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Modulations of visible light irradiance effects the photosynthetic phenotype in UV-B exposed *Arabidopsis thaliana*

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Abstract

Photosynthesis is dependent upon energy provided by visible light from the electromagnetic spectrum. While such wavelengths of light are vital for resource assimilation to take place, we now also understand that other wavelengths of light may likely alter a plant's photosynthetic capability, including the ultraviolet (UV) radiation spectrum. The ultraviolet spectrum includes UV-A (315nm-400nm) and UV-B radiation (280nm-315nm). UV-B light has been of particular interest in recent years as changes in the ozone has resulted in increased UV-B radiation levels reaching the Earth's surface. Such scientific interest has resulted in many subsequent studies trying to understand how plants protect themselves against this powerful waveband. UV-B response in plants has been linked to both physiological and molecular changes in plants. That could be manipulated to protect plants against pathogens and increase crop yields. The quite recent discovery of the UV-B specific photoreceptor UVR8 showed how plants to respond to UV-B. A molecular pathway has begun to take shape for UVR8, with interactions with the transcription factors COP1 and HY5 necessary for activation. What is less understood are the subsequent interactions genes have with UVR8, to cause responses such as flavonoid accumulation and photosynthetic competency.

After previous research showed an increase in photosynthetic rate in lettuce in response to UV-B radiation this study aimed to find the photosynthetic response of *Arabidopsis thaliana* and possibly re-create the increase. To do this the photosynthetic rate was studied under various PAR levels alongside UV-B exposure to characterise the photosynthetic response. The accumulation of photo-protective compounds was also studied to see if their accumulation affected photosynthetic responses. Three different lines were studied; *Columbia-0, Landsberg erecta* and *uvr8-1*. The *uvr8-1* plants provided information on whether UVR8 is necessary for photosynthetic competency in Arabidopsis. qPCR studies of genes linked to the UVR8 pathway were also considered for their role in photosynthetic competency. The results in this thesis will show that manipulations of PAR, changes the UV-B photosynthetic response and that UVR8 is necessary for photosynthetic competency. ELIP1 and SIG5 are not mediated by UVR8 for photosynthetic competency. ELIP1 and SIG5 are possibly involved in UVR8 mediated accumulation of photo-protective compounds.

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Abbreviations

A_{max}	net photosynthetic rate
BLRP	blue light responsive promoter
bp	base pairs
CFCs	chlorofluorocarbons
CHI	CHALCONE ISOMERASE
CHS	CHALCONE SYNTHASE
Col-0	*Columbia-0*
COP1	CONSTITUTIVELY PHOTOMORPHOGENIC 1
CP12	CHLOROPLAST PROTEIN 12
CP12-1	CHLOROPLAST PROTEIN 12-1
CP12-2	CHLOROPLAST PROTEIN 12-2
CP12-3	CHLOROPLAST PROTEIN 12-3
CRY	Cryptochrome
cry1	cryptochrome1
cry2	cryptochrome2
DAS	Days after sowing
ELIP1	EARLY LIGHT-INDUCIBLE PROTEIN 1
ELIP2	EARLY LIGHT-INDUCIBLE PROTEIN 2
FAD	Flavin Adenine Dinucleotide
FLS	FLAVONOL SYNTHASE
FMN	Flavin Mononucleotide
FR	far-red light
GADPH	glyceraldehyde-3-phosphate
HY5	ELONGATED HYPOCOTYL 5
HYH	HY5 HOMOLOG
IRGA	Infra-red gas analyser
Ler	*Landsberg erecta*
LOV	light, oxygen, voltage
MAPK	mitogen-activated protein kinases
MT	Metal halides
MTHF	methenyltetrahydrofolate
MYB111	MYB DOMAIN PROTEIN 111
MYB12	MYB DOMAIN PROTEIN 12
NBI	nitrogen balance index
nm	Nanometers
PAR	Photosynthetically active radiation
PCR	polymerase chain reaction
PHR1	PHOTOLYASE 1
PHY	Phytochrome
PHYA	phytochrome A
PHYB	phytochrome B
PHYE	phytochrome E

PKR	Phosphoribulokinase
PSII	photosystem II
qPCR	quantitative PCR
R	red light
RFR	red: far-red light
ROS	reactive oxygen species
RUP1	REPRESSOR OF UV-B PHOTOMORPHOGENESIS 1
RUP2	REPRESSOR OF UV-B PHOTOMORPHOGENESIS 2
SE	Standard error
SIG5	SIGMA FACTOR 5
TRP	Tryptophan
UV	Ultraviolet
UV-A	Ultraviolet-A
UV-B	Ultraviolet-B
UV_{BE}	Biologically effective UV
UV-C	Ultraviolet-C
UVR3	UV REPAIR DEFECTIVE 3
UVR8	UV RESISTANCE LOCUS 8
VOC	volatile organic compounds
WFT	White fluorescent tubes
WL	White light
WUE	water use efficiency
ZTL	zeitlupes

1. Introduction

1.1. Light in Plants

Light is one of the most important environmental factors for plants; without light photosynthesis cannot occur as discussed by Bryant and Frigaard (2006). For plants, light is a reference to the visible part of the electromagnetic spectrum which lies between the wavelengths of 400-700 nanometres (nm) and ultraviolet (UV) light – which is just below visible light at 100nm-400nm. Both visible light and UV light are divided into smaller wavebands that have specific responses in plants. Visible light is usually broken down into blue light (400nm-500nm), green light (500nm-570nm) and red: far-red light (RFR) (620nm-700nm). Whereas UV light has three wavebands, these are UV-A (315nm-400nm), UV-B (280nm-315nm) and UV-C (100nm-280nm). It is important to note however, that UV-C has generally not studied in plants. This is due to the wavelength not making it through the Earth's ozone layer; therefore plants are never exposed to it naturally.

Extensive research has been completed on how plants respond to the various wavebands (Kami, Lorrain, Hornitschek, & Fankhauser, 2010); with blue light and RFR studied most comprehensively (Hiltbrunner et al., 2006; Lian et al., 2011). Both blue light and RFR have photoreceptors which are proteins that absorb specific wavelengths; thus, activating a signalling cascade which starts and stops many different cellular processes (Kleine, Kindgren, Benedict, Hendrickson, & Strand, 2007; Roig-Villanova, Bou, Sorin, Devlin, & Martinez-Garcia, 2006). All photoreceptors have a region known as the chromophore which specifies the wavelength that is absorbed by that photoreceptor (Bongards & Gartner, 2008).

The RFR photoreceptor group is phytochromes, which has been discussed in greater detail by P.H. Quail et al. (1995), they also absorb small amounts of blue light and UV-A. The chromophore for phytochromes is phytochromobilin. As discussed by Heijde and Ulm (2012); Lin (2000), there are three groups of photoreceptors for blue light and UV-A called: cryptochromes, phototropins and zeitlupes. The chromophores for cryptochromes are Flavin Adenine Dinucleotide (FAD) and methenyltetrahydrofolate

(MTHF) (Liu, Liu, Zhao, Pepper, & Lin, 2011). Whereas, phototropins and zeitlupes have the same chromophore, which is Flavin Mononucleotide (FMN) binding through the light, oxygen, voltage (LOV) domains (Heijde & Ulm, 2012). The ranges of these photoreceptors are illustrated in Figure 1.1.

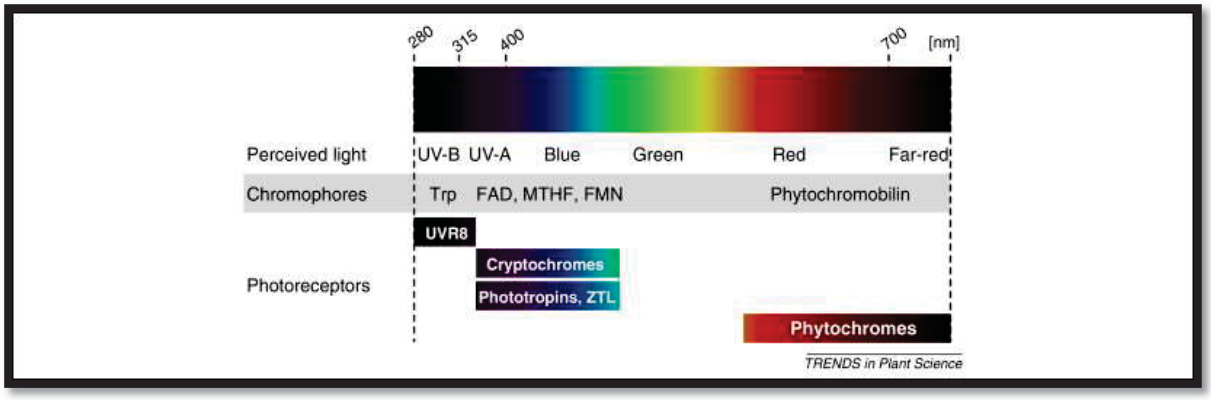

Figure 1.1. Representation of light spectrum and associated photoreceptors. Chromophores are signal receivers of the photoreceptors. Tryptophan (Trp) is the chromophore for UVR8. Cryptochromes bind to Flavin Adenine Dinucleotide (FAD) and methenlytetrahydrofolate (MTHF). Phototropins and zeitlupes (ZTL) proteins bind Flavin Mononucleotide (FMN) through the LOV (light, oxygen, voltage) domains. Phytochromes bind to the chromophore phytochromobilin. From Heijde and Ulm, 2012.

In *Arabidopsis thaliana* two cryptochromes (cry1 and cry2) have been identified which have different cellular functions. cry1 controls hypocotyl extension under blue light (Ahmad & Cashmore, 1993) as well as petiole expansion and expansion of the leaf lamina (Jackson & Jenkins, 1995). Conversely, cry2 controls stem extension at low fluence rates of blue light before the protein is broken down by higher levels post translation level (Lin et al., 1998). In Arabidopsis there are 5 known phytochromes (PHYA-PHYE) that are responsible for controlling different physiological responses (P. H. Quail, 2002; Schafer & Bowler, 2002; Smith & Whitelam, 1997). PHYA controls seedling etiolation under continuous far-red (FR) light whereas PHYB controls etiolation under continuous red light (R) (Chen, Chory, & Fankhauser, 2004; P. H. Quail, 2002; Schafer & Bowler, 2002).

Until recently there was no known photoreceptor for UV-B, however, Rizzini et al. (2011), described a protein; UV Resistance Locus 8 (UVR8) which responds only to UV-B light. The UVR8 protein was known to respond to UV-B radiation (Kliebenstein, Lim, Landry, & Last, 2002), yet until the study undergone by Rizzini et al. (2011) it was not

considered a photoreceptor. Early research on UV-B exposure in plants was often conducted under unrealistic radiation levels, including unnaturally high UV-B:PAR ratios, and over-estimations of local solar UV-B levels, as based on modelled increases under extreme ozone depletion scenarios. Such experiments led to the consensus that UV-B is detrimental to plants due to the severe impairments on physiological functions and plant death (Frohnmeyer & Staiger, 2003; Jansen, Gaba, & Greenberg, 1998; Jordan, 1996; Rozema, van de Staaij, Bjorn, & Caldwell, 1997). These conclusions however, made little sense as plants in nature have not shown these severe impairments to natural levels of UV-B radiation (Jenkins & Brown, 2007; Rozema et al., 2002). Subsequent research has shown that although UV-B does impair plant performance in some ways it can be beneficial (Bornman & Vogelmann, 1991; Cen & Bornman, 1993; Wargent, Elfady, Moore, & Paul, 2011).

1.2. Why look at UV responses in plants?

The importance of understanding the responses of plants to UV is twofold; the changes in stratospheric ozone that has been seen over the last few decades and secondly the demand for crops to yield more for the Earth's increasing population. Firstly, the ozone layer filters the majority of UV that the sun emits; it completely cuts out UV-C radiation and the majority of UV-B radiation. However, with depletions in the ozone layer the amount of UV-B radiation making it to the Earth's surface has increased. The increases in UV-B level were associated to the use of chlorofluorocarbons (CFCs), a widely used organic compound depleting the ozone layer and it is unknown when or if the ozone layer will fully recover (McKenzie, Aucamp, Bais, Bjorn, & Ilyas, 2007). The implementation of the Montreal Protocol by the United Nations banned the production of CFCs, has resulted in reports of some recovery in the ozone layer (Steinbrecht et al., 2009). The lower the latitude and/or the higher the altitude, the more UV-B radiation reaches plants; moreover, in areas of ozone depletion UV-B radiation is higher than normal at any given latitude or altitude(McKenzie, Bodeker, Scott, Slusser, & Lantzc, 2006). As identified by Seckmeyer et al. (2008) latitudes in the Southern Hemisphere receive up to twice as much UV-B as comparable latitudes in the Northern Hemisphere.

The changes in the ozone layer are of significant concern to growers as increased UV exposure is typically associated with negative connotations such as DNA damage, which can greatly decrease the chance of a successful crop (Frohnmeyer & Staiger, 2003; Jordan, 1996). However, as argued by Rozema et al. (1997) plants rarely exhibit visible signs of damage due to UV in nature, as plants have mechanisms to protect themselves. In addition, historical studies involving high ambient, or above ambient light conditions have led to severe impairments reported in plant physiological responses (Frohnmeyer & Staiger, 2003; Jansen et al., 1998; Jordan, 1996; Rozema et al., 1997), holding back research into understanding how a plant can protect itself from UV damage. With increasing levels of UV-B being reported due to depleted ozone, research has begun to look at more natural levels. These newer studies report increases in yield and plant efficiency (Davey et al., 2012; Wargent, Gegas, Jenkins, Doonan, & Paul, 2009).

Secondly, due to the Earth's increasing population the need to produce higher yielding crops is of the utmost importance (TheRoyalSociety, 2009). Over the last few decades increasing crop yields are largely a result of the use of agrichemicals, fertilizers and intense breeding programmes (Evenson & Gollin, 2003; Zhu et al., 2000). However, the increases in crop yields has begun to plateau (Godfray et al., 2010), thus increasing the pressure on researchers to find new ways to maintain yield growth.

Understanding how plants can be manipulated by the growing environment is one way in which researchers have looked to increase yields; another is through the use of genetic modification of plants as discussed in Wargent and Jordan (2013). Genetic modification brings with it many challenges, including its application being restricted due to ethical concerns throughout much of the world. Manipulating the growing environment however, is not restricted and UV radiation is of particular interest due to the responses such as higher yields (Wargent et al., 2011). Figure 1.2 is an example of the many interactions of UV with plants (Wargent & Jordan, 2013) and how the responses could affect overall crop quality. Similarly, wider research has shown changes in response to UV in plant morphology (Davey et al., 2012; Hectors et al., 2010; Wargent et al., 2011; Wargent, Nelson, McGhie, & Barnes, 2015), as well as biotic interactions such as plant pathogen protection (Nigel D. Paul & Gwynn-Jones,

2003; N. D. Paul, Jacobson, Taylor, Wargent, & Moore, 2005). Such previous research and findings has led to a need for a greater understanding of how UV affects plant mechanisms and how such understanding could lead to countless agricultural benefits.

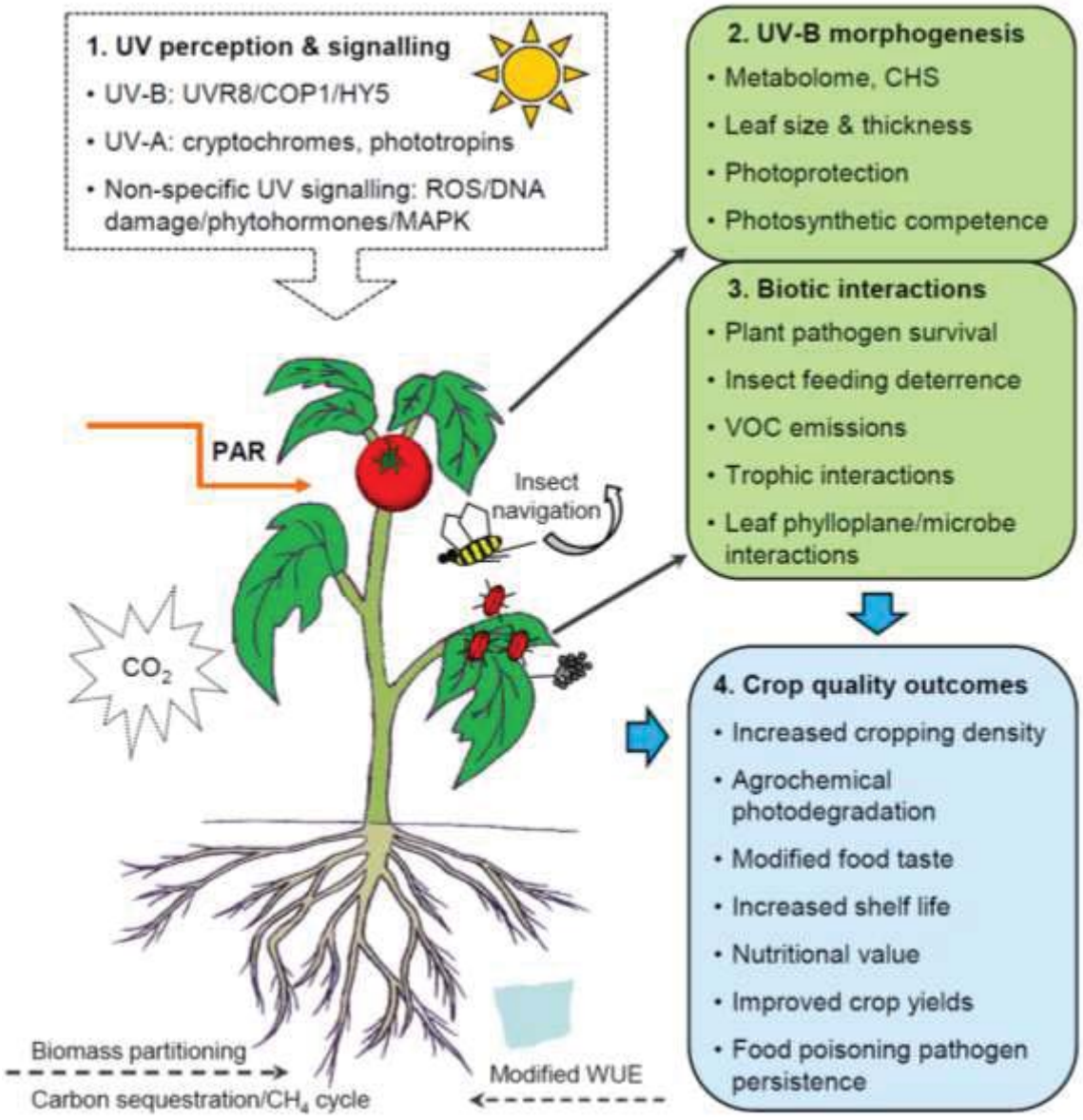

Figure 1.2. Possible interactions of UV with plant morphology and crop quality outcomes. (1) Dashed box; UV-A and UV-B signalling pathways. Thin black arrows indicate early-stage responses to UV, including (2) morphological responses and (3) biotic interactions. (4) Blue arrows indicate crop quality outcomes from responses to UV (hypothesised and observed). Main image highlights typical abiotic factors influencing UV response. Dashed lines below soil describe root-soil processes influenced by UV-plant interactions. CHS, CHALCONESYNTHASE; COP1, CONSTITUTIVELYPHOTOMORPHOGENIC1; HY5, ELONGATEDHYPOCOTYL5; MAPK, mitogen-activated protein kinases; PAR, photosynthetically active radiation; ROS, reactive oxygen species; UVR8, UV RESISTANCE LOCUS 8; VOC,volatile organic compounds; WUE, water use efficiency. From Wargent and Jordan, 2013.

1.3. Classic Responses to UV-B

1.3.1. Morphological responses

As plants are constantly exposed to UV-B radiation in nature, many studies have been carried out to understand the morphological and physiological changes attributed to UV-B exposure (Hectors et al., 2010; Jansen, 2002; Searles, Flint, & Caldwell, 2001). These studies have been conducted under a wide range of conditions and across many different plant species, such as *Arabidopsis thaliana, Lactuca sativa* and *Trifolium repens*. Morphological changes include inhibited leaf expansion (Hectors et al., 2010; Searles et al., 2001), increased leaf thickness (M.E Poulson, Boeger, & Donahue, 2006; Staxen & Bornman, 1994), increased leaf mass per unit area (Laposi et al., 2009; Sprtova, Spunda, Kalina, & Marek, 2003), as well as reduction in the number of leaves plants grow (Hofmann & Campbell, 2011; Krizek, Britz, & Mirecki, 1998; Krizek, Mirecki, & Britz, 1997). Moreover, previous research highlights UV-B radiation reducing hypocotyl growth (Favory et al., 2009) and stimulation of axillary branching in roots and shoots (Jansen, 2002). However, as argued by Hectors et al. (2010) not all morphological changes are positive, as the inhibition of leaf expansion does not affect cell division or shape and is only in pavement cells and is therefore considered a 'negative' morphological trait.

1.3.2. Physiological responses

Classic physiological responses include change in net photosynthetic rate, transpiration rate and metabolomics changes particularly of secondary metabolites (Frohnmeyer & Staiger, 2003; Jansen et al., 1998; Mackerness et al., 1999; Wargent et al., 2011; Wargent et al., 2015). Secondary metabolites such as flavonoids provide a 'sun-screening' effect in plants, protecting them from further damage from UV-B (Bassman, 2004; Wargent et al., 2015). The understanding of this effect is of importance; although plants respond to UV-B radiation, plants still need to protect themselves otherwise UV-B can damage macromolecules and impair cellular processes within plants (Caldwell, Bornman, Ballare, Flint, & Kulandaivelu, 2007; Frohnmeyer & Staiger, 2003; Jordan, 1996). Following Wargent et al. (2015), exposure of *Lactuca sativa* seedlings to UV-B radiation increased the net photosynthetic rate as well as a faster accrual of UV shielding. Whereas Berli, Alonso, Bressan-Smith, and Bottini (2013)

described, in grapes, a decrease in photosynthetic rate. Moreover, Feher et al. (2011), identified that UV-B radiation also plays a part in helping train the circadian clock in plants, possibly through transcriptional activation of various cellular processes. As a result, it has been suggested that applying UV-B radiation helps to save resources within the plant as it adapts to UV (Feher et al., 2011).

1.3.3. Known Molecular responses of genes of interest

1.3.3.1. UVR8

As aforementioned, Rizzini et al. (2011), proposed that the protein UVR8 is the UV-B photoreceptor. However, Kliebenstein et al. (2002), argue that UVR8 induces the flavonoid biosynthesis pathway but does not go as far as to suggest that it is a photoreceptor. Rizzini et al. (2011), propose that the UVR8 is the photoreceptor of UV-B as: (1) no UV-B specific morphological responses in *uvr8* mutant; (2) That tryptophan-285 is the 'chromophore' of the structure and; (3) the tryptophan is excited in the presence of UV-B causing the homodimer to monomerise.

In its inactive form UVR8 is a homodimer and is found in the cytoplasm and the nucleus; upon UV-B irradiation the protein monomerizes and localizes to the nucleus (Favory et al., 2009; Kaiserli & Jenkins, 2007). The monomer then interacts in a UV-B dependent manner with the E3 ubiquitin ligase COP1 (CONSTITUTIVELY PHOTOMORPHOGENIC 1) (Favory et al., 2009). COP1 is an important regulator of UV-B and visible light signalling, interacting with the blue and RFR photoreceptors also (Oravecz et al., 2006; Yi & Deng, 2005). The UVR8-COP1 construct then interacts with HY5 (ELONGATED HYPOCOTYL 5), a promotor, to produce a UV-B mediated photomorphogenic response as shown in Figure 1.3 (Brown et al., 2005; Oravecz et al., 2006; Osterlund, Wei, & Deng, 2000; Saijo et al., 2003; Yi & Deng, 2005) HYH (HY5 HOMOLOG) is another promotor that is partially redundant with HY5, although there is evidence of some genes that are specific to each promotor (Brown & Jenkins, 2008; Feher et al., 2011; Stracke et al., 2010). Moreover, there is evidence of a negative feedback regulation of UVR8 by interaction with RUP1 and RUP2 (REPRESSOR OF UV-B PHOTOMORPHOGENESIS 1/2) (Gruber et al., 2010). *RUP1* and *RUP2* are transcriptionally activated by UV-B in a UVR8/COP1/HY5 dependent manner and

independently of COP1 redimerize UVR8 therefore, stopping UV-B photomorphogenesis (Heijde et al., 2013). Transcriptome analysis of wild-type and mutant *uvr8* Arabidopsis plants found over 100 genes regulated by UVR8 (Wargent & Jordan, 2013). These genes are involved in many processes, such as: DNA repair, chloroplast function and secondary metabolite formation (Figure 1.3).

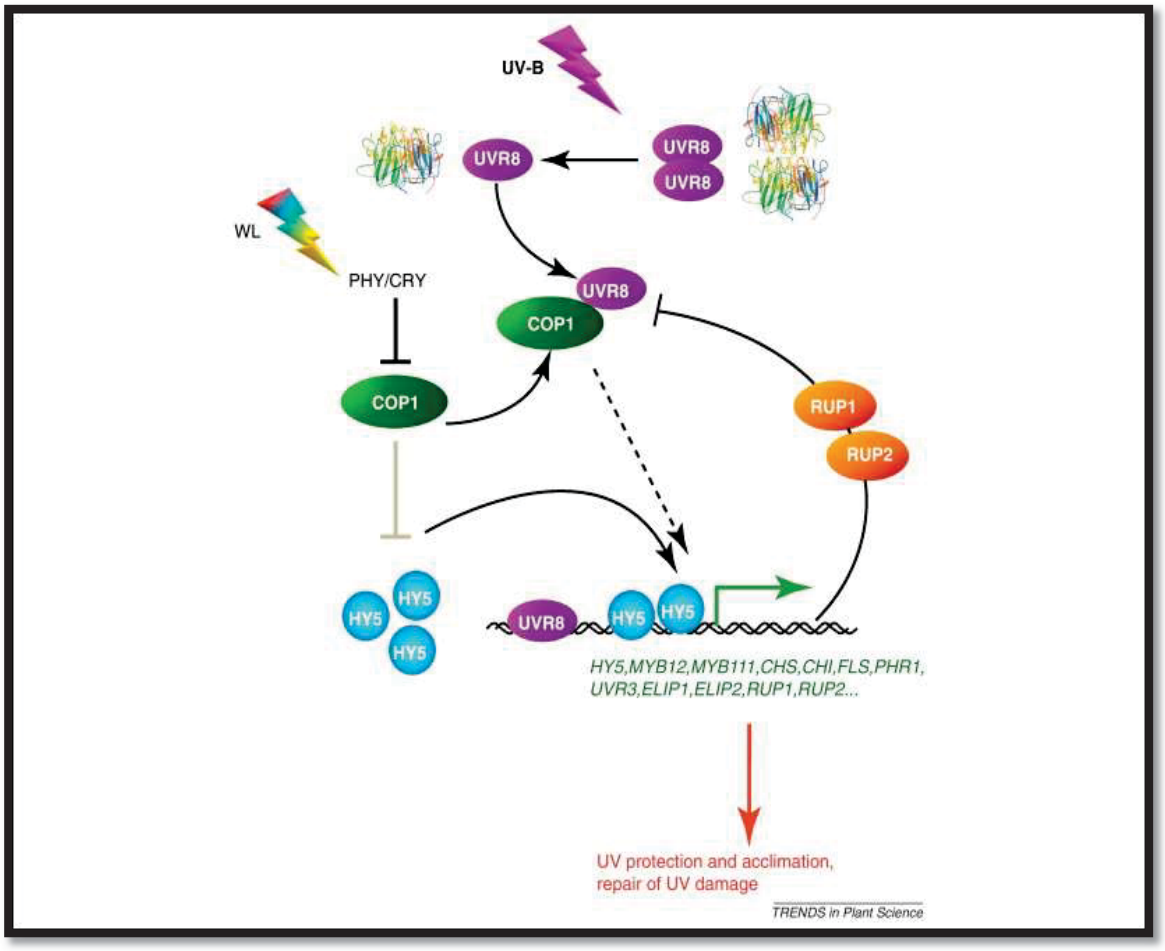

Figure 1.3. Model of UVR8 mediated signalling. In light (WL) conditions with no UV-B present, UVR8 is a homodimer and COP1 promotes the degradation of HY5. COP1 also is negatively controlled by phytochromes and cryptochromes. In the presence of UV-B radiation UVR8 monomerises. COP1 then interacts with the UVR8 monomer and HY5 is stabilised. Gene expression of UV-B specific genes goes ahead including those for flavonoid biosynthesis, DNA repair and also the creation of the negative feedback proteins RUP1 and RUP2. Abbreviations: CHI, CHALCONE ISOMERASE; CHS, CHALCONE SYNTHASE; CRY, cryptochrome; COP1, CONSTITUTIVELY PHOTOMORPHOGENIC 1; ELIP1 and ELIP2, EARLY LIGHT-INDUCIBLE PROTEIN 1 and 2; FLS, FLAVONOL SYNTHASE; HY5, ELONGATED HYPOCOTYL 5; MYB12 and MYB111, MYB DOMAIN PROTEIN 12 and 111; PHR1, PHOTOLYASE 1; PHY, phytochrome; RUP1 and RUP2, REPRESSOR OF UV-B PHOTOMORPHOGENESIS 1 and 2; UV-B, ultraviolet-B radiation; UVR3, UV REPAIR DEFECTIVE 3; UVR8, UV RESISTANCE LOCUS 8; WL, white light. From Heijde and Ulm, 2012.

1.3.3.2. CHS

CHS (CHALCONE SYNTHASE) is the first enzyme in the phenylpropanoid biosynthesis pathway to form flavonoids (Weisshaar & Jenkins, 1998). As established by Jenkins, Long, Wade, Shenton, and Bibikova (2001), CHS has been shown to responds to high light as well as UV-B radiation. Furthermore, Jenkins et al. (2001), postulated that CHS is activated by a UV-B specific photoreception pathway as mutant cryptochrome plants still had a transcriptional response to UV-B exposure. This photoreception pathway was later identified as through the UV-B specific photoreceptor UVR8 (Favory et al., 2009). As a result, CHS is often studied alongside other genes when looking for a UV-B response; as CHS transcription will always increase in the presence of UV-B, thus, it is used to confirm that any responses recorded are due to UV-B radiation.

1.3.3.3. ELIPs

ELIPs (EARLY LIGHT INDUCIBLE PROTEINs) are proteins involved in a plants response to light stress (Rossini et al., 2006). As suggested by Hutin et al. (2003), ELIPs have a photoprotective function, such as protecting chlorophyll from damage. Likewise, Potter and Kloppstech (1993), support the theory that ELIPs have a photoprotective role in plants, as the ELIPs are not found in plants until plants are light stressed. However, the source or process of how ELIPs protect chlorophyll is still unknown, as Davey et al. (2012), identified that ELIP is not needed for the maintenance of the efficiency of photosystem II (PSII). Furthermore, Davey et al. (2012), have reported large fold inductions in transcripts of ELIPs to UV-B exposure which suggests that ELIPs are involved in UV-B photomorphogenesis – yet the knowledge as to how the proteins do this is still unknown.

1.3.3.4. SIG5

SIG5 (SIGMA FACTOR 5) is one of six sigma factors found in Arabidopsis that are induced under various stress conditions (Nagashima et al., 2004). SIG5 is known to respond to blue light, with the induction of *psb*D transcripts to control the response of BLRP (blue light responsive promoter) (Lerbs-Mache, 2011; Nagashima et al., 2004; Tsunoyama et al., 2004). Davey et al. (2012) highlighted that SIG5 responds to UV-B light as well as blue light to mediate the transcription of *psb*D-BLRP. However, there was no impairment of PSII efficiency in sig5 mutants in UV-B, therefore, the role in

which SIG5 plays in UV-B photomorphogenesis is not yet fully comprehended (Davey et al., 2012).

1.3.3.5. CP12

CP12 (CHLOROPLAST PROTEIN 12) is a small regulatory protein that interacts with glyceraldehyde-3-phosphate (GADPH) in chloroplasts (Pohlmeyer, Paap, Soll, & Wedel, 1996). Arabidopsis has three different forms of CP12 (CP12-1, -2 and -3) and each has different expression levels within Arabidopsis (Marri, Sparla, Pupillo, & Trost, 2005; Singh, Kaloudas, & Raines, 2008; Trost et al., 2006). CP12 regulates the formation of GADPH and phosphoribulokinase (PKR) into a supramolecular complex as part of the Calvin cycle (Marri, Trost, Pupillo, & Sparla, 2005; Oesterhelt et al., 2007). CP12-2 is the only CP12 protein that is light dependent, with CP12-1 and CP12-3 only active in dark tissues (Marri, Sparla, et al., 2005; Trost et al., 2006). However, the light signals that activate CP12-2 are yet to be fully understood. Thus, it was theorised that UV-B radiation could play a part in its activation, and therefore, studied as a part of this thesis.

1.4. Effects of PAR on UV-B responses

Plants respond to visible light and UV radiation simultaneously. As such different ratios of visible light to UV radiation cause different molecular and physiological responses. Studies such as Bolink, Schalkwijk, Posthumus, and Hasselt (2001) and M.E. Poulson, Donahue, Konvalinka, and Boeger (2002) have described how a UV-B treatment before high light exposure lowers photoinhibition. This is consistent over a range of plant species including; beans and peas (Bolink et al., 2001), Douglas fir seedlings (M.E. Poulson et al., 2002), *Arabidopsis thaliana* (M.E Poulson et al., 2006) and pumpkins (Hakala-Yatkin, Mantysaari, Mattila, & Tyystjarvi, 2010). Gotz et al. (2010) described how the changes in PAR effected the accumulation of flavonoids and that this accumulation gives Arabidopsis a basic level of protection against UV-B exposure.

1.5. Project and Aims

This project is a follow on from the study by Wargent et al. (2015) on *Lactuca sativa* in which increasing net photosynthesis paralleled by increases in secondary metabolites in the presence of UV-B exposure. To continue this research the plant studied was

changed to *Arabidopsis thaliana* due to the large amount of research already conducted on these plants' responses to UV-B. Thus, the project aims were targeted towards recreating this increase in photosynthetic rate, as well as assessing whether UVR8 regulates certain genes to get the increased photosynthetic rate in *Arabidopsis thaliana.*

Project aims:

1. Quantify the photosynthetic response to UV-B radiation under various light regimes in *Arabidopsis thaliana*
2. Identify signalling changes that occur along a discrete timescale under different growing conditions
3. Compare wild-type Arabidopsis responses with the mutant *uvr8-1* under different UV-B light regimes

2. Materials and Methods

2.1. Plant Material

The plant studied in this research was *Arabidopsis thaliana*. Three different lines were used, including the two wildtype genotypes: *Columbia-0* (*Col-0*), *Landsberg erecta* (*Ler*), and also the *uvr8-1* mutant, in which UVR8 fails to monomerise (Brown et al., 2005). The *Col-0* seeds were provided with the assistance of Professor Paul Barnes of Loyola University, USA. The *Ler* and *uvr8-1* seed were provided with the assistance of Professor Gareth Jenkins, University of Glasgow, Scotland. *uvr8-1* is a mutant in which there is a 15 base pair deletion in the UVR8 gene. The *uvr8-1* seed are from the *Ler* line.

2.2. Growing Environments

2.2.1. Nursery Set-up

The nursery environment was a temperature-controlled room with banks of lights hanging from the ceiling situated at the Plant Growth Unit, Massey University, Palmerston North. The temperature of the room was set to 20 ±3 ^{o}C. Each bank of lights had four white fluorescent tubes; the height at which the lights hung above the table was adjustable (Figure 2.1A). When the photosynthetic active radiation (PAR) in the nursery room was at 50 µmol m^{-2} s^{-1} PAR there was one bank on either side of the room above two tables. To raise the light level to 240 µmol m^{-2} s^{-1} PAR an extra bank was added to each side of the room while also lowering the banks closer to the plants (Figure 2.1B). The PAR was measured using an Optronics 756 spectroradiometer equipped with integrating sphere. Plants were regularly moved around beneath the lights so that the plants got an even spread of PAR. The lights were on for 8 hours a day.

2.2.2. Growth Chamber Set-up

The growth chamber used in this research was a Contherm 630 Environment Controlled Growth Cabinet from Contherm Scientific Ltd (Petone, New Zealand) which was situated at the Plant Growth Unit, Massey University, Palmerston North. The cabinet was equipped with a bank of metal halide (MH) bulbs (Phillips HPI T Plus,

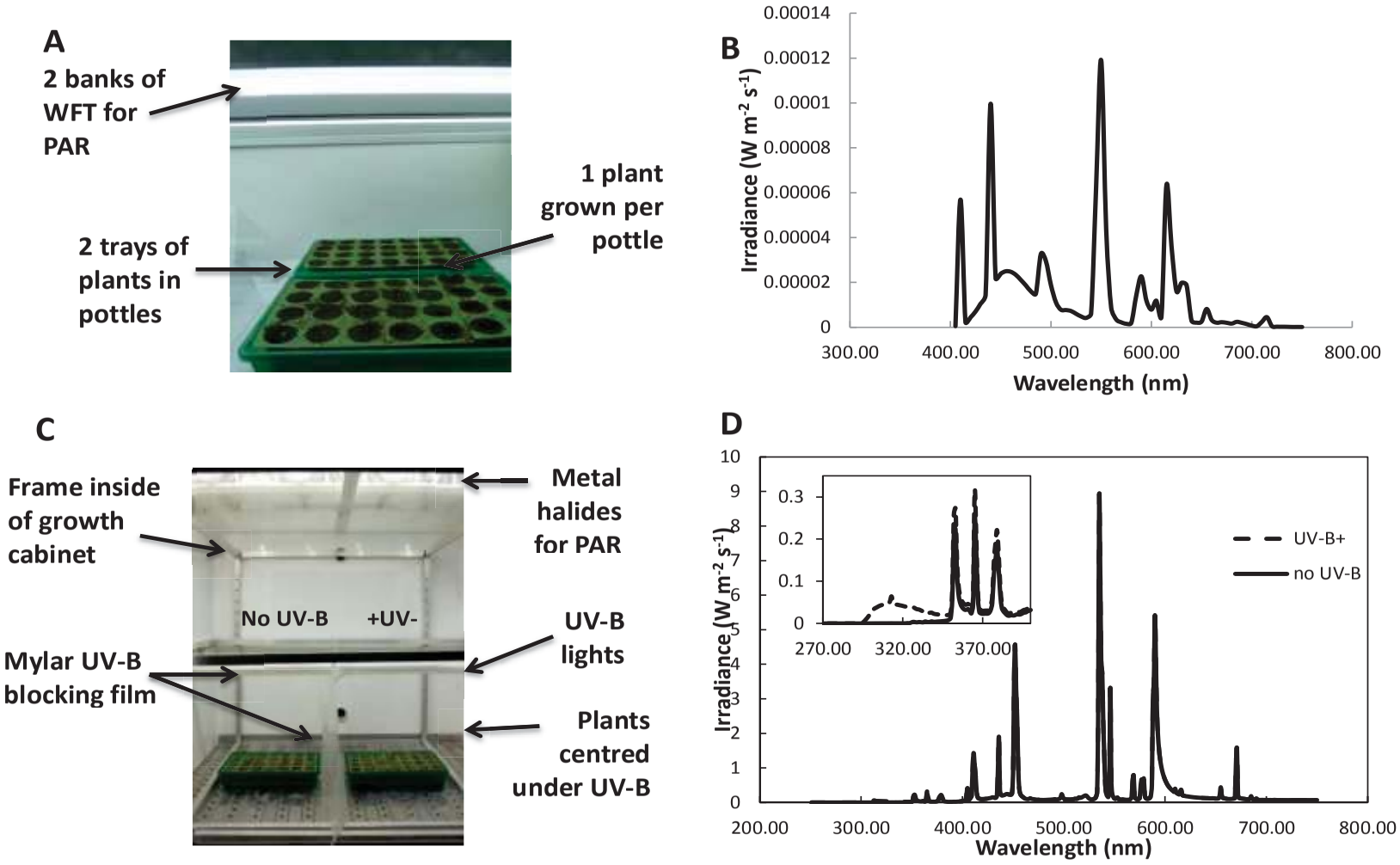

Figure 2.1. Set-up of Nursery room and Growth Chamber and Spectral Irradiances of both environments. (A) Set-up of plants in Nursery room, with two banks of white fluorescent tubes (WFT) above plants. (B) Spectral irradiance of nursery room describing visible light spectra. (C) Set-up of growth Chamber with UV-B lights and plants. (D) Spectral Irradiance of growth chamber. Both UV-B+ (dashed line) and no UV-B (solid line) sides and describe visible light spectra. Insert graph is the UV region of spectra in chamber.

Eindhaven, The Netherlands) that control the amount of photosynthetic active radiation (PAR). Furthermore, the growth chamber controlled the temperature and humidity of the environment. As the lights do not emit ultraviolet-B (UV-B) radiation a specially designed frame was put in so that UV-B fluorescent bulbs (Q-Panel 313; Q-Lab Corp, Cleveland, OH, USA) could be added (Figure 2.1B). The UV-B lights were covered in 0.13mm cellulose diacetate (Clarifoil; Courtlands Ltd, Derby, UK) to cut out any radiation below 290nm. The cellulose diacetate was replaced before every experiment. A non UV-B area was created within the chamber by covering one half of the UV-B bulbs with the UV-B opaque film Mylar (Lee Filters, Andover, UK) as well as a sheet of Mylar down the middle of the cabinet as seen in Figure 2.1B. The Mylar film stops any wavelengths below 320nm, as seen in the spectra in Figure 2.1C. For all experiments the temperature was set to 20^{o}C while the lights were on and 17 ^{o}C when the lights were off. The humidity in the chamber was kept constant at 65%. The MH lights were on for 12 hours every day with the UV-B lights on for 11 hours every day, starting 30 minutes after the MH lamps, the levels of which can be found in Table 2.1. The biologically effective UV-B dose was measured using the spectroradiometer and weighted using the Caldwell (1971) generalized plant action spectrum, normalized to unity at 300nm.

2.3. Experimental Conditions

Over the time of this thesis a variety of different growing and experimental conditions were used. The details of which can be seen in Table 2.1 below.

Table 2.1. Growing conditions of experiments in which data was collected. Experiments conducted in order seen in table.

Plant Lines	Nursery Environment	Pre-treatment Age & Light settings	Age at UV exposure	Growth Chamber Environment during UV exposure	Experimental Question
Col-0	Glasshouse (21days) to growth chamber 360µmol m^{-2} s^{-1} [MH] (5days) to growth room 50µmol m^{-2} s^{-1} [WFT] (13days)		40DAS	7.2 UV_{BE} $kJm^{-2}day^{-1}$ 720µmol m^{-2} s^{-1} [MH]	Does manipulation of PAR levels during plant growth affect UV-B response?
Col-0	Growth room 50 µmol m^{-2} s^{-1}		44DAS	10.2 UV_{BE} $kJm^{-2}day^{-1}$ 720µmol m^{-2} s^{-1} [MH]	
Col-0	Growth room 50µmol m^{-2} s^{-1} for 23days 240µmol m^{-2} s^{-1} for 18days		41DAS	10.2 UV_{BE} $kJm^{-2}day^{-1}$ 360µmol m^{-2} s^{-1} [MH]	
Col-0	Growth room 240µmol m^{-2} s^{-1}		28DAS & 35DAS	10.2 UV_{BE} $kJm^{-2}day^{-1}$ 360µmol m^{-2} s^{-1} [MH]	Does plant age affect the UV-B photosynthesis phenotype, or photo-protecting pigmentation?
Col-0	Growth room 240µmol m^{-2} s^{-1}		28DAS & 35DAS	10.2 UV_{BE} $kJm^{-2}day^{-1}$ 360µmol m^{-2} s^{-1} [MH]	
Col-0	Growth room 240µmol m^{-2} s^{-1}	21DAS 360µmol m^{-2} s^{-1}	28DAS	10.2 UV_{BE} $kJm^{-2}day^{-1}$ 360µmol m^{-2} s^{-1} [MH]	How does pre-treatment with higher PAR affect UV-B photosynthetic response?
Col-0	Growth room 240µmol m^{-2} s^{-1}	23DAS 360µmol m^{-2} s^{-1}	30DAS	10.2 UV_{BE} $kJm^{-2}day^{-1}$ 360µmol m^{-2} s^{-1} [MH]	
Col-0	Growth room 240µmol m^{-2} s^{-1}	23DAS 360µmol m^{-2} s^{-1}	30DAS	10.2 UV_{BE} $kJm^{-2}day^{-1}$ 360µmol m^{-2} s^{-1} [MH]	
Ler & *uvr8-1*	Growth room 240µmol m^{-2} s^{-1}	24DAS 360µmol m^{-2} s^{-1}	31DAS	10.2 UV_{BE} $kJm^{-2}day^{-1}$ 360µmol m^{-2} s^{-1} [MH]	What is the role of the UVR8 photoreceptor in UV-B photosynthetic response?

DAS=Days after sowing, MH= Metal Halides, UV_{BE}= Biologically effective UV, WFT= white fluorescent tubes

2.4. Planting Method

The preparation for planting Arabidopsis seeds was important to this research so that the seeds germinated as evenly as possible. The soil used for this research was Oderings Nurseries Seed Raising Mix. It was sieved to remove the larger soil components and mixed with sand that was also sieved to the same fineness. The soil was then put into detachable seedling pottles. To make sure that the pottles were completely full water was poured over to help make it settle, more soil was then added to the pottles that needed topping up. The pottles were then put into trays lined with moist capillary matting in a 4 by 7 arrangement. The Arabidopsis seeds were then sown with 2-3 seeds per pottle. Once all seeds were sown clear fitted lids were placed with vents closed on top of the trays. The trays were then kept in a refrigerator in the dark for 4 days at 5^{o}C. This was done to simulate a winter chill so that the seeds would germinate evenly. Trays were then moved into the nursery environment with lids on vents closed for one week. After one week the vents are opened for 2 days before lids are removed completely. For consistency whenever days after sowing (DAS) is mentioned this refers to the days from removal from the refrigerator.

2.5. Physiological Measurements

For this research various physiological measurements were taken both destructive and non-destructive.

2.5.1. Photosynthetic Measurements

The infra-red gas analyser (IRGA) used in this research was the Li6400XT Portable Photosynthesis System from Li-Cor Biosciences, Lincoln, Nebraska, USA. To make the system compatible with measuring Arabidopsis plants the attachments 6400-17 Whole Plant Arabidopsis Camber and 6400-18 RGB Light Source were used. The basic set up and warm-up procedures were followed as per the protocol provided with the machine. There were three parameters that had to be set as per the experimental requirements; these were the CO_2 level, temperature of the cuvette and the light level. The first two remained the same for all experiments at 400ppm and 20^{o}C respectively. The light level changed depending on the experimental set up (Table 2.1). When the Arabidopsis were exposed to 750 µmol m^{-2} s^{-1} PAR the light level in the IRGA was set to

1000 µmol m^{-2} s^{-1} PAR. When exposed to 360 µmol m^{-2} s^{-1} PAR the IRGA was set to 500 µmol m^{-2} s^{-1} PAR. This was done to provide a saturating level of PAR to the plants, to get the highest net photosynthetic rate (A_{max}) the plants were capable of. Each plant (in pottle) was placed into the cuvette for at least 5 minutes to ensure a steady state was reached. Plants were removed from the experimental environment just before being measured and then placed back after measurements. All measurements were done during the middle of the light period when plants are the most active.

2.5.2. Leaf metabolism and Secondary metabolite measurements

The Dualex Scientific + from Force-A, Paris, France, was used to non-destructively measure internal leaf metabolism and secondary metabolites. These were anthocyanins, flavinoids, chlorophyll and NBI (nitrogen balance index). All of these were measured using arbitrary units; the NBI index is a ratio of the chlorophyll to flavonoids measured (Cerovic, Masdoumier, Ghozlen, & Latouche, 2012). The cuvette on the Dualex was used on one leaf of each Arabidopsis plant. The leaf measured had to be big enough for the cuvette to fit around without removing it from the plant and also measurable from the first day of the experiment. The measurement is instantaneous with plants measured after the IRGA measurements and before being placed back into the growth cabinet.

2.5.3. Destructive Harvesting

The destructive harvesting in this research involved measuring the Arabidopsis' leaf area and fresh and dry weights. For each *Col-0* experiment a sample of 10 plants was destructively harvested per treatment, before transfer to the growth cabinet; then all plants used in the experiments were harvested on the last day. For the *Ler/uvr8-1* experiment 9 plants were harvested on Day 0 for each treatment; followed by all plants used in the experiment being harvested. The whole rosette was cut from the roots at the soil level and the plants were initially measured for fresh weight before being passed through the leaf area machine. Next, the plants were bagged and placed into a drying oven at 70^{o}C for one week until a constant mass was reached. Once a constant mass was achieved the plants were weighed a second time to obtain the dry weight of each plant.

2.6. Molecular Measurements

2.6.1. Plant Material Preparation

For the molecular measurements the Arabidopsis needed to be harvested using liquid nitrogen to snap freeze the plants. This had to be done promptly so that no degradation of cell contents could occur. Frozen samples were then moved while still in liquid nitrogen to a -80^{o}C freezer. Samples were then prepared for RNA isolation by grinding each plant in liquid nitrogen. The plants used in the molecular measurements were *Ler* and *uvr8-1* plants, whereby four plants were frozen at specific time points. The time points were; before transfer to pre-treatment (24DAS), four hours after pre-treatment (24DAS), and four hours after plants from pre-treatment and nursery were transferred to the experimental environment (UV-B+ and no UV-B, 31DAS). The plants used were grown under the same conditions as described in Table 2.1 for *Ler/uvr8-1* plants.

2.6.2. Primer Design

The primers used in the qPCR for this project were designed around three principles: firstly, a melting point between 55^{o}C and 60^{o}C; secondly, approximately 50% Guanidine and Cytosine content and; thirdly, approximately 20 base pairs (bp) long. Primers were obtained from Sigma-Aldrich New Zealand Ltd (Auckland, NZ). The primer pairs used in this research are listed below in Table 2.2. The last two genes in Table 2.2 are reference genes, selected from Czechowski, Stitt, Altmann, Udvardi, and Scheible (2005) for the qPCR.

Table 2.2. Primer sequences used for qPCR.

Gene	Forward Primer	Reverse Primer	Product Size (bp)
UVR8	GCTCTTCTCTCTGGTGACA	CCACAGGTAACGGAAACAA	140
CHS	AGCGCATGTGCGACAAGTC	TCTGGTGTCCAGAGA AGG	113
ELIP1	CATGGCTGAGGGAGGAC	AACGCTAGCAAGTCGCTAA	192
SIG5	CGAGGTAGTTGAGAGACTCA	TCAATGAATCGAGCACATCG	210
CP12-2	ACAACTAACCGGATGATGAAA	ATCAGCCTTCTTCTTGTCTCTA	201
UBC9	TCACAATTTCCAAGGTGCTGC	TCATCTGGGTTTGGATCCGT	61
AT2G32170	ATCGAGCTAAGTTTGGAGGATGTAA	TCTCGATCACAAACCCAAAATG	61

2.6.3. RNA Isolation

The RNA was isolated from the Arabidopsis samples using the Quick-RNA Mini Prep kit from Zymo Research (Irvine, CA, USA). 50µg of each sample was used in the isolation procedure, with any excess placed back in -80^{o}C freezer. The protocol provided was followed with only two modifications. In the first modification, the samples were kept on ice for 10 minutes after the addition of the RNA Lysis Buffer. The second modification was that the DNAse treatment was not carried out at the point that the protocol suggested but as described in section 2.6.3.2.

2.6.3.1. RNA Quantification

After the RNA was isolated it then had to be quantified so that precise amounts of sample could be used for subsequent steps. This was done using the Nanodrop ND-1000 from Thermo Fisher Scientific (Wilmington, DE, USA). The protocol provided was followed.

2.6.3.2. DNAse Treatment

For the DNAse treatment 5µg of RNA from each sample needed to be re-suspended with DNA/RNA free water to add up to a volume of 42µL. using the equation 5000/x ng/µL=volume. This calculates the amount of sample needed as part of the 42µL. Then 5µL of 10xDNAse Reaction Buffer and 1µL of DNAse I was added to each sample and incubated for 20 minutes at 37^{o}C. As soon as incubation was complete 2µL of 0.2M EDTA was added to each sample and incubated at 75^{o}C for 5mins. Samples were then put back on ice. Before cDNA synthesis each sample was then tested using one of the reference gene primers in a polymerase chain reaction (PCR) and then gel electrophoresis.

2.6.3.3. cDNA synthesis

To create the cDNA for the samples the Transcriptor First Strand cDNA Synthesis kit from Roche Diagnostics Corporation (Indiana, In, USA) was applied. This protocol provided instructions for three different methods. The protocol followed for this research was for the Anchored-oligo(dT)$_{18}$ Primer. Once the samples had been through the protocol the product was checked for the presence of cDNA by PCR and gel electrophoresis.

2.6.4. Polymerase Chain Reaction (PCR)

To test the samples for DNA contamination and also CDNA confirmation PCR amplification was carried out. Each PCR reaction contained; 1µL Forward primer, 1µL Reverse Primer, 10µL of 2x Promega Master Mix for PCR, 7µL PCR water and 1µL sample (10 fold diluted). All reagents were mixed together in a PCR Eppendorf tube and centrifuged to make sure the reagent mixture was all at the bottom. The Eppendorf tubes were then placed into a Biometra Thermocycler from Innovative Sciences Ltd (Dunedin, NZ). The PCR programme was:

95°C for 5min
95°C for 10sec ┐
60°C for 10sec ├ Repeated for 40 cycles
72°C for 10sec ┘
72°C for 10min
Hold at 10°C

Once the PCR programme was completed, samples were then removed from the thermocycler and prepared for gel electrophoresis.

2.6.5. Gel Electrophoresis

For all gels in this research a 1% TBE gel made from TBE buffer was used. To do this 1g of Agarose was added to 100mL of TBE buffer and then heated in microwave until combined. The mixture was then poured whilst still hot into the gel mould and left to set for 20minutes. Once set and just before loading the samples, TBE buffer was poured into the gel mould in the gel dock. The samples were prepared by adding 3µL of loading dye to 4µL of PCR product. A 1kb ladder (Hyper Ladder I, Bioline, UK) was added to the first well of every gel and then prepared samples added to the next wells. The gel was then run for 40mins at 100V. Once the gel finished running, the gel was placed into ethidium bromide for 10 minutes. Following this process, the gel was photographed to show whether any bands were present. For the DNAse contamination no bands should be present, whereas in the cDNA check, bands should be present to confirm that cDNA was created.

2.6.6. Quantitative Polymerase Chain Reaction (qPCR)

For the preparation of the qPCR plates the protocol from the Light Cycler 480 SYBR Green I Master kit from Roche Diagnostics Corporation (Indianapolis, IN, USA) was followed. One adjustment was made to the protocol of halving all of the quantities of reagents used to ensure that the final volume added to 10µL. The plates were then run in a Light Cycler 480 using a pre-set programme. All samples were run on the same Light Cycler 480 at Massey University, Palmerston North. Each replicate was repeated three times on the same plate. The output from this was saved to a USB and converted to a readable formatting using the programmes:

- Convert Light Cycler 480 Raw Data text file into Input Format for LinREG PCR (version 2)
- LinREG PCR: Analysis of quantitative RT-PCR Data (version 2014.4)

These programmes were downloaded from http://www.hartfaalcentrum.nl/index.php?main=files&sub=LinRegPCR

The data was then transformed into a reportable format following the protocol from (Pfaffl, 2001).

2.7. Statistical Analysis

All statistical analysis for this research was done using Microsoft Excel and IBM SPSS Statistics (SPSS Statistics v20; IBM, Armonk, NY, US). When referring to significances through-out this thesis:

- Significant = $P<0.05$
- Very significant = $P<0.01$
- Highly significant = $P<0.001$

2.7.1. Physiological Measurements

A replicate in the physiological measurements refers to one plant. After plants were measured for photosynthetic rate and/or leaf metabolites and secondary metabolism the plants were put back into the Growth Chamber. When a plant was put back it would be placed in a different location from where it was removed, within the no UV-B side or UV-B+ side. No plants were transferred between the no UV-B and UV-B+

treatments during an experiment. Table 2.3 indicates on which day's photosynthetic measurements were taken and also the number of replicates within each treatment. Table 2.4 indicates the days on which measurements were taken and the number of replicates per treatment for leaf metabolism and secondary metabolites.

Each set of data was initially statistically analysed using repeated measures on IBM SPSS Statistics. The within-subjects variables were the days the plants were measured. The between-subjects variables were experimental repeat, line, plant age, pre-treatment and UV treatment; where appropriate for each experiment. To make the correct choice of significance source the following rules were followed from Collier, Baker, Mandeville, and Hayes (1967):

- If Mauchly's test of sphericity was not significant then sphericity is assumed;
- If Mauchly's test was significant then the epsilon values were looked at;
- When the epsilon values were <0.75 then the Green-Giesser source was used;
- If epsilon values were >0.75 then the Huynh-Feldt source was used and;
- If the epsilon values were inconclusive then the source with the most conservative significances, other than the sphericity assumed source, was used.

The graphs found in each figure listed in Tables 2.3 and 2.4 were created using Microsoft Excel. For the leaf area measurements and weights the replicate number was 10 for both Day 0 and harvest day measurements. One way ANOVA tests were completed using Microsoft Excel for any significant differences between treatments on days which showed separation of points on the graphs.

2.7.2. qPCR statistics

In the qPCR data a replicate is one plant, with four replicates harvested for each time point. When carrying out the qPCR 3 technical repeats were used for each replicate. There were 7 different genes measured using qPCR and every replicate was measured for its relative transcription level. Due to the large amount of replicates measured not all replicates for a gene (both *Ler* and *uvr8-1*) could fit on the same qPCR plate. To ensure that run differences were not significant one replicate from each time point was run on every qPCR plate.

The data collected was then transformed using the protocol from Schmittgen and Livak (2008) in Microsoft Excel. The transformed data was then compared using one way ANOVAs to discover significances between the treatments within a gene and between the *Ler* and *uvr8-1* lines.

Table 2.3. Photosynthetic measurements replicate numbers. Highlighted squares indicate the days on which photosynthetic measurements were taken. Treatment numbers refers to the number of graph variables. Replicate number is the number of plants measured for each treatment. Day 0 is the measurement before UV treatment.

Figure (from results)	Days measured							Treatment number	Replicate number (per treatment)
	0	1	2	3	4	5	6		
3.1.								2	4
3.2.								2	5
3.3.								2	5
3.4.								4	10 (2 exact repeats of 5)
3.9.								4	5
3.10.								4	5
3.14.								4	5
3.15.								4	5

Table 2.4. Leaf metabolism and secondary metabolite replicate numbers. Highlighted squares indicate the days on which measurements were taken. Treatment numbers refers to the number of graph variables. Replicate number is the number of plants measured for each treatment. Day 0 is the measurement before UV treatment.

Figure (from results)	Days measured							Treatment number	Replicate number (per treatment)
	0	1	2	3	4	5	6		
3.5.								4	5
3.6.								4	5
3.7.								4	5
3.8.								4	5
3.11.								4	8 (2 repeats; 5,3)
3.12.								4	5, 3
3.13.								4	5,3
3.16.								4	5,5
3.17.								4	5,5
6.18.								4	5,5

3. Results

3.1. Question 1: Does manipulation of PAR levels before or during UV-B exposure affect photosynthetic response?

In order to find out how UV-B exposure affected the photosynthetic performance of wild-type Arabidopsis, nursery and experimental conditions were manipulated in response to photosynthesis measurements where initial plant growth conditions were quite varied. *Col-0* plants were initially grown in a glasshouse for 21 days due to growth space availability. The plants were then transferred to the growth chamber for 5 days at 360 µmol m^{-2} s^{-1} PAR before being transferred into the nursery room at 50 µmol m^{-2} s^{-1} PAR for 13 days. After 13 days the plants had sufficient leaf area for photosynthetic measurements, and were transferred into the growth chamber (i.e. at 40 Days After Sowing; DAS). The growth chamber was configured so that one half of the bench received only PAR, and the other half received identical PAR, plus supplementary UV-B radiation (see Methods 2.2.2). Conditions were 720 µmol m^{-2} s^{-1} PAR, and 7.2 kJ $m^{-2}day^{-1}$ of biologically weighted UV-B (UV_{BE}) using the (Caldwell, 1971) generalized plant action spectrum. Photosynthesis measurements were taken before plants were transferred into the chamber (Day 0) and then each day the plants were in the chamber. The net photosynthetic rate (A_{max}) of the plants increased significantly (P<0.05) over the experimental period of three days (Fig 3.1) with or without the UV-B treatment. There was no significant effect of UV-B treatment on the net photosynthetic rate, either within any individual day, or across the entire experimental period (Increase in A_{max} from Day 0 to Day 3 = 4.26 µmol m^{-2} s^{-1}).

Following this pilot study, a new set of *Col-0* seedlings were grown entirely in the nursery room at 50 µmol m^{-2} s^{-1} PAR, before being transferred into the growth chamber ±UV-B for the experiment at 44 DAS. The UV-B dose in the growth chamber was increased in this experiment to 10.2 UV_{BE} kJ m^{-2} day^{-1} with the PAR remaining at 720 µmol m^{-2} s^{-1} as per the pilot study. The plants remained in the growth chamber for six days. Again we saw a significant increase in the A_{max} rate over the entire experiment (Fig. 3.2) in both the UV-B treated plants and the non UV-B treated plants. Also, the UV-B treated plants were significantly lower in photosynthetic rate over the whole experiment (Fig. 3.2), with the interaction of time and UV-B treatment highly

significant (P<0.001). Some leaf necrosis was seen in the older leaves under both treatments.

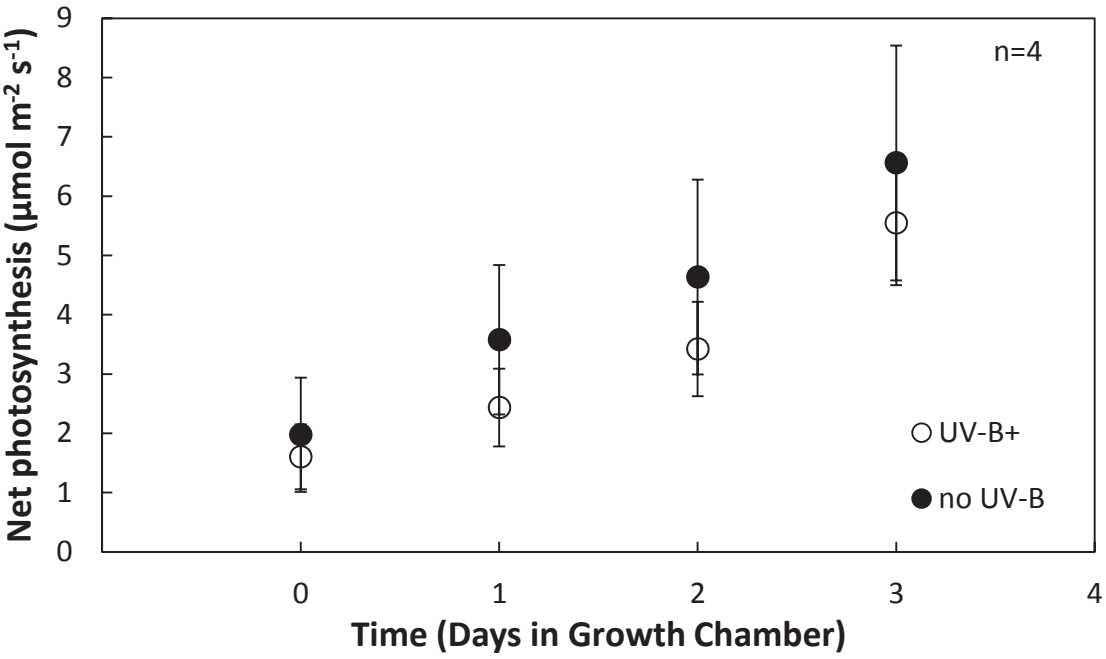

Figure 3.1. Pilot study reveals increased net photosynthetic rate with or without UV-B treatment over time. *Col-0* Arabidopsis plants were exposed at 40 DAS to biologically weighted UV-B radiation for three days at 7.2 UV_{BE} kJm^{-2} day^{-1} in a growth chamber with 720 µmol m^{-2} s^{-1} metal halides. Net photosynthetic rate is measured in µmol m^{-2}s^{-1}. Data points represent means ±1 SE (n=4).

Following this experiment, the PAR in the nursery room and growth chamber were altered to ensure that the nursery environment was not providing, e.g. insufficient PAR, and to provide equivalence between nursery phase, and UV exposure phase. The PAR in the nursery room was increased from 50 µmol m^{-2} s^{-1} PAR to 240 µmol m^{-2} s^{-1} PAR when plants were 23 days old remaining in the nursery for a further 18 days. At 41DAS the plants were transferred into the growth chamber ±UV-B for 4 days. The UV-B dose remained at 10.2 kJ m^{-2} day^{-1}, while the PAR was reduced to 360 µmol m^{-2} s^{-1}. The A_{max} again increased over the entire experiment regardless of the treatment the plants were under (Fig 3.3). Additionally the UV-B treated plants were significantly lower in A_{max} over the entire experiment.

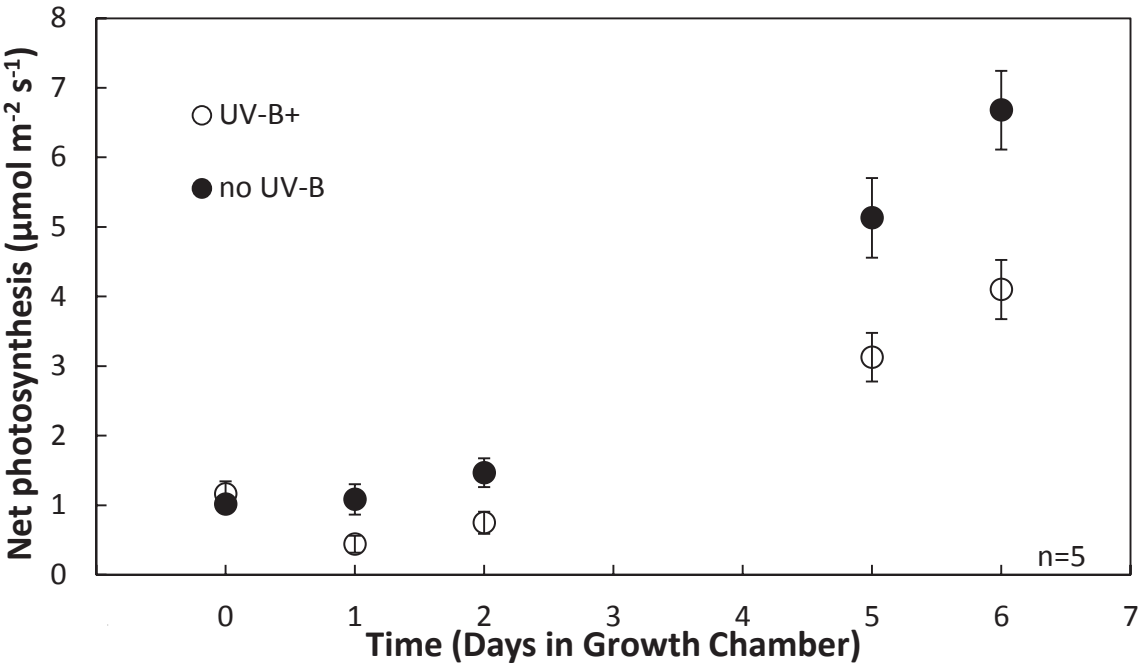

Figure 3.2. UV-B treated Arabidopsis increase in net photosynthetic rate slower than non UV-B treated plants. *Col-0* Arabidopsis plants grown in nursery room at 50 µmol m^{-2} s^{-1} PAR until 44DAS then transferred into growth chamber for 6 days at 720 µmol m^{-2} s^{-1} PAR. Half of the plants were exposed to 10.2 UV_{BE} kJm^{-1} day^{-1}. Net photosynthetic rate is measured in µmol m^{-2} s^{-1}. Data points represent means ±1 SE (n=5).

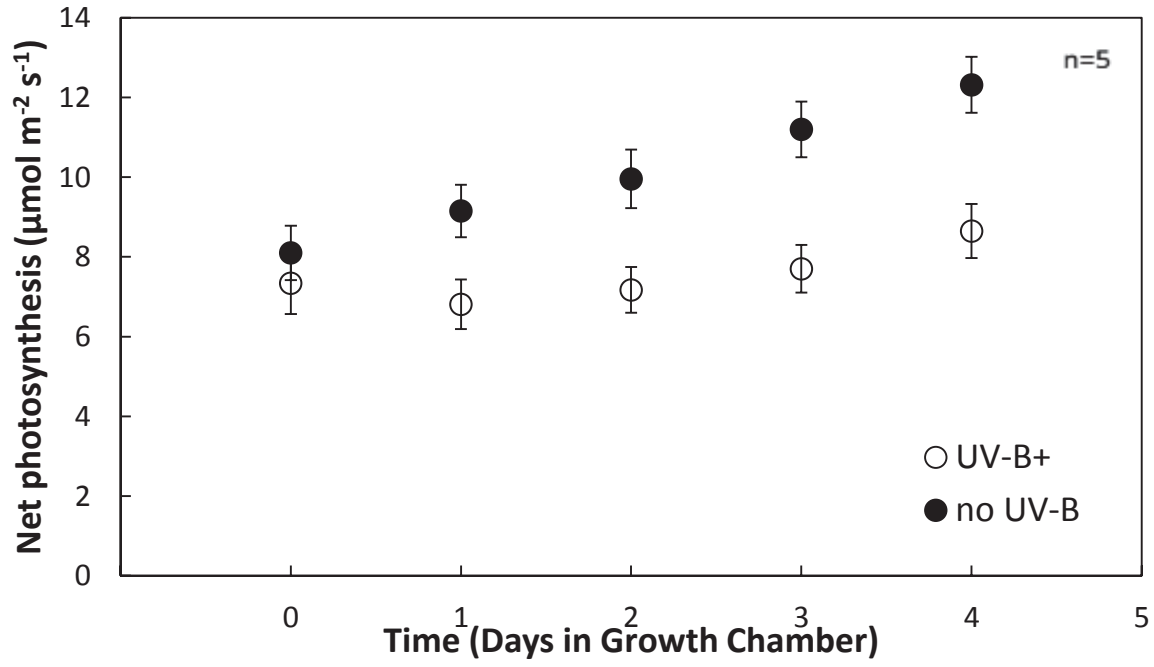

Figure 3.3. UV-B treated Arabidopsis have a lower photosynthetic rate than untreated plants. *Col-0* Arabidopsis plants grown in nursery room at 50 µmol m^{-2} s^{-1} PAR for 23DAS then at 240 µmol m^{-2} s^{-1} PAR for a further 18 days. Plants transferred to growth chamber for 4 days at 360 µmol m^{-2} s^{-1} PAR. Half of plants exposed to 10.2 UV_{BE} kJm^{-1} day^{-1}. Net photosynthetic rate is measured in µmol m^{-2} s^{-1}. Data points represent means ±1 SE (n=5).

3.2. Question 2: Does plant age affect the UV-B photosynthesis phenotype or the level of photo-protective pigmentation?

In order to investigate how plant age affected the photosynthesis phenotype, experiments were conducted comparing the responses of two ages of Arabidopsis. The Arabidopsis plants were grown in a nursery room under white fluorescent tubes at 240 µmol m^{-2} s^{-1} PAR. Two different seedling ages were used in the same experiment; 35DAS and 28DAS. The plants were then transferred into the growth chamber ±UV-B for 6 days, set at 10.2 UV_{BE} kJ m^{-2} day^{-1} and 360 µmol m^{-2} s^{-1} PAR. This experiment was repeated exactly, and with no significant difference observed between experimental iterations, the photosynthetic data were averaged together in Figure 3.4. There was no difference in A_{max} found between the same aged plants, though the 35DAS plants had a higher A_{max} overall. There was a significant interaction effect of time and age (Fig. 3.4).

Leaf areas for both ages of plants were measured, using plants grown alongside the plants put in the experiment from Day 0 and the plants used in the experiment once it was completed. The 35DAS and 28DAS plants were highly significantly different in leaf area on Day 0, in both repeats, with the 35DAS plants larger than the 28DAS plants (see Appendix 1). There was no significant difference in leaf area between 28DAS plants when treated with UV-B or not, in both repeats, at harvest. In the first experimental repeat there was also no significant difference in the 35DAS plants treated with UV-B or no UV-B. There was a very significant ($P<0.01$) difference in the 35DAS plants of the second repeat, with no UV-B treated plants larger at 33.07 ± 0.98 cm^2 than the UV-B treated plants at 27.78 ± 0.98 cm^2 (see Appendix 1). Dry weights in both experimental repeats were highly significantly larger in the 35DAS plants than the 28DAS plants on Day 0 (see Appendix 1). In the first repeat the dry weights, at harvest, of the plants exposed to UV-B or no UV-B were not significantly different, in either 35DAS or 28DAS plants. In the second repeat the 28DAS plants were not significantly different between those treated with UV-B and those not. The 35DAS plants that had no UV-B were very significantly heavier than the UV-B treated plants, with dry weights 0.1769 ± 0.0071 g and 0.1433 ± 0.0062 g, respectively (see Appendix 1).

Non-invasive measurements of leaf nutrition and metabolism were taken as described in Methods 2.5.2. The non-invasive results are from the second repeat of the age comparison. The chlorophyll content in the plants had no overall significant increase or decrease (Fig 3.5). The age of the plants (35DAS or 28DAS) did have a significant effect regardless of UV treatment on the chlorophyll content with the younger plants (28DAS) lower than the older plants.

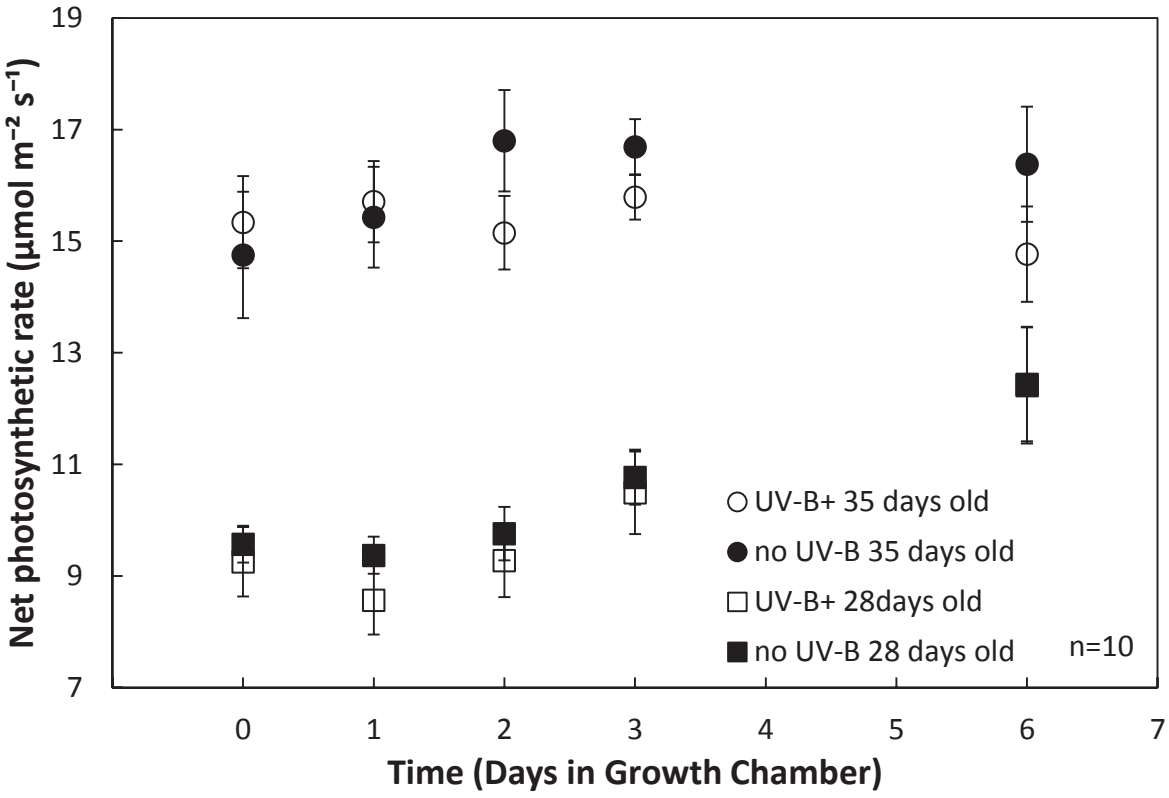

Figure 3.4. 35 day old Arabidopsis have higher photosynthetic rate than 28 day old plants, with no differences between UV treatments. *Col-0* Arabidopsis plants grown in nursery room at 240 µmol m^{-2} s^{-1} PAR for 35 days (DAS) and 28 days (DAS). Plants transferred to growth chamber for 6 days at 360 µmol m^{-2} s^{-1} PAR. Half of plants exposed to 10.2 UV_{BE} kJm^{-1} day^{-1}. Net photosynthetic rate is measured in µmol m^{-2} s^{-1}. Data points represent means ±1 SE (n=10).

The flavonoid content significantly increased in all treatments over the experimental period (Fig. 3.6). 35DAS plants with UV treatment had a 718 ±69.1 % increase from Day 0 to Day 6. 35DAS plants without UV treatment increased 429 ±44.7 %, 28DAS with UV treatment increased 507 ±54.8% and 28DAS without UV treatment increased 207.4 ±31.6 % over the same Day 0 to Day 6 period. UV treatment had a highly significant

effect on the experiment, with both 35DAS and 28DAS plants exposed to UV-B increasing in flavonoids at a faster rate than those not exposed to UV-B. Plant age also had a highly significant effect over the experiment with the 35DAS plants higher in flavonoids than the corresponding 28DAS plants (Fig. 3.6). There was no significant interaction between plant age and UV treatment over the experiment. Time did have a highly significant interaction with both age and UV treatment, as flavonoid content continued to increase. The plants that had no UV treatment from Day 1 have highly significantly lower flavonoid levels than the UV treated plants in their age groups (Fig. 3.6).

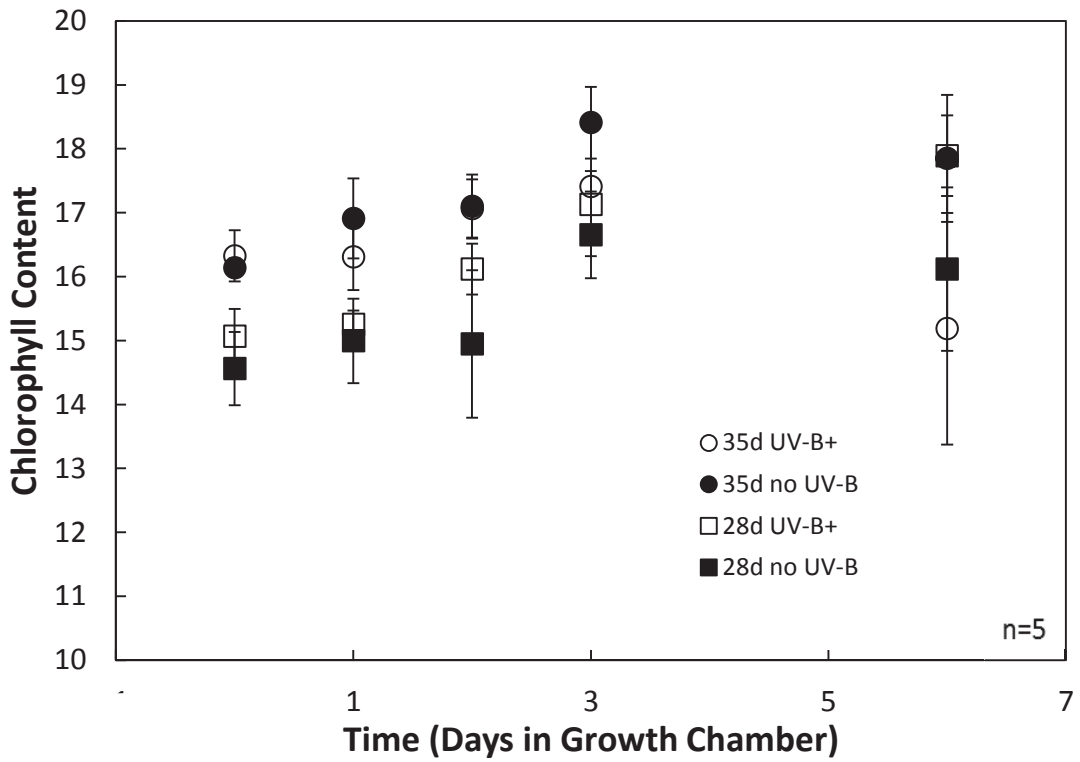

Figure 3.5. Age and UV treatment does not affect chlorophyll accumulation. *Col-0* Arabidopsis plants grown in nursery room at 240 µmol m^{-2} s^{-1} PAR for 35DAS and 28DAS. Plants transferred to growth chamber for 6 days at 360 µmol m^{-2} s^{-1} PAR. Half of plants exposed to 10.2 UV_{BE} kJ m^{-1} day^{-1}. Data points represent means ±1 SE (n=5).

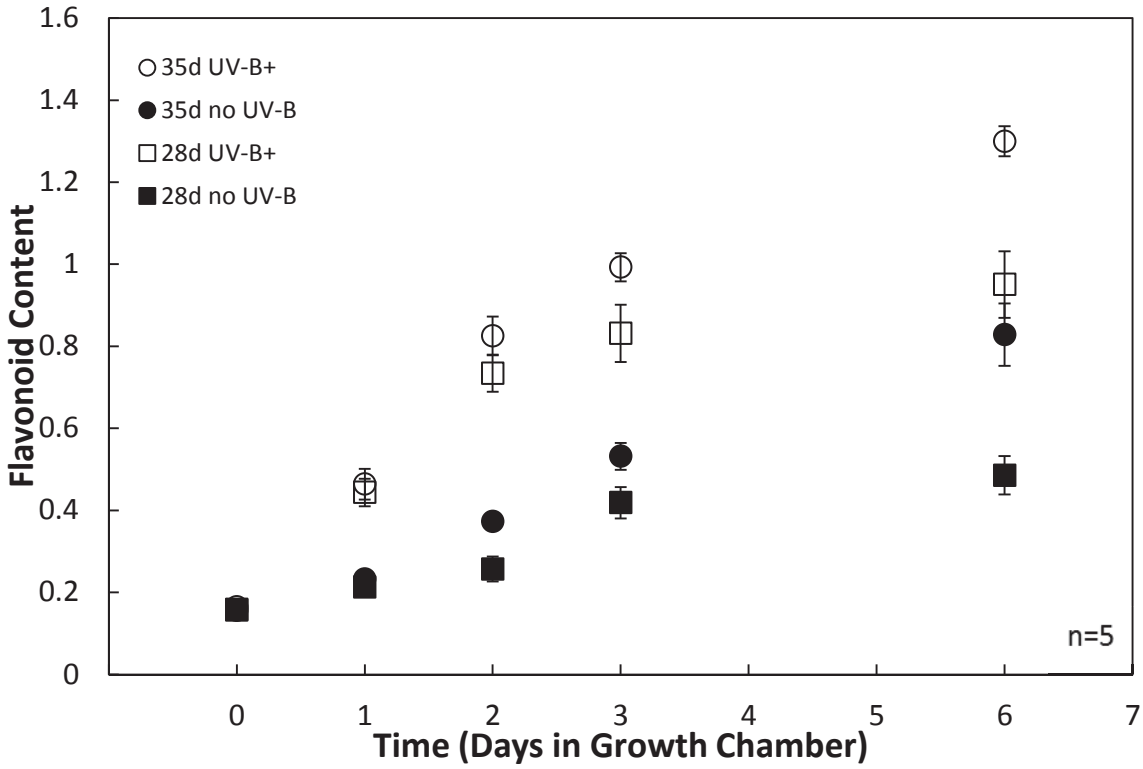

Figure 3.6. Accumulation of flavonoids in Arabidopsis is more rapid in UV treated plants and in older plants after 3 days of higher PAR. *Col-0* Arabidopsis plants grown in nursery room at 240 µmol m^{-2} s^{-1} PAR for 35DAS and 28DAS. Plants transferred to growth chamber for 6 days at 360 µmol m^{-2} s^{-1} PAR. Half of plants exposed to 10.2 UV_{BE} kJm^{-1} day^{-1}. Where there is visible separation of data points within days the difference is significant. Data points represent means ±1 SE (n=5).

Anthocyanin content significantly increased overall across the experiment (Fig. 3.7). The interaction of time with UV treatment was significant and the interaction of time and age was also highly significant. There is no significant interaction between the plant age and UV treatments. On Day 2 the differences between the effects of UV treatment within each age group was significant for the 28DAS plants and very significant for the 35DAS plants (Fig. 3.7). The 35DAS UV treated plants had a significantly higher anthocyanin content than the no UV-B treated plants from Day 2 until Day 6. By Day 6 there was no longer a significant difference in anthocyanin content of 35DAS plants (Fig. 3.7). The 28DAS UV-B treated plants were not significantly different in anthocyanin content from the 28DAS non UV-B treated plants until Day 6. On day 6 the 28DAS UV treated plants were very significantly higher than

the non UV-B treated plants (Fig. 3.7). 28DAS plants were significantly lower than 35DAS plants on Day 6 regardless of UV treatment.

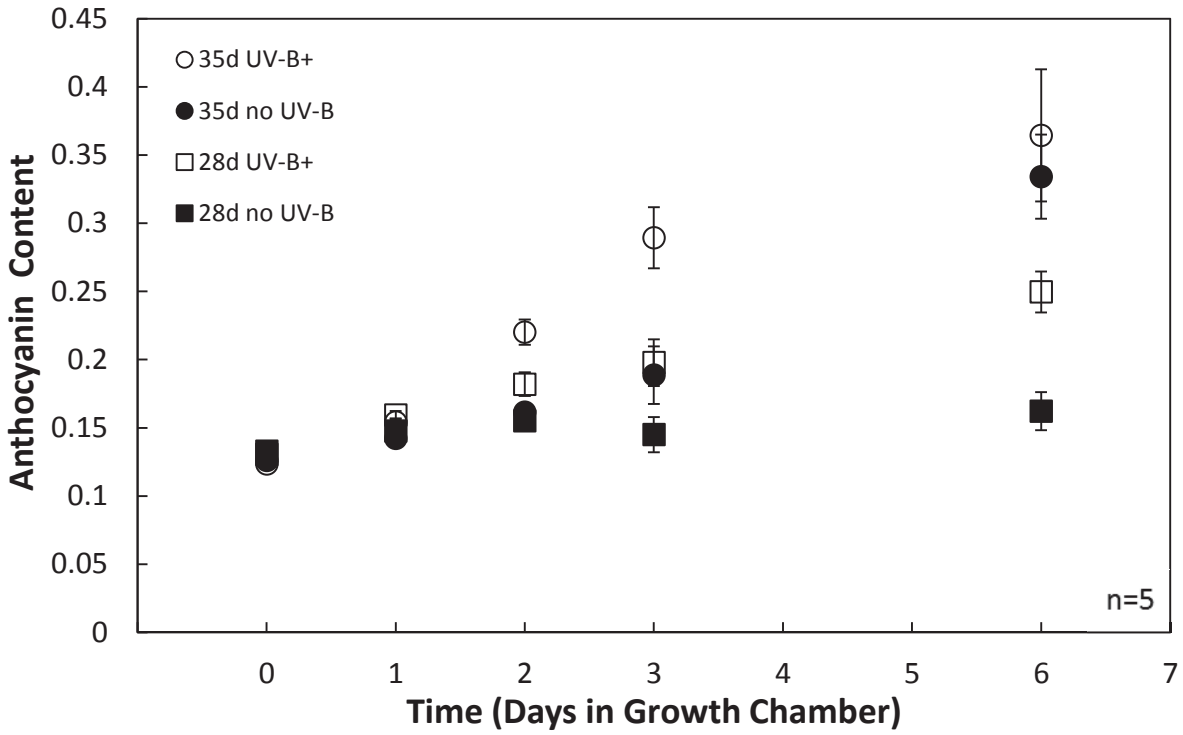

Figure 3.7. 35DAS UV-B treated plants had a significant increase from 35DAS no UV-B plants in anthocyanin content on Day 2. The 28DAS plants didn't have a significant increase due to UV-B until day 6. *Col-0* Arabidopsis plants grown in nursery room at 240 µmol m^{-2} s^{-1} PAR for 35DAS and 28DAS. Plants transferred to growth chamber for 6 days at 360 µmol m^{-2} s^{-1} PAR. Half of plants exposed to 10.2 UV_{BE} kJm^{-1} day^{-1}. Data points represent means ±1 SE (n=5).

The NBI (nitrogen balance index) in the plants significantly decreased over the whole experiment irrespective of plant age or UV treatment (Fig. 3.8). The plants treated with UV-B were highly significantly lower from Day 1 to Day 3 of the experiment. By Day 6 the 35DAS UV-B treated plants were significantly lower than the 35DAS plants without UV. The 28DAS UV-B treated plants were also very significantly lower that the 28DAS treated without UV-B. However there was no longer a significant difference between 35DAS non UV treated plants and the 28DAS UV-B treated plants (Fig. 3.8).

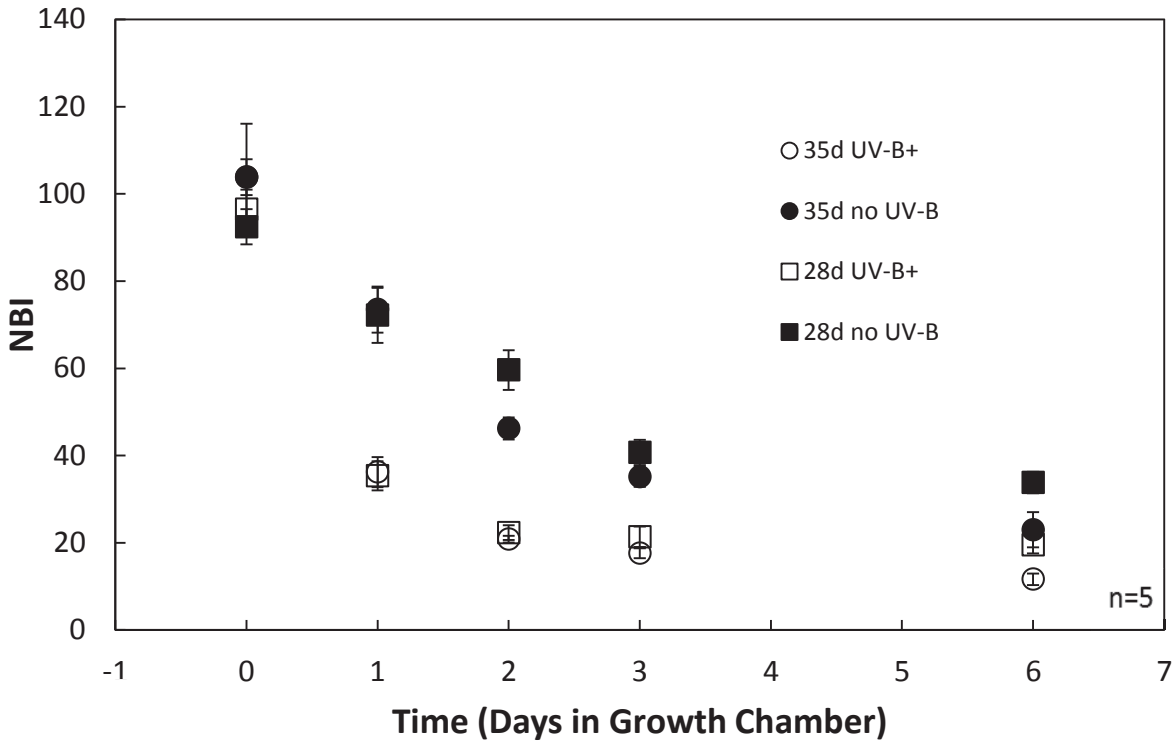

Figure 3.8. UV treatment causes sharper decrease in NBI. *Col-0* Arabidopsis plants grown in nursery room at 240 µmol m^{-2} s^{-1} PAR for 35DAS and 28DAS. Plants transferred to growth chamber for 6 days at 360 µmol m^{-2} s^{-1} PAR. Half of plants exposed to 10.2 UV_{BE} kJm^{-1} day^{-1}. Data points represent means ±1 SE (n=5).

In summary, 35DAS plants had earlier and stronger responses to photo-protective pigments in the UV-B treatment than the 28DAS plants. There was no differentiation of A_{max} due to UV-B in either age, but the 35DAS plants had a higher A_{max} throughout the entire experiment. There was no apparent change in chlorophyll content in either 35DAS or 28DAS plants. Flavonoid content accumulation was stronger in the plants exposed to UV-B, with 35DAS plants more rapidly accumulating flavonoids. 35DAS plants exposed to UV-B also had an earlier response than the 28DAS plants. NBI response to UV-B is the same in both ages of plants initially, by Day 6 though the 35DAS plants have dropped further than the 28DAS plants.

3.3. Question 3: How does pre-treatment with higher PAR affect UV-B photosynthetic response?

As there was no UV-B mediated decrease in A_{max} in the plants grown under a continuous PAR in the nursery a pre-treatment was introduced. For the pre-treatment plants were removed from the nursery a week before UV-B exposure and placed into a growth chamber at the higher PAR the experiment would be conducted at. *Col-0* plants were grown in the nursery room at 240 µmol m^{-2} s^{-1} PAR until 21DAS. At this point half of the plants remained in the nursery room and the rest were transferred into the pre-treatment environment. The pre-treatment environment was a growth chamber with 360 µmol m^{-2} s^{-1} PAR and no UV-B radiation. At 28DAS all the plants were transferred into the growth chamber with UV-B lights. The chamber was configured so that half the bench received only PAR and the other PAR with supplementary UV-B radiation. Conditions were 360 µmol m^{-2} s^{-1} PAR and 10.2 UV_{BE} kJ m^{-2} day^{-1}. There was no significant increase seen in the A_{max} over the experimental period under any of the treatments (Fig. 3.9). Leaf areas of the pre-treatment and no pre-treatment plants were not significantly different on day 0 (see Appendix 2). Also none of the treatments were significantly different in leaf area at harvest. Dry weights of the pre-treatment and no pre-treatment plants were not significantly different on Day 0. There were no significances in dry weight between any treatments at harvest (see Appendix 2).

Following the pre-treatment of 28DAS plants a new group was grown to 30DAS. Half of these plants were put in the pre-treatment growth chamber at 23DAS with 360 µmol m^{-2} s^{-1} PAR. The experimental conditions were again 360 µmol m^{-2} s^{-1} PAR and 10.2 UV_{BE} kJ m^{-2} day^{-1}. This experiment was repeated exactly and the results for both repeats are presented together. In Figure 3.10 the net photosynthetic rates of the repeat experiments are presented individually due to a significant effect of experiment. In Figure 3.10A there is no overall A_{max} change however the interaction between time and UV treatment was very significant. The effect of the pre-treatment over the whole experiment was highly significant with pre-treated plants lower in A_{max} overall. In the repeat (Fig. 3.10B) there was no overall trend in A_{max} but the interaction of time and pre-treatment was highly significant. UV treatment had a significant effect

over the whole experiment, with no PT UV-B+ plants lower in A_{max} as compared to the other treatments (Fig. 3.10B).

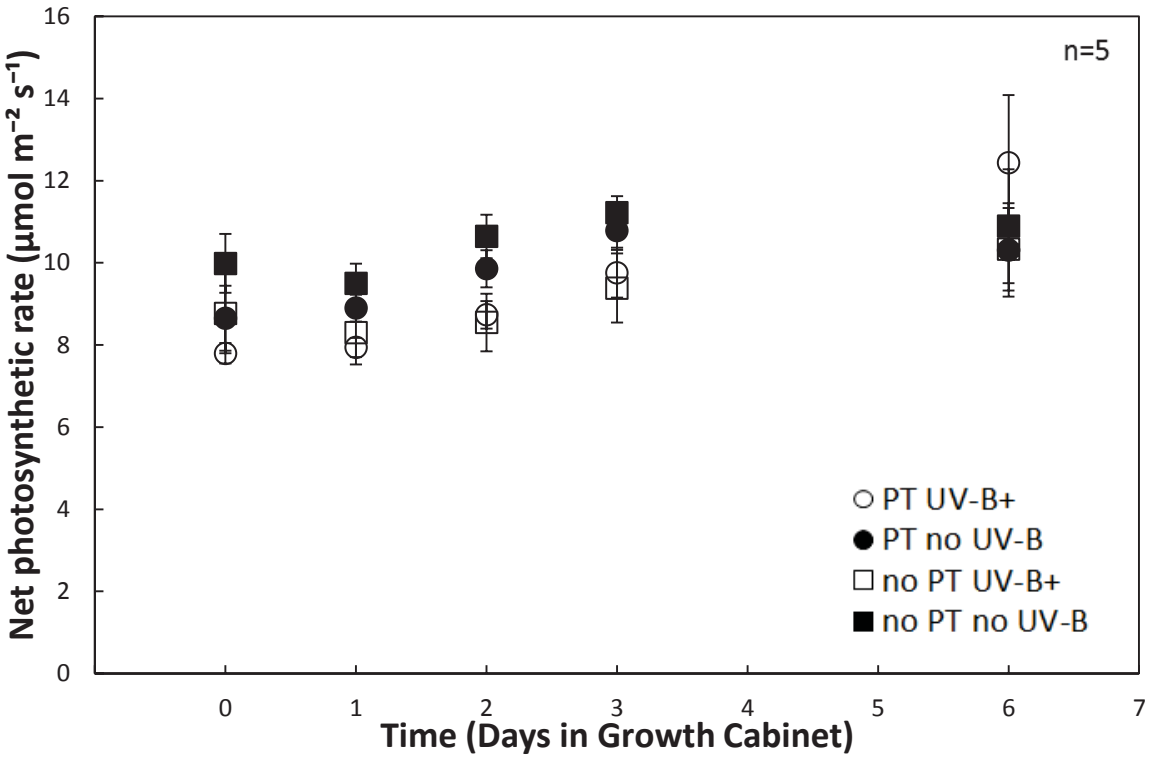

Figure 3.9. No difference seen in net photosynthetic rate of Arabidopsis that had a pre-treatment or UV-B exposure. PT UV-B+= pre-treated plants exposed to UV-B, PT no UV-B= pre-treated plants not exposed to UV-B, no PT UV-B+ = non pre-treated plants exposed to UV-B, no PT no UV-B = non pre-treated plants not exposed to UV-B. *Col-0* Arabidopsis plants grown in nursery at 240 µmol m^{-2} s^{-1} PAR for 21DAS then half of plants transferred to pre-treatment of higher PAR for 1 week at 360 PAR. All plants then transferred to growth chamber for 5 days at 360 µmol m^{-2} s^{-1} PAR. Half of plants exposed to 10.2 UV_{BE} kJm^{-1} day^{-1}. Net photosynthetic rate is measured in µmol m^{-2} s^{-1}. Data points represent means ±1 SE (n=5).

Leaf areas were also measured for both repeats. The first repeat had a highly significant difference between the pre-treated and non-pre-treated plants on Day 0 in leaf area. The average leaf area of the non-pre-treated plants was 9.54 ± 0.74 cm^2 and the pre-treated plants 6.1 ± 0.2 cm^2 (see Appendix 3). In the first repeat the leaf areas, at harvest, of the plants exposed to UV-B or no UV-B were not significantly different, in either pre-treated or non-pre-treated plants. For the second repeat there were no significant differences in leaf area at the end of the experiment between any

treatments. On Day 0 the dry weights of the non-pre-treated plants were significantly heavier than the pre-treated plants, 0.0205 ± 0.0016 g and 0.0164 ± 0.0009 g respectively. By harvest there was no significant difference in dry weights in plants exposed to UV-B or not, whether pre-treated or non-pre-treated. The second repeat had no significant differences in dry weight in any treatment.

Leaf content measurements were taken during both experimental repeats of the 30DAS plants. The chlorophyll content of both repeats is represented as a combined average in Figure 11, due to no significant effect of experiment. The interaction between time and UV treatment is significant (Fig. 3.11).

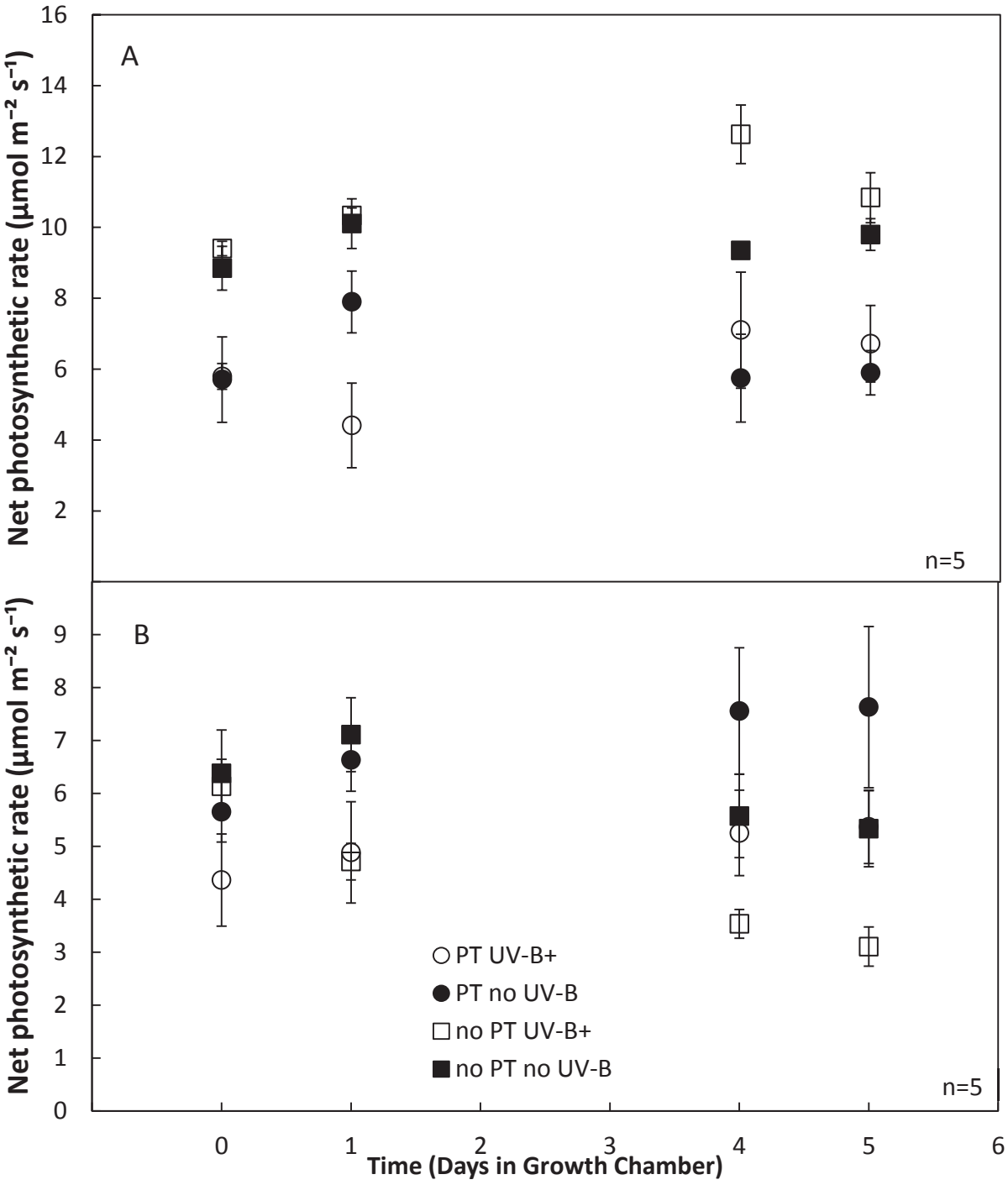

Figure 3.10. Exposure of Arabidopsis plants to higher PAR before UV-B exposure decreases net photosynthetic rate initially. (A) First experimental repeat, (B) Second experimental repeat. PT UV-B+= pre-treated plants exposed to UV-B, PT no UV-B= pre-treated plants not exposed to UV-B, no PT UV-B+ = non pre-treated plants exposed to UV-B, no PT no UV-B = non pre-treated plants not exposed to UV-B. *Col-0* Arabidopsis plants grown in nursery at 240 µmol m^{-2} s^{-1} PAR for 23DAS then half of plants transferred to pre-treatment of higher PAR for 1 week at 360 PAR. All plants then transferred to growth chamber for 5 days at 360 µmol m^{-2} s^{-1} PAR. Half of plants exposed to 10.2 UV_{BE} kJm^{-1} day^{-1}. Net photosynthetic rate is measured in µmol m^{-2} s^{-1}. Data points represent means ±1 SE (n=5).

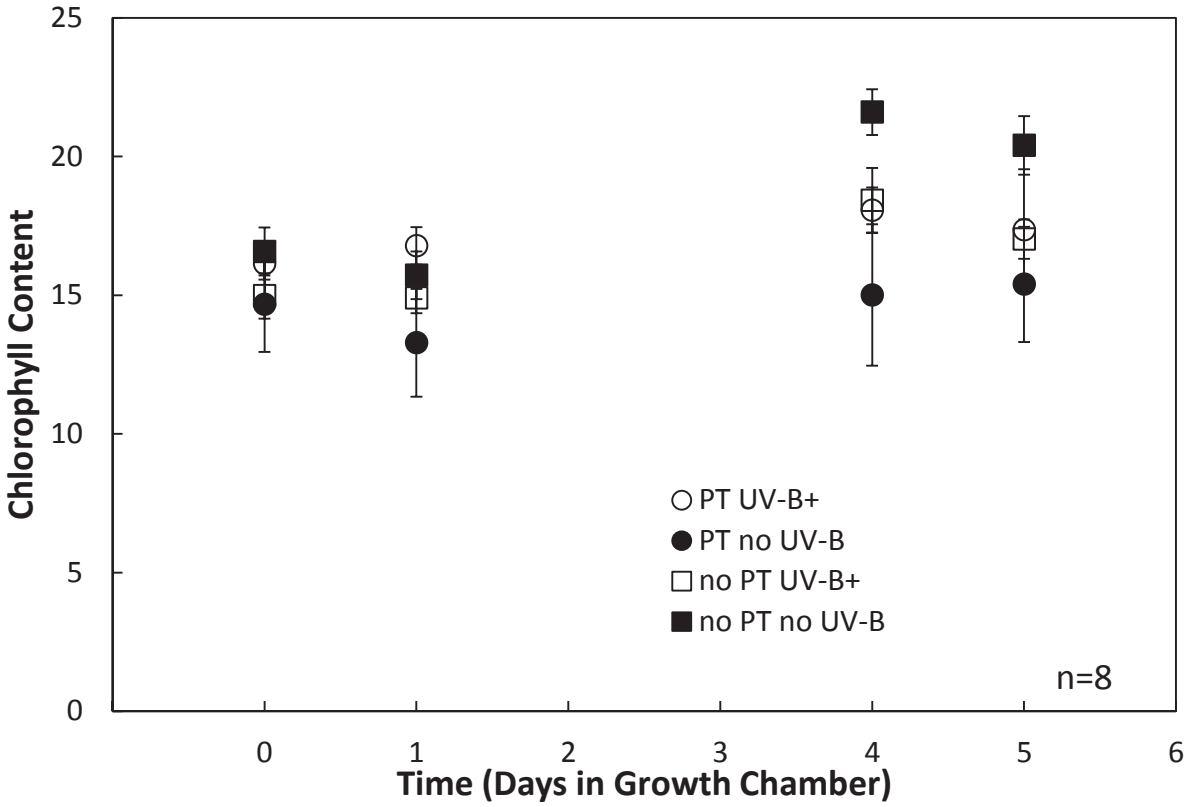

Figure 3.11. Exposure of plants to higher PAR before UV-B treatment does not change chlorophyll accumulation. PT UV-B+= pre-treated plants exposed to UV-B, PT no UV-B= pre-treated plants not exposed to UV-B, no PT UV-B+ = non pre-treated plants exposed to UV-B, no PT no UV-B = non pre-treated plants not exposed to UV-B. *Col-0* Arabidopsis plants grown in nursery at 240 µmol m^{-2} s^{-1} PAR for 23DAS then half of plants transferred to pre-treatment of higher PAR for 1 week at 360 PAR. All plants then transferred to growth chamber for 5 days at 360 µmol m^{-2} s^{-1} PAR. Half of plants exposed to 10.2 UV_{BE} kJm^{-1} day^{-1}. Data points represent means ±1 SE (n=8), 5 in first repeat, 3 in second repeat.

For the flavonoid content the data for the repeats are presented individually due to a significant effect of experiment. The flavonoids in the first repeat had a significant increase over the experiment (Fig. 3.12A). The interaction between time and pre-treatment is significant and the interaction between time and UV treatment is highly significant. UV treated plants had highly significantly increased flavonoids regardless of whether they were pre-treated or not. The second repeat had no overall significant increase (Fig. 3.12B). On Day 1 the plants which were not pre-treated were significantly different with higher flavonoids in the UV treated plants; they were not significantly different on Day 4 or Day 5 (Fig. 3.12A).

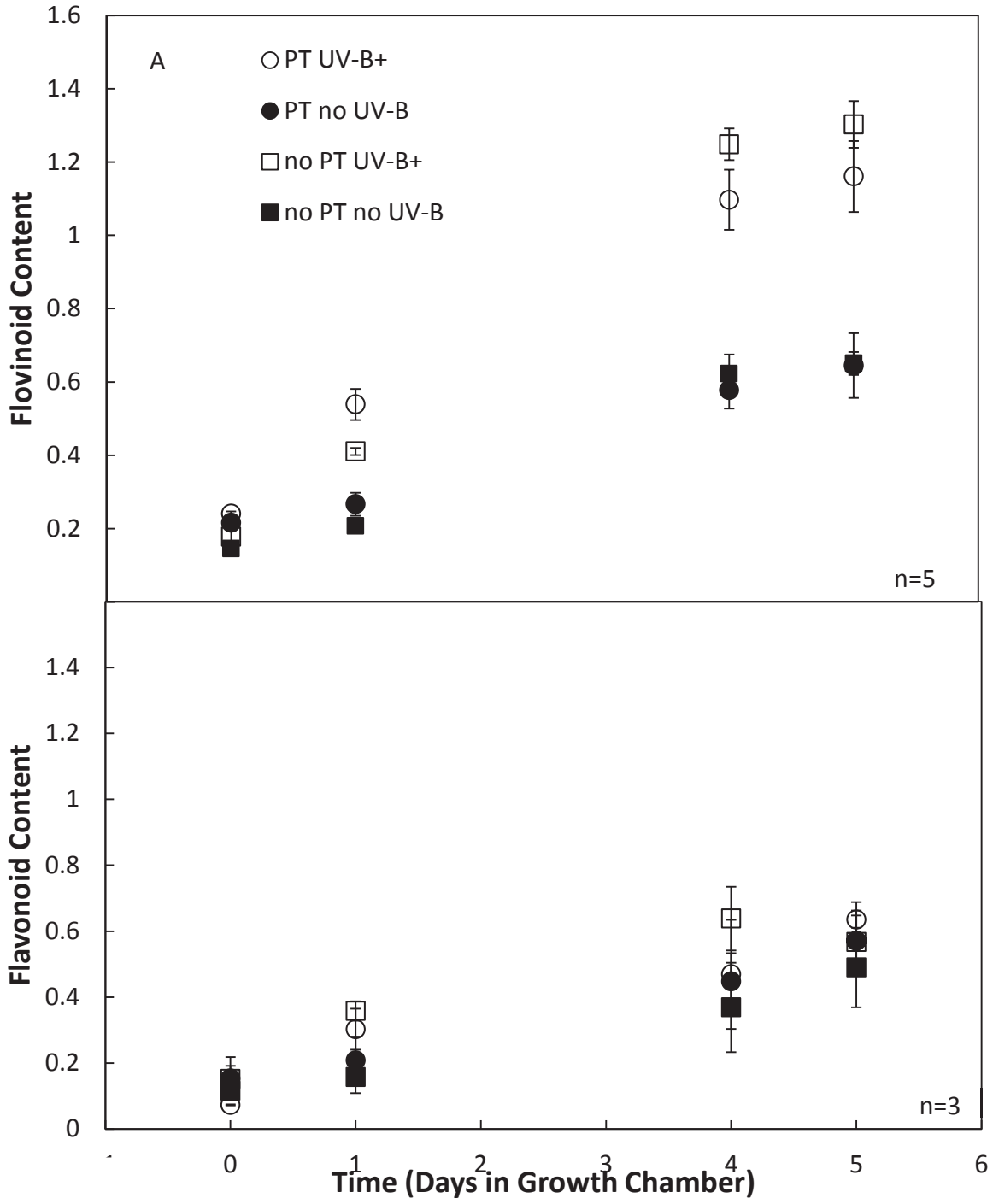

Figure 3.12. UV-B exposure increased the flavonoid accumulation in the first repeat only with plants that had no pre-treatment exhibiting a greater increase than those pre-treated. (A) First experimental repeat, (B) Second experimental repeat. PY UV-B+= pre-treated plants exposed to UV-B, PT no UV-B= pre-treated plants not exposed to UV-B, no PT UV-B+ = non pre-treated plants exposed to UV-B, no PT no UV-B = non pre-treated plants not exposed to UV-B. *Col-0* Arabidopsis plants grown in nursery at 240 µmol m^{-2} s^{-1} PAR for 23DAS then half of plants transferred to pre-treatment of higher PAR for 1 week at 360 µmol m^{-2} s^{-1} PAR. All plants then transferred to growth chamber for 5 days at 360 µmol m^{-2} s^{-1} PAR. Half of plants exposed to 10.2 UV_{BE} kJm^{-1} day^{-1}. Data points represent means ±1 SE (n=5 for A, n=3 for B).

The data for the NBI's of the repeat experiments are presented individually due to a significant effect of experimental iteration (Fig. 3.13). In Figure 3.13A there is a significant decrease in NBI. The interaction of time with both pre-treatment and UV treatment separately is highly significant. Both the UV treatment and pre-treatment have a highly significant effect on decreasing NBI. The plants which had UV treatment are significantly lower in NBI from Day 1 (Fig. 3.13A). Also on Day 1 the plants not treated with UV were very significantly different, by Day 4 there was no significant difference. The second repeat had no overall significant decrease in NBI (Fig. 3.13B). Though on Day 1 the UV-B treated plants were significantly lower than those not treated with UV.

Exposure of the wild-type Arabidopsis to a higher PAR level before exposure to UV-B had an effect on plants that were pre-treated at 23DAS by lowering the A_{max}. There was no difference seen in chlorophyll levels across all treatments. The flavonoid content increased significantly in the first repeat due to the UV-B exposure but there was no increase seen in the second repeat. NBI in the first repeat was decreased initially in the pre-treated plants, with UV-B having a greater effect on NBI by Day 5. The second repeat showed no initial decrease because of the pre-treatment.

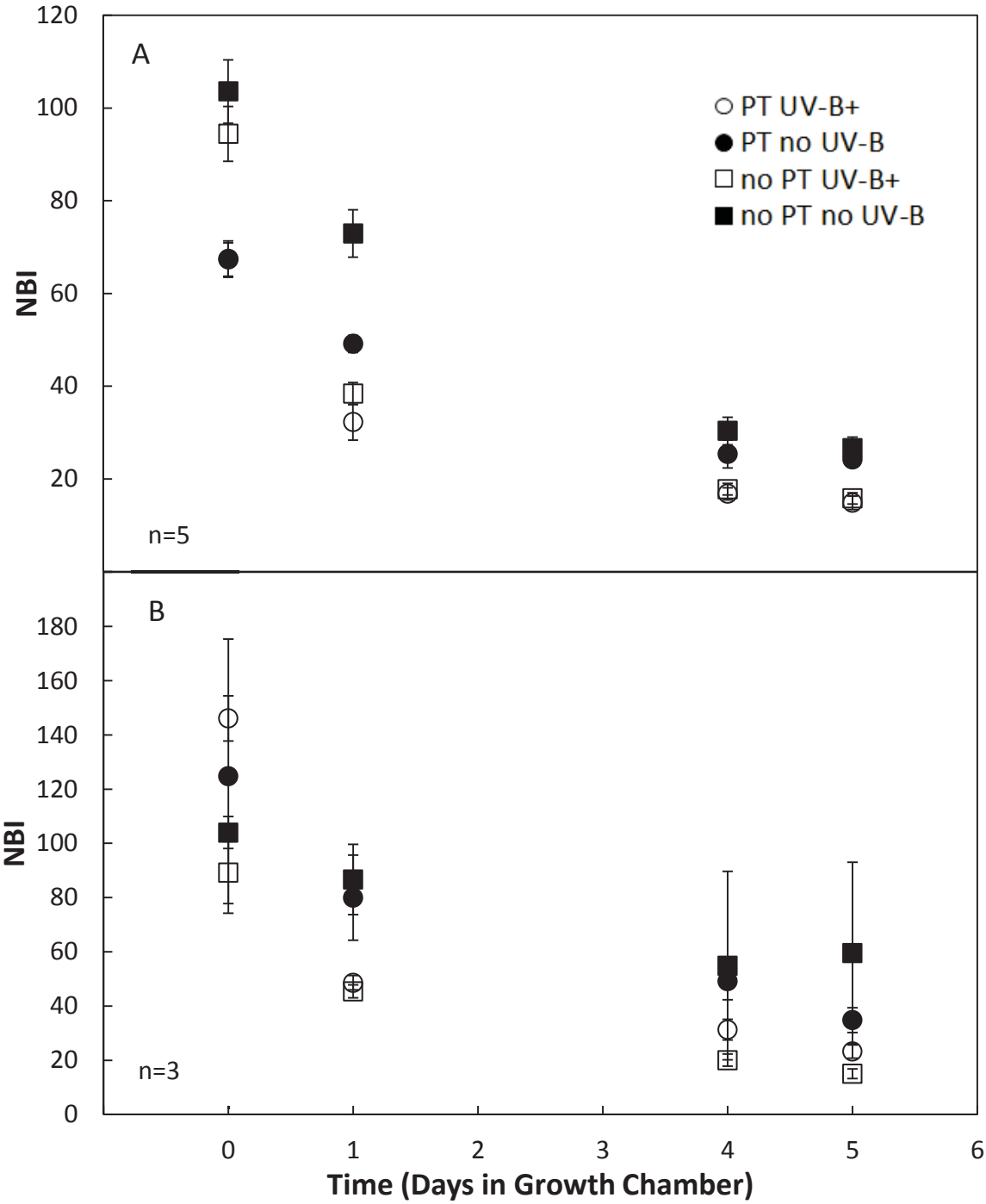

Figure 3.13. UV-B exposure leads to greater decrease in NBI regardless pre-exposure to high PAR. (A) First experimental repeat, (B) Second experimental repeat. PT UV-B+= pre-treated plants exposed to UV-B, PT no UV-B= pre-treated plants not exposed to UV-B, no PT UV-B+ = non pre-treated plants exposed to UV-B, no PT no UV-B = non pre-treated plants not exposed to UV-B. *Col-0* Arabidopsis plants grown in nursery at 240 µmol m^{-2} s^{-1} PAR for 23DAS then half of plants transferred to pre-treatment of higher PAR for 1 week at 360 µmol m^{-2} s^{-1} PAR. All plants then transferred to growth chamber for 5 days at 360 µmol m^{-2} s^{-1} PAR. Half of plants exposed to 10.2 UV_{BE} kJm^{-1} day^{-1}. Data points represent means ±1 SE (n=5 for A, n=3 for B).

3.4. Question 4: What is the role of the UVR8 photoreceptor in UV-B photosynthetic response?

3.4.1. Physiological Measurements

In order to find the role of UVR8 in photosynthetic response the *uvr8-1* mutant with a *Ler* background was grown alongside the *Ler* wild-type. For this experiment the plants were grown in the nursery room with a PAR level of 240 µmol m^{-2} s^{-1}, the same as the experiments using *Col-0*. At 24DAS half of both the *Ler* and *uvr8-1* plants were transferred to the pre-treatment growth chamber with 360 µmol m^{-2} s^{-1} PAR. At 31DAS all the plants were transferred into the growth chamber with 360 µmol m^{-2} s^{-1} PAR and 10.2 UV_{BE} kJ m^{-2} day^{-1}. For the *Ler* wild-type Arabidopsis, time had a very significant effect on A_{max} (Fig. 3.14), with the UV treatment also significantly effecting A_{max}. The pre-treatment highly significantly lowered the A_{max} of the plants and no difference was seen in the A_{max} with or without UV treatment. The A_{max} in the *uvr8-1* mutant significantly decreased over the entire experiment (Fig. 3.15). The interaction between time and pre-treatment was highly significant and the interaction between time and UV treatment very significant. Pre-treatment had a very significant effect on A_{max} separate to any other factors; this was also the same for UV treatment (Fig. 3.15).

On Day 0 *Ler* plants that were not pre-treated were highly significantly bigger in leaf area that the pre-treated plants, 12.56 ± 0.92 cm^2 and 8.03 ± 0.42 cm^2 respectively (see Appendix 4.1). The *Ler* plants that were exposed to UV-B were not significantly different in leaf area to the plants not exposed to UV-B, within either of the pre-treated plants or the non-pre-treated plant treatments. The pre-treated and non-pre-treated plants on Day 0 had no significant differences in dry weight (see Appendix 4.1). At the time of harvest there was no significant difference in dry weight of pre-treated plants exposed to UV-B and pre-treated plants not exposed to UV-B. *Ler* plants that had no pre-treatment and exposed to UV-B were significantly smaller in dry weight than non-pre-treated plants not exposed to UV-B (see Appendix 4.1). On Day 0 the *uvr8-1* plants had no significant difference in leaf area between those plants that were pre-treated and those that were not (see Appendix 4.2). There were no significant differences in leaf area due to UV-B exposure or pre-treatment at the time of harvest. *uvr8-1* plants had no significant differences in leaf area on Day 0. At the time of harvest

there was no significant difference in dry weight of pre-treated plants exposed to UV-B and pre-treated plants not exposed to UV-B. *uvr8-1* plants that had no pre-treatment and exposed to UV-B were significantly smaller in dry weight than non-pre-treated plants not exposed to UV-B (see Appendix 4.2).

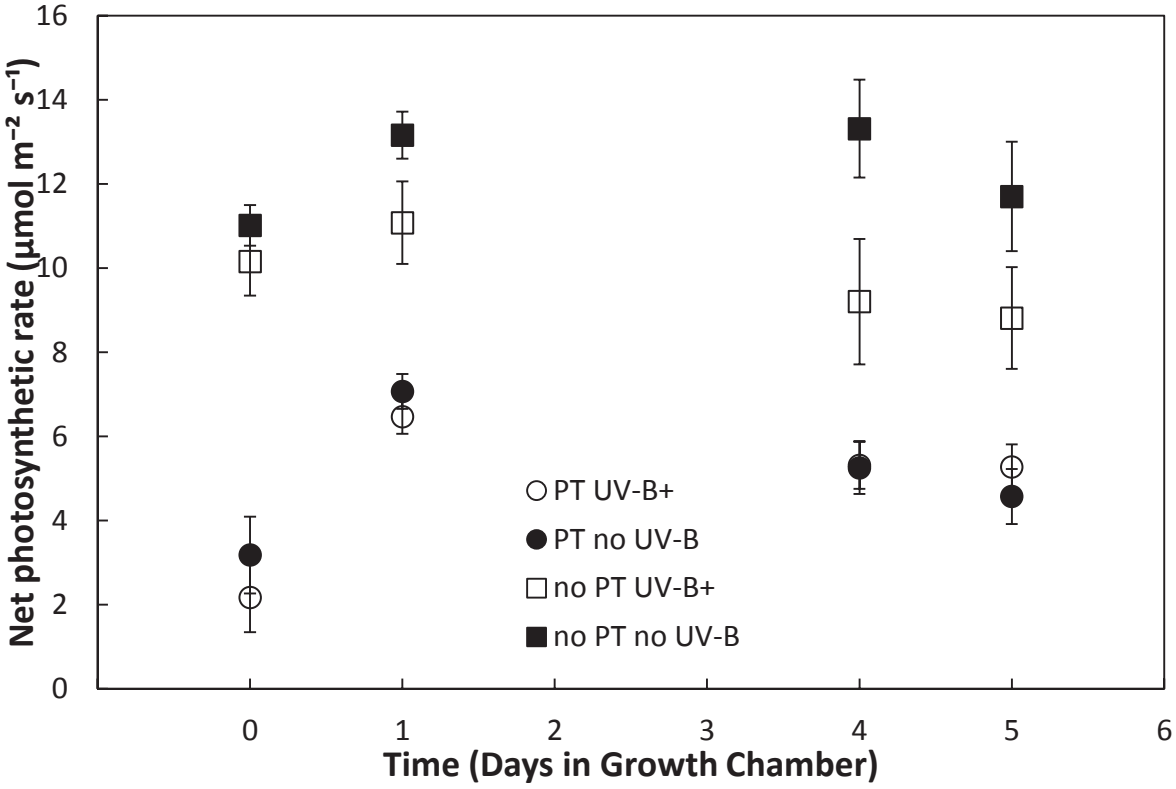

Figure 3.14. Early exposure of Arabidopsis *Ler* plants to higher PAR lowers net photosynthetic rate and no difference is seen due to UV-B treatment unlike those plants that were not pre-treated. PT UV-B+= pre-treated plants exposed to UV-B, PT no UV-B= pre-treated plants not exposed to UV-B, no PT UV-B+ = non pre-treated plants exposed to UV-B, no PT no UV-B = non pre-treated plants not exposed to UV-B. *Ler* plants grown in nursery at 240 µmol m^{-2} s^{-1} PAR for 24DAS then half of plants transferred to pre-treatment of higher PAR for 1 week at 360 µmol m^{-2} s^{-1} PAR. All plants then transferred to growth chamber for 5 days at 360 µmol m^{-2} s^{-1} PAR. Half of plants exposed to 10.2 UV_{BE} kJm^{-1} day^{-1}. Net photosynthetic rate is measured in µmol m^{-2} s^{-1}. Data points represent means ±1 SE (n=5).

The leaf content measurements were taken during the same experiment as the A_{max}. Chlorophyll content in *Ler* had no overall significant increase or decrease over the experiment (Fig. 3.16A). Pre-treatment and UV treatment did have significant effects over chlorophyll content but did not interact together. In *uvr8-1* Arabidopsis there also was no significant increase or decrease in chlorophyll content (Fig. 3.16B). There was a

significant interaction between time and UV treatment though no significant differences could be found within each day.

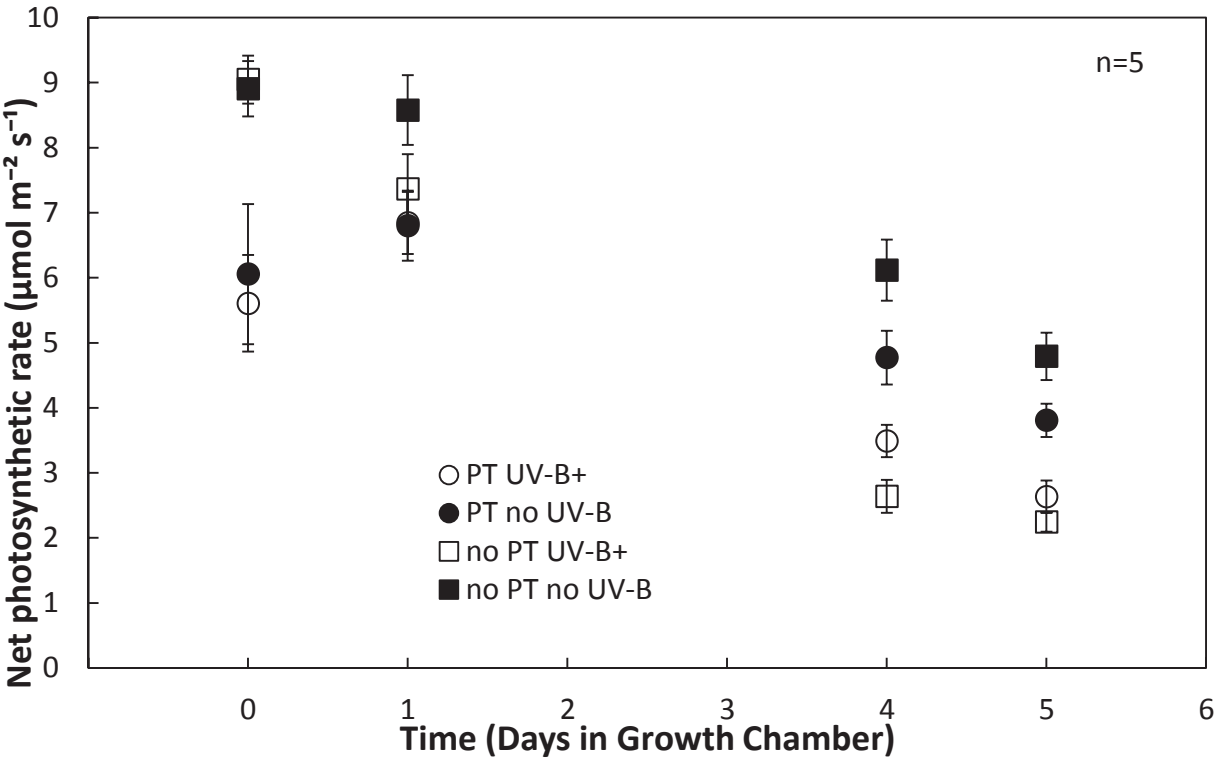

Figure 3.15. Net photosynthetic rate of *uvr8-1* Arabidopsis decreases in presence of higher PAR and UV. PT UV-B+= pre-treated plants exposed to UV-B, PT no UV-B= pre-treated plants not exposed to UV-B, no PT UV-B+ = non pre-treated plants exposed to UV-B, no PT no UV-B = non pre-treated plants not exposed to UV-B. *uvr8-1* plants grown in nursery at 240 µmol m^{-2} s^{-1} PAR for 24DAS then half of plants transferred to pre-treatment of higher PAR for 1 week at 360 µmol m^{-2} s^{-1} PAR. All plants then transferred to growth chamber for 5 days at 360 µmol m^{-2} s^{-1} PAR. Half of plants exposed to 10.2 UV_{BE} kJm^{-1} day^{-1}. Net photosynthetic rate is measured in µmol m^{-2} s^{-1}. Data points represent means ±1 SE (n=5).

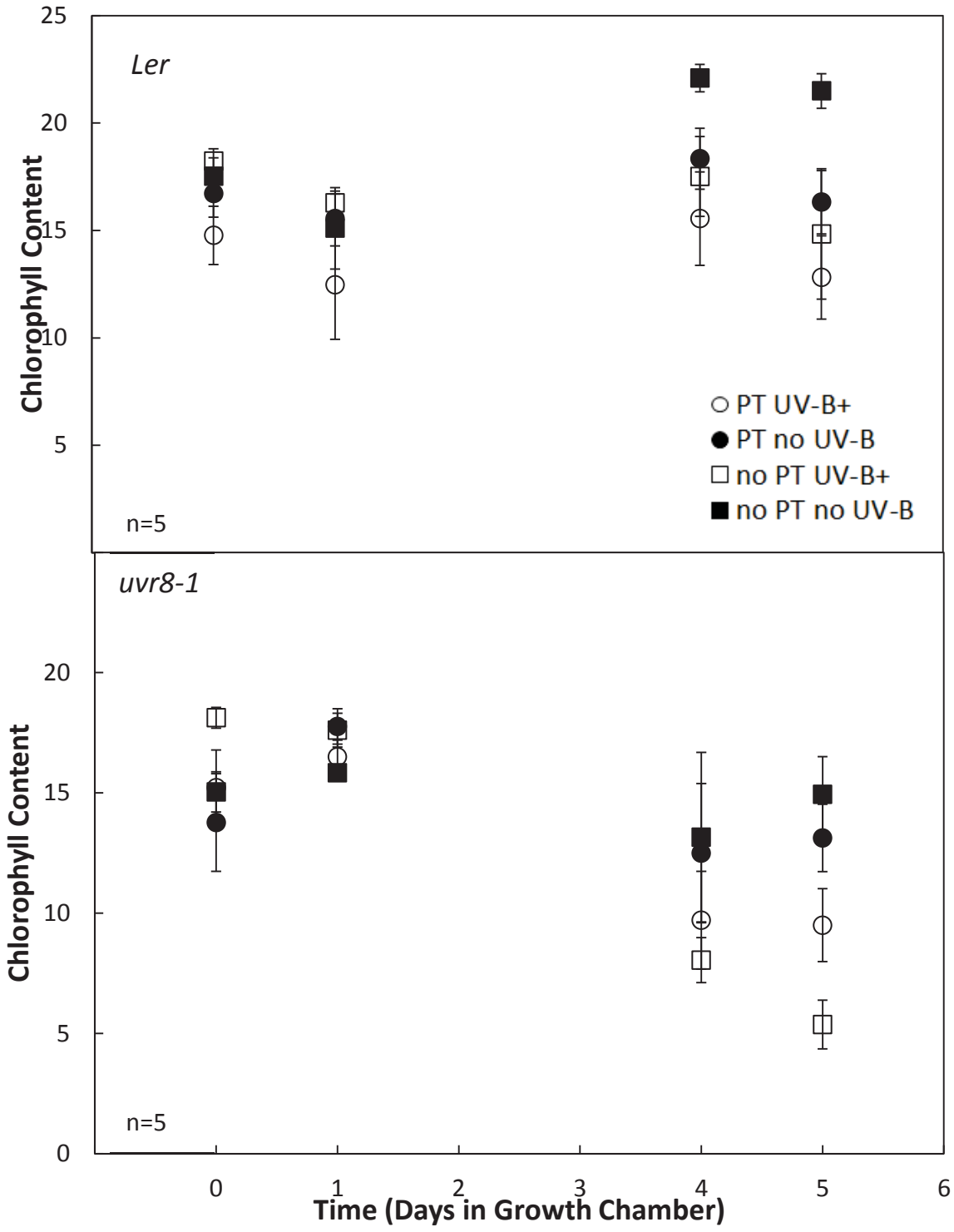

Figure 3.16. Treatments do not affect the amount of chlorophyll in wild-type *Ler* and *uvr8-1* mutant. *Ler* plants, *uvr8-1* plants. PT UV-B+= pre-treated plants exposed to UV-B, PT no UV-B= pre-treated plants not exposed to UV-B, no PT UV-B+ = non pre-treated plants exposed to UV-B, no PT no UV-B = non pre-treated plants not exposed to UV-B. *Ler* and *uvr8-1* plants grown in nursery at 240 µmol m^{-2} s^{-1} PAR for 24DAS then half of plants transferred to pre-treatment of higher PAR for 1 week at 360 µmol m^{-2} s^{-1} PAR. All plants then transferred to growth chamber for 5 days at 360 µmol m^{-2} s^{-1} PAR. Half of plants exposed to 10.2 UV_{BE} kJm^{-1} day^{-1}. Data points represent means ±1 SE (n=5).

The flavonoid content of the *Ler* plants significantly increased over the experiment (Fig. 3.17 *Ler*). The interaction between time and UV was highly significant and UV treatment had a highly significant effect of content regardless of any other treatment (Fig. 3.17 *Ler*). From Day 1 of the experiment the non-pre-treated, UV-B supplemented plants had a very significant increase from the non-pre-treated, minus UV-B. On Day 4 the pre-treated UV-B supplemented plants also had a significantly increased flavonoid content compared with those plants that did not get UV. The flavonoid content of the *uvr8-1* mutant also increased significantly over the experiment however did not increase as much as the *Ler* wild-type did (Fig. 3.17 *uvr8-1*). There was also no significance found between any of the treatments (Fig. 3.17 *uvr8-1*).

Ler plants had a significant decrease in NBI over the whole experiment (Fig. 3.18 *Ler*). The UV treatment had a very significant effect on this decrease with UV irradiated plants very significantly lower from Day 1 than plants not treated by UV (Fig. 3.18 *Ler*). The *uvr8-1* mutant also had a significant decrease in NBI though again no significant differences were found between the treatments used (Fig. 3.18 *uvr8-1*).

In summary, both the *Ler* and *uvr8-1* plants had lower A_{max} in plants pre-treated with higher PAR on Day 0 but the responses after that were different. In *Ler* plants the A_{max} in the pre-treated plants exposed to UV-B was not different from those pre-treated plants not exposed to UV-B. In *uvr8-1* plants decreased in A_{max}, with those plants exposed to UV-B more rapidly decreasing. *Ler* plants had no changes in chlorophyll content but the *uvr8-1* plants had a significant decrease in chlorophyll content due to UV-B by Day 5. Flavonoid content increased in *Ler* plants exposed to UV-B regardless of pre-treatment. There was no change in the flavonoid content of the *uvr8-1* plants. NBI decreased more rapidly in *Ler* plants exposed to UV-B. There was no difference in the rate of NBI depletion in *uvr8-1* plants under any treatment.

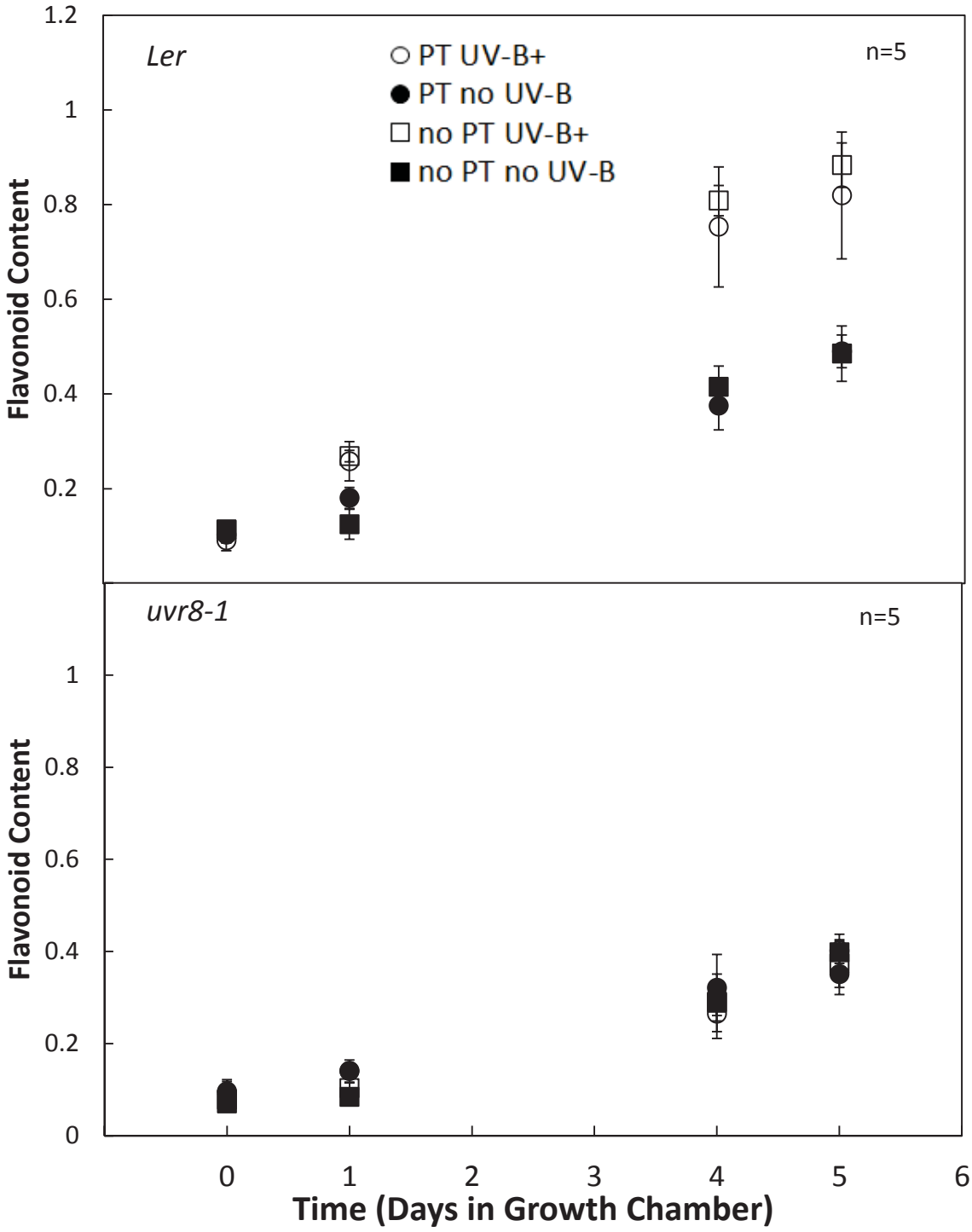

Figure 3.17. Flavonoid content increases during experiment in both *Ler* and *uvr8-1*. *Ler* plants, *uvr8-1* plants. PT UV-B+= pre-treated plants exposed to UV-B, PT no UV-B= pre-treated plants not exposed to UV-B, no PT UV-B+ = non pre-treated plants exposed to UV-B, no PT no UV-B = non pre-treated plants not exposed to UV-B. *Ler* and *uvr8-1* plants grown in nursery at 240 µmol m^{-2} s^{-1} PAR for 24DAS then half of plants transferred to pre-treatment of higher PAR for 1 week at 360 µmol m^{-2} s^{-1} PAR. All plants then transferred to growth chamber for 5 days at 360 µmol m^{-2} s^{-1} PAR. Half of plants exposed to 10.2 UV_{BE} kJm^{-1} day^{-1}. Data points represent means ±1 SE (n=5).

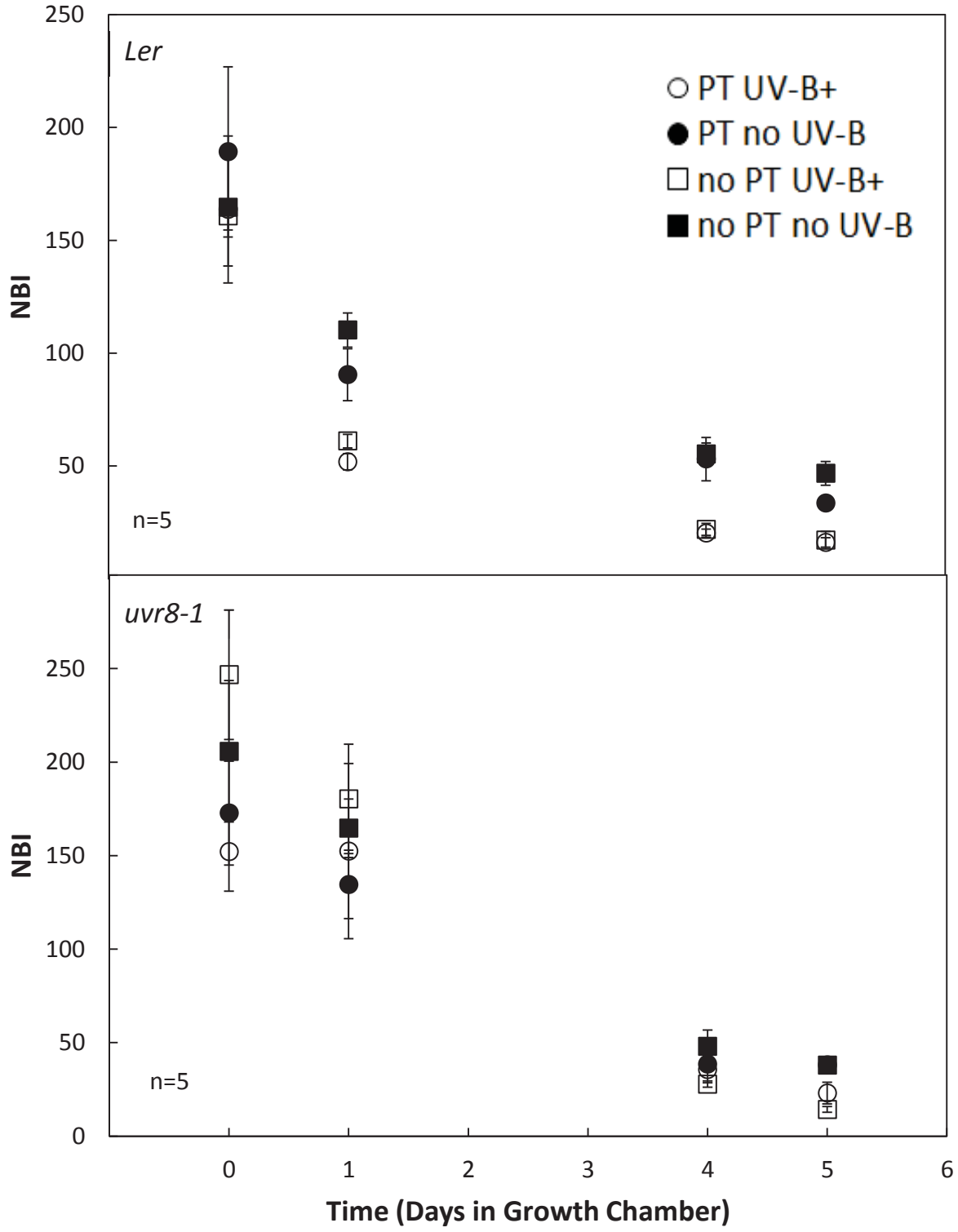

Figure 3.18. NBI decreases in both Ler and *uvr8-1* plants. (A) *Ler* plants, (B) *uvr8-1* plants. PT UV-B+= pre-treated plants exposed to UV-B, PT no UV-B= pre-treated plants not exposed to UV-B, no PT UV-B+ = non pre-treated plants exposed to UV-B, no PT no UV-B = non pre-treated plants not exposed to UV-B. *Ler* and *uvr8-1* plants grown in nursery at 240 µmol m^{-2} s^{-1} PAR for 24DAS then half of plants transferred to pre-treatment of higher PAR for 1 week at 360 µmol m^{-2} s^{-1} PAR. All plants then transferred to growth chamber for 5 days at 360 µmol m^{-2} s^{-1} PAR. Half of plants exposed to 10.2 UV_{BE} kJm^{-1} day^{-1}. Data points represent means ±1 SE (n=5).

3.4.2. Quantitative-PCR Results

To further understand the role of the UVR8 photoreceptor the transcript levels of genes known to be associated with UVR8 metabolic pathway were quantified using qPCR. The plants for this were grown exactly as the plants for the physical measurements. Plants were harvested (as described in methods) at specific time points. The time points were; before half the plants were transferred to the pre-treatment growth chamber (control), four hours after they were put in pre-treatment, and four hours after they were put into the growth chamber with ±UV radiation. The last time point had plants from the pre-treatment and those that were in the nursery room until 31DAS when the UV-B exposure started. The genes looked at were chalcone synthase (*CHS*), early light induced protein 1 (*ELIP1*) and sigma factor 5 (*SIG5*). There are two treatments missing for the uvr8-1 due to not enough plants germinating for all treatments.

CHS is the first enzyme in the phenylpropanoid biosynthesis pathway that is responsible for the induction of flavonoids in Arabidopsis (Weisshaar & Jenkins, 1998). The *Ler* plants that were exposed to UV had a very significantly higher *CHS* transcription level than the plants that did not receive any UV radiation (Fig. 3.19). The *Ler* plants moved from the nursery to the pre-treatment had no significant difference in *CHS* relative transcription level after 4 hours. A week later when the plants were transferred to the growth chamber with UV, the transcript levels of *CHS* had all significantly increased. The pre-treatment did not have a significant effect on the transcript level of *CHS* as the plants that did not receive UV are not significantly different from each other (Fig. 3.19). When UV is present there is no significant difference in *CHS* transcript level whether the plants were pre-treated or not. The *uvr8-1* plants had no significant difference in transcription of *CHS* at any time point regardless of UV treatment.

ELIP1 is an enzyme which responds to light stress in Arabidopsis but its full function is unknown (Rossini et al., 2006). UV treatment had a significant effect on the transcription level of *ELIP1* in both *Ler* and *uvr8-1* plants (Fig. 3.20). In *Ler* the UV treatment resulted in transcript levels very significantly higher than the control plants but there was no significant difference due to the pre-treatment of these plants. The transcript levels of the plants in the pre-treatment for four hours are significantly

decreased compared to the control *Ler* plants. Also the plants that had no pre-treatment and then no UV-B after 31DAS were not significantly different to the control plants and the plants that did receive a pre-treatment but no UV were significantly increased compared to the controls. The plants that had no UV treatment were not significantly different from each other regardless of whether they were pre-treated or not. The *uvr8-1* plants did not have significantly different transcript levels of *ELIP1* at the control stage to the *Ler* plants. The plants put in the pre-treatment for four hours had a significant decrease in *ELIP1* transcripts but by the time the plants were put into the growth chamber with UV-B after a week the transcript level was not significantly different to the control plants. The plants that were exposed to UV-B had significantly higher transcript levels than the plants that were not given UV-B.

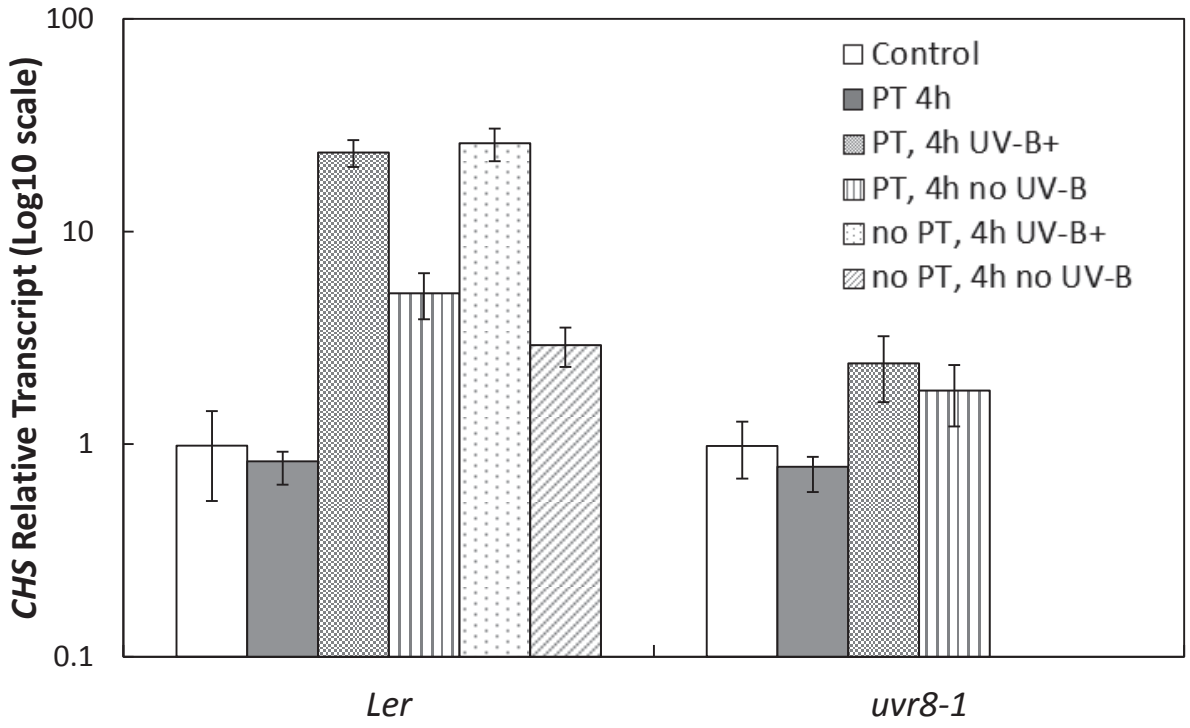

Figure 3.19. UV exposure causes significant increase in *CHS* transcripts. Control = nursery plants, PT 4h = plants 4hours after pre-treatment start, PT 4h UV-B+ = pre-treated plants 4 hours after exposure to UV-B, PT 4h no UV-B = pre-treated plants 4 hours after not being exposed to UV-B, no PT 4h UV-B+ = non pre-treated plants 4 hours after UV-B exposure, no PT 4h no UV-B = non pre-treated plants 4 hours after no exposure to UV-B. *Ler* and *uvr8-1* plants grown in nursery at 240 µmol m^{-2} s^{-1} PAR for 24DAS then half of plants transferred to pre-treatment of higher PAR for 1 week at 360 µmol m^{-2} s^{-1} PAR. All plants then transferred to growth chamber at 360 µmol m^{-2} s^{-1} PAR. Half of plants exposed to 10.2 UV_{BE} kJm^{-1} day^{-1}. Data points represent means ±1 SE (n=4).

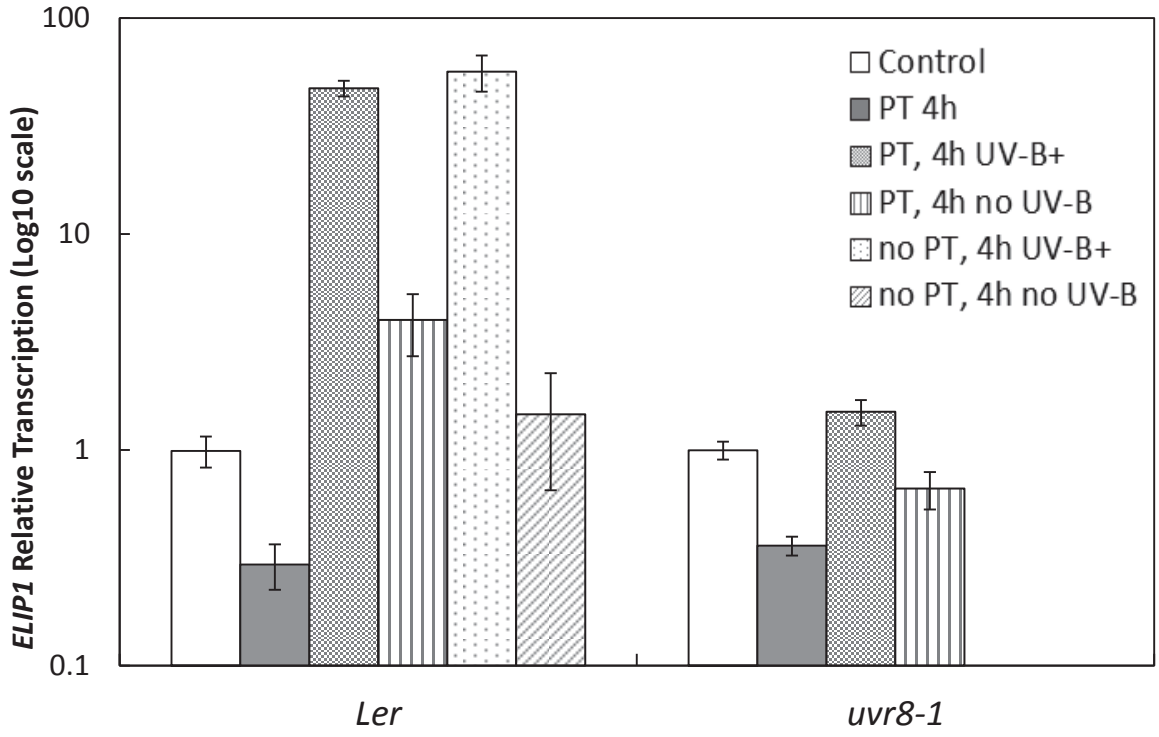

Figure 3.20. UV exposure leads to increased *ELIP1* transcription in *Ler* and a decrease in *uvr8-1*. Control = nursery plants, PT 4h = plants 4hours after pre-treatment start, PT 4h UV-B+ = pre-treated plants 4 hours after exposure to UV-B, PT 4h no UV-B = pre-treated plants 4 hours after not being exposed to UV-B, no PT 4h UV-B+ = non pre-treated plants 4 hours after UV-B exposure, no PT 4h no UV-B = non pre-treated plants 4 hours after no exposure to UV-B. *Ler* and *uvr8-1* plants grown in nursery at 240 µmol m^{-2} s^{-1} PAR for 24DAS then half of plants transferred to pre-treatment of higher PAR for 1 week at 360 µmol m^{-2} s^{-1} PAR. All plants then transferred to growth chamber at 360 µmol m^{-2} s^{-1} PAR. Half of plants exposed to 10.2 UV_{BE} kJm^{-1} day^{-1}. Data points represent means ±1 SE (n=4).

SIG5 is a sigma factor that mediates the production of psbD-BLRP transcripts in Arabidopsis (Davey et al., 2012). The *Ler* plants had a highly significant increase in *SIG5* transcript level in UV treated plants from the control plants (Fig. 3.21). The plants that were pre-treated for four hours decreased significantly from the control plants yet the transcript level after a week in the pre-treatment (31DAS) and then four hours in the growth chamber without UV were not significantly different from the control. Plants that went from the nursery to the growth chamber without UV treatment at 31DAS did not decrease in transcript level significantly and remained similar to the control levels. There is no significant difference in *SIG5* transcript level of plants exposed to UV regardless of pre-treatment and the same occurred with the plants not treated with

UV. The *uvr8-1* mutant also had a very significant decrease in *SIG5* transcript level after four hours in pre-treatment (Fig. 3.21). The control plants were also significantly higher than the plants transferred into UV for four hours and very significantly higher than the plants moved from the pre-treatment to the growth chamber without UV. There is however a significant difference between the transcript levels of *SIG5* in the plants that were exposed to UV and those that were not exposed to UV after the week of pre-treatment.

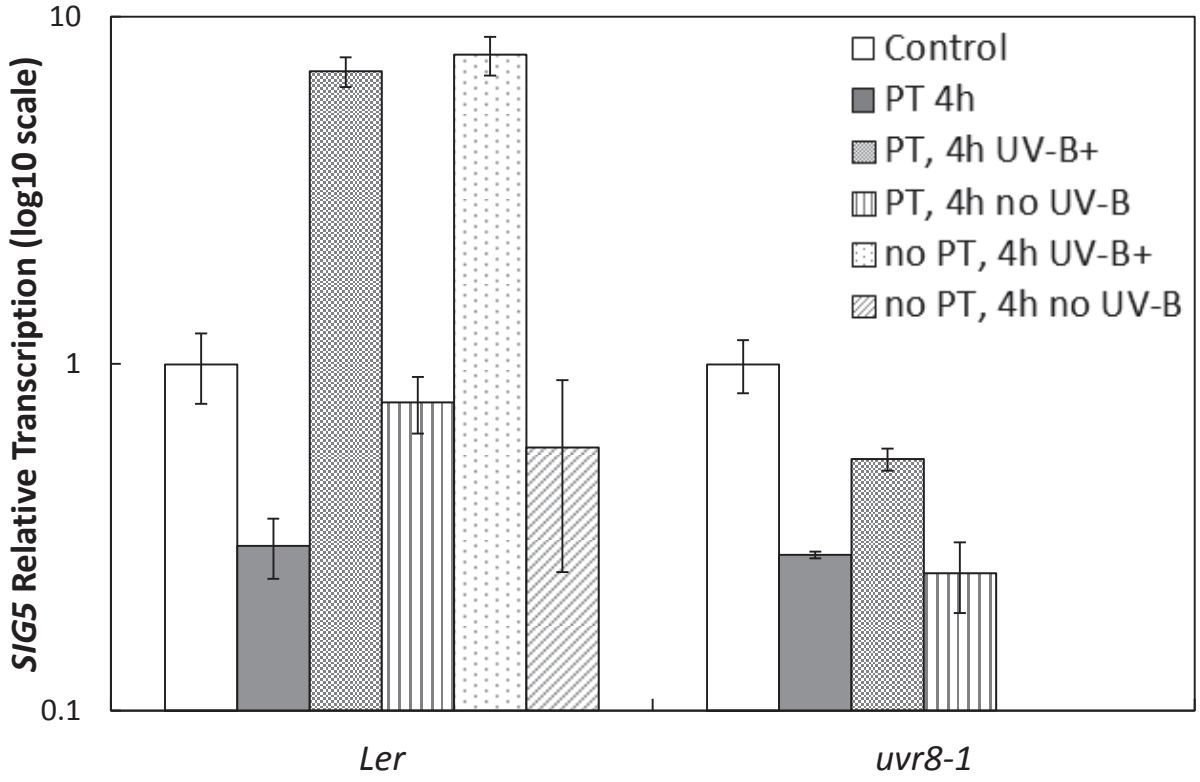

Figure 3.21. UV exposure causes an increased *SIG5* transcript level in *Ler* and decreased transcription in *uvr8-1*. Control = nursery plants, PT 4h = plants 4hours after pre-treatment start, PT 4h UV-B+ = pre-treated plants 4 hours after exposure to UV-B, PT 4h no UV-B = pre-treated plants 4 hours after not being exposed to UV-B, no PT 4h UV-B+ = non pre-treated plants 4 hours after UV-B exposure, no PT 4h no UV-B = non pre-treated plants 4 hours after no exposure to UV-B. *Ler* and *uvr8-1* plants grown in nursery at 240 µmol m^{-2} s^{-1} PAR for 24DAS then half of plants transferred to pre-treatment of higher PAR for 1 week at 360 µmol m^{-2} s^{-1} PAR. All plants then transferred to growth chamber at 360 µmol m^{-2} s^{-1} PAR. Half of plants exposed to 10.2 UV_{BE} kJm^{-1} day^{-1}. Data points represent means ±1 SE (n=4).

The results for the relative transcription levels of both *UVR8* and *CP12-2* can be found in Appendix 5. The *UVR8* transcript results had no significant changes in relative

transcript level as was expected (Fig. A5.1). CP12-2 is a small regulatory protein that interacts with chloroplasts (Pohlmeyer et al., 1996). The qPCR data for *CP12-2* was not included in the results as the values produced were too low and to variable be reliable transcript levels as per the Light cycler 480 parameters (Fig. A5.2).

In summary UV-B exposure in *Ler* plants significantly increases relative transcription levels in *CHS, ELIP1* and *SIG5*. There were no significant differences in relative transcript levels of *CHS* in *uvr8-1*. In *ELIP1* and *SIG5* there was a significant drop in relative transcript level 4 hours after pre-treatment in both *Ler* and *uvr8-1* plants. In *ELIP1* the relative transcript level in *uvr8-1* plants did have a small increase due to UV-B exposure. In *uvr8-1* plants *SIG5* relative transcript levels did not recover to control level but there was an increase in response to UV-B.

4. Discussion

4.1. Manipulation of PAR levels does change the UV-B photosynthetic response

In the first three experiments the PAR at which the plants were grown at in the nursery and the growth chamber was adapted to find the best light conditions of raising Arabidopsis (*Col-0*). In the pilot the photosynthetic rate (A_{max}) increases yet the addition of UV-B radiation had no effect (Fig. 3.1.). This is most likely due to the variation in the growth environments prior to UV-B exposure. In the pilot, plants were originally grown in a glasshouse environment in spring, where the PAR levels were similar to those reported in Wargent et al. (2015). PAR per day in the glasshouse was reported at 1999 ± 56 µmol m^{-2} s^{-1}. At 21DAS the plants were moved into a growth chamber 360µmol m^{-2} s^{-1} PAR before finally moving to a growth room with 50µmol m^{-2} s^{-1} PAR (Table 2.1). The low starting value of A_{max} can be explained by the low PAR environment that the plants were in prior to the start of the experiment. There was no change in response due to UV-B. As although the plants have not been exposed to it before the previous high light exposure meant that the plants had already adapted to deal with that amount of light stress (Chenu et al., 2005).

In the second experiment the nursery treatment of the plants was stable at 50µmol m^{-2} s^{-1} PAR with the experimental PAR again at 700µmol m^{-2} s^{-1} PAR. The plants did exhibit high light stress phenotype in this situation with the A_{max} not increasing for the first few days of high light exposure (Fig. 3.2.). The plants did increase in A_{max} in the last days of the experiment as the plants adapted to the higher PAR available. This increase was due to the fact that A_{max} is dependent on the quantity of light received (Chenu et al., 2005). The decrease in photosynthetic response under UV-B radiation that then increased was similar to the response of the lettuce seen in Wargent et al. (2015). The difference was that the UV-B exposed plants did not have a higher A_{max} than the unexposed plants. The plants were therefore adapting to more than just high PAR, impairing recovery (Hakala-Yatkin et al., 2010). The movement of plants from the low PAR to the high PAR in this case is also an example of the effects of non-ambient responses (Frohnmeyer & Staiger, 2003; Rozema et al., 1997). With the older leaves in both UV-B exposed and non-exposed plants exhibiting leaf necrosis, in older leaves.

To correct these non-ambient light conditions, the PAR of the nursery environment was increased and the growth chamber decreased (see Table 2.1). This was done to try and lower the effect a PAR increase has on the Arabidopsis plants, as otherwise the UV-B specific response could be masked. By raising the nursery room PAR to 240 µmol m^{-2} s^{-1} the initial A_{max} was higher than the previous experiments (Fig. 3.3.). The A_{max} continued to increase in the experiment, which was at 360 µmol m^{-2} s^{-1} PAR, but the increase was not as large as previously. The Arabidopsis plants had a lower overall A_{max} in the presence of UV-B but did increase from the initial A_{max}. As to why this was happening when Wargent et al. (2015) saw a large increase in photosynthesis verses the unexposed UV-B plants, is unknown. It could be that different species of plants have different photosynthetic responses to UV-B, such as the lettuce in Wargent et al. (2015) seeing an increase in A_{max}/photosynthetic rate, while in Berli et al. (2013) saw a decrease in grape A_{max}. Another reason for the decrease could be the due to Arabidopsis being a short day plant and lettuce is a long day plant. Therefore the UV-B level at which there is a positive photosynthetic response is different.

PAR manipulation impacts on the initial photosynthetic response to UV-B by either masking or exacerbating the response. By decreasing the difference in PAR between the nursery and experimental growing environments the impact of PAR on A_{max} was decreased.

4.2. Age had an effect on photosynthetic response but not photo-protective pigmentation.

The stabilisation of PAR in the nursery environment caused the growth of the Arabidopsis (*Col-0*) to become faster, with plants reaching a measurable size by 28DAS. As such, two ages of plants were studied to determine if there was any difference in photosynthetic and photo-protective response. In the results the older 35DAS plants had a higher A_{max} through-out the whole experiment than the 28DAS plants (Fig. 3.4.). However neither age exhibited a response to UV-B exposure in A_{max}, as seen in the two experimental repeats. Why there was no photosynthetic response to UV in these plants is unknown, as similar PAR changes had previously exhibited a decrease in A_{max}. Age also had an effect on leaf area and dry weight with the 35DAS plants larger than the 28DAS plants, both initially and at harvest. Only in the second repeat were there

any differences in leaf area and dry weight. With the 35DAS plants not exposed with UV-B larger than those that were exposed to UV-B. This suggests that there may have been a UV-B response in the plants but the experimental period was too short to confirm this. The 35DAS and 28DAS plants had the same amount of chlorophyll in all treatments and there was no increase or decrease. This could indicate a reason for no change in A_{max} as chlorophyll is found in chloroplasts; the location of photosynthesis (*Biology of Plants*, 2003).

The rapid accumulation in flavonoids due to UV-B seen in this research is a well-documented response (Brown et al., 2005; Gotz et al., 2010; Jenkins et al., 2001) with the 35DAS plants accumulating flavonoids more rapidly than 28DAS plants (Fig. 3.6.). On Day 6 the flavonoid content of the non-UV-B exposed 35DAS plants was the same as the UV-B exposed 28DAS plants. The 28DAS plants appeared to slow in accumulation, but the 35DAS plants did not. It is possible that this happened because the 35DAS plants had begun to display senescence traits, due to their advanced age. John, Morris, Jordan, Thomas, and Mackerness (2001) have shown that when senescence begins to occur there is accumulation of flavonoids in Arabidopsis, this could therefore explain the late accumulation in the non-exposed UV-B 35DAS plants. Had the experiment been longer the senescence response may have been occurred in 28DAS plants also when they reached a similar age. Nitrogen balance index (NBI), is the ratio of chlorophyll to flavonoids within the leaf and gives an estimate of carbon and nitrogen usage in plants (Cartelat et al., 2005). The NBI in the two ages of plants is due UV-B exposure, with the usage of carbon/nitrogen the same until the last day (Fig 3.8.). It was on Day 6 that the 35DAS plants had an increased usage of carbon/nitrogen, probably due to the same reasons explained above for flavonoid content. This is likely due to the carbon/nitrogen being used in the plants for the production of flavonoid compounds.

There was an accumulation of anthocyanins in all treatments but the 35DAS plants exposed to UV-B accumulated them the most rapidly. The differentiation was not within the first 24 hours suggesting that it was a flow on from initial UV-B response. At the end of the experiment there were further indications that there was a senescence response (John et al., 2001) due to the 35DAS plants being significantly higher in

anthocyanins than the 28DAS plants. The 28DAS plants exhibited anthocyanin accumulation from Day 3, but at a much lower rate. In the Wargent et al. (2015) study anthocyanin accumulation was also described as increased under UV-B. However Wargent et al. (2015) discussed how some of the accumulation may be due to a strong green peak in the growth chamber, as anthocyanins readily absorb green light (Lee & Gould, 2002; Neill & Gould, 1999). The initial levels of anthocyanins seen in these experiments could be due to this green peak, as there is a green peak in the nursery room as well as the growth cabinet (Figure 2.1). The accumulation of anthocyanins seen in the UV-B treatment is therefore not impaired by this, as the plants are already acclimated to a high green peak.

Older plants had a higher photosynthetic phenotype due to the plants larger leaf area. Age did not have an effect on the photo-protective compounds as an increases at the end of the experiment, in older plants, was attributed to the initiation of senescence. UV-B exposure had the greatest effect on photo-protective compounds as has been seen many times previously (Bolink et al., 2001; Hakala-Yatkin et al., 2010; M.E. Poulson et al., 2002).

4.3. Pre-treatment at a higher PAR lowers the initial photosynthetic rate

The pre-treatment was where plants were moved into a growth chamber a week before the experiment at the same PAR the experiment would be held at. This was done to the *Col-0* plants to see whether allowing the plants to acclimate to the growth chamber light quality would improve the photosynthetic phenotype. The main difference in light quality the growth chamber had was a peak in the UV-A part of the spectrum (Figure 2.1). The experiment in which 21DAS plants were moved into the pre-treatment had no significant differences in A_{max} response than the plants kept in the nursery until 28DAS (Fig. 3.9.). There were also no differences in leaf area or dry weight. The plants from both the nursery and the pre-treatment were smaller than the previous 28DAS plants used in an experiment.

From these results the decision was made to wait a few extra days until plants were 23 days old before giving them a pre-treatment. This was chosen rather than 28 days as

by the time plants would have finished the pre-treatment they would have been 35 days old. This was the age at which plants seemed to have a senescence response, as discussed earlier. The photosynthetic data from two repeats both showed the pre-treated plants having a lower initial A_{max} than the plants from the nursery (Fig. 3.10.). This was most likely due to the plants adapting to the higher PAR over the pre-treatment and therefore some photoinhibition would have taken place. The first repeat actually had an increase in photosynthesis in the no pre-treatment UV-B plants on Day 4 but this was not repeated. In fact the UV-B exposed plants had a lowered A_{max} phenotype again. Neither repeat had any changes in chlorophyll content in any of the treatments. The leaf areas of the pre-treated plants were smaller, a well-known response to photoinhibition (Jackson & Jenkins, 1995). The plants did not exhibit the increases in dry weight that have been reported on in previous research (Laposi et al., 2009; Sprtova et al., 2003) though this may just be due to the short experimental period not allowing for any changes to occur.

The flavonoid content of the plants again increased more rapidly in response to UV-B exposure in the first repeat. The pre-treated plants were not different in flavonoid content on Day 0 from non-pre-treated plants. However on Day 1 the pre-treated plants exposed to UV-B had a higher accumulation than the non-pre-treated plants. The flavonoid accumulation of the pre-treated and non-pre-treated plants exposed to UV-B was not significantly different in the final days of the experiment. The corresponding NBI data showed that the plants that had a pre-treatment started lower, suggesting that some photo-repair could already have started. The UV-B treated plants still consumed more carbon/nitrogen than the plants not exposed to UV. The flavonoid and NBI data was not as clear in the second repeat, most likely due to the replicate size being smaller, with any variation in response more pronounced.

Pre-treated plants exhibited a lower initial photosynthetic phenotype yet there was no conclusive response to UV-B exposure. The photo-protective compounds also exhibited some pre-treatment effects.

4.4. UVR8 has a role in maintaining photosynthetic competency

It is possible to conclude from the photosynthetic data from the *Ler* and *uvr8-1* plants that UVR8 has a role in maintaining photosynthetic competency. Pre-treatment of the *Ler* and *uvr8-1* plants lowers the initial A_{max}, as is seen in *Col-0*. Of interest is that in the first day of the new treatment all the *Ler* plants increase in A_{max} (Fig. 3.14.). This is a response that had not been seen in the rest of my research and is similar to the response seen in Wargent et al. (2015). It was only similar, as unlike the lettuce, the Arabidopsis plants did not continue to increase in A_{max} but actually decreased. The pre-treated *Ler* plants had no change in A_{max} due to UV-B. However, the plants that did not have a pre-treatment did have a change in A_{max}; with UV-B exposure resulting in a lower A_{max} than non-exposed UV-B plants. The *uvr8-1* plants did not increase in A_{max} at any point and began to rapidly decrease in A_{max} throughout the experiment. The *uvr8-1* plants exposed to UV-B dropped even more rapidly than the plants not exposed to UV-B (Fig. 3.15.). There was an effect of pre-treatment within these plants, as on Day 4 there was a significant decrease in the A_{max} of plants exposed to UV-B that did not have a pre-treatment. This difference is presumably an effect of the pre-acclimation to the higher PAR level (Chenu et al., 2005; Gotz et al., 2010; Kleine et al., 2007). This decrease of A_{max} in *uvr8-1*, while A_{max} was maintained in *Ler*, is why I believe that UVR8 has a role in the photosynthetic competency of Arabidopsis.

While there were no significant differences in chloroplast content in either *Ler* or *uvr8-1* plants, overall UV-B did have some effect. In the *Ler* the non-pre-treated plants exposed to UV-B had a higher number of chloroplast in the last two days (Fig. 3.16.). On day 5 the *uvr8-1* plants exposed to UV-B had significantly lower amounts of chloroplast; which can be attributed to UV-B damaging the chloroplasts and photosystem II (Davey et al., 2012). As no UVR8 means that the plants are protected against UV-B damage. Flavinoid content and NBI in *Ler* had the same response as the *Col-0* plants in the previous experiments. The *uvr8-1* plants do not have a UV-B response in either flavonoid content or NBI. The slight increase that was seen in the flavonoid content is not from the UVR8 pathway but could be due to either senescence or a UV-B independent pathway (John et al., 2001).

The decrease in A_{max} that *uvr8-1* plants exhibited irrespective of UV-B exposure when compared with the *Ler* plants is why I believe that UVR8 maintains photosynthetic competency. As the higher PAR of the experiment would not affect the *uvr8-1* A_{max} if UVR8 was not necessary. The production of photo-protective compounds was also inhibited in *uvr8-1* which allows for significant photoinhibiton due to UV-B radiation.

4.5. ELIP1 and SIG5 do not mediate UVR8 photosynthetic response

ELIP1 and SIG5 are mediated by UVR8, for a purpose, but they are not responsible for the photosynthetic response. Relative transcription of both *ELIP1* and *SIG5* increased in response to UV-B (Figs. 3.20. & 3.21.) in both *Ler* and *uvr8-1* plants. This suggests that there are two different UV-B responses occurring, one that is dependent on UVR8 and one that is independent as discussed by Wargent and Jordan (2013).

Davey et al. (2012) described how the UVR8 photoreceptor mediates a large-scale induction in the transcription of both *ELIP1* and *SIG5*. This was confirmed in my research as the relative transcription of both *ELIP1* and *SIG5* rapidly increased when exposed to UV-B radiation. Interestingly the data showed no difference in response between *Ler* plants that were pre-treated and those that had no pre-treatment. The pre-treatment did have an unexpected response on the relative transcript levels of both *ELIP1* and *SIG5*. There was a sharp decrease in transcripts after four hours of pre-treatment. This seems an odd response to higher PAR as both *ELIP1* and *SIG5* are known to respond to visible light and high light stress (Nagashima et al., 2004; Rossini et al., 2006).

In the *uvr8-1* plants the relative transcription levels of *ELIP1* did increase in response to UV-B. However this response was very small when compared to the UV-B response in *Ler*. This suggests that *ELIP1* may have a role in a UVR8 independent pathway. The response of *SIG5* to UV-B in the *uvr8-1* plants showed a greatly reduced transcription level, as was seen before in Davey et al. (2012). There was however a slight response again to UV-B, suggesting that it may also play a role in a UVR8 independent pathway.

When comparing the response of photosynthesis of both the *Ler* and *uvr8-1* plants to how *ELIP1* and *SIG5* respond to the same treatments, there seems to be no relationship. If *ELIP1* and *SIG5* were mediated by UVR8 photosynthetic competency

then there would have been consistent differences in A_{max}, in both pre-treated and non-pre-treated plants exposed to UV-B. Davey et al, (2012) found no evidence that *ELIP1* and *SIG5* are needed for the efficiency of PSII, which is directly effects photosynthetic competency.

From my research I believe that there is evidence for *ELIP1* and *SIG5* to mediate the photo-protective response in Arabidopsis. As increases in relative transcription level of *ELIP1* and *SIG5* are mirrored by increased *CHS* relative transcription. *CHS* is the first step in the phenylpropanoid biosynthesis pathway that forms flavonoids (Weisshaar & Jenkins, 1998). The relative transcript levels of *CHS* greatly increased in response to UV-B (Fig. 3.19.) in the *Ler* plants. This response was also seen in the leaf metabolite measurements with the *Ler* plants grown under UV-B increasing more rapidly. Therefore ELIP1 and SIG5 are possibly mediated by UVR8 to increase CHS and flavonoid accumulation.

4.6. Future Directions and Conclusion

The original aim for this research was to see if *Arabidopsis thaliana* increased in photosynthetic rate as had been seen in lettuce. This research does not achieve an increase but does show novel responses in photosynthetic rate to PAR and UV-B radiation. Unfortunately there was not time to look at how the *uvr8-1* mutant response changes under various UV-B conditions but it is definitely an area of research worth looking at.

I believe that there are a few adaptations to my experimental design that could improve the understanding of responses seen. This is to use image analysis to track the growth of plants through both the nursery and experimental changes to find any changes in growth rate. As well as, be able to standardise photosynthetic measurements against leaf area to remove any error of plant size. Another adaptation would be to change the ratios of PAR to UV-B radiation to see how else visible light effects the photosynthesis phenotype.

There are many avenues for taking this work further as this work only scratches the surface of what is occurring in Arabidopsis. These include varying the PAR and UV-B radiation as I described above, and also using different mutants in genes that are of

interest to UV-B and UVR8 regulation. Such as using mutants of *ELIP1* and *SIG5* that were described in this research, in the same growing conditions as *uvr8-1*. Another avenue could be comparing the photosynthetic responses of multiple species, at the same time, to UV-B radiation. As this could give provide information on how UV-B photomorphogenesis has evolved in plants. If the different species respond in different ways to UV-B, this knowledge could be used to create species specific UV-B treatments that provide beneficial effects.

In conclusion, I have found that *Arabidopsis thaliana* does not increase in photosynthetic rate to UV-B radiation and that UVR8 has a role maintaining photosynthetic competency. The manipulation of PAR during nursery and experimental growth has an impact on photosynthetic competency and the ability to discern a UV-B photosynthetic response. Photo-protective compounds are not affected by the PAR manipulations but more by UV-B exposure and plant age. There is no evidence to suggest that UVR8 mediated the downstream players, ELIP1 and SIG5, for photosynthetic response but they could mediated by UVR8 for a UV-B specific response in photo-protective compounds.

Appendices

Appendix 1 Age responses physical plant data

Averages for leaf area, fresh weights and dry weights of plants harvested at Day 0 and at the end of the experiment for the 35 day old and 28 day old plants, from section 3.2.

First Experimental Repeat			
Day 0 measurements			
Treatment	Leaf Area (cm^2)	Fresh weight (g)	Dry Weight (g)
35 days old	16.7 ± 1.3	0.5176 ± 0.0494	0.0513 ± 0.0050
28days old	9.31 ± 0.59	0.2181 ± 0.0151	0.0209 ± 0.0019
Harvest measurements –Day 6			
UV-B+ 35 days old	22.03 ± 2	0.0362 ± 0.0847	0.1281 ± 0.0087
No UV-B 35 days old	24.86 ± 1.02	0.9069 ± 0.0552	0.1405 ± 0.0082
UV-B+ 28 day days old	14.84 ± 0.92	0.4839 ± 0.0352	0.0645 ± 0.0047
No UV-B 28 days olds	16.15 ± 0.87	0.5124 ± 0.0337	0.0717 ± 0.0033
Second Experimental Repeat			
Day 0 measurements			
35 days old	24.42 ± 1.62	0.7816 ± 0.663	0.682 ± 0.0055
28days old	9.89 ± 0.51	0.2214 ± 0.0131	0.0178 ± 0.0012
Harvest measurements –Day 6			
UV-B+ 35 days old	27.78 ± 0.98	0.9951 ± 0.0437	0.1433 ± 0.0062
No UV-B 35 days old	33.07 ± 0.98	1.1310 ± 0.0465	0.1769 ± 0.0071
UV-B+ 28 day days old	14.98 ± 0.78	0.5202 ± 0.0264	0.0704 ± 0.0030
No UV-B 28 days olds	15.84 ± 0.88	0.4806 ± 0.0336	0.1383 ± 0.0702

Significances for the first experimental repeat:

Leaf Area			
Treatments used in ANOVA		**P-value**	**Significance**
35DAS -D0	28DAS -D0	6.0696E-05	highly
35DAS -D0	UV-B+ 35DAS	0.03935737	significant
35DAS -D0	no UV-B 35DAS	0.00010775	highly
28DAS -D0	UV-B+ 28DAS	7.8845E-05	highly
28DAS -D0	no UV-B 28DAS	4.1552E-06	highly
UV-B+ 35DAS	no UV-B 35DAS	0.22358546	no
UV-B+ 35DAS	UV-B+ 28DAS	0.00428767	very
no UV-B 35DAS	no UV-B 28DAS	4.0631E-06	highly
UV-B+ 28DAS	no UV-B 28DAS	0.31370664	no

Dry Weight			
Treatments used in ANOVA		**P-value**	**Significance**
35DAS -D0	28DAS -D0	1.9485E-05	highly
35DAS -D0	UV-B+ 35DAS	4.6593E-07	highly
35DAS -D0	no UV-B 35DAS	2.5734E-08	highly
28DAS -D0	UV-B+ 28DAS	9.2411E-08	highly
28DAS -D0	no UV-B 28DAS	8.7358E-11	highly
UV-B+ 35DAS	no UV-B 35DAS	0.31238064	no
UV-B+ 35DAS	UV-B+ 28DAS	5.0272E-06	highly
no UV-B 35DAS	no UV-B 28DAS	3.5036E-07	highly
UV-B+ 28DAS	no UV-B 28DAS	0.22876589	no

Significances for the second experimental repeat:

Leaf area			
Treatments used in ANOVA		P-value	Significance
35DAS -D0	28DAS -D0	3.53477E-08	highly
35DAS -D0	UV-B+ 35DAS	0.229209749	no
35DAS -D0	no UV-B 35DAS	0.000783714	highly
28DAS -D0	UV-B+ 28DAS	3.23724E-05	highly
28DAS -D0	no UV-B 28DAS	1.52568E-05	highly
UV-B+ 35DAS	no UV-B 35DAS	0.001267285	very
UV-B+ 35DAS	UV-B+ 28DAS	6.12773E-09	highly
no UV-B 35DAS	no UV-B 28DAS	1.30247E-10	highly
UV-B+ 28DAS	no UV-B 28DAS	0.472764243	no
Dry weight			
Treatments used in ANOVA		P-value	Significance
35DAS -D0	28DAS -D0	4.22645E-08	highly
35DAS -D0	UV-B+ 35DAS	3.71585E-08	highly
35DAS -D0	no UV-B 35DAS	3.89603E-10	highly
28DAS -D0	UV-B+ 28DAS	2.77142E-12	highly
28DAS -D0	no UV-B 28DAS	0.103130429	no
UV-B+ 35DAS	no UV-B 35DAS	0.002181654	very
UV-B+ 35DAS	UV-B+ 28DAS	3.61332E-09	highly
no UV-B 35DAS	no UV-B 28DAS	0.591602097	no
UV-B+ 28DAS	no UV-B 28DAS	0.346146319	no

Appendix 2 Physical data for plants pre-treated 21DAS

Averages for leaf area, fresh weights and dry weights of plants harvested at Day 0 and at the end of the experiment for the plants pre-treated at 21DAS and moved into the UV-B growth cabinet at 28DAS.

Day 0 measurements			
Treatment	Leaf Area (cm^2)	Fresh weight (g)	Dry Weight (g)
PT-D0	6.57 ± 0.34	0.1690 ± 0.0092	0.0171 ± 0.0011
no PT -D0	7.90 ± .61	0.1953 ± 0.0186	0.0172 ± 0.0014
Harvest measurements –Day 6			
PT UV-B+	13.79 ± 0.79	0.4762 ± 0.0349	0.1018 ± 0.0380
PT no UV-B	12.7 ± 0.59	0.4063 ± 0.0233	0.0597 ± 0.0032
no PT UV-B+	12.74 ± 0.83	0.4389 ± 0.0309	0.0566 ± 0.0045
no PT no UV-B	14.27 ± 0.65	0.4519 ± 0.0274	0.0668 ± 0.0031

Significances for leaf area and dry weight:

Leaf Area			
Treatments used in ANOVA		**P-value**	**Significance**
PT-D0	no PT -D0	0.074806318	no
PT-D0	PT UV-B+	1.17461E-07	highly
PT-D0	PT no UV-B	4.38277E-08	highly
no PT -D0	no PT UV-B+	0.000176485	highly
no PT -D0	no PT no UV-B	1.27329E-06	highly
PT UV-B+	PT no UV-B	0.285284625	no
PT UV-B+	no PT UV-B+	0.3712492	no
PT no UV-B	no PT no UV-B	0.092736301	no
no PT UV-B+	no PT no UV-B	0.16451314	no
Dry Weight			
Treatments used in ANOVA		**P-value**	**Significance**
PT-D0	no PT -D0	0.959531668	no
PT-D0	PT UV-B+	0.038480507	significant
PT-D0	PT no UV-B	2.07565E-10	highly
no PT -D0	no PT UV-B+	1.30392E-07	highly
no PT -D0	no PT no UV-B	1.79648E-11	highly
PT UV-B+	PT no UV-B	0.28318376	no
PT UV-B+	no PT UV-B+	0.251401365	no
PT no UV-B	no PT no UV-B	0.12538545	no
no PT UV-B+	no PT no UV-B	0.076031303	no

Appendix 3 Physical data for plants pre-treated 23DAS

Averages for leaf area, fresh weights and dry weights of plants harvested at Day 0 and at the end of the experiment for the plants pre-treated at 23DAS and moved into the UV-B growth cabinet at 30DAS. There were two repetitions of this experiment. There is no day 0 data for the second repeat.

First Experimental Repeat			
Day 0 measurements			
Treatment	Leaf Area (cm^2)	Fresh weight (g)	Dry Weight (g)
PT-D0	6.1 ± 0.2	0.1615 ± 0.0067	0.0164 ± 0.0009
no PT -D0	9.54 ± 0.74	0.2437 ± 0.0202	0.0205 ± 0.0016
Harvest measurements –Day 5			
PT UV-B+	11.45 ± 0.62	0.3612 ± 0.0223	0.0521 ± 0.0034
PT no UV-B	10.73 ± 0.78	0.3288 ± 0.0312	0.0459 ± 0.0027
no PT UV-B+	14.38 ± 0.64	0.4284 ± 0.0224	0.0617 ± 0.0037
no PT no UV-B	13.68 ± 0.71	0.385 ± 0.0269	0.0561 ± 0.0038
Second Experimental Repeat			
Day 0 measurements			
PT-D0	-	-	-
no PT -D0	-	-	-
Harvest measurements –Day 5			
PT UV-B+	7.23 ± 0.67	0.2148 ± 0.0218	0.0291 ± 0.0029
PT no UV-B	7.51 ± 1.01	0.2166 ± 0.0371	0.0370 ± 0.0042
no PT UV-B+	7.24 ± 0.43	0.1810 ± 0.0115	0.0278 ± 0.0023
no PT no UV-B	7.48 ± 0.75	0.1966 ± 0.0203	0.0355 ± 0.0036

Significances for the first experimental repeat:

Leaf area			
Treatments used in ANOVA		**P-value**	**Significance**
PT-D0	no PT -D0	0.000282668	highly
PT-D0	PT UV-B+	1.73061E-07	highly
PT-D0	PT no UV-B	1.96402E-05	highly
no PT -D0	no PT UV-B+	0.000105405	highly
no PT -D0	no PT no UV-B	0.000749089	highly
PT UV-B+	PT no UV-B	0.481492394	no
PT UV-B+	no PT UV-B+	0.00419308	very
PT no UV-B	no PT no UV-B	0.011936373	significant
no PT UV-B+	no PT no UV-B	0.473853588	no

Dry weight			
Treatments used in ANOVA		**P-value**	**Significance**
PT-D0	no PT -D0	0.035155297	significant
PT-D0	PT UV-B+	6.16572E-09	highly
PT-D0	PT no UV-B	4.40482E-09	highly
no PT -D0	no PT UV-B+	5.59217E-09	highly
no PT -D0	no PT no UV-B	9.54772E-08	highly
PT UV-B+	PT no UV-B	0.16533879	no
PT UV-B+	no PT UV-B+	0.068020589	no
PT no UV-B	no PT no UV-B	0.042490815	significant
no PT UV-B+	no PT no UV-B	0.302947469	no

Significances for the second repeat:

Leaf area			
Treatments used in ANOVA		**P-value**	**Significance**
PT UV-B+	PT no UV-B	0.81940047	no
PT UV-B+	no PT UV-B+	0.99506324	no
PT no UV-B	no PT no UV-B	0.98243589	no
no PT UV-B+	no PT no UV-B	0.77842297	no
Dry weight			
Treatments used in ANOVA		**P-value**	**Significance**
PT UV-B+	PT no UV-B	0.13528451	no
PT UV-B+	no PT UV-B+	0.73491393	no
PT no UV-B	no PT no UV-B	0.78522608	no
no PT UV-B+	no PT no UV-B	0.08569789	no

Appendix 4 Physical Data for *Ler* and *uvr8-1*

Averages for leaf area, fresh weights and dry weights of plants harvested at Day 0 and at the end of the experiment for the plants *Ler* and *uvr8-1* pre-treated at 24DAS and moved into the UV-B growth cabinet at 31DAS.

4.1 *Ler*

Day 0 measurements			
Treatment	Leaf Area (cm^2)	Fresh weight (g)	Dry Weight (g)
PT-D0	8.03 ± 0.42	0.1881 ± 0.0102	0.0255 ± 0.0017
no PT -D0	12.56 ± 0.92	0.3049 ± 0.0271	00.0279 ± 0.0027
Harvest measurements –Day 5			
PT UV-B+	8.73 ± 0.47	0.2331 ± 0.0148	0.0360 ± 0.0025
PT no UV-B	8.18 ± 0.64	0.2385 ± 0.0239	0.0368 ± 0.0031
no PT UV-B+	13.75 ± 1.05	0.4003 ± 0.0884	0.0563 ± 0.0048
no PT no UV-B	16.56 ± 0.78	0.4889 ± 0.0292	0.0701 ± 0.0033

Significances for *Ler* plants:

Leaf area			
Treatments used in ANOVA		**P-value**	**Significance**
PT-D0	no PT -D0	0.000375197	highly
PT-D0	PT UV-B+	0.320374314	no
PT-D0	PT no UV-B	0.841294477	no
no PT -D0	no PT UV-B+	0.433275695	no
no PT -D0	no PT no UV-B	0.012944416	significant
PT UV-B+	PT no UV-B	0.515116047	no
PT UV-B+	no PT UV-B+	0.002439339	very
PT no UV-B	no PT no UV-B	3.46759E-05	highly
no PT UV-B+	no PT no UV-B	0.064432322	no
Dry weight			
Treatments used in ANOVA		**P-value**	**Significance**
PT-D0	no PT -D0	0.472464231	no
PT-D0	PT UV-B+	0.003937335	very
PT-D0	PT no UV-B	0.004476035	very
no PT -D0	no PT UV-B+	0.00011657	highly
no PT -D0	no PT no UV-B	5.13027E-07	highly
PT UV-B+	PT no UV-B	0.857370292	no
PT UV-B+	no PT UV-B+	0.005931959	very
PT no UV-B	no PT no UV-B	7.40914E-05	highly
no PT UV-B+	no PT no UV-B	0.04472019	significant

4.2 *uvr8-1*

Day 0 measurements			
Treatment	Leaf Area (cm^2)	Fresh weight (g)	Dry Weight (g)
PT-D0	7.98 ± 0.44	0.1846 ± 0.0120	0.0226 ± 0.0017
no PT -D0	8.39 ± 0.67	0.1930 ± 0.0199	0.0192 ± 0.0026
Harvest measurements –Day 5			
PT UV-B+	9.46 ± 0.6	0.2504 ± 0.0151	0.0369 ± 0.0021
PT no UV-B	9.73 ± 0.74	0.2480 ± 0.0197	0.0446 ± 0.0037
no PT UV-B+	9.91 ± 0.47	0.2251 ± 0.0113	0.0327 ± 0.0020
no PT no UV-B	10.83 ± 0.99	0.2789 ± 0.0226	0.0436 ± 0.0038

Significances for *uvr8-1* plants:

Leaf area			
Treatments used in ANOVA		**P-value**	**Significance**
PT-D0	no PT -D0	0.620560171	no
PT-D0	PT UV-B+	0.070237975	no
PT-D0	PT no UV-B	0.051147323	no
no PT -D0	no PT UV-B+	0.143164882	no
no PT -D0	no PT no UV-B	0.055897227	no
PT UV-B+	PT no UV-B	0.784147976	no
PT UV-B+	no PT UV-B+	0.569220967	no
PT no UV-B	no PT no UV-B	0.395627449	no
no PT UV-B+	no PT no UV-B	0.4249876	no
Dry weight			
Treatments used in ANOVA		**P-value**	**Significance**
PT-D0	no PT -D0	0.286583705	no
PT-D0	PT UV-B+	0.000221498	highly
PT-D0	PT no UV-B	4.0357E-05	highly
no PT -D0	no PT UV-B+	1.80754E-12	highly
no PT -D0	no PT no UV-B	0.000141542	highly
PT UV-B+	PT no UV-B	0.10661002	no
PT UV-B+	no PT UV-B+	0.006269085	very
PT no UV-B	no PT no UV-B	0.857605062	no
no PT UV-B+	no PT no UV-B	0.034383561	significant

Appendix 5 Relative transcript levels of *UVR8* and *CP12-2*.

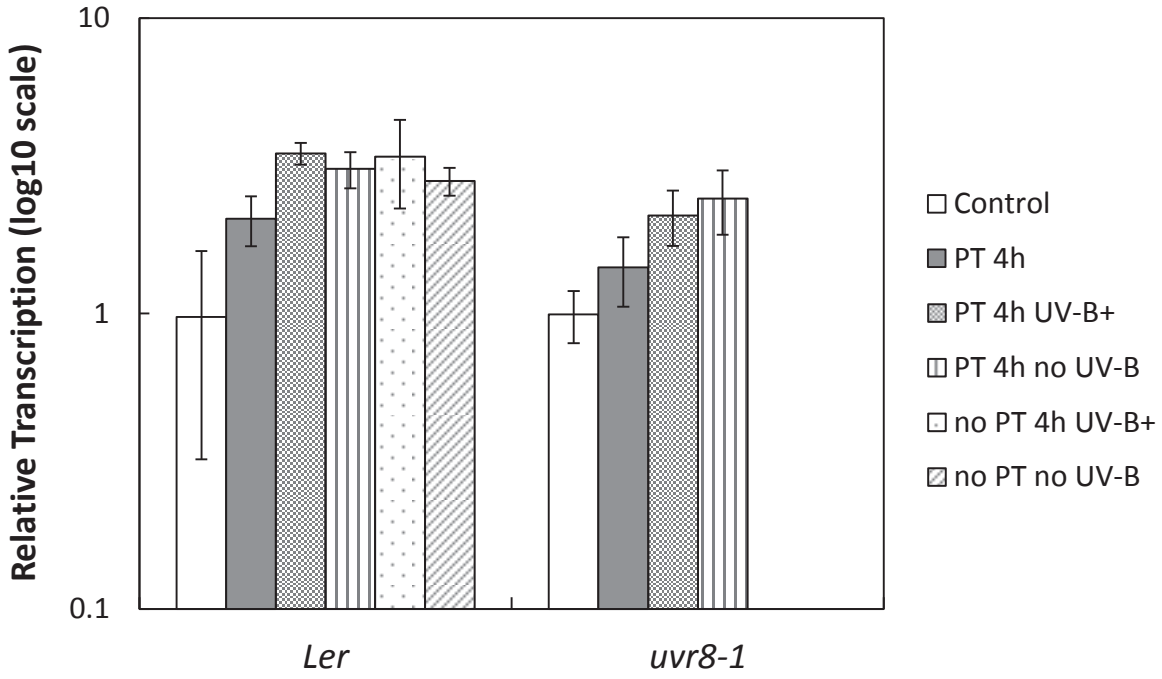

Figure A5.1. Relative transcript levels of *UVR8* did not change in any treatment from control. Control = nursery plants, PT 4h = plants 4hours after pre-treatment start, PT 4h UV-B+ = pre-treated plants 4 hours after exposure to UV-B, PT 4h no UV-B = pre-treated plants 4 hours after not being exposed to UV-B, no PT 4h UV-B+ = non pre-treated plants 4 hours after UV-B exposure, no PT 4h no UV-B = non pre-treated plants 4 hours after no exposure to UV-B. *Ler* and *uvr8-1* plants grown in nursery at 240 µmol m^{-2} s^{-1} PAR for 24DAS then half of plants transferred to pre-treatment of higher PAR for 1 week at 360 µmol m^{-2} s^{-1} PAR. All plants then transferred to growth chamber at 360 µmol m^{-2} s^{-1} PAR. Half of plants exposed to 10.2 UV_{BE} $kJm^{-1}day^{-1}$. Data points represent means ±1 SE (n=4).

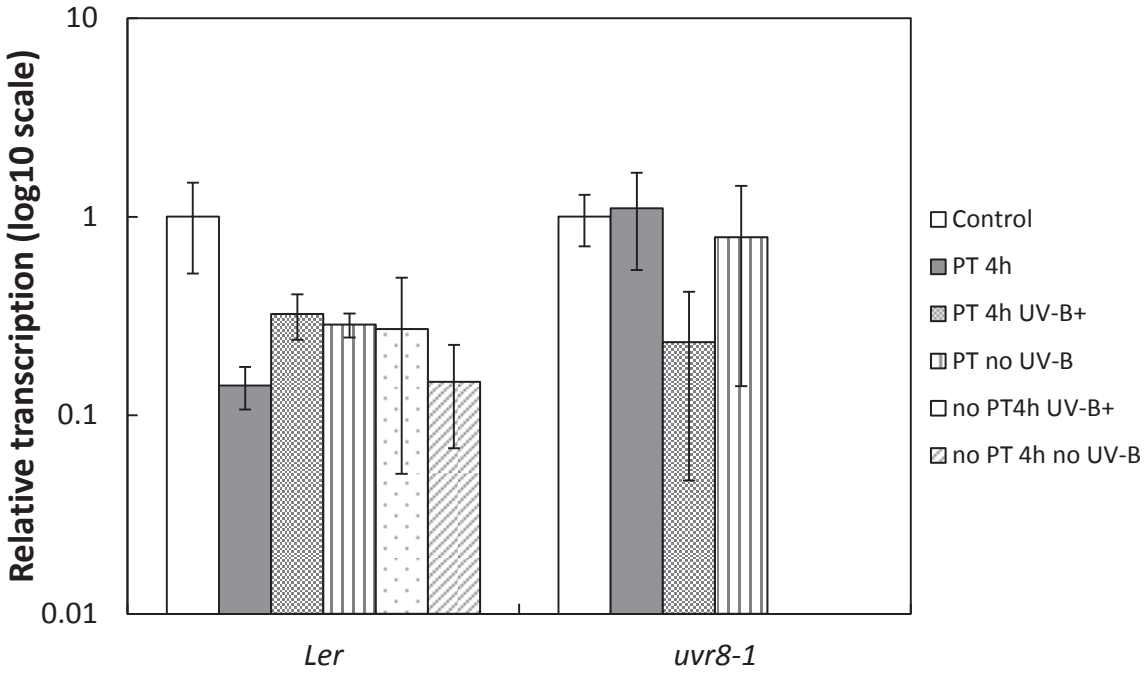

Figure A5.2. Relative transcript levels of *CP12-2* relative transcript levels, unreliable due to too much variation in data. Control = nursery plants, PT 4h = plants 4hours after pre-treatment start, PT 4h UV-B+ = pre-treated plants 4 hours after exposure to UV-B, PT 4h no UV-B = pre-treated plants 4 hours after not being exposed to UV-B, no PT 4h UV-B+ = non pre-treated plants 4 hours after UV-B exposure, no PT 4h no UV-B = non pre-treated plants 4 hours after no exposure to UV-B. *Ler* and *uvr8-1* plants grown in nursery at 240 µmol m^{-2} s^{-1} PAR for 24DAS then half of plants transferred to pre-treatment of higher PAR for 1 week at 360 µmol m^{-2} s^{-1} PAR. All plants then transferred to growth chamber at 360 µmol m^{-2} s^{-1} PAR. Half of plants exposed to 10.2 UV_{BE} $kJm^{-1}day^{-1}$. Data points represent means ±1 SE (n=4).

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