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Adding Far-Red Light to White LEDs: Implications for Cucumber Seedling Morphology, Growth, and Photosynthesis

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MSc in Plant Sciences – Plant Production Systems

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Abstract

Far-red (FR) light (700–800 nm) affects photosynthetic efficiency and regulates shade responses, which influences plant morphology and growth. However, plant responses to FR vary widely between environmental conditions, species, and even genotypes. Here, we investigated the responses of cucumber (*Cucumis sativus* L. 'Hi Light' and 'Imea') seedlings to the addition of FR (peak = 732 nm) under a common background of white light by using light-emitting diodes (LEDs). In three controlled environment experiments, seedlings were subjected for ten days to either a white light control or one of three FR treatments: high intensity continuous FR in the photoperiod (110 μmol m^{-2} s^{-1} of FR), low intensity continuous FR (50 μmol m^{-2} s^{-1} of FR), or end-of-day FR (EOD-FR; 1.5 h of 110 μmol m^{-2} s^{-1} of FR) added to a common background of white light (photosynthetic photon flux density of 240 μmol m^{-2} s^{-1}). Plant morphology, growth components, and leaf photosynthetic light responses were analysed and compared with plants exposed to corresponding white light controls.

Adding FR commonly resulted in increased shoot length, leaf expansion, and improved growth when compared to white light treatments. Relative growth rates (RGRs) increased by 8–18% as a result of large increases in net assimilation rates (NARs) and in spite of decreased leaf area ratios (LARs). Decreased LARs were largely caused by decreased leaf mass ratios (LMRs) due to greater dry mass partitioning to stem and petioles at the expense of leaves. Under EOD-FR, the negative influence of LMR on LAR was partly mitigated as specific leaf areas (SLAs), the other component of LAR, slightly increased. Continuous FR commonly resulted in increased leaf photosynthetic capacity but no changes were observed following EOD-FR. Moreover, FR decreased total chlorophylls and carotenoids concentrations. Despite differences in morphological and physiological traits, like leaf area and photosynthetic capacity, the cultivars 'Hi Light' and 'Imea' responded similarly to supplemental FR in terms of relative changes in plant morphology and growth components. We conclude that supplemental FR, either continuously in the photoperiod or as EOD-FR, under sole-source lighting can improve cucumber plant growth through complex plant morphological and physiological changes.

List of Abbreviations

P_N net photosynthesis. 20, 21, 26–29, 33, 34, 38, 42, 44, 50, 51

DAT days after treatment start. 18, 19

EC electrical conductivity. 15, 16

EOD end-of-day. 2, 3, 12, 48, 51, 52

ETR electron transport rate. 20, 28, 29, 34, 42, 50, 51

FR far-red. iv

HPS high-pressure sodium. 1, 2, 6, 15

LAR leaf area ratio. iv, 24, 31, 38, 48, 51, 54

LED light-emitting diode. iv, 1–3, 6, 14, 16, 19, 52–54

LMR leaf mass ratio. iv, 24, 31, 38, 51

NAR net assimilation rate. iv, 23, 24, 31, 38, 48–51, 54

PAR photosynthetic active radiation. 1–3, 5, 9, 52

PPE phytochrome photoequilibrium. 13, 14, 16–18, 46, 47

PPFD photosynthetic photon flux density (400–700 nm). 16, 19–21, 26–28, 33, 34, 38, 39, 41, 42, 47, 48, 50–52

QY quantum yield (moles of CO_2 assimilated per mole of photons). 7, 9, 49

QY_{abs} absolute quantum yield (moles of CO_2 assimilated per mole of absorbed photons). 7

RGR relative growth rate. iv, 23, 31, 38, 48, 51, 54

RH relative air humidity. 15, 16, 18, 20

SLA specific leaf area. iv, 24, 31, 38, 48

TPFD total photon flux density (400–800 nm). 16, 18

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1 Introduction

Light is fundamental to crop production as it acts as a fuel for photosynthesis enabling plant growth but also as an environmental signal that triggers changes to plant morphology and development. At high northern latitudes, solar radiation, however, varies vastly throughout the year to the detriment of both yield and the production season (Moe *et al.*, 2006). Use of supplemental lighting during periods of low or no irradiance has therefore become increasingly common practice in protected cultivation since the early twentieth century (Pinho & Halonen, 2017). In Scandinavia, artificial lighting is often essential for year-round production in greenhouses, where the glazing material further limits sunlight transmission to the crop canopy (Moe *et al.*, 2006). The beneficial effects of supplemental light on growth in numerous species are therefore well established, but plant responses vary depending on species as well as light intensity and spectral quality of the light source (Snowden *et al.*, 2016).

The most common types of supplemental lighting in controlled environment agriculture include gas-discharge type lamps like fluorescent, metal-halide or high-pressure sodium (HPS), and solid-state lighting like light-emitting diodes (LEDs) (Moe *et al.*, 2006; Kusuma *et al.*, 2020). LEDs boast many advantages over the widely used HPS lamps, including better photon efficacy, more precise control, ability to be placed closer to the canopy, and longer longevity (Kusuma *et al.*, 2020). Importantly, LEDs are also tuneable in their spectral output, unlike the largely fixed spectral composition of gas-discharge lamps (Pattison *et al.*, 2018). This suggests that the light quality of LEDs can be adapted to best match plant absorption in order to evoke desirable morphological responses or even improve photosynthetic efficiency.

Spectral light quality influences photosynthetic responses in plants to a great extent. Photosynthetic active radiation (PAR) is typically regarded as limited to wavelengths between 400–700 nm (McCree, 1971; Liu & Iersel, 2021). Within this range, the different wavelengths of photons are absorbed with varying efficiency by mass pigments in leaves, and thus the photosynthetic efficiency, or quantum yield, varies under illumination with different narrow wavebands (McCree, 1971; Hogewoning *et al.*, 2012). Generally, red light (600–700 nm) is the most efficient driver of photosynthesis, while green (500–600 nm) and blue light (400–500 nm) are less efficient (McCree, 1971; Evans, 1987). But, this categorisation does not take into account synergistic effects among wavelengths (Emerson & Rabinowitch,

1960; Zhen & Iersel, 2017) and disregards the effects of absorption depth at higher light intensities (Sun *et al.*, 1998; Terashima *et al.*, 2009). Far-red radiation (700–800 nm), in particular, is poorly absorbed in leaves but it may enhance leaf photosynthesis by increasing the quantum yield of PAR wavelengths (Emerson & Rabinowitch, 1960; Zhen & Iersel, 2017; Zhen & Bugbee, 2020a).

Photomorphogenic responses to light quality vary notably between species and environmental conditions (e.g., Snowden *et al.*, 2016). These responses, ranging from germination, elongation, leaf expansion, phototropism, stomatal opening to flowering and change in growth of particular organs, are mediated by several classes of photoreceptors (Franklin *et al.*, 2005; Davis & Burns, 2016). Far-red and red radiation are the primary regulators of the state of phytochromes, which exist in two interconvertible isoforms: an inactive red absorbing form (Pr) and a biologically active far-red absorbing form (Pfr) (Franklin *et al.*, 2005). Consequently, a low R/FR-ratio induces phytochrome-mediated shade-avoidance responses, which include promotion of stem elongation, leaf expansion, plant growth (Ruberti *et al.*, 2012; Demotes-Mainard *et al.*, 2016), and, in some long-day plant species, acceleration of flowering (Park & Runkle, 2017). Furthermore, short-term end-of-day (EOD) treatments with low R/FR-ratios have been effective in inducing similar responses in regards to promotion of stem and petiole elongation and, in some cases, leaf expansion (López-Juez *et al.*, 1990; Yang *et al.*, 2012; Kalaitzoglou *et al.*, 2019). Plant responses to far-red light may, however, not only vary between species but also genotypes. Ji *et al.* (2021), for instance, found that tomato genotypes varied significantly in growth responses to far-red light.

Cucumber is a species known to be sensitive to changes in spectral quality (Snowden *et al.*, 2016; Hernández & Kubota, 2016). Cucumber is also a widely grown greenhouse crop that is often cultivated intensively using the high-wire method with high plant and canopy density (Pettersen *et al.*, 2010b). As the shortage of light during late autumn to early spring necessitates use of artificial lighting in Norway, cultivation is at times also energy-intensive. As a result, some commercial growers have started to utilise the more energy-efficient LEDs, largely containing no far-red light, as the sole source of overhead supplemental lighting. But, this practice has resulted in issues with maintaining growth and production during winter-time (growers in Rogaland, pers. comm.). In addition, some cucumber cultivars seemingly respond better to LED lighting than others when compared with HPS lighting (growers in Rogaland, pers. comm.). In periods of low natural irradiance, plants cultivated under artificial light, especially under narrow bandwidth LEDs, may experience very different light quality to that found in natural sunlight, especially in terms of far-red radiation (Kusuma & Bugbee, 2021a). It, however, remains unclear whether growth is

largely affected by lack of far-red light when cultivated under sole-source LED lighting and how responses differ between cucumber cultivars.

The aim of this study was to better understand how far-red and PAR interacts to influence growth and physiology of cucumber plants under sole-source LED lighting. Our specific objectives were to investigate how adding far-red light to a background of white light influences morphology, growth, and photosynthesis of cucumber plants. Through analysis of plant development, underlying growth components, and single leaf photosynthetic light responses, we tested the hypothesis that supplemental far-red light would promote shoot extension, leaf expansion, and increase plant growth. Cucumber seedlings of two cultivars, 'Hi Light' and 'Imea', were therefore grown in controlled environment chambers with and without additional far-red light for ten days in three successive experiments. The experiments utilised three different supplemental far-red treatments, including high and low intensity continuous far-red and an EOD far-red treatment.

2 Background

2.1 Light as energy and a signal

Light acts as fuel for photosynthesis that enables the production of adenosine triphosphate and similar molecules that direct plant growth. In addition, light also acts as an environmental signal that regulates plant morphology and development.

Light, however, varies in its energetic content depending on the wavelength emitted. The Planck-Einstein relation states that the energy of light is inversely proportional to its wavelength ($E = \frac{hc}{\lambda}$, where E is the energy, h is the Planck constant (6.63×10^{-34} J s), c is the speed of light in vacuum (3.0×10^{8} m s^{-1}) and λ is the wavelength). This means that light at shorter wavelengths contain more energy than light at longer wavelengths. As photosynthesis is a quantum storage process (Davis & Burns, 2016), the energy that drives the photochemical processes within the plant is supplied in discrete light packets, or photons. When a photon is absorbed by an electron of a molecule, energy from the photon is communicated to it (Walker, 1992, ch. 3). If the photon carries sufficient energy, the absorbing electron becomes excited, in that it is lifted from its ground state of lowest energy, but highest stability, to an energy-rich, unstable state. At the same time, if the photon has excess energy that lifts the electron to a higher, more unstable, second excited state, it will essentially instantaneously 'fall' to the first excited state. This first excited state can then be used to transfer energy to neighbouring molecules via resonance, which, in turn, can use the energy to drive other processes.

In plants, these photon-absorbing molecules are the mass and sensor pigments, which drive photosynthesis, screen potentially harmful excess photons, or regulate photomorphogenesis (Walker, 1992, ch. 3). In particular, the photosynthetic mass pigments include chlorophyll *a* as the primary pigment but the range of light absorption (that is, which wavelengths can be absorbed and utilised) is extended by chlorophyll *b*, β-carotene, and other accessory pigments (Smith *et al.*, 2017). These photosynthetic pigments are organised in photosystems, PSII and PSI, and associated light harvesting chlorophyll-protein complexes, which act as antennae to funnel excitation energy to the photosystem reaction centres in order to drive linear electron transport and further photochemical processes (Walker, 1992, ch. 3).

The primary wavelengths that drive photosynthesis are referred to as PAR, and this range is generally considered limited to 400–700 nm (McCree, 1971; Liu & Iersel, 2021). Wavelengths outside of this range are known to induce changes to development and morphology (e.g., Jenkins, 2014), but certain wavelengths may also affect photosynthetic efficiency (Zhen & Iersel, 2017).

2.1.1 Solar radiation

Solar radiation is relatively constant in its spectral composition in space, but it varies slightly throughout the day (Holmes & Smith, 1977). Red light constitutes the majority of the emitted PAR at *ca.* 39%, while green and blue light constitute around 36% and 26%, respectively (Figure 2.1A). However, the amount of red and far-red light is for the most part nearly equal as indicated by a typical R/FR-ratio of 1–1,2 at noon (Holmes & Smith, 1977).

At high latitudes the amount of sunlight varies vastly throughout the year (Moe *et al.*, 2006). In Eastern Norway, the daily light integral, that is the sum of light in a day, typically ranges from above 40 mol m^{-2} s^{-1} in mid summer to largely negligible amounts of 1–2 mol m^{-2} s^{-1} during winter (Figure 2.1B). In protected cultivation, the cover material further limits light transmission to just 60–80% of the outside radiation, depending on the specific material (Hemming *et al.*, 2016).

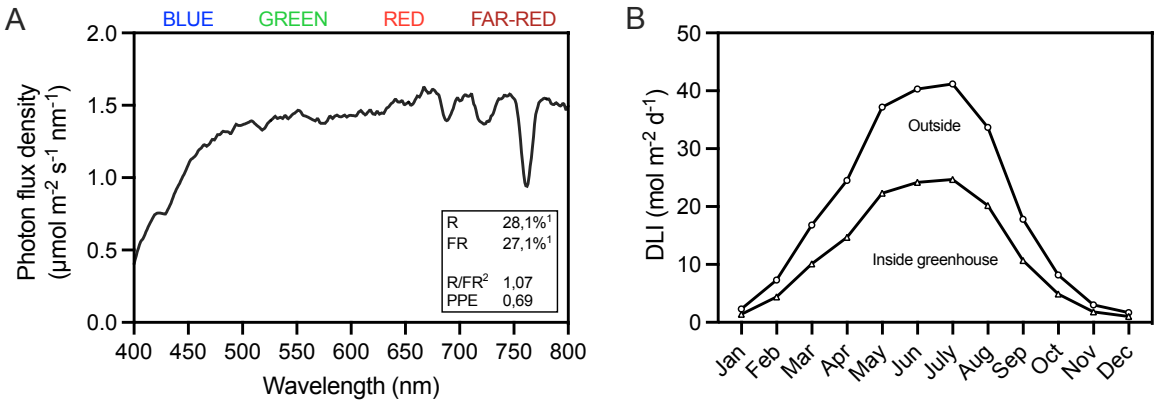

Figure 2.1.: Solar radiation in Ås, Norway. (A) The spectral composition of solar radiation as measured at noon on February 18, 2022. (B) The variation in sunlight throughout the year, both outside and inside a greenhouse with 60% light transmission, shown as daily light integrals and based on data from Moe *et al.* (2006). [1]Percentage of total photon flux density (400–800 nm). [2]For the calculation of R/FR-ratio, PFD was integrated over 20 nm intervals for red (650–670 nm) and far-red (720–740 nm).

2.1.2 Artificial lighting

As plants must receive sufficient light to drive growth and maintain plant quality, the natural variations in sunlight availability limits both yield and the growing season (Moe *et al.*, 2006; Verheul *et al.*, 2012). Year-round production in greenhouses in Norway is therefore dependent on supplemental light supplied artificially (Moe *et al.*, 2006). Similarly, indoor cultivation, like vertical farming, is completely dependent on artificial lighting. The conversion rate from electricity to photons, or the photon efficacy (expressed in μmol photons J^{-1}), is therefore often one of the most critical elements in cost-effective production.

In protected cultivation, high-intensity-discharge lamps, and in particular HPS, have typically been among the most widely used sources of supplemental lighting (Pattison *et al.*, 2018). HPS lamps characteristically emit a lot of radiant heat due to their high operating temperature (Nelson & Bugbee, 2015). As a result, HPS lamps have to be placed at good distances from the plant canopy as to avoid leaf burn and negative influence on growth (Tewolde *et al.*, 2016). These more traditional lighting technologies are also limited in the ability to control both the intensity and spectrum (Pattison *et al.*, 2018). The efficacy of HPS lamps are typically around 1.7–1.8 μmol J^{-1} (Kusuma *et al.*, 2020; Katzin *et al.*, 2021).

In contrast, the efficacy of solid-state LED lighting varies but it often ranges from 2.1–3.0 μmol J^{-1} (Kusuma *et al.*, 2020), and it is expected to increase in the future. Several studies have found that switching from HPS lamps to LEDs increases energy efficiency, in spite of greater heating demand, and also improves productivity and profitability (Wacker *et al.*, 2022; Katzin *et al.*, 2021). Furthermore, LEDs emit much less radiant heat and dissipate much of the heat away from the plane which they illuminate (Nelson & Bugbee, 2015). LED lamps can therefore be placed closer to the canopy to improve light distribution (e.g., as intercanopy lighting; Pettersen *et al.*, 2010a). LEDs also generally offer greater longevity and maintain their efficacy over longer periods compared to HPS lamps (Kusuma *et al.*, 2020). Importantly, while gas discharge-types are largely fixed in their spectral output, LEDs are, to a large extent, tuneable allowing output of narrow bands of wavelengths (Pattison *et al.*, 2018; Kusuma *et al.*, 2020).

The flexibility in the spectral composition of the multitude of LED offerings (`https://qpl.designlights.org/horticulture`) also mean that plants cultivated under sole-source artificial lighting may experience very different light quality to that of natural sunlight. Nonetheless, this customisation allows for the light quality to be adapted to provide wavelengths that best induce desirable morphological responses or are more efficient in photosynthesis.

2.2 Light quality in photosynthesis

2.2.1 Efficiency of blue, green, and red light in photosynthesis

The effects of spectral light quality on photosynthesis in leaves vary considerably between wavelengths. The prominent studies of McCree (1971) and Inada (1976) demonstrated that single leaves subjected to narrow wavebands of light at low intensity differed in their efficiency to drive photosynthesis, or quantum yield (moles of CO_2 assimilated per mole of photons) (QY). In general, red light had the highest QY, while green and blue light were markedly less efficient in photosynthesis (Figure 2.2; McCree, 1971; Inada, 1976).

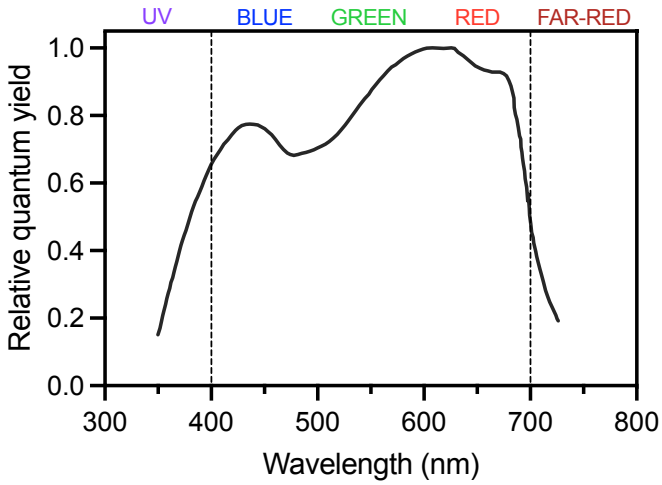

Figure 2.2.: Relative quantum yields considering leaf absorptance and based on single-point measurements of photosynthetic rate at low photon flux density by narrow band light sources (McCree, 1972)

Light is, however, absorbed to different extents in leaves depending on the wavelength as determined by the absorption ranges of the pigment protein complexes within the plant cells (Hogewoning *et al.*, 2012). In green leaves, blue light is absorbed the most followed closely by red light (Figure 2.3). Green light, on the other hand, is absorbed considerably less than both blue and red light (Hogewoning *et al.*, 2012; Liu & Iersel, 2021).

The low absolute quantum yield (moles of CO_2 assimilated per mole of absorbed photons) (QY_{abs}) of blue light thus cannot be attributed to low leaf absorption. Instead, blue light is absorbed by photosynthetic carotenoids and non-photosynthetic pigments to a larger degree than red or green light (Hogewoning *et al.*, 2012). Photosynthetic carotenoids, like

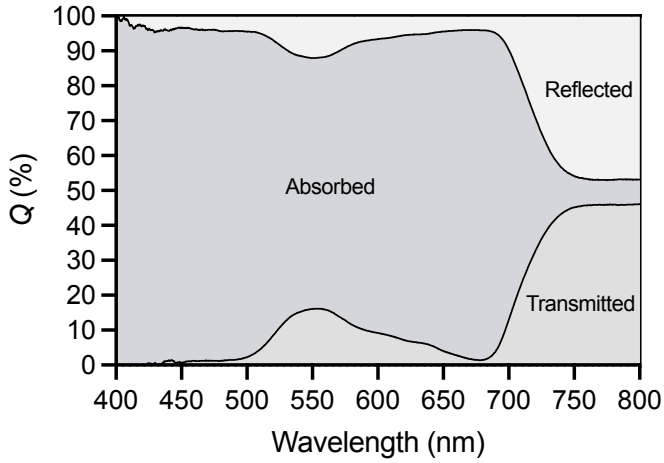

Figure 2.3.: Absorptance, reflectance, and transmittance of fully expanded leaves of 5-week-old cucumber 'Quarto' plants when illuminated on the adaxial surfaces (data from Anthony and Wennerberg, 2021). Areas between the curves show percentage of light absorbed, reflected, and transmitted at a given wavelength.

β-carotene, are located primarily in the light-harvesting antennae complexes but transfer only 35–90% of their excitation energy to chlorophylls, depending on the specific carotenoid and its location (Hogewoning *et al.*, 2012; Liu & Iersel, 2021). In contrast, the chlorophyll-to-chlorophyll transfer efficiency within the antennae complexes is 100% (Hogewoning *et al.*, 2012). In addition, blue light is also absorbed by non-photosynthetic pigments that divert energy away from photochemistry and instead act as screening pigments in order to avoid potentially damaging effects of excess photons (Hogewoning *et al.*, 2012). Free carotenoids, for instance, do not contribute to photosynthesis (Hogewoning *et al.*, 2012), and anthocyanins are located primarily in the vacuole and thus cannot transfer absorbed light energy to use in photochemistry (Sun *et al.*, 1998). As a result, blue photons are inherently less efficient in photosynthesis due to capture by inefficient accessory pigments and non-photosynthetic pigments.

In contrast, red light is absorbed much more strongly by photosynthetic pigments, and, in particular, chlorophylls (Hogewoning *et al.*, 2012). However, the strong absorption of both red and blue light means that these wavelengths easily attenuate in the upper chloroplasts of the pallisade mesophyll (Sun *et al.*, 1998). Consequently, non-photochemical quenching (i.e., heat dissipation) may be up-regulated in the upper part of the leaves in response to strong red or blue light (Sun *et al.*, 1998; Liu & Iersel, 2021).

On the other hand, green light, that is absorbed considerably less by photosynthetic pigments, can penetrate deeper into leaf tissues (Sun *et al.*, 1998; Terashima *et al.*, 2009). As such, green light is less likely to dissipate as heat and can excite photosystems in the

chloroplasts of the deeper pallisade and spongy mesophyll cells that would otherwise receive little excitation energy (Sun *et al.*, 1998; Liu & Iersel, 2021). Liu and Iersel (2021) found that the QY was lower under green light than red or blue light when lettuce plants were subjected to low light intensities. However, when plants were subjected to high light intensities, green light had comparable QY to that of red light, likely resulting from a more uniform distribution of light in the leaves. There is therefore an interaction between light quality and intensity on photosynthesis (Sun *et al.*, 1998; Terashima *et al.*, 2009; Liu & Iersel, 2021).

2.2.2 The red drop and Emerson enhancement effects — the case for far-red in photosynthesis

When narrow waveband light, used to illuminate leaves, approaches the range of far-red radiation, the corresponding QY decreases rapidly. This occurs at wavelengths above 680 nm and it has therefore become known as the red drop effect (Emerson & Lewis, 1943). In general, far-red light is also poorly absorbed in green leaves compared to PAR wavelengths (Figure 2.3; Hogewoning *et al.*, 2012).

However, later studies observed that when far-red light was applied simultaneously with shorter wavelength red light, the corresponding rate of photosynthesis increased considerably (Emerson *et al.*, 1957; Emerson & Rabinowitch, 1960). This effect was greater than what you would expect based on the sum of the gross photosynthesis rates when the two wavelengths were applied separately (Figure 2.4). Emerson and Rabinowitch (1960) therefore concluded that far-red acted synergistic with red light when the two were combined, which later became known as the Emerson enhancement effect.

This synergistic phenomenon is the result of the absorption ranges of the two photosystems (Hogewoning *et al.*, 2012; Laisk *et al.*, 2014). Specifically, longer wavelengths (685–730 nm) overexcite PSI and to a very small extent excite PSII (Hogewoning *et al.*, 2012). Due to the cyclical nature of linear electron transport between the two photosystems, monochromatic far-red light effectively limits CO_2 assimilation (Hogewoning *et al.*, 2012; Laisk *et al.*, 2014). However, when PSI-preferential light is combined with shorter wavelength light (400–670 nm) that overexcite PSII, it results in an improved balance in excitation between the two photosystems thus increasing the QY (Hogewoning *et al.*, 2012; Zhen & Iersel, 2017). In contrast, there are no interactive effects among other PAR wavelengths on photosynthetic efficiency (McCree, 1972; Zhen & Iersel, 2017; Liu & Iersel, 2021).

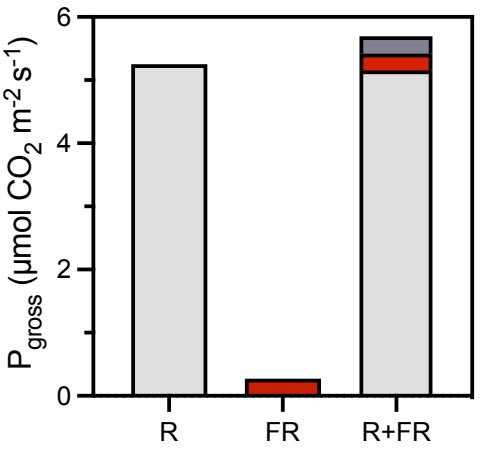

Figure 2.4.: Emerson enhancement effect as observed in single fully expanded leaves of cucumber 'Quarto' (Anthony & Wennerberg, 2021). Leaves were exposed to photon flux densities of 100 μmol m^{-2} s^{-1} of red (R; peak = 657 nm), 100 μmol m^{-2} s^{-1} of far-red (FR; peak = 732 nm), or both simultaneously for a total of 200 μmol m^{-2} s^{-1} (R+FR).

Nonetheless, absorption and the consequent quantum yield therefore depend on the spectral light quality emitted by the source. Far-red lamps extending into shorter wavelengths may emit light that is both more absorbed by the leaf and, consequently, also utilised more efficiently in photochemistry. Extrapolating measurements of photosynthesis on single leaves to whole plants or plant communities is, however, difficult as light quality may also alter plant response (Snowden *et al.*, 2016).

2.3 Plant responses to light quality

2.3.1 Photoreceptors and photosynthetically active radiation

The light quality of the immediate growth environment also strongly influences plant morphological and developmental responses. Several major classes of photoreceptors are involved in sensing changes to the light environment and, consequently, trigger changes to modify plant morphology and development in order to enhance plant survival (Galvão & Fankhauser, 2015). The plant responses induced by the different photoreceptors may overlap to some degree (Davis & Burns, 2016).

UV-A and blue light responses in plants are mediated by three classes of these photoreceptors: cryptochromes, phototropins, and zeitlupe (Galvão & Fankhauser, 2015; Huché-Thélier

et al., 2016). Cryptochromes are involved in de-etiolation, circadian entrainment, and flowering, while phototropins regulate a wide range of responses, including stomatal opening, chloroplast movement, and phototropism (Davis & Burns, 2016). Zeitlupe is involved in daylength perception and the circadian rhythm (Galvão & Fankhauser, 2015).

Conversely, there has not been identified any photoreceptor specific to green wavelengths (Davis & Burns, 2016). Unlike plants subjected to spectral distributions deficient in blue light, the absence of green light does not necessarily result in abnormal plant morphology (Kusuma *et al.*, 2021). In some species, increasing the fraction of green light may induce stem elongation or leaf expansion (Kusuma *et al.*, 2021).

Plants grown in the absence of blue light become etiolated and leaves often remain curled (Davis & Burns, 2016). Moreover, increasing the fraction of blue photons may decrease stem height and leaf area (Kusuma *et al.*, 2021), but it has also been found to increase photosynthetic efficiency in several species (Terfa *et al.*, 2013; Hogewoning *et al.*, 2010). Importantly, some blue light is qualitatively required in order to avoid dysfunctional stomata and photosynthetic operation if plants are grown under primarily red light (Hogewoning *et al.*, 2010). In cucumber, plants cultivated under purely red light develop 'red light syndrome' characterised by unresponsive stomata, low photosynthetic capacity, and impaired growth (Hogewoning *et al.*, 2010; Trouwborst *et al.*, 2016). At the same time, red light is also important for proper function of the photosynthetic apparatus (Trouwborst *et al.*, 2016).

2.3.2 Phytochromes and far-red radiation

Phytochromes are a class of photoreceptors that primarily absorb light in the red and far-red regions (Franklin *et al.*, 2005). Phytochromes can exist in two photoconvertible isoforms: an inactive red absorbing form (Pr) and a physiologically active far-red absorbing form (Pfr). While the absorption of these two isoforms peak in different regions (within red and far-red for Pr and Pfr, respectively), both isoforms extend their absorption to 300–800 nm and, to some extent, overlap (Kreslavski *et al.*, 2018). The equilibrium between these two phytochrome isoforms therefore depend on the immediate light environment.

Phytochromes are synthesised in the inactive Pr form and convert into active Pfr primarily by absorption of red light. The active Pfr are then translocated to the nucleus where Pfr specific interactions initiate downstream signaling cascades and subsequently affect photomorphogenesis (Franklin *et al.*, 2005; Ruberti *et al.*, 2012). Oppositely, the far-red antagonism results in Pfr being reverted to inactive Pr mainly by far-red light but also

in darkness due to thermal reversion (Klose *et al.*, 2020). Phytochromes are involved in seed germination, de-etiolation, circadian entrainment, flowering, and shade-avoidance (Demotes-Mainard *et al.*, 2016; Davis & Burns, 2016)

In naturally shaded environments, the relative fraction of far-red is markedly enriched compared to red light. This is caused by the filtering action of pigments like chlorophylls that strongly absorb red light while far-red is largely reflected or transmitted (Kusuma & Bugbee, 2021a). Consequently, the selective attenuation of red light lowers the R/FR-ratio underneath, especially in dense vegetation or within canopies. Shade can therefore also be simulated by lowering the R/FR-ratio with artificial lighting. In turn, the low R/FR-ratio, or the absence of Pfr, induces certain phytochrome-mediated responses like the shade-avoidance syndrome (Franklin *et al.*, 2005).

The shade-avoidance syndrome typically includes rapid elongation of internodes and petioles, leaf hyponasty, reduced chlorophyll content, accelerated flowering, and increased apical dominance (Franklin *et al.*, 2005; Ruberti *et al.*, 2012; Kusuma *et al.*, 2021). In more shade tolerant species, plants may respond to low R/FR-ratios with promotion of leaf expansion whilst, to a larger extent, suppressing other responses like stem elongation (Gommers *et al.*, 2013; Park & Runkle, 2017; Zhen & Bugbee, 2020b). In addition, plants often also develop thinner leaves, lower the chlorophyll *a*/*b*-ratio, and increase PSII:PSI-ratio in order to increase light capture and utilisation (Gommers *et al.*, 2013).

The thermal reversion of Pfr to Pr in darkness varies in magnitude, but biologically meaningful amounts of Pfr can persist for hours despite lack of light stimuli (Casal, 2012). In turn, an EOD input of far-red light just prior to the beginning of the night may reduce Pfr levels to a minimum (Casal, 2012). Such EOD treatments with far-red light can therefore induce shade-avoidance responses of varying strength in several species (Kasperbauer, 1971; López-Juez *et al.*, 1990; Yang *et al.*, 2012; Kalaitzoglou *et al.*, 2019).

R/FR-ratios have previously been used to describe these phytochrome-mediated responses, but the use of narrow-band artificial lighting, both in research and commercial practice, has highlighted several problems in using this measure to interpret such responses (Kusuma & Bugbee, 2021a).

2.4 Phytochrome photoequilibrium and red/far-red ratio

Phytochrome photoequilibrium (PPE), or photostationary state, is a model for estimating the proportion of biologically active phytochrome (Pfr) relative to total phytochrome (Ptotal = (Pr + Pfr)) within the plant (Kusuma & Bugbee, 2021a). The model is calculated on the basis of the spectral light distribution above the canopy within the biologically relevant wavelengths (300–800 nm) that are then weighted using photoconversion coefficients derived from the absorption of the two phytochrome isoforms (Figure 2.5). As the two isoforms primarily absorb light in the red and far-red regions, the PPE is in large part determined by the red/far-red-ratio (Kusuma & Bugbee, 2021a). The PPE is usually confined to values between 0–0.89 due to the overlapping absorption spectra of the phytochrome forms (Lagarias *et al.*, 1987; Kusuma & Bugbee, 2021a).

There are several commonly used photoconversion coefficients derived from different studies on the photochemical properties of the phytochrome isoforms (notably, Kelly and Lagarias, 1985; J. C. Sager *et al.*, 1988; Lagarias *et al.*, 1987). While these coefficients vary slightly, the coefficients are largely similar when normalized to the Pr peak (Kusuma & Bugbee, 2021b). The absolute magnitudes are only important when also considering other phytochrome dynamics like thermal reversions (Kusuma & Bugbee, 2021b). Moreover, the PPE may also be estimated using measurements of absorbance from chlorophyll-deficient tissue, but this method is more time-consuming (Kusuma & Bugbee, 2021a).

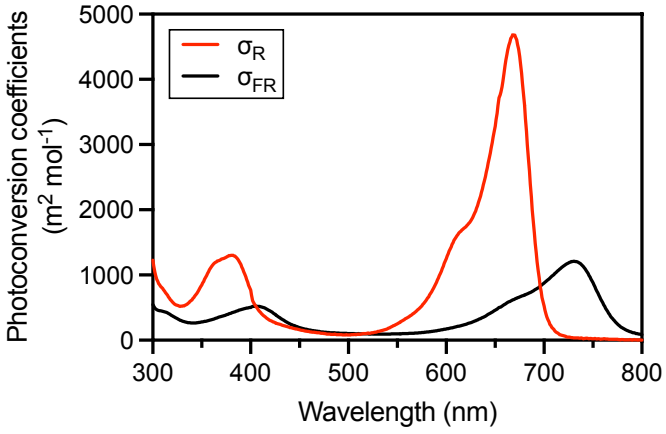

Figure 2.5.: Normalised photoconversion coefficients for estimation of PPE by spectral distribution above the canopy. Data were derived from Lagarias *et al.* (1987) by Kusuma and Bugbee (2021b). σ_R are photoconversion coefficients for conversion of inactive Pr into active Pfr, and σ_{FR} are coefficients for conversion of Pfr into Pr.

In any case, estimates of PPE may be used to predict phytochrome-mediated responses in plants. Specifically, stem extension rate and stem height of several species show an inverse linear or log linear relationship with the estimated PPE (e.g., Park and Runkle, 2017). Additionally, estimates of PPE may be important when considering sole-source use of narrow-banded lights that differ considerably from sunlight in spectral distribution. For instance, in LEDs that incorporate a high amount of red but negligible amounts of far-red light, the R/FR-ratio may approach infinity (Kusuma & Bugbee, 2021b). As a result, the PPE can to some extent be more appropriate for quantifying certain phytohrome-mediated photomorphogenic responses to light quality.

3 Materials and Methods

Three experiments were performed with seedlings of two cucumber cultivars in growth chambers at Norwegian University of Life Sciences, Ås, Norway, during spring 2022. Each experiment lasted ten days and consisted of two overhead LED lighting treatments that utilised either white light or white light in combination with a form of supplemental far-red.

3.1 Plant material and pre-cultivation

Plants of cucumber (*Cucumis sativus* L.) 'Hi Light' (Nunhems Netherlands BV, Haelen, the Netherlands) and 'Imea' (Enza Zaden, Enkhuizen, the Netherlands) were grown from seed in 12 cm pots containing fertilized Sphagnum peat medium, electrical conductivity (EC) 1.0–1.5 dS m^{-1}, pH 5.0–6.0 (Veksttorv, Norgro AS, Lier, Norway) at the Centre for Plant Research in Controlled Climate, Norwegian University of Life Sciences, Ås, Norway (59°40'05.7"N, 10°46'16.5"E) prior to each experiment. Once seeded, plants were placed in a greenhouse compartment with glass roof and walls at 20°C, 65% relative air humidity (RH), and ambient CO_2 under supplemental HPS lighting (GAN 4-550 AL 400W, Gavita International b.v., Rozenburg, the Netherlands), controlled by a PRIVA system (Priva, De Lier, the Netherlands). During pre-cultivation, plants were watered daily with tap water. When the first true leaf measured approximately 1.5 cm in length (ten days after sowing in all experiments), plants were moved to the experimental controlled environment chambers for lighting treatments.

3.2 Growth chamber conditions and treatments

Three separate experiments were carried out in succession using factorial 2×2 designs, where the same two cultivars were subjected to different lighting conditions for ten days. For every experiment, 24 uniformly developed seedlings per cultivar were chosen as replicates and randomisation was used to determine treatment and placement within growth chambers upon start ($n = 12$ plants per cultivar per treatment). Plants were routinely rotated between places within the chambers to minimise border effects.

Each experiment utilised two separate growth chambers with two different lighting regiments for 18-h photoperiods followed by 6-h dark periods. Plants were subjected to overhead lighting, mounted above a clear glass barrier, from either white LEDs (EPX FS, model 2021, Evolys AS, Oslo, Norway; 10% B, 20% G, 70% R) or white LEDs in combination with far-red LEDs (OEM, EAX 7M O1 730 nm, Evolys AS). The white spectra peaked at 655 nm, while the far-red spectra peaked at 732 nm. Light intensity at the top of the canopy was measured regularly using a handheld spectroradiometer (SpectraPen mini, Photon Systems Instruments, Drasov, Czech Republic) in order to maintain similar photosynthetic photon flux density (400–700 nm) (PPFD) throughout experiments and between treatments. When the mean PPFD ($n = 5$ measurements within each chamber) varied more than 10 μmol m^{-2} s^{-1} from the desired PPFD, the height of the growth chamber platforms were adjusted as needed. For all experiments, chambers were set to 23 ± 1°C day and night, 75% RH, and ambient CO_2, controlled by a PRIVA system (Priva).

In all experiments, plants were watered daily with a 50/50% solution mixture of YaraTera® Calcinit™ (14.4% NO_3, 1.1% NH_4, 19.0% Ca, Yara Norge AS, Oslo, Norway) and Kristalon™ Indigo (7.5% NO_3, 1.0% NH_4, 4.9% P, 24.7% K, 4.2% Mg, 5.7% S, 0.027% B, 0.004% Cu, 0.2% Fe, 0.06% Mn, 0.004% Mo, 0.027% Zn, Yara Norge AS), EC 2.0 dS m^{-1}.

3.2.1 Experiment one — high intensity continuous far-red

In experiment one, plants were subjected to one of two lighting treatments: white light (white) or white light with supplemental high intensity continuous far-red (high CFR) for the entire duration of the photoperiod (Figure 3.1A). In the high CFR treatment, far-red light constituted *ca.* 31.3% of the total photon flux density (400–800 nm) (TPFD) and PPE was 0.74 (Table 3.1). Spectral distribution, PPFD, and TPFD of the treatments were measured using a spectroradiometer (SpectraPen mini, Photon Systems Instruments) on five different locations at the top of the canopy in each growth chamber and five times during the experiment. PPE was estimated based on the mean spectra of all 25 measurements and calculated as Pfr/(Pfr+Pr) following the methods of Kusuma and Bugbee (2021b) and using the photoconversion coefficients derived from Lagarias *et al.* (1987). The height of the platforms supporting the plants treated with supplemental high CFR were adjusted twice in order to maintain the desired PPFD.

During the experiment, air temperature was 22.6 ± 0.3°C (mean ± s.d.) and RH was 75.0 ± 2.1% in the growth chamber containing the white treatment. In the high CFR treatment chamber, air temp. was 22.3 ± 0.6°C and RH was 74.8 ± 1.7%.

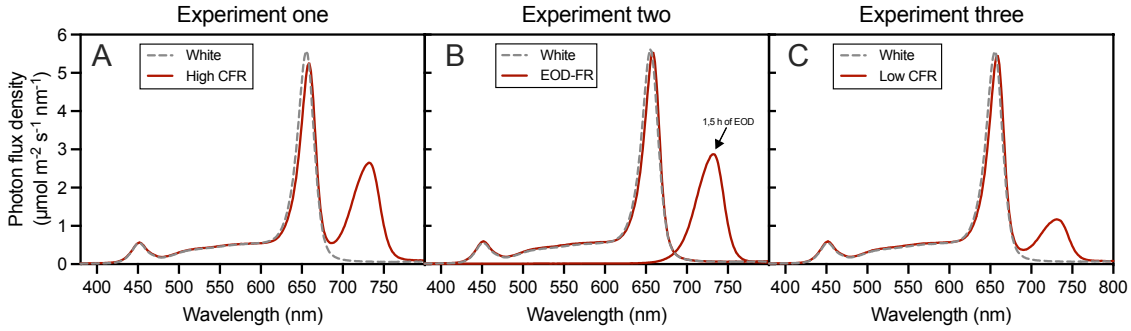

Figure 3.1.: Spectral distribution of lighting treatments in all experiments with white light (white) or white light in combination with supplemental far-red as measured at the top of the canopy ($n=5$). Supplemental far-red treatments were high intensity continuous far-red (high CFR) in experiment one (A), 1.5 h of end-of-day far-red (EOD-FR) in exp. two (B), and low intensity continuous far-red (low CFR) in exp. three (C).

Table 3.1.: Photosynthetic photon flux density (PPFD; 400–700 nm), photon flux density (PFD) of far-red (700–800 nm), red/far-red ratio (R/FR), phytochrome photoequilibrium (PPE), and daily light integral (DLI) of lighting treatments in all experiments with white light (white) or white light in combination with supplemental far-red as measured at the top of the canopy. Supplemental far-red treatments were high intensity continuous far-red (high CFR) in experiment one, 1.5 h of end-of-day far-red (EOD-FR) in exp. two, and low intensity continuous far-red (low CFR) in exp. three. Values are means ± 1 SEM ($n=5$).

Experiment	Treatment	PPFD (μmol m^{-2} s^{-1})	Far-red (μmol m^{-2} s^{-1})	R/FR-ratio[1]	PPE[2]	DLI[3] (mol m^{-2} d^{-1})
Exp. one	White	240.9 ± 2.0	7.1 ± 0.1	62.7 ± 1.0	0.86	15.6 ± 0.1
	High CFR	239.6 ± 2.2	109.1 ± 2.8	1.7 ± 0.0	0.74	15.5 ± 0.1
Exp. two	White	243.7 ± 2.2	7.2 ± 0.1	62.3 ± 0.9	0.86	15.8 ± 0.1
	EOD-FR[4]	16.3 ± 0.2	113.2 ± 0.8	0.1 ± 0.0	0.21	15.7 ± 0.2
Exp. three	White	242.0 ± 2.3	7.6 ± 0.3	59.8 ± 1.7	0.86	15.7 ± 0.1
	Low CFR	242.6 ± 2.9	51.5 ± 0.9	3.94 ± 0.1	0.81	15.7 ± 0.2

[1]For the calculation of R/FR-ratios, PFD was integrated over 20 nm intervals for red (650–670 nm) and far-red (720–740 nm).
[2]SEM was <0.01.
[3]DLI was calculated as the integral of the mean photosynthetic photon flux density (400–700 nm) for the duration of 18-h photoperiods.
[4]Values represent light conditions only during end-of-day treatment (except DLI) as conditions during photoperiods were similar to that of the white light treatment.

3.2.2 Experiment two — end-of-day far-red

In experiment two, plants were also subjected to one of two treatments: white light (white) or white light with 1.5 h of end-of-day far-red (EOD-FR) (Figure 3.1B). In the EOD-FR treatment, far-red light was provided with a 10 min overlap with white light to ensure a continuous light period with no dark interruption, thus resulting in solely far-red light for roughly 1 h and 20 min following the 18-h photoperiod. During the photoperiod, light conditions were similar between treatments, while PPE decreased from 0.86 to 0.21 under

EOD-FR (Table 3.1). For the duration of the experiment, air temps. were 22.8 ± 0.2°C and 22.8 ± 0.1°C for the white and EOD-FR treatment chambers, respectively, while RHs were 75.1 ± 1.1% and 74.9 ± 1.2%, respectively.

3.2.3 Experiment three — low intensity continuous far-red

In experiment three, plants were again subjected to one of two treatments: white light (white) or white light with supplemental low intensity continuous far-red (low CFR) for the entire duration of the photoperiod (Figure 3.1C). In the low CFR treatment, far-red light constituted only *ca.* 18% of the TPFD and PPE was 0.81 (Table 3.1).

During the experiment, air temps. were 22.7 ± 0.2°C for both treatment chambers. RHs were 75.1 ± 1.4% and 74.8 ± 1.2% in the white and low CFR treatment chambers, respectively.

3.3 Plant growth measurements

Six plants per cultivar per treatment were randomly selected for destructive harvest after three and ten days of treatment. For each plant, shoot length was measured with a ruler and as the length from the base of the stem to the top of the stem or petiole, whichever was greatest. Additionally, number of true leaves (≥ 3 cm in length) was counted, and total leaf area (including cotyledons) was determined using an area meter (LI-3100 Area Meter, LI-COR Biosciences, Lincoln, NE, USA). Stem, petioles and leaf materials were dried separately in a drying cabinet at 70°C for at least four days before measuring dry mass (DM).

3.4 Growth analysis

The dried plant material harvested at three and ten days after treatment start (DAT) were used to calculate the relative growth rate (RGR; d^{-1}), net assimilation rate (NAR; g m^{-2} d^{-1}), leaf area ratio (LAR; cm^{-2} g^{-1}), specific leaf area (SLA; cm^{-2} g^{-1}), and leaf mass ratio (LMR; g g^{-1}) using the equations (Radford, 1967; Shibuya *et al.*, 2016):

$$\text{RGR} = \frac{ln(W_2/W_1)}{t_2 - t_1} = \text{NAR} \times \text{LAR} \tag{3.1}$$

$$\text{NAR} = \frac{W_2 - W_1}{A_2 - A_1} \times \frac{ln(A_2) - ln(A_1)}{t_2 - t_1} \tag{3.2}$$

$$\text{LAR} = \frac{A_2 - A_1}{ln(A_2) - ln(A_1)} \times \frac{ln(W_2) - ln(W_1)}{W_2 - W_1} = \text{SLA} \times \text{LMR} \tag{3.3}$$

$$\text{SLA} = \frac{A_2 - A_1}{ln(A_2) - ln(A_1)} \times \frac{ln(L_2) - ln(L_1)}{L_2 - L_1} \tag{3.4}$$

$$\text{LMR} = \frac{L_2 - L_1}{ln(L_2) - ln(L_1)} \times \frac{ln(W_2) - ln(W_1)}{W_2 - W_1}, \tag{3.5}$$

where W_2 and W_1 are total above-ground DM at times t_2 and t_1 (ten and three DAT, respectively), A_2 and A_1 are the corresponding total leaf areas, and L_2 and L_1 are the corresponding leaf DM. Prior to analysis, replicates within the same treatment $\times$ cultivar were paired across harvest times by sorting total above-ground DM from low to high.

3.5 Leaf gas exchange measurements

After subjecting seedlings to nine days of treatment, photosynthetic light responses to determine the rate of CO_2 exchange, stomatal conductance, intercellular CO_2 concentration, transpiration rate, and electron transport rate at six PPFD levels were measured on the second youngest fully expanded true leaf of four randomly selected plants per cultivar per treatment ($n = 4$ per cultivar per treatment) using a portable infrared gas analyser (LI-6400 XT Portable Photosynthesis System, LI-COR Biosciences) connected to a 2 cm^2 leaf chamber fluorometer (6400-40. LI-COR Biosciences) with a built-in LED light source (providing 90% red light (R) and 10% blue light (B)). Light intensity was decreased in the following six steps of decrements: 600, 300, 150, 100, 50, and 0 μmol m^{-2} s^{-1}. Prior to recording measurements, leaves were placed inside the leaf chamber at a PPFD of 600 μmol m^{-2} s^{-1} until they reached steady-state. During measurements, the entire system was placed wholly inside the growth chamber. Measurements were carried out between 6 and 14 hours after beginning of the photoperiod.

For the measurements, air flow was set to 300 μmol s^{-1}, reference CO_2 concentration was maintained at 415 μmol CO_2 (mol air)$^{-1}$, and the leaf chamber block temperature at 23°C.

RH was kept at chamber conditions, averaging 69.3 ± 4.2% inside the leaf chamber during measurements. Measurements were performed using the auto-programme LightCurve2 with one recording per light intensity level, a minimum wait time of 90 s and a maximum wait time of 150 s before recording, and whilst matching IRGAs before every change in light intensity. For measurements of steady state fluorescence, the measuring light was modulated at 0.25 kHz, the signal was filtered at 5 Hz, and the gain factor was set to 10. For maximal fluorescence, the measuring light was instead modulated at 20 kHz whilst filtering at 50 Hz and using rectangular saturating flashes of *ca.* 10 400–10 800 μmol m^{-2} s^{-1}.

Electron transport rate (ETR) was calculated following the LI-6400 XT infrared gas analyser user manual (LI-COR Biosciences, 2012) using the equation:

$$\text{ETR} = (\frac{F_m{}' - F_s}{Fm'}) \times f \times I \times \alpha_{\text{leaf}}, \tag{3.6}$$

where $F_m{}'$ is maximal fluorescence, F_s is 'steady-state' fluorescence, f is the factor for photosystem partitioning to PSII (assumed to be 0.5), I is incident light, and α_{leaf} is leaf absorbance. Leaf absorbance was estimated based on assumptions of 87% absorption of red light and 92% absorption of blue light, whilst correcting for the proportions used in measuring (here, red:blue of 9:1), which varied only very slightly. Hence, leaf absorbance was estimated to *ca.* 87.5%.

3.5.1 Light response curves and apparent quantum yield

Based on net photosynthesis (P_{N}) light response data from leaf gas exchange measurements, photosynthetic light response (P_{N}/I) curves for each replicate were fitted using a non-rectangular hyperbola-based model (Prioul & Chartier, 1977) following the procedure of Lobo *et al.* (2013):

$$P_{\text{N}} = \frac{\phi_{(I_0)} \times I + P_{\text{gmax}} - \sqrt{(\phi_{(I_0)} \times I + P_{\text{gmax}})^2 - 4\theta \times \phi_{(I_0)} \times I \times P_{\text{gmax}}}}{2\theta} - R_{\text{D}}, \tag{3.7}$$

where P_{N} is net photosynthesis, $\phi(I_0)$ is the apparent quantum yield at a PPFD of 0, I is incident PPFD, P_{gmax} is the asymptotic estimate of the maximum gross photosynthetic rate, θ is convexity, and R_{D} is dark respiration. In short, $\phi(I_0)$, P_{gmax}, θ, and R_{D} were estimated using non-linear optimisation to minimise the sum of the squares of the errors for the fitted function in Microsoft Excel.

Based on the estimated parameters for the function above, the apparent quantum yield [μmol CO_2 m^{-2} s^{-1} per incident photon] (AQY) was calculated as the slope of a linear regression line of estimated P_N at PPFDs from 0 until 150 μmol m^{-2} s^{-1} for each replicate. AQYs were estimated based on these PPFDs values as, within these values, P_N/I curves displayed linear responses, and it avoided inflation resulting from decreased respiration in light compared to darkness.

3.6 Chlorophyll and carotenoid extraction and concentrations

At the final destructive harvest (after ten days of treatment), two leaf discs were cut randomly from the second youngest fully developed true leaf from each plant using a 8 mm diameter cork borer. Each leaf disc (0.503 cm^2) was then immediately suspended in 1.5 mL of dimethylsulfoxide (DMSO) magnesium carbonate solution in Eppendorf tubes and kept away from sunlight. Samples were subsequently cold-stored in the dark for 72 h to allow for extraction of chlorophylls and carotenoids. Prior to measurement, the samples were centrifuged for two min at 8200 rpm. For each sample, 1 mL of extract was transferred to a cuvette and absorbance was measured at 665, 649, 480, and 750 nm (for chl *a*, chl *b*, carotenoids, and background noise, respectively) using a scanning spectrophotometer (UV-1800. Shimadzu Corporation, Kyoto, Japan) with DMSO as a reference.

Chl *a*, chl *b* and total carotenoid concentrations were calculated following the equations of Wellburn (1994) for a 1-4 nm resolution spectrophotometer whilst correcting for background noise (absorbance at 750 nm) in each absorbance value:

$$C_a = 12.19A_{665} - 3.45A_{649} \tag{3.8}$$

$$C_b = 21.99A_{649} - 5.32A_{665} \tag{3.9}$$

$$C_{x+c} = \frac{(1000A_{480} - 2.14C_a - 70.16C_b)}{220}, \tag{3.10}$$

where A are absorbances at the respective wavelengths, C_a is chl *a* concentration, C_b is chl *b* concentration, and C_{x+c} is total carotenoid concentration. In order to avoid pseudoreplication, the mean of the two leaf discs for each plant were used in further analysis.

3.7 Data analysis

Data were analysed in GRAPHPAD PRISM 9.3.1 (GraphPad Software, San Diego, CA, USA) for macOS at significance levels of $p < 0.05$, unless otherwise stated. For all data, homogeneity of variances were tested using the Brown-Forsythe test for equal variances. Normality of residuals were checked by inspection of Q-Q plots and tested with Shapiro-Wilk and D'Agostino-Pearson omnibus K2 tests of normality. Two-ways ANOVA with treatment and cultivar as fixed factors were performed to determine their effects on morphology, growth, gas exchange, apparent quantum yield, and chlorophyll and carotenoid concentrations. When there were significant effects, *post hoc* Tukey's honestly significant difference (HSD) tests were carried out to identify significant differences between treatments $\times$ cultivars. In the case of experiment one, where there were unequal sample sizes for morphological and growth measurements due to plant damage, the Tukey-Kramer method was used. When residuals were not normally distributed, and no transformation could restore normality, cultivars or treatments were grouped and non-parametric Mann-Whitney U-tests were performed to determine treatment and cultivar effects. If variances were not equal, but residuals were normally distributed, cultivars or treatments were grouped and Welch's t-tests were carried out to determine effects. Relationships between selected parameters were analysed using simple linear regression.

4 Results

4.1 Experiment one — high intensity continuous far-red

4.1.1 Shoot length, leaf area expansion, and leaf count

Shoot length of both cultivars increased significantly when seedlings were subjected to supplemental high CFR compared to solely white light (Figure 4.1A, Tukey's HSD test, $p < 0.05$). Whilst there were no significant differences between the two cultivars, there was a significant treatment $\times$ cultivar interaction (two-way ANOVA, $p < 0.05$). In general, treatment with high CFR increased shoot length approximately 2.7-fold and 3.2-fold for 'Hi Light' and 'Imea', respectively, compared to white light treatment.

Total leaf area was affected significantly by treatment and, to a lesser degree, cultivar (Figure 4.1B, two-way ANOVA, $p < 0.05$). In particular, total leaf area increased by *ca.* 40 and 35% for 'Hi Light' and 'Imea', respectively, following high CFR relative to white light treatment. Under both treatments, 'Imea' tended to have greater total leaf area than 'Hi Light'. Furthermore, number of true leaves (≥ 3 cm) increased significantly with high CFR treatment (Figure 4.1C, two-way ANOVA, $p < 0.01$).

4.1.2 Relative growth rate and other growth components

Relative growth rates (RGRs) of 'Hi Light' and 'Imea' were both affected significantly by treatment, but overall growth did not differ between the two cultivars (Figure 4.2A, two-way ANOVA, $p < 0.05$). In general, RGRs increased 15 and 18% for plants of 'Hi Light' and 'Imea', respectively, treated with high CFR relative to white light treatment.

Both treatment and cultivar affected net assimilation rate (NAR) significantly (Figure 4.2B, two-way ANOVA, $p < 0.001$). Overall, 'Imea' had lower NARs under both treatments com-

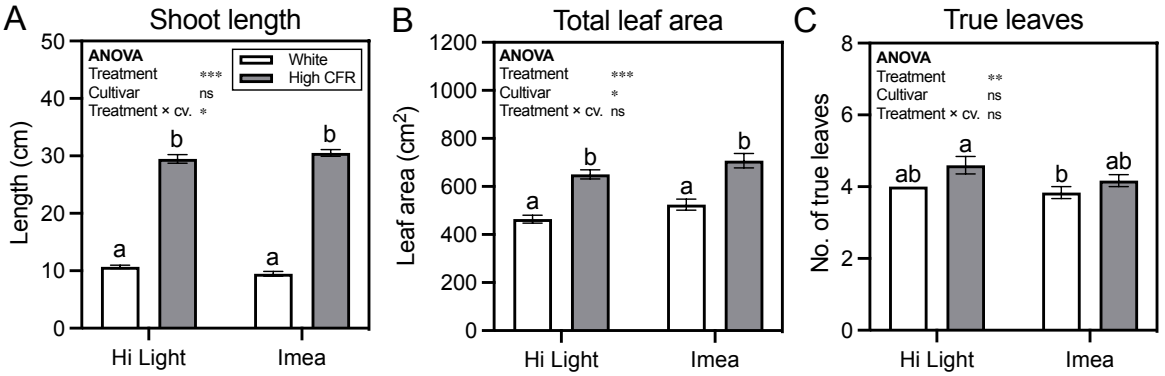

Figure 4.1.: Shoot length (A), total leaf area (B), and no. of true leaves (≥ 3 cm) (C) per plant in cucumber 'Hi Light' and 'Imea' after ten days of treatment with white light (white) or white light with supplemental high intensity continuous far-red (high CFR). Data are means ± 1 SEM ($n = 6$, except for White×'Hi Light' where $n = 5$). Asterisks denote significant differences tested by two-way ANOVA: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant. Different letters denote significant differences in means (Tukey's HSD test, $p < 0.05$).

pared to 'Hi Light'. Nonetheless, both cultivars generally increased NARs by 34–36% as a result of high CFR.

Similarly, leaf area ratio (LAR) was affected by both treatment and cultivar (Figure 4.2C, two-way ANOVA, $p < 0.001$). In contrast to NAR, LAR showed an opposite response to far-red light. Specifically, high CFR lead to significantly reduced LARs in both cultivars, which were just 86–87% of the values under white light treatment (Tukey's HSD test, $p < 0.05$). At the same time, 'Imea' had greater LARs than 'Hi Light' within treatments. Specific leaf area (SLA) was affected significantly by both treatment and cultivar, and, on the whole, high CFR reduced SLA by 3–4%, potentially indicating an increase in leaf thickness (Figure 4.2D, two-way ANOVA, $p < 0.01$). 'Imea' generally had higher SLAs than 'Hi Light' (Tukey's HSD test, $p < 0.05$) but differences between treatments were minor. In addition, whilst leaf mass ratios (LMRs) did not differ significantly between 'Hi Light' and 'Imea', it was, in both cultivars, reduced significantly by high CFR (Figure 4.2E, Mann-Whitney U-tests, $p < 0.05$). Hence, the reduction in LARs under high CFR can largely be attributed to decreased LMRs.

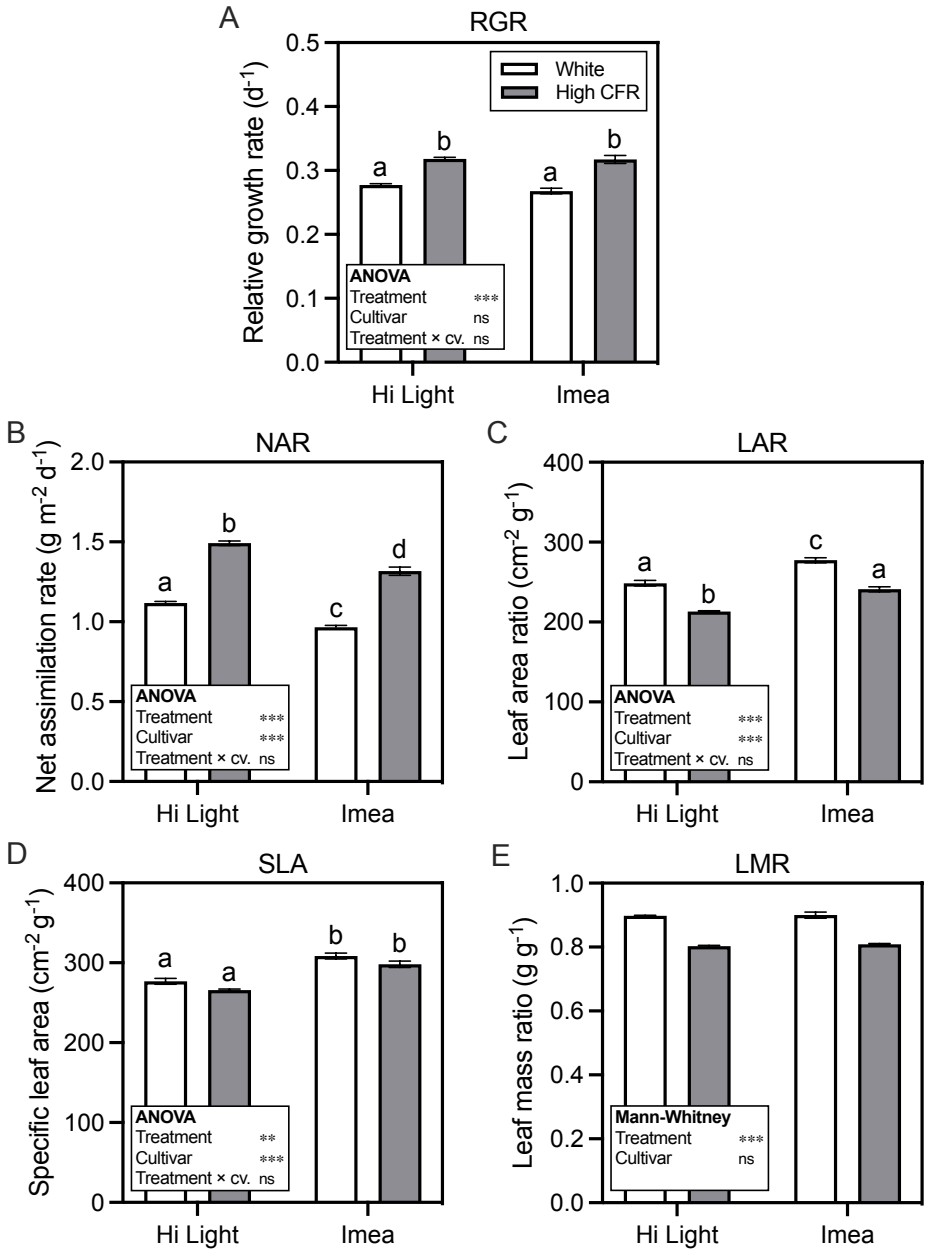

Figure 4.2.: Effects of adding high intensity continuous far-red (high CFR) to white light (white) and cultivar on relative growth rate (RGR) (A), net assimilation rate (NAR) (B), leaf area ratio (LAR) (C), specific leaf area (SLA) (D), and leaf mass ratio (LMR) (E) in seedlings of cucumber 'Hi Light' and 'Imea'. Data are means ± 1 SEM ($n=6$, except for White×'Hi Light' where $n=5$). Asterisks denote significant differences tested by two-way ANOVA: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ns, not significant. Different letters denote significant differences in means (Tukey's HSD test, $p<0.05$). For LMR, residuals were not distributed normally, hence cultivars or treatments were grouped and Mann-Whitney U-tests were performed to determine treatment and cultivar effects.

4.1.3 Leaf CO_2 exchange responses

In terms of photosynthetic light response measurements, there were significant differences in P_N between treatments at PPFDs near light saturation (as the P_N/I response curves begin to tail off, Figure 4.3) and near darkness (Table 4.1, two-way ANOVA, $p < 0.05$). Specifically, P_N at a PPFD of 600 μmol m^{-2} s^{-1} was *ca.* 26–27% greater in plants subjected to high CFR relative to white light treatment. At light intensities of 100 and 150 μmol m^{-2} s^{-1}, there were similar tendencies for plants subjected to high CFR to have greater P_N but differences were not significant (two-way ANOVAs, $p > 0.05$). Furthermore, dark respiration was significantly greater in seedlings treated with white light than in those subjected to high CFR (i.e., P_N at a PPFD of 0 μmol m^{-2} s^{-1} was more negative under white light treatment; two-way ANOVA, $p < 0.05$).

P_N also differed significantly between cultivars but only at a PPFD of 600 μmol m^{-2} s^{-1} (Table 4.1, two-way ANOVA, $p < 0.01$). Here, P_N was 22–23% higher in 'Hi Light' when compared to 'Imea' within treatments.

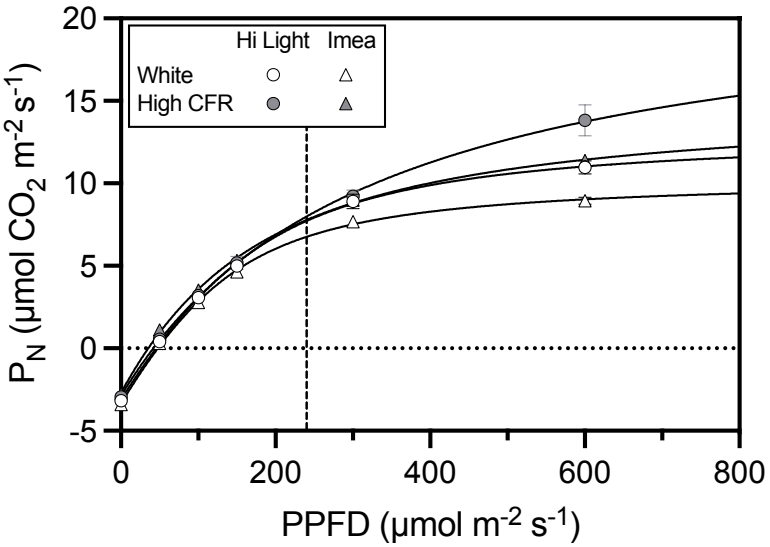

Figure 4.3.: Single leaf photosynthetic light response in seedlings of cucumber 'Hi Light' and 'Imea' as measured after nine days of treatment with white light (white) or white light with supplemental high intensity continuous far-red (high CFR). Net photosynthesis (P_N) was measured on the second youngest fully expanded true leaf at six levels of photosynthetic photon flux density (0, 50, 100, 150, 300, and 600 μmol m^{-2} s^{-1}) using an infrared gas analyser system connected to a leaf chamber fluorometer with a built-in light source (90% R, 10% B light). Data are means ± 1 SEM ($n = 4$). Vertical line shows mean PPFD in growth chamber conditions for reference (*ca.* 240 μmol m^{-2} s^{-1}). Curves were fitted based on mean light response data of treatment × cultivar using a non-rectangular hyperbola-based model (Prioul & Chartier, 1977).

Table 4.1.: Single leaf net photosynthesis (P_N) at six levels of photosynthetic photon flux density (PPFD) in seedlings of cucumber 'Hi Light' and 'Imea' as measured after nine days of treatment with white light (white) or white light with supplemental high intensity continuous far-red (high CFR). Leaf CO_2 exchange was measured on the second youngest fully expanded true leaf using an infrared gas analyser system connected to a leaf chamber fluorometer with a built-in light source (90% R, 10% B light). Values are means ± 1 SEM ($n=4$).

Cultivar	Treatment	P_N (μmol CO_2 m^{-2} s^{-1})					
PPFD (μmol m^{-2} s^{-1})		0[1]	50	100	150	300	600
Hi Light	White	-3.2a ± 0.2	0.4a ± 0.2	3.1a ± 0.3	5.0a ± 0.2	8.9ab ± 0.5	11.0ab ± 0.4
	High CFR	-3.0a ± 0.1	0.5a ± 0.3	3.2a ± 0.2	5.2a ± 0.4	9.2a ± 0.4	13.8c ± 1.0
Imea	White	-3.4a ± 0.2	0.3a ± 0.2	2.8a ± 0.2	4.6a ± 0.3	7.7b ± 0.3	9.0a ± 0.2
	High CFR	-2.8a ± 0.1	1.1a ± 0.1	3.5a ± 0.2	5.3a ± 0.2	9.0ab ± 0.3	11.4b ± 0.3
Treatment effect[2]		*	*	ns	ns	*	***
Cultivar effect		ns	ns	ns	ns	ns	**
Treatment × cultivar		ns	ns	ns	ns	ns	ns

[1]Within a column, different letters denote significant differences tested by Tukey's HSD test, $p<0.05$.
[2]Asterisks denote significant differences tested by two-way ANOVA: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ns, not significant.

Comparing differences in P_N to stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) measured at the same time does not point to either as the likely cause of the increased P_N near light saturation in high CFR treated seedlings (Figure 4.4A, B). In particular, differences in g_s and C_i between treatments but within cultivars were much smaller at a PPFD of 600 μmol m^{-2} s^{-1}, as well as not significant (Table A.1), than at 300 μmol m^{-2} s^{-1}, which is in contrast to the relationship found in P_N. Differences in g_s and C_i between treatments were in large part only noticeable for 'Hi Light' and not for 'Imea'. For instance, g_s at all levels of PPFD, except at 600 μmol m^{-2} s^{-1}, were significantly affected by both treatment and cultivar (Table A.1, two-way ANOVA, $p<0.05$), which could mainly be attributed to much greater values of high CFR × 'Hi Light'.

Transpiration rate was affected significantly by only cultivar at all measured levels of PPFD, except that of 600 μmol m^{-2} s^{-1} which was not significant (Table A.1, two-way ANOVA, $p<0.05$). Whilst transpiration rate, similar to g_s, increased up to 300 μmol m^{-2} s^{-1} and then declined, it was also coupled to large inter-replicate variation (Figure 4.4C).

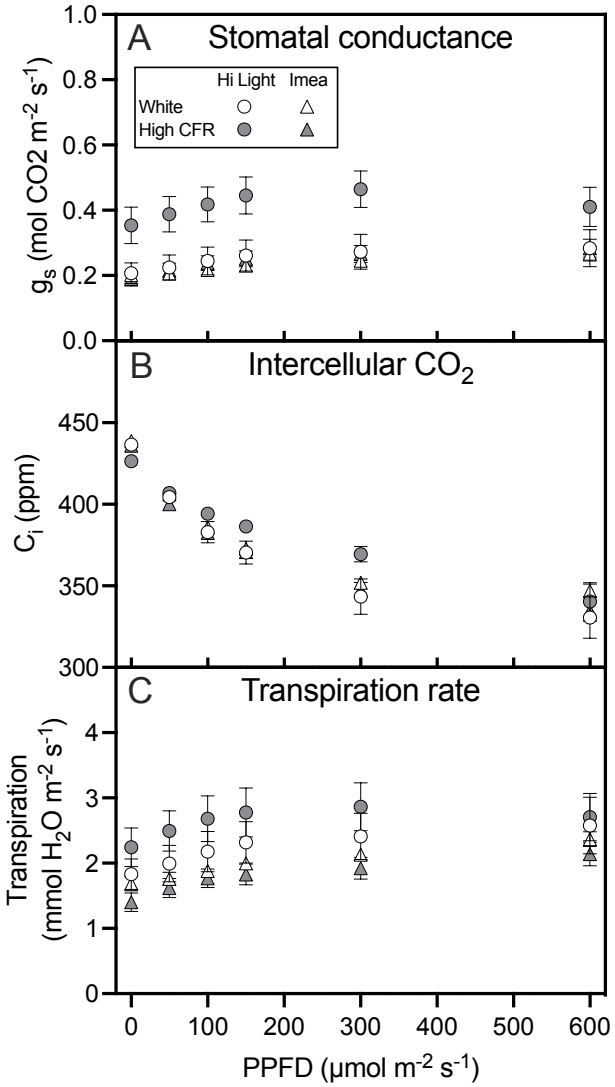

Figure 4.4.: Effects of adding high intensity continuous far-red (high CFR) to white light (white) and cultivar on stomatal conductance (A), intercellular CO_2 (B), and transpiration rate (C) in seedlings of cucumber 'Hi Light' and 'Imea'. Parameters were measured when establishing leaf photosynthetic light response curves after nine days of treatment and using an infrared gas analyser system connected to a leaf chamber fluorometer with a built-in light source (90% R, 10% B light). Data are means ± 1 SEM ($n=4$).

4.1.4 Electron transport rate and the relationship to net photosynthesis

As expected, ETR followed a similar relationship as the P_N/I response curve, where it tailed off as PPFD increased and leaves approached light saturation (Figure 4.5A). ETR also increased significantly with high CFR relative to white light treatment but this was only true at a PPFD of 600 μmol m^{-2} s^{-1} (Table A.1, two-way ANOVA, $p<0.05$). Moreover, ETR

was only greater for 'Hi Light' when compared to 'Imea' at 600 μmol m^{-2} s^{-1} (two-way ANOVA, $p < 0.05$).

When all treatments $\times$ cultivars were grouped, ETR was highly and linearly correlated with P_N ($r^2 = 0.97$; Figure 4.5B).

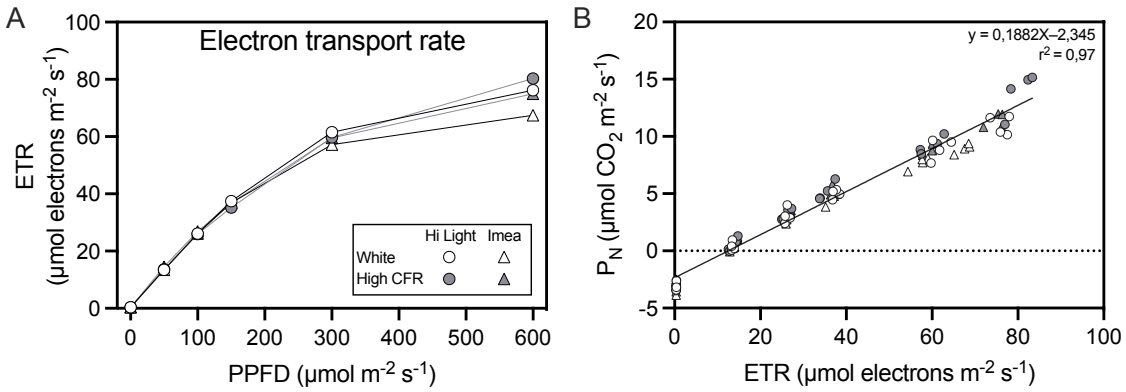

Figure 4.5.: Electron transport rate (ETR) (A) and the relationship between ETR and leaf net photosynthetic rate (B) in seedlings of cucumber 'Hi Light' and 'Imea' subjected to white light (white) or white light with supplemental high intensity continuous far-red (high CFR). Parameters were measured when establishing leaf photosynthetic light response curves after nine days of treatment and using an infrared gas analyser system connected to a leaf chamber fluorometer with a built-in light source (90% R, 10% B light). For ETR (A), data are means ± 1 SEM ($n = 4$). For P_N/ETR (B), each data point represent P_N and corresponding ETR value at six PPFDs from 0–600 μmol m^{-2} s^{-1} for each replicate. All data were grouped for linear regression.

4.1.5 Changes in leaf chlorophylls and carotenoids concentrations

Total chlorophylls and total carotenoids concentrations decreased significantly under treatment with high CFR when compared with white light treatment (Table 4.2, two-way ANOVA, $p < 0.001$). Under high CFR, total chlorophylls concentrations decreased with 19 and 17% for 'Hi Light' and 'Imea', respectively, whilst total carotenoids decreased with 14 and 17%, respectively. But, as chl *a* declined relatively less than chl *b* in the case of both cultivars, this resulted in greater chl *a*/*b*-ratios under high CFR compared to white light treatment (two-way ANOVA, $p < 0.05$).

Furthermore, total chlorophylls, chl *a*/*b*-ratio, and total carotenoids concentrations were also affected by cultivar (two-way ANOVA, $p < 0.05$). Within treatments, 'Hi Light' had generally significantly greater total chlorophylls and total carotenoids concentrations than

'Imea' (Tukey's HSD test, $p < 0.05$). In contrast, chl *a*/*b*-ratio was significantly greater in 'Imea' than 'Hi Light' (two-way ANOVA, $p < 0.05$).

Table 4.2.: Effects of adding high intensity continuous far-red (high CFR) to white light (white) and cultivar on total chlorophylls concentration, chl *a*/*b*-ratio, and total carotenoids concentration in the second youngest fully expanded true leaves of cucumber 'Hi Light' and 'Imea' seedlings. Values are means ± 1 SEM ($n = 6$, except for White×'Hi Light' where $n = 5$).

Cultivar	Treatment	Total chlorophylls[1] (μg cm^{-2})	Chl *a*/*b*-ratio	Total carotenoids (μg cm^{-2})
Hi Light	White	39.95a ± 0.74	3.50a ± 0.05	6.58a ± 0.18
	High CFR	32.31b ± 0.55	3.67ab ± 0.05	5.64bc ± 0.07
Imea	White	34.35b ± 0.77	3.68b ± 0.07	6.18ab ± 0.15
	High CFR	28.40c ± 0.74	3.80b ± 0.07	5.16c ± 0.09
Treatment effect[2]		***	*	***
Cultivar effect		***	*	**
Treatment × cultivar		ns	ns	ns

[1]Within a column, different letters denote significant differences tested by Tukey's HSD test, $p < 0.05$.
[2]Asterisks denote significant differences tested by two-way ANOVA: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant.

4.2 Experiment two — end-of-day far-red

4.2.1 Shoot length, leaf area expansion, and leaf count

In experiment two, shoot length was affected significantly only by treatment (Figure 4.6A, two-way ANOVA, $p < 0.001$). Seedlings subjected to EOD-FR generally increased shoot length by 87–89% relative to white light treatment.

Total leaf areas were also significantly greater under EOD-FR (Figure 4.6B, Tukey's HSD test, $p < 0.05$). Specifically, total leaf areas ranged from 40% greater in 'Hi Light' to 29.1% greater in 'Imea' under EOD-FR compared to plants grown under solely white light. However, the total leaf area was also significantly greater in 'Imea' than 'Hi Light' when compared within treatments (Tukey's HSD test, $p < 0.05$). The number of true leaves were similar in both cultivars but it also increased following EOD-FR when compared to white light treatment (Figure 4.6C, two-way ANOVA, $p < 0.001$).

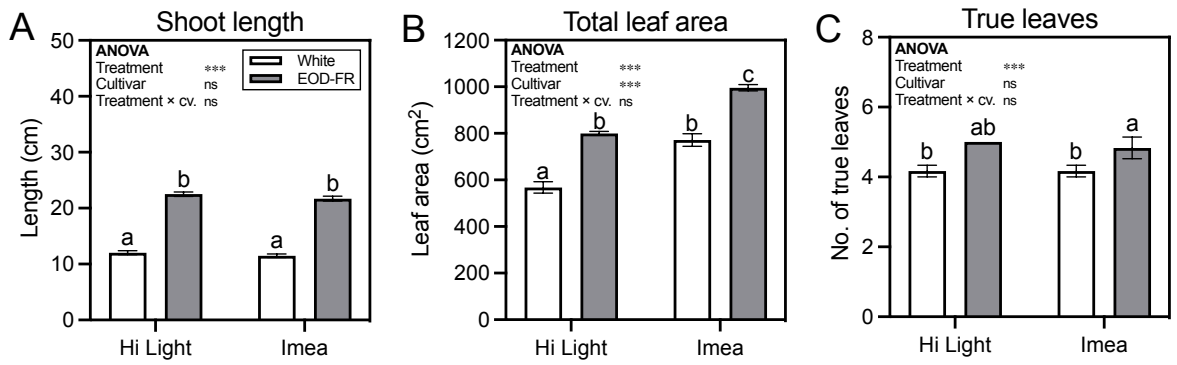

Figure 4.6.: Shoot length (A), total leaf area (B), and no. of true leaves (≥ 3 cm) (C) per plant of cucumber 'Hi Light' and 'Imea' after ten days of treatment with white light (white) or white light with supplemental 1.5 h of end-of-day far-red (EOD-FR). Data are means $\pm$ 1 SEM ($n = 6$). Asterisks denote significant differences tested by two-way ANOVA: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant. Different letters denote significant differences in means (Tukey's HSD test, $p < 0.05$).

4.2.2 Relative growth rate and other growth components

When seedlings of both cultivars were subjected to EOD-FR, RGRs increased significantly compared to white light (Figure 4.7A, two-way ANOVA, $p < 0.001$). Whilst the relative response was slightly greater in 'Hi Light' than in 'Imea' (15 and 12% increase relative to white light treatment, respectively), there were no significant differences between the two cultivars (two-way ANOVA, $p > 0.05$).

Contrastingly, NAR was affected significantly by both treatment and cultivar (Figure 4.7B, two-way ANOVA, $p < 0.001$). NAR increased similarly in response to EOD-FR treatment in both cultivars (16 and 17% for 'Imea' and 'Hi Light', respectively), but NAR was generally greater in 'Hi Light' than in 'Imea' when compared within treatments (Tukey's HSD test, $p < 0.05$).

Conversely, LAR was greater in 'Imea' than in 'Hi Light' (Figure 4.7C, Tukey's HSD test, $p < 0.05$). But, in both cultivars, LARs declined only slightly under EOD-FR treatment (two-way ANOVA, $p < 0.05$). The slight decline in LARs can be attributed to a moderate increase in SLAs (Figure 4.7D, two-way ANOVA, $p < 0.01$), which outweighed the lower LMRs across cultivars (Figure 4.7E, two-way ANOVA, $p < 0.001$).

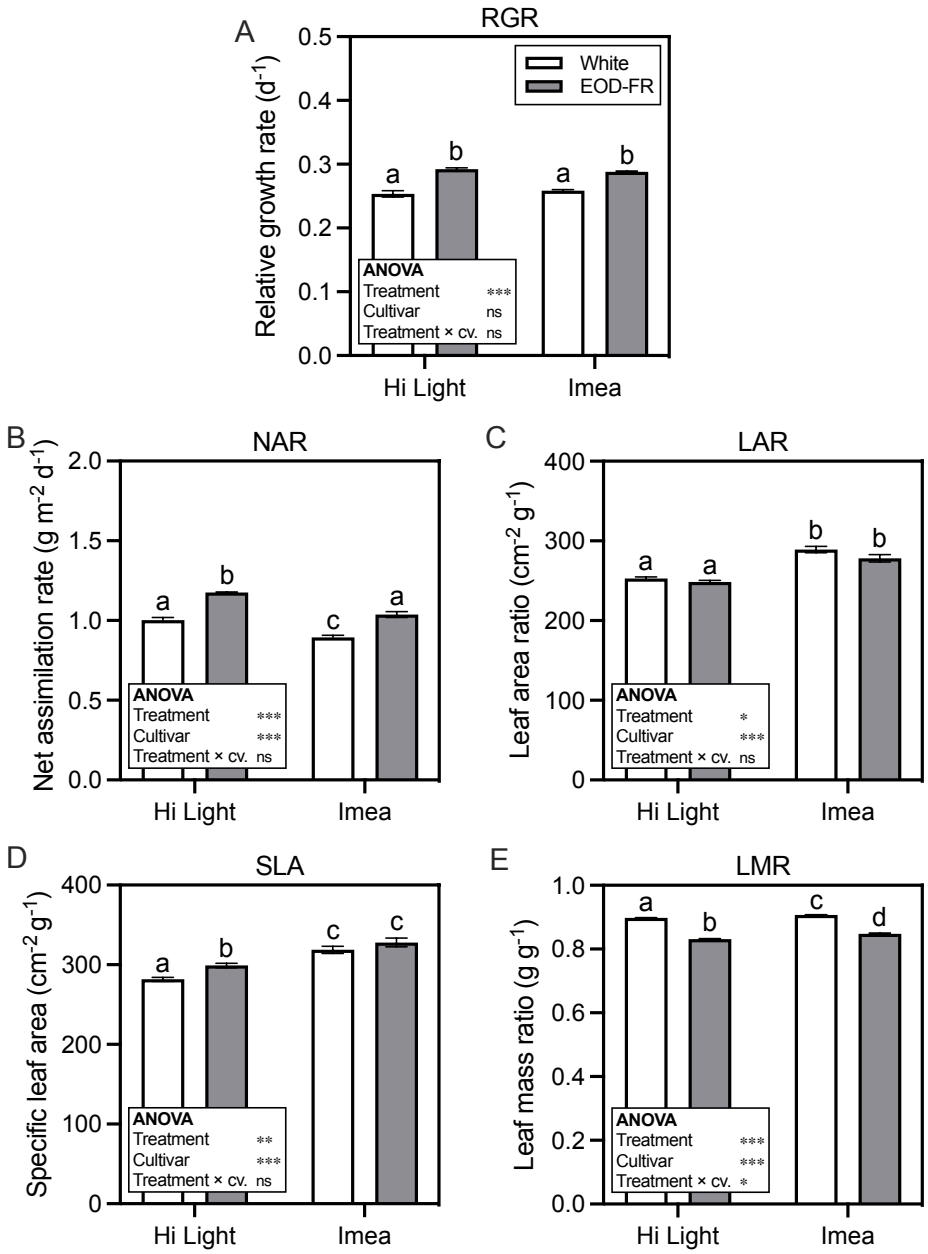

Figure 4.7.: Effects of adding 1.5 h of end-of-day far-red (EOD-FR) to white light (white) and cultivar on relative growth rate (RGR) (A), net assimilation rate (NAR) (B), leaf area ratio (LAR) (C), specific leaf area (SLA) (D), and leaf mass ratio (LMR) (E) in seedlings of cucumber 'Hi Light' and 'Imea'. Data are means ± 1 SEM ($n=6$). Asterisks denote significant differences tested by two-way ANOVA: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ns, not significant. Different letters denote significant differences in means (Tukey's HSD test, $p<0.05$).

4.2.3 Leaf CO_2 exchange responses

In large part, leaf photosynthetic light response did not differ significantly between treatments (two-way ANOVAs, $p>0.05$, Table 4.3). In contrast to the previous experiment,

seedlings of 'Imea' treated with white light tended to have greater P_N at high light intensities than those treated with EOD-FR but differences were largely negligible and not significant (Table 4.3, two-way ANOVAs, $p > 0.05$). At the three highest levels of PPFD, 'Hi Light' had significantly greater P_N than 'Imea', which ranged from 29 to 50% greater in 'Hi Light' relative to 'Imea' at 600 μmol m^{-2} s^{-1} (two-way ANOVAs, $p < 0.01$). In general, the fitted mean P_N/I response curves of all four treatment $\times$ cultivar began to tail off at relatively low PPFDs (Figure 4.8).

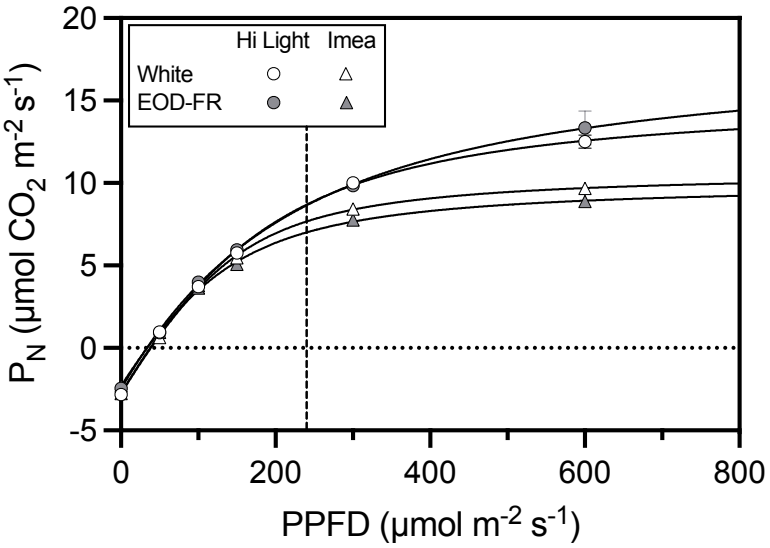

Figure 4.8.: Single leaf photosynthetic light response in seedlings of cucumber 'Hi Light' and 'Imea' as measured after nine days of treatment with white light (white) or white light with 1.5 h of end-of-day far-red (EOD-FR). Net photosynthesis (P_N) was measured on the second youngest fully expanded true leaf at six levels of photosynthetic photon flux density (0, 50, 100, 150, 300, and 600 μmol m^{-2} s^{-1}) using an infrared gas analyser system connected to a leaf chamber fluorometer with a built-in light source (90% R, 10% B light). Data are means ± 1 SEM ($n = 4$). Vertical line shows mean PPFD in growth chamber conditions for reference (*ca.* 240 μmol m^{-2} s^{-1}). Curves were fitted based on mean light response data of treatment $\times$ cultivar using a non-rectangular hyperbola-based model (Prioul & Chartier, 1977).

g_s and C_i measured simultaneously as P_N did not reflect similar relationships to that found in P_N light responses (Figure 4.9A, B). Whilst not significant, seedlings of 'Hi Light' subjected to EOD-FR had a tendency to have much larger values of g_s than those under white light treatment (Table A.2, Tukey's HSD tests, $p > 0.05$). Additionally, there were significant effects of cultivar on g_s at all PPFDs, except at 600 μmol m^{-2} s^{-1} (Table A.2). However, these differences between cultivars were only evident within the EOD-FR treatment, unlike the differences found in P_N.

Table 4.3.: Single leaf net photosynthesis (P_N) at six levels of photosynthetic photon flux density (PPFD) in seedlings of cucumber 'Hi Light' and 'Imea' as measured after nine days of treatment with white light (white) or white light with 1.5 h of end-of-day far-red (EOD-FR). Leaf CO_2 exchange was measured on the second youngest fully expanded true leaf using an infrared gas analyser system connected to a leaf chamber fluorometer with a built-in light source (90% R, 10% B light). Values are means ± 1 SEM ($n=4$).

Cultivar	Treatment	P_N (μmol m^{-2} s^{-1})					
PPFD (μmol m^{-2} s^{-1})		0[1]	50	100	150	300	600[2]
Hi Light	White	-2.8a ± 0.1	1.0a ± 0.3	3.7a ± 0.2	5.8ab ± 0.2	10a ± 0.2	12.5a ± 0.4
	EOD-FR	-2.5a ± 0.1	0.9a ± 0.2	4.0a ± 0.1	6.0a ± 0.1	9.9a ± 0.2	13.3a ± 1.0
Imea	White	-2.8a ± 0.2	0.6a ± 0.3	3.8a ± 0.1	5.5ab ± 0.2	8.4b ± 0.3	9.7b ± 0.3
	EOD-FR	-2.3a ± 0.1	1.0a ± 0.1	3.6a ± 0.2	5.1b ± 0.2	7.8b ± 0.1	8.9b ± 0.3
Treatment effect[3]		*	ns	ns	ns	ns	ns
Cultivar effect		ns	ns	ns	**	***	***
Treatment × cultivar		ns	ns	ns	ns	ns	ns

[1]Within a column, different letters denote significant differences tested by Tukey's HSD test, $p<0.05$.
[2]Data were reciprocity transformed for testing in order to fulfill assumption of homogeneity of variances (Brown-Forsythe test, $p<0.05$).
[3]Asterisks denote significant differences tested by two-way ANOVA: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ns, not significant.

Transpiration rates followed the same relationship as g_s (Figure 4.9C). Comparably, transpiration was significantly affected by cultivar at all light intensities, except at 600 μmol m^{-2} s^{-1} (Table A.2), but this could also largely be attributed to differences within the EOD-FR treatment.

4.2.4 Electron transport rate and the relationship to net photosynthesis

Again, ETR increased with increasing PPFD in the same manner as P_N (Figure 4.10A), but there were largely no differences between treatments (Table A.2, two-way ANOVAs, $p>0.05$). However, ETR of 'Hi Light' was significantly greater than that of 'Imea' but only at PPFDs of 300 and 600 μmol m^{-2} s^{-1} (Tukey's HSD tests, $p<0.05$). ETR was also highly and linearly correlated with P_N ($r^2=0.98$; Figure 4.10B).

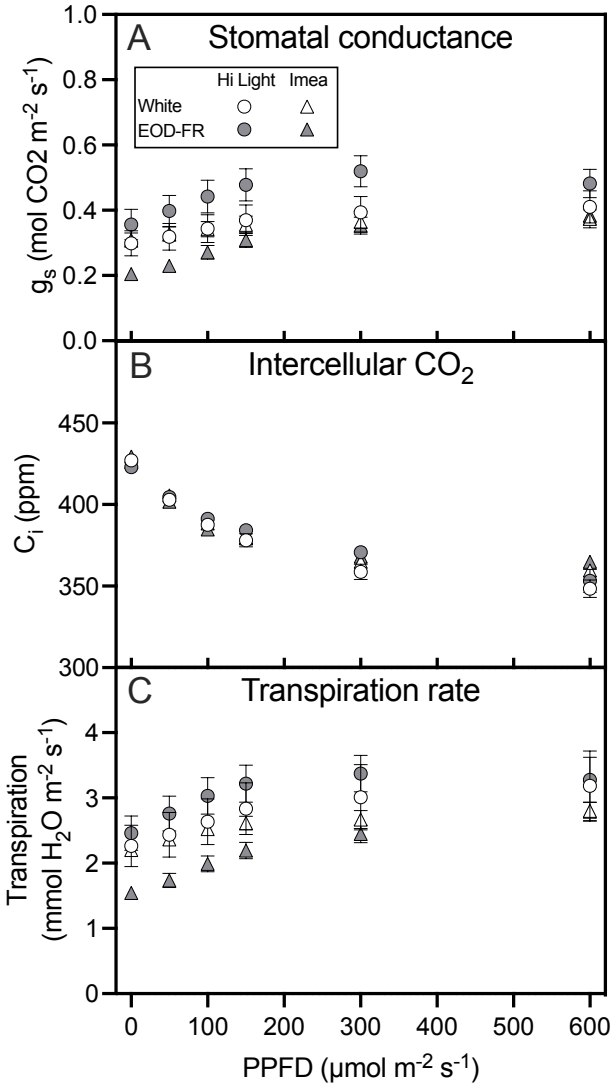

Figure 4.9.: Effects of adding 1.5 h of end-of-day far-red (EOD-FR) to white light (white) and cultivar on stomatal conductance (A), intercellular CO_2 (B), and transpiration rate (C) in cucumber 'Hi Light' and 'Imea'. Parameters were measured when establishing leaf photosynthetic light response curves after nine days of treatment and using an infrared gas analyser system connected to a leaf chamber fluorometer with a built-in light source (90% R, 10% B light). Data are means ± 1 SEM ($n=4$).

4.2.5 Changes in leaf chlorophylls and carotenoids concentrations

Overall, there were no significant effects of treatment or cultivar, nor any interaction between the two, in total chlorophylls, chl *a*/*b*-ratio or total carotenoids concentrations (Table 4.4, two-way ANOVAs, $p>0.05$). Seedlings of 'Imea' did, however, tend to decrease

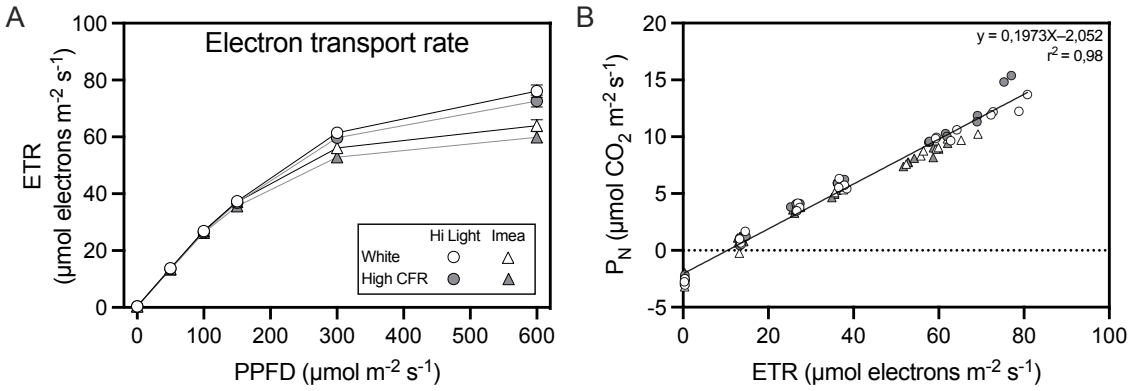

Figure 4.10.: Electron transport rate (ETR) (A) and the relationship between ETR and leaf net photosynthetic rate (B) in seedlings of cucumber 'Hi Light' and 'Imea' subjected to white light (white) or white light with 1.5 h of end-of-day far-red (EOD-FR). Parameters were measured when establishing leaf photosynthetic light response curves after nine days of treatment and using an infrared gas analyser system connected to a leaf chamber fluorometer with a built-in light source (90% R, 10% B light). For ETR (A), data are means ± 1 SEM ($n=4$). For P_N/ETR (B), each data point represent P_N and corresponding ETR value at six PPFDs from 0–600 μmol m^{-2} s^{-1} for each replicate. All data were grouped for linear regression.

in chlorophylls and carotenoids concentrations when subjected to EOD-FR but differences were not significant (two-way ANOVAs, $p>0.05$).

Table 4.4.: Effects of adding 1.5 h of end-of-day far-red (EOD-FR) to white light (white) and cultivar on total chlorophylls concentration, chl *a*/*b*-ratio, and total carotenoids concentration in the second youngest fully expanded true leaves of cucumber 'Hi Light' and 'Imea' seedlings. Values are means ± 1 SEM ($n=6$).

Cultivar	Treatment	Total chlorophylls (μg cm^{-2})	Chl *a*/*b*-ratio	Total carotenoids (μg cm^{-2})
Hi Light	White	36.70 ± 1.11	3.40 ± 0.04	6.24 ± 0.10
	EOD-FR	36.22 ± 2.46	3.49 ± 0.02	6.09 ± 0.32
Imea	White	35.97 ± 1.23	3.52 ± 0.06	6.15 ± 0.22
	EOD-FR	31.49 ± 0.87	3.51 ± 0.04	5.41 ± 0.15
Treatment effect[1]		ns	ns	ns
Cultivar effect		ns	ns	ns
Treatment × cultivar		ns	ns	ns

[1]Asterisks denote significant differences tested by two-way ANOVA: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ns, not significant. *Post-hoc* Tukey's HSD tests revealed no significant differences between treatment × cultivar in any of the measured parameters.

4.3 Experiment three — low intensity continuous far-red

4.3.1 Shoot length, leaf area expansion, and leaf count

In general, seedlings that were subjected to low CFR significantly increased in shoot length by a moderate 45–50% relative to plants of the white light treatment (Figure 4.11A, two-way ANOVA, $p < 0.001$).

Similarly, low CFR also resulted in significantly increased total leaf area by 11–15% dependent on cultivar (Figure 4.11B, Mann-Whitney U-test, $p < 0.05$). Moreover, total leaf area differed significantly between the two cultivars, which was generally 21–25% greater in 'Imea' than in 'Hi Light' (Mann-Whitney U-test, $p < 0.001$). Number of true leaves showed significant effects of both treatment and cultivar (Figure 4.11C, two-way ANOVA, $p < 0.05$). Generally, no. of true leaves increased with low CFR treatment when compared to white light treatment.

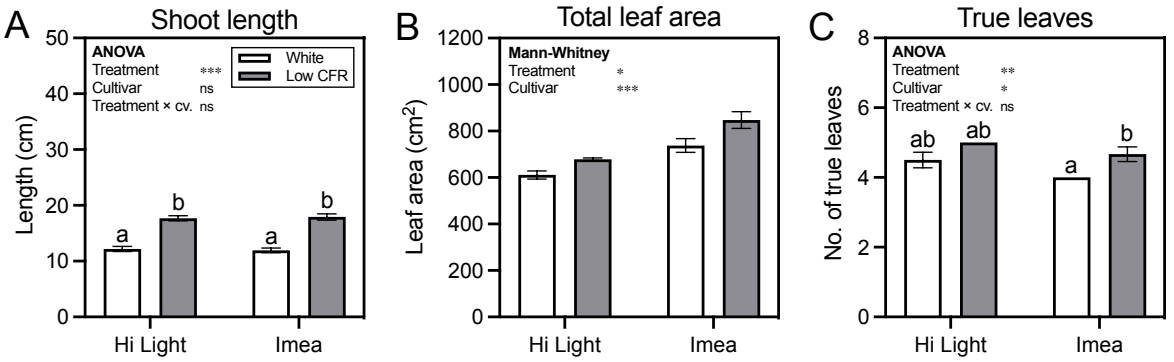

Figure 4.11.: Shoot length (A), total leaf area (B), and no. of true leaves (≥ 3 cm) (C) per plant of cucumber 'Hi Light' and 'Imea' after ten days of treatment with white light (white) or white light with supplemental low intensity continuous far-red (low CFR). Data are means ± 1 SEM ($n = 6$). Asterisks denote significant differences tested by two-way ANOVA: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant. Different letters denote significant differences in means (Tukey's HSD test, $p < 0.05$). For total leaf area, residuals were not distributed normally, hence cultivars or treatments were grouped and Mann-Whitney U-tests were performed to determine treatment and cultivar effects.

4.3.2 Relative growth rate and other growth components

Plant responses in regards to growth components were generally more moderate following low CFR than those observed in experiment one. Nonetheless, RGR was affected significantly by treatment and cultivar (Figure 4.12A, two-way ANOVA, $p < 0.01$). In general, RGRs increased by 8–10% when seedlings were subjected to low CFR compared to the corresponding white light treatment and differences between cultivars were overall minor.

NAR was also affected significantly by both treatment and cultivar (Figure 4.12B, two-way ANOVA, $p < 0.001$). For both cultivars, NARs increased by 14–15% following low CFR treatment when compared with white light treatment. Within treatments, NARs were significantly lower for 'Imea' than 'Hi Light' (Tukey's HSD test, $p < 0.05$).

Likewise, LARs varied significantly with treatment and cultivar (Figure 4.12C, two-way ANOVA, $p < 0.05$). LARs were greater for 'Imea' than 'Hi Light', but LARs nonetheless decreased in both as a result of low CFR treatment (Tukey's HSD test, $p < 0.05$). The reduction in LARs across cultivars following low CFR were primarily caused by reduction in LMRs (Figure 4.12D, Tukey's HSD test, $p < 0.05$), while SLAs remained unchanged (Figure 4.12E, two-way ANOVA, $p < 0.05$). Specifically for 'Imea', a lower NAR was generally compensated by a higher LAR than that observed in 'Hi Light', which ultimately resulted in RGRs of comparable magnitudes in the two cultivars.

4.3.3 Leaf CO_2 exchange responses

In general, P_N was affected significantly by treatment, as well as cultivar, only at high light intensities near light saturation (i.e., 300 and 600 μmol m^{-2} s^{-1}; Figure 4.13 and Table 4.5, two-way ANOVA, $p < 0.05$). Moreover, dark respiration was also affected significantly by treatment (Table 4.5, two-way ANOVA, $p < 0.05$). However, while seedlings subjected to low CFR treatment had a tendency to have greater dark respiration and P_N, differences were mostly not significant when tested by *post-hoc* multiple comparisons (Tukey's HSD test, $p > 0.05$).

Differences in P_N light response measurements were not consistent with those found in g_s and C_i across measured PPFDs (Figure 4.14A, B). Whilst there was a single significant effect of cultivars on C_i at a PPFD of 600 μmol m^{-2} s^{-1} (Table A.3, two-way ANOVA, $p < 0.05$), it was the opposite of that found in P_N. For g_s, there were rather consistent significant effects

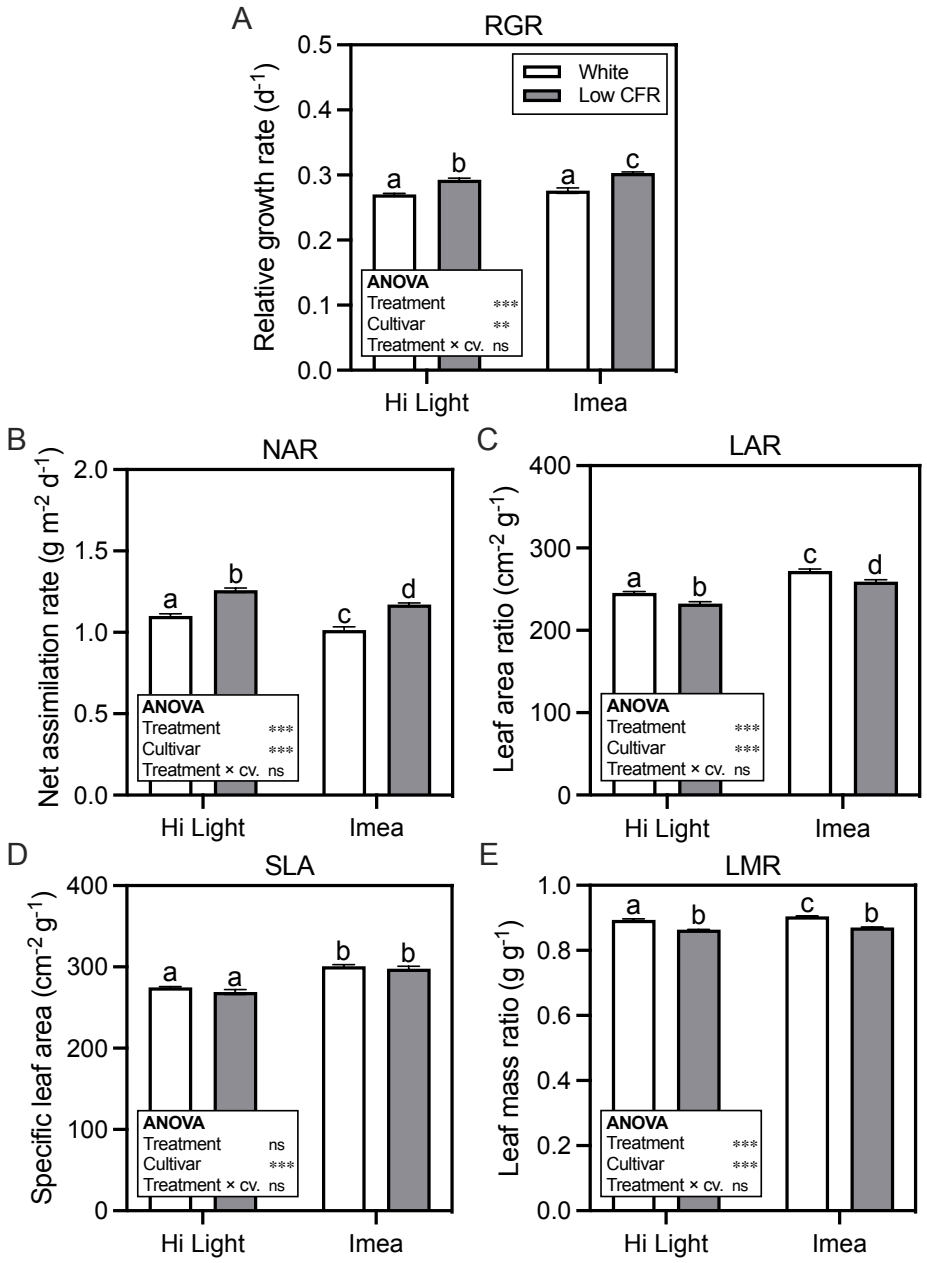

Figure 4.12.: Effects of adding low intensity continuous far-red (low CFR) to white light (white) and cultivar on relative growth rate (RGR) (A), net assimilation rate (NAR) (B), leaf area ratio (LAR) (C), specific leaf area (SLA) (D), and leaf mass ratio (LMR) (E) in seedlings of cucumber 'Hi Light' and 'Imea'. Data are means ± 1 SEM ($n=6$). Asterisks denote significant differences tested by two-way ANOVA: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant. Different letters denote significant differences in means (Tukey's HSD test, $p<0.05$).

of treatment at nearly all PPFDs (two-way ANOVAs, $p<0.05$), where g_s were greater for low CFR treatment.

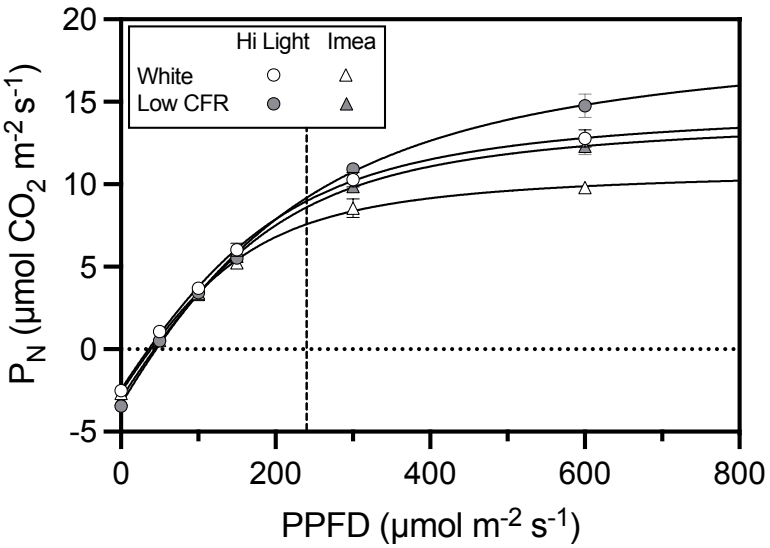

Figure 4.13.: Single leaf photosynthetic light response in seedlings of cucumber 'Hi Light' and 'Imea' as measured after nine days of treatment with white light (white) or white light with supplemental low intensity continuous far-red (low CFR). Net photosynthesis (P_N) was measured on the second youngest fully expanded true leaf at six levels of photosynthetic photon flux density (0, 50, 100, 150, 300, and 600 μmol m^{-2} s^{-1}) using an infrared gas analyser system connected to a leaf chamber fluorometer with a built-in light source (90% R, 10% B light). Data are means ± 1 SEM ($n=4$). Vertical line shows mean PPFD in growth chamber conditions for reference (*ca.* 240 μmol m^{-2} s^{-1}). Curves were fitted based on mean light response data of treatment × cultivar using a non-rectangular hyperbola-based model (Prioul & Chartier, 1977).

Table 4.5.: Single leaf net photosynthesis (P_N) at six levels of photosynthetic photon flux density (PPFD) in seedlings of cucumber 'Hi Light' and 'Imea' as measured after nine days of treatment with white light (white) or white light with supplemental low intensity continuous far-red (low CFR). Leaf CO_2 exchange was measured on the second youngest fully expanded true leaf using an infrared gas analyser system connected to a leaf chamber fluorometer with a built-in light source (90% R, 10% B light). Values are means ± 1 SEM ($n=4$).

Cultivar	Treatment	P_N (μmol CO2 m^{-2} s^{-1})					
PPFD (μmol m^{-2} s^{-1})		0[1]	50	100	150	300	600
Hi Light	White	-2.5a ± 0.3	1.1a ± 0.3	3.7a ± 0.3	6.0a ± 0.4	10.3a ± 0.4	12.8ab ± 0.5
	Low CFR	-3.5a ± 0.4	0.5a ± 0.2	3.4a ± 0.1	5.5a ± 0.2	10.9a ± 0.3	14.8a ± 0.7
Imea	White	-2.7a ± 0.2	0.9a ± 0.2	3.4a ± 0.3	5.2a ± 0.3	8.6b ± 0.6	9.8c ± 0.3
	Low CFR	-3.1a ± 0.2	0.5a ± 0.2	3.3a ± 0.1	5.6a ± 0.3	9.9ab ± 0.2	12.3b ± 0.5
Treatment effect[2]		*	ns	ns	ns	*	**
Cultivar effect		ns	ns	ns	ns	**	***
Treatment × cultivar		ns	ns	ns	ns	ns	ns

[1]Within a column, different letters denote significant differences tested by Tukey's HSD test, $p<0.05$.
[2]Asterisks denote significant differences tested by two-way ANOVA: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ns, not significant.

Generally, transpiration rate increased up to a PPFD of 300 m^{-2} s^{-1} and then tailed off (Figure 4.14C). In addition, transpiration rate was affected significantly only by treatment at all PPFDs but 150 μmol m^{-2} s^{-1} (Table A.3, two-way ANOVAs, $p<0.05$).

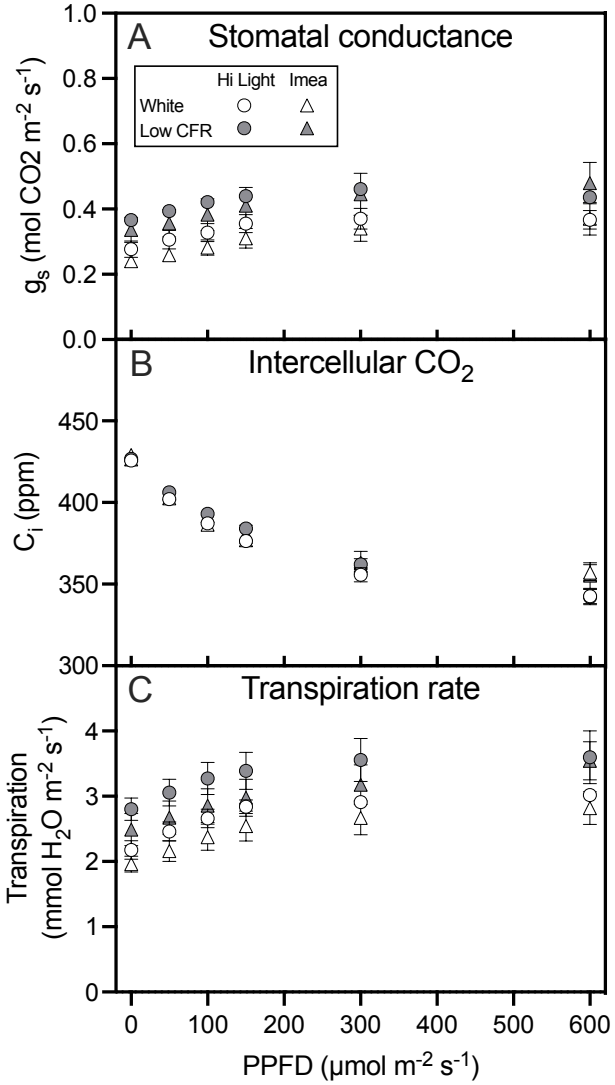

Figure 4.14.: Effects of adding low intensity continuous far-red (low CFR) to white light (white) and cultivar on stomatal conductance (A), intercellular CO_2 (B), and transpiration rate (C) in cucumber 'Hi Light' and 'Imea'. Parameters were measured when establishing leaf photosynthetic light response curves after nine days of treatment and using an infrared gas analyser system connected to a leaf chamber fluorometer with a built-in light source (90% R, 10% B light). Data are means ± 1 SEM ($n=4$).

4.3.4 Electron transport rate and the relationship to net photosynthesis

At most PPFDs, ETR was greater in seedlings subjected to low CFR treatment than those in white light treatment (Figure 4.15A and Table A.3, Mann-Whitney U-tests and two-way ANOVAs, $p<0.05$). At the same time, ETR was greater in 'Hi Light' than 'Imea' at PPFDs of 300 and 600 μmol m^{-2} s^{-1} (Mann-Whitney U-tests, $p<0.05$), which is consistent with that found in P_N. Identical to the previous experiments, ETR was highly and linearly correlated with P_N ($r^2=0.98$; Figure 4.15B).

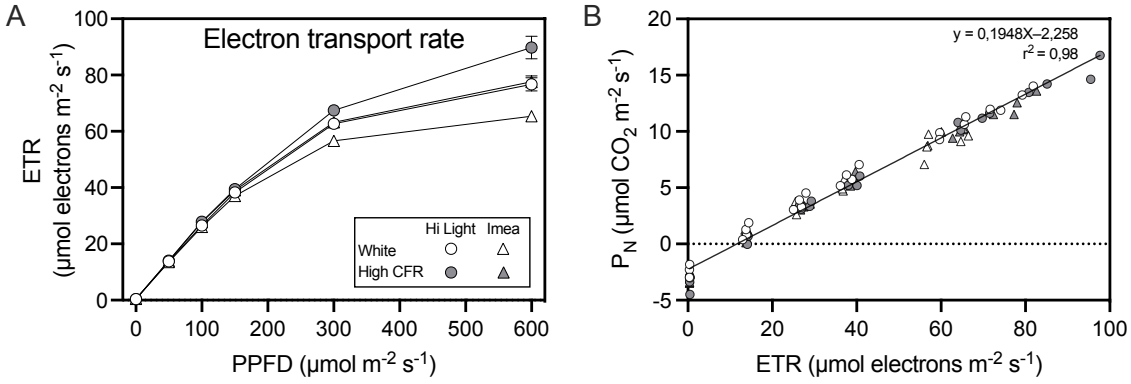

Figure 4.15.: Electron transport rate (ETR) (A) and the relationship between ETR and leaf net photosynthetic rate (B) in seedlings of cucumber 'Hi Light' and 'Imea' subjected to white light (white) or white light with supplemental low intensity continuous far-red (low CFR). Parameters were measured when establishing leaf photosynthetic light response curves after nine days of treatment and using an infrared gas analyser system connected to a leaf chamber fluorometer with a built-in light source (90% R, 10% B light). For ETR (A), data are means ± 1 SEM ($n=4$). For P_N/ETR (B), each data point represent P_N and corresponding ETR value at six PPFDs from 0–600 μmol m^{-2} s^{-1} for each replicate. All data were grouped for linear regression.

4.3.5 Changes in leaf chlorophylls and carotenoids concentrations

There were significant interactions between treatment and cultivar in total chlorophylls, and total carotenoids concentrations (Table 4.6, two-way ANOVAs, $p<0.05$). In general, treatment with low CFR decreased concentrations in 'Hi Light', while 'Imea' remained unaffected (Tukey's HSD tests, $p<0.05$). When comparing cultivars, total chlorophylls and carotenoids concentrations tended to be greater in 'Hi Light' than in 'Imea', but differences were only significant within the white light treatment (Tukey's HSD tests, $p<0.05$). Con-

versely, chl *a*/*b*-ratio did not differ significantly between treatments (Mann-Whitney U-test, $p > 0.05$), but it was, in general, greater in 'Imea' than 'Hi Light' ($p < 0.001$).

Table 4.6.: Effects of adding low intensity continuous far-red (low CFR) to white light (white) and cultivar on total chlorophylls concentration, chl *a*/*b*-ratio, and total carotenoids concentration in the second youngest fully expanded true leaves of cucumber 'Hi Light' and 'Imea' seedlings. Values are means ± 1 SEM (n = 6).

Cultivar	Treatment	Total chlorophylls[1] (μg cm^{-2})	Chl *a*/*b*-ratio[2]	Total carotenoids (μg cm^{-2})
Hi Light	White	51.02a ± 3.30	3.37 ± 0.02	7.93a ± 0.32
	Low CFR	39.96b ± 1.45	3.44 ± 0.02	6.86b ± 0.17
Imea	White	36.71b ± 1.00	3.54 ± 0.02	6.39b ± 0.09
	Low CFR	35.04b ± 1.05	3.55 ± 0.06	6.25b ± 0.14
Treatment effect[3]		**	ns	**
Cultivar effect		***	***	***
Treatment cultivar		*		*

[1]Within a column, different letters denote significant differences tested by Tukey's HSD test, $p < 0.05$.
[2]Residuals were not normally distributed, hence cultivars or treatments were grouped and Mann-Whitney U-tests were performed to determine treatment and cultivar effects.
[3]Asterisks denote significant differences tested by two-way ANOVA: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant.

4.4 Comparison of apparent quantum yields

Based on linear regression of non-rectangular hyperbola-based models to P_N light response data, the apparent quantum yield (AQY) did not change in response to high CFR treatment nor was there any effect of cultivar (Table 4.7, two-way ANOVA, $p > 0.05$). Similarly, there were no significant differences in AQY between cultivars in experiment two or experiment three (Mann-Whitney U-test and two-way ANOVA, $p > 0.05$, respectively). Hence, in experiment one, AQY was generally constant at *ca.* 0.05 (μmol CO_2 m^{-2} s^{-1}) photon^{-1}. In contrast, AQY decreased significantly with EOD-FR treatment when compared to white light treatment in experiment two (Mann-Whitney U-test, $p < 0.01$), while AQY was greater overall following low CFR treatment in experiment three (two-way ANOVA, $p < 0.05$). Differences in AQY between treatments and experiments were nonetheless very minor.

Table 4.7.: Apparent quantum yield (AQY) based on leaf photosynthetic light response of cucumber seedlings of 'Hi Light' and 'Imea' subjected to white light (white) or white light with a form of supplemental far-red (FR; high intensity continuous far-red in exp. one, 1.5 h of end-of-day far-red in exp. two, and low intensity continuous far-red in exp. three). AQYs were calculated by linear regression of estimated net photosynthesis (P_N) values at photosynthetic photon flux densities from 0–150 μmol $m^{m\text{-}2}$ s^{-1} for non-rectangular hyperbola based P_N/I response curves fitted to measured leaf P_N light response data for each replicate. Data are means ± 1 SEM ($n = 4$).

		Apparent quantum yield ((μmol CO_2 m^{-2} s^{-1}) photon^{-1})		
Cultivar	Treatment	Exp. one[1]	Exp. two[2]	Exp. three
Hi Light	White	0.055a ± 0.001	0.058 ± 0.001	0.057ab ± 0.001
	FR	0.053a ± 0.001	0.056 ± 0.000	0.061a ± 0.003
Imea	White	0.054a ± 0.001	0.056 ± 0.001	0.053b ± 0.001
	FR	0.054a ± 0.001	0.050 ± 0.002	0.058ab ± 0.001
Treatment effect[3]		ns	**	*
Cultivar effect		ns	ns	ns
Treatment cultivar		ns		ns

[1]Within a column, different letters denote significant differences tested by Tukey's HSD test, $p < 0.05$.
[2]Residuals were not normally distributed, hence cultivars or treatments were grouped and Mann-Whitney U-tests were performed to determine treatment and cultivar effects.
[3]Asterisks denote significant differences tested by two-way ANOVA: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant.

4.5 Estimated PPE and shoot length

Shoot length decreased linearly with increasing photoperiod PPE (i.e., as the amount of far-red decreased) from 0.74–0.86 ($r^2 = 0.97$; Figure 4.16).

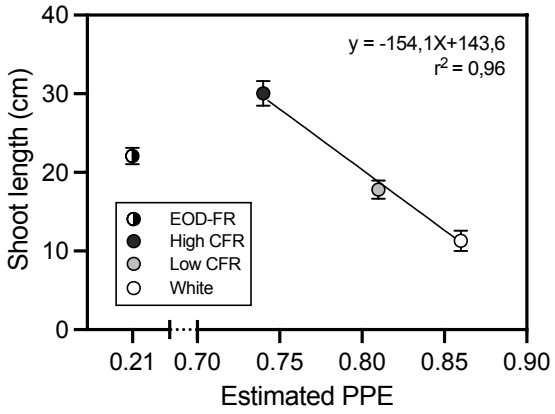

Figure 4.16.: The relationship between estimated phytochrome photoequilibrium (PPE) and shoot length in seedlings of cucumbers 'Hi Light' and 'Imea' subjected to white light (white) or white light with supplemental far-red in the form of high intensity continuous far-red (high CFR), 1.5 h of end-of-day far-red (EOD-FR), or low intensity continuous far-red (low CFR). PPE was estimated based on spectral distributions at the top of the canopy in each treatment. Data are means ± 1 SEM ($n = 11$ for high CFR, $n = 12$ for EOD-FR and low CFR, and $n = 36$ for white). Data for 'Hi Light' and 'Imea' were treated as one. Data for white light treatments were grouped from all experiments. Linear regression did not include data from EOD-FR.

5 Discussion

Far-red light has been increasingly investigated for its importance in regulating plant growth through its effects on morphology and photosynthesis (e.g., Zhen and Iersel, 2017; Zou *et al.*, 2019; Zhen and Bugbee, 2020b; Ji *et al.*, 2021). Here, we showed that adding far-red light to a background of white light, either continuously during the photoperiod or as an end-of-day treatment, significantly increased shoot length, leaf area, and growth of cucumber seedlings in a controlled environment (Table 5.1). The magnitude of the responses depended mainly on the form and intensity of supplemental far-red light as responses were largely similar in the two cultivars 'Hi Light' and 'Imea'.

Table 5.1.: Relative changes (%) in shoot length, total leaf area, relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA), leaf mass ratio (LMR), estimated leaf net photosynthesis (P_N) at a PPFD of 240 μmol m^{-2} s^{-1}, and measured P_N at a PPFD of 600 μmol m^{-2} s^{-1} in cucumber 'Hi Light' and 'Imea' plants when supplemental far-red light treatments were compared to corresponding white light treatments for each experiment.

	Hi Light			Imea		
	High CFR	EOD-FR	Low CFR	High CFR	EOD-FR	Low CFR
Shoot length	175.9	87.0	45.1	222.0	89.0	49.9
Total leaf area	40.1	40.8	11.1	34.9	29.1	14.8
RGR	14.7	15.3	8.3	18.4	11.6	9.9
NAR	33.6	17.3	14.4	36.4	16.0	15.3
LAR	−14.2	−1.7	−5.3	−13.1	−3.8	−4.8
SLA	−4.0	6.2	−2.0	−3.4	2.9	−1.0
LMR	−10.6	−7.5	−3.4	−10.1	−6.5	−3.8
Estimated $P_{N(I=240)}$	3.5	−0.5	2.2	15.1	−8.6	13.6
Measured $P_{N(I=600)}$	26.0	6.6	15.5	27.0	−8.2	25.4

Note: high CFR, EOD-FR, and low CFR refers to the supplemental far-red light treatments in experiment one, exp. two, and exp. three, respectively. denotes a significant increase, denotes a significant decrease, and denotes no significant change compared to corresponding white light treatments as tested by *post-hoc* Tukey's HSD tests or Mann-Whitney U-tests, $p < 0.05$.

5.1 Far-red induces changes to plant morphology

Decreasing PPE or R/FR-ratio results in phytochrome-mediated plant morphological adaptations to optimise radiation capture (Franklin *et al.*, 2005; Gommers *et al.*, 2013). Responses are typically characterised by stem and petiole elongation and, in some cases, leaf expansion,

dependent on species, shade-tolerance, and growth environment (Demotes-Mainard *et al.*, 2016). In this study, shoot length increased linearly with decreasing PPE in the photoperiod (i.e., increasing far-red light) as expected (Figure 4.16). In comparison, EOD-FR also promoted shoot extension but the relative response was lower and higher than that of high CFR and low CFR, respectively (Table 5.1). As there were few differences in number of true leaves, increases in shoot length were likely the result of internode and petiole elongation. Similar results with increasing stem elongation following decreasing PPEs have been observed in numerous species (Kalaitzoglou *et al.*, 2019; Park & Runkle, 2017; Demotes-Mainard *et al.*, 2016). However, Park and Runkle (2019) noted that whilst lowering PPE by adding far-red light stimulated stem elongation, the response was attenuated by blue light, presumably due to co-action between phytochromes and cryptochromes. Taken together, photoperiod PPE may be an appropriate predictor of changes to shoot length if only the R/FR-ratio is manipulated.

Similar to shoot length, total leaf area increased substantially following far-red treatments, though the dry mass fraction allocated to leaves decreased (Table 5.1). The observed increases in total leaf area across far-red treatments could primarily be attributed to an increase in individual leaf expansion and not total leaf count. Interestingly, EOD-FR and high CFR promoted leaf area expansion to similar extents when compared with corresponding white light treatments. This large increase in leaf area following EOD-FR may reflect the competition for resources between stem and leaves. In general, greater stem elongation is typically accompanied by greater dry mass partitioning to stems, often at the expense of growth of organs like leaves (Park & Runkle, 2017; Demotes-Mainard *et al.*, 2016). The large increase in shoot extension under high CFR could therefore have inhibited further leaf expansion by reducing assimilates available for leaf growth. Conversely, due to less shoot extension, more resources may comparably have been available for leaf expansion under EOD-FR. Earlier studies have indicated that a low PPE only promotes leaf area expansion when the PPFD is high enough to adequately support growth (Casal *et al.*, 1987; Zhen & Bugbee, 2020a). For instance, Heraut-Bron *et al.* (1999) observed that decreasing R/FR-ratio by adding far-red light only stimulated leaf expansion in white clover plants subjected to a high PPFD and not in plants subjected to a low PPFD. Far-red radiation may therefore interact with PPFD to affect leaf expansion and subsequently plant growth.

5.2 Continuous and end-of-day supplemental far-red increased plant growth by improving net assimilation rate

In our experiments, subjecting seedlings to far-red light significantly increased RGRs (Table 5.1), which is the product of whole-plant NAR and LAR. Doubling the far-red light intensity in the continuous far-red treatments (from 51.5 μmol m^{-2} s^{-1} in low CFR to 109.1 μmol μmol m^{-2} s^{-1} in high CFR at a constant background PPFD) effectively doubled RGR, but it simultaneously caused non-proportional decreases to LARs. EOD-FR treatment resulted in increased RGRs that were comparable to increases under high CFR but only in seedlings of 'Hi Light'. In both cultivars, LARs declined comparably less following EOD-FR treatment than continuous far-red light treatments. For EOD-FR, a slight increase in SLAs, presumably indicating thinner leaves, mitigated the decrease in fraction of dry mass partitioned to leaves. In agreement with other studies, EOD far-red light appeared relatively effective, and at times as effective as continuous far-red, in improving plant growth (Zhang *et al.*, 2019; Zou *et al.*, 2019; Zou *et al.*, 2021).

As LARs consistently declined following all far-red light treatments, the increased RGRs can solely be attributed to increased NARs, reflecting that an improved whole-plant dry-matter assimilation per leaf area outweighed the decreases in LARs. In other words, whilst the total leaf area per plant increased following far-red light treatments, it did not increase proportionally to the increase in plant above-ground dry mass. However, plants assimilated more dry mass per leaf area under far-red treatments, indicating that plants more efficiently utilised incident radiation due to 1) direct effects of far-red radiation on photochemistry and/or 2) morphological or other physiological adaptations.

5.3 Possible mechanisms for increased net assimilation rates

Leaf photosynthesis depends both on quantity and spectral quality of the incident light. Far-red light, in particular, is both poorly absorbed in leaves and a poor driver of photosynthesis on its own as wavelengths above 685 nm strongly overexcite PSI (Hogewoning *et al.*, 2012; Zhen & Iersel, 2017). Due to the cyclical nature of photochemistry, illumi-

nation with monochromatic far-red light results in insufficient excitation of PSII, which in turn limits electron supply to PSI, drastically reducing photosynthetic efficiency, or QY. Conversely, wavelengths between 400–670 nm more-or-less over-excite PSII (Hogewoning *et al.*, 2012; Laisk *et al.*, 2014). Overexcitation of PSII compared to PSI instead causes the plastoquinone (PQ) pool, or the intermediate electron transporter, to gradually become reduced as electrons donated from PSII to PSI are not able to be transferred away from PSI fast enough (Zhen & Iersel, 2017; Allen, 2003). In turn, the reduction of the PQ pool prevents the transfer of electrons away from PSII, which ultimately 'closes' the reaction centres as they become unable to use absorbed light energy for further electron transport. The simultaneous application of far-red and shorter wavelength light can therefore act synergistically to enhance the QY by optimising the excitation balance between the two photosystems leading to faster re-oxidiation of PQs and, consequently, improved electron transport (the Emerson effect; Hogewoning *et al.*, 2012; Zhen and Iersel, 2017).

Supplemental far red light may enhance photosynthesis in a dose-dependent manner as long as it improves the excitation balance, or reduces the restrictions on photosynthesis imposed by PSI excitation (Zhen & Bugbee, 2020a). Several studies found that adding far-red light to backgrounds of red-blue or white light increased leaf (and canopy) photosynthesis in numerous species under a wide range of far-red intensities (Zhen & Iersel, 2017; Zou *et al.*, 2019; Zhen & Bugbee, 2020b). Zhen *et al.* (2019), however, suggested that the upper wavelength limit of light that acts in photochemistry, and also preferentially excites PSI, are within the 731–752 nm range due to decreasing absorptance and energy with increasing wavelength. Altogether, it is likely that continuous supplemental far-red light in the photoperiod in experiment one and exp. three (high and low CFR) directly increased leaf photosynthesis, thus partly contributing to increased NARs.

NAR may also be influenced by morphological or other physiological adaptations that could increase light use efficiency per leaf area (Zou *et al.*, 2019). Far-red light may, for instance, facilitate changes to canopy architecture by affecting internode and petiole length as well as petiole angle to improve light distribution and interception in younger plants with limited self-shading (Kalaitzoglou *et al.*, 2019). Furthermore, far-red light may influence chloroplast structure, increase the level of soluble carbohydrate, reduce starch grain size (Kasperbauer & Hamilton, 1984), and affect source-sink relations to alter growth (Ji *et al.*, 2020). It is likely that a number of such adaptations affected NAR to a large degree when considering that plants subjected to EOD-FR, where direct effects of far-red light on photochemistry are unlikely, also increased significantly in NAR.

Far-red treated plants in this study, however, also had reduced leaf chlorophyll concentrations (Table 4.2, 4.4, and 4.6), which is a common shade-avoidance response elicited by low R/FR-ratios in many species (Casal *et al.*, 1987; Zou *et al.*, 2019; Franklin *et al.*, 2005). In addition, far-red treatments also decreased total carotenoids similar to Li and Kubota (2009) and Kalaitzoglou *et al.* (2019). It is not clear what causes the decrease in chlorophyll concentration, but it has been suggested that it, among others, may be the result of a dilution effect upon far-red promoted leaf expansion or changes to biosynthesis following phytochrome-mediated effects on gene expression (Casal *et al.*, 1987; Meng *et al.*, 2019). Nonetheless, decreases in mass pigment concentration following far-red treatments have often been coupled to decreased leaf absorptance (Kalaitzoglou *et al.*, 2019; Zou *et al.*, 2019; Zhen & Bugbee, 2020b). However, Heraut-Bron *et al.* (1999) notably found that whilst a low R/FR-ratio decreased chlorophyll concentration in white clover, it did not change rubisco carboxylase activity nor maximum photosynthesis under high and low irradiance.

We found that plants grown under continuous far-red light (high and low CFR) increased in single leaf P_N at high PPFDs when illuminated by a red-blue light source (Table 4.1 and 4.5). Estimated P_N at growth chamber PPFD (*ca.* 240 μmol m^{-2} s^{-1}) also increased slightly for continuous far-red treated plants when compared to white light treatments (Table 5.1), but it is important to note that estimates were based on leaf photosynthesis measured under red-blue light. Photosynthesis was therefore likely even greater under chamber conditions due to the photosynthetic activity of far-red light. There were not any great effects on apparent QY [μmol CO_2 m^{-2} s^{-1} per incident photon] under red-blue light (Table 4.7). Taken together, this may indicate that far-red light acclimation potentially improves photosynthetic efficiency, and capacity, compared to white light treated plants but only under higher PPFDs. This is in large contrast to previous studies that have largely found that plants grown under continuous far-red decreased or did not change in leaf P_N when illuminated with red-blue light (Zhang *et al.*, 2019; Ji *et al.*, 2019; Zou *et al.*, 2019, studies on tomato and lettuce). On the contrary, there were no significant changes to measured P_N under red-blue illumination for our EOD-FR treated plants, which is consistent with other studies (Zou *et al.*, 2019; Zhang *et al.*, 2019; Zou *et al.*, 2021). However, changes to NAR, which is the integration of daily carbon gain and respiratory losses, may not necessarily be reflected in individual leaf P_N as it is determined only on a small fraction of a single leaf and over a very short period (Bugbee, 2016).

In all three experiments, we observed linear relationships between ETR and P_N, which were generally lower than the theoretical maximum efficiency of CO_2 fixation as indicated by linear regression slopes lower than 0.250. In theory, if all incident light is absorbed by photosystems and there are no other electron sinks, four electrons are used to generate two

NADPH used to assimilate one molecule of CO_2 in the Calvin cycle (Baker, 2008). However, alternative electron sinks to CO_2 fixation in the chloroplasts reduce this efficiency, which include different processes such as photorespiration, nitrate metabolism, sulfate assimilation, and cyclic electron flow (Baker, 2008; Liu & Iersel, 2021). Nonetheless, CO_2 fixation efficiency per transported electron [through PSII] remained relatively constant across all treatments, indicating that it is likely that no competiting processes, or alternative electron sinks, were down- or upregulated in response to far-red light treatments. In turn, P_N could potentially be estimated using measurements of ETR. Overall, seedlings exposed to far-red light may become more efficient in whole-plant net assimilation due to complex morphological and physiological changes and interactions that have yet to be fully elucidated.

5.4 Similarities and variation in cultivar responses to supplemental far-red light

We observed largely similar relative responses to supplemental far-red light in the two cultivars across the different far-red treatments (Table 5.1). Following continuous and EOD far-red light treatments, cultivars increased in total dry mass, shoot length, and stimulated leaf expansion compared to white light treatments. For both 'Hi Light' and 'Imea', RGRs increased significantly due to improved NARs, and LARs declined mainly as a result of decreased LMRs. In contrast, in a study on the response of 33 tomato genotypes to continuous far-red light, Ji *et al.* (2021) found that whilst genotypes responded similarly in terms of increased plant height, stem dry mass, and decreased LMRs with increasing far-red light, there were large differences in terms of total plant dry mass. Some genotypes increased strongly in NAR resulting in large increases in RGRs, whilst other genotypes increased only in LAR, due to increased SLA, resulting in weaker growth. The authors concluded that, generally, tomato genotypes that increased strongly in NAR following far-red light treatment achieved the greatest increase in total plant dry mass. Growth responses to supplemental far-red light may therefore depend both on species and genotype, but there were no apparent differences between the two cultivars used in this study.

However, whilst relative responses to far-red light largely did not differ between the two cultivars used here, there were some absolute differences in morphology and photosynthetic efficiency. In general, total leaf area was greater in 'Imea' than in 'Hi Light' when compared both within far-red light and white light treatments. Conversely, the opposite was true for leaf photosynthetic capacity. Here, P_N at high PPFDs was consistently greater in 'Hi Light' than 'Imea', which would suggest that leaves of 'Hi Light' may, in fact, be more adapted

to high light [intensity] than 'Imea'. Both transpiration rate and stomatal conductance also increased at most measured levels of PPFD in 'Hi Light' and not in 'Imea' following far-red light treatments. Although transpiration and stomatal opening were measured over a short period and thus likely did not reach steady-state, it could potentially have implications for water- and nutrient uptake under far-red light. Moreover, total chlorophylls and carotenoids concentrations were similarly higher in 'Hi Light' compared to 'Imea' but differences were not apparent nor consistent when correcting for differences in SLA. Taken together, this may indicate that 'Imea' is more dependent on total leaf area and total radiation capture for growth, whilst 'Hi Light' is inherently more efficient in CO_2 assimilation per leaf area.

Differences in plant morphology and physiology between genotypes may reflect how well they respond to supplemental far-red light. The few differences between the cultivars used in this study, in combination with other studies like Ji *et al.* (2021) that found large variation in growth response between tomato genotypes, nonetheless suggest that there is a need for further study to better characterise the variation in growth under far-red light between cucumber genotypes.

5.5 Practical implications of supplemental far-red light

In LEDs, far-red photons can be generated with higher efficacy than photons of traditional PAR (Kusuma *et al.*, 2020). Far-red light supplementation nevertheless comes at an energy cost that must be justified by the benefits to crop production. Here, additional far-red light was found to increase growth of cucumber seedlings but it also substantially increased shoot length. Importantly, EOD-FR was similarly effective in improving growth as continuous far-red light treatments. Treatments with EOD far-red light may therefore prove to be a more energy-efficient alternative to improve production. It must, however, be noted that our experiments were conducted under sole-source lighting in controlled environments with rather broad-spectra far-red LEDs. Hence, our results may be mostly applicable to cultivation under limited influence of natural sunlight as in vertical farms or during winter in greenhouses.

The use of supplemental far-red light may nonetheless improve transplant production of cucumbers by increasing plant growth rate and leaf expansion. It could also help ensure better uniformity in plant morphology throughout the year by minimising the variation in light quality when artificial lighting is used in combination with sunlight. However, the associated increase in shoot extension, if excessive, could take up more space and

potentially lead to stem or petiole breakage during transport. On the other hand, the greater dry mass partitioning to stems and hypocotyls following far-red light treatment may be useful in rootstock production (Chia & Kubota, 2010; Yang *et al.*, 2012). Here, sufficient length of hypocotyls is critical to avoid the scion coming into unwanted contact with the soil (Chia & Kubota, 2010). Moreover, greater hypocotyl elongation may help improve grafting success and reduce rooting from the scion (Chia & Kubota, 2010).

Far-red light supplementation may also improve vegetable and fruit crop production, particularly if the benefits to plant growth also transfer to fruit yield. This appears to be the case in a fruiting crop like tomato. Recent studies found that supplementation with far-red light over longer terms significantly increased fruit yield and size in greenhouse tomato crops due to changes in, among others, dry mass partitioning and sink strength (Ji *et al.*, 2020; Kalaitzoglou *et al.*, 2019; Zhang *et al.*, 2019). Similar research is needed to further explore the potential of far-red light in cucumber production.

Collectively, these results demonstrate that far-red light can promote plant growth, change morphology, and, in some cases, improve yield. The benefits of improved growth, and potentially yield, must nevertheless be weighed against the changes, for better or worse, to plant morphology, energy consumption, and investments when considering the implementation of supplemental far-red light. As more research on plant responses accumulate and LED technology advances, advantages of supplemental, or even substitutional, far-red light may become more apparent.

6 Conclusion

Adding far-red light to white light either during the photoperiod or as an end-of-day treatment profoundly altered plant morphology and improved growth of cucumber plants under sole-source lighting in controlled environments. Supplemental far-red light promoted phytochrome-mediated shoot extension and leaf expansion. Far-red light, however, also increased dry mass partitioning to stem and petioles at the expense of leaves, which in large part resulted in decreased LARs. Substantially increased RGRs in plants subjected to supplemental far-red light were therefore the result of greatly improved NARs. For continuous far-red light treatments, increased NARs can in part be attributed to the synergistic effects of far-red light on leaf photosynthesis. Other physiological or morphological adaptations likely also improved the utilisation of incident radiation. In particular, EOD-FR was similarly effective in stimulating plant growth as continuous far-red treatments, which may be coupled to trade-offs between changes to plant morphology and growth. Despite differences in morphological and physiological traits, particularly in terms of leaf area and photosynthetic efficiency, cultivars 'Hi Light' and 'Imea' responded similarly in terms of relative changes to plant morphology and growth components following far-red light treatments.

We conclude that growing cucumber plants under supplemental far-red light can improve plant growth through complex morphological and physiological changes and interactions that are yet to be fully elucidated. The addition of far-red light to white LEDs can benefit many aspects of horticultural production, but further research is needed to minimise undesirable effects and to investigate the applicability to cucumber fruit production.

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Supplementary information

A

A.1 Results of two-way ANOVAs on stomatal conductance, intercellular CO_2 concentration, transpiration rate and electron transport rate in all experiments

Table A.1.: Results of two-way ANOVAs on stomatal conductance (g_s), intercellular CO_2 concentration (C_i), transpiration rate, and electron transport rate (ETR) in seedlings of cucumber 'Hi Light' and 'Imea' in experiment one (high intensity continuous far-red). Parameters were measured when establishing leaf photosynthetic light response curves at six levels of photosynthetic photon flux density after nine days of treatment. Data was measured using an infrared gas analyser system connected to a leaf chamber fluorometer with a built-in light source (90% R, 10% B light) placed wholly inside the growth chambers.

Experiment one — high intensity continuous far-red							
PPFD (μmol m^{-2} s^{-1})		0	50	100	150	300	600
g_s	Treatment effect[1]	*	*	*	*	*	ns
	Cultivar effect	*	*	*	*	*	ns
	Treatment × cultivar	ns	ns	ns	ns	ns	ns
C_i	Treatment effect	*	ns	ns	ns	ns[2]	ns[2]
	Cultivar effect	*	ns	ns	ns	ns[2]	ns[2]
	Treatment × cultivar	ns	*	ns	ns		
Transpiration	Treatment effect	ns	ns	ns	ns	ns	ns
	Cultivar effect	*	*	*	*	*	ns
	Treatment × cultivar	ns	ns	ns	ns	ns	ns
ETR	Treatment effect	ns	ns[2]	ns[2]	ns	ns	***
	Cultivar effect	ns	ns[2]	ns[2]	ns	ns	***
	Treatment × cultivar	ns			ns	ns	ns

[1]Asterisks denotes significant differences tested by two-way ANOVA: *, $p<0{,}05$; **, $p<0{,}01$; ***, $p<0{,}001$; ns, not significant.
[2]Assumption of homogeneity of variances was not met (Brown-Forsythe test, $p<0{,}05$), hence cultivars or treatments were grouped and Welch's t-tests were performed to determine treatment and cultivar effects.

Table A.2.: Results of two-way ANOVAs on stomatal conductance (g_s), intercellular CO_2 concentration (C_i), transpiration rate, and electron transport rate (ETR) in seedlings of cucumber 'Hi Light' and 'Imea' in experiment two (end-of-day far-red). Parameters were measured when establishing leaf photosynthetic light response curves at six levels of photosynthetic photon flux density after nine days of treatment. Data was measured using an infrared gas analyser system connected to a leaf chamber fluorometer with a built-in light source (90% R, 10% B light) placed wholly inside the growth chambers.

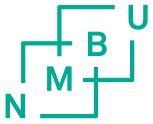
Experiment two — end-of-day far-red							
PPFD (μmol m^{-2} s^{-1})		0	50	100	150	300	600
g_s	Treatment effect[1]	ns[2]	ns[2]	ns	ns	ns	ns
	Cultivar effect	*[2]	*[2]	*	*	*	ns
	Treatment × cultivar	*[2]	*[2]	*	ns	ns	ns
C_i	Treatment effect	ns[3]	ns	ns	ns[3]	*	ns[3]
	Cultivar effect	ns[3]	ns	ns	ns[3]	ns	*[3]
	Treatment × cultivar		ns	ns		ns	
Transpiration	Treatment effect	ns	ns	ns	ns	ns	ns
	Cultivar effect	*	*	*	*	ns	ns
	Treatment × cultivar	*	ns	ns	ns	ns	ns
ETR	Treatment effect	ns	ns	ns	*	ns	ns
	Cultivar effect	ns	ns	ns	ns	***	***
	Treatment × cultivar	ns	ns	ns	ns	ns	ns

[1]Asterisks denotes significant differences tested by two-way ANOVA: *, $p<0{,}05$; **, $p<0{,}01$; ***, $p<0{,}001$; ns, not significant.
[2]Data was reciprocity transformed for testing in order to fulfill assumption of homogeneity of variances (Brown-Forsythe test, $p<0{,}05$).
[3]Assumption of homogeneity of variances was not met (Brown-Forsythe test, $p<0{,}05$), hence cultivars or treatments were grouped and Welch's t-tests were performed to determine treatment and cultivar effects.

Table A.3.: Results of two-way ANOVAs on stomatal conductance (g_s), intercellular CO_2 concentration (C_i), transpiration rate, and electron transport rate (ETR) in seedlings of cucumber 'Hi Light' and 'Imea' in experiment three (low intensity continuous far-red). Parameters were measured when establishing leaf photosynthetic light response curves at six levels of photosynthetic photon flux density after nine days of treatment. Data was measured using an infrared gas analyser system connected to a leaf chamber fluorometer with a built-in light source (90% R, 10% B light) placed wholly inside the growth chambers.

Experiment three — low intensity continuous far-red							
PPFD (μmol m^{-2} s^{-1})		0	50	100	150	300	600
g_s	Treatment effect[1]	**	**	**	*	*	ns
	Cultivar effect	ns	ns	ns	ns	ns	ns
	Treatment × cultivar	ns	ns	ns	ns	ns	ns
C_i	Treatment effect	ns	ns	*	*[2]	ns	ns
	Cultivar effect	ns	ns	ns	ns[2]	ns	*
	Treatment × cultivar	ns	ns	ns		ns	ns
Transpiration	Treatment effect	**	*	*	ns	*	*
	Cultivar effect	ns	ns	ns	ns	ns	ns
	Treatment × cultivar	ns	ns	ns	ns	ns	ns
ETR	Treatment effect	ns	ns	*	*	*[3]	*[3]
	Cultivar effect	ns	ns	ns	ns	*[3]	*[3]
	Treatment × cultivar	ns	ns	ns	ns		

[1]Asterisks denotes significant differences tested by two-way ANOVA: *, $p<0{,}05$; **, $p<0{,}01$; ***, $p<0{,}001$; ns, not significant.
[2]Assumption of homogeneity of variances was not met (Brown-Forsythe test, $p<0{,}05$), hence cultivars or treatments were grouped and Welch's t-tests were performed to determine treatment and cultivar effects.
[3]Residuals were not normally distributed, hence cultivars or treatments were grouped and non-parametric Mann-Whitney U-tests were carried out to determine treatment and cultivar effects.



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