A Comparison of the Molecular Mechanisms Underpinning High-Intensity, Pulsed Polychromatic Light and Low-Intensity UV-C Hormesis in Tomato Fruit

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Abstract

Postharvest treatment of tomato fruit with high-intensity, pulsed polychromatic light (HIPPL) has previously been shown to induce delayed ripening and disease resistance comparable to that of low-intensity UV-C (LIUV). Little, however, is known of the mechanisms underpinning postharvest HIPPL hormesis in tomato fruit. Expression of genes involved in plant hormone biosynthesis, defence, secondary metabolism and ripening were monitored 24 h post treatment (24 HPT), 10 d post treatment (10 DPT) and 12 h post inoculation with *Botrytis cinerea* (12 HPI). All genes monitored were constitutively expressed and changes in expression profiles following treatment were highly similar for both HIPPL and LIUV treatments. Expression of pathogenesis-related proteins P4, β-1,3,-Glucanase and Chitinase 9 and a jasmonate biosynthesis enzyme (OPR3), were significantly upregulated at 10 DPT and 12 HPI. Both treatments significantly downregulated the expression of polygalacturonase and flavonol synthase at 10 DPT and 12 HPI. Ethylene biosynthesis enzyme ACO1 and β-carotene hydroxylase were significantly upregulated at 24 HPT, and phenylalanine ammonia-lyase (PAL) was significantly upregulated at 12 HPI. Both HIPPL and LIUV treatments stimulate

defence responses that are mediated by salicylic acid, jasmonic acid and ethylene. This may lead to broad range resistance against both necrotrophic and biotrophic pathogens as well as abiotic stresses and herbivorous pests. Following inoculation with *B. cinerea* only *PAL* showed indication of a gene priming response for HIPPL- and LIUV-treated fruit.

Key words: Hormesis; *Solanum lycopersicum*; pulsed light; gene expression, polychromatic light.

1. Introduction

Wavelengths of light that fall between 100 and 280 nm are referred to as UV-C. The application of high-dose germicidal UV-C is extensively used in decontamination processes due to its ability to directly inactivate a range of micro-organisms. Lu *et al.,* (1987) published the first studies utilising UV-C for inducing hormesis in fresh produce. During the following three decades hormetic UV-C treatment was successfully performed on a wide range of fresh produce including climacteric and non-climacteric fruit, tubers, salads and brassicas (Ranganna *et al.,* 1997; D'Hallewin *et al.,* 1999, Costa *et al.,* 2006; Pongprasert *et al.,* 2011; Kasim & Kasim, 2012). The beneficial effects of UV-C hormesis include pathogen resistance, delayed chlorophyll degradation and improved nutritional content, all of which have been reviewed in depth by Shama & Alderson (2005), Ribeiro *et al.* (2012) and Turtoi (2013).

The majority of previous studies have been conducted with conventional low-pressure mercury sources that emit low-intensity UV-C light (LIUV) with peak emission at 254 nm. Recently, however, a number of publications have shown that high-intensity, pulsed polychromatic light (HIPPL) also induces similar hormetic benefits to that of LIUV (Oms-Oliu *et al.,* 2010; Koyyalamudi *et al.,* 2011; Rodov *et al.,* 2012; Pataro *et al.,* 2015; Scott *et al.,* 2017). In a previous study of ours, it was found that a 16-pulse treatment at 4.6 kJ/m^2/pulse of HIPPL induced both delayed ripening and increased disease resistance on tomato fruit at levels comparable levels to those achieved at a dose of 3.7

50 kJ/m^2 of LIUV (Scott *et al.,* 2017). The use of HIPPL reduced treatment times from 350 s to 10 s per
51 fruit when LIUV treatments were delivered at an intensity of 20 W m^{-2}.

52 One of the major benefits of HIPPL and LIUV hormesis is that of induced disease resistance.
53 Resistance is achieved through the upregulation of defence responses alongside alterations to
54 physiology and metabolism. Such changes include phytoalexin production, delayed ripening and
55 senescence, production of pathogenesis-related (PR) proteins and establishment of physical barriers
56 that inhibit pathogen progression (Ben-Yehoshua *et al*., 1992; D'Hallewin *et al.,* 1999; D'Hallewin *et*
57 *al.,* 2000; Mercier *et al.,* 2000; Romanazzi *et al.,* 2006; Charles *et al.,* 2008a; Charles *et al.,* 2009). PR
58 proteins that have been shown to be induced or increase in concentration following LIUV treatment
59 include chitinases and β-1,3-glucanases (Charles *et al.,* 2009). Such PR proteins interact directly with
60 pathogens causing cleavage of their cell wall components leading to loss of viability (Ebrahim *et al.,*
61 2011).

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

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

corymbosum), grapefruit (*Citrus paradisi*) and mango (*Mangifera indica*) (D'Hallewin *et al.,* 2000; González-Aguilar *et al.,* 2007, Perkins-Veazie *et al.,* 2008; Jagadeesh *et al*., 2011). 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.

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

2. Materials and Methods

2.1 Plant Material

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

2.2 LIUV and HIPPL Treatment

Upon arrival, tomatoes were randomly assigned to treatment groups and treated at room temperature on the same day. Fruit received exposure on two sides through 180 ° axial rotation following the protocols described by Scott *et al.* (2017). LIUV treatments were carried out using a U-shaped amalgam UV source (UVI 12OU2G11 CP15/469) housed within an anodised aluminium

parabolic reflector. The source was obtained from Dr Hőnle AG, Gräfelfing, Germany. Doses of 3.7 kJ/m^2 were delivered at an intensity of 20 W m^{-2} based upon the findings of Charles *et al.,* (2008b).

HIPPL treatments were carried out with a XENON LH-840 16" ozone-free B lamp. The lamp was powered and controlled by the RT-847 cabinet and RC-802 controller, supplied by Lambda Photometrics (Harpenden, UK). The source emitted 505 J of energy per pulse with a pulse width of 360 µs at 3.2 pulses/s. Spectral emissions of the source ranged from 240 nm to 1050 nm. Fruit were placed at a distance of 10 cm from the window of the lamp housing. Using information provided by the manufacturer it is estimated that 4.6 kJ/m^2/pulse was delivered at fruit level.

After treatment, fruit were stored in the dark until sterilisation. Sterilisation was performed immediately following the completion of treatments. Tomatoes were immersed in 2 % Sodium hypochlorite (Sigma-Aldrich) for approximately 5 to 10 s. This prevented the growth of naturally occurring phytopathogens 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 at a relative humidity > 98 %. Sterilisation was performed in indirect ambient light to prevent photoreversal.

2.4 Pathogen Maintenance, Inoculum Preparation and Inoculation

A *Botrytis cinerea* culture, originally isolated from a plant of the genus *Rosa*, was supplied from the University of Nottingham's fungal collection. Cultures were grown at room temperature on potato dextrose agar (Sigma-Aldrich) supplemented with Penicillin G sodium salt (Sigma-Aldrich) at 33 mg/L and Streptomycin sulphate salt (Sigma-Aldrich) at 133 mg/L. A calibrated spore solution was made from 10 - 14 d old cultures following Scott *et al*. (2017). At 10 d post treatment (10 DPT) artificial inoculations were performed on control and treated fruit. This interval was chosen based upon Charles *et al.,* (2008b) who demonstrated near optimal disease control at 10 d following LIUV treatment. Inoculations were performed by wounding the fruit with a sterile hypodermic needle to a depth of 3 mm. A 10 µl aliquot of *B. cinerea* spores at 1x10^6 spores/ml was pipetted into the

wound. Fruit were stored at 21 °C following inoculation.

2.5 Sampling, RNA Extraction and Reverse Transcription

A No.2 cork borer (6.25 mm outer diameter) was used to take a 50 to 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 required. Twenty four hours before tissue homogenisation a single 4 mm steel bead (Qiagen) was cooled in liquid nitrogen and added to each microcentrifuge tube. 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 30 Hz for 1 min. Homogenised samples were stored at - 80 °C until RNA extraction was performed.

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

2.6 qPCR

Two technical replicates were performed for each sample. Each 10 µl reaction contained 5 µl of 2x Fast SYBR® Green master mix (Applied Biosystems) and 2 µl of template cDNA. Primer concentrations and annealing temperatures were as stated in Table 1. Reactions were run on a LightCycler 480 ® (Roche) with a two-step amplification cycle. The cycle was as follows; a pre-

incubation of 10 min at 95 °C followed by 40 cycles of 95 °C for 5 s and the anneal for 45 s. Cycle threshold (Ct) values were calculated utilising the second derivative maximum method. A melting curve was run between 90 °C and 60 °C following amplification to check product specificity. Primers were optimised utilising a pooled sample and a 5-point 5-fold dilution series from which efficiency was calculated (Eq.1). Specificity of products from each primer pair was confirmed by sequencing and NCBI basic local alignment search tool (BLAST) analysis.

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

Equation 1. Amplification efficiency showing efficiency (AE), fold dilution (D) and gradient of the logarithmically plotted dilution curve (∇) (Pfaffl, 2004).

Table 1: Details of the primers used in qPCR

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

2.7 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 h post treatment (HPT), 10 d post treatment (DPT) and 12 h post inoculation (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 (Eq1). Following amplification 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 unfit for further analysis and the data was re-collected. Interplate calibration was performed with a pooled sample to correct for interplate bias (Eq.2). Amplification efficiency was then used to correct Ct values following Eq. 3. Technical replicates were then averaged before further analysis.

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

Equation 2. Interplate calibration equation. 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 3. Efficiency correction of cycle threshold (Ct) values. CtE is the efficiency corrected Ct value and AE is the efficiency of amplification (Kubista & Sindelka, 2007).

Actin was used as the reference gene as in previous UV-C 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). Data was then normalised to baseline (pre-treatment) gene expression and fold change between treatment groups was calculated following Eq.5. For experiments utilising theoretical copy number, a copy number of 100 was assigned to the baseline gene expression levels and the further data was adjusted accordingly.

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

Equation 4. Normalisation of gene of interest with reference gene. CtE(goi) is the efficiency corrected Ct value for the gene of interest and CtE(ref) is the efficiency corrected Ct value for the reference gene (Pfaffl, 2004).

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

Equation 5. Calculating fold change. ΔCtET is the normalised and efficiency corrected mean Ct value for the treatment group and ΔCtEC is the normalised and efficiency corrected mean Ct value of the control group (Livak & Schmittgen, 2001).

Statistical analysis was performed on the efficiency corrected and 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 $p \leq 0.05$.

3 Results and Discussion

Expression profiles of genes involved in plant defence, secondary mtabolism and ripening were analysed and compared for HIPPL- and LIUV-treated fruit. 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 the baseline expression before treatment.

3.1 Phytohormones and Disease Resistance

Ethylene (ET) is a plant hormone that plays a significant role in the control of ripening and ripening-related susceptibility to *B. cinerea* in tomato fruit (Cantu *et al.,* 2009). ACO (1-aminocyclopropane-1-carboxylic acid oxidase) is involved in the final oxygen-dependant step converting ACC (1-aminocyclopropane-1-carboxylic acid) to ethylene (Hamilton *et al.,* 1991 & Dong *et al.,* 1992). ACO1 is one of five identified ACO enzymes involved in ethylene biosynthesis in tomato (Hamilton *et al.,* 1991; Bouzayen *et al.,* 1993; Sell & Hehl, 2005). In our study, the expression of *ACO1* in control fruit increased during the 10 d storage by approximately 8-fold, which is consistent with *ACO1* increases during 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 (Figure 1). Conversely, at 10 DPT and 12 HPI the levels of *ACO1* in control fruit were 1.2- to 2.2-fold lower. The differences, however, were not statistically significant.

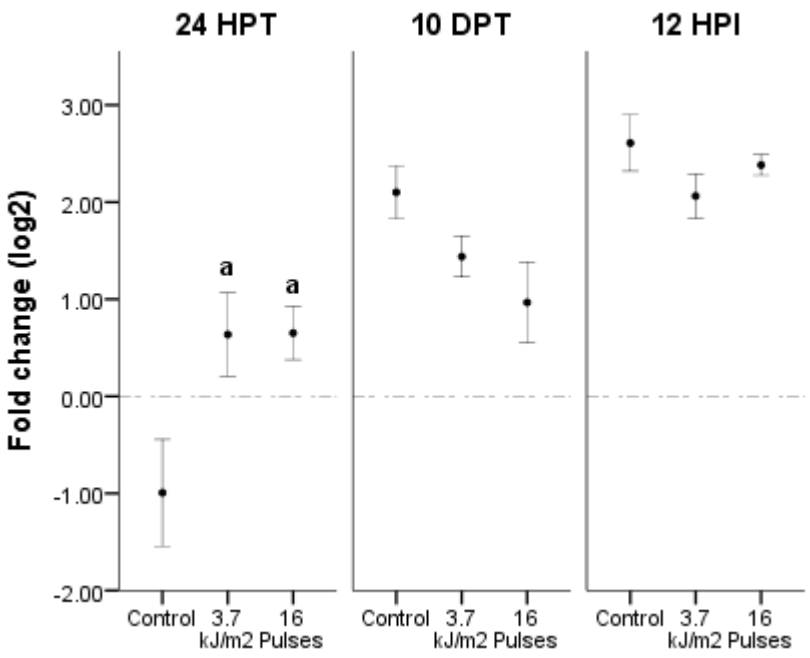

Figure 1: Relative expression of *ACO1* (1-aminocyclopropane-1-carboxylic acid oxidase 1), a bottleneck enzyme in ethylene biosynthesis, following treatment with either 16 pulses from a high-intensity, pulsed polychromatic light (HIPPL) source or 3.7 kJ/m^2 from a low-intensity UV-C source (LIUV). Samples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

The downregulation of *ACO1* at 10 DPT and 12 HPI contributes towards the delayed ripening phenotype observed following HIPPL and LIUV treatment of tomato fruit (Liu et al., 1993; Scott et al., 2017). This is supported by two studies. Firstly, Zhefeng et al., (2008) observed that a reduction in ACO1 mRNA led to delayed ripening (colour change). Secondly, inhibition of *ACO1* was shown to lead to a reduction in ethylene biosynthesis and a prolonged shelf life (Behboodian *et al.,* 2012).

Our results are consistent with those of Maharaj *et al.* (1999) who observed a transient peak in ethylene production at 3 and 5 d after LIUV treatment followed by a lag in ethylene production and a lower maximum ethylene level from the seventh day following treatment. Similarly, Tiecher *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 plants adaptation to herbivorous pests and necrotrophic plant pathogens (Spoel & Dong, 2012). OPR3 (12-oxophytodienoate reductase 3) is the major enzyme catalysing the penultimate enzymatic step in JA biosynthesis, where 9S, 13S-12-oxophytodienoate is reduced to a cyclopentane JA precursor (Schaller *et al.,* 2000; Breihaupt *et al.,* 2006; Bosch *et al.,* 2014).

In HIPPL-treated fruit, a slight downregulation of *OPR3* (<2-fold) at 24 HPT was detected (Figure 2). Expression in control fruit remained at the baseline levels. At 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 for HIPPL- and LIUV-treated fruit, respectively. The initial reduction in *OPR3* expression was analogous to the results observed by Liu *et al.* (2011) which showed a 3.9-fold reduction in *OPR2* at 24 HPT following LIUV treatment; no further time points were monitored.

OPR3 upregulation following LIUV and HIPPL treatments can result in increased JA levels and activation of JA-inducible plant defences which are involved in resistance against necrotrophic pathogens (Glazebrook, 2005). This is supported by Scalschi *et al.,* (2015) who showed that *OPR3* 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* 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,* therefore, contributes towards the control of *B. cinerea* following HIPPL and LIUV treatment; observed previously by Liu *et al.,* (1993) and Scott *et al.,* (2017).

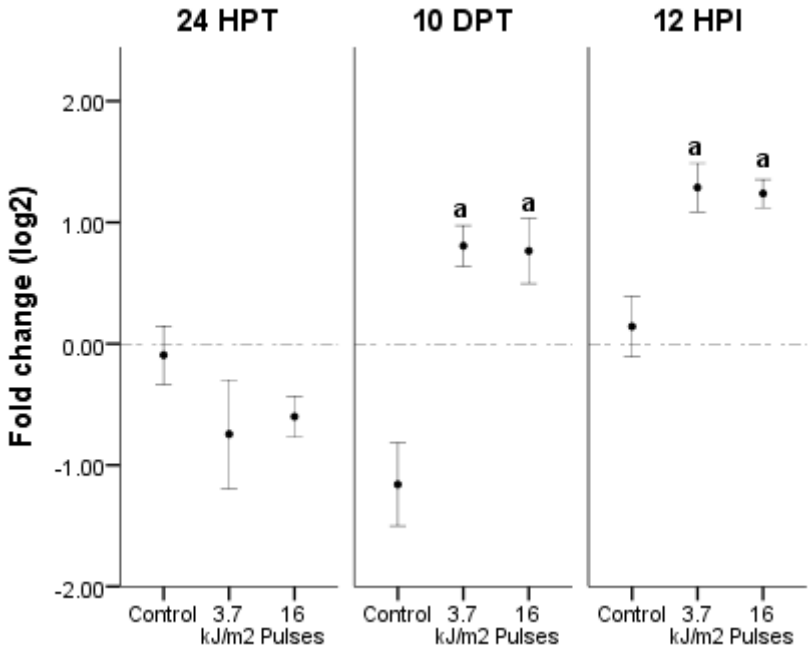

Figure 2: The relative expression of *OPR3* (12-Oxophytodienoate reductase 3), a jasmonate biosynthesis enzyme, following treatment with either 16 pulses from a high-intensity, pulsed polychromatic light (HIPPL) source or 3.7 kJ/m^2 from a low-intensity UV-C (LIUV) source. Samples were taken before treatment,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

SA is a phytohormone which plays a major role in defence against biotrophic pathogens, insect pests and abiotic stress, it is also involved in 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 in 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 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. Our results indicate that both HIPPL and LIUV treatments induce SA signalling following treatment.

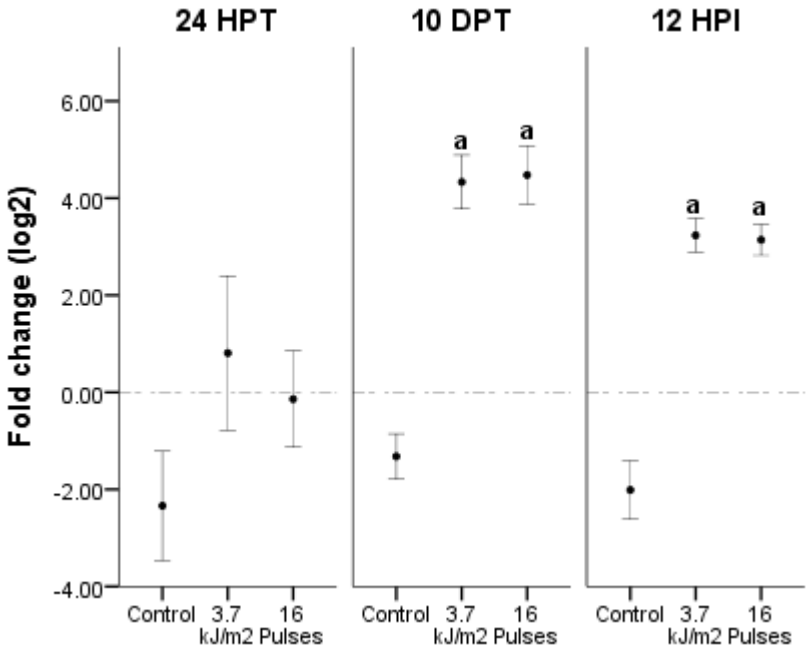

Figure 3: The relative expression of *P4* (*PR1a*), a salicylic acid-inducible pathogenesis-related protein and marker of systemic acquired resistance (SAR), following treatment with either 16 pulses from a high-intensity, pulsed polychromatic light source (HIPPL) or 3.7 kJ/m^2 from a low-intensity UV-C source (LIUV). Samples were taken before treatment, 24 h post treatment (HPT), 10 d post

treatment (DPT), immediately before inoculation, and 12 h post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). Labelling indicates statistical significance, within a given time point, where groups sharing labels are not significantly different at $p< 0.05$. N=6. Bars show ± 1S.E.M.

SA, however, has been shown to only play a small part in resistance against *B. cinerea*. In work undertaken by Asselbergh *et al. (*2007) tomato plants expressing the bacterial gene *nahG*, which cannot accumulate SA, were shown to be slightly more susceptible to *B. cinerea*. SA and P4, however, play a greater role in protecting the plant 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 a number of important roles in the plant from regulating germination to defence against pathogen attack. Here we observed significant upregulation in the expression of a basic, intracellular, 33 kDa, ethylene-inducible and PR β-1,3,-Glucanase (*GLUB*) (van Kan *et al.,* 1992; Aimé *et al.,* 2008).

Levels of *GLUB* were similar in all groups at 24 HPT (Figure 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 approximately 32-fold and 2-fold for control and treated samples following inoculation (12 HPI). Expression levels in both HIPPL- and LIUV-treated fruit, however, remained significantly higher than the control with 2.1- and 2.2-fold differences, respectively. A similar pattern in protein expression was observed by Charles *et al.* (2009) on LIUV-treated tomato fruit. They reported the induction of a basic, 33.1 kDa β-1,3,-Glucanase which increased in concentration between 3 and 10 d after treatment and following inoculation with *B. cinerea*. Increased expression of *GLUB* before and after the inoculation may contribute towards the increased disease resistance we observed previously in HIPPL- and LIUV-treated fruit (Scott *et al.,* 2017)

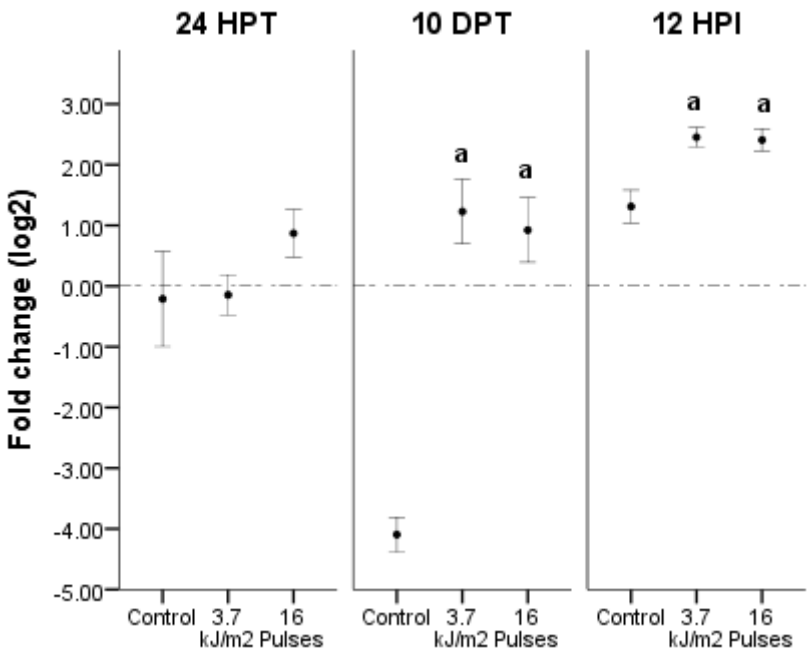

Figure 4: Relative expression of *GLUB* (β-1,3,-Glucanase), an ethylene-inducible pathogenesis related protein transcript, following treatment with either 16 pulses from a high-intensity, pulsed polychromatic light (HIPPL) source or 3.7 kJ/m^2 from a low-intensity UV-C (LIUV) source. Samples were taken before treatment, 24 h post treatment (HPT), 10 d post treatment (DPT), immediately before inoculation, and 12 h post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). Labelling indicates statistical significance, within a given time point, where groups sharing labels are not significantly different at $p< 0.05$. N=6. Bars show ± 1S.E.M.

PR chitinases are involved in the breakdown of glycosidic bonds in the cell wall of fungal pathogens. In this work we monitored the ET-, 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 *GLUB*. At 24 HPT a slight increase in *CHI9* expression was detected in HIPPL- and LIUV-treated fruit, while expression in the control decreased below baseline (Figure 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. The concentration of two chitinases observed by Charles *et al.* (2009) also showed a similar pattern of expression to those observed here. Little change in expression was reported at 3 DPT with upregulation occurring at 10 DPT and following inoculation (Charles *et al.,* 2009). Similarly, we observed an aproximately a 2-fold increase in control fruit expression following inoculation.

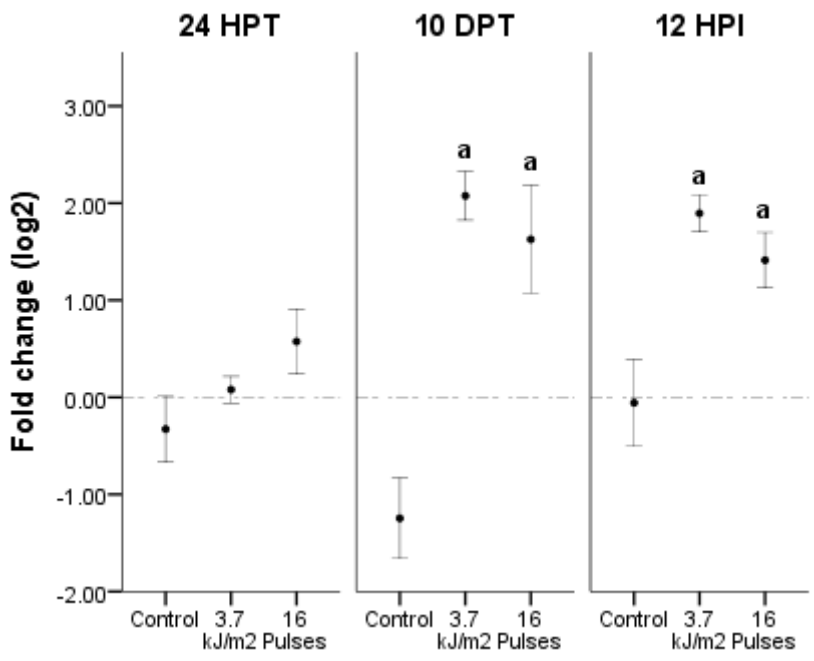

Figure 5: Relative expression of *CHI9* (Chitinase 9), a jasmonic acid-inducible pathogenesis-related protein, following treatment with either 16 pulses from a high-intensity, pulsed polychromatic light

(HIPPL) source or 3.7 kJ/m^2 from a low-intensity UV-C (LIUV) source. Samples were taken before treatment, 24 h post treatment (HPT), 10 d post treatment (DPT), immediately before inoculation, and 12 h post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). Labelling indicates statistical significance, within a given time point, where groups sharing labels are not significantly different at $p < 0.05$. N=6. Bars show ± 1S.E.M.

The upregulation of JA synthesis gene *OPR3* and PR proteins *P4*, *GLUB* and *CHI9* following HIPPL and LIUV treatment supports the hypothesis that the control of *B. cinerea* is achieved through induced resistance mediated by SA and JA pathways (Liu *et al.,* 1993; Scott *et al.,* 2017). Furthermore, the postulated broad-range resistance is further supported as all three PR proteins are also upregulated in tomato's defence 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.

3.2 Ripening and Secondary Metabolism

A delay in ripening, through both delayed colour change and texture softening, is a further benefit of LIUV hormesis which leads to extended shelf life and reduced 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 elevates tomato's susceptibility to *B. cinerea* (Bennett *et al.,* 1993).

At 24 HPT, *PG* expression was at baseline levels (Figure 6) which then increased at 10 DPT for all groups. In HIPPL- and LIUV-treated fruit, however, levels of *PG* were significantly lower than the

418 control with 6.1- and 32.2-fold decreases, respectively. *PG* levels decreased in response to
419 inoculation (12 HPI) with *B. cinerea* in all groups. Fruit from both treated groups, however, still
420 showed significantly lower expression than control fruit with 15.4- and 3.0-fold less *PG* in LIUV- and
421 HIPPL-treated fruit, respectively. Reduced expression of *PG* in HIPPL-treated fruit supports our
422 observations that control fruit were 14.6 and 22.4 % softer than HIPPL-treated fruit at 14 and 21 DPT
423 (unpublished data). Furthermore, Barka *et al.,* (2000) showed a reduction in PG activity following
424 LIUV treatment. The reductions in *PG* are, therefore, likely to play a role in the delayed tissue
425 softening observed following LIUV (Liu *et al.,* 1993) and HIPPL treatments. This is supported by
426 Langley *et al.,* (1994) who showed that silencing of *PG* reduced tissue softening of tomato fruit.

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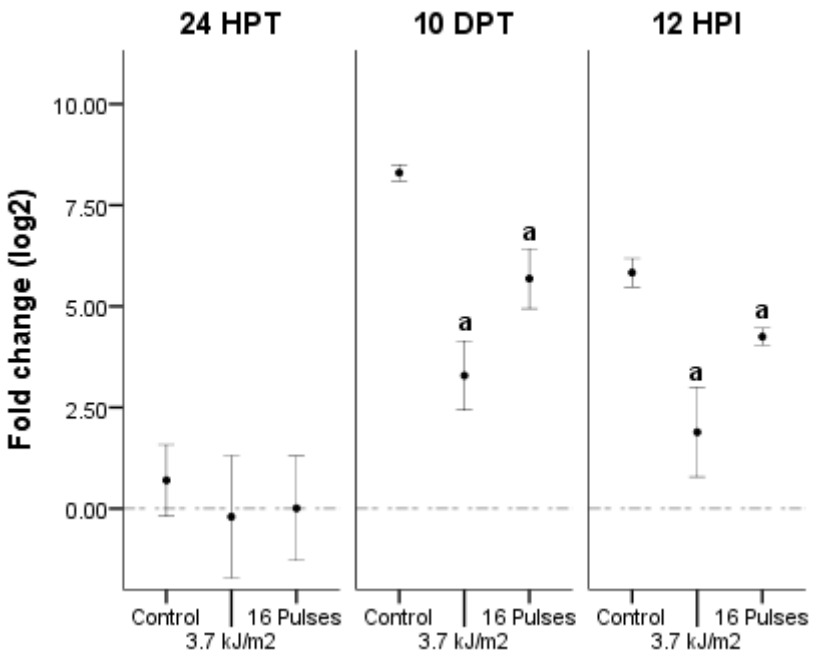

436 **Figure 6:** The relative expression of *PG* (polygalacturonase) following treatment with either 16 pulses
437 from a high-intensity, pulsed polychromatic light (HIPPL) source or 3.7 kJ/m^2 from a low-intensity UV-
438 C (LIUV) source. Samples were taken before treatment, 24 h post treatment (HPT), 10 d post
439 treatment (DPT), immediately before inoculation, and 12 h post inoculation (HPI). Fold changes (log_2)
440 are relative to baseline expression before treatment (dotted line). Labelling indicates statistical

441 significance, within a given time point, where groups sharing labels are not significantly different at
442 $p < 0.05$. N=6. Bars show ± 1S.E.M.

443

444 Carotenoids are organic molecules responsible for the red, orange and yellow pigmentations found
445 in flowers and fruits (Yuan *et al.,* 2015). The carotenoid, β-carotene, gives rise to the orange
446 pigmentation in tomato fruit and is synthesised from the cyclisation of lycopene; the major
447 carotenoid in tomato fruit which gives rise to their red colour (Pecker *et al.,* 1996; Tadmor *et al.,*
448 2005; Yuan *et al.,* 2015). Here, we monitored the expression of β-carotene hydroxylase (*CRTR-B1*)
449 involved in β-carotene modification producing the xanthophylls zeaxanthin and lutein which impart
450 a yellow pigmentation to plant organs (Galpaz *et al.,* 2006). These carotenoids are also found in the
451 retina of the human eye, and their uptake through food can lower the risk of age-related macular
452 degeneration of retina (Mares-Perlman *et al.,* 2002).

453 We have shown a significant 1.7-fold increase in *CRTR-B1* expression in HIPPL- and LIUV-treated fruit
454 24 HPT (Figure 7). At 10 DPT and 12 HPI, however, expression of *CRTR-B1* was not significantly
455 different from that of the control. Analogous patterns of *CRTR-B1* expression along with zeaxanthin
456 and lutein concentrations were observed by Tiecher *et al.* (2013) who reported increases in both at 1
457 d following LIUV treatment, and similar levels to the control at 7 DPT.

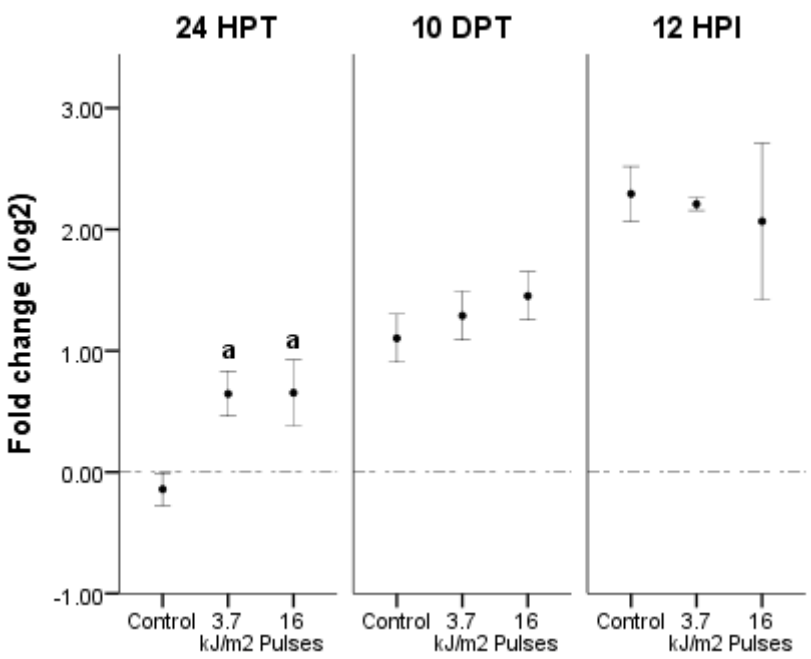

Figure 7: Relative expression of *CRTR-B1* (β -carotene hydroxylase) following treatment with either 16 pulses from a high-intensity, pulsed polychromatic light (HIPPL) source or 3.7 kJ/m^2 from a low intensity UV-C (LIUV) source. Samples were taken before treatment, 24 h post treatment (HPT), 10 d post treatment (DPT), immediately before inoculation, and 12 h post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). Labelling indicates statistical significance, within a given time point, where groups sharing labels are not significantly different at $p< 0.05$. N=6. Bars show ± 1S.E.M.

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. It also plays an important role in SA biosynthesis. Furthermore, phenolic compounds can act as phytoalexins involved in pathogen defence, free radical absorbers and light quenchers (Pietta, 2000; Sourivong *et al.,* 2007; Lev-Yadun & Gould, 2009).

At 24 HPT, expression of *PAL* was approximately at baseline levels in all groups (Figure 8). Following 10 d of storage and immediately before inoculation (10 DPT) a slight increase in *PAL* expression, in

comparison to the control, was observed for the treated fruit with 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. 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 effective disease control as observed by Liu *et al.,* (1993) and Scott *et al.,* (2017).

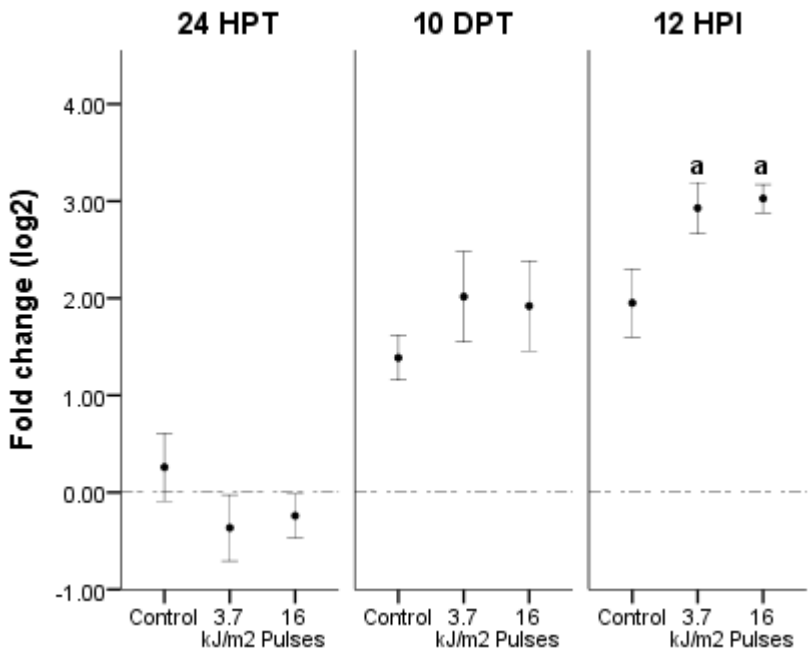

Figure 8: The relative expression of *PAL* (phenylalanine ammonia lyase) following treatment with either 16 pulses from a high-intensity, pulsed polychromatic light (HIPPL) source or 3.7 kJ/m^2 from a low-intensity UV-C (LIUV) source. Samples were taken before treatment, 24 h post treatment (HPT), 10 d post treatment (DPT), immediately before inoculation, and 12 h post inoculation (HPI). Fold changes (log_2) are relative to baseline expression before treatment (dotted line). Labelling indicates

504 statistical significance, within a given time point, where groups sharing labels are not significantly
505 different at $p < 0.05$. N=6. Bars show ± 1S.E.M.

506

507 The results of this study are in agreement with Tiecher *et al.* (2013) who showed an approximately
508 2- to 3-fold increase in *PAL* in the mesocarp of tomato fruit following LIUV treatment at both 1 and 7
509 DPT. The exocarp, however, showed no increase in *PAL* at either 1 or 7 DPT. *PAL* expression,
510 however, was not monitored following inoculation.

511

512 Flavonols are a group of phenolic flavonoid antioxidants which have recently been targeted for
513 enrichment in genetically modified tomato for their health-promoting benefits (Choudhary *et al.,*
514 2016). Following LIUV treatment, total phenolic and flavonoid concentrations have been shown to
515 increase. Flavonol synthase (*FLS*) is directly involved in biosynthesis of flavonols, compounds with
516 important roles in plant-pathogen interactions due to their antioxidant properties.

517 *FLS* expression was decreased at 24 HPT with 5.8- and 2.5-fold higher concentration in the control
518 fruit when compared to the LIUV and HIPPL treatments, respectively (Figure 9). Only the LIUV
519 treatment was significantly different from the control. At 10 DPT, *FLS* expression further decreased
520 with the HIPPL- and LIUV-treated fruit showing 100.3- and 109.1-fold differences when compared to
521 the control. At 12 HPI, *FLS* expression in the control fruit decreased by approximately 4-fold to
522 baseline levels. Expression for both treatments increased to 8.9- and 10.8-fold below the control for
523 HIPPL- and LIUV-treated fruit, respectively. This was still significantly lower than the control.

524 Downregulation of *FLS* would result in decreased biosynthesis of flavonols such as myricetin,
525 quercetin and kaempferol. A previous study by Tiecher *et al.* (2013) reported similar results in LIUV-
526 treated tomato fruit where querecetin concentration was measured by HPLC. Decreased levels were
527 observed in both the exocarp and mesocarp at 1 DPT and 7 DPT with an approximately 4-fold

decrease in treated fruit in comparison to the control at 7 DPT. 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.

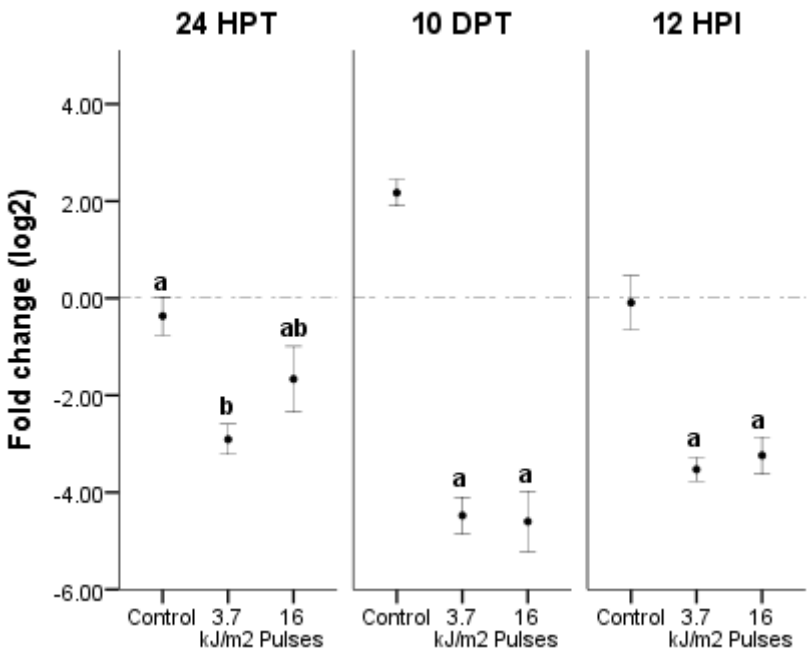

Figure 9: Relative expression of *FLS* (flavonol synthase) following treatment with either 16 pulses from a high-intensity, pulsed polychromatic light (HIPPL) source or 3.7 kJ/m^2 from a low-intensity UV-C (LIUV) source. Samples were taken before treatment, 24 h post treatment (HPT), 10 d post treatment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

3.3 Gene Priming

It has been shown that both biotic and abiotic inducers of disease resistance can prime plant defences, reducing the impact of subsequent phytopathogen attack (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 this case HIPPL and LIUV treatments. In primed plants, transcriptional responses are deployed in a faster, stronger or more sustained manner following the perception of a secondary stress (Martinez-Medina *et al.,* 2016).

Martinez-Medina *et al.,* (2016) defined a number of priming-related expression profile criteria. Firstly, a small or transient change in expression following the initial priming stimulus should be present. To identify this change, we 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 1-9). Excluding *ACO1*, *CRTR-B1* and *PAL*, all of the genes from LIUV- and HIPPL-treated samples, however, showed an increased change in expression at 10 DPT. This indicates that the changes were not transient and may have an increased fitness cost, this is 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 10). *P4*, however, also exhibited an increase in expression at 10 DPT indicating direct induction (Figure 3). Expression levels of *PAL* at 10 DPT, from LIUV and HIPPL

575 treated fruit, is similar to that of the control and, therefore, meets the criteria of a priming-
576 associated expression profile outlined by Martinez-Medina *et al.,* (2016) (Figure 8).

577 With exception of *PAL* all genes investigated in this study appear to be directly induced and fail to
578 meet the expression profile of gene priming; a summary of the results is available in table 2. Further
579 investigations, however, are required to provide conclusive evidence on whether or not priming is
580 following the secondary stimulus, analyses of histone modifications and DNA methylation and
581 monitoring the expression of transcription factors (WRKYs and MYC2) and mitogen-activated protein
582 kinases MPK3 and MPK6 for changes that are associated with priming (Conrath *et al.,* 2015). An
583 involvement for priming in LIUV and HIPPL hormesis, however, is supported by further criteria
584 outlined in Martinez-Medina *et al.,* (2016) such as a more robust defence response and broad-
585 spectrum activity. LIUV hormesis has been shown to induce resistance against a number of
586 pathogens on tomato fruit including *B. cinerea, Rhizopus stolonifer*, *Penicillium expansum* and
587 *Alternaria alternata* (Liu *et al.,* 1993; Stevens *et al.,* 1997). Furthermore, HIPPL hormesis can induce
588 resistance against *B. cinerea* and *P. expansum* on tomato fruit (Scott *et al.,* 2017; unpublished data).
589 This is supported by previous work carried out on *Arabidopsis thaliana* in which LIUV-induced
590 resistance to both downy mildew (*Hyaloperonospora parisitica*) and grey mould (*B. cinerea*) was
591 observed (Kunz *et al.,* 2008; Stefanato *et al.,* 2009).

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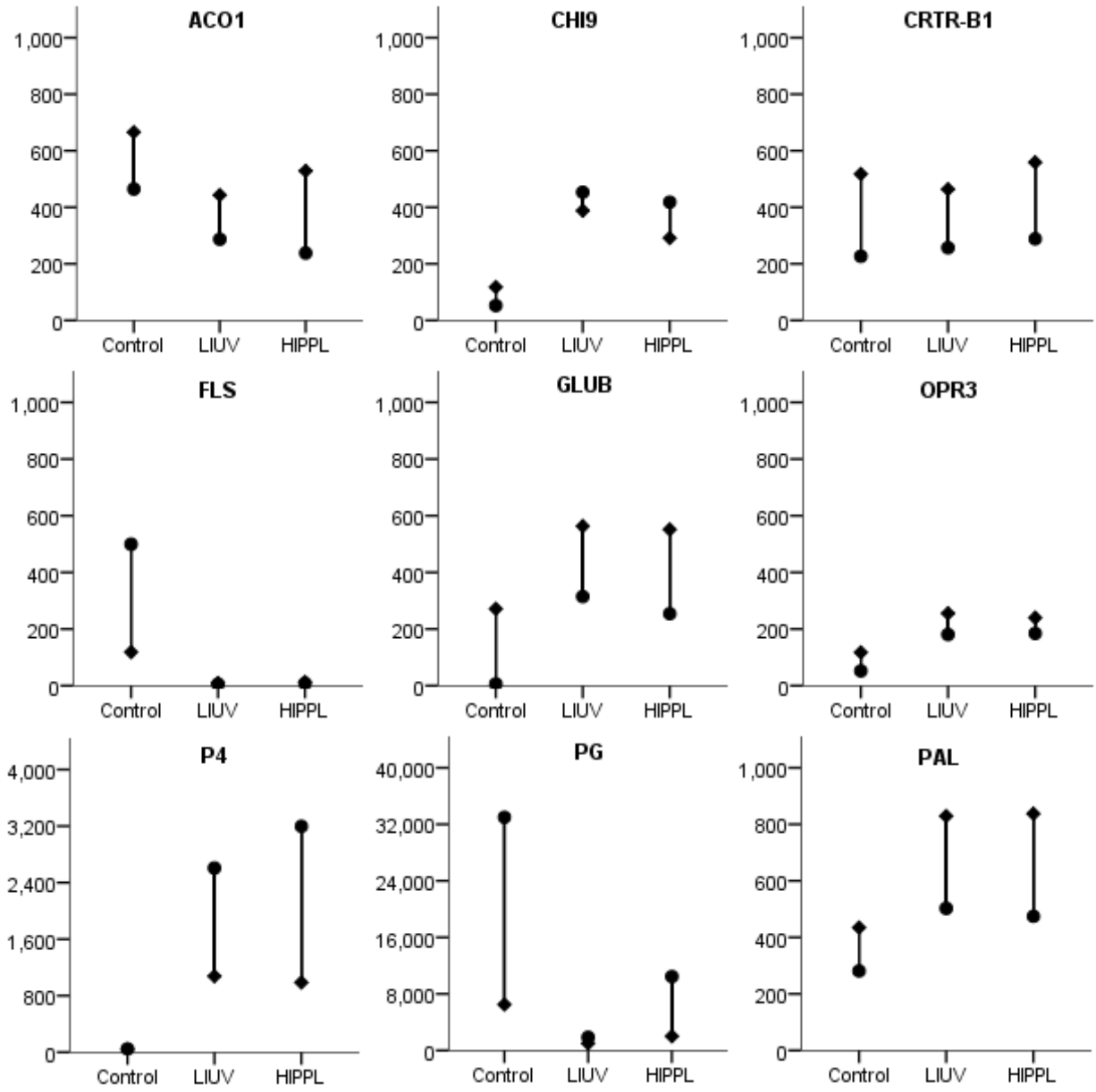

Figure 10: Gene expression levels shown as the change theoretical copy number between samples taken at 10 days post treatment (●) and 12 h post inoculation with *Botrytis cinerea* (♦). The vertical line denotes the magnitude of change. Fruit were treated with either 16 pulses from a high-intensity, pulsed polychromatic light (HIPPL) source or 3.7 kJ/m^2 from a low-intensity UV-C (LIUV) source and compared to the untreated control. Graphs show the following genes; ***ACO1*** (1-aminocyclopropane-1-carboxylic acid oxidase; a bottleneck enzyme in ethylene biosynthesis), ***GLUB*** (β-1,3,-Glucanase an ethylene-inducible pathogenesis-related protein) , ***CHI9*** (chitinase 9 a jasmonic acid-inducible pathogenesis-related protein) ***CRTR-B1*** (β -carotene hydroxylase), ***FLS*** (flavonol synthase), ***OPR3*** (12-

621 Oxophytodienoate reductase 3, a jasmonate acid biosynthesis protein), ***PAL*** (phenylalanine
622 ammonia lyase), ***PG*** (polygalacturonase), ***P4*** (a salicylic acid-inducible pathogenesis-related protein).

623

624 **Table 2:** Gene priming expression profile identifier summary. Criteria are defined as A) a small
625 change following the priming stimulus B) a transient change following the priming stimulus and C) a
626 stronger response following the triggering stimulus; as defined in Martinez-Medina *et al.,* (2016).

Gene	A	B	C	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

627 0 = No and 1 = Yes

628

629 The observed HIPPL- and LIUV-induced resistance may, therefore, be mainly due to increased
630 expression and/or accumulation of transcripts between treatment and the day of inoculation (10
631 DPT). This would result in a gradual increase in resistance following light treatment, similar to that
632 observed by Charles *et al.* (2008) following LIUV treatment of tomatoes. Priming, however, may also
633 play a role in the induction of resistance as an expression profile analogous to that of a priming
634 response can be seen for *PAL.* It is also possible that the priming may have occurred before or after
635 12 HPI was, therefore, not identified in our study. Priming responses have shown greater levels of
636 protein activity and gene expression > 3 h following inoculation (Mur *et al.,* 1996; Latunde-Dada &
637 Lucas, 2001; Cools & Ishii, 2002; Yang *et al.,* 2015). Further investigation is required to elucidate the
638 full extent to which priming may play a role in LIUV- an HIPPL-induced resistance.

639

4. Conclusions

In our previous study (Scott *et al.,* 2017) we showed that 16 pulses of HIPPL induced similar hormetic benefits to a 3.7 kJ/m^2 LIUV treatment on both mature green and ripe tomatoes. Utilising HIPPL reduced treatment times by 97.3 % to only 10 s. In this study, we have monitored the expression of genes involved in ripening, secondary metabolism and defence following HIPPL and LIUV treatments.

On the basis of the genes monitored here, we are now able to 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 *CRTR-B1* were only significantly upregulated at 24 HPT whereas *PAL* was significantly upregulated at 12 HPI. Following inoculation, only *PAL* showed an expression profile analogous to that of a gene priming response. Further investigation is required to conclusively confirm the presence of gene priming.

Importantly, we can infer that HIPPL-induced resistance, similarly to that of LIUV, is due to the upregulation of PR proteins including P4, GLUB and CHI9. 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.,* 2017).

Changes in the expression of phytohormone biosynthesis genes *OPR3* and *ACO1* and 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 HIPPL and LIUV hormesis may provide broad range pathogen resistance against biotrophic and necrotrophic pathogens and also abiotic stressors.

Acknowledgements

The study was financed by the Agriculture and Horticulture Development Board's Horticulture

664 Department (AHDB Horticulture) Project Code- PE 023 and Loughborough University. Tomato fruit
665 were provided by APS Salads (UK) to whom thanks are extended. All experimental studies were
666 carried out in The University of Nottingham's Plant Science department. Finally, thanks are due to
667 Abra Millar for her grammatical input.

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670 **5. References**

671 Aimé S., Cordier, C., Alabouvetter, C. & Olivain, C. (2008). Comparative Analysis of PR Gene
672 Expression in Tomato Inoculated with Virulent Fusarium Oxysporum f.sp. Lycopersici and the
673 Biocontrol Strain F. Oxysporum Fo47. Physiological and Molecular Plant Pathology, 73, pp.9-15.

674 Asselbergh, B. Curvers, K., Franca, S.C., Audenaert, K., Vuylsteke, M., van Breusegem, F. & Hofte, M.
675 (2007). Resistance to *Botrytis cinerea* in *sitiens*, an Abscisic Acid-Deficient Tomato Mutant, Involves
676 Timely Production of Hydrogen Peroxide and Cell Wall Modifications in the Epidermis. Plant
677 Physiology, 144, pp.1863-1877.

678 Barka, E.A., Kalantari, S., Makhlouf, J. & Arul. J. (2000). Impact of UV-C Irradiation on the Cell Wall-
679 Degrading Enzymes During Ripening of Tomato (Lycopersicum esculentum L.) Fruit. Journal of
680 Agricultural and Food Chemistry. 48, pp. 667-671

681 Behboodian, B., Ali Z.M., Ismail, I. & Zainal, Z. (2012). Postharvest Analysis of Lowland Transgenic
682 Tomato Fruit Harbouring hpRNAi-ACO1 Construct. The Scientific World Journal, 2012 [online]
683 doi:10.1100/2012/439870 [Accessed 31/07/2017]

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

687 Bennett, A.B., Chetelat, R., Klann, E., Lashbrook, C., Martin, R. and Gilchrist, D., 1993. Physiologically
688 directed molecular approaches to plant improvement. Trans Malaysian Soc Plant Physiol, 3, pp.200-
689 209.

690 Blanco-Ulate, B., Vincenti, E., Powell, A.L. & Cantu, D. (2013). Tomato Transcriptome and Muntant
691 Analyses Suggest a Role for Plant Stress Hormones in the Interaction Between Fruit and Botrytis
692 Cinerea. Frontiers in Plant Science, 4, online doi: 10.3389/fpls.2013.00142

693 Bosch, M., Wright, L.P., Gershenzon, J., Wasternakc, C., Hause, B., Schaller, A. & Stintzi, A. (2014).
694 Jasmonic Acid and Its Precursor 12-Oxophytodienoic Acid Control Different Aspects of Constitutive
695 and Induced Herbivore Defences in Tomato. Plant Physiology, 166, pp.396-410

696 Bouzayen, M., Cooper, W., Barry, C., Zegzouti, H., Hamilton, A.J. & Grierson, D. (1993). EFE Multigene
697 Family in Tomato Plants: Expression and Characterisation. Current Plant Science and Biotechnology
698 in Agriculture, 16, pp.76-81

699 Bovy, A., de Vos, R., Kemper, M., Schijlen, E., Almenar Pertejo, M., Muir, S., Collins, G., Robinson, S.,
700 Verhoeyen, M., Hughs, S., Santos-Buelga, C. & can Tunen, A. (2002). High-Flavonol Tomatoes
701 Resulting from the Heterologous Expression of the Maize Transcription Factor Genes LC and C1.
702 Plant Cell, 14, pp.2509–2526.

703 Breithaupt, C., Kurzbauer, R., Lile, H., Schaller, A., Strassner, J., Huber, R., Macheroux, P. & Clausen,
704 T. (2006). Crystal Structure of 12-Oxophytodienoate Reductase 3 From Tomato: Self-Inibition by
705 Dimerisation. PNAS, 103, pp.14337-14342

706 Cantu, D., Blanco-Ulatem B., Yang, L, Labavitch, J.M., Bennett, A.B. & Powell, A.L.T. (2009). Ripening-
707 Regulated Susceptibility of Tomato Fruit to Botrytis cinerea Requires NOR But Not RIN or Ethylene.
708 Plant Physiology, 150, pp.1434-1449

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

712 Charles, M.T., Mercier, J., Makhlouf, J., & Arul, J. (2008b). Physiological Basis of UV-C Induced
713 Resistance to Botrytis cinerea in Tomato Fruit. I. Role of Pre- and Post-Challenge Accumulation of the
714 Phytoalexin-Rishitin. Postharvest Biology and Technology, 47, pp.10–20

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

718 Choudhary, D., Pandey, A., Adhikary, S., Ahmad, N., Bhatia, C., Bhambhani, S., Trivedi, P.K. & Trivedi,
719 R. (2016). Genetically Engineered Flavonol Enriched Tomato Fruit Modulates Chondrogenesis to
720 Increase Bone Length in Growing Animals. Scientific Reports, 6: 21668 doi: 10.1038/srep21668

721 Conrath, E., Beckers, G.J.M., Langenback, C.J.G. & Jaskiewicz, M.R. (2015). Priming for Enhanced
722 Defence. The Annual Review of Phytopathology, 53, pp.97-119

723 Cools, H.J. & Ishii, H. (2002). Pre-Treatment of Cucumber Plants with Acibenzola-S-Methyl
724 Systemically Primes a Phenylalanine Ammonia Lyase Gene (PAL1) for Enhanced Expression Upon
725 Attack with a Pathogenic Fungus. Physiological and Molecular Plant Pathology, 61, 273-280

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

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

731 D'Hallewin, G., Schirra, M., Pala, M. & Ben-Yehoshua, 2000. Ultraviolet C Irradiation at 0.5 kJ/m-2
732 Reduces Decay without Causing Damage or Affecting Postharvest Quality of Star Ruby Grapefruit (C.
733 paradisi Macf.). Journal of Agricultural and Food Chemistry, 48, pp.4571–4575

734 Diaz, J., ten Have, A. & van Kan, A.L. (2002). The Role of Ethylene and Wound Signalling in Resistance
735 of Tomato to Botrytis cinerea. Plant Physiology, 129, pp.1314-1315

736 Dong, J.G., Fernández-Maculet, J.C. & Yang, S.F. (1992). Purification and characterization of 1-
737 aminocyclopropane-1-carboxylate oxidase from apple fruit. Proceedings of the National Academy of
738 Sciences, 89, pp.9789–9793

739 Dowen, R.H., Pelizzola, M., Schmitz, R.J., Lister, R., Dowen, J.M., Nery, J.R., Dixon, J.E. & Ecker, J.R.
740 (2012). Widepread Dynamic DNA Methylation in Response to Biotic Stress. PNAS. Online doi:
741 10.1073/pnas.1209329109

742 Ebrahim, S., Usha, K. & Singh, B. (2011). Pathogenesis Related (PR) Proteins in Plant Defence
743 Mechanism. Science Against Microbial Pathogens : Communicating Current Research and
744 Technological Advances (Ed. Mendez-Vilas, A.). Formatex Research Center, Spain.

745 Espinas, N.A., Saze, H. & Saijo, Y.(2016). Epigenetic Control of Defence Signalling and Priming in
746 Plants. Frontiers in Plant Science. Online doi: 10.3389/fpls.2016.01201

747 Galpaz, N., Ronen, G., Khalfa, Z., Zamir, D. & Hirschberg. (2006). A Chromoplast-Specific Carotenoid
748 Biosynthesis Pathways is Revealed by Cloning of the Tomato White-Flower Locus. The Plant Cell, 18,
749 pp.1947-1960

750 Glazebrook, J. (2005). Contrasting Mechanisms of Defence Against Biotrophic and Necrotrophic
751 Pathogens. Annual Review of Phytopathology, 43, pp.205-227.

752 Goellner, K. & Conrath, U. (2008). Priming: It's All the World to Induced Resistance. European Journal
753 of Plant Pathology, 121, pp.233-242

754 González-Aguilar, G. a., Zavaleta-Gatica, R. & Tiznado-Hernández, M.E. (2007). Improving
755 Postharvest Quality of Mango “Haden” by UV-C Treatment. Postharvest Biology and Technology, 45,
756 pp.108–116

757 Hamilton, A.J., Bouzayen, M., & Grierson, D. (1991). Identification of a tomato gene for the ethylene
758 forming enzyme by expression in yeast. Proceedings of the National Academy of Sciences, 88,
759 pp.7434–7437

760 Jagadeesh, S. L., Charles, M. T., Gariepy Y., Goyette B.,. Raghavan, G. S. V,& Vigneault, C. 2011.
761 Influence of postharvest UV-C hormesis on the bioactive components of tomato during post-
762 treatment handling. Food Bioprocess Technol. 4:1463-1472

763 Kasim, R. & Kasim, M.U. (2012). UV-C Treatments on Fresh-Cut Garden Cress (Lepidum sativum L.)
764 Enhanced Chlorophyll Content and Prevent Leaf Yellowing. World Applied Sciences Journal. 17, 509-
765 515.

766 Kauss, H., Theisinger-Hinkel, E., Mindermann, R. & Conrath, U. (1992). Dichloroisonicotinic and
767 Salicylic Acid, Inducers of Systemic Acquired Resistance, Enhance Fungal Elicitor Responses in Prasley
768 Cells. The Plant Journal, 2, pp.655-660

769 King, G.A. & O’Donoghue, E.M. (1995). Unravelling Senescence: New Opportunities for Delaying the
770 Inevitable in Harvested Fruit and Vegetables. Trends in Food Science & Technology, 6, pp.385-389

771 Koyyalamudi, S.R., Jeong, S.C., Pang, G., Teal, A., & Biggs, T. (2011). Concentration of Vitamin D2 in
772 White button Mushrooms (Agaricus bisporus) Exposed to Pulsed UV Light. Journal of Food
773 Composition and Analysis, 24, pp.976-979

774 Kubista, M. & Sindelka, R. (2007). The Prime Technique: Real-Time PCR Data Analysis. G.I.T.
775 Laboroatory Journal, 9-10, pp.33-35

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

779 Langley, K.R., Martin, A., Stenning, R., Murray, A.J., Hobson, G.E., Schuch, W.W. & Bird, C.R. (1994).
780 Ripening of Tomato Fruit with Reduced Polygalacturonase Activity. Journal of the Science of Food
781 and Agriculture, 66, pp.547-554.

782 Latunde-Dada, A.O. & Lucas, J.A. (2001). The Plant Defence Activator Acibenzolar-S-Methyl Primes
783 Cowpea [Vigna unguiculta (L.) Walp.] Seedlings for Rapid Induction of Resistance. Physiological and
784 Molecular Plant Pathology, 58, pp.199-208

785 Lee, H-I., Leon, J. & Raskin, I. (1995). Biosynthesis and Metabolism of Salicylic Acid. Proceedings of
786 the National Academy of Sciences, 92, pp.4076-4079

787 Levy, N.O. Harel, Y.M., Haile, Z.M., Elad, Y., Rav-David, E., Jurkevitch, E. & Katan, J. (2015). Induced
788 Resistance to Foliar Diseases by Soil Solarization and Trichoderma Harzianum. Plant Pathology, 64,
789 pp.365-374

790 Lev-Yadun, S. & Gould, K.S., (2009). Role of Anthocyanins in Plant Defence. Anthocyanins:
791 Biosynthesis, Functions and Applications. (Winefield, C., Davies, K., Gould, K. Eds) Springer; New
792 York, USA

793 Liu, C., Cai, L., Han, X. & Ying, T. (2011). Temporary Effect of Postharvest UV-C Irradiation on Gene
794 Expression Profile in Tomato Fruit. Gene, 486, pp.56-64

795 Liu, C-H., Cai, L-Y., Lu, X-Y., Han, X-X. & Ying, T-J., 2012. Effect of Postharvest UV-C Irradiation on
796 Phenolic Compound Content and Antioxidant Activity of Tomato Fruit During Storage. Journal of
797 Integrative Agriculture, 11, pp.159-165

798 Liu, J., Stevens, C., Khan, V.A., Lu, J.Y., Wilson, C.L., Adeyeye, O., Kabwe, M.K., Pusey, P.L., Chalutz, E.,
799 Sultana, T., Droby, S. (1993). Application of Ultraviolet-C light on storage rots and ripening of
800 tomatoes. Journal of Food Protection, 56, pp.868–872.

801 Liu, L.H., Zabaras, D., Bennett, L.E., Aguas, P. & Woonton, B.W., (2009). Effects of UV-C, Red Light
802 and Sun Light on the Carotenoid Content and Physical Qualities of Tomatoes During Post-Harvest
803 Storage. Food Chemistry, 115, pp. 495-500

804 Livak, K.J. & Schmittgen, T.D. (2001). Analysis of Relative Gene Expression Data Using Real-Time
805 Quantitative PCR and the 2-ΔΔCT Method. Methods, 25, pp.402-408

806 Lu, J., Stevens, C., Yakabu, P., Loretan, P., Eakin, D. (1987). Gamma, Electron Beam and Ultraviolet
807 Radiation on Control of Storage Rots and Quality of Walla Onions. Journal of Food Processing and
808 Preservation, 12, pp. 53-62

809 Maharaj, R., Arul, J. & Nadeau, P. (1999). Effect of Photochemical Treatment in the Preservation of
810 Fresh Tomato (Lycopersicon esculatum cv. Capello) by Delaying Senescence. Postharvest Biology and
811 Biotechnology, 15, pp.13-23

812 Mares-Perlman, J.A., Millen, A.E., Ficek, T.L. & Hankinson, S.E. (2002). The body of evidence to
813 support a protective role for lutein and zeaxanthin in delaying chronic disease. Journal of Nutrition,
814 132 pp.518S-524S

815 Martinez-Medina, A., Flors, V., Heil, M., Mauch-Mani, B., Pieterse, C.M.J., Pozo, M.J., Ton, J., van
816 Dam, N.M. & Contrath, U. (2016). Recognizing Plant Defence Priming. Cell Press: Trends in Plant
817 Science, 21, pp.818-822

818 Mercier, J., Roussel, D., Charles, M-T. & Arul, J. (2000). Systemic and Local Responses Associated with
819 UV-C and Pathogen-Induced Resistance to Botrytis cinerea in Stored Carrot. Phytopathology, 90,
820 pp.981–986

821 Mur, L.A.J., Naylor, G., Warner, S.A.J, Sugars, J.M., White, R.F. & Draper. (1996). Salicylic Acid
822 Potentiates Defence Gene Expression in Tissue Exhibiting Acquired Resistance to Pathogen Attack.
823 The Plant Journal, 4, 559-571

824 Oms-Oliu, G., Aguilo-Aguayo, I., Martin-Belloso, O., & Soliva-Fortuny, R. (2010). Effects of Pulsed
825 Light Treatments on Quality and Antioxidant Properties of Fresh-Cut Mushrooms (Agaricus bisporus).
826 Postharvest Biology and Technology, 56, pp.216-222

827 Pataro, G., Sinik, M., Capitoli, M.M., Donsi, G. & Ferrari, G. (2015). The Influence of Post-Harvest UV-
828 C and Pulsed Light Treatments on Quality and Anitoxidant Properties of Tomato Fruits During
829 Storage. Innovative Food Science and Emerging Technologies, 30, pp.103-11

830 Pecker, I., Gabbay, R., Cunningham, F.X. & Hirschberg, J. (1996). Cloning and Characterization of the
831 cDNA for Lycopene beta-cyclase from Tomato Reveals Decrease in its Expression During Fruit
832 Ripening. Plant Molecular Biology, 30, 807-819

833 Perkins-Veazie, P., Collins, J.K. & Howard, L. (2008). Blueberry Fruit Response to Postharvest
834 Application of Ultraviolet Radiation. Postharvest Biology and Technology, 47, pp.280–285

835 Pfaffl, M.W. (2004). Quantification Strategies in Real-Time PCR. A-Z of Quantitative PCR. (Bustin, S.A.
836 Eds.) USA, pp.87-112.

837 Pietta, P.G., (2000). Flavonoids as Antioxidants. Journal of Natural Products, 63, pp.1035-1042.

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

841 Puthoff, D.P., Holzer, F.M., Perring, T.M. & Walling, L.L. (2010). Tomato Pathogenesis-Related Protein
842 Genes are Expressed in Response to Trialeurodes vaporium and Bemisia tabaci Biotype B Feeding.
843 Journal of Chemical Ecology, 36, pp.1271-1285

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

846 Ribeiro, C., Canada, J., & Alvarenga, B. (2012). Prospects of UV Radiation for Application in
847 Postharvest Technology. Emirates Journal of Food and Agriculture, 24, pp.586-597

848 Rodov, V., Vinokur, Y. & Horev, B. (2012). Brief Postharvest Exposure to Pulsed Light Stimulates
849 Coloration and Anthocyanin Accumulation in Fig Fruit (*Figus carica L.).* Postharvest Biology and
850 Technology, 68, pp.43-46.

851 Romanazzi, G., Gabler, F.M. & Smilanick, J.L. (2006). Preharvest Chitosan and Postharvest UV
852 Irradiation Treatments Suppress Gray Mold of Table Grapes. Plant Disease, 9, pp.445–450.

853 Scalschi, L., Sanmartin, M., Camanes, G., Troncho, P., Sanchex-Serrano, J.J., Garcia-Augustin, P. &
854 Vicedo, B. (2015). Silencing of OPR3 in Tomato Reveals the Role of OPDA in Callose Deposition
855 During the Activation of Defence Responses against *Botrytis cinerea*. The Plant Journal, 81, pp.304-
856 315.

857 Schaller, F., Biesgen, C., Mussig, C., Altmann, T. & Weiler, E.W. (2000). 12-Oxophytodienoate
858 reductase 3 (OPR3) is the Isoenzyme Involved in Jasmonate Biosynthesis. Planta, 210, pp.979-984

859 Scott, G., Rupar, M., Fletcher, A.G.D., Dickinson, M. & Shama, G. (2017). A Comparison of Low
860 Intensity UV-C and High Intensity Pulsed Polychromatic Sources as Elicitors of Hormesis in Tomato
861 Fruit. Postharvest Biology and Technology, 125, pp.52-58

862 Sell, S. & Hehl, R. (2005). A Fith Member of the Tomato 1-Aminocyclopropane-1-Carboxylic Acid
863 (ACC) Oxidase Gene Family Harbours a Leucine Zipper and is Anaerobically Induced. DNA Seq, 16,
864 pp.80-82

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

867 Song, J. & Bent, A.F. (2014). Microbial Pathogens Trigger Host DNA Double-Strand Breaks Whose
868 Abundance Is Reduced by Plant Defense Responses. PLoS Pathogens, 10. Online
869 doi:10.1371/journal.ppat.1004030

870 Sourivong, P., Schronerova, K. & Babincova, M. (2007). Scoparone Inhibits Ultraviolet Radiation-
871 Induced Lipid Peroxidation. Naturforsch, 62, pp.61-64.

872 Spoel, S.H. & Dong, X. (2012). How do plants achieve immunity? Defence without specialized
873 immune cells. Nature Reviews Immunology, 12, pp.89–100.

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

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

880 Tadmor, Y., King, S., Levi, A., Davis, A., Meir, A., Wasserman, B., Hirschberg, J. & Lewinsohn, E.
881 (2005). Comparative fruit colouration in watermelon and tomato. Food Research International, 38,
882 pp.837–841

883 TATAABiocenter. (2012). TATAA Interplate Calirator: SYBR protocol. Online
884 http://www.tataa.com/wp-content/uploads/2012/10/TATAA-
885 Manual_Interplate_Calibrator_SYBR_v01_1.pdf

886 Tiecher, A. Arantes de Paula, L., Chaves, F.C. & Rombaldi, C.V. (2013). UV-C Effect on Ethylene,
887 Polyamines and the Regulation of Tomato Fruit Ripening. Postharvest Biology and Technology, 86,
888 pp.230–239

889 Turtoi, M. (2013). Ultraviolet Light Treatment of Fresh Fruits and Vegetables Surface: A Review.
890 Journal of Agroalimentary Processes and Technologies, 19, pp.325-337

van de Poel, B., Bulens, I., Markoula, A., Hertog, M.L.A.T.M., Dreesen, R., Wirtz, M., Vandoninck, S., Oppermann, Y., Keulemans, J., Hell, R., Waelkens, E., de Proft, M.P., Sauter, M., Nicolai, B.M. & Geeraerd, A.H. (2012). Targeted Systems Biology Profiling of Tomato Fruit Reveals Coordination of the Yang Cycle and a Distinct Regulation of Ethylene Biosynthesis During Postclimacteric Ripening. Plant Physiology, 160, pp.1498-1514

Van Hulten, M., Pelser, M., van Loon, L.C., Pieterse, C.M.J., Ton, J. (2006) Costs and benefits of priming for defence in Arabidopsis. Proceedings of the National Academy of Sciences U.S.A., 103, pp.5602–5607

van Kan, J.A.L., Joosten, M.H.A.J., Wagemakers, C.A.M., van den Berg-Velthuis, G.C.M. & de Wit P.J.G.M. (1992). Differential Accumulation of mRNAs Encoding Extracellular and Intracellular PR Proteins in Tomato Induced by Virrulent and Avirrulent Races of Cladosporium fulvum. Plant Molecular Biology, 20, pp.513-527

Virk, N., Liu, B., Zhang, H., Li, X., Zhang, Y., Li, D., Song, F. (2013). Tomato SlMPK4 is Required for Resistance Against Botrytis Cinerea and Tolerance to Drought Stress. Acta Physiologiae Plantarum, 35, pp.1211-1221

Walters, D.R. & Fountain, J.M. (2009). Practical Application of Induced Resistance to Plant Diseases: An Appraisal of Effectiveness under Field Conditions. Journal of Agricultural Science, 147, pp.523-535.

Walters, D.R., Ratsep, J. & Havis, N.D. (2013). Controlling Crop Diseases Using Induced Resistance: Challenges for the Future. Journal of Experimental Botany, 64, pp.1263–1280

Wu, C-T., & Bradford, K.J. (2003). Class I Chitinase and β-1,3-Glucanase Are Differentially Regulated by Wounding, Methyl Jasmonate, Ethylene and Giberellin in Tomato Seeds and Leaves. Plant Physiology, 133, pp.263-273

914 Xie, Q., Hu, Z., Zhu, Z., Dong, T., Zhao, Z., Cui, B. & Chen, G. (2014). Overexpression of a Novel MADS-
915 box gene SlFYFL Delays Senescence, Fruit Ripening and Abscission in Tomato. Scientific Reports, 4.
916 Online doi:10.1038/srep04367

917 Yan, S., Wang, W., Marques, J., Mohan, R., Saleh, A., Durrant, W.E., Song, J. & Dong, X. (2013).
918 Salicylic Acid Activates DNA Damage Responses to Potentiate Plant Immunity. Molecular Cell, 52,
919 pp.602-610

920 Yang, W., Xu, X., Li, Y., Wang, Y., Li, M., Wang, Y., Ding, X. & Chu, Z. (2015). Rutin-Mediated Priming
921 of Plant Resistance to Three Bacterial Pathogens Initiating the Early SA Signal Pathway. PLOS ONE.
922 Online DOI:10.1371/journal.pone.0146910

923 Yuan, H., Zhang, Z., Nageswaran, D. & Li, L. (2015). Carotenoid Metabolism and Regulation in
924 Horticultural Crops. Horticulture Research, 2. Online doi:10.1038/hortres.2015.36

925 Zhefeng, L., Hong, Y., Yin, M., Li, C., Zhang, K. & Grierson, D. (2008). A Tomato HD-Zip Homeobox
926 protein, LeHB-1, Plays an Important Role in Floral Organogenesis and Ripening. The Plant Journal, 55,
927 pp. 301-310.