UV-C source or a high-intensity, pulsed polychromatic light source. Measurements were taken from tissue either directly facing the light sources or unexposed tissue (U) at 90 degrees from that facing the sources. Box plots show the distribution of data from the independent experimental replicates. Asterisks represent observed outliers. N= 10.

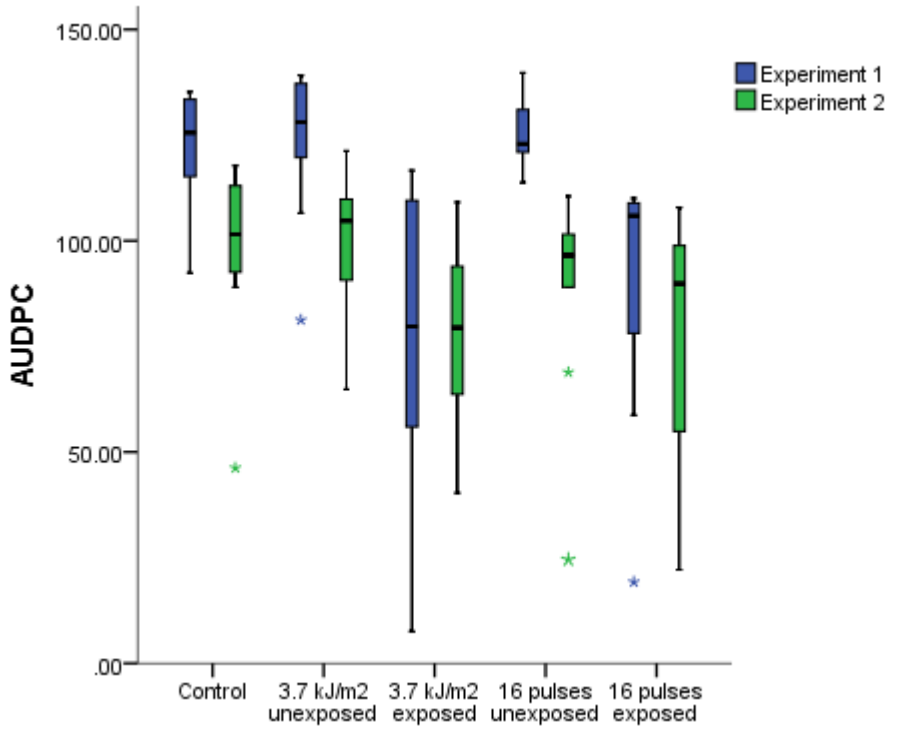

Figure A4.2. Area underneath the disease progression curve (AUDPC) of tomatoes, cv. Mecano, treated on a single side and inoculated with *B. cinerea* at 10 d post treatment (DPT). Fruit were treated with an established low intensity UV-C treatment of 3.7 kJ/m^2 and a high intensity pulsed polychromatic treatment of 16 pulses. Measurements were taken from either directly exposed or unexposed tissue, N=10. Box plots show the distribution of data from the independent experimental replicates. Asterisks represent observed outliers.

Appendix 5

Appendix 5 gives the data from the independent replicate experiments of Chapter 6 "Assessing the importance of UV-C, B and A and visible light within the high intensity pulsed polychromatic light source, for inducing the hormetic effects observed on tomato fruit". Tomato fruit (cv. Mecano) were treated (postharvest) with a high-intensity, pulsed polychromatic light source with the addition of a UV-C filter to determine whether UV-C radiation was essential for disease control and delayed ripening. Technical information on the glass filter can be found in Chapter 6 section 6.2.

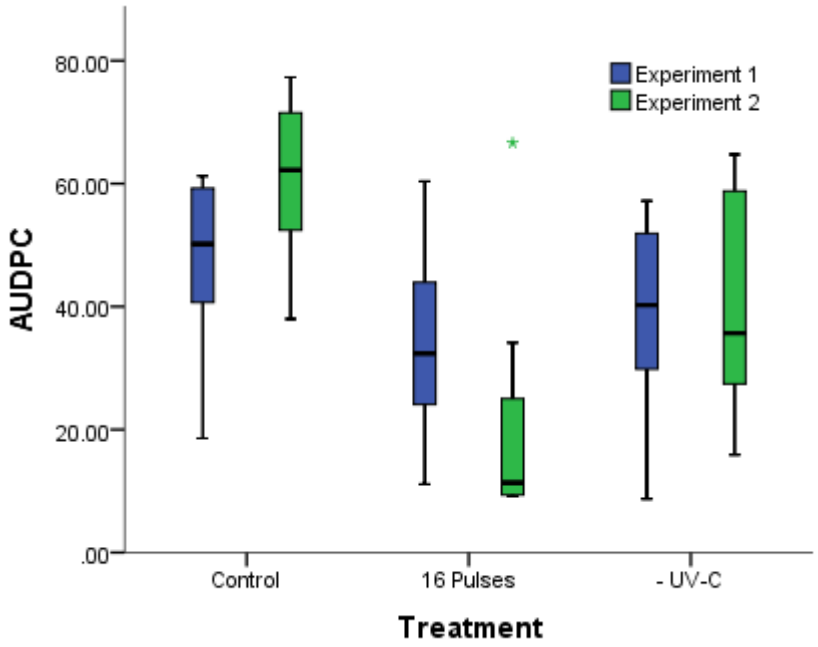

Figure A5.1: Area underneath the disease progression curve (AUDPC) of mature green tomato fruit (cv. Mecano) treated a high-intensity, pulsed polychromatic treatment of 16 pulses with or without UV-C filtering glass. N = 10. Box plots show the distribution of data from the independent experimental replicates. Asterisks represent observed outliers.

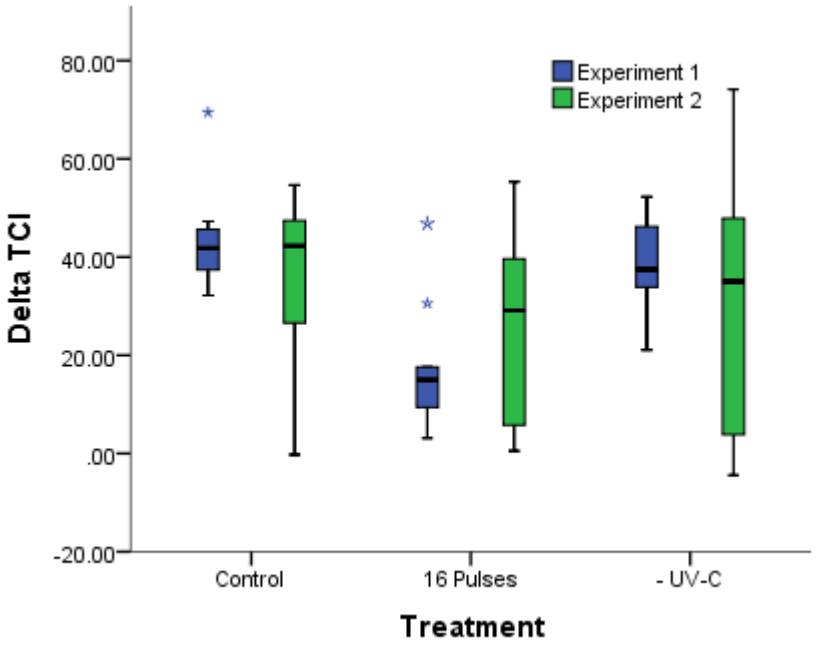

Figure A5.2: The change (delta) in tomato colour index of mature green tomato fruit (cv. Mecano) over 0 – 10 days following treatment with a high-intensity, pulsed polychromatic light source without (16 pulses) or with UV-C filtering glass in place (- UV-C). Box plots show the distribution of data from the independent experimental replicates. Asterisks represent observed outliers. N=10.

Appendix 6

Appendix 6 contains the full experimental data from Chapter 7 "Investigation into LIUV and HIPPL treatments for the control of disease through pre-harvest foliar treatments of lettuce in the glasshouse environment". Chapter 7 was concerned with elucidating whether low-intensity UV-C and high-intensity, pulsed polychromatic light could be used as an alternative to chemical control of preharvest pathogens on glasshouse grown, soilless lettuce plants. The following graphs provide the complete experimental data which is summarised in Tables 7.1, 7.2, 7.3 and 7.4. Each of the experiments are provided as both bar charts and box plots which express the mean values and distribution of the data, respectively.

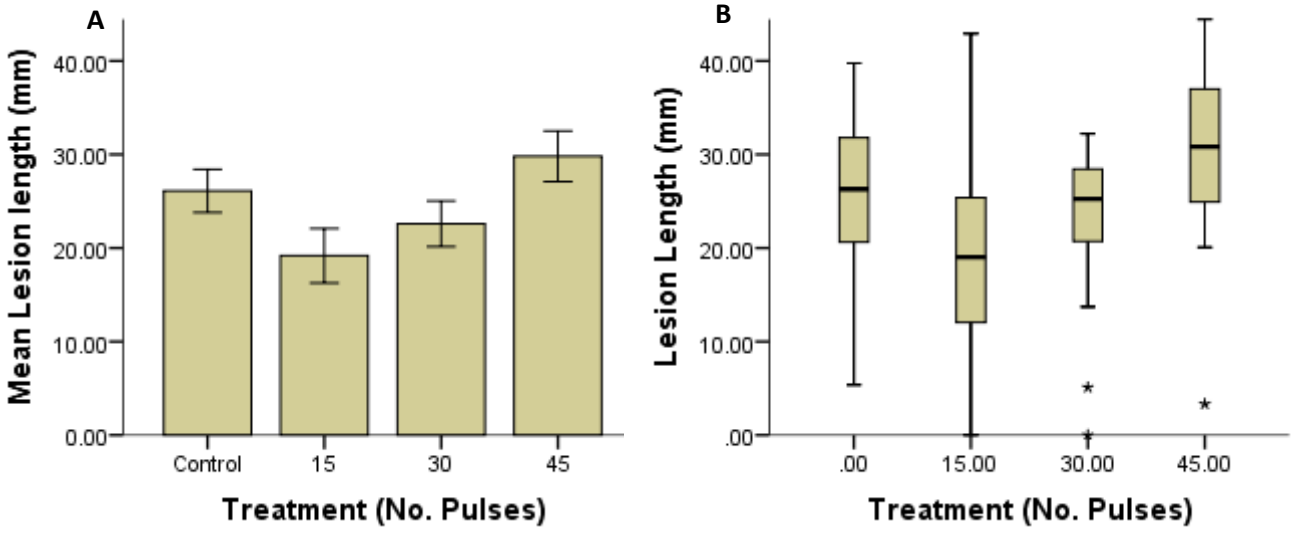

Figure A6.1: Disease control assays performed in April 2015 (14.04.2015) on the butterhead lettuce variety Amica. Plants were treated at early head formation and inoculated 9 days after treatment with 4mm agar plugs from 5 day old *B.cinerea* cultures. Lesion lengths were measured at 3 days after inoculation with Vernier callipers. (**A**) Shows the mean lesion length and ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 5.

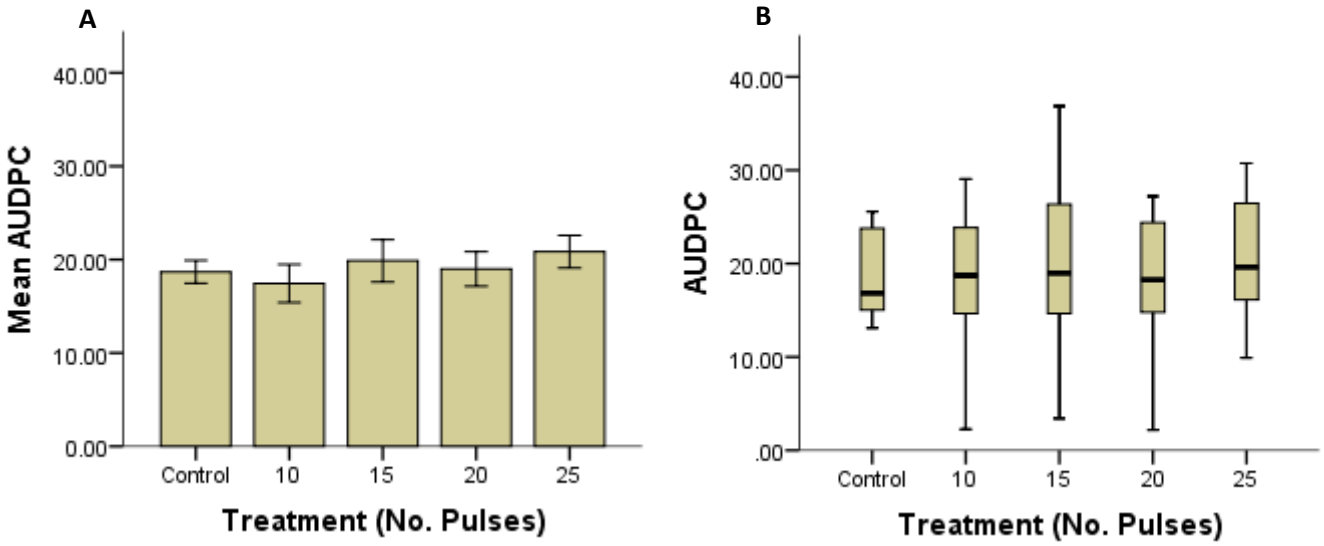

Figure A6.2: Disease control assays performed in May 2015 (05.05.2015) on the butterhead lettuce variety Temira. Plants were treated at early head formation and inoculated with *B. cinerea* 6 days after treatment with 10 µl of $1x10^6$ spores/ml amended with 50 % potato dextrose broth. Lesion lengths 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.

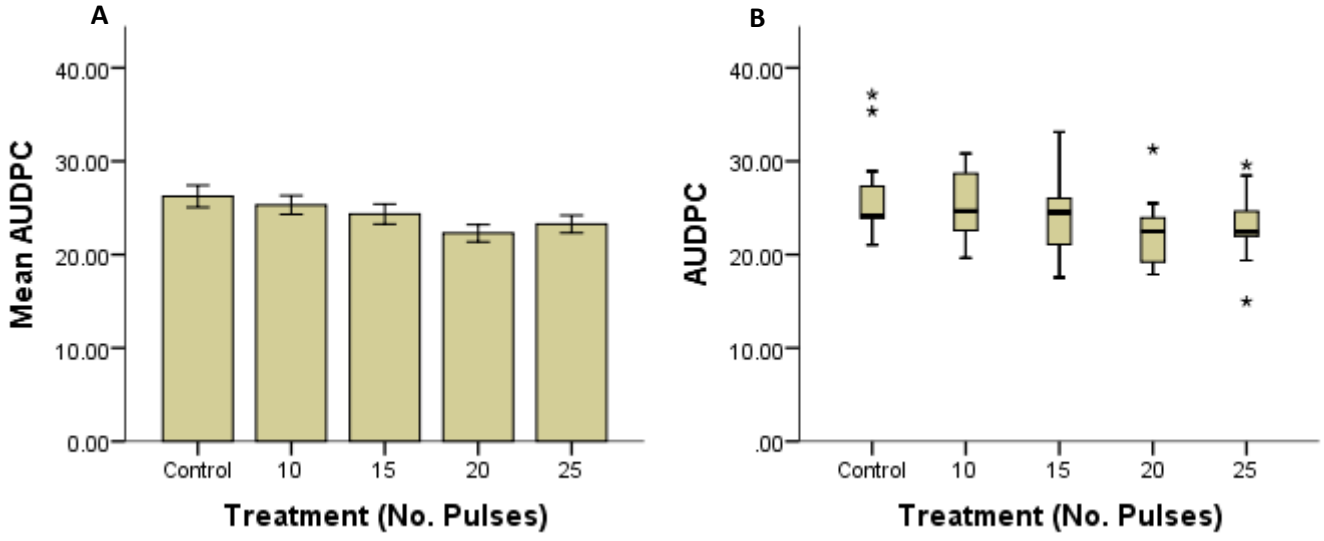

Figure A6.3: Disease control assays performed in May 2015 (05.05.2015) on the butterhead lettuce variety Amica. Plants were treated at early head formation and inoculated with *B. cinerea* 6 days after treatment with 10 µl of $1x10^6$ spores/ml amended with 50 % potato dextrose broth. Lesion lengths were measured at 2 and 3 days after inoculation with Vernier callipers to allow calculation of AUDPC. (**A**) Shows the mean lesion length ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 5.

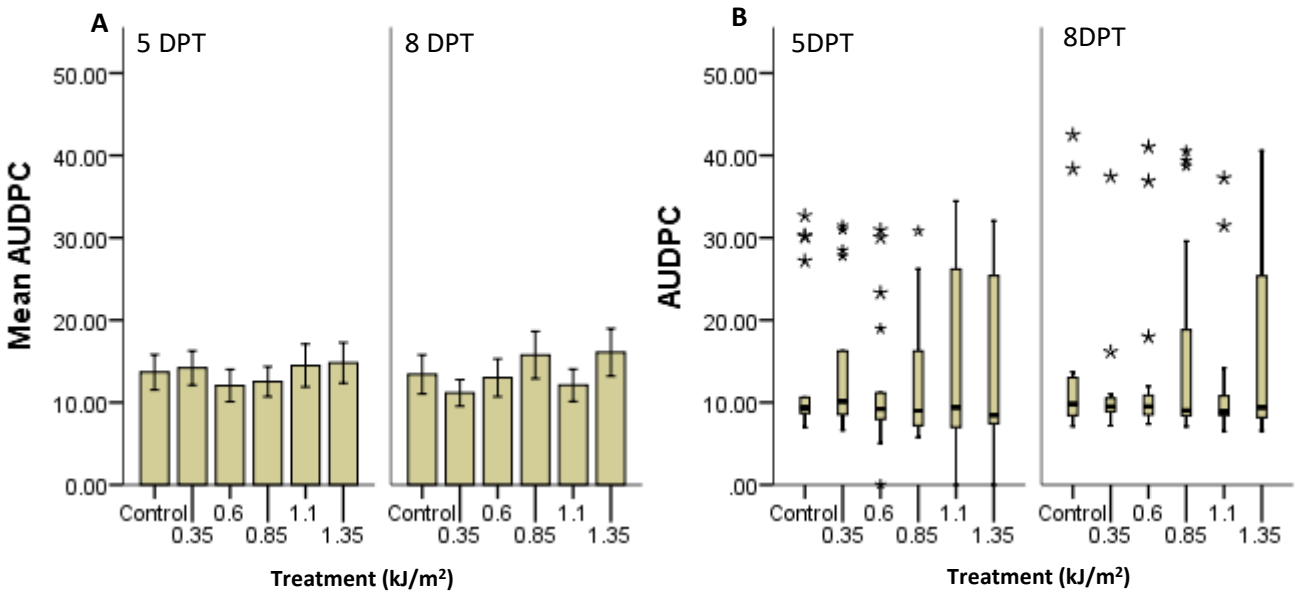

Figure A6.4: Disease control assays performed in September 2015 (10.09.2015) on the butterhead lettuce variety Amica treated with the conventional UV-C source. Plants were treated at 3-5 true leaves and inoculated 5 and 8 days with 10 µl of 1x10^6 spores/ml of *B. cinerea* amended with 50 % potato dextrose 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.

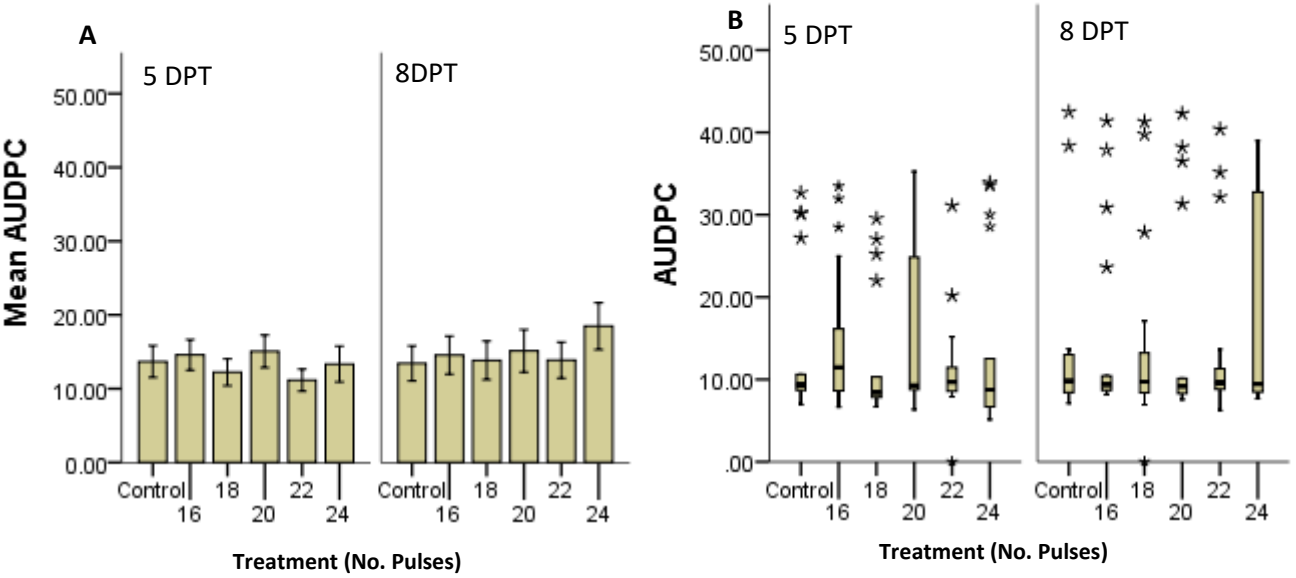

Figure A6.5: Disease control assays performed in September 2015 (10.09.2015) on the butterhead lettuce variety Amica treated with the high intensity pulsed polychromatic source. Plants were treated at 3-5 true leaves and inoculated 5 and 8 days after treatment with 10 µl of 1x10^6 spores/ml of *B. cinerea* 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.

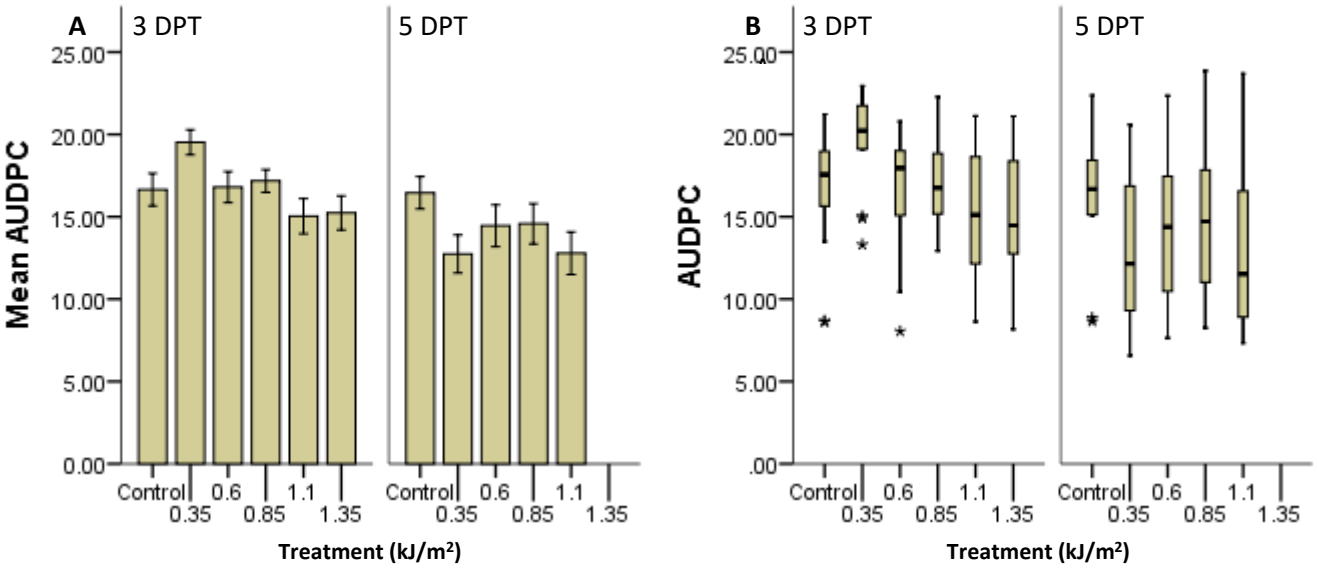

Figure A6.6: Disease control assays performed in September 2015 (29.09.2015) on the butterhead lettuce variety Temira treated with the conventional UV-C source. Plants were treated at 3-5 true leaves and inoculated 3 and 5 days after treatment with 10 µl of $1x10^6$ spores/ml of *B. cinerea* amended 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.

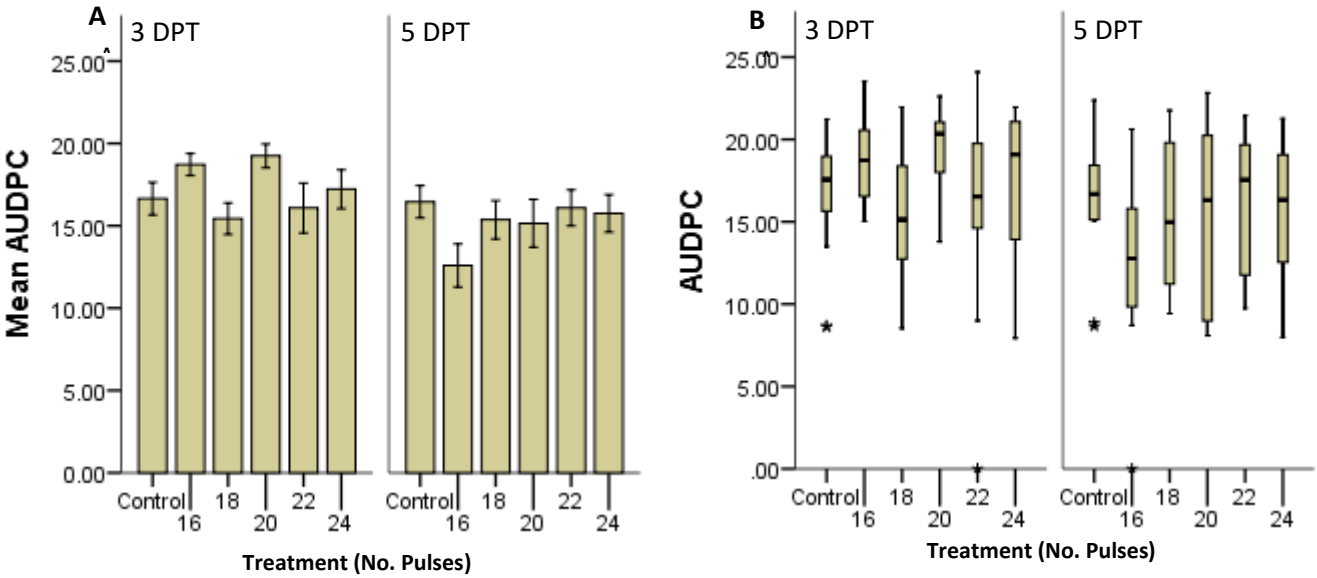

Figure A6.7: Disease control assays performed in September 2015 (29.09.2015) on the butterhead lettuce variety Temira treated with the high intensity pulsed polychromatic source. Plants were treated at 3-5 true leaves and inoculated 3 and 5 days after treatment with 10 µl of $1x10^6$ spores/ml of *B. cinerea* amended with 50 % potato dextrose broth. Lesion lengths were measured at 2 and 3 days after inoculation with Vernier callipers to allow calculation of AUDPC. (**A**) Shows the mean lesion length ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 6.

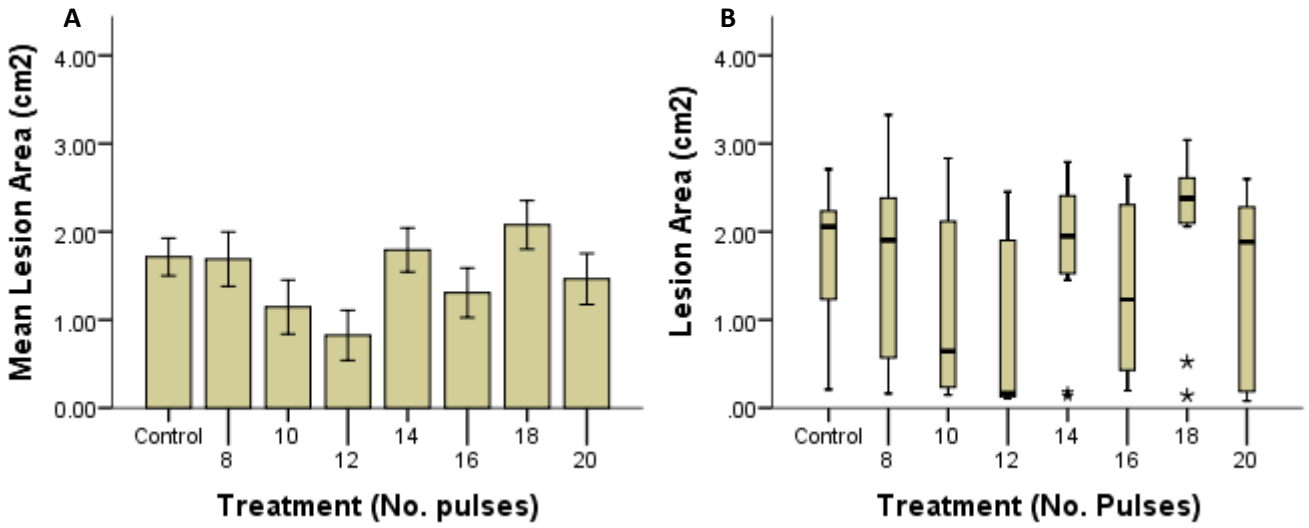

Figure A6.8: Disease control assays performed in October 2015 (28.10.2015) on the butterhead lettuce variety Temira treated with the high intensity pulsed polychromatic source. Plants were treated at late head formation and inoculated 2 days after treatment with 10 µl of 1x10^6 spores/ml of *B. cinerea* 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.

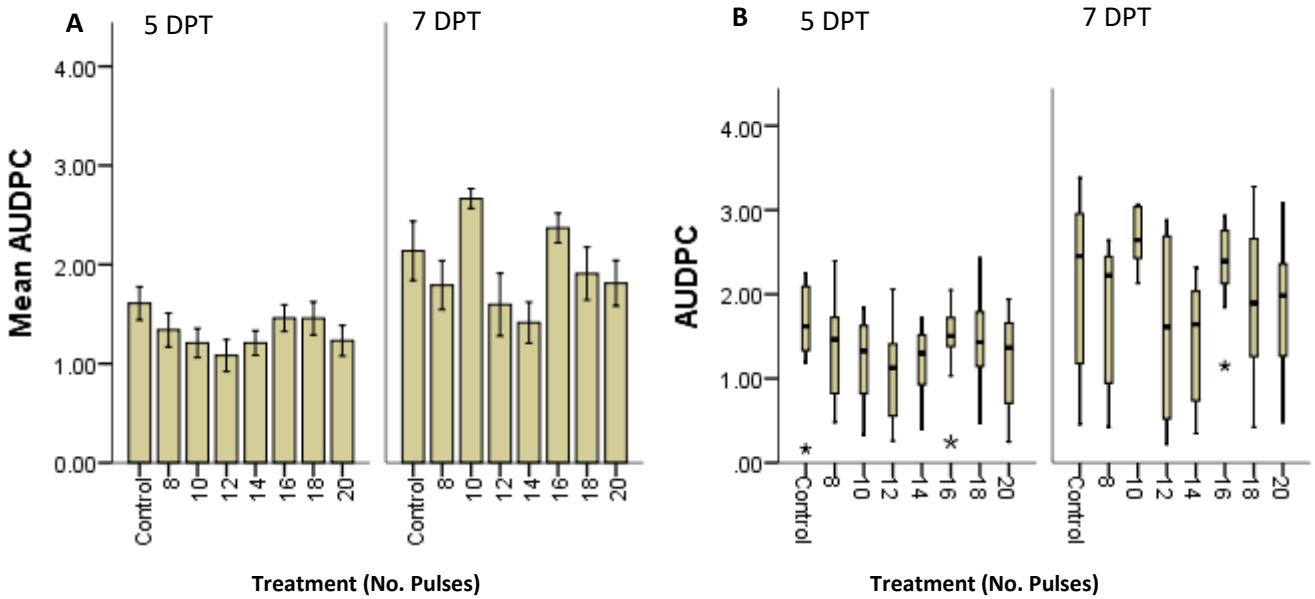

Figure A6.9: Disease control assays performed in October (28.10.2015) on the butterhead lettuce variety Temira treated with the high intensity pulsed polychromatic source. Plants were treated at late head formation and inoculated 5 and 7 days after treatment with 10 µl of 1x10^6 spores/ml of *B. cinerea* amended with 50 % potato dextrose broth Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. (**A**) Shows the mean lesion length ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 5.

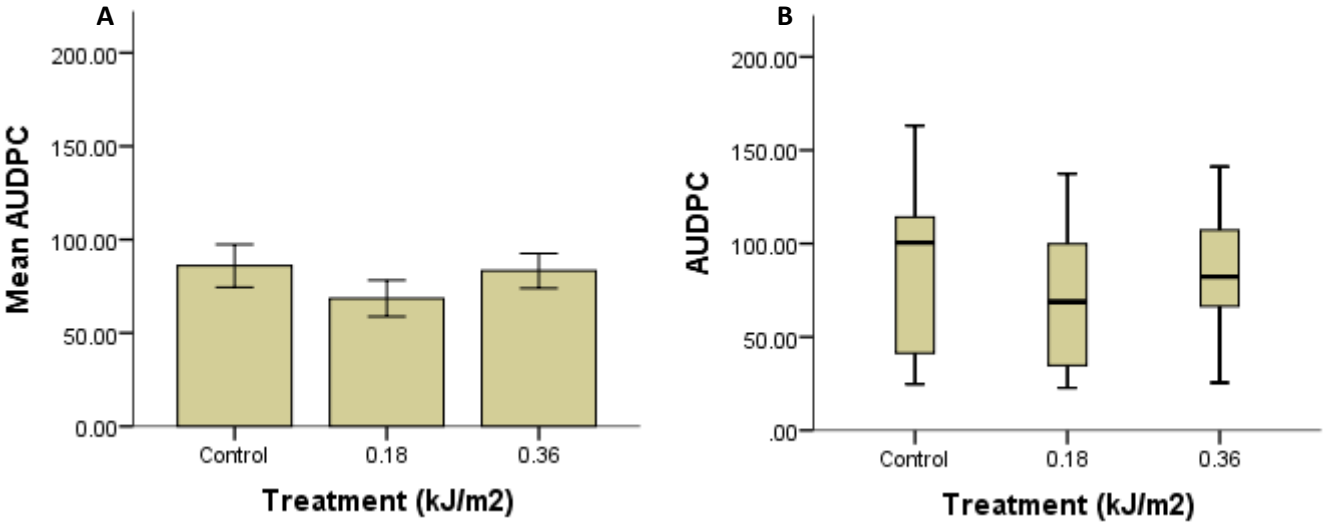

Figure A6.10: Disease control assays performed in November 2015 (18.11.2015) on the butterhead lettuce variety Amica treated with the conventional UV-C source. Plants were inoculated 2 days after treatment with 10 µl of $1x10^6$ spores/ml of *B. cinerea* amended with 50 % potato dextrose broth. Lesion 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.

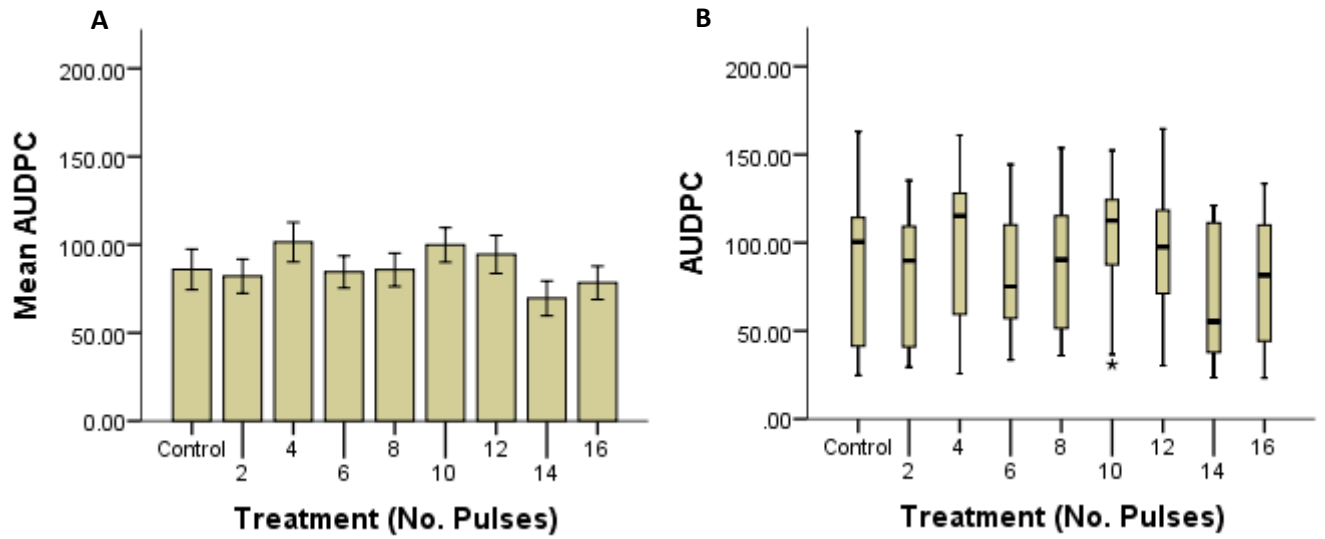

Figure A6.11: Disease control assays performed in November 2015 (18.11.2015) on the butterhead lettuce variety Amica treated with the pulsed polychromatic source. Plants were treated at 3-5 true leaves and inoculated 2 days after treatment with 10 µl of $1x10^6$ spores/ml of *B. cinerea* amended with 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.

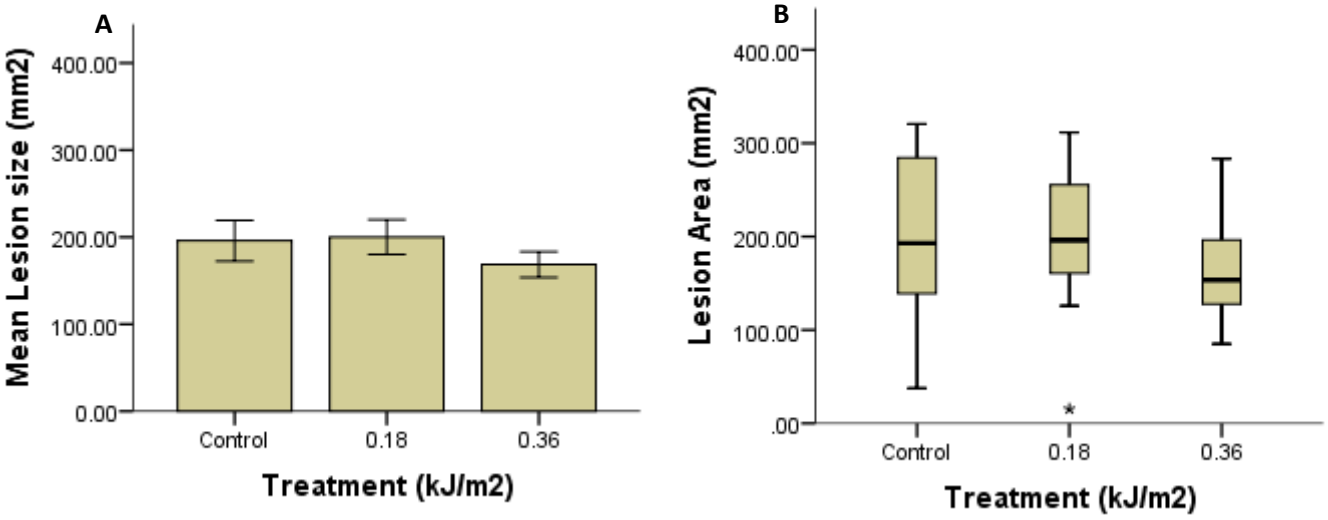

Figure A6.12: Disease control assays performed in November 2015 (18.11.2015) on the butterhead lettuce variety Amica treated with the conventional UV-C source. Plants were treated at 3-5 true leaves and inoculated 2 days after treatment with 4mm agar plugs from 3 day old *Rhizoctonia solani* cultures. 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.

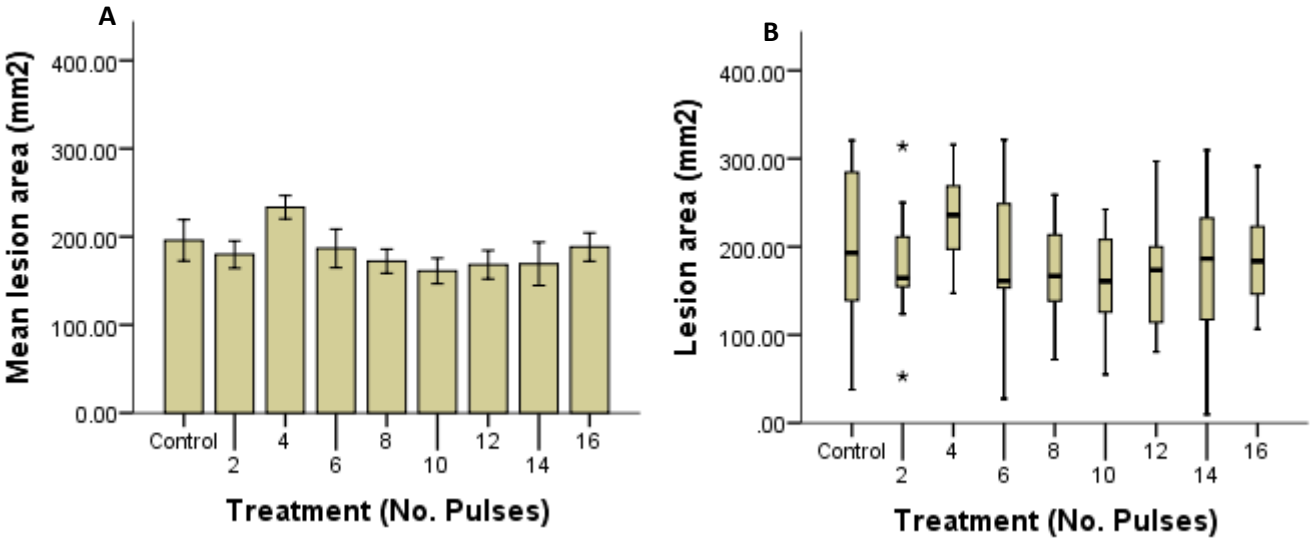

Figure A6.13: Disease control assays performed in November 2015 (18.11.2015) on the butterhead lettuce variety Amica treated with pulsed polychromatic source. Plants were treated at 3-5 true leaves and inoculated 2 days after treatment with 4mm agar plugs from 3 day old *Rhizoctonia solani* cultures. Lesion areas were measured at 3 days after inoculation with ImageJ. (**A**) Shows the mean lesion length ± 1 standard error of the mean. (**B**) Shows the distribution of the lesion length data. Asterisks represent observed outliers. N= 5.

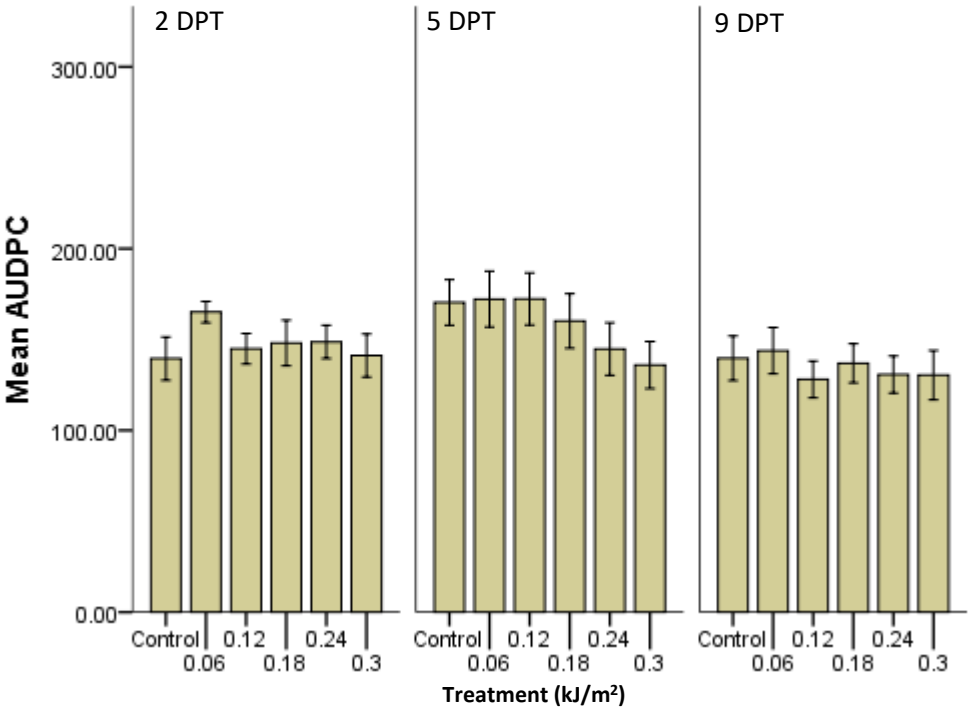

Figure A6.14: Disease control assays performed in December 2015 (09.12.2015) on the butterhead lettuce variety Amica treated with the conventional UV-C source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 10 µl of 1x10^6 spores/ml of *B. cinerea* amended 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.

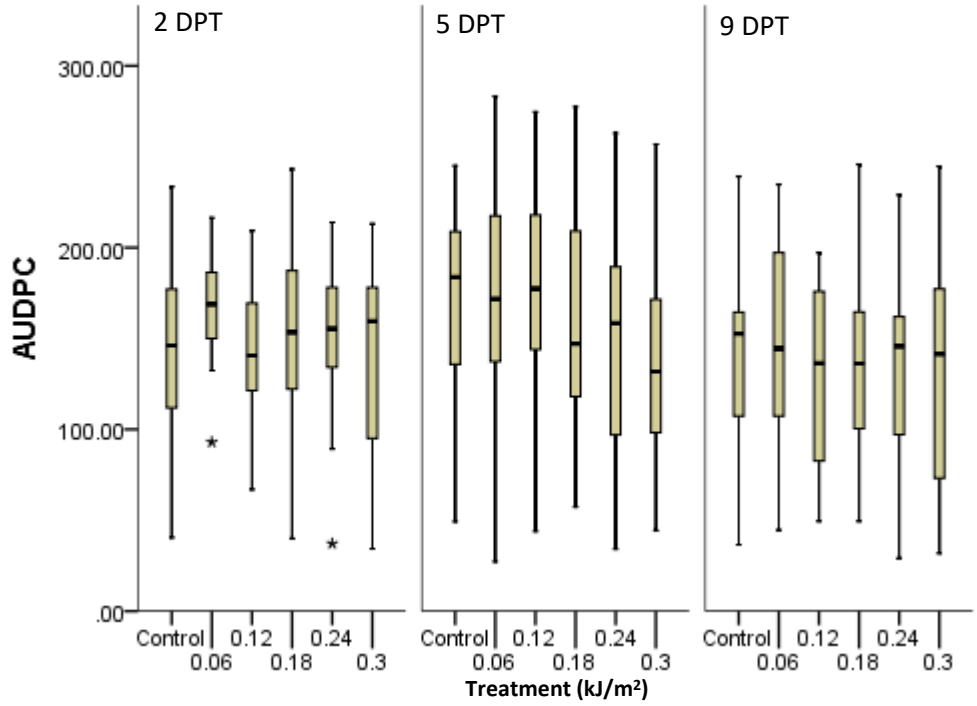

Figure A6.15: Disease control assays performed in December 2015 (09.12.2015) on the butterhead lettuce variety Amica treated with the conventional UV-C source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 10 µl of 1x10^6 spores/ml of *B. cinerea* amended 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.

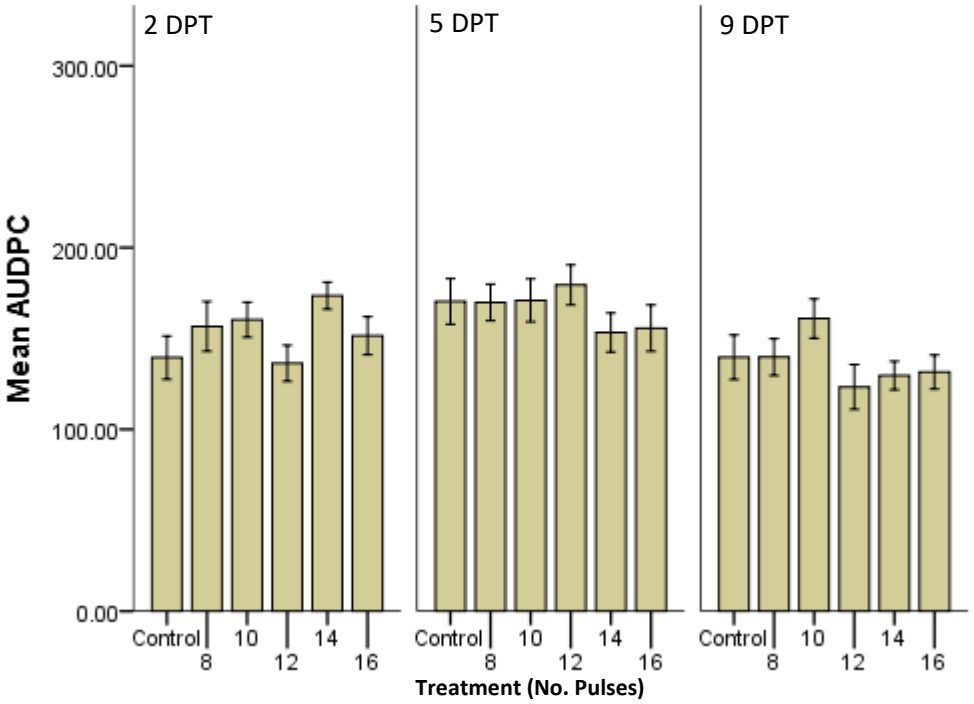

Figure A6.16: Disease control assays performed in December 2015 (09.12.2015) on the butterhead lettuce variety Amica treated with the pulsed polychromatic source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 10 µl of $1x10^6$ spores/ml of *B. cinerea* amended 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.

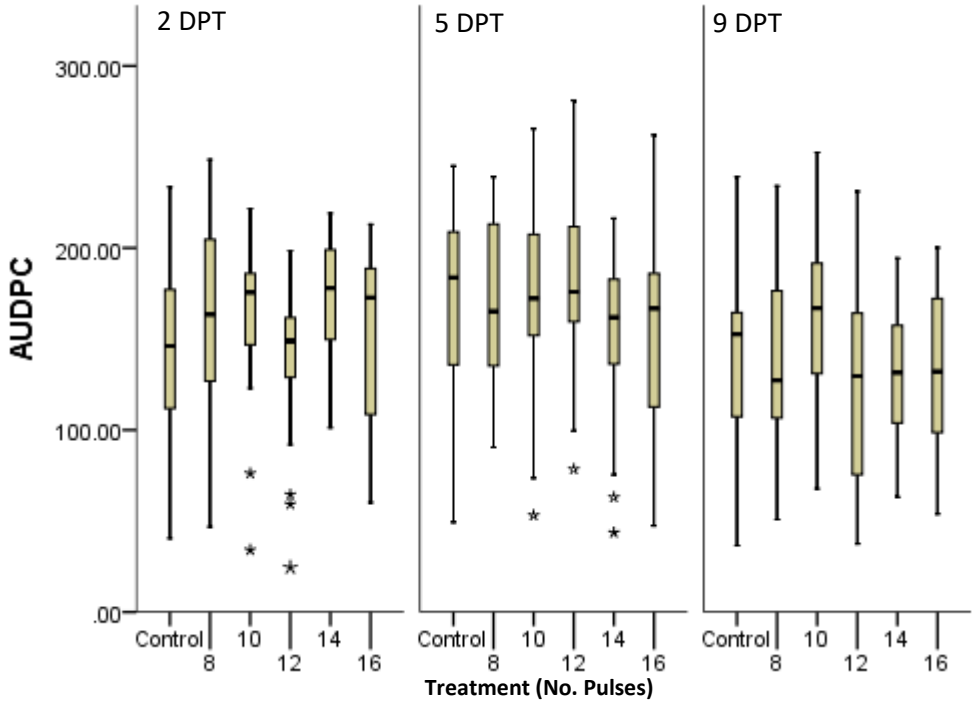

Figure A6.17: Disease control assays performed in December 2015 (09.12.2015) on the butterhead lettuce variety Amica treated with the pulsed polychromatic source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 10 µl of $1x10^6$ spores/ml of *B. cinerea* amended 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.

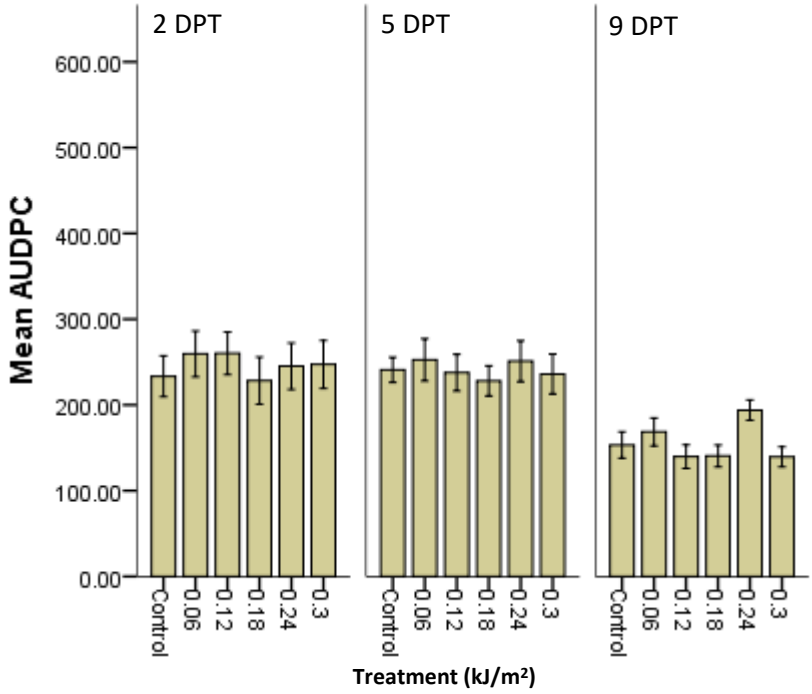

Figure A6.18: Disease control assays performed in December 2015 (09.12.2015) on the butterhead lettuce variety Amica treated with the conventional UV-C source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 4mm agar plugs of *R. solani*. Lesion areas were 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.

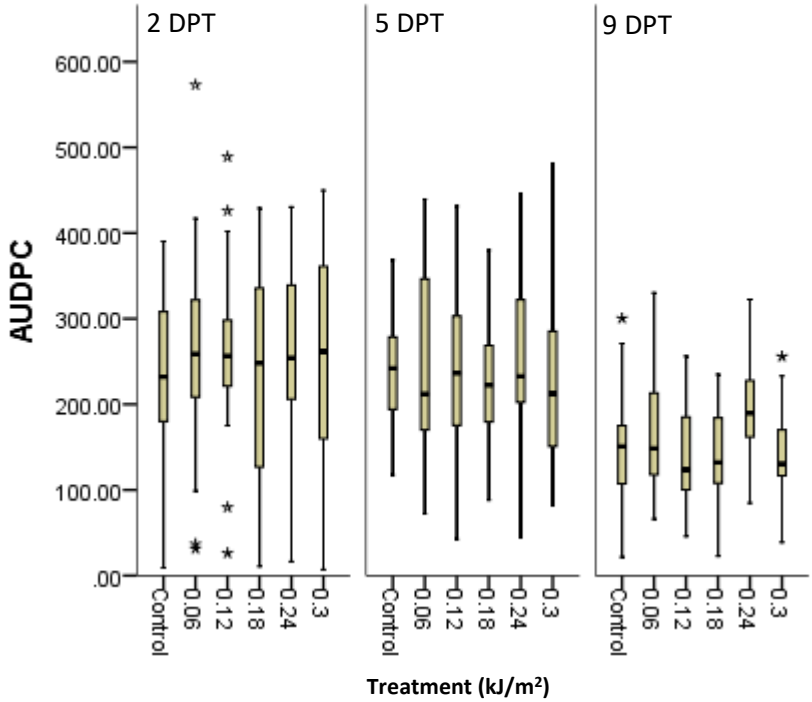

Figure A6.19: Disease control assays performed in December 2015 (09.12.2015) on the butterhead lettuce variety Amica treated with the conventional UV-C source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 4mm agar plugs of *R. solani*. Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. Asterisks represent observed outliers. N= 7

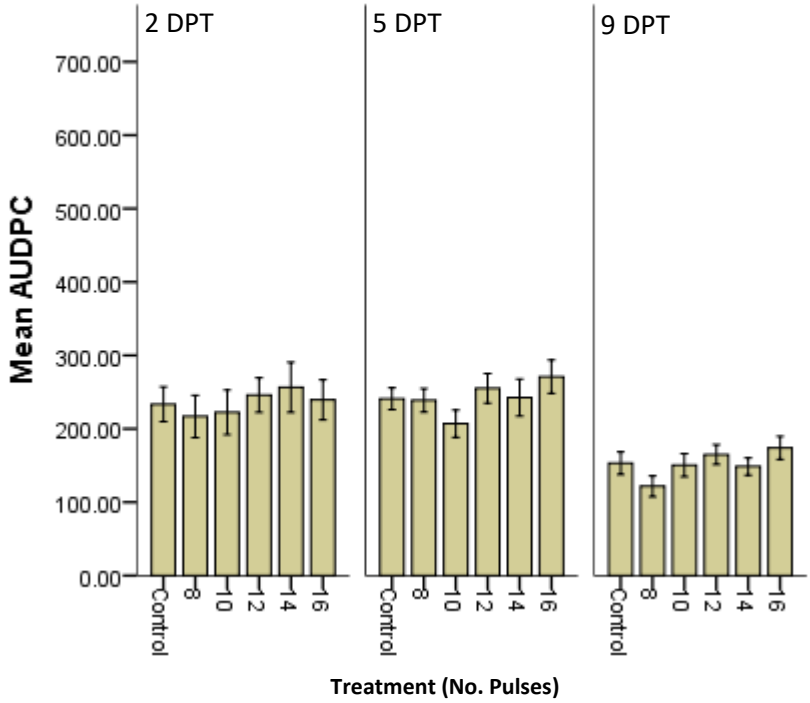

Figure A6.20: Disease control assays performed in December 2015 (09.12.2015) on the butterhead lettuce variety Amica treated with the pulsed polychromatic source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 4mm agar plugs of *R. solani*. Lesion areas were 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.

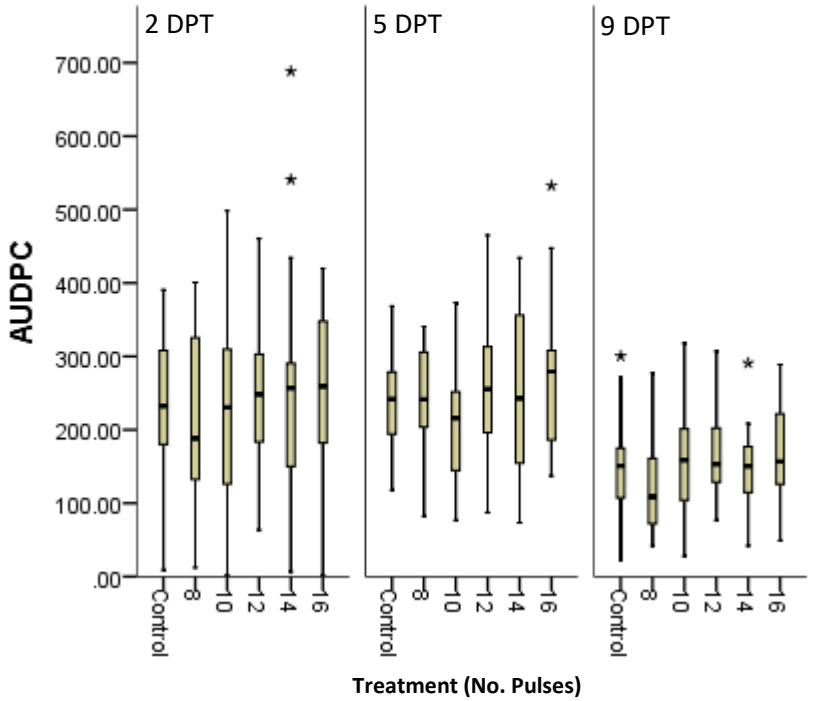

Figure A6.21: Disease control assays performed in December 2015 (09.12.2015) on the butterhead lettuce variety Amica treated with the pulsed polychromatic source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 4mm agar plugs of *R. solani*. Lesion areas were measured at 2 and 3 days after inoculation with ImageJ to allow calculation of AUDPC. Asterisks represent observed outliers. N= 7.

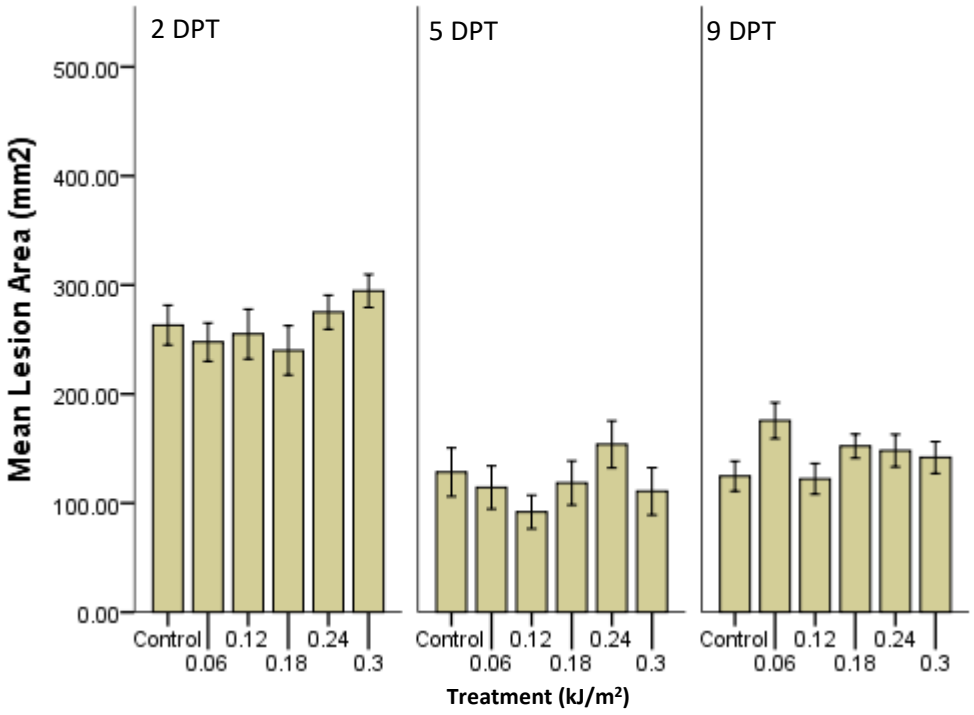

Figure A6.22: Disease control assays performed in December 2015 (09.12.2015) on the butterhead lettuce variety Amica treated with the conventional UV-C source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 4mm agar plugs of *S. sclerotiorum*. Lesion areas were measured at 2 days after inoculation with ImageJ. Bars shows the mean lesion length ± 1 standard error of the mean. N= 7.

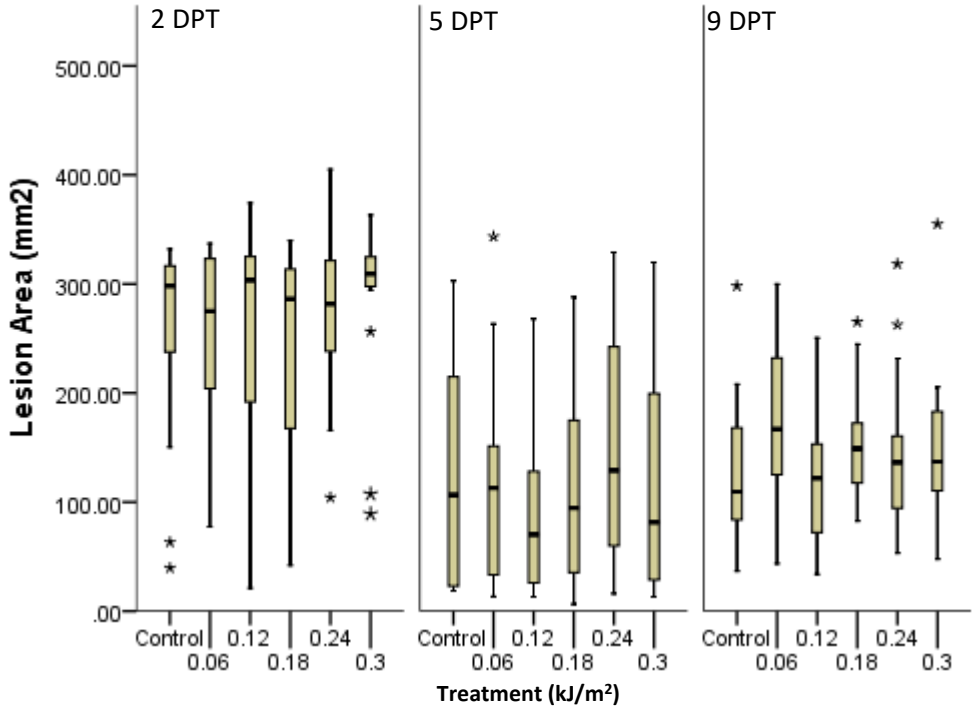

Figure A6.23: Disease control assays performed in December 2015 (09.12.2015) on the butterhead lettuce variety Amica treated with the conventional UV-C source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 4mm agar plugs of *S. sclerotiorum*. Lesion areas were measured at 2 days after inoculation with ImageJ. Asterisks represent observed outliers. N= 7

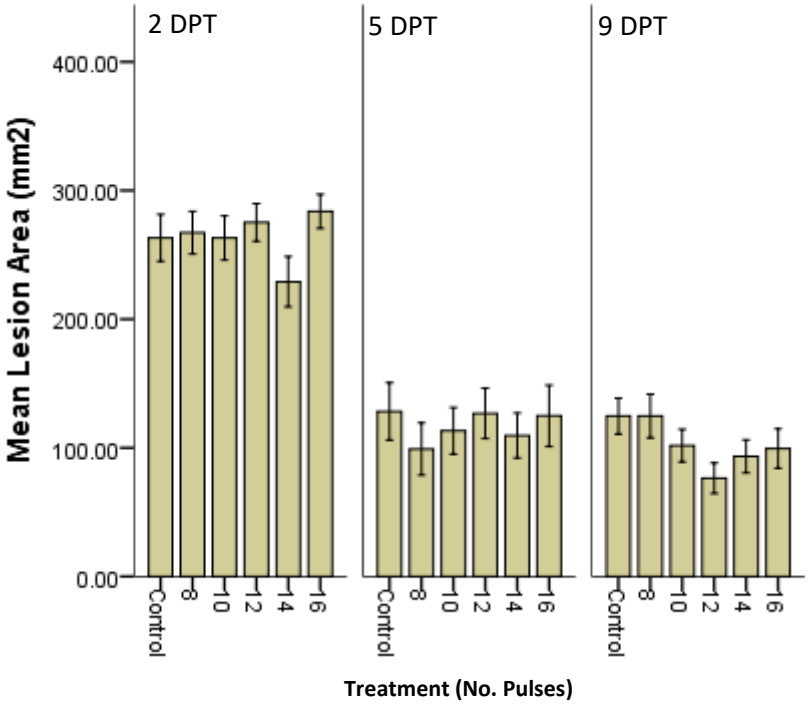

Figure A6.24: Disease control assays performed in December 2015 (09.12.2015) on the butterhead lettuce variety Amica treated with the pulsed polychromatic source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 4mm agar plugs of *S. sclerotiorum*. Lesion areas were measured at 2 days after inoculation with ImageJ. Bars shows the mean lesion length ± 1 standard error of the mean. N= 7.

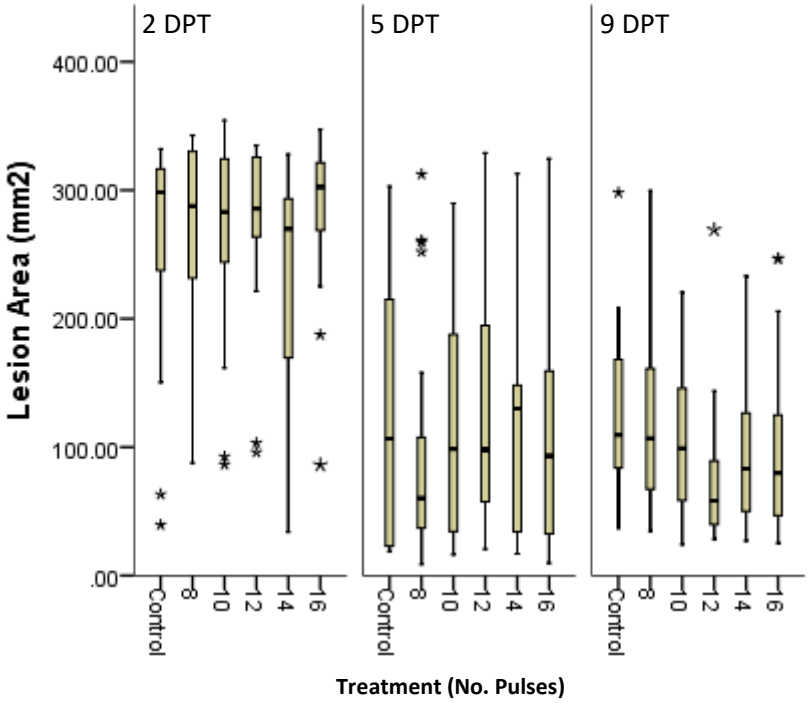

Figure A6.25: Disease control assays performed in December 2015 (09.12.2015) on the butterhead lettuce variety Amica treated with the pulsed polychromatic source. Plants were treated at 8-10 true leaves and inoculated 2, 5 and 9 days after treatment with 4mm agar plugs of *S. sclerotiorum*. Lesion areas were measured at 2 days after inoculation with ImageJ. Asterisks represent observed outliers. N= 7.

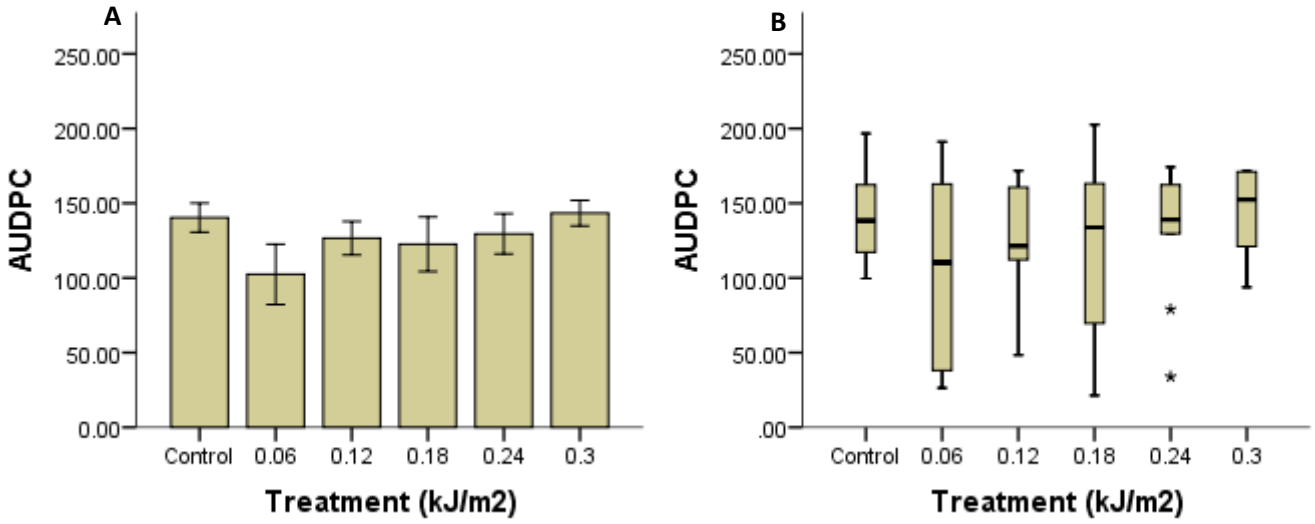

Figure A6.26: Disease control assays performed in February 2016 (12.02.2016) on the butterhead lettuce variety Amica treated with the conventional UV-C source. Plants were treated at 6-8 true leaves and inoculated 2 days after treatment with 10 µl of $1x10^6$ spores/ml of *B. cinerea* amended with 50 % 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.

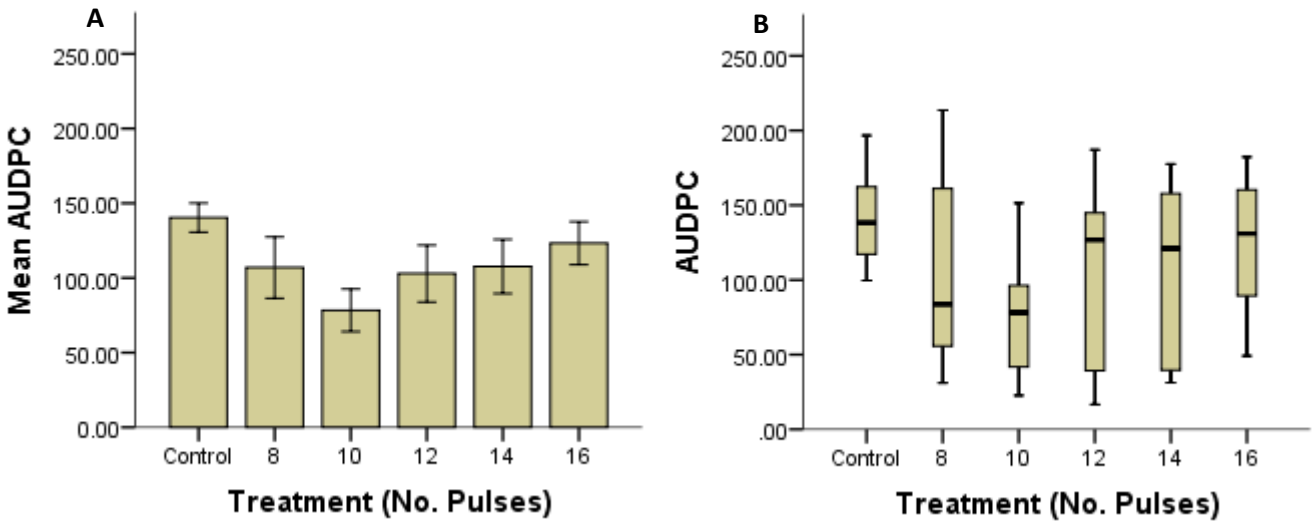

Figure A6.27: Disease control assays performed in February 2016 (12.02.2016) on the butterhead variety Amica treated with the conventional UV-C source. Plants were treated at 6-8 true leaves and inoculated 2 days after treatment with 10 µl of $1x10^6$ spores/ml of *B. cinerea* amended with 50 % potato 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.

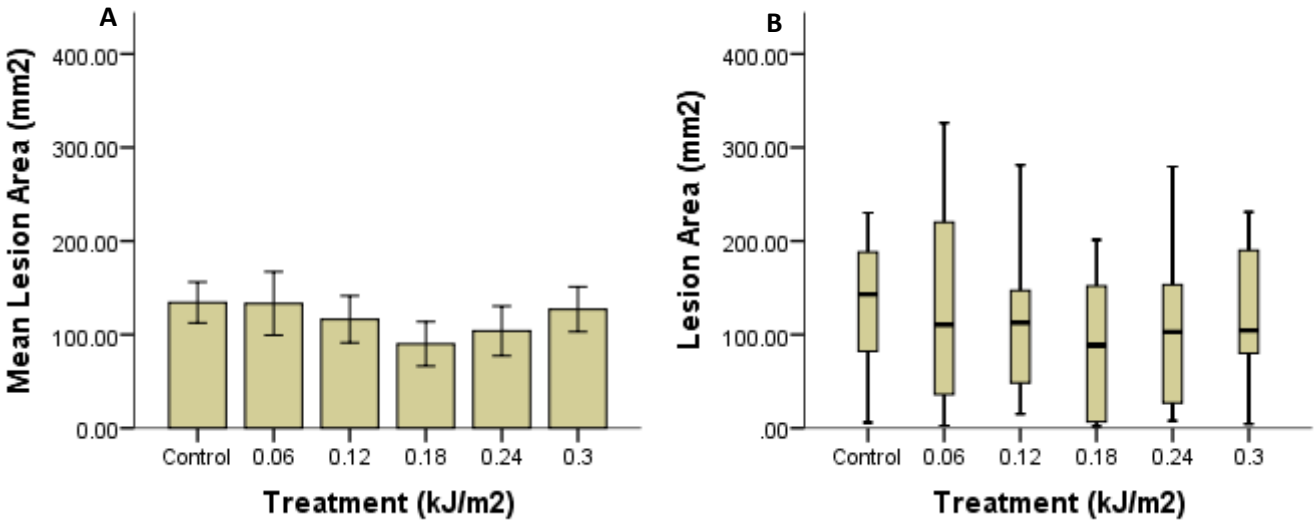

Figure A6.28: Disease control assays performed in February 2016 (12.02.2016) on the butterhead variety Amica treated with the conventional UV-C source. Plants were treated at 6-8 true leaves and inoculated 2 days after treatment with 4 mm agar plugs of *R. solani*. Lesion areas were measured at 2 and 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.

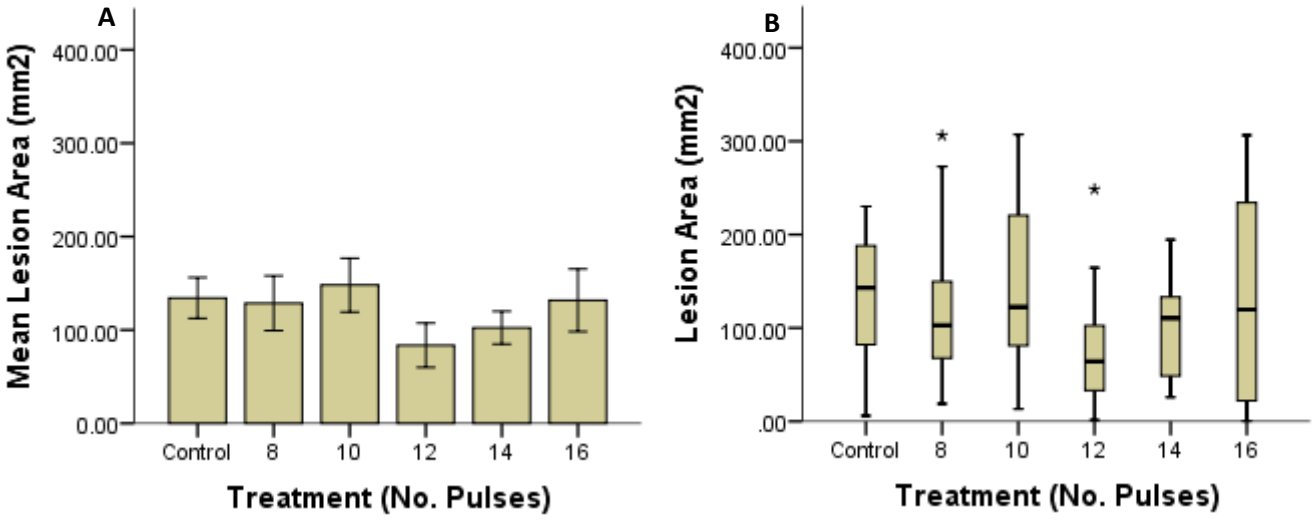

Figure A6.29: Disease control assays performed in February 2016 (12.02.2016) on the butterhead lettuce variety Amica treated with the pulsed polychromatic source. Plants were treated at 6-8 true leaves and inoculated 2 days after treatment with 4 mm agar plugs of *R. solani*. Lesion areas were measured 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.

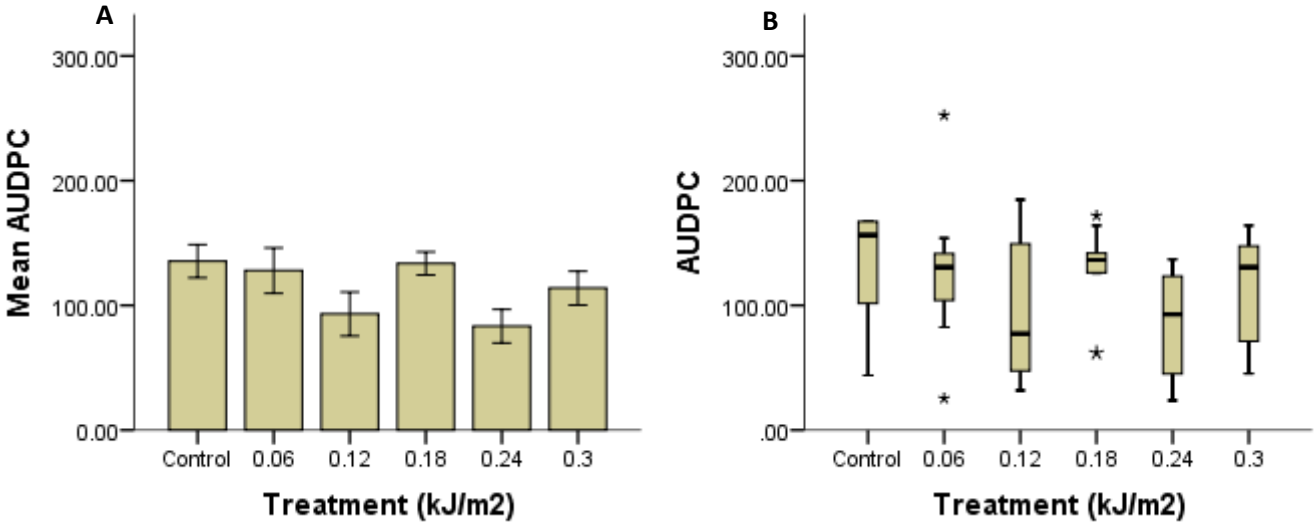

Figure A6.30: Disease control assays performed in February 2016 (12.02.2016) on the butterhead lettuce variety Temira treated with the conventional UV-C source. Plants were treated at 6-8 true leaves and inoculated 2 days after treatment with 10 µl of $1x10^6$ spores/ml of *B. cinerea* amended with 50 % 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.

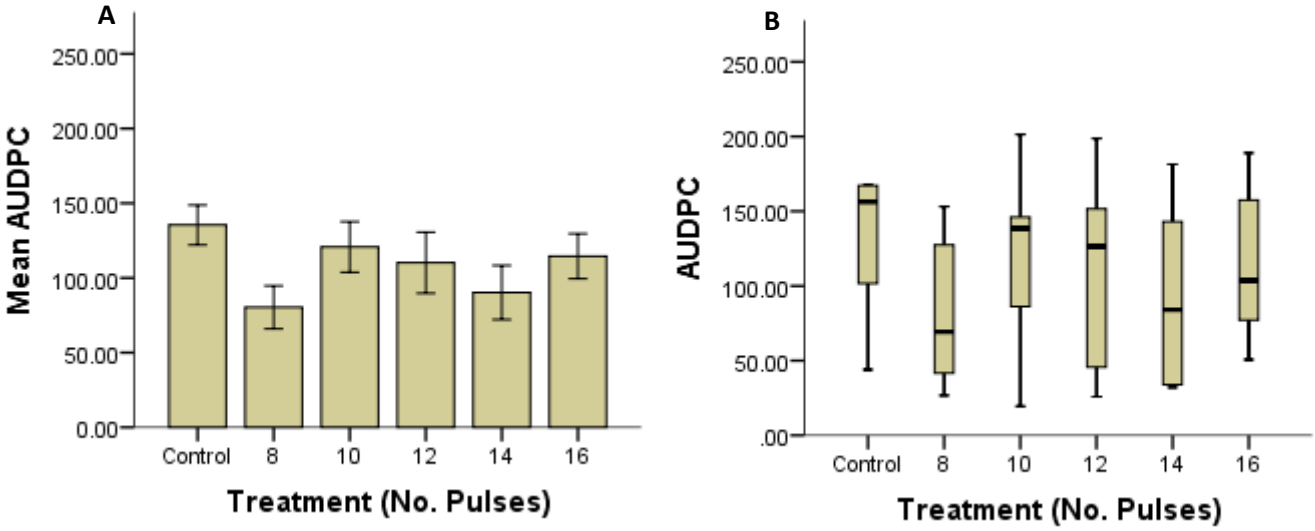

Figure A6.31: Disease control assays performed in February 2016 (12.02.2016) on the butterhead lettuce variety Temira treated with the conventional UV-C source. Plants were treated at 6-8 true leaves and inoculated 2 days after treatment with 10 µl of $1x10^6$ spores/ml of *B. cinerea* amended with 50 % 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.

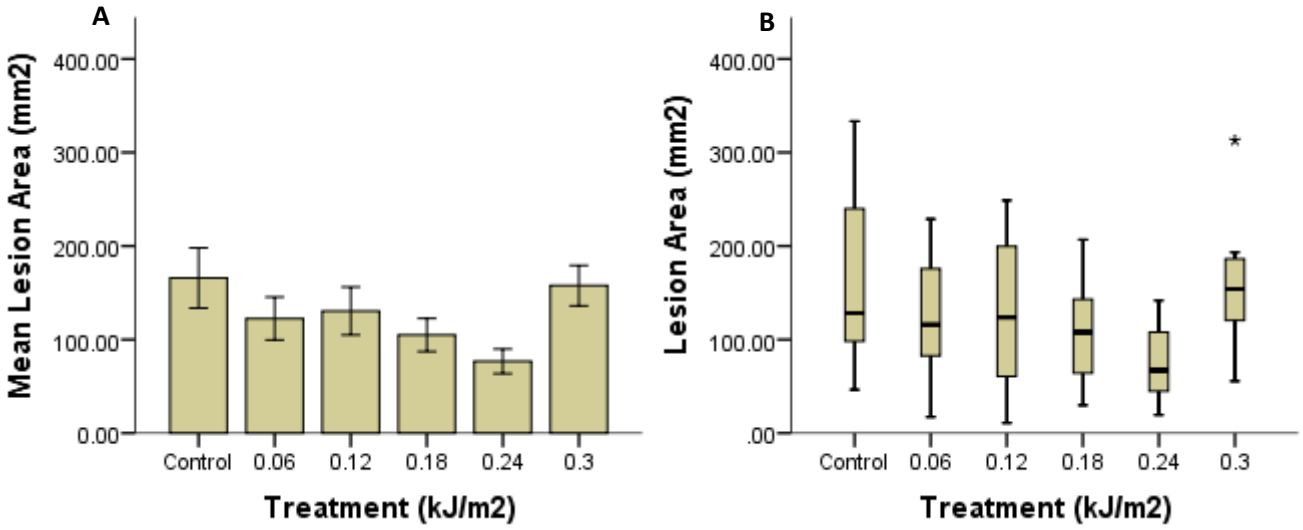

Figure A6.32: Disease control assays performed in February 2016 (12.02.2016) on the butterhead lettuce variety Temira treated with the conventional UV-C source. Plants were treated at 6-8 true leaves and inoculated 2 days after treatment with 4 mm agar plugs of *R. solani*. Lesion areas were measured 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= 7.

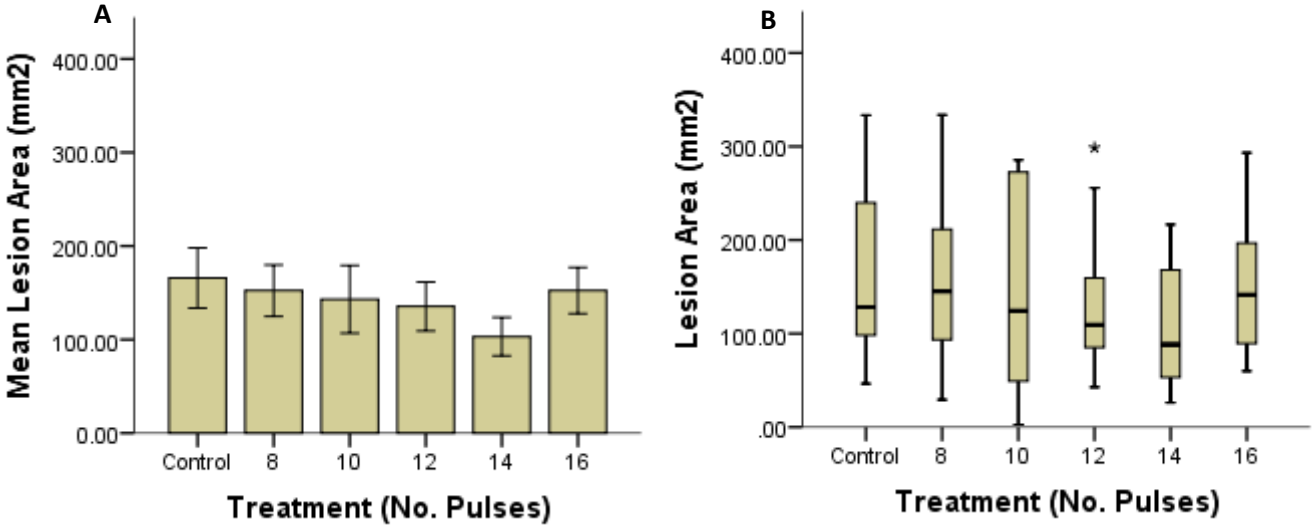

Figure A6.33: Disease control assays performed in February 2016 (12.02.2016) on the butterhead lettuce Temira treated with the pulsed polychromatic source. Plants were treated at 6-8 true leaves and inoculated 2 days after treatment with 4 mm agar plugs of *R. solani*. Lesion areas were measured at 2 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.

Appendix 7

Appendix 7 contains the data from the independent experimental replicates in Chapter 8 "Multiple low-dose preharvest LIUV and HIPPL treatments for the control of disease on lettuce in the Glasshouse". Here, we are assessing whether low-dose treatments that were not damaging at any point of the year, may provide disease control when applied on either single or multiple occasions.

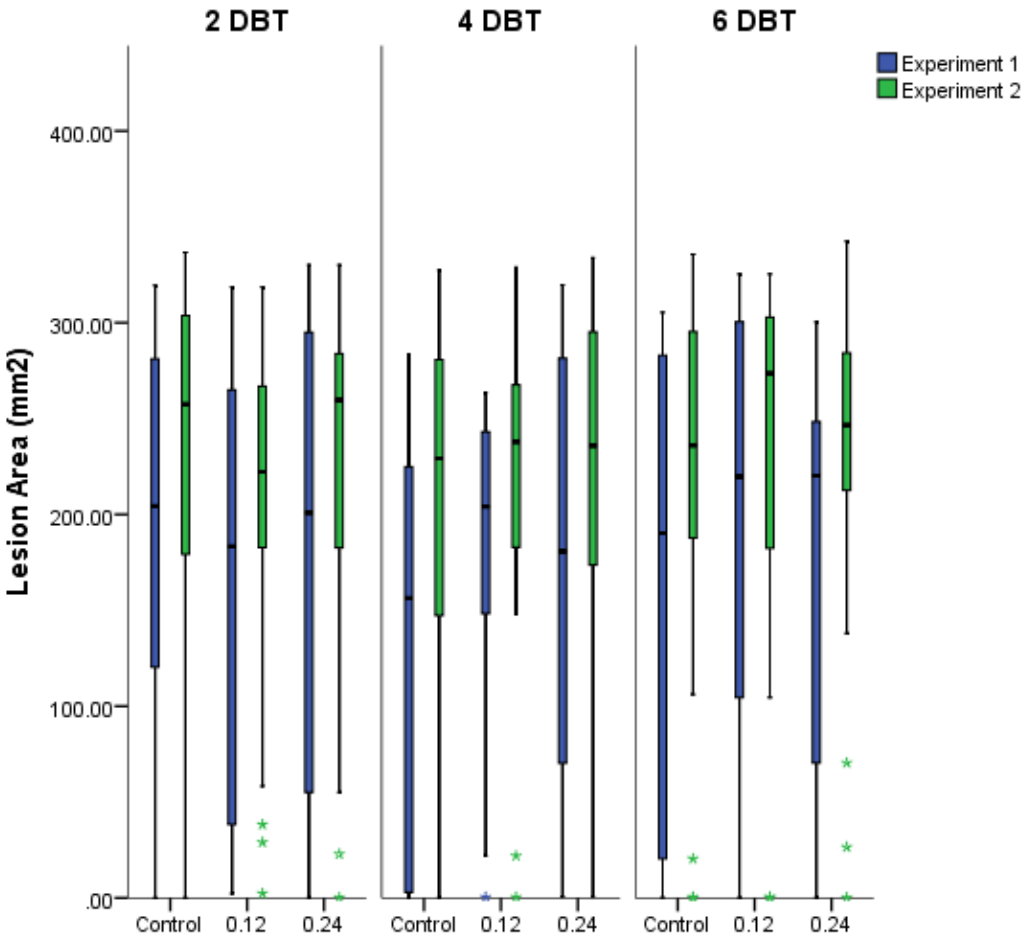

Figure A7.1: The mean lesion area from lettuce (cv. Amica) treated with multiple low-dose, low-intensity UV-C treatments (kJ/m^2) at the 6-8 true leaf stage, followed by inoculation with *R. solani.* Treatments were applied with either 2, 4 or 6 days between treatments (DBT). N=5 and 7 for experimental replicates 1 and 2, respectively. Box plots show the distribution of data from the independent experimental replicates. Asterisks represent observed outliers.

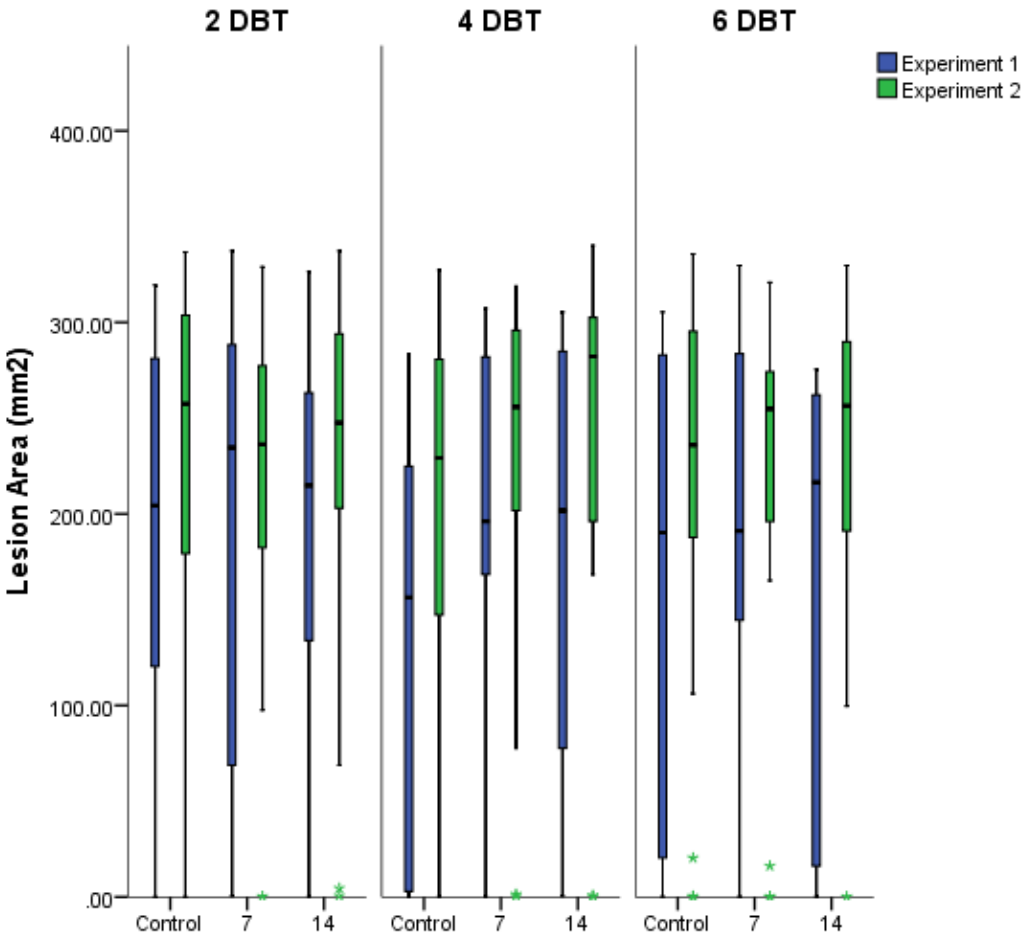

Figure A7.2: The mean lesion area from lettuce (cv. Amica) treated with multiple low-dose, high-intensity, pulsed polychromatic treatments (No. pulses) at the 6-8 true leaf stage, followed by inoculation with *R. solani.* Treatments were applied with either 2, 4 or 6 days between treatments (DBT). N=5 and 7 for experimental replicates 1 and 2, respectively. Box plots show the distribution of data from the independent experimental replicates. Asterisks represent observed outliers.

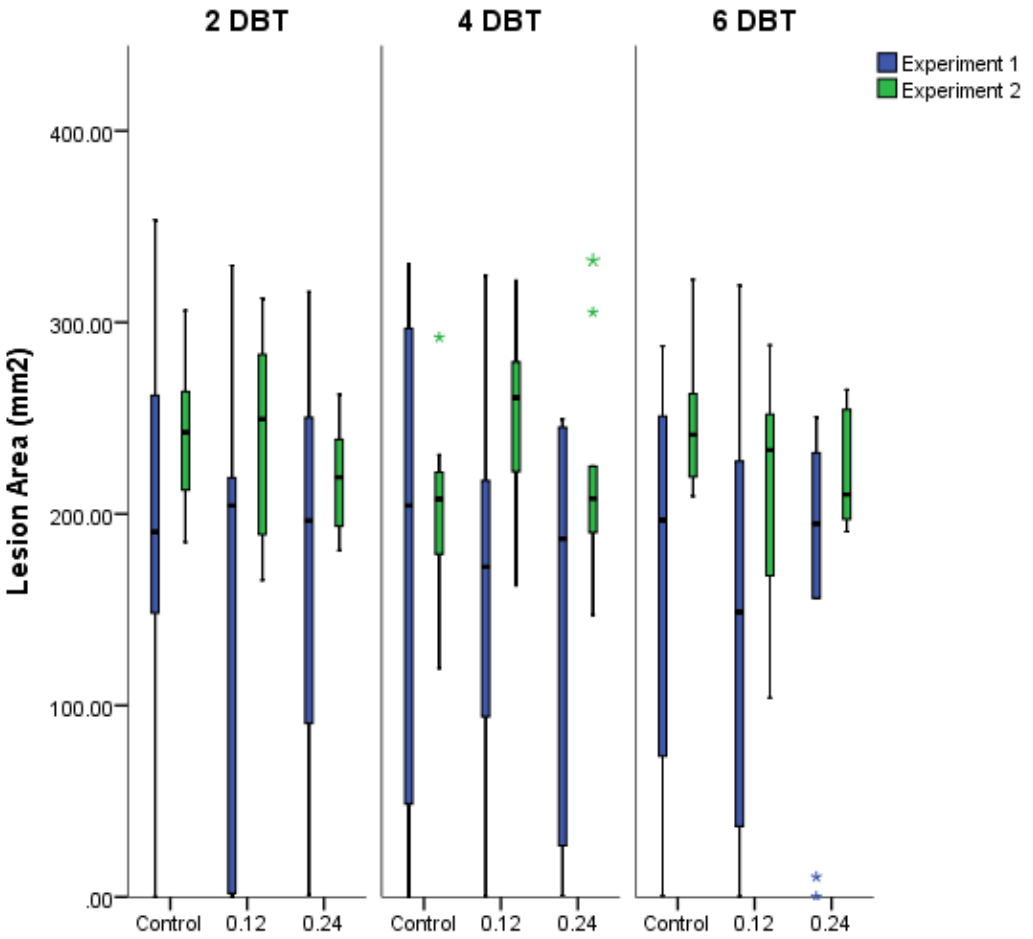

Figure A7.3: The mean lesion area from lettuce (cv. Temira) treated with multiple low-dose, low-intensity UV-C treatments (kJ/m^2) at the 6-8 true leaf stage, followed by inoculation with *R. solani.* Treatments were applied with either 2, 4 or 6 days between treatments (DBT). N=5 and 7 for experimental replicates 1 and 2, respectively. Box plots show the distribution of data from the independent experimental replicates. Asterisks represent observed outliers.

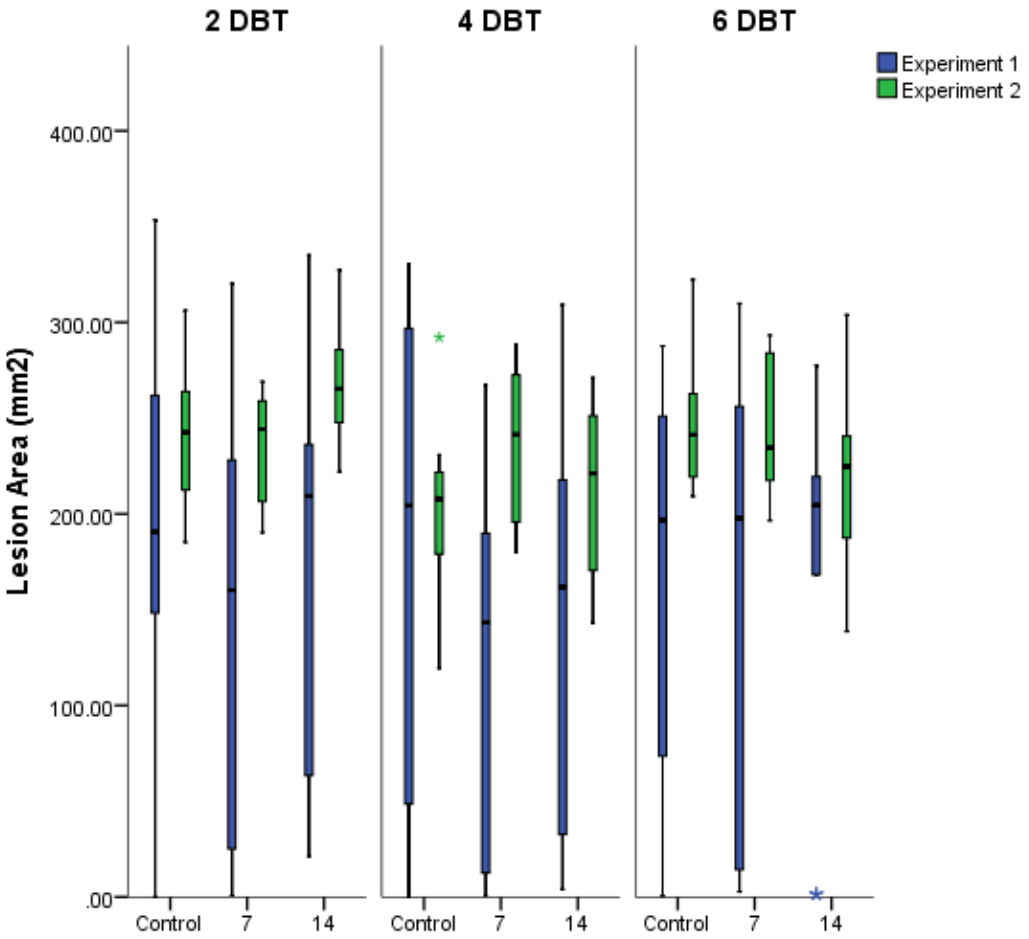

Figure A7.4: The mean lesion area from lettuce (cv. Temira) treated with multiple low-dose, high-intensity, pulsed polychromatic treatments (No. pulses) at the 6-8 true leaf stage, followed by inoculation with *R. solani.* Treatments were applied with either 2, 4 or 6 days between treatments (DBT). N=5 and 7 for experimental replicates 1 and 2, respectively. Box plots show the distribution of data from the independent experimental replicates. Asterisks represent observed outliers.

Appendix 8

Appendix 8 contains the data from the independent replicate experiments carried out within Chapter 9 “Foliar LIUV and HIPPL treatments of lettuce in a controlled environment”. Low-intensity UV-C and high-intensity, pulsed polychromatic light treatments were applied to lettuce crops grown on an NFT system in a controlled environment. Damage assessments and disease control assays with *B. cinerea* were then performed.

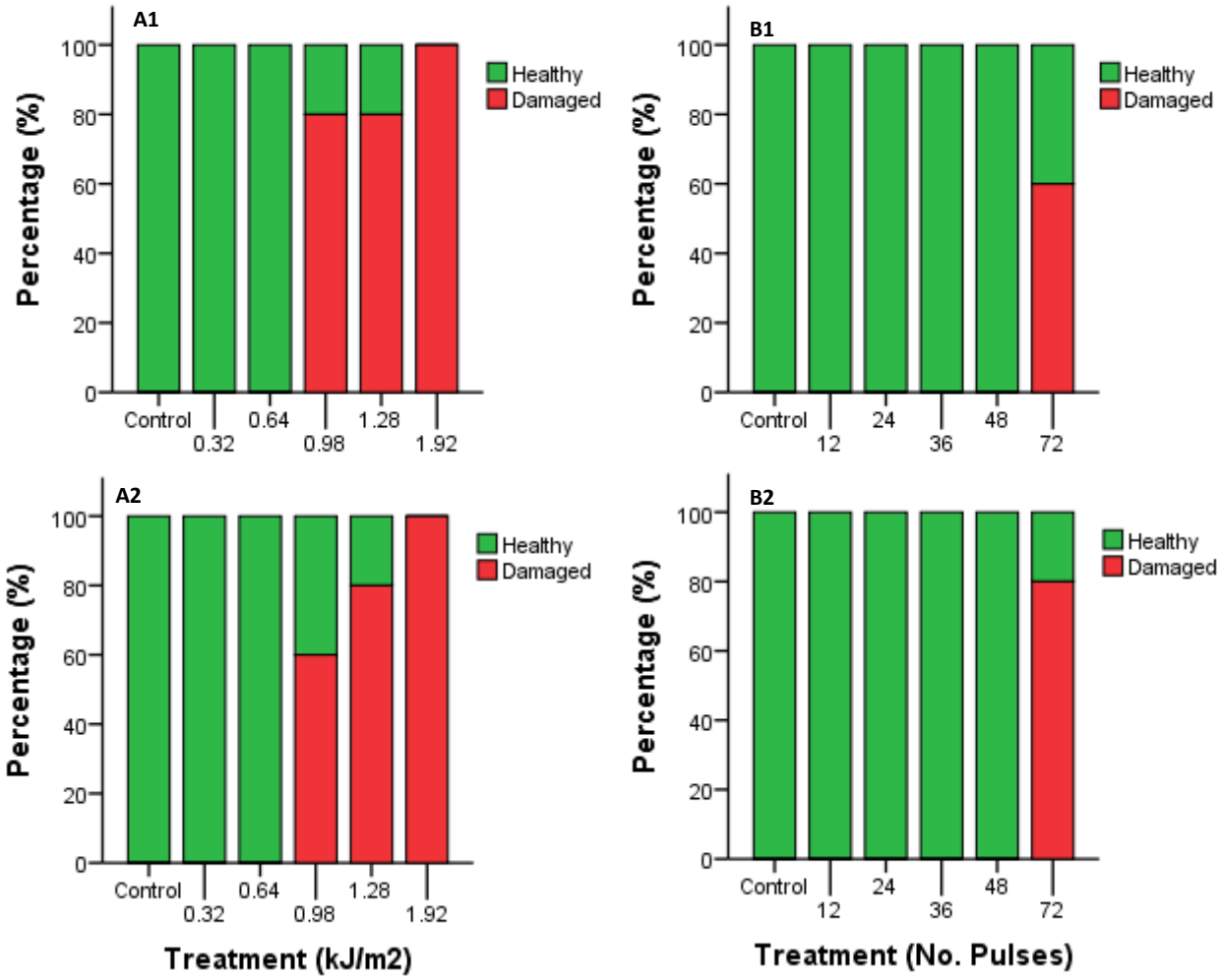

Figure A8.1. The percentage of healthy and damaged plants for the lettuce variety Temira. Plants were either treated with a conventional low-intensity mercury UV-C source (**A**) or a high-intensity, pulsed polychromatic source (**B**) at the 6-8 true leaf stage. Independent experimental replicates performed during May (**1**) and June (**2**) 2017 N=5. Damage assessments were performed two days post-treatment.

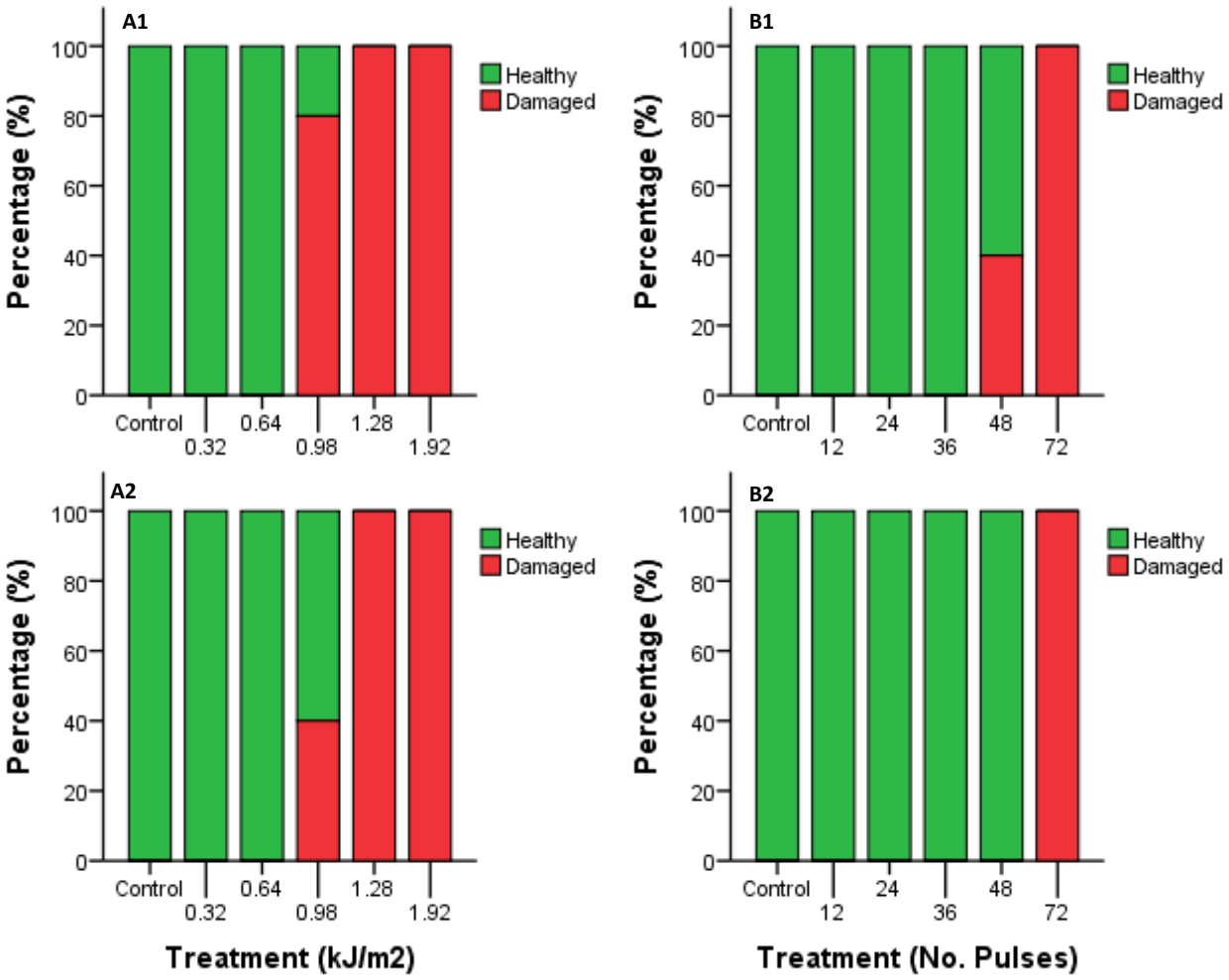

Figure A8.2. The percentage of healthy and damaged plants for the lettuce variety Amica. Plants were either treated with a conventional low-intensity mercury UV-C source (**A**) or a high-intensity, pulsed polychromatic source (**B**) at the 6-8 true leaf stage. Independent experimental replicates performed during May (**1**) and June (**2**) 2017 N=5. Damage assessments were performed two days post-treatment.

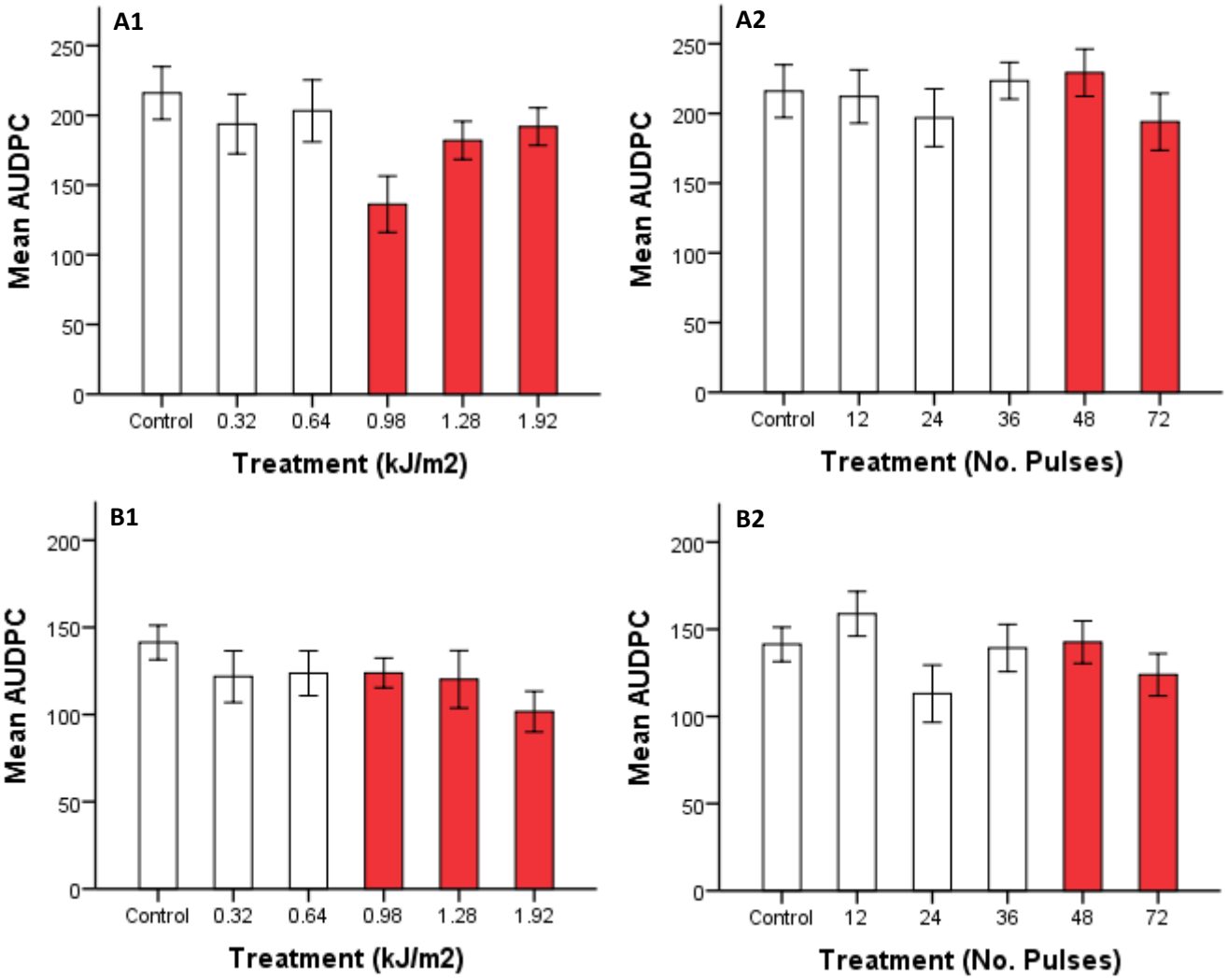

Figure A8.3: The area underneath the disease progression curve (AUDPC) from lettuce plants (Amica) treated with either low-intensity UV-C (**A**) or high-intensity, pulsed polychromatic light (**B**) and inoculated with *Botrytis cinerea* at 2 days post-treatment. Disease progression measurements were taken at 2 and 3 days post inoculation to allow calculation of AUDPC. Graphs show the data from two independent replicate experiments one performed during May (**1**) and June (**2**) 2017 N=5.

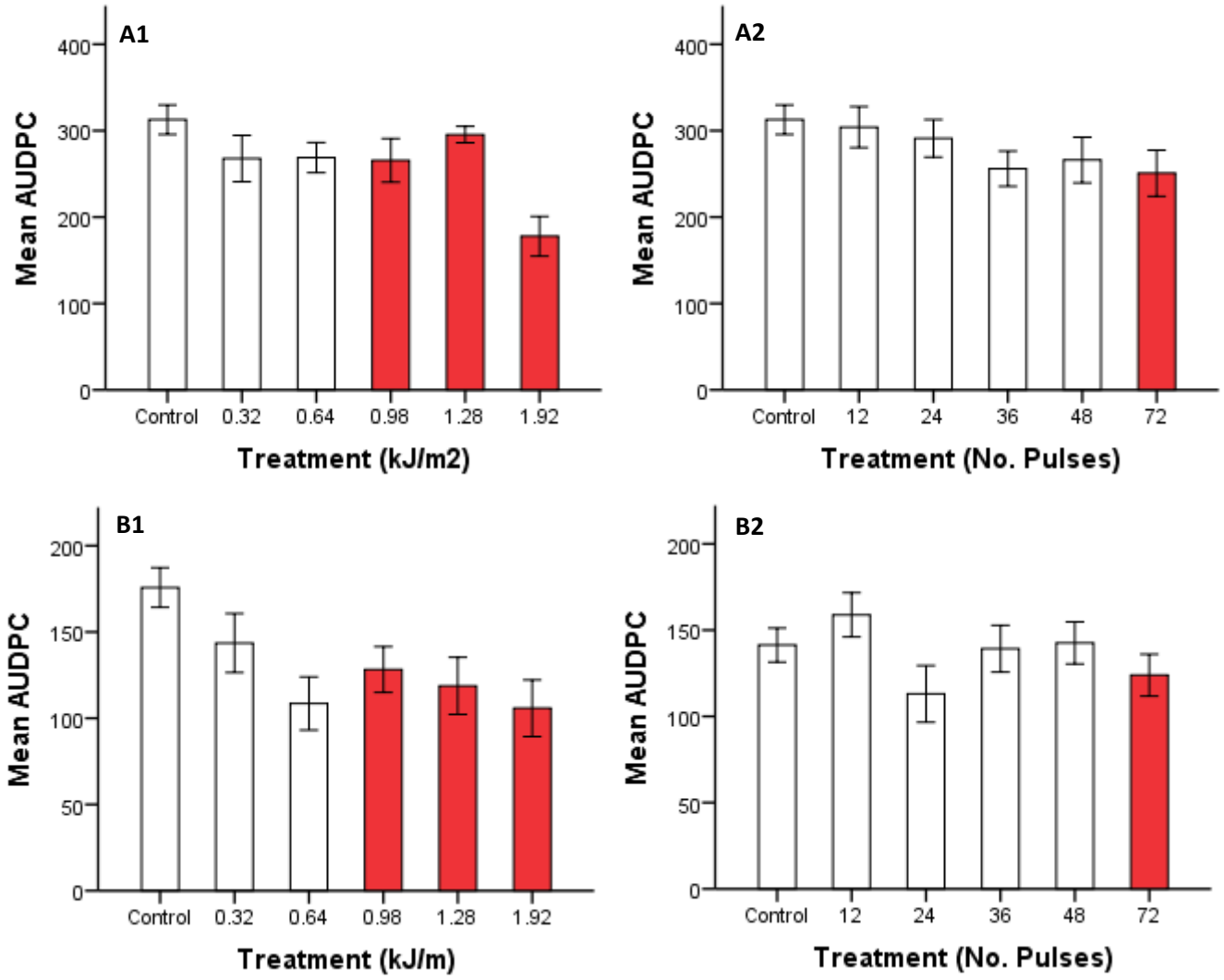

Figure A8.4: The area underneath the disease progression curve (AUDPC) from lettuce plants (Temira) treated with either low-intensity UV-C (**A**) or high-intensity, pulsed polychromatic light (**B**) and inoculated with *Botrytis cinereal* at 2 days post-treatment. Disease progression measurements were taken at 2 and 3 days post inoculation to allow calculation of AUDPC. Graphs show the data from two independent replicate experiments one performed during May (**1**) and June (**2**) 2017 N=5.

Appendix 9

Appendix 9 contains the results from the independent replicate experiments which were combined for data analysis in Chapter 10 “Investigating UV-C seed treatments for the control of disease on tomato crops”. In Chapter 10 tomato seeds were treated with a range of low-intensity UV-C exposures and disease control assays with *B. cinerea* were performed when the plant reached the initiation of the flowering developmental stage.

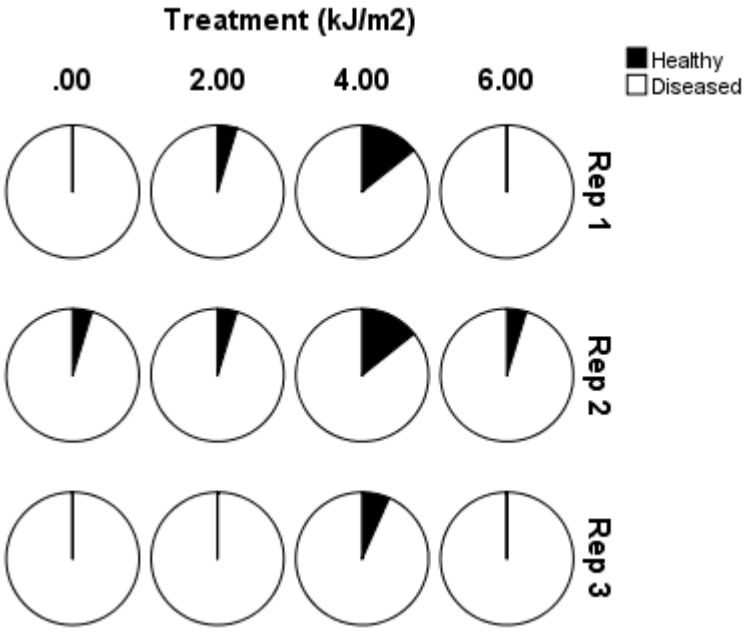

Figure A9.1: Disease incidence of tomato plants (cv. Shirley) grown from UV-C treated seeds and inoculated with *B. cinerea* at flowering. N= 7, 10 and 10 for independent experimental replicates 1, 2 and 3, respectively.

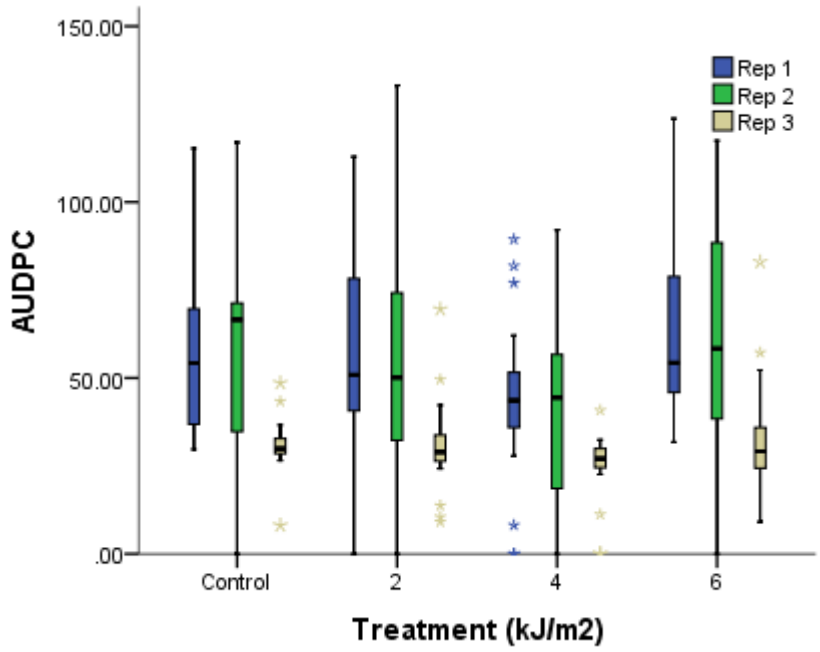

Figure A9.2: Area underneath the disease progression curve of tomato plants (cv. Shirley) grown from UV-C treated seeds and inoculated with *B. cinerea* at flowering. N= 7, 10 and 10 for independent replicate experiments 1, 2 and 3, respectively.

Appendix 10

Appendix 10 contains the data from the independent experimental replicates in Chapter 11 "Effect of UV-C seed treatment on early seedling growth and development". Tomato seeds (cv. Shirley) were treated with low-intensity UV-C and then grown in a controlled environment on Murashige and Skoog media in vertical Petri dishes. This allowed germination and seedling growth and development to be monitored with the aim to assess the impact of treatment on the plant.

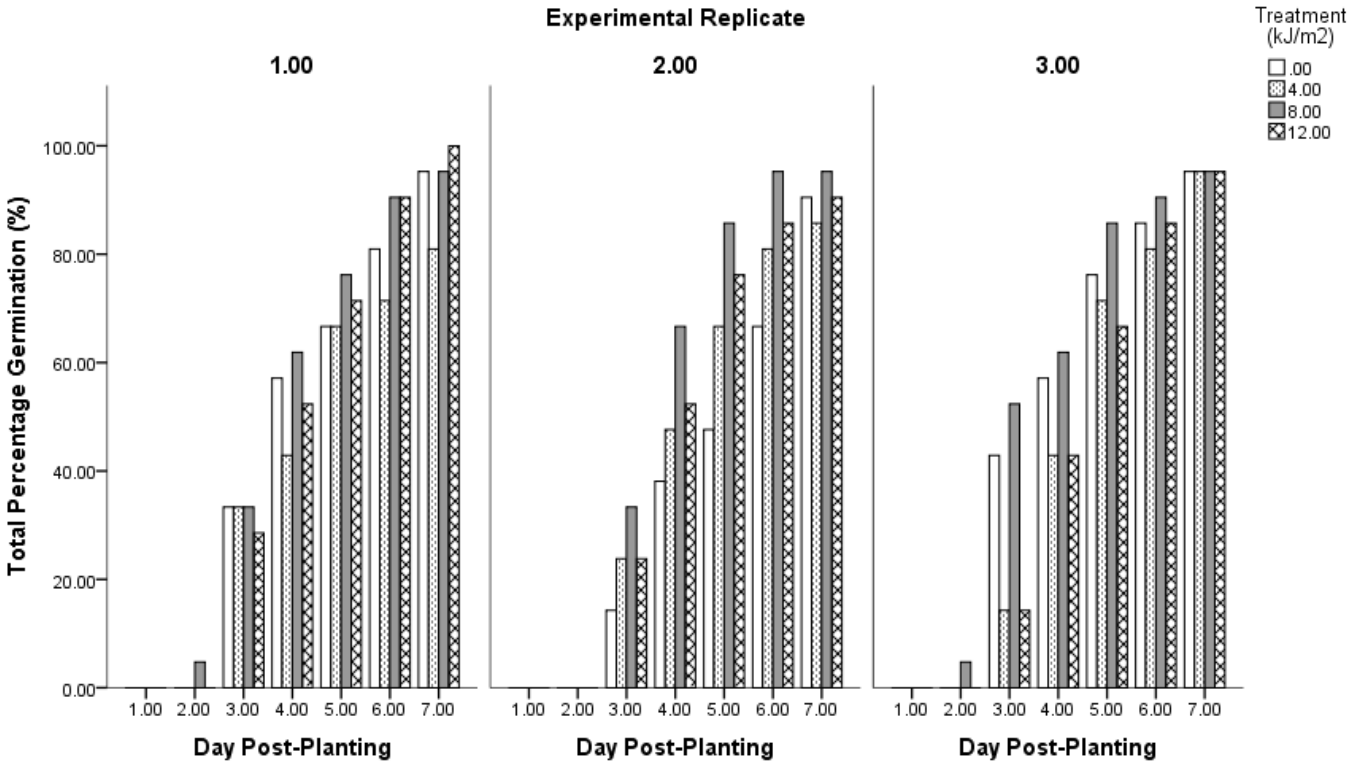

Figure A10.1: The total germination percentage of UV-C treated tomato (cv. Shirley) seeds from three experimental replicates. Seeds were grown in a controlled environment on Murashige and Skoog media in vertical Petri dishes. N= 21.

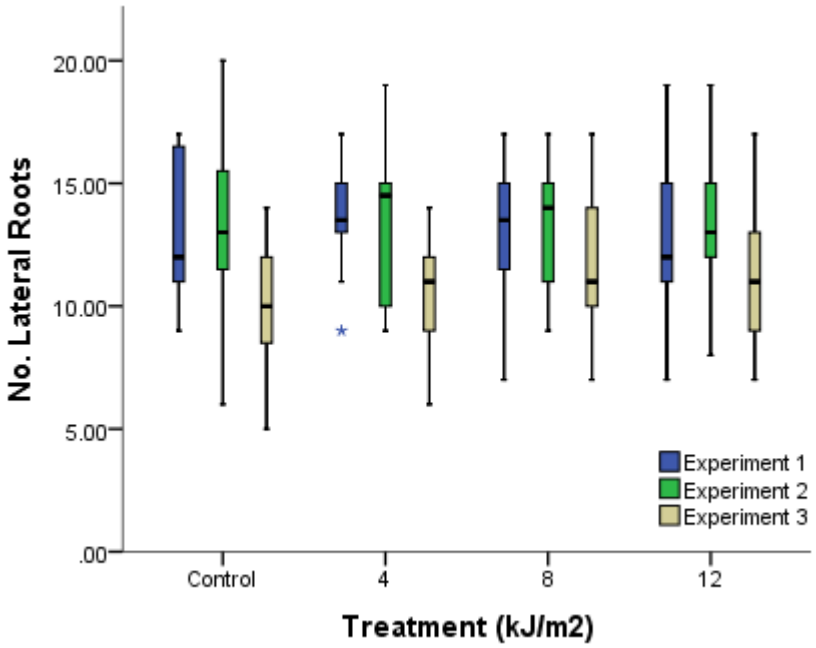

Figure A10.2: The number of lateral roots, at 5 days post germination, of UV-C treated tomato (cv. Shirley) seeds from three experimental replicates. The number of lateral roots was measured with a dissection microscope. Seeds were grown in a controlled environment on Murashige and Skoog media in vertical Petri dishes. N= 21.

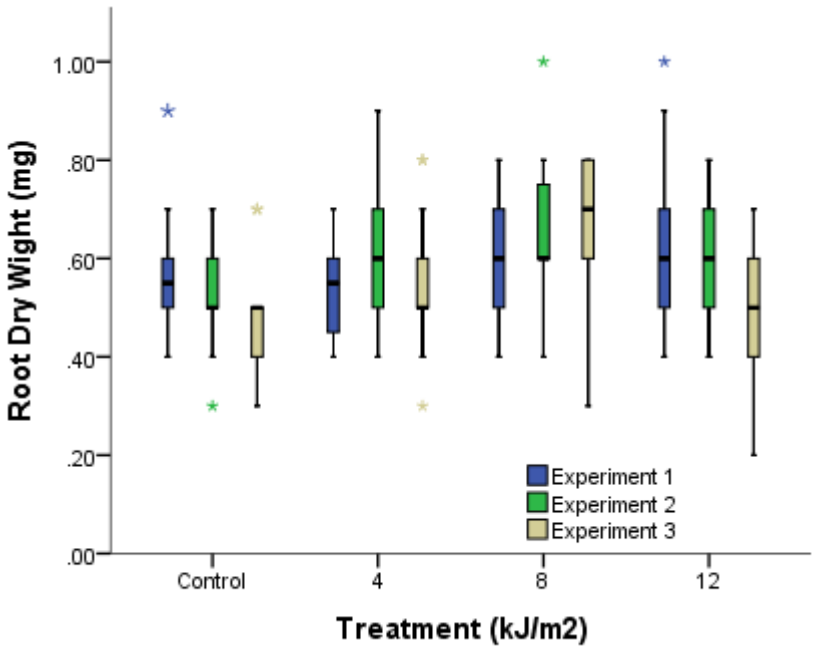

Figure A10.3: The dry root weight, at 5 days post germination, of UV-C treated tomato (cv. Shirley) seeds from three experimental replicates. Seeds were grown in a controlled environment on Murashige and Skoog media in vertical Petri dishes. N= 21.

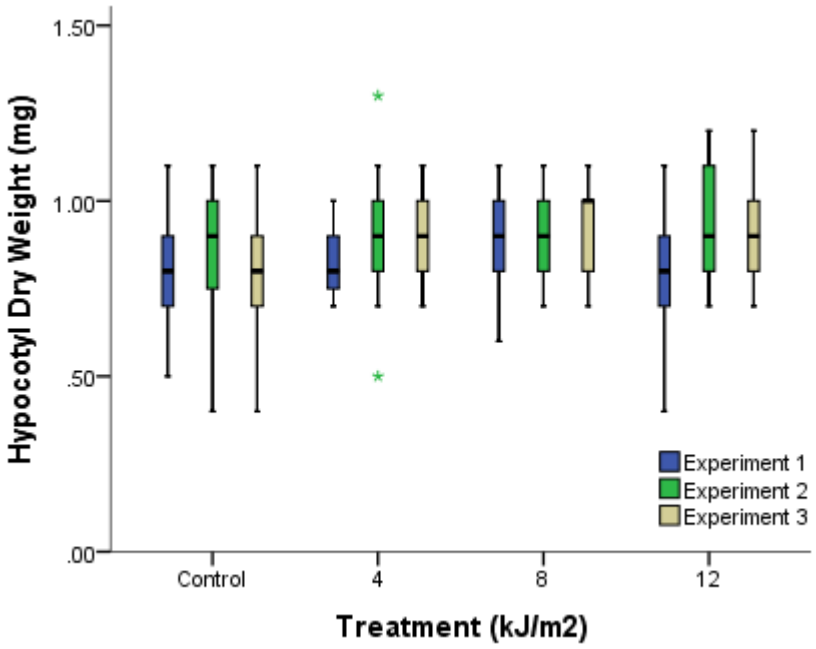

Figure A10.4: The dry hypocotyl weight, at 5 days post germination, of UV-C treated tomato (cv. Shirley) seeds from three experimental replicates. Seeds were grown in a controlled environment on Murashige and Skoog media in vertical Petri dishes. N= 21.

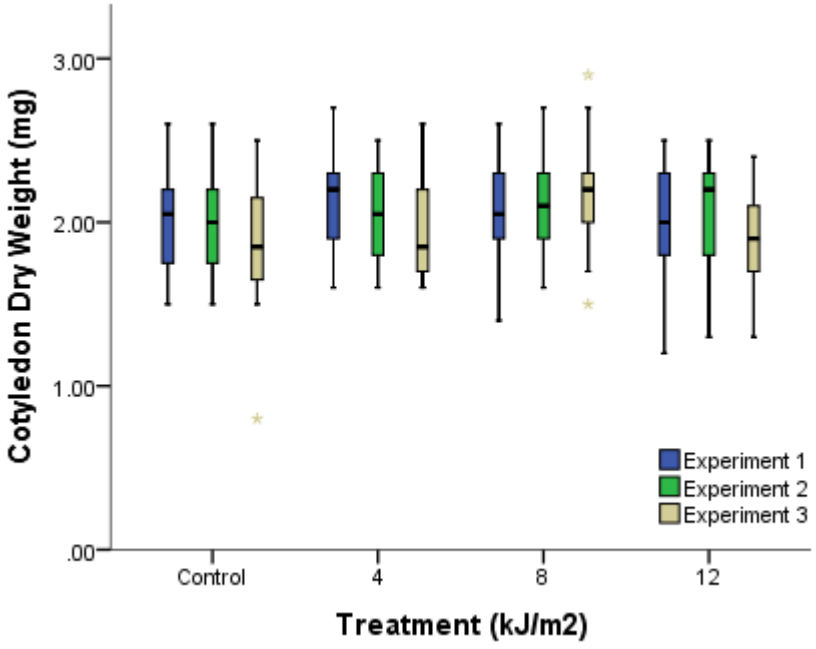

Figure A10.5: The dry cotyledon weight, at 5 days post germination, of UV-C treated tomato (cv. Shirley) seeds from three experimental replicates. Seeds were grown in a controlled environment on Murashige and Skoog media in vertical Petri dishes. N= 21.

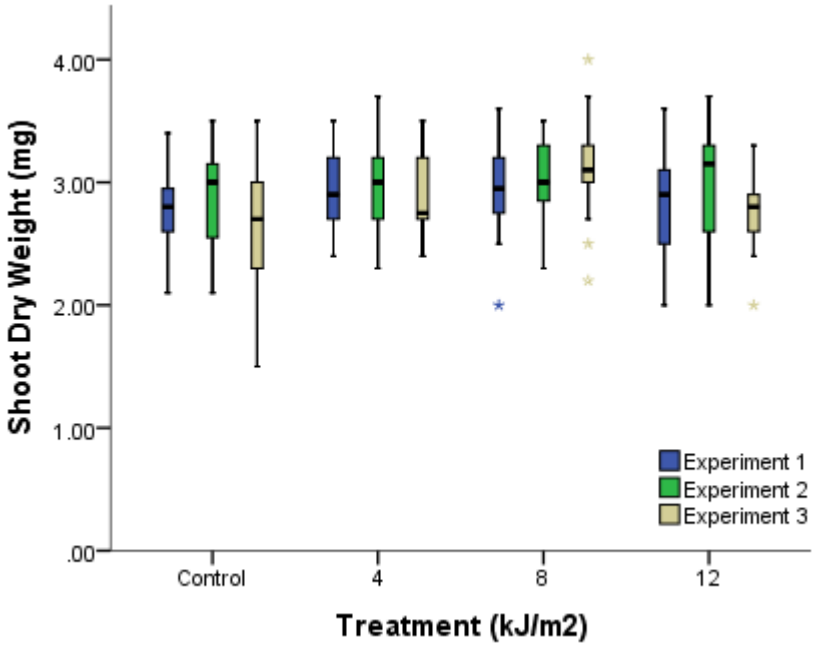

Figure A10.6: The dry shoot weight, at 5 days post germination, of UV-C treated tomato (cv. Shirley) seeds from three experimental replicates. Seeds were grown in a controlled environment on Murashige and Skoog media in vertical Petri dishes. N= 21.

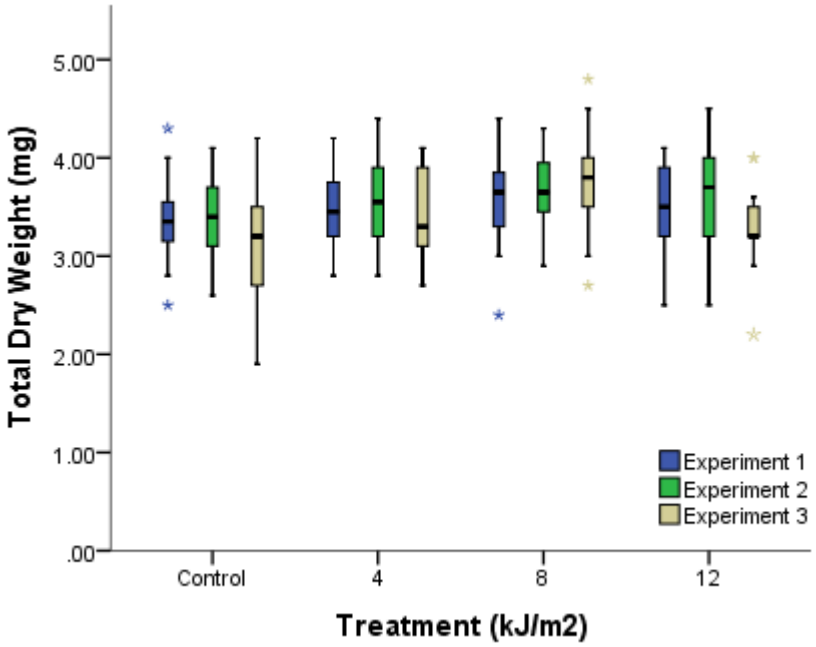

Figure A10.7: The total dry weight, at 5 days post germination, of UV-C treated tomato (cv. Shirley) seeds from three experimental replicates. Seeds were grown in a controlled environment on Murashige and Skoog media in vertical Petri dishes. N= 21.

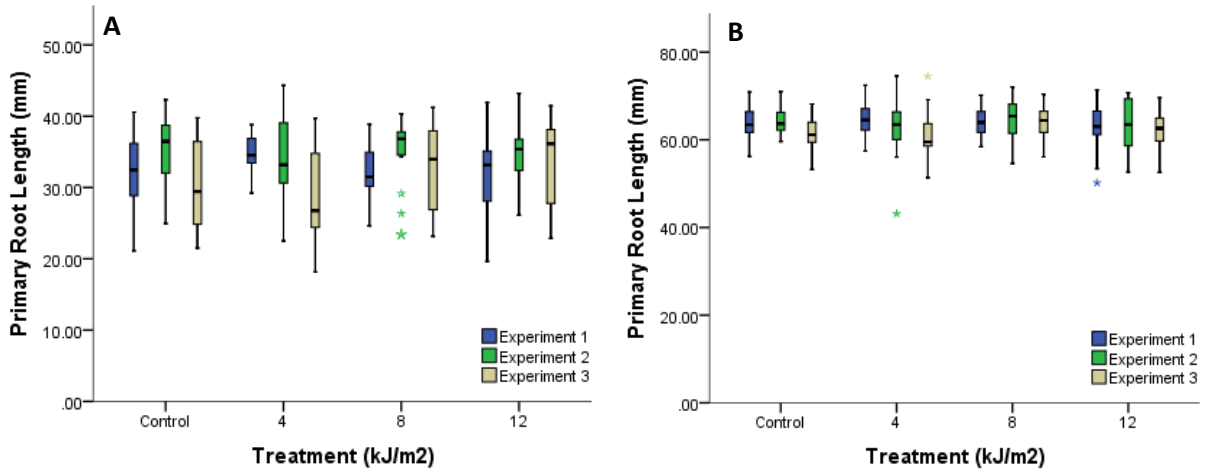

Figure A10.8: The primary root length at 2 (**A**) and 5 (**B**) days post germination of UV-C treated tomato (cv. Shirley) seeds from three experimental replicates. Root length was measured with imageJ. Seeds were grown in a controlled environment on Murashige and Skoog media in vertical Petri dishes. N= 21.

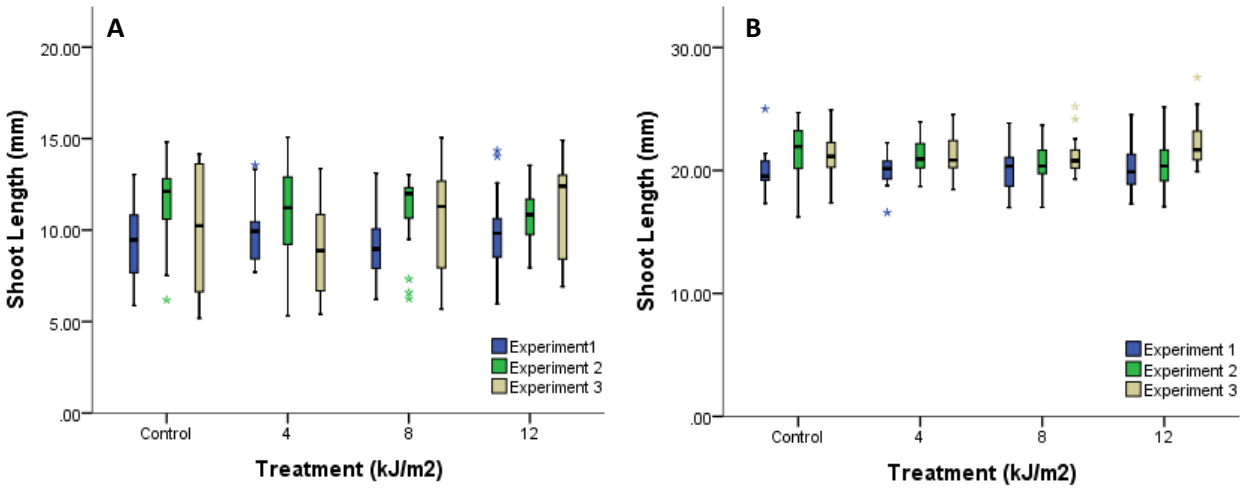

Figure A10.9: The shoot length at 2 (**A**) and 5 (**B**) days post germination of UV-C treated tomato (cv. Shirley) seeds from three experimental replicates. Shoot length was measured with imageJ. Seeds were grown in a controlled environment on Murashige and Skoog media in vertical Petri dishes. N= 21.

Appendix 11

Appendix 11 contains the copyright information and written confirmation of reuse for images used in Chapter 1.

All images used in Figure 1.2 were created by Dr. Scot Nelson a Phytopathologist from the University of Hawaii and are available at www.flickr.com/photos/scotnelson. All images are held under a creative commons licence (CC BY 2.0). Information on the licence terms and conditions can be found at https://creativecommons.org/licenses/by/2.0/. Images can be found at the following URLs:

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URL: https://www.flickr.com/photos/scotnelson/36529624215/

B) Common fig (Ficus carica): Gray mold caused by Botrytis cinerea
URL: https://www.flickr.com/photos/scotnelson/33841046942/

C) Powdery mildew Acacia mangium
URL: https://www.flickr.com/photos/scotnelson/5830549554/

D) Downy mildew of collard (Brassica oleracea) caused by Peronospora parasitica
URL: https://www.flickr.com/photos/scotnelson/15320370712/

E) Napa Cabbage (Won Bok, Chinese Cabbage, Celery Cabbage): Bacterial soft rot
URL: https://www.flickr.com/photos/scotnelson/34795134544/

F) Tomato (Solanum lycopersicum): Root-knot nematodes
URL: https://www.flickr.com/photos/scotnelson/26930345099/

Personal communications between myself (George Scott) and Scot Nelson regarding re-use of the images used in Figure 2.1 can be found below.

On Thu, Jan 11, 2018 at 12:38 AM, George Scott <G.Scott@lboro.ac.uk> wrote:

Dear Dr Scot C Nelson,

My name is George Scott and I am currently a PhD Student at Loughborough University.

I am writing for permission to use images available on your flickr account (Scot Nelson), for inclusion in the digital version of my PhD thesis, which is being submitted for examination at Loughborough University.

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Thanks for your time.

Yours sincerely,
George Scott

From: Scot Nelson <snelson@hawaii.edu.
Sent: 11 January 2018 11:40
To: George Scott

Subject: Re: Copyright permission request (flickr)

Dear Mr. Scott:

Thank you for contacting me.

Yes, you may include the images in your published thesis. I do not require any payment for the images.

Regards, Scot Nelson