LIGHT QUALITY AFFECTS LEAF PIGMENTS
AND LEAF PHENOLOGY

Craig C. Brelsford

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ACADEMIC DISSERTATION
Supervisor:
Dr T Matthew Robson, University of Helsinki, Finland

Advisory committee:
Prof. Paula Elomaa, University of Helsinki, Finland
Assoc. Prof. Markku Larjavaara, Peking University, China
Prof. Anders Lindfors, Finnish Meteorological Institute, Finland

Pre-examiners:
Prof. Eva Rosenqvist
Assoc. Prof. Carla Valeria Giordano

Opponent:
Prof. Gareth Phoenix

Custos:
Prof. Kurt Fagerstedt, University of Helsinki, Finland

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“Live in each season as it passes; breathe the air, drink the drink, taste the fruit, and resign yourself to the influence of the earth.”

- Henry David Thoreau, Walden
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CCB: Craig C Brelsford  
JN: Jakub Nezval  
LOM: Luis O Morales  
MT: Marieke Trasser  
PJA: Pedro J Aphalo  
SMH: Saara M Hartikainen  
TK: Titta Kotilainen  
TP: Tom Paris  
TMR: T Matthew Robson
ABSTRACT

Light quality varies in space and time, and plants are able to detect and respond to these environmental cues. Plants must time when their leaves come out in spring and fall off in autumn, to maximise opportunities for photosynthesis whilst conditions are favourable. Similarly, they must optimise the amount of sun-screening pigments in their leaves, to minimise the harmful effects of ultraviolet radiation at high irradiance.

Solar radiation reaching the Earth, as well as its composition, vary diurnally and seasonally with solar angle. During twilight, plants are able to detect changes in red:far-red light, and use this to help time their spring and autumn phenology. When forest canopies leaf out in spring, and cause canopy closure, the understorey becomes mostly covered in shade. This shade also causes a low red: far-red ratio, that plants are able to detect and increase their stem elongation. However, the amount of blue and UV radiation also varies in space and time, and we know considerably less about how plants respond to these changes in the blue-and-UV region.

Using a combination of controlled indoor experiments, literature review, and manipulative field experiments, we set out four aims. 1) How do blue and UV-A radiation affect leaf pigments under controlled conditions? 2) How does blue light affect spring bud burst under controlled conditions? 3) How do blue and UV radiation affect leaf pigments and leaf phenology for understorey plant species? 4) How important is light quality as a phenological cue?

We found that both under controlled conditions and in the field, blue light had a large positive effect on the accumulation of flavonoids, most likely governed by cryptochrome photoreceptors. Interestingly, the flavonols in more light-demanding species of plants were more responsive to changes in light quality, particularly blue light. Similarly, blue light advanced spring bud burst in tree species both in the lab and in the field. We also report that both blue light and UV radiation can advance autumn leaf senescence in understorey plants. Lastly, when critically comparing the effect sizes of light quality treatments on phenological responses in trees, we found that light quality effects on spring phenology are generally small. However, the effects reported on autumn phenology are much larger. This adds to the complexity of drivers affecting autumn phenology, and may be one reason why autumn phenology is typically much harder to forecast compared to spring.

Future work should seek to understand how other environmental drivers such as temperature will interact with light quality to affect leaf pigments and leaf phenology. It will be important to understand how climate change could produce potential phenological mismatches in cues between the canopy and understorey, and even between different organisms such as plants, herbivores, and pollinators.
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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:


The publications are referred to in the text by their Roman numerals.
ABBREVIATIONS

CRY1 – cryptochrome 1
CRY2 - cryptochrome 2
CRYs – cryptochrome
$F_{m}/F_{m}^{0}$ – maximal quantum efficiency of PSII photochemistry
$F_{q}^{0}/F_{m}^{0}$ – operating efficiency of PSII photochemistry
LAI – leaf area index
PHOT1 – phototropin 1
PHOT2 – phototropin 2
PHOTs – phototropins
PSII – photosystem II
PHYs – phytochromes
PHYA – phytochrome A
PHYB – phytochrome B
R:FR – red:far-red
SAS – shade avoidance syndrome
UV-A – ultraviolet radiation A
UV-B – ultraviolet radiation B
UVR8 – ultraviolet resistance locus 8
WT – wild type
1. INTRODUCTION

1.1 PHENOLOGY

Organisms time their life cycle events to exploit natural resources, and ultimately to successfully reproduce. However, unlike organisms that are able to migrate or travel, plants are sessile. Because of this, they must sense seasonal changes in environmental cues, and use them to time life-cycle events to the time of year. For instance, come springtime, increasing solar radiation, and thus temperature, mean that conditions for photosynthesis become suitable. As such, annual, perennial and woody plant species in temperate and boreal regions, time their leaf out in spring to exploit these conditions which are suitable for photosynthesis (Hänninen 1991; Augspurger 2009; Bennie et al. 2010). Come autumn time, there is a decrease in solar radiation, and lower temperatures which could cause frost damage. Consequently, deciduous plants will go through leaf senescence, and remobilize nutrients from leaves, before shedding their leaves and entering dormancy over winter (Lang 1987, Hänninen 1995; Cesaraccio et al. 2004), before spring returns the next year.

The timing of biological events is known as ‘phenology’. Monitoring phenology is widely accessible to both the public and scientists alike, and thus plays an important role in scientific communication (Cooper et al. 2014; Bestelmeyer et al. 2015). Accordingly, it is a commonly used metric for reporting the effects of climate change (Menzel 2002; Cleland et al. 2007). It has cultural and commercial importance ranging from records made by Henry David Thoreau in New England, to the timing of grape ripening in vineyards (Chuine et al. 2004; Primack et al. 2012). Beyond this, plant phenology determines the length of the growing season, and thus the period that plants are able to photosynthesize and store carbon. In this respect, understanding phenology can help inform our predictions of how carbon sequestration will be affected by climate change (Peñuelas and Filella, 2009).

What are the major cues governing leaf phenology of plants in temperate and boreal regions?

For temperate and boreal tree species, the major cues governing bud burst and leaf out in spring are accumulated chilling temperatures over winter, forcing temperatures in spring, and to some extent increasing photoperiod (Körner 2007; Körner and Basler 2010; Caffarra and Donnelly 2011). However, these cues can interact. If a small amount of chilling has accumulated, then the effect of photoperiod and forcing temperatures can be greater to compensate for low chilling, so that the plant may still reach bud burst (Flynn and Wolkovich, 2018). Likewise, if forcing temperature is low and photoperiod short, then a high amount of chilling during winter can compensate for this (Flynn and Wolkovich, 2018). For autumn, our understanding of leaf senescence and bud set is more limited, and it has been aptly
named the ‘forgotten season’ with respect to climate change research (Gallinat et al. 2015). A meta-analysis showed that the major cues governing autumn leaf senescence were October temperatures, cooling degree-days, latitude, photoperiod, and lastly precipitation (Gill et al. 2015). High latitude sites were also more sensitive to photoperiod (Gill et al. 2015). Furthermore, there was a different effect of environmental drivers on leaf fall compared to their effect on the point at which 50% of leaves had turned yellow (Gill et al. 2015); with latitude having the greatest effect on the prediction of 50% of leaves turning yellow. This suggests that there may be different environmental factors governing leaf colouration in autumn, and final leaf fall. For some tree species, accumulated chilling temperatures have been found to largely predict the date of leaf senescence, whilst for other tree species photoperiod can be a better predictor (Delpierre et al. 2009; Vitasse et al. 2011). There is also experimental evidence that decreasing photoperiod advances leaf senescence in several tree species (Li et al. 2003; Welling and Palva 2006; Lagercrantz 2009).

How do phenological strategies differ?

Phenological strategies can also differ according to functional strategy. For instance, it has been proposed that more light-demanding tree species often leaf out earlier, and that this response is dominated mostly by forcing temperatures rather than photoperiod (Basler and Körner, 2012). In contrast, species which leaf out later would be more responsive to photoperiod cues. However, as more recent studies have shown that different phenological cues can compensate for each other (Flynn and Wokovich, 2018), a more updated synthesis of how different functional types of plants integrate these cues is needed. Likewise, many herbaceous species and woody species in forest understoreys exhibit phenological avoidance, whereby they time leaf out earlier than the canopy trees above, or leaf senescence later in autumn, to exploit periods of light in the understorey for photosynthesis (Uemura 1994; Augspurger et al. 2005; Richardson and O’Keefe, 2009). Earlier leaf out in understorey tree species has been shown to be due to ontogenic factors (Vitasse, 2013), whereas the environmental drivers that cause leaf out in herbaceous species has been less well studied compared to tree species, but include snowmelt, and forcing temperatures (Price and Waser 1998; Rice et al. 2018). Elevated temperatures have been shown to advance leaf senescence in the understorey herb Panax quinquefolius (Jochum et al. 2007). For some spring ephemeral species, shading has been shown to induce earlier leaf senescence (Augspurger and Salk, 2017). However, beyond these studies, there is limited information on the environmental cues which specifically affect leaf senescence in understorey plant species.

Whilst phenological differences exist between the overstorey and the understorey, they also vary according to latitude. For autumn phenology, it has been shown that bud set in ecotypes from higher latitudes is more responsive to photoperiod (Ekberg et al. 1979, Li et al. 2003). However, for spring phenology this issue remains contentious, as there is evidence to suggest that bud burst in more northern ecotypes is more responsive to photoperiod (Vaartaja 1959; Myking and
Heide 1995; Robson et al. 2013; reviewed by Way and Montgomery 2015; Cooper et al. 2018), as well as evidence to suggest that more southern species are more responsive to photoperiod (Kriebel 1957; Olson et al. 2013; Zohner et al. 2016; Osada et al. 2018). As such, it is not known whether bud burst of northern ecotypes of trees is more sensitive to changes in photoperiod, or whether southern ecotypes are more sensitive.

1.2 THE LIGHT ENVIRONMENT

Not only do the amount of light and day length vary over the course of a year, but also the light quality. That is to say, the different colours or wavelengths of solar radiation that a plant receives. There is growing evidence that plants can detect these changes in light quality throughout the year and adjust their phenology accordingly.

*How does the light environment vary over the year?*

Light quality changes diurnally over the course of a day, and thus with day length, latitude, and season. For instance, as solar elevation decreases, and the sun dips below the horizon, the light quality during this twilight period before night-time changes substantially. There is a drop in the ratio of red light relative to far-red light, known as the R:FR ratio (Figure 1) (Robertson 1966; Smith 1982; Chambers and Spence 1984; Hughes et al. 1984). This is because the lower elevation of the sun means that longer wavelengths of FR light are refracted over the horizon and are thus enriched during twilight hours (Smith 1982). Whereas the ratio of blue to red light (B:R) increases during twilight (Johnson et al. 1967, Hughes et al. 1984). This is due to the Chappuis bands in the ozone, which preferentially absorb red light, and also because blue light is scattered across the sky to a greater extent than red light (Hulburt 1953; Johnsen 2012). Of course, overall irradiance also changes drastically over the course of a day, being higher at noon, along with the proportion of ultraviolet-B (UV-B) radiation. Atmospheric conditions such as cloud cover and water vapour can affect the composition of solar radiation reaching the ground as well. For example, cloud cover can increase the amount of diffuse radiation, thus proportionally enriching blue and UV radiation in comparison to total PAR (Urban et al. 2007; Dengel et al. 2015). Furthermore, water vapour in the atmosphere has also been suggested to be responsible for a peak in R:FR which is sometimes observed during sunrise and sunset (Lee and Downum, 1991). Changes in ozone also lead to changes in the amount of UV radiation reaching the ground, and areas where the ozone is thinnest are around the equatorial belt, and also lead to the highest irradiances of UV-B radiation there (Caldwell et al. 1980; Blumthaler et al. 1997; McKenzie et al. 2001a; 2001b).
Figure 1. A schematic showing changes in light quality that occur diurnally, and thus vary with day length across the year.

**Light quality affects phenology**

Evidence is also amounting that shows plants respond to these temporal changes in light quality. For example, low R:FR during twilight has been shown to advance bud burst in *Betula pendula* (Linkosalo and Lechowiz, 2006), as well as delay bud set in *P. abies* (Mølmann et al. 2006). In this sense, changes in the duration of low R:FR during twilight hours, aid the perception of changes in photoperiod. Similarly, day length extensions with blue light have also been shown to delay bud set in *P. abies*, albeit not as effectively as low R:FR (Mølmann et al. 2006). Furthermore, UV-B radiation has been shown to advance bud-set in *P. tremula* (Strømme et al. 2015). Whilst these studies show that light quality can affect phenology, there is a lack of information on the response of bud burst to blue light, as well as leaf senescence in response to blue light and UV radiation.

**How does light environment vary in the understorey?**

Light quality not only varies temporally, but also spatially. Underneath canopies, there is a reduction in overall irradiance and photosynthetically active radiation (PAR), as well as a low R:FR ratio (Figure 2) (Ballaré and Pierik 2017). The low R:FR
ratio is produced because FR light is scattered by vegetation to a greater extent than red light, and so is enriched in the understorey (Ballaré et al. 1987). Similarly, blue light is absorbed by the canopy and so also becomes greatly depleted in the forest understorey (Casal 2013). UV radiation is also absorbed by the canopy, but because UV radiation is more diffuse, it scatters through the understorey, and so is proportionally enriched compared to the amount of PAR (Flint and Caldwell 1998, Grant et al. 2005). In response to low R:FR light, plants exhibit a shade avoidance syndrome (SAS, Ballaré et al. 1987), whereby they induce hypocotyl extension to outcompete neighbouring vegetation by growing towards light. Similarly, it has also been shown that blue light and UV radiation negatively regulate this response, i.e. high blue or UV radiation downregulates SAS (Casal 2013, Fraser et al. 2016).

Whilst the role of light quality in SAS response has been well studied, the effects of understorey light quality on plants where shade-avoidance is absent and would be detrimental are largely unknown. Lee et al. (2003) observed that understorey shade delayed leaf senescence in understorey woody species but found that R:FR had no effect. This suggests that other regions of the solar spectrum, or overall irradiance, are responsible for this phenological response in understorey woody species.

Figure 2. A schematic demonstrating changes in understorey light quality that occur from canopy opening and canopy closure.
1.3 PHOTORECEPTORS

Photoreceptors enable plants to detect changes in the composition of solar radiation. This is important to plants, because it allows them to adjust their development and growth to suit their particular environment. For instance, seed-germination, flowering, stem elongation, and organelle movement are all affected by light quality (Kong and Okajima, 2016). Plants are able to detect and integrate different light cues because they are equipped with an array of photoreceptors to detect different regions of light. Phytochromes (PHYs) are R:FR-detecting photoreceptors, and are synthesized in the dark in their red light absorbing form (Pr, $\lambda = 660$ nm). When exposed to light, they are converted to their far-red light absorbing form (Pfr, $\lambda = 730$ nm; Smith 1982; Smith and Morgan 1983). The two main groups of photoreceptors that underpin blue and UV-A responses are cryptochromes (CRYs) and phototropins (PHOTs) (Casal 2000; Briggs and Christie 2002). Lastly, the UVR8 photoreceptor underpins responses to UV-B, and possibly some responses to UV-A (Rizzini et al. 2011; Morales et al. 2013).

1.4 SECONDARY LEAF PIGMENTS

Function and response

Plants also modulate the concentration and content of secondary leaf pigments in response to changes in light intensity and light quality. Flavonoids are a group of photoprotective pigments which screen UV radiation, and reduce ROS stress, although to varying degrees based upon the particular composition of these compounds (Agati and Tattini 2010). Carotenoids and xanthophylls also play important roles in photoprotection, by dissipating excess light energy produced from photoinhibition of PSII (Young, 1991; Middleton and Teramura 1993). CRYs govern the accumulation of flavonoids in response to blue and UV-A radiation, and UVR8 governs the accumulation of flavonoids in response to UV-A and UV-B radiation (Christie and Jenkins 1996; Fuglevand et al. 1996; Morales et al. 2013; Rai et al. 2019).

Biochemistry

Flavonoids are present throughout the plant kingdom. They are synthesized from $p$-coumaroyl-CoA with three malonyl-CoA molecules by a chalcone synthase (Roy et al. 2016). This in turn produces a flavonone with a 2-phenylchroman backbone. This 2-phenylchromen backbone gives rise to flavonols, isoflavonoids, flavones and anthocyanidins in the phenylpropanoid pathway (Stafford, 1991). The different carbons of the flavonoid skeleton can be substituted and catalysed by isomerases, reductases, hydroxylases, methylases and glycosyltransferases (Harborne and Williams, 2001; Ferrer et al., 2008). In turn, these changes lead to a high structural
diversity of flavonoids, which modifies the solubility, stability, antioxidant activity, UV-screening and compartmentalisation (Roy et al. 2016) (see Figure 3).

Flavonoids can be sub-divided into two major groups: anthocyanins and flavonols (Agati et al. 2012). Flavonols tend to have higher UV screening ability, whereas both flavonols and anthocyanins possess antioxidant activity (Liakoura et al. 2003). Sun-exposed leaves of plants normally have higher amounts of flavonols compared to shaded leaves (Liakoura et al. 2003; Wang et al. 2012). In many plant species the accumulation of anthocyanins in leaves occurs during senescence in autumn (Feild et al. 2001; Lee 2003; Hoch et al. 2003). Shading has been found to delay the autumnal increase in anthocyanins (Lee et al. 2003), however similarly to its effect on leaf senescence, it is unsure whether there is a particular region of the spectrum underpinning this response, or whether it is a response to reduced irradiance (Lee et al. 2003).

Figure 3. The phenylpropanoid pathway leading to the production of different flavonols and anthocyanins.

Location in the leaf

Flavonoid location can vary within the leaf, and between different plant structures. For instance, there is large accumulation of flavonoids in external appendages, such as trichomes (Rozema et al. 1997). This is consistent with the role of surface flavonoids primarily as effective UV-B absorbers and anti-herbivory agents (Rozema et al. 1997; Close and McArthur 2002). The composition of flavonoids can also vary.
between locations. More than 90% of the total flavonoids pool which is distributed in the nucleus, chloroplast, and vacuole of mesophyll cells, comprises compounds which have higher anti-oxidant properties (Brunetti et al. 2018). Whereas monohydroxy-substituted flavonoids, which are poor antioxidants, are mostly located in the adaxial epidermal cells of shade-adapted plants (Brunetti et al. 2018).

The location within the cell of UV-screening compounds such as flavonoids can differ between different plant species. For example, it has been shown that two species of Antarctic moss have different strategies relating to the location of their UV-screening compounds (Clarke and Robinson, 2008). *Byrum pseudotriquestrum* has equal proportions of cell-wall-bound and –unbound UV-screening compounds. In comparison the moss *Ceratodon purpureus* has a smaller proportion of cell-wall-bound UV-screening compounds. Similarly, the deciduous *Vaccinium vitis-idaea* has much more substantial cell-wall flavonoids in comparison to flavonoids throughout the rest of the leaf (Day 1993; Semerdjieva et al. 2003). A member of the same genus, the evergreen *Vaccinium mytrillus*, has no cell-wall-bound flavonoids at all (Semerdjieva et al. 2003). Furthermore, different growth forms within the same species can even differ in the location of their flavonoids. The red growth form of *C. purpureus* has a higher concentration of cell-wall-bound UV-screening compounds in comparison to the green growth form (Waterman et al. 2018). Considered together, we can expect that not only do different plant species have different flavonoid locations, but also different plant growth forms may also differ in the location of their flavonoids.

**Seasonal variation of flavonoids**

Plants must optimise the accumulation of flavonoids throughout the seasons. Leaves of *Vaccinium vitis-idaea* have higher flavonols and anthocyanins after snowmelt in early spring (Solanki et al. 2019). Leaves beneath the snowpack, which are insulated from colder temperatures and higher irradiance, have lower flavonoid content. In comparison, after snowmelt in spring, leaves are exposed to high irradiance and low temperatures, and so higher flavonoids can provide both better UV-screening capabilities and higher antioxidant activity. Further into summer, with increasing temperature, flavonoids of *V. vitis-idaea* begin to decrease (Solanki et al. 2019). The composition of flavonoids can also change throughout the year. For example, in walnut (*Juglans regia*), the flavonoids catechin and myricetin increase from spring up until August (Solar et al. 2006). In contrast, phenolic acids were highest in spring and lowest in summer. It has also been shown that for *B. pendula* and *A. glutinosa*, flavonoids are highest in early summer, and decrease towards the end of the season (Kotilainen et al. 2010). Not only do abiotic variables such as temperature and solar radiation affect the seasonal dynamics in phenolic profiles in walnut, but pathogens such as blight (*Xanthomonas campestris* pv. juglandis) also affect the seasonal dynamics of flavonoids (Solar et al. 2006).
For many plant species, leaf anthocyanins have been shown to increase in autumn, giving characteristically red colours. In contrast, yellow colouration comes from yellow carotenoid pigments which become visible after the degradation of chlorophyll (Goodwin, 1958). There are many hypotheses as to why anthocyanins increase in autumn. These include: reducing photoinhibition and ROS generated at low temperatures under which light-saturation more easily occurs; as a red colouration signal to prevent herbivory; and to provide protection against pathogens (review by Wilkinson et al. 2002). Perhaps, most aptly, Gould (2004) described anthocyanins as “Nature’s Swiss army knife”, in relation to the cross-tolerance they provide against a number of different environmental stressors. Interestingly, only recently was it shown that flavonols in the leaves of Sorbus aucuparia and Betula pendula increase in autumn, just before and during leaf senescence (Mattila et al. 2018).

Leaf pigments in forest understories

Flavonoids can vary in space as well as time. When forest canopies leaf out in spring, the understory becomes covered in shade. Shade has been shown to reduce flavonoid content in several plant species (Liakoura et al. 2003; Wang et al. 2012). Furthermore, the flavonoids in plant leaves in the forest understory have important interactions within the forest understory ecosystem. For instance, leaves of understory shrub species growing in high light conditions have higher concentrations of defence metabolites such as flavonoids, and are less damaged by folivorous insects (Karolewski et al. 2013). Flavonoids in leaves that senesce in autumn can inhibit litter decomposition via collembola (Das and Joy, 2009). Although flavonoids are important physiologically, and affect ecosystem processes, there are no studies we are aware of demonstrating how light quality in the forest understory affects their seasonal dynamics.

Functional strategies

Relatively little is known about how functional types of plants differ in their flavonoid response to different light treatments, and which environmental cues are the most important in determining variation in their seasonal flavonols. Most evidence suggests that epidermal UV-screening is highest in evergreen plants, intermediate in woody plants and lowest in herbaceous plants (Day et al. 1992; Day 1993; Semerdjieva et al. 2003; Li et al. 2010). In contrast, a study comparing different plant growth forms in a subtropical alpine area found no difference between the UV-screening ability of woody plants and herbaceous plants (Barnes et al. 2017). Thus, there is a need to investigate whether there are any differences in UV-screening among plant functional types.
1.5 CLIMATE CHANGE

Different understorey plants adopt different light strategies in the forest understorey. For instance, some may emerge in early spring, or remain in late autumn to exploit periods of light availability whilst the canopy is open (Kudo et al. 2008; Richardson and O'Keefe 2009). Conversely, shade-tolerant plants may adopt a strategy to not invest resources during periods of light availability in spring and autumn, but to photosynthesise at a steady rate during canopy closure whilst they are covered in shade (Augspurger et al. 2005; Heberling et al. 2019). These strategies may also dictate not only when plants time their leaf emergence and leaf senescence in the forest understorey, but also when they invest in the accumulation of flavonoids in their leaves, and how much they invest.

Climate change is increasing the period of canopy duration (Vitasse et al. 2009), and so these interactions between the overstorey and understorey may be becoming unhinged. Already it has been shown that the canopy is leafing out at a faster rate than the forest understory, and so understorey plants will be at risk of lower carbon capture throughout the year (Heberling et al. 2019). However, it is not certain how the change in light quality that this will bring throughout the year will affect different functional types of plants. Regions in the blue and UV part of the solar spectrum have received little attention thus far. How these changes will affect leaf out and leaf senescence in different understorey species, and how it will affect their leaf pigmentation throughout the year is not comprehensively understood.

1.6 AIMS

Our aim was to combine three approaches of literature review, controlled laboratory studies, and field experiments to answer the following questions:

Which photoreceptors regulate flavonoids in response to low irradiance blue and UV-A radiation? (I)

How does blue light affect spring bud burst under controlled conditions? (II)

Does light quality influence leaf phenology and leaf pigments throughout spring and autumn? (IV)

How important is light quality as a phenological cue, compared to other environmental drivers? (III)
2. MATERIALS AND METHODS

To allow easy comparison between the results, I took a consistent approach where possible to address the aims of my dissertation across multiple experiments and papers. To show the overlap between them, I list the main methods and devices used across the paper in Table 1 below.

Table 1. Table summarising which studies used which methods.

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Environmental variables

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2.1 EXPERIMENTAL DESIGN

2.1.1 EXPERIMENT I – PIGMENTS IN THE LAB

Plant material

*Arabidopsis thaliana* in the background accession of Landsberg erecta were used, and all genotypes were sourced from seeds of plants grown under standard growth conditions in Viikki greenhouses, Helsinki. We used the following photoreceptor mutants: *phot1* (Inada et al. 2004), *cry1* and *cry2* (Mazzella et al. 2001), and *uwr8-2* (Brown et al. 2005).
Five seeds per pot (6 x 6 cm) were sown into well-soaked 1:1 pre-fertilised peat to vermiculite (Agra-vermiculite; Pull Rhenen, TX Rhenen, the Netherlands). A thin layer of peat, sieved through a 2 mm gauge, was added to provide a smooth even surface for germinating seeds. Following this, pots were placed for 1 day in the dark at 15°C to allow seeds to become imbibed. Then they were placed under light treatment conditions for 3 days at 5°C day/2°C night for cold stratification. Temperature was then increased to approximate spring temperatures for northern temperate latitudes (15°C day/10°C night). Water vapour pressure deficit was 0.17 kPa day/0.18 kPa night for germination and growth.

When the first pair of true leaves became visible, seedlings were thinned so that there was one seedling per pot. In total, this left 288 plants in the experiment. Plants were given 50ml of tap water per pot every 5-7 days. Pots were also rotated within compartments to reduce any unknown gradients in temperature, relative humidity and irradiance.

**Light treatments – Growing conditions and high-light conditions**

This experiment consisted of two phases: growth conditions and high-light conditions. For growth conditions, six compartments of equal size were used inside a temperature-controlled growth room. Each compartment was of equal size (97 cm wide x 57 cm deep x 57 cm high). The six compartments were arranged into three blocks, with each block consisting of two compartments. Each was randomly allocated a light treatment, either a broad-spectrum LED containing blue light, or a broad spectrum LED with blue light filtered out (attenuated). These lamps were Valoya AP67, 400–750 nm, PAR 168 μmol m⁻² s⁻¹ plus 32 μmol m⁻² s⁻¹ FR, (Helsinki, Finland) and in the other three compartments blue light was attenuated by wrapping film Rosco #313 Canary Yellow (Westlighting, Helsinki, Finland) around the LED lamps. The difference in PAR this created between the treatments was adjusted for by controlling the height of the lamps, so that PAR was homogenous between compartments. Each compartment was protected from outside light entering the compartment by using white-black plastic film that blocked both visible and UV radiation. The number of plastic sheets used was also adjusted to ensure equal temperature between compartments. Each compartment was also given a split-plot, with one half of the compartment having a UV-A treatment (LED arrays with peak emission at 365 nm (mean and SE of 15.0 ± 0.6 μmol m⁻² s⁻¹: Z1-Z1-10UV00, LED Engin, San Jose, CA)). UV-A was filtered out of the other half of the compartment using Rosco #226 (Westlighting) which attenuated all wavelengths below 400 nm. The irradiance and ratio of blue light:UV-A in the experiment was the same as that measured under canopy shade of a B. pendula stand at Lammi Biological station (Table S3 supplementary material, I). To reflect more realistic spring conditions, a 10-hour photoperiod was used, and UV-A treatment was applied for 4 hours centred around noon, when UV radiation is its highest during the day (Flint and Caldwell 1998).
For high-light treatments, half of the plants in each treatment combination were randomly allocated to receive high light. High light treatments were made at midday in winter in a temperature-controlled greenhouse (25°C air temperature). Almost no natural light entered the greenhouse and ambient light was through high-pressure sodium (HPS) growth lights. The high light treatments were provided by Osram Powerstar HQI-E 400 W/D stadium lamps (Osram GmbH, Munich, Germany). They were warmed up for at least 1 hour prior to treatment, to ensure emission was stable. 75 mm-deep baths of flowing cold water were placed between the lamps and plants to serve as a heat sink to reduce the warming effect of the lamps. Plants were kept under high light treatment for exactly 30 minutes, directly following transfer from the growth room, and were returned to the growth room immediately after the measurements. Photon irradiance incident on the leaves of plants receiving the high-light treatment was 1800–2100 μmol m⁻² s⁻¹ PAR and the incident temperature measured on the leaves was about 35°C.

2.1.2 EXPERIMENT II – PHENOLOGY IN THE LAB

Plant material

We collected dormant branches of *Alnus glutinosa*, *B. pendula*, and *Quercus robur* from local populations which were co-occurring in a natural forest stand in southern Finland (60°13′04.2″N, 25°00′31.0″E). The branches were cut from lower canopy branches on trees (2-3 m above ground) with sterile garden secateurs on 6th February 2017. We sampled from eight trees per species, and collected 10-15 branches from each tree, making sure that each branch was dormant, free of pests and free of disease symptoms. Several studies have exemplified the reliability of this method (Basler and Körner 2012; Laube et al. 2014; Polgar et al 2014; Vitasse and Basler 2014; Primack et al. 2015). We followed a similar protocol, whereby we placed the cut end of the dormant branches into test tubes of water immediately after being re-cut, and continuously monitored stages of their bud development.

Prior to sampling, trees received 111 chilling days, calculated as the number of days from the 1st of November the previous year, with a mean temperature ≤5°C (Murray et al. 1989). All trees were facing the east side of a mixed forest stand, and branches that were receiving sunshine for the majority of the day were selected. All branches presented at least five buds including the terminal bud. Subsequently, the branches were immediately taken to the greenhouse into an ambient temperature room (0°C). Here, they were re-cut so that all branches were of a similar length (28.35 ± 0.34 cm (mean ± SE)). Immediately after this they were placed into sterile clear plastic tubes (10.26 cm long, 15 ml volume) which were then filled with tap water.
Light treatments

Branches were moved into a temperature-controlled growth room with six compartments (97 cm wide x 57 cm deep x 57 cm high). The branches were randomly assigned into each compartment, with each compartment having 16 branches of *Q. robur*, 20 of *B. pendula*, and 16 of *A. glutinosa*. Test tubes were rotated in the compartments every 2 days, avoiding any bias coming from unknown microclimatic variation. Lights were kept the same distance from branches in every compartment.

Three compartments were randomly allocated to receive a blue light LED spectrum (Valoya AP67, 400–750 nm, Table S1: mean PAR among compartments of $161 \pm 10 \mu\text{mol m}^{-2}\text{s}^{-1}$, plus $28 \mu\text{mol m}^{-2}\text{s}^{-1}$ far red). Another three compartments were allocated to receive a no blue treatment, in which blue light was filtered out from the broad-spectrum lamps using Rosco #313 Canary Yellow (Westlighting, Helsinki, Finland, PAR $156 \pm 10 \mu\text{mol m}^{-2}\text{s}^{-1}$, plus $31 \mu\text{mol m}^{-2}\text{s}^{-1}$ far red). A 12h photoperiod was used to simulate similar photoperiod conditions at the beginning of spring in southern Finland (60°13’04.2″N 25°00’31.0″E).

2.1.3 EXPERIMENT IV – PIGMENTS AND PHENOLOGY IN THE FIELD

Site information and climate

The experiment was conducted in the grounds of Lammi Biological station (61.05°N, 25.05°E). Average mean monthly temperature is 5.1°C and average mean monthly precipitation was 47.7 mm between 2017 and 2018.

Plot design

Experimental plots were set up underneath two stand types: the first type being deciduous stands dominated by *B. pendula* or *Q. robur*, and the second type being an evergreen stand of *P. abies*. We set up nine split-plots in the deciduous stands, and six plots in the evergreen stand. The plots were established according to space that was available in the forest understory. Plots were adjacent to areas chosen by Hartikainen et al. (2018), and with similar spacing, architecture, light and LAI (IV, supplementary material).

Each plot was split into four filter treatments which attenuated different regions of the solar spectrum. These were made from polycarbonate (Foïteck Oy, Vantaa, Finland). This consisted of a control filter which was transparent to all wavelengths of solar radiation (PLEXIGLAS 2458 GT SOLARIUMLAATU), a filter attenuating UV radiation below 350 nm (PLEXIGLAS oZ023), a filter attenuating all UV radiation (ARLA MAKROLIFE), and a filter attenuating blue light and all UV radiation (PLEXIGLAS 1C33 (303)). Thus, any differences between the blue and the UV treatments can be attributed to the effect of blue light. Every filter had the same dimensions (88 cm length x 60 cm width x 40 cm height). Each filter was placed so
that the longest side faced south. Protocol was followed according to Aphalo et al. (2012), whereby a 10-cm border inside each filter was excluded, leaving a central area inside where plants were measured, thus avoiding any edge effects. Air flow was facilitated by raising the filters 10 cm by placing them on wooden blocks, and via a gap at the top of the north-facing panel. Every filter was randomly assigned a position within each plot.

**Selection of understorey species**

We used both volunteer plant species already growing beneath the installed filters, and transplanted plant species from within the same stand type, canopy architecture, and stand spacing, underneath the filters. This enabled us to capture responses from different functional plant types represented in the forest understoreys at our field site in southern Finland. Germinating seedlings of *Acer platanoides* were transplanted in 2016 a year prior to the experiment. At the end of 2017, the 1st year of the experiment, germinating acorns of *Q. robur* were transplanted under the filters. Volunteer plants of the following species were also used: *Oxalis acetosella*, *Fragaria vesca*, *Aegopodium podagraria*, *Anemone nemorosa*, *Maianthemum bifolium*, and *Ranunculus catus*.* A. podagararia*, *R. cassubicus*, and *Q. robur* were only present in deciduous stands, and *M. bifolium* was only present in the evergreen stand.

**Care of plots**

Size, reproductive status, and time of year, were similar for all plants that were transplanted (April 2016 following snowmelt for *A. platanoides*, and September 2017 for *Q. robur*). During transplanting, original vegetation in the plot was left intact as much as possible. Four transplants of *A. platanoides* were placed under each filter, and four transplants of *Q. robur* under each filter in deciduous stands, because this was the only stand type where *Q. robur* seedlings were naturally occurring.

Filters reduced precipitation reaching the ground beneath, so additional watering was given to plants every 3 days. Soil moisture of plots was monitored with a TDR probe (SM200 Moisture Sensor with HH2 Moisture Meter, Delta-T Devices, Cambridge, UK), ensuring that watering was consistent between treatments. Temperature was an average of 0.3°C higher under filters compared to understorey ambient conditions, but was consistent between treatments.
2.2 PLANT RESPONSES

2.2.1 OPTICAL ASSESSMENT OF PIGMENTS

A Dualex Scientific+ (Force-A TM, Paris, France) was used for non-destructive \textit{in vivo} measurements of leaf pigments, derived from their optical properties. Chlorophyll content in the leaf is calculated by the Dualex, based on the difference in leaf transmission at 710nm and 850nm, compared with transmission at 650nm (Cerovic et al. 2012). Relative leaf adaxial-epidermal UV-A absorbance (flavonol content) at 375nm is calculated by the Dualex, derived from chlorophyll fluorescence at 375nm and 650nm, whilst also accounting for chlorophyll content. Relative leaf adaxial-epidermal absorbance at 515nm, measuring anthocyanin content, was calculated by the Dualex, derived from chlorophyll fluorescence at 375nm and 650nm, whilst also accounting for chlorophyll content.

2.2.2 CHLOROPHYLL FLUORESCENCE OF PSII

Chlorophyll fluorescence was used a diagnostic tool to assess the efficiency of PSII, and whether any damage to PSII may have occurred. This was measured with a PAM fluorometer (mini-PAM, Heinz-Walz GmbH, Effeltrich, Germany). We used $\phi_{\text{PSII}}$ (calculated as $F_q`/F_m`$; Murchie and Lawson 2013) as an indicator of the operating efficiency of PSII. Electron transport rate (ETR) was also calculated assuming a leaf absorption of 0.84 and an even energy partitioning between PSII and PSI using the equation from (Maxwell and Johnson 2000; Murchie and Lawson 2013):

$$\text{ETR} = \phi_{\text{PSII}} \times \text{absorbed PAR} \times (0.5)$$

To assess damage to PSII, $F_v/F_m$ of all plants was measured from the same leaves as $\phi_{\text{PSII}}$ measurements, after they had been dark-adapted for at least 30 min immediately follow each measurement of $\phi_{\text{PSII}}$, in the growth room (I) or field (IV). Non-photochemical quenching (NPQ) was also calculated from pairs of measurements of $F_v/F_m$ and $\phi_{\text{PSII}}$ from the same leaves (Maxwell and Johnson 2000).

$$\text{NPQ} = \left( F_m - F_m` \right) / F_m`$$

In the growth room (I), chlorophyll fluorescence measurements were performed: (1) before the high light treatments to assess baseline response to the spectral irradiance treatments during growth; (2) during and immediately after the high-light treatments to assess photoinhibition and photodamage resulting from excess irradiance; and (3) following a period of recovery from the high-light treatments. Fully expanded horizontal \textit{Arabidopsis} leaves that were fully exposed to
the light treatments and were large enough to hold a leaf clip went through these same measurements. The same leaves were used for the Dualex measurements.

In the forest stands (IV), only $F_v/F_m$ was measured on fully expanded leaves of *A. platanoides* on two consecutive sunny days in late June, to assess baseline effects on growth under spectral-irradiance treatments which attenuated different regions of solar radiation. Leaves of the same age and size, and similar exposure to sunlight, were chosen for measurement.

2.2.3 PHENOLOGICAL OBSERVATIONS OF BUD BURST AND LEAF SENESCENCE

Bud burst and leaf out of tree species was measured every 2-3 days along a scale of 1-7 adapted from Teissier du Cros et al. (1981), whereby 1 = dormant, 2 = bud swelling, 3 = bud split, 4 = leaf tip protruding, 5 = leaf mostly out, 6 = leaf out but not fully expanded, and 7 = fully expanded leaves. For the herbaceous species, emergence of new leaves was measured once a week as 1 = shoot visible, 2 = expanded leaf. Leaf senescence for all tree and herbaceous species was measured on a scale of 1-5, whereby 1 = fully green, 2 = starting to yellow, 3 = mostly yellow, 4 = turning brown, 5 = all leaves fallen. Leaf senescence was measured for all the leaves on each plant every 2 weeks.

2.3 ENVIRONMENTAL VARIABLES

2.3.1 SPECTRAL IRRADIANCE

Spectral irradiance was measured with a portable CCD array spectroradiometer Maya 2000 pro (Ocean Optics, Dunedin, FL, USA) with a D7-H-SMA cosine diffuser (Bentham Instruments Ltd., Reading, UK) with spectral range of 200-1100 nm. Calibration was done by the Finnish Radiation and Nuclear Safety Authority (Aphalo, 2017; Ylianttila et al. 2005). The integration time was set manually to provide maximum resolution without saturating the array. Each measurement was immediately followed by one measurement with a polycarbonate filter (attenuating UV radiation) to correct for stray radiation on the array in the UV range, and one dark signal measurement blocking all UV and visible radiation, to account for temperature-dependent noise (details in Aphalo 2017). The diffusor was aligned horizontally, and the spectrometer was wrapped with a white cotton cloth to decrease the exchange of radiation between the spectrometer and its environment, (e.g. reducing any increase in the temperature of the spectroradiometer caused by direct heating by sunlight).
2.3.2 TEMPERATURE

Temperature was continuously monitored with iButton sensors (Maxim Integrated, San Jose, CA), which were set to 0.065 °C resolution, and recorded temperature integrated over 10-minute intervals. Heat shields made from duct tape were used to block any direct solar radiation hitting the iButton causing it to directly heat up, as opposed to measuring ambient temperature. Data from iButtons were downloaded once every two months, and then wiped, to prevent the iButtons memory running out.

2.3.3 SOIL MOISTURE

The soil moisture in the plot was also monitored with a TDR probe (SM200 Moisture Sensor with HH2 Moisture Meter, Delta-T Devices, Cambridge, UK). Soil moisture at 0-15-cm depth was recorded three times inside each filter for experiment IV, and three times for soil conditions with no filter in each plot.

2.3.4 RADIATIVE TRANSFER MODELLING

To calculate solar angles and solar noon for different locations, we used the photobiology packages in R (Aphalo, 2016). Twilight length was defined as civil twilight, including solar angles from -6° and 0°. From this information, libRadtran was used to simulate spectral composition at solar angles of -6° and at solar noon for the spring equinox, summer solstice, autumn equinox, and winter solstice, across the locations of Oulu, Helsinki and Madrid, spanning the range of latitudes covered in publication III.

libRadtran is a library of radiative transfer equations that allows solar radiation to be simulated at different locations and solar angles. There are different models of radiative transfer available, each presenting their own advantages. For example, the TUV model provides a simpler but more rapid calculator for spectral composition over a shorter wavelength range (150 nm-750 nm) (Madronich et al. 1997). libRadtran, in contrast, has the advantage that it can calculate any wavelength between 120 nm-100,000 nm (Brelsford, 2017). To incorporate changes in FR light, as well as other spectral regions, we chose to use libRadtran.

In particular, we used the uvspec model from libRadtran, version 2.0.1, radiative transfer package (Emde et al., 2016). The uvspec model allows for the calculation of the radiation field in the Earth’s atmosphere, accounting for atmospheric composition, including parameters such as water vapour and ozone column. The absorption and scattering of solar radiation based on these constituents of the atmosphere are taken from algorithms and databases provided in the libRadtran package.
The parameters that we adjusted in our model were total ozone column and column-integrated water vapour. We used the DISORT radiative transfer solver (Stamnes et al. 1988) for daytime irradiance, and the SDISORT solver for twilight solar angles, which includes pseudospherical geometry to account for the curvature of the Earth (Dahlback and Stamnes, 1991). Water column data was taken from Källberg et al. (2005), and ozone column thickness data from Experimental Studies Unit, Environment Canada (http://exp-studies.tor.ec.gc.ca/e/index.htm).

For constructing figures, ratios of spectral integrals were defined as follows: B:R (410-500 nm/610-700 nm, Johnson et al. 1967); R:FR (650–670/720 nm–740 nm, Sellaro et al. 2010); and R:FR (655–665 nm/725–735 nm, Smith, 1982). Spectral regions were defined as follows: blue light (420–490 nm); UV-A radiation (315–400 nm); and UV-B (280–315 nm). Figures were then produced from irradiance data using photobiology packages in R (Aphalo, 2015).
3. RESULTS AND DISCUSSION

3.1 CRYS AND UVR8 BOTH AFFECT THE ACCUMULATION OF FLAVONOIDS IN RESPONSE TO UV-A RADIATION

By combining laboratory and field manipulations, here we are able to show that light quality affects pigment accumulation throughout spring and autumn (I, IV). Although, as demonstrated by our field manipulations (IV), the extent of this response is dependent on the specific plant functional type, or species, being considered. Controlled laboratory experiments using the model species *A. thaliana* point to a significant role of CRYS and UVR8 regulating the response of seasonal variation in flavonols to changes in blue and UV radiation (I).

Using controlled laboratory conditions simulating understorey UV-A: BL, we found that CRYS affect the accumulation of flavonol content. The *cry1cry2* mutant had the lowest flavonol content of all mutants when grown under blue light (24.5% lower in comparison to WT). Similarly, blue light increased flavonol content in all genotypes apart from *cry1cry2*. The effects of blue light on phenolic content quantified from leaf extracts were generally consistent with the optically assessed flavonol content, however this trend was not as consistent across genotypes. Typically, studies reporting large increases in the accumulation of phenolic compounds and flavonoids in response to blue light have either used solar blue light (Siipola et al. 2015), or controlled conditions using high irradiances (Hoffmann et al. 2015; Taulavuori et al. 2015). Surprisingly, we found that in the absence of blue light, phot1 mutants had lower flavonol content. However, it is not certain to what extent this effect would be pleiotropic or not, and to what extent PHOT1 affects the constitutive levels of flavonols in the absence of blue light.

Interestingly, growth under UV-A caused a decrease in epidermal flavonol content in *cry1cry2* and *uvr8-2* mutants, although did not alter flavonol content in WT. Nevertheless, this suggests that CRYS and UVR8 are involved in the regulation of flavonols in response to UV-A (Figure 4). In contrast to our results, previous solar UV-A attenuation studies conducted outdoors have reported an increase in flavonols in response to UV-A (Ibdah eh et al. 2002; Kotilainen et al. 2008; Kotilainen et al. 2009; Morales et al. 2013). There are several possible explanations for this apparent discrepancy between our findings and those of previous studies. 1) We used a UV-A LED source with a narrow band centred around 365nm, whereas the majority of UV-A studies using lamps/solar UV-A have a broader spectrum (Joshi et al. 2007; Victorio et al. 2011; Štroch et al. 2015). 2) There may be overlap at different regions/different photoreceptor interactions e.g. Rai et al. (2019). 3) Not increasing flavonol content in response to UV-A, and allowing transmittance to the chloroplasts of non-damaging amounts of UV-A may in fact be useful to the plant. UV-A can be absorbed by chlorophyll and induce chlorophyll fluorescence (McCree 1981; Lang et al. 1991), and thus may be utilised for photosynthesis (Bilger et al. 2007; Turnbull et
This could be especially relevant for understorey shade environments, where there is a limited amount of light and UV-A irradiance is unlikely to be high enough to induce stress or photodamage (Štroch et al. 2015; Casal 2013), and is proportionally enriched in comparison to PAR (Flint and Caldwell 1998; Leuchner et al. 2011; Urban et al. 2012; Dengel et al. 2015).

Figure 4. A schematic representation of the pathways which affect the accumulation of epidermal flavonol content in response to UV-A and blue light, as well as the effect of blue light and high light on the $F_v/F_m$ of PSII.

We assessed the recovery of $F_v/F_m$ as a measure of resistance to high light stress. In contrast to other studies (Goins et al. 1997; Matsuda et al. 2004; Terfa et al. 2013; Hoffmann et al. 2015; Štroch et al. 2015), we did not find a role for UV-A or blue light in improving resistance to high light stress under controlled conditions for mutants of *A. thaliana*. Similarly, although other studies have described a role for flavonoids in providing photoprotection and antioxidant properties which can improve $F_v/F_m$ in response to stress (Wargent et al. 2011; Klem et al. 2015; Robson et al. 2015), we found no correlation between flavonoids and $F_v/F_m$. However, our results do suggest a role for CRYs in ameliorating the effects of high light stress. CRYs improved $F_v/F_m$ in response to blue light under growing conditions, and also in response to high light stress.

There are several mechanisms through which it may be possible that CRYs could lead to an increase in $F_v/F_m$ both under blue light and in response to high light.
stress. 1) CRYs could affect leaf morphology so that the absorption of light through the leaf, or the ratio of absorption between PSI and PSII reaction centres, changes (Murchie and Lawson, 2013; Miao et al. 2016). 2) CRYs increase activation of D1 and D2 proteins of PSII in response to blue light, leading to an increase in the amount of proteins available for PSII repair (Thum et al. 2001; Tsunoyama et al. 2004; Onda et al. 2008). In this way, CRYs may be vital for the normal functioning of PSII. 3) CRY1 activates 77 of 996 genes that respond to high light, including the gene for Vitamin B6, which provides antioxidant activity against ROS stress (Kleine et al. 2007).

Accordingly, Boccalandro et al. (2012) suggested that non-stomatal limitations, such as reduced electron transport rate in PSII, were the most likely cause of reduced photosynthetic capacity in cry1cry2.

### 3.2 BLUE LIGHT ADVANCES BUD BURST IN THE LAB

For the three tree species we measured, we found that blue light advanced the date of 50% bud burst in branches kept under controlled conditions. This effect was greatest in *Q. robur* (6.3 days), followed by *A. glutinosa* (6 days), and *B. pendula* (3 days). These results are consistent with studies showing that early-successional species achieve bud burst sooner than late-successional species (Wesołowski and Rowiński 2006; Basler and Körner, 2012), as *B. pendula* and *A. glutinosa* reached bud burst before the later-successional *Q. robur*. We also showed the same effect when expressed as degree-days, meaning that the effect was not due to any undetected microclimatic differences in temperature between different chambers.

We present two hypotheses as to why blue light might advance bud burst. The first is that enriched blue light could be a signal for increased twilight hours during spring, as twilight hours are enriched in blue light (Robertson et al. 1966, Johnson et al. 1967, Hughes et al. 1984). However, extending day length with narrow band blue LEDs did not advance bud burst in *P. abies* (Mølmann et al. 2006), suggesting that diurnal changes in blue light may not affect bud burst. Alternatively, blue light has been shown to enhance photosynthesis in several plant species (Goins et al. 1997; Matsuda et al. 2004; Hogewoning et al. 2010). Thus, it might be the case that blue light is a cue for conditions which promote photosynthesis.

Previous research has shown that members of the Rosacea family have a higher percentage of bud burst for axillary shoots under enriched blue light (Muleo et al. 2001; Girault et al. 2008). Whilst this is a different process to spring bud burst, it suggests possible mechanisms by which blue light advances springtime bud burst in trees, for example by increasing the allocation of sugars to the growing bud (Girault et al. 2010). Interestingly, several tree species, such as *Q. robur*, *Fagus sylvatica*, *Betula pubescens*, *Fraxinus excelsior* and *Acer pseudoplatanus* (Catesson 1964; Barnola et al. 1986; Cottignies 1986; Kelner et al. 1993; Rinne et al. 1994a, b), increase mobilisation of stem carbohydrates towards buds when they are close to bud burst. Thus, it appears that sugar metabolism plays a part in this process, and could be an
interesting line of inquiry with regards to blue light-mediated mechanisms which promote bud burst.

### 3.3 Light Quality Influences the Seasonal Dynamics of Flavonoids

Flavonol response in more light-demanding species is more sensitive than shade-tolerant species to changes in light quality

We installed filters attenuating different wavelengths in the blue-and-UV region over plants in a forest understorey in southern Finland. This enabled us to test how leaf pigments and leaf phenology in different plant species responded to blue and UV radiation, scaling from our laboratory studies, and testing these ideas in a more ecologically relevant context.

Consistent with our laboratory study (I), we found that for most species in the understorey, blue light had the largest effect on flavonol content, when compared to longwave and shortwave UV radiation. This is also consistent with Siipola et al. (2015) who found that solar blue radiation had the largest effect on flavonol content when compared with solar UV radiation. Whilst experiments such as ours, and that used by Siipola et al. (2015), resulted in a reduction of PAR in treatments attenuating blue light, our results from (I) demonstrate the effects of blue light under equal PAR conditions.

For most species, the effect of shortwave UV and longwave UV radiation was smaller than the effect of blue light on flavonol content (see Table 2 in IV). However, for *Q. robur*, shortwave UV radiation had the largest effect on flavonol content. One reason this species-specific effect may occur is because *Q. robur* is at the edge of its northern range limit at our Finnish field site, meaning that it is typically exposed to higher UV radiation at more southern latitudes in its distribution range core. In this way, *Q. robur* may be more sensitive to UV radiation as a cue for flavonol content, in comparison to blue light.

Anthocyanin content was largely unaffected by filter treatments throughout most of the season. Thus, we may expect that seasonal dynamics in anthocyanin content are affected more by cues other than light quality, such as temperature (Pescheck and Bilger 2019; Renner and Zohner, 2019).

The more light-demanding species in our study were most responsive to our filter treatments. In addition, more light-demanding species tended to have higher flavonol content throughout the season, when compared to most of the species which had overwintering leaves (e.g. *O. acetosella* and *M. bifolium*). Previous research suggested that evergreen and woody species would have higher constitutive contents of UV-B absorbing compounds in comparison to herbaceous species, because they have a longer leaf life-span (Semerdjieva et al. 2003; Li et al. 2010). However, one study comparing the UV-screening ability of different growth forms in a sub-tropical...
alpine environment found no differences (Barnes et al. 2017). Here, we suggest that despite having shorter leaf life-spans, more light-demanding species tend to have higher induced flavonol content, and are more sensitive to changes in light quality, so that higher photoprotection can allow them to more readily exploit periods of light availability (Takahashi and Badger, 2011).

**Light quality affects flavonol and anthocyanin content in autumn**

Until recently, it was generally considered that flavonol content did not increase during autumn (Wilkinson et al. 2002). However, Mattila et al. (2018) showed that flavonols increase during autumn senescence in the tree species *B. pendula* and *Sorbus aucuparia*. Similarly, we report an autumn increase in adaxial epidermal flavonol content for *A. platanoides*, *A. podagraria*, *F. vesca*, and *Q. robur* (weeks 35-40). This increase coincided with a period of canopy opening during autumn in the deciduous stands, and was absent, or much smaller, under filters attenuating blue and UV radiation. A similar trend was observed with anthocyanins, although anthocyanins were generally less responsive than flavonols to light quality for most species, apart from *A. platanoides* and *A. podagraria*.

Figure 5. Schematic of the factors affecting seasonal dynamics in understorey flavonoids. The figure shows the phenological progress of a typical deciduous tree from our experiment, and the phenology of species present in the understorey: forbs, *Anemone nemorosa*, *Aegopodium podagraria* and seedling *Acer platanoides*. “+”
means that particular cue or environmental stressor is increasing during that period in the understorey, whereas “-” means the opposite. Colour coding is purely aesthetic.

It is possible that the increased solar radiation in the blue and UV region reaching the understorey during autumn could lead to this increase in flavonoids. However, this pattern was also present in the evergreen stands, suggesting that colder temperatures are also a contributing factor. The accumulation of autumn flavonoids in response to colder temperatures would be consistent with their role as antioxidants (Pescheck and Bilger 2019; Renner and Zohner, 2019). In addition, it could be that the increase in anthocyanins is part of the leaf senescence timetable, also supporting their role as antioxidants to remove ROS produced during chlorophyll breakdown (Figure 5) (Archetti et al. 2009).

3.4 HOW IMPORTANT IS LIGHT QUALITY COMPARED TO OTHER ENVIRONMENTAL FACTORS AFFECTING PHENOLOGY?

Spring Phenology

Under field conditions, we found that blue light advanced bud burst by an average 1.4 days, and final leaf out by 2.8 days in A. platanoides seedlings in a forest understorey. This is equivalent of 0.8% and 1.5% of the average growing season length for A. platanoides seedlings growing under the ambient control filter in our experiment (182 days). The blue light effect we report is consistent with the effects that we found in (II). Although experiments such as ours attenuating blue light also reduce the amount of PAR (Siipola et al. 2015), we demonstrated this effect of blue light was consistent under controlled PAR conditions, which provided equal PAR to both treatments in (II). We did not find evidence showing that UV radiation affects bud burst. Similarly, long-term UV-B supplementation had no effect on the bud burst of four dwarf shrub species (Phoenix et al. 2001).

The evidence from our review and meta-analyses conducted in (III) support the larger effect of blue light, in comparison to UV radiation, on bud burst that we report in (IV). In general, both the mean and median effect sizes for different light quality treatments on spring bud burst were of a similar magnitude to the size of effects which have been reported for photoperiod (2.1 days advanced bud burst per hour photoperiod increase, and 4–6 days earlier bud burst in treatments of enriched blue light and twilight R:FR). We also found that previously reported significant effects of UV radiation (reviewed in III) are negligible when considered in terms of biological effect size (e.g. an average of 0.67 days earlier under UV-B radiation). In comparison, the mean effect sizes of chilling and forcing temperatures were 1.0 days advanced bud burst per one chilling day increase (III: Table 3), and 2.0 days advanced bud burst per 1°C increase in forcing temperature (III: Table 3), respectively. Considering the relatively large responses to an increase in chilling days or forcing
temperatures compared with photoperiod and spectral composition, we might expect chilling days and forcing temperature to have a greater potential to affect the bud burst of trees than other cues (Figure 6).

We did not find evidence that the spring leaf out of herbaceous species is affected by light quality. This is because they are covered by snow during winter, and so ground temperature and snowmelt are probably more important environmental factors affecting their leaf out (Price and Waser 1998; Rice et al. 2018). One caveat here is that herbaceous species were not sampled as often as *A. platanoides* in our field study due to time constraints, so this could mean that small differences in leaf out between filter treatments of herbaceous species were not detected.

**Autumn Phenology**

Attenuating blue light delayed leaf senescence in *A. platanoides* (14.3 days for stage 2; 7.6 days for stage 5), *A. podagraria* (5.6 days for stage 2; 0 days for stage 5), and *R. cassubicus* (7.4 days for stage 2; 7.0 days for stage 5). These differences can also be expressed as a percentage of the growing season length (*A. platanoides* – 7.8% for stage 2; 4.2% for stage 5; *A. podagraria* – 3.8% for stage 2; *R. cassubicus* – 5.5% for stage 2; 5.3% for stage 5). Blue light enhances photosynthesis in several plant species (Sæbø et al. 1995; Goins et al. 1997; Matsuda et al. 2008; Košvancová–Zitová et al. 2009; Hogewoning et al. 2010). This is because blue light allows rapid stomatal opening to exploit sunflecks, and also enables rubisco activation (Košvancová-Zitová et al. 2009). It is possible that by attenuating blue light, we reduced photosynthesis in these plants, and so they compensated for reduced carbon gain by extending their leaf phenology further into autumn (Chabot and Hicks, 1982; Zhang et al. 2013). As previously discussed, in agreement with other filter experiments attenuating solar blue light (Siipola et al. 2015), there was a reduction in PAR (34.4%). This means that delayed leaf senescence in response to attenuated blue light could be due to the reduction in PAR (Lee et al. 2003). Accordingly, attenuating blue light delayed leaf senescence the most for *A. platanoides* seedlings growing in the evergreen stand, where PAR was also the lowest. However, we found no difference in $F_v/F_m$ or stomatal conductance between our filter treatments to support the idea that the reduction in PAR or blue light might have affected gas exchange or photosynthetic capacity. Furthermore, CRYs are involved in the regulation of leaf senescence in *Glycine max* (Meng et al. 2013), which may indicate a role for blue light in advancing leaf senescence.

Attenuating UV radiation below 350 nm delayed leaf senescence in *A. platanoides* (4.1 days for stage 2; 0 days for stage 5). Supplementary UV-B radiation from lamps can advance leaf senescence in saplings of *F. sylvatica*, and it has been suggested that this effect is due to photodamage to the leaves (Zeuthen et al. 1997). As such, it is possible that attenuating UV radiation below 350 nm delayed leaf senescence, because of reduced photodamage throughout the year. Conversely, long term UV-B supplementation had no effect on the leaf senescence of *Vaccinium*
myrtillus and Vaccinium uliginosum (Phoenix et al. 2001). This suggests that more research is required to understand why autumn leaf senescence of different plant species is affected by UV radiation.

Figure 6. Conceptual schematic of environmental factors affecting leaf out in the forest understorey. Changes in solar radiation also result in changes in light quality entering the forest understorey, as displayed in Figure 2. “+” means that particular cue or environmental stressor is increasing in the understorey, whereas “-” means the opposite. Colour coding is purely aesthetic.

We found that the effects of light quality were larger for autumn leaf senescence in comparison to spring bud burst and leaf out. This is one of the few studies to conduct experiments on the effects of light quality on leaf senescence. However, when conducting our literature review (III), we found a similar general trend to that observed in our experiments that the effects of light quality were greater for autumn phenology (e.g. bud set), than for spring phenology (bud burst). For instance, twilight R:FR light can advance bud burst by 4 days in B. pendula, but daylength extension with R:FR can nearly prevent bud set occurring altogether (III). The mean and median percentage of trees reaching bud set after daylength extension with FR light was 2.4% and 0% (III). Beyond these findings, there is currently a paucity of studies that have been conducted on both bud set and leaf senescence, meaning that the effects of light quality in comparison to other environmental drivers cannot presently be ranked (Gallinat et al. 2015, III).
3.5 HOW WILL CLIMATE CHANGE AFFECT UNDERSTOREY LIGHT INTERACTIONS?

Climate change is advancing spring leaf-out in trees in the north-east USA, faster than the emergence and leaf out of understorey plants (Heberling et al. 2019). This mismatch in phenology means that the carbon assimilation of many herbaceous species is being restricted by earlier canopy shading. The inability of understorey plants to respond as much as canopy trees to rising temperatures in spring may be the result of soil temperatures being cooler than air temperatures in the canopy (Coulson et al. 1995; Ling & Zhang 2003). The lag in understorey springtime warming relative to the canopy would be further exacerbated by earlier canopy closure, reducing the transmission of IR radiation to the ground.

We found that changes in light quality did not affect the spring emergence of herbaceous species, because these species principally rely on timing their phenology according to ground temperatures and snowmelt (Augspurger and Salk 2017). Changes in blue light in particular can accelerate the spring bud burst of trees (II) and tree seedlings (IV). The treatments we used excluded blue light completely, whereas we found that in nature, the amount of blue light is reduced by 50.5% under canopy shade compared to sun gaps in the forest understorey (IV, supplementary material). Furthermore, tree seedlings have ontogenic safeguards against being overshadowed by adult trees (Vitasse et al. 2013). Altogether, this makes it less likely that seedlings of the same species will experience overshadowing and reduced blue light resulting from advanced canopy spring leaf-out.

It has been suggested that plants covered by snow in early spring may also be exposed to different spectral cues (Robson and Aphalo, 2019). There is a much larger (98.9%) reduction of blue light 30cm under the snowpack, compared to the surface (Robson and Aphalo, 2019). Earlier snow melt in spring under climate change could expose plants to a higher irradiance of blue light, which could accelerate their phenology even further, although many tree seedlings have buds above the snowpack which are exposed to ambient light conditions. Furthermore, it is probable that for tree seedlings, factors such as chilling and forcing temperatures have a larger role to play under these conditions (III).

Whilst we did not assess leaf senescence of spring ephemeral species in this study, there is some evidence suggesting that earlier shade can induce earlier leaf senescence in spring ephemerals (Augspurger and Salk, 2017). This is very interesting to note, as other studies have suggested that for woody species which senesce in autumn, shading delays leaf senescence even further (Lee et al. 2003). As such, it would be interesting to investigate whether changes in blue and UV radiation underpin this response as well. Furthermore, this could demonstrate that different functional plant types may respond differently to changes in canopy duration under climate change, with spring ephemerals reaching leaf senescence earlier, and autumnal species reaching leaf senescence later.
There have been range expansions under climate change for some temperate tree species, with them shifting either further north in the Northern Hemisphere, or to higher elevations (Beckage et al. 2008, Harsch et al. 2009; Vitasse et al. 2012). It has been proposed that phenology in more northern ecotypes of trees is more sensitive to changes in photoperiod than more southern trees (Vaartaja 1959, Myking and Heide 1995, Robson et al. 2013, review by Way and Montgomery 2015, Cooper et al. 2018). If this is the case, greater variation in photoperiod at higher latitudes may constrain northwards range-shifts of southern trees under climate change (Way and Montgomery, 2015). There have, however, also been studies suggesting the opposite, that more southern trees have a greater sensitivity to photoperiod changes (Kriebel 1957, Olson et al. 2013, Zohner et al. 2016, Osada et al. 2018).

In addition to possibly being more sensitive to changes in photoperiod, studies have also suggested that more northern trees are more sensitive to changes in light quality (III). As such, we may expect that the greater variation in seasonal and diurnal changes in light quality at higher latitudes could also be a factor limiting northwards range shifts. Failure to adapt to these changes in light quality at higher latitudes could result in frost damage (if for instance spring bud burst is too early, or autumn leaf senescence/bud-set occurs too late). Conversely, if spring bud burst occurs too late or autumn leaf senescence occurs too early, then this constitutes a missed opportunity for photosynthesis. Either way, this failure to correctly time phenology could result in reduced fitness. Whilst the latitudinal effects of light quality may correlate with the effects of photoperiod, light quality, unlike photoperiod, can also vary longitudinally. Changes in atmospheric conditions, water vapour, cloud cover, and ozone all vary with location, and can affect light quality reaching the ground (Emde et al. 2016). Considering this, it is important to understand plant phenological responses to light quality in different biomes and regions beyond temperate and boreal regions in North America and Europe.

3.6 METHODOLOGICAL CONSIDERATIONS

In the field experiment conducted for this Thesis (IV), we used an optical leaf sensor to assess the differences in seasonal leaf epidermal flavonol and anthocyanin content among species. This comparison was based on the assumption that the relationship between the absorbance indices are approximately linear with their respective leaf pigment contents (unpublished data; Hartikainen et al. 2020). The Dualex absorbance indices have a positive correlation with leaf flavonoid content for some plant species (Agati et al. 2008, Morales et al. 2010), but some alpine plant species do not exhibit this positive correlation (Lefebvre et al. 2016). Accordingly, it is possible that species-specific differences in the relationship between Dualex absorbance indices and total flavonoid content, could contribute to the species-specific patterns in optically assessed flavonoids we observed throughout the growing season.
Using filters attenuating solar radiation, as we did (IV), can also increase the temperature of the plants beneath. This is why it was essential that we incorporated a transparent control filter. While the average increase in temperature due to heating was very small in our experiment (an average of 0.3°C higher under all filters compared to ambient understorey conditions), it is possible that filter presence in the understorey could alter the responses of underlying plants, for example by changing air flow or herbivore access. The leaf epidermal flavonol and anthocyanin contents in our study fell within the normal range of values reported by Hartikainen et al. (2020) in the same forest stands. Similarly, the timing of leaf senescence for plants beneath the filters was similar to that of plants in ambient forest understorey conditions (Fig. S10, IV). Considering the controls in place and comparisons with ambient conditions given here, any effect of the filters altering plant responses is probably of insufficient magnitude to interfere with our conclusions derived from the comparison of spectral attenuation treatments.
3.7 CONCLUSIONS AND FUTURE RESEARCH

Conclusions

CRYS and UVR8 both regulate flavonols in response to UV-A. The effects of blue light on the accumulation of flavonols were larger than those of UV radiation, both under controlled conditions and under field conditions using understorey plants. Interestingly, flavonols in the more-light-demanding species we studied were more sensitive to changes in light quality, despite having shorter leaf life-spans than shade-tolerant species. We found that blue light can advance both bud-burst and autumn leaf senescence. While the effects of UV radiation on spring phenology were negligible, UV radiation may affect autumn leaf senescence. In general, most published studies have found that light quality has a small effect on spring phenology, however the limited number of studies on light quality related to autumn phenology suggests its effect could be greater at the end of the growing season.

Future research

Future work should endeavour to understand the processes by which blue light and UV radiation affect leaf senescence. This will reveal the reasons for, and mechanisms by which, different species’ autumn phenology follows different patterns. In addition, this will reveal whether prolonged canopy shade can produce phenological mismatches between understorey and overstorey species, or segregate different plant functional types within the understorey community. The template provided by this research could also be expanded to studying different plant communities in different plant biomes around the globe. For instance, studying responses between understorey plant communities in American and European temperate and boreal regions, which have different seasonality, and so may receive differing amounts of solar radiation throughout the year.

Whilst it may be ambitious to attempt to introduce the effects of spectral composition into phenological models, doing this may help us understand the mechanisms by which commonly used phenological model parameters such as photoperiod, solar irradiance, solar insolation, and daily light integral (DLI) can affect plants (Foggo and Warrington, 1989, Borchert et al. 2015, Calle et al. 2010, Liu et al. 2015, Fu et al. 2015, Liu et al. 2016). Understanding the mechanisms by which solar insolation and DLI could affect phenology, would provide further insight into their application into phenological models. Similar approaches could also be applied to integrating processes and models that underpin new leaf production in tropical trees, and flowering in trees in general.
4. REFERENCES


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