

**On the photosynthetic and developmental responses
of leaves to the spectral composition of light**

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Abstract

A wide range of plant properties respond to the spectral composition of irradiance, such as photosynthesis, photomorphogenesis, phototropism and photonastic movements. These responses affect plant productivity, mainly via changes in the photosynthetic rate per unit leaf area, light interception, and irradiance distribution through the canopy. The spectral environment of plants is dependent on location (e.g. latitude), changes over time (e.g. Sun-angle), shading by other leaves, and, in the case of protected cultivation, the use of growth lamps. Therefore, not only the acclimation of developing leaves to light spectrum is important for plant productivity and survival, but also the capability of mature leaves to respond to changes in spectrum. This thesis focuses on the acclimation of photosynthesis per unit leaf area to the growth-light spectrum, the consequences of spectral acclimation for the wavelength dependence of photosynthetic quantum yield, and photomorphogenetic versus leaf photosynthetic acclimation in relation to biomass production. *Cucumis sativus* is used as a model plant. Additionally, the consequences of the choice and quality of the actinic light used during photosynthesis measurements are explored.

By growing plants under seven different combinations of red and blue light, blue light is shown to have both a qualitative and a quantitative effect on leaf development. Only leaves developed under red alone (0% blue) displayed a dysfunctional photosynthetic operation, which was largely alleviated by only 7% blue. Quantitatively, leaf responses to an increasing blue light percentage resembled responses associated with an increase in irradiance.

Next, the wavelength dependence of the quantum yield for CO₂ fixation (α) is analysed in detail. Leaves grown under artificial shadelight, which overexcites photosystem I (PSI), had a higher α at wavelengths overexciting PSI (≥ 690 nm) and a lower PSI:PSII ratio compared with artificial sunlight and blue light grown leaves. At wavelengths overexciting PSII, α of the sun and blue grown leaves was higher. The photosystem excitation balance is quantitatively shown to determine α at those wavelengths where absorption by carotenoids and non-photosynthetic pigments is insignificant (≥ 580 nm). The wavelength dependences of the photosystem excitation balance calculated via an *in vivo* and an *in vitro* approach were substantially in agreement with each other, and where not, carotenoid absorption and state transitions are likely to play a role.

Not only is the photosynthetic rate per unit leaf area important for plant productivity, but also photomorphogenesis. We have engineered an artificial solar (AS) spectrum under which plants produced a dry weight that was, respectively, 2.3 and 1.6 times greater than that of plants grown under fluorescent tubes and high pressure sodium light. This striking difference was due to a morphology of the AS-plants that was more efficient in light interception, and not related to photosynthesis per unit leaf area. These results highlight the importance of a spectrum that is more natural than that of usual growth-lamps for research and possibly also for horticultural production.

A technically orientated part of this thesis presents a simple method to quantify the light distribution in leaf chambers, which is shown to be important for the accuracy of photosynthesis measurements by gas-exchange. The match between growth-light and measuring-light spectrum is likewise shown to be important. A mismatch can have significant consequences for the estimate of α *in situ*, but only minor consequences for the estimate of the light-saturated photosynthetic rate. The relationship between the electron transport rate calculated using chlorophyll fluorescence measurements and the CO₂ fixation rate also changed considerably with changes in measuring-light spectrum. The use of erroneous estimates of α as input for crop growth models is shown to have disproportionately large consequences for predictions of plant growth.

Key words: action spectrum, artificial solar spectrum, blue light, *Cucumis sativus*, gas-exchange, light-emitting diodes (LEDs), light interception, light quality, non-photosynthetic pigments, photosynthetic capacity, photomorphogenesis, photosystem excitation balance, quantum yield, red light.

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Abbreviations

a_{leaf}	absorbed light fraction
$a_{\text{PSII}}/(a_{\text{PSI}} + a_{\text{PSII}})$	absorptance by PSII/ absorptance by (PSI + PSII)
A_{gross}	gross assimilation
A_{max}	light saturated assimilation
A_{net}	net assimilation
AL	actinic light
AS	artificial solar
B	blue light (%)
BR	30% blue light/ 70% red light
$C_i C_a^{-1}$	$[\text{CO}_2]$ in leaf (i.e. C_i) relative to $[\text{CO}_2]$ in leaf chamber air
CF	chlorophyll fluorescence
Chl(s)	chlorophyll(s)
DW	dry weight
ETR	electron transport rate
F'	steady-state light-adapted fluorescence
F_0	dark-adapted minimum fluorescence
F_0'	light-adapted minimum fluorescence
F_v/F_m	ratio of variable to maximum fluorescence; i.e. Φ_{PSII} if all PSII reaction centers are open
F_v'/F_m'	maximum light-adapted relative efficiency of PSII
FR	far-red
FT(s)	fluorescent tube(s)
g_{sw}	stomatal conductance
g_{sw} ratio	ratio of g_{sw} on the adaxial and abaxial surface of the leaf
GL	growth-light
HPS	high pressure sodium
LAI	leaf area index (m^2 leaf/ m^2 ground area)
LED(s)	light emitting diode(s)
LMA	leaf mass per unit leaf area
LUR	leaf unfolding rate (number of leaves per day)
ML	measuring-light
NIR	near infrared
PNUE	photosynthetic nitrogen use efficiency
PPFD	photosynthetic photon flux density
PSI	photosystem I
PSII	photosystem II
PSS	phytochrome photostationary state
Q_A	primary quinone acceptor of PSII
q_p	PSII efficiency factor (also known as F_q'/F_v')
q_L	fraction of oxidized Q_A
R_{dark}	dark respiration
R	red light (%)
SD	standard deviation
α	quantum yield for CO_2 fixation (either on an absorbed or incident light basis, depending on the context)
α_{est}	α estimated via the photosystem efficiency balance
ΔA_{820}	absorption changes at 820 nm
Φ_{PSI}	relative quantum yield of PSI electron transport
Φ_{PSII}	relative quantum yield of PSII electron transport (also known as F_q'/F_m').

CHAPTER 1

General Introduction

Green plants convert the light energy provided by the Sun into chemical energy via the process of photosynthesis. The energy provided by light is employed to drive the reduction of CO₂ that diffuses into the leaf via its stomata, to synthesize carbohydrates. Oxygen is released in this process following the oxidation of H₂O. The assimilated carbohydrates are subsequently processed further into numerous other biochemical building blocks. Various biotic and abiotic factors influence the efficiency with which photosynthesis fixes CO₂, such as the intensity and quality of irradiance, water and nutrient availability, temperature, atmospheric pressure and composition, and environmental contaminants. This thesis offers new insights into the effects of light quality (i.e. spectral composition) on photosynthesis and the development of plants, using *Cucumis sativus* as a model plant.

Variations in the spectral quality of irradiance depend on factors such as habitat, time of the day and season, weather, and the shade cast by other plants. Whereas differences between some environmental factors have a rather constant effect on the light spectrum (e.g. latitude), other factors change continuously (e.g. Sun-angle and cloud cover) within a short time-frame. The shading of leaves by neighbouring vegetation or self-shading by younger leaves can lead to substantial, long-term changes in spectrum. The spectral changes produced by plant-shade result in a large relative increase in far-red (FR) wavelengths and a smaller relative increase of green (e.g. Whitelam and Halliday, 2007). The opposite effect will occur when shaded leaves are exposed to full sunlight, e.g. after gap formation in a canopy (e.g. Turnbull *et al.*, 1992), or for evergreens when neighbouring deciduous trees lose their leaves. In protected cultivation plants are grown under a variety of lamp-types emitting spectra which are dissimilar to natural daylight spectra (Hogewoning *et al.*, 2010a). Whereas in climate chambers the growth-light spectrum is often constant, in greenhouse cultivation natural daylight is sometimes supplemented with light provided by growth-lamps, so that the crop is subjected to strong changes in its spectral environment. Especially at latitudes with short natural photoperiods in winter, assimilation lamps are used in greenhouses to lengthen the photoperiod and increase the total irradiance at times when natural irradiance is relatively low (Heuvelink *et al.*, 2006). High pressure sodium (HPS) lamps are the supplemental light sources that are commonly used in greenhouses, though recently light-emitting diodes (LEDs) have been proposed as potential replacements for HPS lamps (Hogewoning *et al.*, 2007). The large diversity of narrow-band spectra emitted by LEDs creates a challenge for

their efficient use in horticulture, because plant responses to their restricted spectra are poorly understood.

A large bibliography of studies on plant responses to the intensity of irradiance has become available over the years. Plant responses to the light spectrum are relatively less well understood, despite detailed studies on specific features such as red:FR ratios (R:FR; e.g. Smith, 2000) or short term responses of relative photosynthetic efficiency to different narrow-band spectra (e.g. Balegh and Biddulph, 1970; McCree, 1972a; Inada, 1976; Evans, 1987). In general, a wide range of plant properties respond to the spectral environment, such as photosynthesis, photomorphogenesis, phototropism and photonasty. The limitless range of spectral compositions, and changes in spectrum, make a comprehensive, overall understanding of plant responses to their spectral environment a virtually impossible task. A subdivision of the broad range of plant responses to the spectral environment into different levels of time and function helps to structure the various ways that irradiance-spectra can influence plant growth. A schematic overview is given in Fig. 1. The subjects covered in this thesis focus on the responses of leaf photosynthesis and development to the spectral environment. In particular this study is focuses on:

1. The adaptation of leaf photosynthesis and related leaf morphological and chemical characteristics to the spectral environment.
2. The acclimation of leaf photosynthetic quantum yield and photosystem stoichiometry of leaves which have developed under different spectra and the consequences of acclimation for the wavelength dependence of photosynthetic quantum yield.
3. The consequences for plant production of spectrally induced plant morphological changes in relation to leaf photosynthesis.

1.1 Leaf photosynthesis and related characteristics in response to growth-spectrum

The irradiance-spectrum of a plant's growth environment affects a range of leaf characteristics which influence the photosynthetic rate per unit area of leaf surface. These characteristics can be related to leaf anatomy, the content and composition of proteins, protein complexes, cofactors and other essential biochemicals in the leaf, and stomatal conductance. The effects of growth-light spectrum on the assimilation-irradiance relationship for that particular spectrum are introduced separately here for photosynthesis under light-limited (1.1.1) and light-saturated irradiance (1.1.2); leaf responses at intermediate irradiances are a hybrid of the light-limited and light-saturated responses.

1.1.1 Effect of growth-spectrum on light-limited quantum yield

The light-limited quantum yield for CO₂ fixation or O₂ evolution on an incident light basis, i.e. the maximum light use efficiency at relatively low incident irradiances, is linearly related to the fraction of incident light that the leaf absorbs. Leaf light-absorption depends on the fraction of light reflected and transmitted by the leaf, which in turn depends on the leaf pigment content, composition and spatial distribution through the leaf. Growth-light spectrum can affects these factors, e.g. via blue light induced chloroplast movements (Jarillo *et al.*, 2001) or spectral effects on leaf anatomy (see 1.1.2). In a healthy, non-stressed

leaf, the light-limited quantum yield for CO₂ fixation or O₂ evolution on an absorbed light basis is expected to be influenced predominantly by pigment composition. Carotenoids and non-photosynthetic pigments such as flavonoids and anthocyanins absorb light but do not, or only partially (carotenoids), transfer excitation energy to photosystem reaction centres (Inada 1976, Evans 1986, Nishio 2000), whereas chlorophyll excitation energy transfer is highly efficient (e.g. Croce *et al.*, 2001; van Amerongen and van Grondelle, 2001). Therefore a relatively higher content of carotenoids and non-photosynthetic pigments decreases the quantum yield for CO₂ fixation or O₂ evolution of those wavelengths absorbed by these pigments (i.e. UV, blue and green). Carotenoids protect against photodamage by quenching triplet state chlorophyll, scavenging of reactive oxygen species (ROS) and via the xanthophyll cycle (Young, 2001). Non-photosynthetic pigments protect against photodamage by screening out incident irradiance by absorption and also via ROS-scavenging (Edreva, 2005), and protect plants against herbivores (Treutter, 2006). Notably, flavonoids are found mainly in the adaxial epidermis of leaves (e.g. Hutzler *et al.*, 1998), and arboreal species usually have a lower quantum yield in the UV/ blue than herbaceous species (Inada, 1976), probably because of the high levels of phenolics in their leaves (Bate-Smith, 1962). A higher content of protective non-photosynthetic pigments in arboreal species at the cost of a lower quantum yield is a more functional investment for trees than for herbs, as leaf lifetime is usually longer for trees. As non-photosynthetic pigments have a photoprotective function, a high irradiance is a trigger for the synthesis of these pigments (Lambers *et al.*, 2008).

The growth-spectrum also affects the leaf pigment content and composition and therefore the light-limited quantum yield. As the energy of photons depends linearly on the reciprocal of wavelength (Planck, 1901), light with a relatively short wavelength such as UV and blue is potentially more damaging than light with a relatively long wavelength such as red, and these shorter wavelengths are also more absorbed by proteins and nucleic acids. UV and blue light have been reported to stimulate the transcription of flavonoid synthesis genes in order to protect the plant against photodamage (e.g. Kubasek *et al.* 1992, Jackson and Jenkins 1995). Indeed leaves of field grown plants tend to have lower quantum yields for UV and blue wavelengths than plants grown under a spectrum containing no UV (e.g. fluorescent tubes in a growth chamber; McCree, 1972a). The excitation balance of the two photosystems also affects the light-limited quantum yield (e.g. Pfannschmidt, 2005; Dietzel *et al.*, 2008). However, it is widely assumed that plants tune their photosystem stoichiometry to their growth-light spectrum such that the two photosystems are excited in balance (e.g. Chow *et al.*, 1990; Kim *et al.*, 1993), and that light-limited quantum yield is maximised. Changes in spectrum will, however, affect the photosystem excitation balance, at least in the short term (see 1.2.2).

It is not known yet whether leaves may display stress reactions in response to growth under specific unnatural spectra, such as narrow-band light provided by LEDs. In a stressful environment irradiance results more often in photoinhibition, compared to non-stressful conditions (e.g. Hogewoning and Harbinson, 2007; Baker, 2008). In the case of photoinhibition, light-limited quantum yield will be reduced. An indication of stress effects in response to growth under red light alone has been reported for the green alga *Acetabularia* (Wennicke and Schmid, 1987; Schmid *et al.* 1990a, b). For the application of LEDs as growth-light in protected plant cultivation systems more knowledge on the response of leaves to narrow-band lighting is highly relevant.

1.1.2 Effect of growth-spectrum on light-saturated photosynthetic capacity

Photosynthetic capacity, i.e. the maximum photosynthetic rate per unit area of leaf at light-saturated irradiance, can be affected by a wide range of leaf characteristics which may be altered by the plant's growth spectrum. Photosynthetic capacity can be limited by physical restrictions in intercellular space per unit leaf area, which limit the area of cell membrane against which chloroplasts can abut (Oguchi *et al.* 2003). Molecules necessary for the operation of photosynthesis, many of which require nitrogen (e.g. Rubisco), can also limit photosynthetic capacity. Leaf mass per unit area of leaf (LMA) is a parameter often measured in growth analyses and is affected by both anatomy (the number of cell layers and cell size) and cell content (Poorter *et al.*, 2009). At sub-saturating and saturating irradiance the intercellular CO₂ concentration is an important limiting factor for photosynthesis and therefore the stomatal and mesophyll conductance are important determinants of the CO₂ fixation rate as well. Stomatal conductance is determined by the number, size and aperture of stomata. In the short term, blue light has been shown to stimulate stomatal opening (Sharkey and Raschke, 1981; Zeiger, 1990) while green light can reverse stomatal opening (Frechilla *et al.*, 2000; Talbott *et al.*, 2002). Effects of growth-spectrum on the number and size of stomata are, however, not known to date. The effects of light intensity on leaf traits affecting photosynthetic capacity have been studied extensively (e.g. Evans and Poorter, 2001; Poorter *et al.*, 2009, 2010). However, little is known about spectral effects on these leaf traits. Spectral effects on leaf development may be mediated via wavelength sensitive photoreceptors. The limited number of published studies focussing on spectral effects on photosynthetic capacity have mainly investigated blue light effects. Blue light has been associated with 'sun-type' chloroplasts with a relatively high photosynthetic capacity (Lichtenthaler *et al.*, 1980). Usually the responses of plants grown under blue-deficient light and plants grown under a spectrum containing blue have been compared (e.g. Voskresenskaya *et al.*, 1977; Britz and Sager, 1990; Brown *et al.*, 1995; Bukhov *et al.*, 1995; Goins *et al.*, 1997; Yorio, 2001; Matsuda *et al.*, 2004, 2007, 2008; Ohashi *et al.*, 2006). Most of these studies have a qualitative character, i.e. a growth spectrum with or without blue. The overall trend is that a growth spectrum deficient in blue results in a lower photosynthetic capacity and less plant dry weight production. A thorough quantitative analysis of the blue light responses of the various leaf characteristics affecting photosynthetic capacity is lacking, as are the responses to other wavelength ranges.

1.2 Responses of fully grown leaves to changes in spectrum

Both the light-limited quantum yield and the light-saturated photosynthetic capacity of mature leaves can respond to changes in the incident irradiance spectrum by means of rapid physiological responses, and by slower acclimatory adaptations occurring over a range of time-scales. In this section the rapid physiological (1.2.1) and slower acclimatory responses (1.2.2) of the light-limited quantum yield of mature leaves to spectral changes will be reviewed separately, and the responses of light-saturated photosynthesis will be dealt with thereafter (1.2.3). Additionally, the role of photoreceptors in spectral acclimation (1.2.4) and the implications of leaf responses to spectral changes for photosynthesis measurements (1.2.5) will be briefly discussed.

1.2.1 Light-limited quantum yield of mature leaves: rapid responses to spectral changes

The light-limited quantum yield for CO₂ fixation or O₂ evolution is wavelength dependent (e.g. Hoover, 1937) and can change rapidly (in the order of one second) following changes in the incident irradiance spectrum. Different wavelengths are absorbed by leaves with different efficiencies and therefore the absorbed light fraction is wavelength dependent, with blue and red light being absorbed more efficiently by green leaves than green and FR light (e.g. McCree, 1972a). Second, the quantum yield of absorbed light is wavelength dependent due to the different absorption spectra of the different leaf pigments (as described in 1.1.1) and wavelength dependent ratios of excitation of the two photosystems (Terashima, 2009). If it is assumed that the photosystem stoichiometry in leaves is tuned to maximise the light use efficiency for photosynthesis, then a change in spectrum will usually lead to an imbalance in photosystem excitation which could reduce photosynthetic quantum yield (e.g. Pfannschmidt, 2005). The wavelength dependence of the light-limited quantum yield of leaf photosynthesis on an incident or absorbed light basis for a variety of broad-band spectrum grown species has been published by e.g. Balegh and Biddulph (1969), McCree (1972a), Inada (1976) and Evans (1987). However, the effects of growth-light spectrum on the wavelength dependence of photosynthetic quantum yield have not yet been explored in detail. Although some species and environment dependent variation in the wavelength dependence for photosynthetic quantum yield has been described (e.g. McCree, 1972), the highest quantum yields were always found for red light. The maximum absolute quantum yield in the absence of photorespiration has been found to vary little between C₃ species (Singsaas *et al.*, 2001) and was reported to be close to 0.093 CO₂ fixed per absorbed photon, as measured by Long *et al.* (1993), or 0.106 O₂ evolved per absorbed photon (Björkmann and Demmig, 1987). This is a lower quantum yield than the theoretical maximum of 0.125 CO₂ molecules fixed per absorbed photon, derived from a simplified view of light-harvesting, photochemistry, the Z-scheme and metabolism. The quantum yields of UV and blue wavelengths are, at least partly, reduced due to partial absorption by carotenoids and non-photosynthetic pigments (Terashima, 2009). The quantitative contribution of imbalances in photosystem excitation *in vivo* to wavelength dependent efficiency losses of quantum yield has not yet been elucidated. Qualitatively, it has been shown that over-excitation of PSII is significant where chlorophyll b has its absorption peaks (around 480 and 660 nm; Evans, 1987; Evans and Anderson, 1987), as relatively more chlorophyll b is associated with PSII than with PSI. Above 690 nm quantum yield is progressively lost due to over-excitation of PSI (e.g. Evans, 1987). Wavelengths beyond the range 350-730 nm are not considered to contribute significantly to plant photosynthesis.

As imbalances in photosystem excitation reduce quantum yield, a combination of wavelengths over-exciting PSI and wavelengths overexciting PSII can produce a photosynthetic rate which is higher than the sum of the two parts. This is called 'the Emerson enhancement effect' (Emerson *et al.*, 1957). Surprisingly, however, no evidence has been found for an enhancement of the quantum yield efficiency of broad-band light, compared with the weighted sum of the quantum yield efficiencies of each of the component wavelengths of the broad-band light (McCree, 1972b). Note that the wavelength range associated with the highest quantum yield measurements (i.e. red light) does not necessarily produce the most productive plants when applied as a growth-light (e.g. Brown *et al.*, 1995; Goins *et al.*, 1997; Yorio, 2001; Matsuda *et al.*, 2004; Ohashi *et al.*, 2006).

1.2.2 Light-limited quantum yield of mature leaves: acclimation to spectral changes

In the short term, quantum yield losses due to imbalances in photosystem excitation caused by changes in the irradiance spectrum can be counteracted, at least partly, by state transitions. State transitions occur in the order of minutes and are believed to involve redistribution of excitation energy between the photosystems via a mobile part of light harvesting complex II (LHCII), which is nominally a PSII antenna (Allen, 1992; Haldrup *et al.*, 2001). A reduced plastoquinone pool, which is expected to develop at a low irradiance when PSII is overexcited, leads to activation of a kinase which phosphorylates the mobile part of LHCII. Following phosphorylation, the mobile part of LHCII migrates from PSII and all, or part of the energy absorbed by the mobile LHCII is redirected to PSI (i.e. state 2; Bennett *et al.*, 1980; Horton and Black, 1981). If PSI is overexcited and therefore the plastoquinone pool is oxidized, the opposite reaction occurs, resulting in dephosphorylation of mobile LHCII and subsequent re-attachment to PSII (i.e. state 1). Note that in darkness LHCII is in state 1, so no state transitions occur when a dark-adapted leaf is illuminated with light preferentially exciting PSI (PSI-light). The extent to which state-transitions are significant in leaves is still a matter debate. For example, in spinach and pea chloroplasts a state 1-state 2 transition did result in dissociation of LHCII from PSII, but the absorption cross section of PSI did not increase (Haworth and Melis, 1983; Deng and Melis, 1986). However, Telfer *et al.* (1984) did report an increase in the PSI absorption cross section in pea chloroplasts following phosphorylation of LHCII. In wheat leaves a state 1-state 2 transition was associated with a decrease in PSII antenna size, but not with an increase in quantum yield for CO₂ fixation (Andrews *et al.* 1993). Therefore, although state transitions increase the efficiency with which photons absorbed by PSII are used for electron transport, it is uncertain whether state transitions ultimately function to increase the light use efficiency for photosynthesis in leaves.

If plants are exposed to a different spectrum for a longer period (hours to days), imbalances in excitation can also be counteracted by changes in photosystem stoichiometry (e.g. Anderson, 1995). While adjustments of photosystem stoichiometry in chloroplasts are believed to allow plants to retain a high quantum yield for photosynthesis in a changed spectral environment (Chow *et al.*, 1990), only a few studies have shown this principle for higher plants. *Pisum sativum* grown under PSI- and PSII-light had a higher quantum yield for O₂ evolution when illuminated with the spectrum under which they were grown, and the PSI:PSII ratio was more than two-fold higher in the thylakoids of the PSII-light grown leaves (Chow *et al.*, 1990). Likewise, an increased quantum yield for O₂ evolution was found in *Arabidopsis* leaves grown under 'white' and FR-enriched light when they were illuminated with the growth-light, and this was paralleled with a higher electron transport efficiency through PSI and PSII (Walters and Horton, 1995). No detailed studies, however, on the quantitative relationship between photosystem stoichiometry, photosystem efficiency balance *in vivo*, and leaf photosynthetic quantum yield have so far been published. Similarly, the consequences of acclimation to growth-light spectrum for the wavelength dependence of photosynthetic quantum yield, whether that be for CO₂ fixation or the efficiencies of PSI or PSII, has not yet been described. The wavelength dependence of the photosystem excitation balance has been estimated *in vitro* via absorption measurements of pigment-protein complexes (Evans and Anderson, 1987). Such an approach would allow some insight into the effects of acclimation to growth-light spectrum on the photosystem efficiency balance *in vivo*. However, the absorption balance

of pigment-protein complexes is not necessarily the same as a more functional photosystem efficiency balance. Discrepancies between the two approaches may occur due to processes *in vivo* which do not apply for absorption *in vitro*, such as inefficiencies in excitation energy transfer of photons absorbed by carotenoids, different efficiencies of charge separation, cyclic electron transport (e.g. Baker *et al.*, 2007), back-reactions (Quigg *et al.*, 2006) or electron transfer to O₂ (Pospíšil, 2009). An evaluation of the influence of such processes on the functionality of *in vitro* measurements for estimates of the *in vivo* photosynthetic operation would be valuable.

Using evidence derived from plant growth experiments, it has been suggested that state transitions and photosystem stoichiometry acclimation provide an evolutionary advantage for plants. An *Arabidopsis* mutant deficient in the kinase (STN7) required for both state transitions and photosystem stoichiometry acclimation produced 50% less seed than the wild type when grown under conditions where the growth-irradiance fluctuated between PSI- and PSII-light every 20 minutes or every 2-3 days (Wagner *et al.*, 2008). The wild-type was more productive under long-term fluctuations than under short-term fluctuations, which indicated that the capacity for re-balancing excitation energy distribution between the two photosystems is greater for photosystem stoichiometry acclimation than it is for state transitions.

Long term acclimation to changes in growth-light spectrum may also affect photosynthetic quantum yield due to changes in the carotenoid and non-photosynthetic pigment content in the leaf (as described in 1.1.1).

1.2.3 Photosynthesis of mature leaves at light-saturated irradiance: Rapid responses and acclimation to spectral changes

Not all of the factors that produce rapid changes in the light-limited quantum yield following changes in the incident irradiance spectrum (1.2.1) will produce comparable changes in the light-saturated rate of photosynthesis. Changes in the fraction of light absorbed, the relative absorption by pigments other than chlorophyll and imbalances in photosystem excitation, which have a large impact on the light-limited quantum yield for incident irradiance, are not expected to result in any change in the light-saturated rate of photosynthesis. For example, a greater absorption fraction following a change in spectrum can not increase the photosynthetic rate at light-saturation and possibly even have a negative effect due to an increased chance of photodamage (e.g. Kasahara *et al.*, 2002), whereas a smaller absorption fraction may only slightly decrease the photosynthetic rate as the irradiance level can become sub-saturating. Spectral effects on stomatal opening (see 1.1.2) would be expected to affect light-saturated photosynthesis under photorespiratory conditions, as carboxylation by Rubisco is a limiting factor under such conditions (e.g. Lawlor, 2001).

Long term acclimation of mature leaves to spectral changes is likely to be paralleled by changes in the content and composition of components that can be limiting for the photosynthetic capacity per unit leaf area, likewise growth-spectrum during leaf development (see 1.1.2). However, where the growth spectrum during leaf development also affects leaf anatomy (1.1.2), no large effects on the anatomy of mature leaves are expected following spectral changes. In the case of leaves with 'high irradiance' properties, photosynthetic capacity may decrease upon e.g. less blue light. On the other hand, more blue light may increase the photosynthetic capacity only to a certain extent, as the

intercellular space per unit leaf area is already determined in mature leaves, analogous to the transition of leaves from low to high irradiance reported by Oguchi *et al.* (2003) or Trouwborst *et al.* (submitted).

1.2.4 Role of photoreceptors in acclimation to spectral changes

Changes in the growth-irradiance spectrum may not only result in changes in cell signalling due to changes in the balance of photosystem excitation, but also as a result of changes in the excitation of non-photosynthetic photoreceptors (Dietzel *et al.*, 2005). The role that photoreceptor signalling plays in photosynthetic acclimation is not known in any detail. Studies with photoreceptor deficient mutants suggest that acclimation of photosystem stoichiometry does not depend on the presence of phytochromes or cryptochromes Walters *et al.*, 1999; Fey *et al.*, 2005). The acclimation of photosynthesis to the spectral environment of plants is explored in detail in this thesis, but not the possible interaction with processes mediated via photoreceptors.

1.2.5 Practical challenges in photosynthesis research related to rapid responses of leaf photosynthesis to spectral changes

It is noteworthy that red or mixed red/blue LEDs are commonly used as measuring-light source in modern photosynthesis-measuring devices. Photosynthesis measurements using such equipment, which are important for understanding the physiological functioning of plants, are also widely used as input for plant productivity models. Discrepancies between the spectral conditions in the environment where plants grow and those provided by the measuring equipment may result in a wrong estimate of photosynthetic rates *in situ*. Such discrepancies are generally not taken into account and neither have the consequences for the validity of the outcome of plant productivity models been explored. As the use and the quality of plant productivity models increases, then so does the demand on the validity of the basic model inputs. Ideally, the spectrum used during measurements should match the growth-light spectrum *in situ*. Practically this is, however, difficult to realize, especially because both in the field, and in glasshouses where daylight is combined with supplemental lighting, the spectrum over time is not stable. Methods to limit the discrepancy between measured photosynthesis and that occurring *in situ* would be valuable in order to improve the relevance of the measurements and their usefulness in predictive models for plant productivity.

1.3 Photomorphogenesis versus photosynthesis per unit leaf area: Consequences for biomass production

Spectral effects on photosynthesis per unit leaf area have been introduced in detail in the previous two sections. All other things being equal, a higher photosynthetic rate per unit leaf area will increase biomass production. Photosynthesis is not, however, the only processes to respond to the irradiance spectrum. Photomorphogenesis, phototropism and photonastic movements respond to the irradiance spectrum as well, mediated via phytochromes, cryptochromes, phototropines and possibly other as yet unidentified photoreceptors (Whitelam and Halliday, 2007). All these responses affect the light-interception of a plant and therefore the biomass production. Especially in an open

canopy, plant responses that affect the effectiveness of light interception may be even more important for biomass production than the photosynthetic rate per unit leaf area. In a dense canopy with a high leaf area index the light interception efficiency of an individual leaf will not significantly influence the biomass production of the canopy. Under such circumstances the photosynthetic efficiency per unit leaf area is the key factor for production.

A low R:FR ratio is a well known example of a spectrum inducing an overall shade-type morphology in a wide range of species, typically characterized by etiolation so that plants can reach above neighbouring plants (e.g. Grime, 1981). Individual plants can benefit from such a response as it increases the plant's light interception. Plant photomorphogenetic properties such as long petioles preventing self-shading, a large leaf area at the expense of leaf thickness and thus light-saturated photosynthesis, and a high leaf unfolding rate increase the light interception of an open canopy.

The consequences of the differences between natural daylight spectra and spectra produced by growth-lamps, such as fluorescent tubes, high pressure sodium lamps, metal halide lamps and LEDs, have not been explored in detail. Especially in older research light sources in growth-chambers were often combined with incandescent lamps in order to add FR wavelengths to the growth-light spectrum. The aim of such lighting modifications was to produce morphologically 'normal' appearing plants (see e.g. Deutch and Rasmussen, 1973). The lack of a growth-lamp producing a spectrum similar to that provided by the Sun makes it difficult to evaluate the consequences of differences between natural spectra and growth-lamp spectra for plant morphology and production. A comparison between plant responses to light provided by lamps and by natural daylight inherently implies an interaction between effects of light intensity and spectrum, as the intensity of natural light is not stable. This is a gap in the eco-physiological understanding of plant development. Spectral effects other than those on photosynthesis per unit leaf area are also not taken into consideration in greenhouse production models, despite the potentially large effects of supplemental lighting on plant properties affecting light interception and therefore productivity in an early growth stage.

Contents of this thesis

Chapter 2 describes the responses of photosynthesis and related developmental characteristics per unit area of *Cucumis sativus* leaves that were grown at an equal photon flux under seven different combinations of red and blue light provided by LEDs. Both qualitative spectral effects on the intrinsic photosynthetic functioning of the leaves and quantitative effects of the different blue light doses on photosynthetic capacity are analysed. Differences in photosynthetic capacity are quantitatively related to leaf properties such as pigment content, nitrogen content, LMA and stomatal functioning.

Chapter 3 focuses on the light-limited quantum yield of *Cucumis sativus* leaves grown under equal photon fluxes (400-700 nm) of artificial sunlight, artificial shadelight and blue light. In the first sub-chapter a simple method for the quantification of the light distribution within leaf chambers is described, which supports the materials and methods section of the second subchapter, and an analysis of the consequences of a heterogeneous light distribution for the validity of photosynthesis measurements is made. The second

sub-chapter shows the consequences of growth under irradiance-spectra exciting PSI and PSII in different ratios for the wavelength dependence of light-limited quantum yield for CO₂ fixation. The acclimation of photosynthetic quantum yield is related quantitatively to the photosystem efficiency balance *in vivo* and photosystem stoichiometry. Spectroscopic measurements *in vivo* and absorption measurements on photosystem supercomplexes are both used to calculate the wavelength dependence of the photosystem excitation balance after acclimation to different growth-light spectra and the agreement between the results is discussed.

Chapter 4 shows the consequences of a photosynthesis measuring-light spectrum deviating from the plant's growth-light spectrum on the validity of measured photosynthetic rates and the consequences for the outcome of plant productivity models using such measurements as input.

Chapter 5 demonstrates the differences in photosynthesis per unit leaf area in combination with the morphological properties of *Cucumis sativus* grown under different lamp types. The consequences for biomass production are analysed. An artificial solar spectrum is used in one of the treatments, which produces surprising differences in plant development compared to the other lamp types. The results are relevant to the interpretation of research using climate room grown plants and offer perspectives for supplemental lighting in protected cultivation.

Chapter 6 is the general discussion. The research results described in chapter 2-5 are placed in a more general (eco)-physiological perspective and practical implications of this study are discussed.

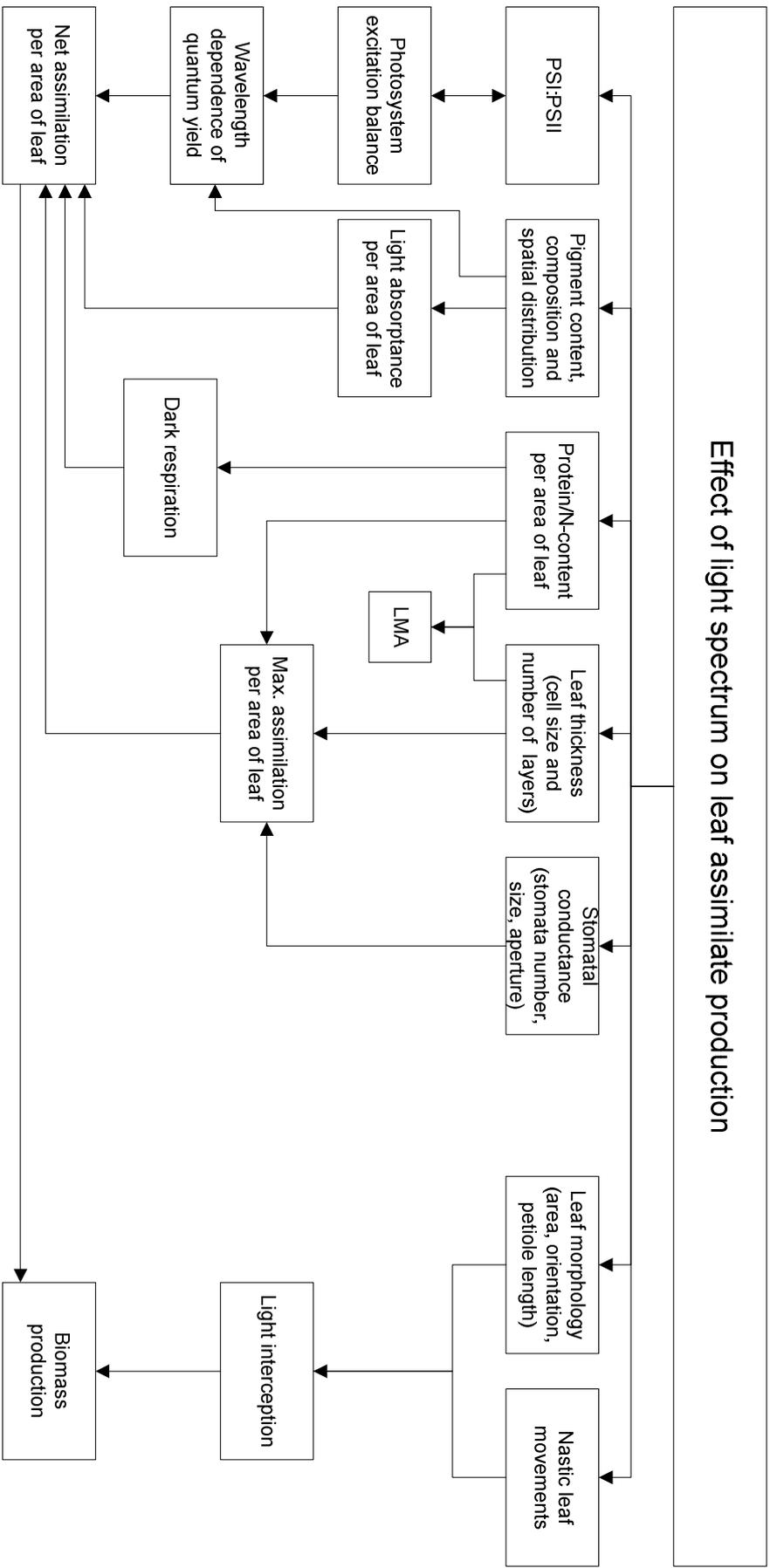


Figure 1. Schematic overview of the responses of leaf functioning to the spectral environment and its consequences for leaf assimilate production.

CHAPTER 2

Blue light dose-responses of leaf photosynthesis, morphology, and chemical composition of *Cucumis sativus* grown under different combinations of red and blue light

Abstract

The blue part of the light spectrum has been associated with leaf characteristics which also develop under high irradiances. In this study blue light dose-response curves were made for the photosynthetic properties and related developmental characteristics of cucumber leaves that were grown at an equal irradiance under seven different combinations of red and blue light provided by light emitting diodes. Only the leaves developed under red light alone (0% blue) displayed a dysfunctional photosynthetic operation, characterized by a sub-optimal and heterogeneously distributed dark-adapted F_v/F_m , a stomatal conductance unresponsive to irradiance and a relatively low light-limited quantum yield for CO₂ fixation. Only 7% blue light was sufficient to prevent any overt dysfunctional photosynthesis, which can be considered a qualitatively blue light effect. The photosynthetic capacity (A_{max}) was two times higher for leaves grown at 7% blue compared with 0% blue and continued to increase with increasing blue percentages during growth measured up to 50% blue. At 100% blue A_{max} was lower but photosynthetic functioning was normal. The increase in A_{max} with blue percentage (0-50%) was associated with an increase in leaf mass per unit leaf area (LMA), N content per area, Chl content per area and stomatal conductance. Above 15% blue the parameters A_{max} , LMA, Chl content, photosynthetic N use efficiency and the Chl:N ratio had a comparable relationship as reported for leaf responses to irradiance intensity. It is concluded that blue light during growth is qualitatively required for normal photosynthetic functioning and quantitatively mediates leaf responses resembling those to irradiance intensity.

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Introduction

Plant development and physiology are strongly influenced by the light spectrum of their growth environment. The underlying mechanisms of the effect of different growth-spectra on plant development are not known in detail, although the involvement of photoreceptors has been demonstrated for a wide range of spectrum-dependent plant responses. Cryptochromes and phototropins are specifically blue-light sensitive, whereas phytochromes are more sensitive to red than to blue (Whitelam and Halliday, 2007). Blue light is involved in a wide range of plant processes such as phototropism, photomorphogenesis, stomatal opening and leaf photosynthetic functioning (Whitelam and Halliday, 2007). At the chloroplast level blue light has been associated with the expression of 'sun-type' characteristics such as a high photosynthetic capacity (Lichtenthaler *et al.*, 1980). Most studies assessing blue light effects on leaf or whole plant level have either compared responses to a broad-band light source with responses to blue-deficient light (e.g. Britz and Sager, 1990; Matsuda *et al.*, 2008), or compared plants grown under blue or a combination of red and blue light with plants grown under red light alone (e.g. Brown *et al.*, 1995; Bukhov *et al.*, 1995; Yorio, 2001; Matsuda *et al.*, 2004; Ohashi *et al.*, 2006). Overall there is a trend to higher biomass production and photosynthetic capacity in a blue light containing irradiance. Before the development of light emitting diodes (LEDs) that were intense enough to be used for experimental plant cultivation (Tenessen *et al.*, 1994), light sources emitting wavelengths in a broader range than strictly the red (i.e. 600-700 nm) or blue (i.e. 400-500 nm) region were often used (e.g. Voskresenskaya *et al.*, 1977). Other wavelengths can interact with blue light responses. For example, green light has been reported to antagonize some blue light responses, such as stomatal opening and inhibition of hypocotyl elongation in seedlings (Folta and Maruhnich (2007). The blue light enhancement effect on photosynthetic capacity appears to be greater when using combinations of red and blue light produced by LEDs than when broad-band light is made deficient in blue by a filter (e.g. for spinach compare Matsuda *et al.*, 2007 and 2008). This raises the question whether plants exposed to red light alone suffer a spectral 'deficiency' syndrome, which may be undone by blue light as well as by longer wavelengths.

Poorter *et al.* (2010) stress the importance of dose-response curves for quantitative analysis of environmental factors on plant phenotypes, allowing a better understanding of plant-environment interactions than the comparison of two treatments only. It is not clear whether the enhancement effect of blue light on leaf photosynthetic capacity is a qualitative threshold response or a quantitative progressive response, or a combination of both. Only few specific processes in leaves have been identified as quantitative blue light responses, such as chloroplast movement (Jarillo *et al.*, 2001) and stomatal conductance (Sharkey and Raschke, 1981). Matsuda *et al.* (2007) found a higher photosynthetic capacity for spinach leaves grown under $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ mixed red/blue irradiance containing $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ blue than for leaves grown under red alone. A higher blue light fraction did not yield a significant further enhancement in A_{max} , which may be interpreted as a qualitative blue light effect. However, a quantitative blue light effect at quantum fluxes below $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ cannot be excluded.

A diverse choice of LEDs powerful enough for use as a growth-irradiance source in controlled environments has recently become available (e.g. Massa *et al.*, 2008). These LEDs allow the effect of light quality to be investigated independently of the amount of

photosynthetic irradiance. We have used LED illumination to study the response curves of a range of parameters related to leaf photosynthesis of plants that were grown at an irradiance with a proportion of blue light ranging from 0 to 100%. We also determined a range of other leaf characteristics important for the functioning of photosynthesis, such as stomatal development and behaviour, leaf mass per area (LMA), and the content of N, pigments and carbohydrates. The spectra and the extent of variation in the ratio of red and blue irradiance that can be achieved with LED lighting are dissimilar to field conditions. However, the responses of leaves to these unnatural environments enables the possibility to unravel the complex developmental and functional interactions that normally occur in the natural light environment.

Materials and methods

Plant material and growth conditions

Cucumber plants (*Cucumis sativus* cv. Hoffmann's Giganta) were sown in vermiculite and germinated under $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ cool white fluorescent lamps (TLD 50W 840 HF, Philips, The Netherlands) in a climate chamber. After one week, when the cotyledons had just opened, the seedlings were transferred to a hydroponic system (Hoagland's solution, pH = 5.9 ± 0.2 ; EC = 1.2 mScm^{-1}) in a climate chamber. The day/night-temperature was $25 \text{ }^\circ\text{C}/23 \text{ }^\circ\text{C}$, the relative humidity was 70% and the CO_2 concentration was ambient. All plants were subjected to $100 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$ irradiance (16 h/8 h day/night) provided by a mixture of blue and red LEDs with dominant wavelengths of 450 and 638 nm, respectively (types Royal Blue and Red Luxeon K2, Lumileds Lighting Company, San Jose, Ca. USA). The LEDs were equipped with lenses (6° exit angle) and the arrays were suspended about one meter above the plants, so irradiance from the two LED types was well mixed. The lenses ensured that small differences in leaf height had only minor effects on the irradiance received. The seven different spectral treatments are expressed as the blue (B) light percentage: 0B, 7B, 15B, 22B, 30B, 50B and 100B; the remaining percentage was red. Irradiance was measured routinely using a quantum sensor (LI-COR, Lincoln, Nebraska USA), but was also verified with a spectroradiometer (USB2000 spectrometer, Ocean Optics, Duiven, The Netherlands, calibrated against a standard light-source). The difference in irradiance measured with the two devices was $< 2 \%$ for the spectra used.

The plants were allowed to grow until the second leaf was fully mature (17-22 days after planting the seedlings) when it could be used for photosynthesis measurements. If necessary, the second leaf, which was the leaf used for all measurements, was supported in a horizontal position during growth to ensure that it received the specified irradiance.

Stomata analysis

The stomatal conductance (g_{sw}) was measured on three positions on each leaf surface using a leaf porometer (model SC-1, Decagon Devices, Inc, Pullman, WA, USA) prior to the gas-exchange measurements (see below). The ratio of the average g_{sw} of the abaxial and adaxial leaf surface (g_{sw} ratio) was used in the calculations of the gas exchange parameters ($n=6$). Additionally, silicon rubber impressions were made (see Smith *et al.*, 1989) on both the ad- and abaxial surface of the leaves grown under 0B, 15B, 30B and 50B ($n \geq 3$). Stomatal

density, length and aperture were determined from images of the impressions using the procedure described in Nejad and van Meeteren (2005).

Leaf gas exchange and fluorescence measurements

Gas exchange and chlorophyll fluorescence were measured using a custom made leaf chamber within which 4.52 cm² of leaf surface was illuminated. A LI-7000 CO₂/H₂O gas analyzer (LI-COR, Lincoln, Nebraska USA) measured the CO₂ and H₂O exchange of the leaf and ambient atmospheric pressure. Leaf temperature was monitored by a thermocouple pressed against the abaxial leaf surface. A custom made measuring light source comprised of independently controllable red and blue LEDs with attached lenses, emitting a spectrum similar to that of the LEDs used for growth light, was used to provide the required red/blue combination in the irradiance range 0-1700 μmol m⁻² s⁻¹. A polished steel reflector in the form of an inverted truncated cone (i.e. the inlet to the reflector was larger than the outlet) allowed the irradiance to be well mixed and equally distributed over the leaf surface. The gas mix used contained 380 μmol mol⁻¹ CO₂, 20.8 ± 0.4 mmol mol⁻¹ H₂O and either 210 or 20 mmol mol⁻¹ O₂ (ambient O₂ or low O₂), dependent on the type of measurement. A flow rate of 200-700 ml min⁻¹ was used, depending on the CO₂ depletion which ranged from 18 to 26 μmol mol⁻¹ at saturating irradiance. The equations developed by von Caemmerer and Farquhar (1981) were used to calculate assimilation, g_{sw}, and the CO₂ concentration in the sub-stomatal cavity of the leaf relative to that in the leaf chamber air (C_i:C_a⁻¹) from the gas-exchange data. The boundary layer resistance of both leaf surfaces in the leaf chamber during gas exchange measurements was estimated using the method of Jarvis (1971). Chlorophyll fluorescence was measured using a PAM 101 chlorophyll fluorometer with an emitter detector unit (model 101 ED; Heinz Walz, Effeltrich, Germany). The modulated red measuring-light intensity was <0.5 μmol m⁻² s⁻¹. A 250 W quartz-halogen lamp connected to an additional optical fiber provided a saturating light pulse (7500 μmol m⁻² s⁻¹) to allow measurement of the F_m or F_m' relative fluorescence yield (Baker *et al.*, 2007). The fibers were fixed about four centimeter above the leaf chamber at such an angle that they did not interfere with the actinic light beam.

Irradiance response curves were measured on fully expanded second leaves and each growth-light treatment was performed twice. As there were no significant differences between the two repetitions, the individual plants from the two repetitions were treated as independent repetitions (n=6) in the analysis. An ambient O₂ concentration was used for these measurements. After clamping a leaf in the leaf chamber, it was dark-adapted for 30 min and dark-respiration (R_{dark}) and the dark-adapted F_v/F_m (Baker *et al.*, 2007) were measured. The irradiance-response curve was measured using a spectrum identical to that under which the plants were grown, using 14 intensities in the range 0-1700 μmol m⁻² s⁻¹. The leaves were subjected to each irradiance for at least 20 minutes, when steady-state assimilation was amply reached. The highest irradiances were omitted if CO₂ fixation clearly became light-saturated at lower irradiances. At an irradiance of 100 μmol m⁻² s⁻¹, which is equal to the irradiance during growth, the relative quantum yield of PSII electron transport (Φ_{PSII}) was measured using the method of Genty *et al.* (1989). After measuring the irradiance response curve, the plant was left over-night in the dark in a climate room and the following day samples were taken from the measured leaf in order to measure the light absorptance spectrum, leaf mass per area (LMA), and pigment- and N-content (see below).

In order to assess the possibility that C_i was limiting assimilation at low irradiance, the relationship between assimilation and electron transport rate (ETR) was investigated in more detail. Under photorespiratory conditions a lower assimilation per unit ETR is expected for a leaf with a C_i that is limiting for assimilation than for a leaf with no limiting C_i . Under non-photorespiratory conditions no difference is to be expected (Harbinson *et al.*, 1990). Additional gas exchange and fluorescence measurements were made on leaves grown under 0B and 30B using seven different incident irradiances ($0-100 \mu\text{mol m}^{-2} \text{s}^{-1}$) and both ambient and low O_2 ($n=3$). Chlorophyll fluorescence measurements were made at each irradiance to determine Φ_{PSII} once CO_2 fixation had stabilized, after which the actinic irradiance was switched off to measure R_{dark} . Gross assimilation (A_{gross}) was calculated as net assimilation (A_{net}) plus R_{dark} , which assumes, as is commonly done, that R_{dark} is a reasonable estimate of respiration in the light. Light absorbance (see below) was measured directly after measuring the photosynthesis-irradiance response. The product of the absorbed actinic irradiance and Φ_{PSII} serves as an index for ETR (e.g. Kingston-Smith *et al.*, 1997). The distribution of dark-adapted F_v/F_m over these 0B and 30B grown leaves was measured by means of chlorophyll fluorescence images. Images of three different leaves from each treatment were made using a PSI Fluorcam 700MF chlorophyll fluorescence imaging system (PSI, Brno, Czech Republic), using the procedure described in Hogewoning and Harbinson (2007).

Measurement of leaf light absorbance

Leaf light-absorbance was calculated in one nm steps in the range 400-800 nm from measurements of leaf reflectance and transmittance made on 12 leaf discs per leaf. Details of the procedure and measurement system, which consisted of two integrating spheres, each connected to a spectrometer and a custom made light source, are described in Hogewoning *et al.* (2010a) and Zheng *et al.* (2010). The integrated absorbance of the actinic measuring irradiance used during gas exchange measurements was subsequently calculated by multiplying the relative leaf absorbance spectrum with the spectrum of the measuring-light.

LMA, nitrogen, pigment and carbohydrate analysis

From each leaf, ten leaf discs (1.28 cm^2) were cut randomly over the leaf area, avoiding the leaf margins and main veins. The discs were stored at $-22 \text{ }^\circ\text{C}$, freeze dried and weighed, and LMA was calculated. After weighing, the C and N content were determined for all treatments by C/N-analyzer ($n=5$) and the nitrate content was determined for the treatments 0B and 30B ($n=4$) according to Trouwborst *et al.* (2010).

An additional eight leaf discs (0.65 cm^2) were cut from the same leaf and stored in 10 ml DMF in dark at $-22 \text{ }^\circ\text{C}$. The absorbance of the extract was measured in the range 400-750 nm using a Cary 4000 spectrophotometer (Varian Instruments, Walnut Creek, Ca, USA) and the chlorophyll and carotenoid concentrations were calculated using the equations of Wellburn (1994).

The carbohydrate content of leaves grown under 0B, 30B and 100B was measured by cutting 10-15 discs (1.28 cm^2) from one side of the main vein at the end of the photoperiod and 10-15 discs from the other side of the main vein just before the start of the photoperiod ($n=4$). Soluble carbohydrate and starch concentrations were analyzed as described in Hogewoning and Harbinson (2007).

Curve fitting and statistics

The photosynthesis data measured to obtain light-response curves of the leaves grown under different blue/red combinations were fitted with a non-rectangular hyperbola (Thornley, 1976) using the non-linear fitting procedure NLIN in SAS (SAS Institute Inc. 9.1, Cary, NC, USA) in order to determine the light-limited quantum yield for CO₂-fixation (α).

Tukey's HSD was used to make post-hoc multiple comparisons among spectral treatment means from significant one way ANOVA tests ($P < 0.05$) and regression analysis was used to test for significant differences ($P < 0.05$) between the slope of the $A_{\text{gross}} - \Phi_{\text{PSII}} \cdot \text{absorbed}$ measuring-light relationship using Genstat (release 9.2, Rothamsted Experimental Station, Harpenden, UK).

Results

Leaf photosynthesis

The light-saturated net assimilation (A_{max}) significantly differed for the leaves grown under different blue (B) light percentages (Fig. 1). Increasing the blue light fraction from 0% to 50% resulted in an increasing A_{max} , with the greatest increase occurring at the increase from 0% to 7% blue. The 100B grown leaves had an A_{max} that was lower than that of the 50B leaves. The light-limited quantum yield for CO₂-fixation (α) was lowest for 0B and 100B leaves and highest for the 7B- 30B leaves (within this range there was no significant difference in α ; Table 1). Dark respiration was lowest for 0B leaves and tended to increase with blue light percentage, except for 100B (Table 1), similar to the pattern found for A_{max} .

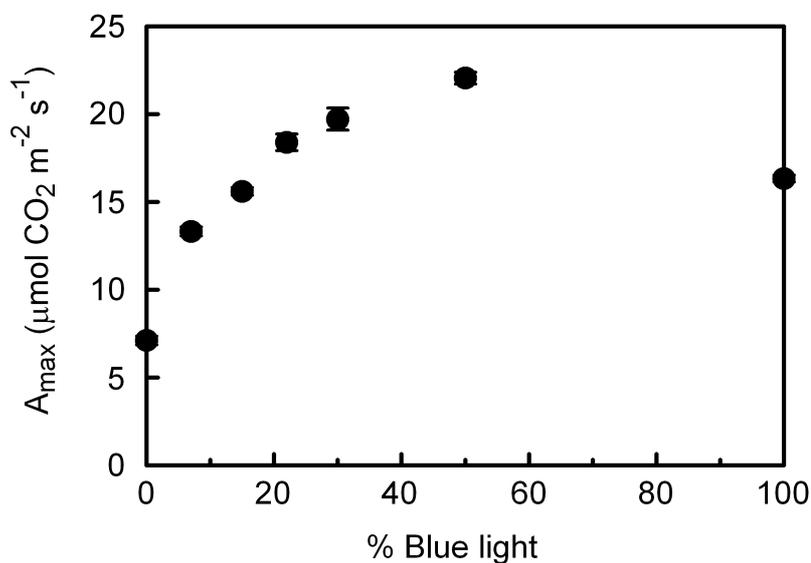


Fig. 1. The effect of light quality (the proportion of total PAR that is from the blue rather than from the red part of the spectrum) during growth on the photosynthetic capacity (A_{max}) of cucumber leaves. Error bars indicate the s.e.m. ($n=6$).

Table 1. Different parameters measured or calculated on leaves grown under different light qualities (the proportion of total PAR that is from the blue rather than from the red part of the spectrum). Different letters indicate significant differences ($P \leq 0.05$; $n=5$ or $n=6$, no variation for PSS).

Blue light percentage	0	7	15	22	30	50	100
F_v/F_m	0.76 ^b	0.80 ^a	0.80 ^a	0.80 ^a	0.81 ^a	0.81 ^a	0.81 ^a
Φ_{PSII}	0.65 ^d	0.74 ^c	0.76 ^b	0.76 ^{ab}	0.76 ^{ab}	0.77 ^a	0.76 ^{abc}
$F_v/F_m - \Phi_{PSII}$	0.110 ^a	0.055 ^b	0.044 ^c	0.040 ^c	0.042 ^c	0.034 ^c	0.044 ^{bc}
Quantum yield CO ₂ fixation	0.045 ^c	0.052 ^{ab}	0.053 ^a	0.053 ^a	0.053 ^a	0.048 ^{bc}	0.045 ^c
R_{dark} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.93 ^d	1.17 ^c	1.29 ^{abc}	1.39 ^{ab}	1.27 ^{bc}	1.45 ^a	1.33 ^{abc}
g_{sw} ratio (abaxial: adaxial)	2.7 ^a	2.6 ^a	2.1 ^{ab}	1.7 ^{bc}	1.7 ^{bc}	1.4 ^c	1.7 ^{bc}
Integrated absorptance	90.0 ^d	92.1 ^c	92.4 ^{bc}	93.1 ^{bc}	94.0 ^{ab}	93.7 ^b	95.4 ^a
Chl-a: Chl-b (g g^{-1})	3.24 ^d	3.36 ^c	3.51 ^{ab}	3.48 ^{ab}	3.42 ^{bc}	3.54 ^a	3.54 ^a
N (% DW)	5.7 ^a	6.0 ^a	5.7 ^a	6.0 ^a	6.1 ^a	6.0 ^a	6.2 ^a
C (% DW)	39.6 ^a	38.0 ^a	36.8 ^a	38.7 ^a	37.7 ^a	37.6 ^a	37.7 ^a
C:N (g g^{-1})	6.9 ^a	6.4 ^{ab}	6.5 ^{ab}	6.4 ^{ab}	6.2 ^b	6.2 ^b	6.1 ^b
Chl: N (g g^{-1})	5.1 ^a	4.3 ^{bc}	4.6 ^{ab}	4.1 ^{bcd}	4.3 ^{bc}	3.9 ^{cd}	3.7 ^d
PSS (phytochromes)	0.89	0.89	0.89	0.89	0.88	0.87	0.51

The dark adapted F_v/F_m was typical for an unstressed leaf (i.e. ≥ 0.8) in all treatments, except 0B, where it was significantly reduced (Table 1). The Φ_{PSII} measured at growth-light intensity (i.e. $100 \mu\text{mol m}^{-2} \text{s}^{-1}$) and spectrum was similar for the 15B- 100B leaves, but was markedly lower for 0B leaves and slightly, but significantly, lower for 7B leaves.

Concerning the more detailed measurements of the photosynthesis-irradiance response between 0 and $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ incident irradiance on 0B and 30B grown leaves, gross assimilation (A_{gross}) was markedly higher for the low O₂ measurements than it was for the ambient O₂ measurements (Fig. 2). At all light intensities Φ_{PSII} was consistently lower for the 0B leaves than it was for the 30B leaves. In both treatments the O₂ concentration did not affect Φ_{PSII} (not shown). The absorptance in the green region of the spectrum was 5-10% lower for the 0B and 100B grown leaves than for the other treatments, whereas differences in absorptance between the growth-light treatments were negligible for the blue and red region (not shown). Only the red and blue wavelength regions are relevant for integrated absorbed irradiance in this experiment. The integrated absorptance of the growth- and measuring-light increased with the percentage of blue light (Table 1), as the blue light was better absorbed than the red light. At both low and ambient O₂ concentration there were no significant differences between 0B and 30B for the linear regression between A_{gross} and the product of Φ_{PSII} and absorbed actinic irradiance (Fig. 2).

The images of dark-adapted F_v/F_m obtained via chlorophyll fluorescence imaging showed conspicuous differences between the 0B and 30B leaves. Whereas the images from 30B grown leaves were perfectly homogeneous with an $F_v/F_m > 0.8$, the images of the 0B

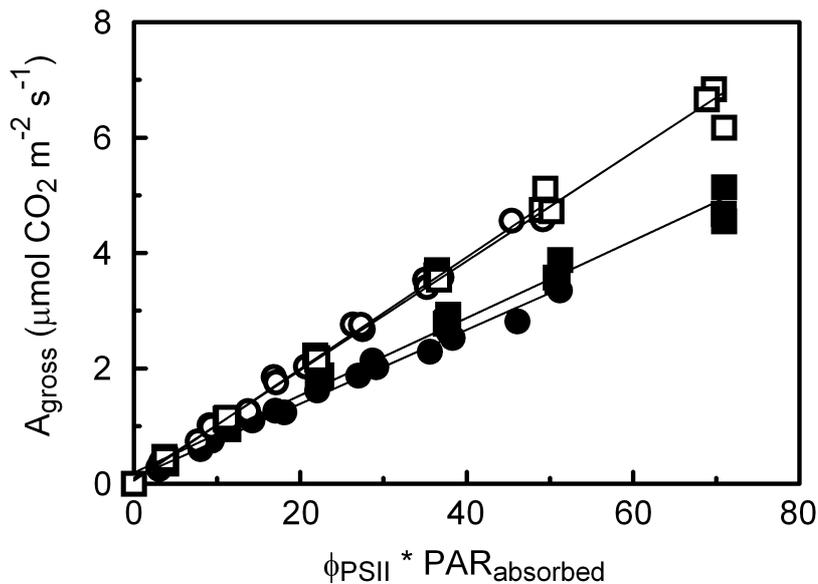


Fig. 2. Relationship between gross CO₂ assimilation (A_{gross}) and the product of Φ_{PSII} and the actinic measuring-light absorbed by the leaves, which serves as an index of electron transport (e.g. Kingston-Smith *et al.*, 1997), at an incident irradiance $\leq 100 \mu\text{mol m}^{-2} \text{s}^{-1}$. The cucumber leaves were grown under and also measured with 0B (=100% red; circles) and 30B (squares) irradiance and gas exchange was measured under low (open symbols) and ambient O₂ (closed symbols). Gross assimilation was calculated as dark respiration plus net assimilation. The slopes of the regression lines are significantly different for the two O₂ levels ($P < 0.001$), but not for the spectral treatments ($P \geq 0.23$).

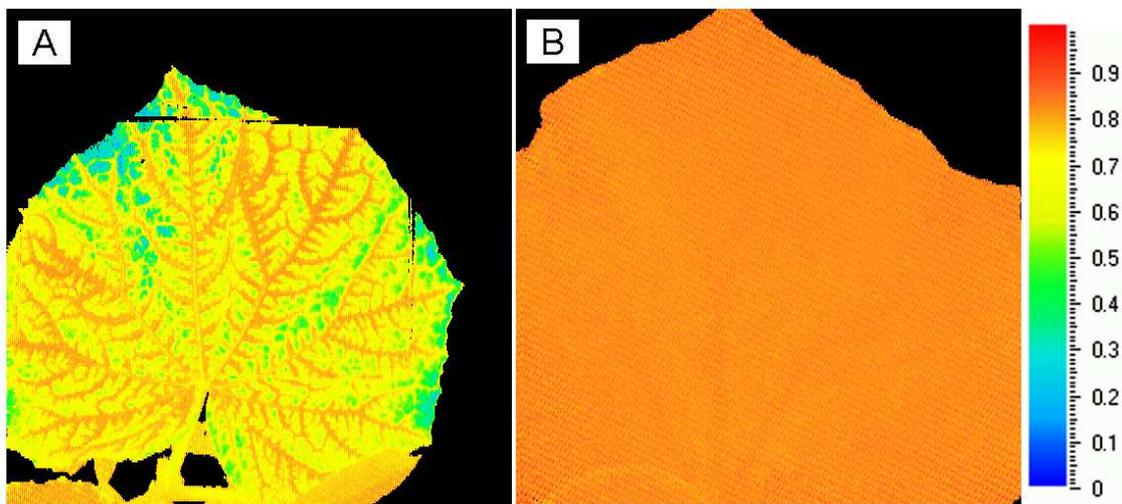


Fig. 3. Image of the dark-adapted F_v/F_m distribution over a 0B (=100% red; A) and 30B (B) irradiance grown cucumber leaf. The mixed blue-red grown leaf (B) has a homogeneous F_v/F_m distribution centered around an F_v/F_m of 0.82, whereas the 0B grown leaf (A) has a heterogeneous distribution with a high F_v/F_m around the veins and lower values between the veins.

grown leaves showed a heterogeneous distribution with dark-adapted F_v/F_m values of around 0.8 adjacent to the veins and with zones of lower F_v/F_m (typically 0.55- 0.70) between the veins (Fig. 3). The 0B leaves also occasionally appeared slightly chlorotic between the veins.

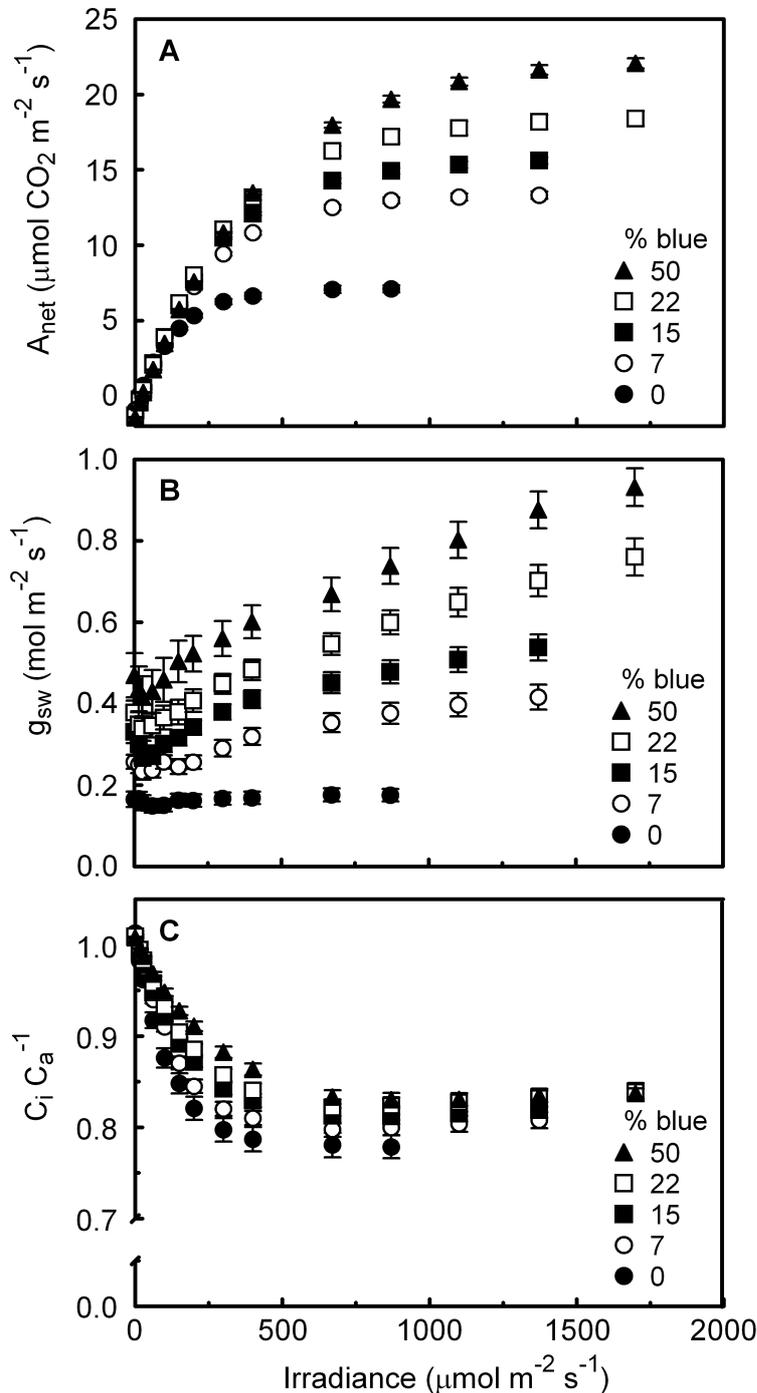


Fig. 4. Response of net assimilation (A_{net} ; A), stomatal conductance (g_{sw} ; B) and leaf internal CO_2 concentration relative to that of the leaf chamber air (C_i/C_a ; C) to irradiance for cucumber leaves grown under different light qualities (the proportion of total PAR that is from the blue rather than from the red part of the spectrum). The actinic-light quality was identical to that during growth. Error bars indicate the s.e.m. (n=6).

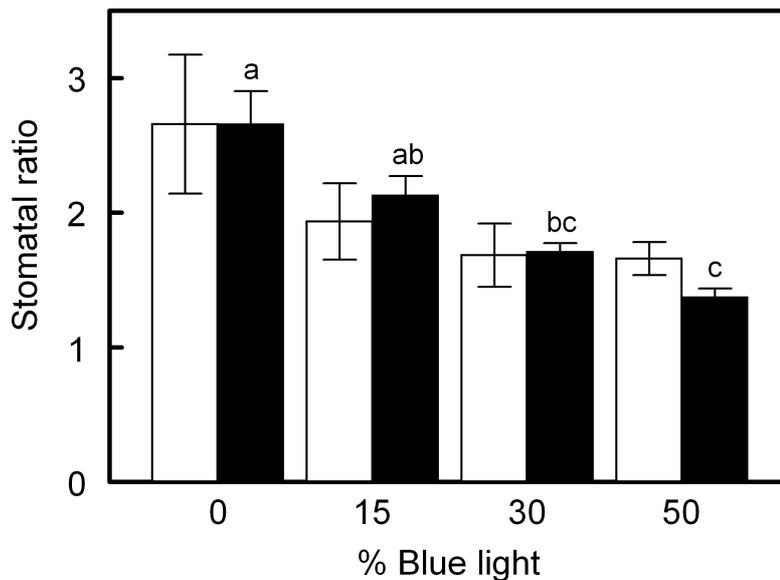


Fig. 5. Ratio of stomatal density (open bars; $n \geq 3$) and stomatal conductance measured with a porometer (filled bars; $n=6$) for the abaxial and adaxial leaf surface of cucumber leaves grown under different light qualities (the proportion of total PAR that is from the blue rather than from the red part of the spectrum; both parameters are labeled 'stomatal ratio' in the plot). Error bars indicate the s.e.m. and letters indicate significant differences ($P \leq 0.05$). No significant differences between the individual means of the stomatal density ratio were found, however, the linear component of the stomatal density ratio-blue light percentage relationship was significant ($P = 0.04$). The decrease in stomatal density ratio with increasing blue light percentage was due to an increasing stomatal density on the adaxial leaf surface.

Stomatal effects

There was a considerable stomatal conductance (g_{sw}) calculated from gas-exchange data in dark-adapted state (Fig. 4B). As the photoperiod of the plants in their growth-environment started 1 h before leaves were dark-adapted in the leaf-chamber, the absence of complete stomatal closure may be due to the diurnal rhythm of the stomata. Also, a significant nighttime g_{sw} is not unusual, especially for leaves with a high daytime g_{sw} (Snyder *et al.*, 2003), such as cucumber. Moreover, a substantial nighttime g_{sw} has been reported to occur in many horticultural species and ample water availability (e.g. hydroponics as used here) can increase nighttime g_{sw} (Caird *et al.*, 2007). The g_{sw} of leaves grown and measured using 0B was lowest of all the treatments and did not respond to increases in measuring-irradiance intensity. Even using 30B or 100B as a measuring-irradiance spectrum on the 0B grown leaves at either $100 \mu\text{mol m}^{-2}\text{s}^{-1}$ irradiance or saturating irradiance had no effect on their g_{sw} (data not shown). In all other treatments g_{sw} increased with increasing irradiance ($> 100 \mu\text{mol m}^{-2}\text{s}^{-1}$). Consistent with the low and constant g_{sw} , the $C_i C_a^{-1}$ of the 0B grown leaves decreased more with increasing irradiance than that of the other treatments (Fig. 4C). Data of g_{sw} and $C_i C_a^{-1}$ for the 30B and 100B leaves are not shown in Fig. 4 due to instrument failure.

The g_{sw} measured using a porometer also increased with increasing blue light in the growth spectrum (not shown). The ratio of g_{sw} on the abaxial and the adaxial leaf

surface (g_{sw} ratio) became smaller with increasing percentage of blue light (Table 1). The stomatal counts on both leaf sides paralleled these results, as the number of stomata on the adaxial leaf surface significantly increased with increasing blue percentage, whereas on the abaxial leaf surface no significant changes were found (not shown), resulting in a decreasing stomatal ratio with increasing blue light (Fig. 5). No significant changes in stomatal length and guard cell width were found for the different treatments (not shown).

LMA and nitrogen, pigment and carbohydrate content

The LMA increased with increasing percentage of blue up to 50% (Fig. 6A). Similar to the A_{max} - blue percentage relationship (Fig 1), the increase in LMA was relatively greatest when the growth irradiance was changed from 0% blue to 7% blue. The total chlorophyll content (Chl a + Chl b; Fig. 6A) and total carotenoid content (not shown) per unit leaf area increased in a similar way to LMA, increasing with percentage blue up to 50%. The Chl a:b ratio was significantly lower for 0B and 7B than at higher blue percentages (Table 1).

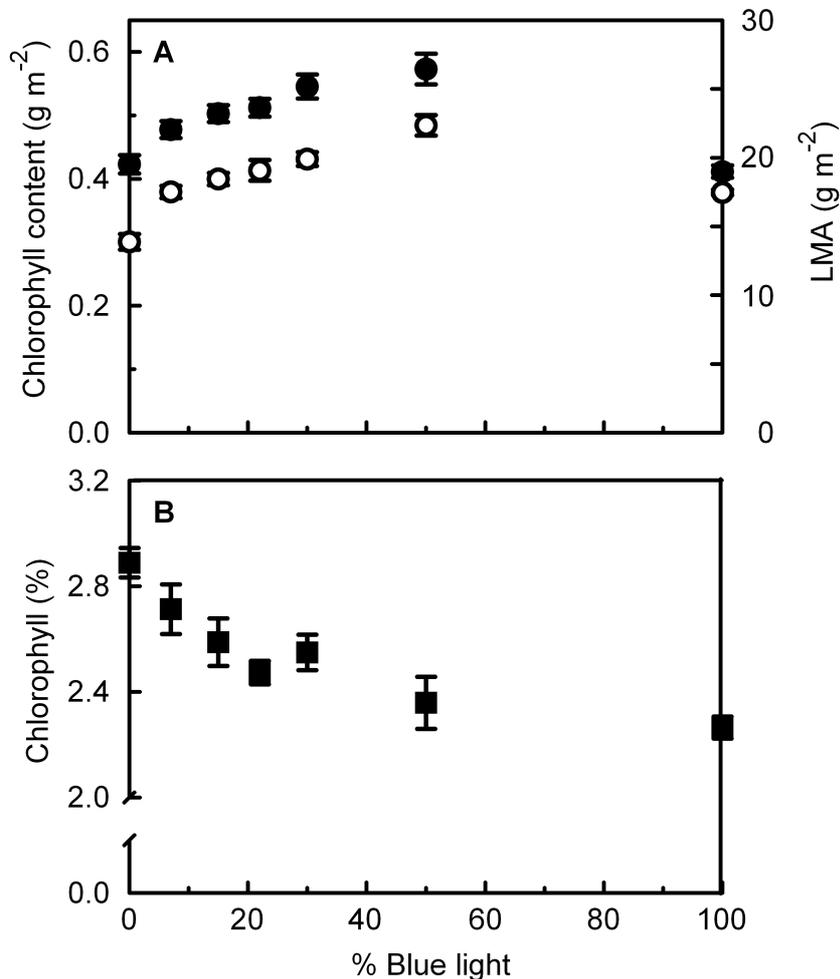


Fig. 6. The effect of light quality (the proportion of total PAR that is from the blue rather than from the red part of the spectrum) during growth on the chlorophyll content per unit leaf area (A, closed symbols, left axis), leaf mass per unit leaf area (LMA; A, open symbols, right axis) and the percentage chlorophyll in the leaf on a dry weight basis (B, squares).

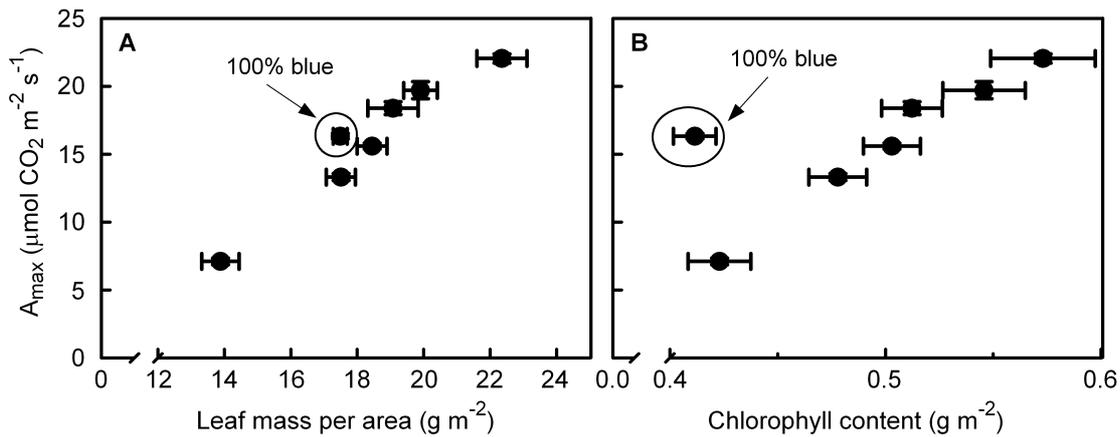


Fig. 7. Relationship of leaf photosynthetic capacity (A_{max}) with leaf mass per unit leaf area (A) and chlorophyll content per unit leaf area (B) of cucumber grown under different combinations of red and blue light at an equal irradiance. The order of the values related to the data-points correspond with the blue light percentage the leaves were grown under, except for the encircled data-point which refers to the 100% blue treatment.

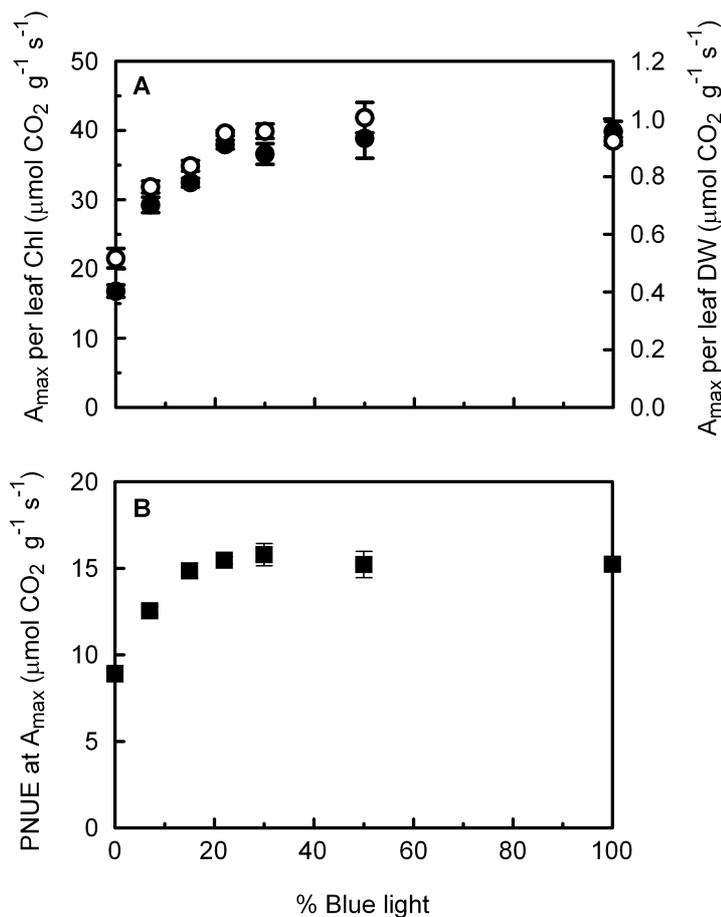


Fig. 8. The effect of light quality (the proportion of total PAR that is from the blue rather than from the red part of the spectrum) during growth of cucumber on leaf photosynthetic capacity (A_{max}) reached per unit chlorophyll (A, closed symbols, left axis), per unit leaf dry weight (A, open symbols, right axis) and per unit N (B, squares).

Leaf N content and C content per unit DW did not differ significantly between the treatments (Table 1). When expressed per unit leaf area the N- and C content therefore depended on the percentage blue light in a way that was similar to LMA (Fig 6A). The C:N ratio however was significantly higher for the 0B treatment than it was for the 30B, 50B and 100B treatments. The nitrate part of total leaf N was not significantly different for the 0B and 30B leaves and was only 8.8% and 6.4%, respectively.

Chlorophyll content per unit leaf area correlates well with LMA (Fig. 6A), though there is a small but significant decrease in the Chl content per unit leaf DW as the percentage blue light in the growth irradiance increases (Fig. 6B). For all treatments A_{max} correlated positively with LMA and Chl content per area leaf, except for Chl content of the 100B leaves (Fig. 7). With an increasing percentage blue light during growth A_{max} per unit Chl increases up to 22% blue, whereas at higher percentages blue there are no differences between the treatments (Fig. 8A). A similar pattern can be seen for A_{max} per unit leaf DW (Fig. 8A) and A_{max} per unit N, which is the photosynthetic N use efficiency (PNUE; Fig. 8B). On a DW basis, the Chl: N ratio decreases significantly with increasing percentage blue (Table 1).

The leaf carbohydrate content (on a unit weight basis) was negligibly low at the end of the night period for all treatments (Table 2). At the end of the photoperiod a considerable amount of carbohydrates, which were mainly comprised of starch and smaller quantities of sucrose, was present in the leaves, with highest values in the leaves grown under 30% blue light.

Table 2. Carbohydrate content (mg g^{-1} DW) of leaves grown under different light qualities (the proportion of total PAR that is from the blue rather than from the red part of the spectrum). Different letters indicate significant differences ($P \leq 0.05$; $n=4$).

Blue %	End dark period			End photoperiod		
	0	30	100	0	30	100
glucose	0.4 ^a	0.2 ^a	0.4 ^a	0.5 ^a	0.4 ^a	0.4 ^a
sucrose	0.5 ^a	0.3 ^a	0.4 ^a	8.4 ^b	9.6 ^b	13.2 ^a
starch	1.1 ^a	0.6 ^a	0.8 ^a	45.1 ^b	55.8 ^a	39.5 ^b

Discussion

Peculiarly, whereas parameters such as A_{max} , leaf composition and LMA depended on the percentage of blue light during growth, only the leaves that developed under 0B (100% red light) had a suboptimal F_v/F_m , a low light-limited quantum efficiency for CO_2 fixation (α ; Table 1) and a stomatal conductance (g_{sw}) that was unresponsive to irradiance (Fig. 4). Such effects on leaves have, to the best of our knowledge, not been reported before and highlight the fundamental difference between leaf adaptation to growth spectrum and instantaneous spectral effect on photosynthesis. Instantaneous photosynthetic rates are relatively high when a leaf is illuminated with red light (e.g. McCree, 1972a, Inada, 1976).

Disorders in leaf physiology associated with growth under red light alone

A lower photosynthetic rate in plants grown under red light alone has been shown for several crop plants. Matsuda *et al.* (2004) found a lower photosynthetic rate for rice grown under red LEDs alone than for plants grown under a mixture of red and blue LEDs. Similar results were found for wheat (Goins *et al.*, 1997), which had a lower photosynthesis and DW accumulation when grown under red alone compared with growth under white fluorescent tubes or under red light supplemented with blue. While Yorio *et al.* (2001) reported a lower DW accumulation in radish, spinach and lettuce grown under red LEDs alone than under white fluorescent tubes or red supplemented with blue, only radish developed a lower photosynthetic rate when grown under red LEDs (as we also found for cucumber; Figs 1 and 4A). This suggests that vulnerability to decreases in photosynthetic rate associated with growth under red light alone may be subject to genetic variation.

The low A_{\max} of the leaves that developed under 0B (Fig. 1) cannot be attributed to a low leaf N content, as the PNUE at A_{\max} is lower for the 0B treatment than for the other treatments (Fig. 8B). Chlorophyll content and LMA can also be ruled out, as A_{\max} expressed per unit leaf DW and per unit Chl is also lower for the 0B leaves (Fig. 8A). The nitrate fraction of leaf N content has been reported to be relatively higher in leaves grown under low irradiance than those grown under a high irradiance (e.g. Felipe, *et al.*, 1975). In the present study this nitrate effect on PNUE can be excluded as in both in the 0B and 30B leaves N in the form of nitrate was <10% of the total N content. The unresponsiveness of the 0B grown leaves' stomata did limit A_{\max} due to a more restricted CO₂ diffusion into the leaf, as reflected by the lower $C_i C_a^{-1}$ with increasing measuring irradiance in the 0B leaves compared with the other treatments (Fig. 4).

In contrast to A_{\max} , the low α found for the 0B treatment (Table 1) is entirely related to a lower Φ_{PSII} and not to a low C_i due to a low g_{sw} (Fig. 4), as under both ambient O₂ and non-photorespiratory conditions the relationship between A_{gross} and an index of ETR (the product of Φ_{PSII} and absorbed irradiance) did not differ significantly for the 0B and the 30B leaves (Fig. 2). If C_i were to be limiting assimilation of the 0B leaves at low irradiance, A_{gross} per unit ETR would have been lower for 0B than for 30B at ambient O₂ but not at low O₂ (e.g. Harbinson *et al.*, 1990). Therefore the underlying cause of the relatively low photosynthetic rates at low irradiance of the 0B grown leaves may be due to disorders in the development and functioning of the photosynthetic machinery itself. During our photosynthesis measurements the measuring-light spectrum was identical to the growth-light, so a higher α would be expected for the 0B treatment as the quantum yield for incident red light is known to be higher than that of blue light (McCree, 1972a; Inada, 1976). Where the relatively low α measured for the treatments containing a high blue light percentage (50B, 100B) was to be expected based on the differences in quantum yields for the different wavelengths, the low α for the 0B treatment is unexpected and points to problems in the development and operation of photosynthesis. An F_v/F_m below 0.8, as measured for the 0B leaves, is normally associated with damage or long-term down-regulation of PSII in response to stress (e.g. Baker, 2008). Evidently red light alone, or the absence of blue light during growth, results in a dysfunction of the photosynthetic machinery with a particularly adverse effect on leaf tissue regions between the veins (Fig. 3). Matsuda *et al.* (2008) reported an $F_v/F_m \geq 0.8$ for spinach leaves grown under white fluorescent light deficient in blue, so wavelengths beyond the blue region may also prevent a loss of F_v/F_m as found for 100% red in this study.

Several diverse, spectrally related factors have been associated with inhibition of photosynthesis. Feedback down-regulation of photosynthesis is associated with carbohydrate accumulation in leaves (e.g. Stitt, 1991; Paul and Foyer, 2001). Britz and Sager (1990) found lower leaf photosynthesis associated with higher starch content at the end of the night period in soybean and sorghum leaves grown under low pressure sodium lamps emitting very little blue light and mainly amber/red light (~595 nm), compared with leaves grown under daylight fluorescent tubes. In the case of the present experiments any such effects on carbohydrate transport and metabolism can be discounted as no differences in carbohydrate content at the end of the dark period were found between the treatments (Table 2). In wheat seedlings inhibition of PSI and PSII development and Chl synthesis was reported upon exposing the root-shoot transition zone to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ pure red light (Sood *et al.*, 2004), suggesting an unidentified problem related to transport of substances within the plant. In our experiment Chl content on leaf DW basis was not impaired in the 0B treatment (Fig. 6), however, the higher F_v/F_m adjacent to the veins (Fig. 3) and occasional chlorotic appearance between the veins also point to a potential transport problem. Schmid and co-workers related a depressed F_v/F_m and photosynthesis in chloroplasts of red light grown green algae *Acetabularia* to uncoupling of antennae and PSII reaction centers due to reduced amounts of core antenna chlorophyll-protein complexes (Wennicke and Schmid, 1987; Schmid *et al.* 1990a, b). The involvement of a blue light/UV-A photosensory pathway in the maintenance of PSII core protein synthesis has been postulated by Christopher and Mullet (1994) and Mochizuki *et al.* (2004) found a threshold intensity of 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ blue light (470 nm) for activation of the PSII core protein D2 encoding gene *psbD* in *Arabidopsis* acting via cryptochromes, along with a non-blue-specific activation signal. An impaired ability to synthesize core proteins may be related to the low F_v/F_m and α that we found for the 0B grown cucumber leaves, however, this theory cannot be directly linked to a problem with transport within the plant as indicated by the heterogeneous F_v/F_m .

Blue light dose responses

The physiological disorders associated with leaf development under red light alone were eliminated by adding only a small amount of blue light (7% or 7 $\mu\text{mol m}^{-2} \text{s}^{-1}$; Fig. 1). Beside this response to blue, which may be characterized as a “qualitative” or “threshold” effect, the increase in A_{max} upon increasing the blue light percentage up to 50B clearly indicates that leaf photosynthesis also responds quantitatively to blue light.

The quantitative increase in A_{max} with an increasing proportion of blue light was associated with an increase in LMA (Fig.7A), Chl content and N per unit area (Table 1; Fig. 7B) and g_{sw} at saturating irradiance (Fig. 4B). The larger g_{sw} is both due to a larger number of adaxial stomata (Fig. 5) and a greater stomatal aperture. Blue light deficiency has been associated with a lower LMA in soybean (Britz and Sager, 1990), consistent with the lowest LMA that we found for the 0B grown leaves here. A higher irradiance is usually found to lead to both a higher LMA and A_{max} (Poorter *et al.* (2009). Our results show that the quantitative relationship between LMA and A_{max} with increasing irradiance (Poorter *et al.*, 2009, 2010) is also found for a varying blue percentage at a constant irradiance (Fig. 7A). In general, in parallel with leaf responses to irradiance, blue light is shown to stimulate “sun-type” characteristics on leaf level, even at the relatively low growth irradiance used in this study.

The question remains which blue light regulated response(s) can explain the differences in A_{\max} of leaves grown under different blue light percentages? At a blue light percentage $\geq 22\%$ A_{\max} appears to change proportionally to changes in LMA, Chl and PNUE (Fig. 8), although Chl per leaf DW (Fig. 6B) and Chl:N (Table 1) decrease slightly with an increasing percentage of blue light. Similar relations between these leaf traits are usually observed with increasing irradiances, where A_{\max} increases proportionally with LMA and N content per unit leaf area, and Chl:N decreases (e.g. Evans and Poorter, 2001). Leaf N content may therefore indeed be a limiting factor for A_{\max} of leaves grown at an irradiance $\geq 22B$. Regulation of potential A_{\max} due to restrictions in cell size and the number of cell layers in a mature leaf as proposed by Oguchi *et al.* (2003) is also well in line with the correlation found between LMA and A_{\max} in our experiment. A restriction in intercellular space per unit leaf area may be expected to be associated with a limitation of N-requiring components of the photosynthetic machinery per unit leaf area. More unusual is the lower A_{\max} per unit LMA, Chl and N found for leaves grown under an irradiance containing $\leq 15B$ (Fig. 8). These results indicate that cell space within the leaf, N availability and pigment content were sufficiently large to allow a higher A_{\max} . Hogewoning *et al.* (2010a) likewise found a lower A_{\max} per unit LMA for cucumber leaves grown under high pressure sodium light (5% blue) compared with leaves grown under fluorescent tubes (23% blue) and an artificial solar spectrum (18% blue). Apparently leaves grown at an irradiance containing $\leq 15B$ are subject to limitations which may be related to the disorders associated with 0B leaves as discussed above, whereas $\geq 22B$ the relationships between A_{\max} and LMA, N and Chl are very similar to usual leaf responses to irradiance.

The Chl a:b ratio was also conspicuously lower for 0B and 7B leaves, but remained stable $>15B$ (Table 1). This response is not in accordance with the usually measured increasing Chl a:b ratio with increasing irradiance during growth (Evans and Poorter, 2001), in contrast to the responses of the other leaf traits measured, which are in accordance with usual responses to irradiance.

Leaf responses to growth under blue light alone

Though the responses of A_{\max} (Fig 1), LMA and Chl content (Fig. 6A) in the range 0B to 50B display clear progressive trends, the results for the 100B treatment deviate from those trends. In contrast to 0B, 100B leaves did not show any signs of dysfunctional photosynthesis. One conspicuous contrast between red and blue light is the absence of cryptochrome and phototropin stimulation in pure red, whereas pure blue does stimulate cryptochromes, phototropins and also phytochromes (Whitelam and Halliday, 2007). The 100B leaves invested relatively little in Chl considering their A_{\max} (Fig. 7). The relative amount of active phytochrome expressed as phytochrome photostationary state (PSS; calculated according to Sager *et al.*, 1988) of the 100B leaves is also markedly lower than that of the other red/blue combinations (Table 1), which may indicate a role of phytochrome activity in the regulation of the Chl content- A_{\max} relationship. As LMA has been shown to be much less affected than A_{\max} at spectra containing relatively little blue (Fig. 8A; high pressure sodium light grown leaves in Hogewoning *et al.*, 2010a), the lower A_{\max} of 100B leaves compared to 50B leaves may be related to a limitation in LMA due to the absence of responses regulated by red light.

Conclusions

In this study blue light has been shown to trigger both a qualitative, signaling effect enabling normal photosynthetic functioning of cucumber leaves and a quantitative response stimulating leaf development normally associated with acclimation to irradiance intensity. Leaf acclimation to irradiance intensity may therefore be regulated by a limited range of wavelengths instead of the full PAR spectrum. Varying the blue light fraction offers the possibility to manipulate leaf properties under a low irradiance such that they would normally be associated with high irradiances. The possibility to grow plants under relatively low irradiance in a plant growth facility, with a relatively high photosynthetic capacity able to withstand irradiances under field conditions, is a useful practical consequence for research and agriculture.

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CHAPTER 3

Effects of light-spectrum on photosynthetic quantum yield

- 3.1 Light distribution in leaf chambers and its consequences for photosynthesis measurements¹

- 3.2 A quantitative molecular and physiological analysis of photosynthetic quantum yield dynamics

¹ Chapter 3.1 supports the materials and methods section of chapter 3.2.

CHAPTER 3.1

Light distribution in leaf chambers and its consequences for photosynthesis measurements

Abstract

The impact of a heterogeneous distribution of actinic light within a leaf chamber for photosynthesis measurements by gas-exchange on the photosynthesis-irradiance relationship was investigated. High resolution light distributions were measured over the area of a commercially available clamp-on leaf chamber equipped with build-in red and blue LEDs, as well as over the area of a custom-made leaf chamber with external light source, using a low-cost digital camera and freely available software. The impact of the measured heterogeneity on the photosynthesis-irradiance response curve was calculated for two realistic scenarios. When the average light intensity over the leaf chamber area was estimated accurately, heterogeneity had minor effects on the photosynthesis-irradiance response curve. However, when the irradiance was measured in the chamber centre, which is common practice, and assumed to be homogeneous, for both leaf chambers the photosynthesis-irradiance response curve was subject to considerable error and led to serious underestimation of the light-limited quantum yield of photosynthesis. Additionally, mixed light-sources with different heterogeneity patterns per light-source, such as in the clamp-on leaf chamber, potentially increase errors due to heterogeneous physiological responses to light spectrum. High resolution quantification of the leaf chamber light distribution enables calculation of the correct average light intensity and already resolves the most pressing problems associated with heterogeneity. To exclude any light-distribution related errors in gas-exchange measurements a leaf chamber and actinic irradiance source design with a homogeneous light distribution is an absolute requirement.

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Introduction

Leaf chambers are widely used for photosynthesis measurements by gas-exchange. For a correct presentation of the photosynthesis-irradiance relationship, the correct light intensity does need to be known and the distribution of the light projected on the leaf-area in the chamber should be homogeneous. Heterogeneity of light-distribution in a leaf chamber is undesirable for a number of reasons. In the first place, heterogeneity in light distribution easily leads to a wrong estimate of the average light intensity over the leaf chamber area. Many leaf chambers have an area of only a few square centimeters. It is common practice to calibrate the average light intensity over a leaf area in the chamber by measuring light intensity in the center of the leaf chamber using a well calibrated device (e.g. thermopile or PAR-sensor). In case of a heterogeneous light distribution over the leaf chamber area this will inevitably lead to an erroneous estimate of the actual average light intensity where the leaf area in the chamber is subjected to. An important problem that arises from such an error is a wrong estimate of maximal quantum yield for CO₂ fixation (α), which has been suggested to have frequently occurred in the past (Singsaas *et al.* 2001).

Second, a heterogeneous light distribution has consequences for the interpretation of photosynthesis measurements, even when the average amount of light over the leaf surface is known. When measuring at an irradiance which is strictly light-limited for all chloroplasts in the leaf so that α is maximal, a correct photosynthesis-irradiance relationship can still be calculated from data measured using a chamber with a heterogeneous light distribution. This requires that heterogeneity has been accounted for so that the average light intensity is correct and nowhere exceeds the light-limited range. Beyond the light-limited irradiance range photosynthesis measurements will also be erroneous when the correct average light intensity over a heterogeneously illuminated leaf area is used, as α will also become heterogeneous. The significance of such errors for the interpretation of photosynthesis-irradiance response data has not yet been explored.

In systems using combined light sources, such as mixed red and blue LEDs, the situation is more complicated, as the light distribution for the different light sources can be different. This may result in spatial differences in spectral composition of incident light, which can contribute to heterogeneous photosynthesis rates over the measured leaf area, caused by spectral effects on e.g. stomatal opening (e.g. Zeiger 1990, Willmer and Fricker 1996) and photosynthetic quantum yield (e.g. McCree 1972a, Inada 1976, Evans, 1987).

Several recent studies have contributed to the improvement in accuracy of photosynthesis measurement and its consequent calculations: Pons and Welschen (2002) studied effects of respiration rates under the seal of leaf chambers on net photosynthesis, Flexas *et al.* (2007) and Rodeghiero *et al.* (2007) analyzed the effect of diffusion leakage in clamp-on leaf cuvettes and Dubois *et al.* (2007) optimized the statistical estimation of the parameters of the Farquhar, von Caemmerer and Berry model (Farquhar *et al.* 1980). We present an example of a relatively easy, low-cost method for measuring the light distribution in a leaf chamber, and show the light distribution over the area of a custom made leaf chamber and a widely used commercially available clamp-on chamber. The difference between the photosynthesis-irradiance relationship associated with the measured and an ideal, homogeneous light distribution is explored via a photosynthesis-irradiance response simulation. The consequences of a heterogeneous light distribution for the interpretation of measured photosynthesis data are discussed.

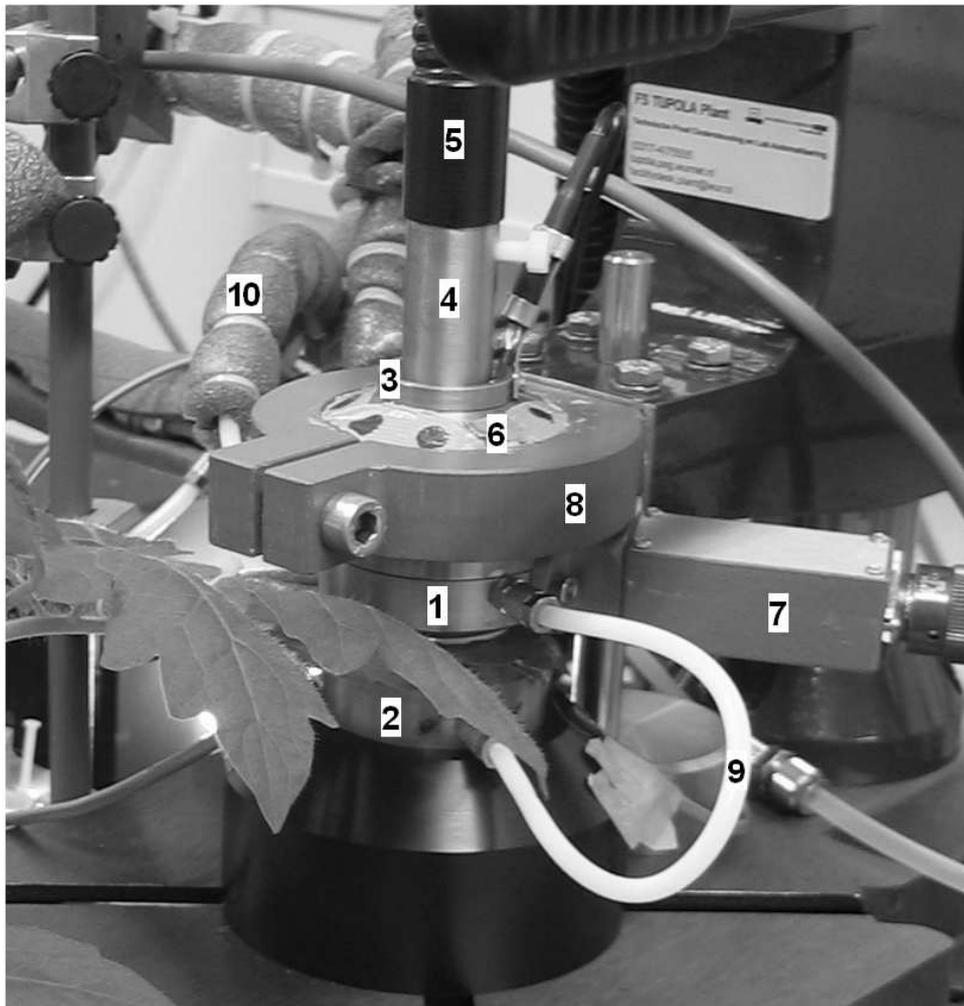


Fig. 1. Custom made leaf chamber with enclosed tomato leaf. The numbers correspond with different parts of the leaf chamber and accessories: Upper leaf chamber part (1), lower leaf chamber part (2), brass ring equipped with 16 LEDs (640 nm peak wavelength, Luxeon Rebel, Philips Lumileds Lighting Company, San Jose, Ca. USA) which can provide a $10000 \mu\text{mol m}^{-2} \text{s}^{-1}$ light pulse to obtain a maximum fluorescence signal (3), brass holder allowing the fiber to be positioned at different distances from the leaf (4), randomized optical fiber providing actinic light (5), one of the nine apertures for an additional fiber (6), light-proof box with photodiode in contact with a light pipe picking up actinic irradiance in the chamber, connected to a multimeter (7), lab stand holding the upper leaf chamber in position (8), tube leading gas from the lower to the upper chamber part (9), and insulated tube through which water is pumped into channels in both chamber part walls to control the leaf chamber temperature (10).

Materials and methods

Description leaf chambers

The custom made leaf chamber is of a conventional design: It is comprised of two separate round chamber parts (upper and lower) made from nickel plated brass, mounted in a lab stand (numbers 1, 2, and 8 in Fig. 1). The 5.2 cm^2 chamber area is covered with a quartz

window for the upper chamber half. For the lower chamber half a perspex window is used, through which an infrared leaf temperature sensor is mounted. A leaf can be clamped between the two parts by lowering the upper chamber part onto the lower, so that the leaf is sealed gas-tight between two rings of white, flexible foam. Light was provided using a randomized optical fiber which was split into four fibers (Heinz Walz GmbH, Effeltrich, Germany; number 5 in Fig. 1) allowing four different light sources to be used for leaf illumination simultaneously. The single fiber end rested in the upper chamber part at 4.5 cm from the leaf and had an effective diameter of 1.2 cm. The light sources used were two projector lamps equipped with 250W halogen lamps. One lamp was used to obtain narrow-band light using a near-infrared cut-off filter and bandpass filters (10nm width at half maximum, range 400-740nm, every 20 nm; Thorlabs, Newton NJ, USA). The other lamp provided a broad-band spectrum, using a near-infrared cut-off filter in combination with a tungsten-to-day-light conversion filter (Full C.T. Blue; Lee Filters, Hampshire, UK). Light intensity was monitored using a computing multimeter (Thurlby Thandar Instruments Ltd., Huntingdon, Cambs, UK), connected to a photodiode (OSD15-5T, Centronic) in a light-proof box mounted on the outside of the upper leaf chamber part (number 7 in Fig. 1). The photodiode was in contact with a light guiding pipe (3 mm Ø, Mentor GmbH & CO, Erkrath, Germany) which was mounted in an opening drilled through the chamber wall, picking up light via a 45° cut, polished end above the upper chamber window. The light pipe did not interfere with the actinic light beam. The multimeter output was calibrated using a thermopile (type PS10Q, Moletron Detector Inc., Portland, USA), which was calibrated using a quantum sensor (LI-COR Lincoln, Nebraska USA). The calibration was repeated with a spectroradiometer (USB2000 spectrometer, Ocean Optics, Duiven, The Netherlands), which produced identical results. The sensors used for calibration were placed at the position where the leaf would be clamped in, in the centre of the chamber.

The commercial leaf chamber tested was a LI-6400-40 Leaf Chamber Fluorometer (LI-COR, Lincoln, Nebraska, USA) equipped with independently controllable red (27) and blue (3) LEDs as actinic light source, with peak wavelengths of 640 nm and 464 nm, respectively. This chamber has a 1.59 cm diameter (2.0 cm² area), black foam as a seal for the lower chamber part and white foam for the upper chamber part.

Light distribution imaging procedure

For determining the light distribution in the leaf chambers, the upper parts of the leaf chambers were placed upside-down, placing a piece of thin white filter paper (Whatman 589/ 1) in place of the leaf. This type of filter paper does not produce much scattering which can alter the light distribution pattern of the incident light. The filter paper was illuminated by the actinic light source and imaged in a dark room using a digital camera (P&S Canon 590IS) expanded with software allowing RAW-format imaging (appendix). Shutter time and aperture were set manually, so that no pixels were saturated or underexposed. The illuminated filter paper circle was imaged in the middle of the image (Fig. 2), to prevent a reduction in brightness or saturation at the periphery compared to the image centre (i.e. vignetting). Some images were made closer to the margin of the image, to be able to test whether vignetting effects were significant. No vignetting was observed in the image area used for analysis.

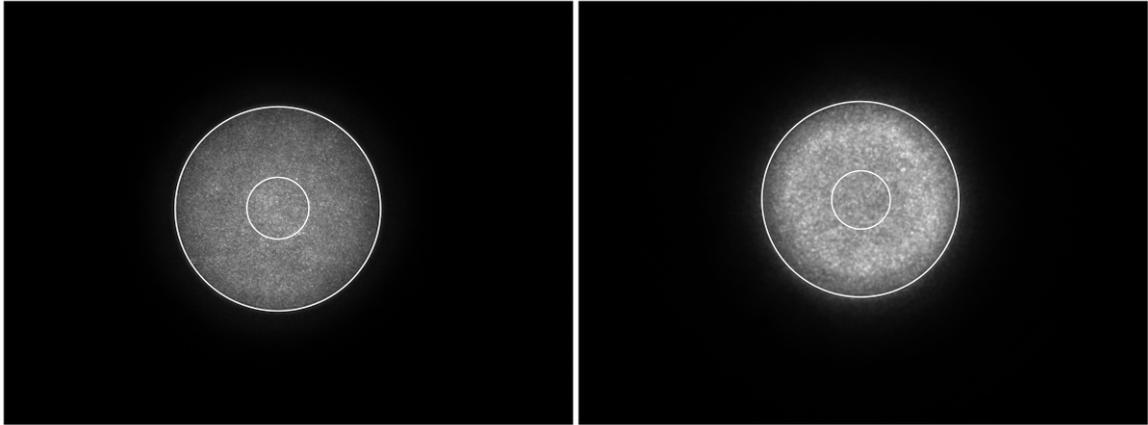


Fig. 2. Gray-scale images of custom made (white light, left) and LI-6400 leaf chamber (red LEDs, right). The outer white circles represent the whole leaf chamber area, the inner white circles the “centre-area”, representative for the area commonly used for light intensity calibration, as used in Table 1.

When using a common digital camera as we did, the option to use a RAW-image-format is required to obtain quantitative data on light-intensity. A usual jpg-format does not represent light intensity linearly, as the camera software tends to dim bright spots and make dark spots brighter. Imaging methods using more specialized equipment can also be used (e.g. a technical camera), providing no information is lost due to image-processing by the camera software.

For the custom made leaf chamber, images were made using blue (445 nm), green (560 nm), red (620 nm) and broad-band (“white”) light, at different light intensities for each color ($n=4$). For the LI-6400 leaf chamber images were made using the red and the blue LEDs at different light intensities for both colors ($n\geq 5$). For each different color, shutter time and aperture were optimized and kept unchanged for the different light intensities imaged for each color. An image was also made with a reference object with known area placed in the centre of both leaf chambers to allow the dimensions to be scaled to millimeters.

Quantification of the light distribution in a leaf chamber

The images of the illuminated filter paper placed in the leaf chamber were processed such that the intensity value of the pixels corresponded linearly with light intensity (see appendix). The reliability of the procedure was determined by comparing the different light intensities as measured by the measuring device ($\mu\text{mol m}^{-2} \text{s}^{-1}$) per color of actinic light imaged, with the mean pixel intensity of the corresponding images after processing. The relationships were always perfectly linear (Fig. 3), hence proving that the relative light intensities obtained from the image analysis procedure (appendix) are representative for the real light intensity in the leaf chamber.

The mean pixel intensity was measured for the centre of both leaf chambers, which is usually used for light-intensity calibration, and the whole area, representative for the light intensity that a clamped leaf would receive. The standard deviation (SD) of the mean light intensity was also determined for the total leaf chamber area. Furthermore, the mean pixel intensity was measured for the area of nine circular bands around the centre, up to

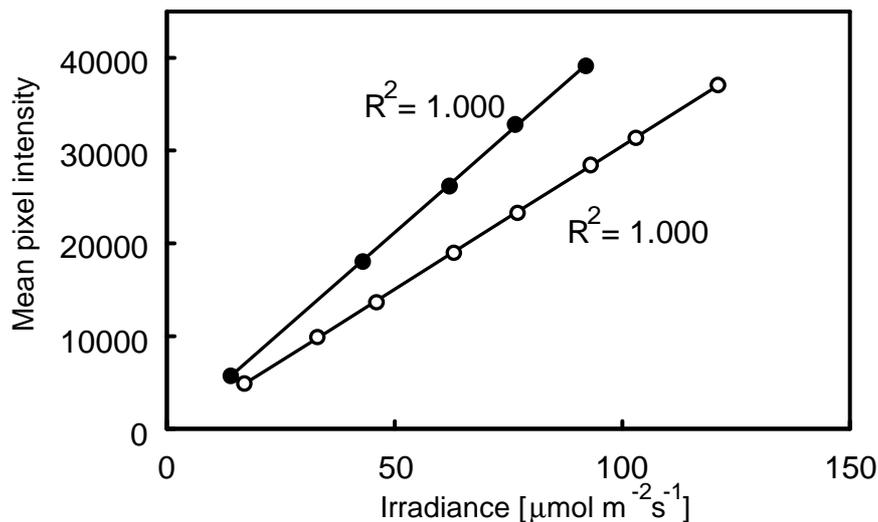


Fig. 3. Light intensity in the LI-6400 leaf chamber as indicated by the LI-6400 system versus the mean pixel intensity of the centre circle (3.4 mm width) in the image, as analyzed in imageJ (see appendix). Closed circles: Red LEDs; open circles: Blue LEDs. For the custom made chamber a similar linearity was observed (not shown).

the margin of the chamber area. As the width of each band was equal, the area of the bands analyzed increased from the centre towards the margin of the chamber area. For each band the relative light intensity was calculated separately, which allowed the light distribution from the centre to the margin of the leaf chamber to be mapped in 10 intervals. This approach requires a centrally symmetrical distribution of the light intensity, as was the case for the two chambers tested. In the case that a distribution is not centrally symmetrical, a map of isophots would be more appropriate (see e.g. Laisk and Oja 1998, p.24).

Quantification of the impact of a heterogeneous light distribution on the photosynthesis-irradiance response curve

To assess the impact of the measured light distribution in both leaf chambers on the validity of photosynthesis measurements the response curve was simulated for three situations. Photosynthesis-irradiance response curves were produced for (1) an ideal, homogeneous light distribution, for (2) the measured light distribution assuming the light intensity in the chamber centre to be representative for the entire chamber area and for (3) the measured light distribution using the correct average light intensity over the entire chamber area (see appendix for procedure). Note that the maximum intensity of the blue LEDs (LI-6400) is $<300 \mu\text{mol m}^{-2} \text{s}^{-1}$ and therefore insufficient to measure a complete photosynthesis-irradiance response curve on leaves of most plant species. The irradiance provided by the blue LEDs is often mixed with irradiance provided by red LEDs when measuring photosynthesis-irradiance response curves.

Results

Light distribution in the leaf chambers

For both leaf chambers, the mean light intensity in the centre of the leaf chamber (inner circles Fig. 2) was different from the mean light intensity over the entire chamber area (Table 1A). Over the entire area of the custom made chamber, the light intensity was only 77% of that in the centre of the chamber. Differences for the different colors used were negligible. In the LI-6400-40 leaf chamber the light intensity over the entire area compared to the centre deviated much more for the blue LEDs than for the red LEDs (respectively 80% and 94% of the centre intensity). The SD from the mean light intensity over the entire chamber area was in a much closer range (22-27%) than the ratios of light intensity over the entire area compared to the centre (77-94%; Table 1). The granularity of the filter paper is visible in the image (Fig. 2), but does not obstruct the analysis of the light distribution pattern over the chamber area.

Table 1. A: Relative light intensity of total leaf chamber area compared to the chamber centre (as represented by the inner circles in Fig. 2). B: Standard deviation of mean light intensity for the total chamber area (mean intensity total area=1).

	Custom made chamber				LI-6400 chamber	
	red	blue	green	“white”	red	blue
A:	0.78	0.77	0.77	0.77	0.94	0.80
B:	0.23	0.25	0.24	0.24	0.22	0.27

The more detailed analysis of light distribution over the leaf chambers makes clear why the SD of the light intensity produced by the red LEDs was relatively large compared with the ratio of light intensity over the entire chamber area and that in the centre (Table 1). Whereas the light intensity over the entire area produced by the red LEDs (LI-6400) was only slightly smaller than in the centre, local differences over the area were considerable (Fig. 4). The light intensity was >30% lower in the outer margin of the chamber, compared to the centre, whereas slightly higher in the area between the centre and the margin (about 10%). The blue LEDs (LI-6400) produced the highest light intensity in the chamber centre, gradually decreasing away from the centre and dropping rapidly close to the chamber margin. A comparable pattern as for the blue LEDs (LI-6400) was observed for the light distribution in the custom-made leaf chamber (Fig. 4; white, red, blue, and green light had a similar distribution). The relative light distribution was similar for the different light intensities tested per color/ leaf chamber combination, as indicated by the very small standard errors in Fig. 4.

Consequences of a heterogeneous light distribution

The simulated photosynthesis-irradiance response curves show a considerable difference between an ideal homogeneous light distribution and the measured actual light distribution assuming the light intensity in the leaf chamber centre is representative for the entire chamber area, especially for the custom made chamber and the blue light in the LI-6400 chamber (Fig. 5). A reduction in the slope of the light-limited part of the

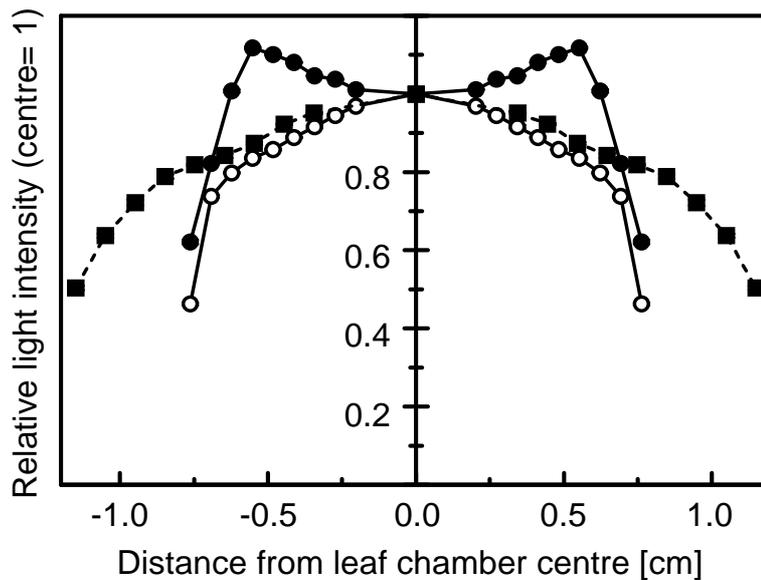


Fig. 4. Relative light distribution over the custom made leaf chamber area (squares, dotted line) and the LI-6400 leaf chamber area (red light: closed circles; blue light: open circles). For the custom made chamber, the distribution of the white light is shown. The other three colours analyzed (blue, green, red) had similar distributions. Zero on the x-axis represents the centre of the chamber, each following point indicates the relative light intensity of bands with an equal width, so the outer bands have a greater area than those closer to the chamber centre. Error bars indicate the s.e. of the mean relative light intensity over the images taken at different light intensities ($n \geq 4$) of actinic light per color/leaf chamber combination (not visible in graphs as $se \leq 0.007$).

photosynthesis-irradiance response curve (i.e. the maximum quantum yield for CO_2 fixation) of 23%, 6%, and 20% was found for, respectively, the custom-made leaf chamber, the LI-6400 red LEDs and the blue LEDs (see insets Fig. 5). These simulated differences in α are proportional to the ratio of average light intensity in the leaf chamber centre versus that of the entire chamber area (Table 1). The curve resulting from the correct light intensity distribution (dashed curve) over the entire chamber area is also different from the curve representing a homogeneous light distribution (upper curve), however, the difference is small. In this case there is no significant effect of the heterogeneity in light distribution on the light-limited part of the curve (Fig. 5, insets). Clearly, a heterogeneous light distribution has a considerable effect on the validity of photosynthesis-irradiance measurements which can largely be resolved by measuring and correcting for heterogeneity.

Discussion

Our results show that whenever accurate quantitative data on the relationship between irradiance and photosynthesis are required, the light-distribution in a leaf chamber becomes important to know. For a custom made leaf chamber, knowing the light

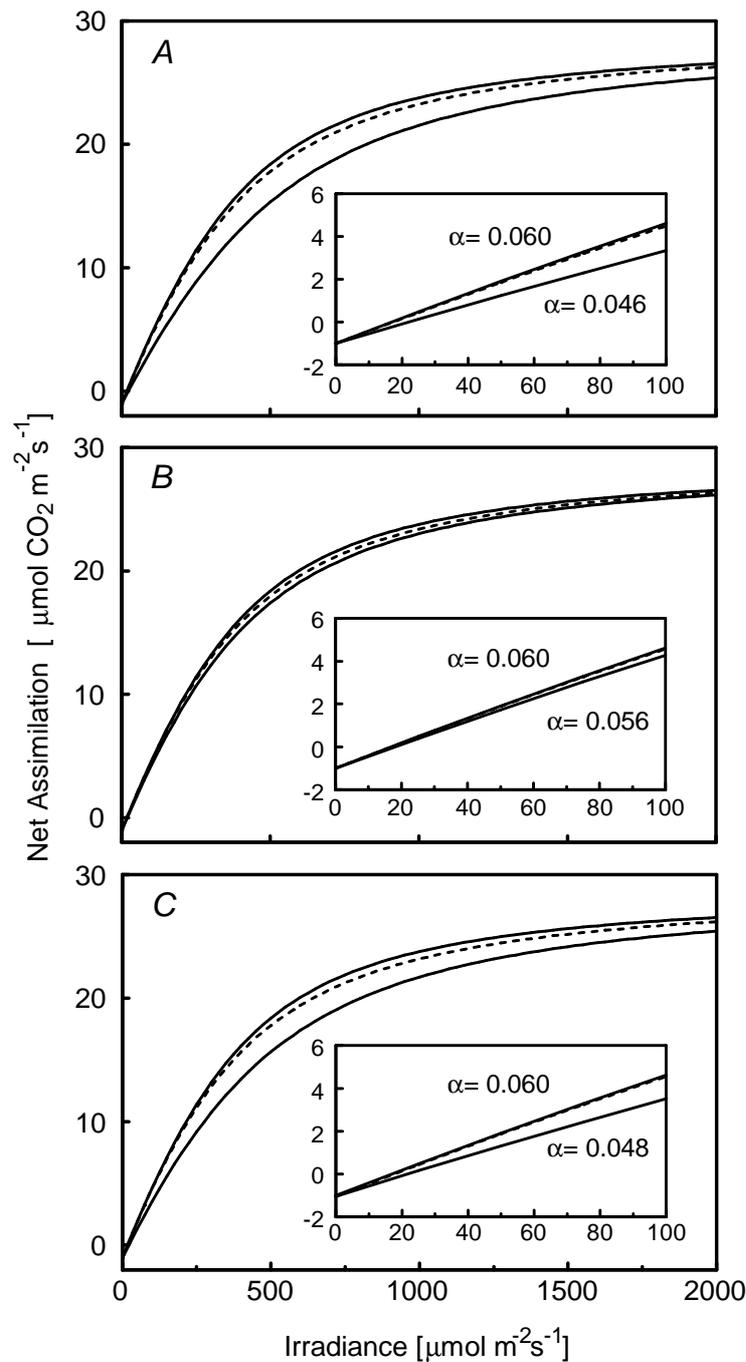


Fig. 5. Simulated photosynthesis-irradiance response curves for the custom leaf chamber (A) and the red (B) and blue (C) light in the LI-6400 leaf chamber. The upper solid lines represent an ideal, homogeneous light distribution, the lower solid lines the real light distribution assuming the chamber centre light intensity to be representative for the entire chamber area and the dashed lines the real light distribution using the correct average light intensity over the entire chamber area. The same graphs are shown on a different scale in the insets, where α indicates the maximum quantum yield for CO₂ fixation. Note that the maximum intensity of the blue light in the LI-6400 chamber (C) is $<300 \mu\text{mol m}^{-2} \text{s}^{-1}$ and therefore insufficient to measure a complete photosynthesis-irradiance response curve on leaves of most plant species.

distribution is crucial for the reliability of quantitative measurements. When a usual light intensity calibration in the centre of the chamber deviates from the light intensity over the entire chamber area, the error of calculated quantum yields for CO₂ fixation (α) in the light-limited range will be proportional. In our example that would imply measurements of α of only 77% of those obtained using the correct light intensity the leaf received (Table 1). In commercial systems heterogeneity may have been corrected for by the manufacturer, so that the read-out indicates a correct average light intensity. This is the case for the LI-6400 system tested (LI-COR, pers. comm.). A disadvantage of measuring α using a chamber with a heterogeneous light distribution which is corrected for so that the average intensity is correct, is that the strictly light-limited irradiance range will be smaller. This implies that some chloroplasts in the leaf may already be subjected to irradiance levels that start becoming non-light-limited, whereas the average light intensity would be well within the light-limited range for an individual chloroplast. Especially shade-plants can become non-light-limited at low irradiances, even below 20 $\mu\text{molm}^{-2}\text{s}^{-1}$ (Singsaas *et al.* 2001). In such leaves a heterogeneous light-distribution further limits the already narrow range of average irradiances low enough to measure the light-limited part of the photosynthesis-irradiance response curve. Quantum yield measurements made with an Ulbricht sphere leaf chamber on 11 diverse C₃ species showed a high quantum yield compared to many earlier studies and low variance among species (Long *et al.* 1993). Singsaas *et al.* (2001) studied variation in the light-limited quantum yields for photosynthesis found in literature and concluded that in numerous studies the presented quantum yields were too low due to methodological errors. They concluded that photosynthesis measurements beyond the strictly light-limited range are a main source of error. We conclude that a heterogeneous light distribution in the leaf chamber used can also easily be a potential source of error in quantum yield studies. A heterogeneous light distribution indeed does greatly limit the strictly light-limited measuring range, so that already at relatively low irradiances an error as concluded by Singsaas *et al.* (2001) is made.

At irradiances beyond the light-limited range quantum yields remain heterogeneous when the correct average light intensity over a heterogeneously illuminated leaf chamber area is used. For example, when red light becomes saturating in the chamber centre of the LI-6400 chamber, the light intensity in the outer margin of the chamber is only 62% of that required for saturation (Fig. 4). In fact, all chloroplasts, both over the leaf surface and in the leaf cross-section, need to be subjected to a saturating irradiance for a correct measurement of light saturated photosynthesis. Simultaneous illumination of both leaf sides greatly reduces inhomogeneous illumination of chloroplasts through the leaf cross-section, as applied by e.g. Oya and Laik (1976) and Terahshima (1986). Nevertheless, in contrast to heterogeneity in light distribution over the surface without making a correction for the average light intensity, the overall effect of a heterogeneous light distribution on the photosynthesis-irradiance response curve using the correct average light intensity value was small in the examples we presented (Fig. 5). An error of such magnitude may be acceptable for the majority of users and therefore a correction for heterogeneity using a method as we presented may be sufficient in most cases.

Physiological effects of differences in light distribution of different light sources used for mixed actinic light will further complicate the accuracy of photosynthesis-irradiance relationship measurements. Especially under conditions where the internal leaf

CO₂ concentration is limiting for photosynthetic rate, blue-light induced stomatal opening (e.g. Sharkey and Raschke 1981, Zeiger 1990) will directly affect photosynthesis. A heterogeneous photosynthetic rate associated with heterogeneous stomatal conductance has been shown making use of chlorophyll fluorescence images (Morison *et al.* 2005, Nejad *et al.* 2006). Blue light which is heterogeneously distributed over a leaf may therefore affect the photosynthesis-irradiance response curve not only by the distribution of irradiance intensity (as simulated in Fig. 5C), but also by other physiological responses of the leaf. The most commonly used blue/red ratio in the LI-6400 chamber is 0.1. Based on the distribution of the two colors as presented in Fig. 4, this ratio will deviate up to 24% over the leaf area. Chen *et al.* (2008) showed that heterogeneity of photosynthetic parameters in leaves during gas-exchange measurements affects biochemical parameter estimates using the Farquhar-model (Farquhar *et al.* 1980). An increased heterogeneity of photosynthetic parameters due to a heterogeneous light distribution will make biochemical parameter estimates less reliable. Note that parameters other than those from gas-exchange measured in relatively large leaf chambers with a heterogeneous light distribution may also be susceptible to errors when measurements are made from a part of the clamped leaf area. Parameters often measured simultaneously with gas-exchange involve photosystem I and photosystem II electron transport, measured respectively by 820 nm absorbance changes at P700 and chlorophyll fluorescence (Baker *et al.* 2007).

The most pressing problems associated with a heterogeneous light distribution in a leaf chamber can be resolved by using the correct average light intensity over the leaf area (Fig. 5). The method we presented offers the advantage over more specialized equipment (e.g. a technical camera) that the investment in a simple digital camera suffices. However, heterogeneous light distributions in leaf chambers can affect the reliability of photosynthesis measurements in numerous ways, even when using the correct average light intensity. Calculations from crop and canopy growth models which make use of photosynthesis-irradiance relations will also be affected by such errors. Therefore the use of leaf chambers with a well distributed light intensity would be the simplest way to improve the accuracy of such data. Laisk and Oja (1998) described a leaf chamber design with a notably homogeneous distribution of irradiance ($\pm 10\%$) and provided an exemplary mapping of the distribution. Recent technological developments resulting in smaller, more powerful LEDs offer opportunities to improve the configuration of light sources for leaf chambers. We consider that the distribution of light intensity in a leaf chamber deserves more attention in research using photosynthesis measurements.

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Appendix

Image analysis procedure

Installation of the free software “Canon Hack Development Kit” (CHDK) on the camera enabled us to use the RAW-format modus. When using a simple digital camera other lossless image formats than RAW, such as TIF, can not be used for a quantitative analysis of light intensity via imaging. As the original 10 bit RAW image is processed by the camera into a readable 8 bit format, information necessary for quantitative analysis will be lost. Note that a home-use digital camera as we used is equipped with a Bayer-filter, which inherently leads to a loss of genuinely recorded data. The Bayer-filter records 25% in blue, 50% in green and 25% in red. Therefore, when imaging e.g. red light, 75% of the pixels are acquired via interpolation. To be able to process the RAW-format (CRW-format) images produced, we made use of the open source software “dcraw”, which converted the images in a PPM image format.

The PPM-format images (10 bit) were processed in ImageJ (<http://rsbweb.nih.gov/ij/>) including a plug-in allowing 16-bit images to be read. Images were split into three channels (RGB) and the brightest channel was used for analysis. Knowing the size of the reference object and chamber diameter, the exact area corresponding with the chamber could easily be determined. The pixel intensity over the image represented the light intensity over the imaged area. ImageJ also allows the SD of the mean pixel intensity over an image area to be calculated.

Procedure photosynthesis-irradiance response curve simulation

Photosynthesis-irradiance response curves were simulated for (1) an ideal, homogeneous light distribution, (2) a heterogeneous distribution assuming the light intensity in the chamber centre is representative for the entire chamber and (3) a heterogeneous distribution using the correct average light intensity over the entire chamber area. The leaf chamber area on images processed in ImageJ as described above was exported as a numerical histogram consisting of 256 pixel intensity classes with corresponding occurrence counts. For the heterogeneous distribution simulations, the different classes of pixel intensities were set as relative pixel intensities compared to the intensity in the chamber centre (simulation 2; chamber centre intensity= 1) or as relative pixel intensities compared to the correct average intensity of the entire chamber area (simulation 3; average entire chamber area intensity= 1) . The relative pixel (or light) intensity classes with corresponding occurrence counts were imported in SAS (release 9.1.3; SAS Institute, Cary, NC, USA). Both relative pixel intensity classes (256) were multiplied by light-intensity steps needed to simulate a photosynthesis-irradiance response curve (range 0-2000 $\mu\text{molm}^{-2}\text{s}^{-1}$ using 25 $\mu\text{molm}^{-2}\text{s}^{-1}$ intervals, i.e. 80 light steps). This produced two data sets, each consisting of 20480 light intensities. For all these light intensities the net assimilation rate (P_n) was calculated via eq. 1 (Thornley 1976; input parameters: $\alpha = 0.06$, $R_d = 1$, $\theta = 0.7$ and $P_{\max} = 30$, where α is the light-limited slope, R_d dark respiration, θ scaling constant for curvature and P_{\max} the light saturated gross assimilation rate).

$$P_n = -R_d + \frac{\alpha \cdot PPF + P_{\max} - \sqrt{(\alpha \cdot PPF + P_{\max})^2 - 4\theta \cdot PPF \cdot P_{\max}}}{2\theta} \quad (1)$$

For each of the 80 light steps a weighed mean of P_n associated with the real light distribution was calculated and the non-rectangular hyperbola (eq. 1) was fitted to these weighed means by non-linear fitting (PROC NLIN) in SAS.

CHAPTER 3.2

A quantitative molecular and physiological analysis of photosynthetic quantum yield dynamics

Abstract

Leaf photosynthetic quantum yield is wavelength dependent due to inefficient energy transfer, absorption of light by non-photosynthetic pigments and imbalances in excitation of the two photosystems. We investigated photosystem excitation balance in relation to wavelength dependency of the quantum yield for CO₂ fixation in plants grown in different spectral environments (artificial SUN, SHADE and BLUE). The quantum yield for photosystem I and II electron transport and CO₂ fixation measured on leaves (*in vivo*) were related to the content, composition and spectroscopic properties of the two photosystems. Leaves grown in SHADE, which tends to overexcite PSI, had a higher quantum yield for CO₂ fixation at wavelengths overexciting PSI (≥ 700 nm) and a lower PSI:PSII ratio compared with the SUN and BLUE leaves. At wavelengths overexciting PSII, the quantum yield for CO₂ fixation of the SUN and BLUE leaves was higher. In the spectral region where absorption by pigments other than chlorophyll is insignificant (≥ 580 nm) the quantum yield for CO₂ fixation could be estimated from the photosystem excitation balance, whereas at lower wavelengths it was overestimated. The wavelength dependence of the photosystem excitation balance calculated via *in vitro* and *in vivo* approaches were substantially in agreement with each other. Where they were not, carotenoid absorption and state transitions are likely to play a role. We show for the first time how the wavelength dependence of the quantum yield for CO₂ fixation relates quantitatively to photosystem composition and excitation balance, and how these properties acclimate to the plant's growth-light spectrum.

Hogewoning SW, Wientjes E, Douwstra P, Trouwborst G, van Ieperen W, Croce R and Harbinson J, submitted

Introduction

For over half a century it has been known that the energy conversion efficiency of incident photons to chemical energy by leaves is wavelength dependent (e.g. Hoover, 1937). This is due to several processes that can be divided into two classes. First, the absorption of incident irradiance by a leaf is wavelength dependent due to the different absorption spectra of the different leaf pigments. Second, even if measured on an absorbed light basis, different wavelengths have different quantum yields for CO₂ fixation or O₂ evolution. On an absorbed light basis red light (600-640 nm) has the highest quantum yield, whereas blue and green light (400-570 nm) is considerably less efficient in driving photosynthesis (McCree, 1972a; Inada, 1976; Evans, 1987). There is a large variation in the reported maximum photosynthetic quantum yield for CO₂ fixation, which Singaas *et al.* (2001) attributed to errors in the measurement procedure. They conclude that the maximum quantum yield on an absorbed light basis under non-photorespiratory conditions varies little from 0.093 for CO₂ fixation (Long *et al.*, 1993) or 0.106 for O₂ evolution (Bjorkman and Demmig, 1987).

Three major causes for the wavelength dependence of the quantum yield for absorbed photons have been identified, i.e. absorption by carotenoids, absorption by non-photosynthetic pigments and an imbalanced excitation of the two photosystems (Terashima, 2009). First, energy transfer from carotenoids to chlorophylls (Chl) has an efficiency of 70-90%, whereas the energy transfer efficiency from Chl to Chl is 100% (Croce *et al.*, 2001; de Weerd *et al.*, 2003; van Oort *et al.*, 2008). Carotenoids have absorption maxima for blue wavelengths. Second, leaves contain pigments such as flavonoids and anthocyanins which absorb photons but do not transfer the absorbed energy to the photosynthetic apparatus. Flavonoids absorb predominantly in the UV/ blue part of the spectrum and anthocyanins in the green part. Third, the pigment composition of PSI and that of PSII differs and consequently the balance of excitation between the two photosystems is wavelength dependent (Evans 1986, Evans 1987, Chow *et al.*, 1990, Melis 1991, Walters and Horton 1995). Any imbalance in excitation of the two photosystems results in quantum yield losses (Pfannschmidt 2005). Although the factors causing quantum yield losses have been identified, a quantitative understanding of the relative contribution of each of these factors is still lacking.

Plants are continuously exposed to spectral changes, in the short term due to changes in weather and sun-angle, and in the longer term when leaves become shaded by other leaves or when shaded leaves are suddenly exposed to full sun (e.g. after canopy gap formation). In greenhouse cultivation plants also experience substantial spectral changes when supplemental lighting is used in order to enhance crop production. Spectral changes can directly alter photosynthetic quantum yield via changes in the relative absorption by the carotenoids and non-photosynthetic pigments, and via changes in photosystem excitation balance. On a scale of minutes, state transitions are believed to re-direct excitation energy from one photosystem to another (Haldrup *et al.*, 2001), although in intact leaves no subsequent increase in the quantum yield for CO₂ fixation has been found (Andrews *et al.*, 1993). Plants and other photosynthetic organisms can adapt to longer term spectral changes by altering the relative antenna size of the two photosystems, thus at least partly restoring the excitation balance between the photosystems (e.g. Chow *et al.*, 1990; Melis *et al.*, 1996; Fujita, 1997). Plants have been shown to acclimate to growth-light spectra

resulting in an increased quantum yield for CO₂ fixation, more efficient linear electron transport (Walters and Horton, 1995) and changes in the PSI:PSII ratio (Chow *et al.*, 1990). The consequences of the acclimation of electron transport efficiency and photosystem composition for the wavelength dependence of quantum yield for CO₂ fixation and how this relates quantitatively to the photosystem excitation balance have, however, not been explored in these studies. Measurements of electron transport *in vivo* using chlorophyll fluorescence in combination with 820 nm absorption changes (Baker *et al.*, 2007) can be used to estimate the functional photosystem efficiency balance (Eichelmann and Laisk, 2000). Absorption measurements on isolated pigment-protein complexes (i.e. *in vitro*) can likewise be used to estimate the photosystem excitation balance (Evans and Anderson, 1987). Due to inefficiencies in excitation energy transfer and charge separation, and non-linear electron transport processes such as cyclic electron transport, back-reactions or transfer to O₂, the relationship between excitation balance, absorption balance and a more functional photosystem efficiency balance is not necessarily simple. Whether or not the *in vivo* and *in vitro* methods can be used reliably to estimate the functional wavelength dependence of photosystem efficiency balance *in vivo* has not been explored, nor has a comprehensive combined approach been used to explore photosynthetic acclimation to different growth-light spectra.

In this study we show for the first time how the spectral growth environment affects the wavelength dependence of leaf photosynthetic quantum yield for CO₂ fixation and photosystem excitation balance, and how this relates to photosystem composition. By combining both an *in vitro* approach on isolated pigment-protein complexes and an *in vivo* approach on intact leaves we unambiguously show how acclimation to growth-spectrum results in changes in the relative absorption of the two photosystems which are directly linked to changes in photosystem excitation balance and quantum yield for CO₂ fixation.

Materials and methods

Plant material and growth conditions

Cucumber plants (*Cucumis sativus* cv. Hoffmann's Giganta) were sown in vermiculite and germinated under 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ cool white fluorescent lamps (TLD 50W 840 HF, Philips, The Netherlands) in a climate chamber. After one week, when the cotyledons had just opened, the seedlings were transferred to a hydroponic system (Hoagland's solution, pH 5.9 ± 0.2 ; EC 1.2 mS cm^{-1}) in a climate chamber. The day/night-temperature was 25 °C/23 °C, the relative humidity was 70% and the CO₂ concentration was ambient. All plants were subjected to $100 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$ irradiance (16/ 8 h day/ night) provided by three light sources with distinct differences in their spectrum (Fig. 1): artificial sunlight (SUN), artificial shadelight (SHADE; expected to over-excite PSI) and blue light (BLUE; expected to over-excite PSII). The SUN system provided a spectrum that, except for a deficiency in blue, closely matched the calculated direct and circumsolar irradiance spectrum for the 48 contiguous states of the USA, and which is available for download in a tabular form (www.astm.org/Standards/G173.htm). Details of the SUN system are described in Hogewoning *et al.* (2010a). The SHADE system was comprised of quartz-halogen lamps filtered with a tungsten-to-daylight conversion filter (Full C.T. blue, Lee filters, Hampshire, UK) and a dielectric multilayer film reflecting near infrared wavelengths

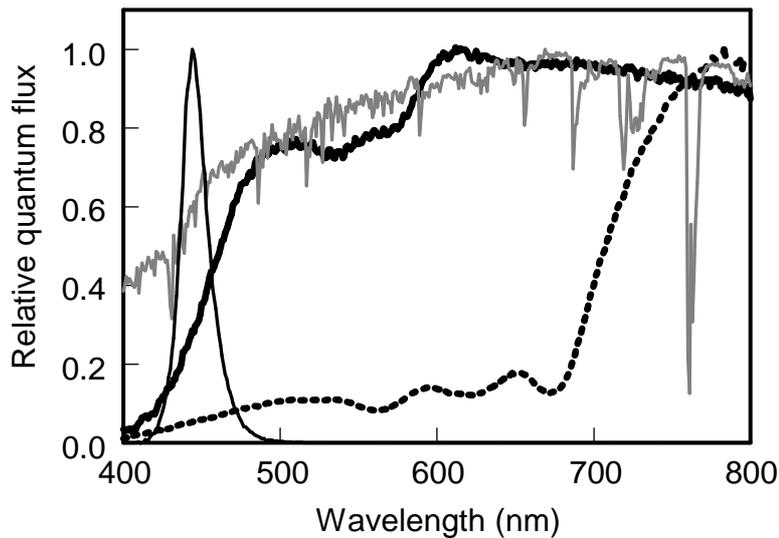


Fig. 1. Spectral distribution of the artificial sunlight (SUN; thick solid line), artificial shadelight (SHADE; dotted line) and blue light (BLUE; thin solid line) used as growth-light sources during leaf development. The grey line represents a standard solar spectrum (ASTM, 2003).

(NIR; 900-1200 nm; Sonneveld *et al.*, 2009). BLUE was provided by light emitting diodes (LEDs; weighted mean wavelength 447 nm; details in Hogewoning *et al.*, 2010c). Irradiance was measured routinely using a quantum sensor (LI-COR, Lincoln, Nebraska, USA), but was also checked with a spectroradiometer (USB2000, Ocean Optics, Duiven, The Netherlands, calibrated against a standard light-source). The difference in irradiance measured with the two devices was less than 2 % for the spectra used. All measurements were done on fully mature second leaves (17-22 days after planting the seedlings), which were supported in a horizontal position during growth if necessary, to ensure that the specified irradiance was received.

Finally, cucumber plants derived from a F3 population in which one third of the plants developed an albino phenotype in the tissue between the veins during and after leaf expansion (De Ruiter Seeds, Bergschenhoek, The Netherlands) were grown under similar conditions but using red LEDs as growth light. Absorption spectra (see below) were measured on the albino leaves in order to obtain a qualitative measure of absorption by non-photosynthetic pigments (N=10).

Gas-exchange

Gas-exchange was measured on leaves from intact plants using a lab-built two-part leaf chamber. Chlorophyll fluorescence (CF) and absorption changes at 820 nm (ΔA_{820}) were measured simultaneously (see below). A gas mix containing $380 \mu\text{mol mol}^{-1} \text{CO}_2$, $20.8 \pm 0.4 \text{ mmol mol}^{-1} \text{H}_2\text{O}$ and $20 \text{ mmol mol}^{-1} \text{O}_2$ in N_2 was used at a flow rate sufficient for CO_2 depletion to remain below $10 \mu\text{mol mol}^{-1}$. Leaf CO_2 and H_2O exchange and the atmospheric pressure were measured using a LI-7000 gas analyzer (LI-COR, Lincoln, Nebraska USA) and CO_2 assimilation was determined according to von Caemmerer and Farquhar (1981). Leaf temperature was maintained $25 \pm 1 \text{ }^\circ\text{C}$ by circulating temperature conditioned water through channels in the upper and lower leaf chamber halves and was monitored from below the leaf using a non-contact temperature sensor (Micro IRT/c,

Exergen, Watertown, MA, USA). No significant leaks in the leaf chamber seal, which might have affected the gas-exchange measurements, were found. Further details on the leaf chamber are described in Hogewoning *et al.* (2010b).

Actinic light

Actinic light (AL) was projected onto the leaf from above via a randomized optical fiber split into four fibers (Heinz Walz GmbH, Effeltrich, Germany) allowing the projection of up to four different light sources onto the leaf. The light sources used were filtered 250W quartz-halogen lamps and a blue LED of the same type as used for BLUE growth-light. To provide narrow band light with 19 different weighted mean wavelengths in the range 400-740 nm, a quartz halogen lamp was filtered using a NIR cut-off filter in combination with different bandpass filters: 400 nm with a 40 nm full width at half maximum (FWHM; Thorlabs, Newton, NJ, USA); 427 nm with 15 nm FWHM (Semrock, Rochester, NY, USA); 445 nm with 25 nm FWHM (Semrock); and 460 nm-740 nm with 10 nm FWHM (Thorlabs). The filters from Semrock were used in combination with an additional NIR filter. In the UV-A region of the spectrum AL was provided using three LEDs (380 nm weighted mean wavelength; H2A1-H375, Roithner Lasertechnik, Vienna, Austria) coupled to three fibers which illuminated the leaf via ports in the side of the leaf chamber. Broad-band AL was provided by filtering a quartz-halogen lamp with a tungsten-to-daylight conversion filter (similar to the SHADE treatment) and by adding an additional NIR filter to remove the longer wavelengths from the spectrum. Rotary-solenoid driven shutters were used to interrupt the AL and different light intensities of the AL provided by the filtered quartz halogen lamps were set using neutral density filters.

Accurate calibration of AL is crucial for a reliable quantitative determination of the wavelength dependence of photosynthetic quantum yield. The incident AL intensity was measured using the photocurrent output of a photodiode (OSD15-5T, Centronic, Croydon, UK) that sampled the irradiance in the leaf chamber via a light guide. The light guide-photodiode system was calibrated *in situ* for each wavelength using a thermopile calibrated against both a quantum sensor (LI-COR) and the spectroradiometer (USB2000, see above), the sensors being placed in the chamber at the same level as the leaf. In case of the spectroradiometer the fiber-optic probe was held in a black holder in order to replicate the arrangement of the quantum sensor, where the optical window of the sensor is located in a black background. The area surrounding the optical window of the thermopile was also painted black. The two calibration methods produced identical results. The calibration with the spectroradiometer was repeated with a cucumber leaf as a background (i.e. the fiber projected through the leaf), producing differences with the results obtained with a black background consistent with the reflectance spectrum of the cucumber leaf. The calibration with a black background underestimated the irradiance a leaf receives more at those wavelengths where reflectance is greater (e.g. 1% at 520 nm, 5 % at 560 nm, 1% at 620 nm, 10% at 720 nm, 22% at 740 nm). This is due to the radiation reflected from the leaf being reflected from the chamber interior back onto the leaf surface. The error was corrected for. The light distribution over the leaf chamber area was measured (Hogewoning *et al.*, 2010b) in order to determine the average irradiance that the leaf received.

Chlorophyll fluorescence

Chlorophyll fluorescence was measured using red and green excitation wavelengths as measuring-light (ML). Green excitation was obtained from a 530 nm peak emission LED (Luxeon K2) filtered by a 560 nm 10 nm FWHM bandpass filter (Thorlabs). Red excitation was obtained from a 640 nm peak emission LED (Luxeon K2) filtered by a 660 nm short-pass filter. Both sources were modulated at different frequencies of about 1000 Hz and applied simultaneously to the leaf. The ML was projected onto the leaf via optical fibers inserted into ports in the side of the leaf chamber. A saturating light-pulse (1.2 s; 10,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) was provided by 16 LEDs (640 nm; Luxeon Rebel) mounted on a brass ring which supported and surrounded the optical fiber for the AL and served as a heat sink for the LEDs. The CF signal was detected from below the leaf by three photodiodes (GaAsP G1736, Hamamatsu, Hamamatsu Japan) mounted on a custom made detector board and wavelengths $<700 \text{ nm}$ were filtered (RG9, 3 mm, Schott, Mainz, Germany). The signal from the photodiodes was demodulated using two lab-built demodulators, one for each of the two excitation wavelengths. Light- and dark-adapted minimum fluorescence (F_0' and F_0 , respectively) were measured in the absence of AL by first closing the shutters and then applying a 1s pulse of far-red (FR) to oxidize any primary quinones (Q_A) and ensure the measurement of a real F_0' . Short FR pulses were provided by a quartz-halogen lamp with a bandpass filter (710 nm, 10 nm FWHM, Thorlabs) coupled into an optical fiber inserted into a port in the side of the leaf chamber. Measurements of light-adapted steady state CF (F'), light-saturated CF (F_m') and F_0' (nomenclature as in van Kooten and Snel, 1990) were used to calculate the PSII operating efficiency (F_q'/F_m' , for which we use Φ_{PSII}), the electron transport efficiency by open PSII traps (F_v'/F_m'), the PSII efficiency factor (F_q'/F_v' , for which we use q_p) and the fraction of Q_A oxidized (i.e. 'open'; q_L) according to Kramer *et al.*, (2004) and Baker (2008). Dark-adapted (20 mins) F_0 and F_m measurement allowed the calculation of the maximum quantum efficiency of PSII (F_v/F_m). In the results only data obtained from measurements with red ML are shown, as the parameters calculated from measurements with red and green ML did not differ significantly, indicating that over the irradiances and wavelengths used any differences in the operational parameters of PSII arising from a different light penetration into the leaves were minimal.

820 nm absorption changes

The ML for ΔA_{820} was provided by an LED (ELD-810-525, Roithner Lasertechnik) coupled into an optical fiber which was inserted into a port in the side of the leaf chamber. The 820 nm signal was detected by three silicon photodiodes (BPW 34 FA, Osram, Regensburg, Germany) mounted on the same circuit board as the photodiodes detecting CF and demodulated using a lab-built demodulator. The signal changes produced by interrupting the AL and applying and removing a 10s FR pulse were recorded. During the FR pulse a saturating light pulse (5ms) was applied to the leaf to ensure a complete oxidation of the P700 pool (see Kingston-Smith *et al.*, 1999) and the rapid signal changes produced were recorded using a USB oscilloscope module (Mephisto Scope 500 kHz, Meilhaus GmbH, Germany). A saturating light pulse (400 ms) from the red LED array was used to accelerate complete reduction of P700 during dark measurement in case P700 reduction was slow (usually at wavelengths $\geq 680 \text{ nm}$). From the signal recorded during light-adapted steady, fully reduced and fully oxidized state of the P700 pool the relative PSI operating efficiency was calculated according to Baker *et al.* (2007).

Measurement procedure for gas-exchange, chlorophyll fluorescence, 820nm absorption changes, light absorption and subsequent calculations

Intact plants were removed from their growth environment and transferred to the laboratory for measurements. A CF image of dark-adapted F_v/F_m was made of each second fully expanded leaf according to Hogewoning and Harbinson (2007). Leaves with a homogeneously distributed $F_v/F_m \geq 0.8$ were clamped in the custom built temperature controlled leaf chamber capable of measuring gas-exchange, CF and ΔA_{820} simultaneously. Gas-exchange was measured under non-photorespiratory (2% O_2) conditions for 20 different spectral regions (in the range 380-740 nm) at five strictly light-limited irradiances per wavelength range. The incident irradiance range used was approximately 10-60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for most wavelength ranges and higher for wavelengths that were relatively poorly absorbed (540-560 nm and >700nm). A minimum irradiance of 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was used to prevent the 'Kok effect' (Kok, 1948; Sharp *et al.*, 1984). The quantum yield for CO_2 fixation was determined by calculating the slope of the linear regression between net assimilation and incident and absorbed (see below) irradiance. Only for 380 nm no quantum yield is shown in the results, due to an error in the irradiance calibration of this wavelength. When gas-exchange was in steady-state, steady-state fluorescence (F'), the maximum fluorescence (F_m') and ΔA_{820} were recorded at each of the five irradiances. The light-adapted minimum fluorescence (F_0') was recorded at three irradiances. Before the leaf was subjected to a different spectrum the leaf was always exposed to an AL spectrum similar to the spectrum used during growth and gas-exchange was allowed to stabilize. This was a precaution to prevent possible effects of adaptation to one spectrum on the response to another spectrum. At the start, middle and end of each day the gas-exchange, CF and ΔA_{820} were measured at the AL spectrum similar to the spectrum used during growth to identify any changes in the leaf response arising during the day. The dark-adapted F_v/F_m was also measured at the start and end of each day. At most eight wavelengths could be measured on a single day and no plant was used for more than one day, so three plants were needed to measure the full sequence of 20 wavelength ranges and per growth-light treatment each sequence was measured three times. As three leaves were required for a full measuring sequence of 20 wavelength ranges, a correction for the calculated quantum yields was made based on the average quantum yield measured for the AL similar to the spectrum used during growth. The correction never exceeded 6%.

After measuring gas-exchange and related parameters, the absorption spectrum of each leaf was calculated in nanometer steps from reflectance and transmittance measurements using two integrating spheres connected to spectrometers, as in Hogewoning *et al.* (2010a). The fraction of the incident AL that was absorbed was calculated for each AL wavelength range by multiplying the absorption spectrum with the AL spectrum. Chloroplast movements from the periclinal to the anticlinal cell walls can be induced by exposure to blue light (Jarillo *et al.*, 2001). The effect of chloroplast movements on the leaf absorption spectrum was checked by comparing the absorption spectrum of a dark-adapted leaf with that measured after exposing the leaf for one hour to 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ blue LED light (weighted mean wavelength 463 nm). The difference was negligible (< 1 %).

The loss of quantum yield for CO_2 fixation on an absorbed light basis (α) that can be attributed to an imbalanced excitation of the two photosystems was estimated for each wavelength using Φ_{PSI} and Φ_{PSII} measured at the highest irradiance for that wavelength to

which the leaf was exposed. The relative quantum efficiencies of electron transport through PSI and PSII were normalized by dividing by the Φ_{PSI} and Φ_{PSII} by the highest efficiency measured across the spectrum, usually 0.80 for Φ_{PSII} measured at 700-720nm and 0.99 for Φ_{PSI} measured at 480 nm. These normalized relative electron transport efficiencies were then used to determine the excitation balance for each wavelength. To estimate the effect of photosystem excitation imbalance on α , the highest measured α (at 620 or 640 nm) was taken as a reference and an adjusted value for α was calculated (α_{est}) for each wavelength.

Quantification of photosystem composition and excitation balance

Thylakoids were prepared from snap-frozen leaves as described by Bassi *et al.* (1988) and the number of LHCII complexes per PSII core was evaluated after SDS-PAGE by colorimetric detection of the amount of Coomassie stain bound to each band. Gel staining bands were imaged (LAS-3000 imaging system, Fujifilm, Japan) and the optical density integrated on the area of the band was quantified using GEL-PRO ANALYZER software (Media Cybernetics Inc., Silver Spring, MD, USA). Eight repetitions from three different leaves per growth light treatment were used. The Chl a:b ratio of the samples was determined by fitting the absorption spectrum of the acetone extract with the spectra of individual pigments in acetone (Croce *et al.*, 2002). The PSI:PSII ratio was calculated from the measured Chl a:b ratio, using the pigment-protein stoichiometry of the individual complexes (Table 1) and the number of LHCII complexes per core. A stoichiometry of 1:1 between the core and the minor antenna was used (Yakushevskaya *et al.*, 2001).

Table 1. The chlorophyll content values of PSI-LHCI, PSII core, LHCII trimer and minor Lhcb (sum of CP29, CP26 and CP24) that were used to calculate the PSI:PSII ratio from the Chl a:b ratio of the membranes. For the calculation a 1:1 ratio for PSII core:minor Lhcb was used for all samples, while the LHCII:PSII core ratio was obtained for each sample from the analysis of the SDS page.

	# Chl	Chl a:b	Chl a	Chl b
PSI-LHCI	168	8.5	150.3	17.7
PSII core	37	∞	37	
LHCII trimer	42	1.3	23.7	18.3
Minor Lhcb	27	2.7	17	10

The excitation balance of the two photosystems was also determined using the absorption spectra of the different photosystem components. First LHCII and PSI (including LHCI) were purified from chloroplasts as in Caffarri *et al.*, 2001. Next, absorption of these components was measured in the range 360-750 nm using a Cary 4000 spectrophotometer. Additionally the absorption spectrum of the PSII C₂S₂M₂ supercomplex of *Arabidopsis* (Caffarri *et al.*, 2009) was used as no significant differences between C₂S₂M₂ in *Arabidopsis* and our leaf samples are expected. From the determination of the number of LHCII per PSII core and the PSI:PSII ratio, the ratio of light absorbed by PSI to that absorbed by PSII was scaled for the photosystem composition corresponding to each of the three different growth light treatments at each wavelength. This calculation assumes that all LHCII remained associated with PSII and therefore takes no account of state-transitions.

Results

Leaf absorption spectrum

The absorption spectra of the leaves that developed under a SUN, SHADE and BLUE spectrum (Fig. 1) followed a similar pattern and differences were small (Fig. 2). At those wavelengths that are absorbed least efficiently (around 560 nm and >700 nm) the absorption was somewhat higher for the BLUE leaves and lowest for the SHADE leaves. The absorption of the albino leaves, which had a white visual appearance and were largely free of Chl and carotenoids, is shown to illustrate the pattern of absorption by non-photosynthetic pigments qualitatively. The albino leaves absorbed substantially at wavelengths <520 nm, whereas at higher wavelengths absorption was close to zero.

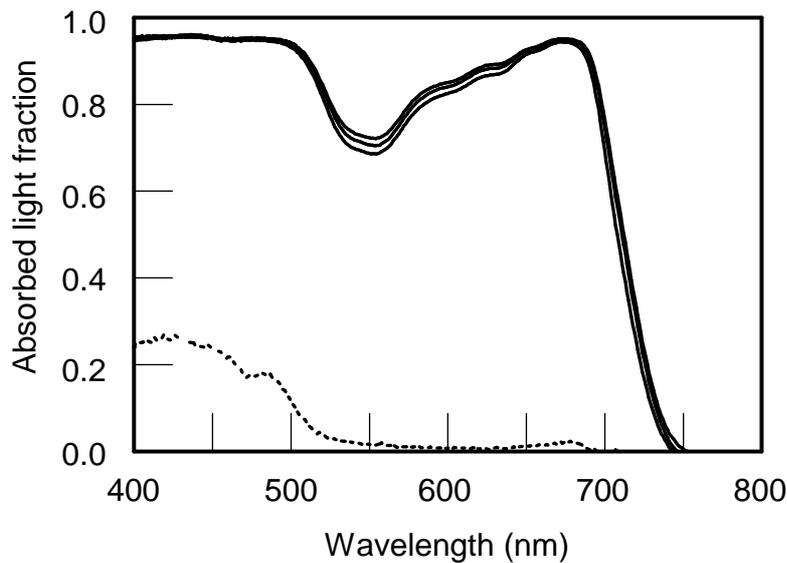


Fig. 2. Absorption spectra of green cucumber leaves grown under SUN (middle solid line), SHADE (lower solid line) and BLUE (upper solid line), and absorption spectrum of albino cucumber leaves (dotted line).

Quantum yield for CO₂ fixation

The assimilation-irradiance response measured in order to calculate the light-limited quantum yield for CO₂ fixation was highly linear ($R^2 \geq 0.997$) at all wavelengths, except at wavelengths ≥ 710 nm where assimilation became so low that the signal-to-noise ratio of the gas-analyzer started affecting the quality of the linear regression (not shown). Therefore the range of irradiances used were strictly in the light-limited part of the CO₂ fixation/irradiance curve. The quantum yield for CO₂ fixation on an incident irradiance basis, which is an eco-physiologically relevant parameter, was highest in the range 620-680nm (Fig. 3A; Tables S2). From 427-560 nm the yield changed relatively little and was approximately 70% that of the highest yield measured, but at 400 nm the yield was higher than in the range 427-560nm. Above 680 nm the yield declined rapidly down to zero at 736 nm. The quantum yield for CO₂ fixation on an absorbed light basis (α), which is physiologically more relevant than on an incident light basis, was maximally 0.093 (Fig. 3B, Tables S1). The wavelengths producing the highest α (580-640 nm for SUN and BLUE) shifted to lower wavelengths on an absorbed light basis compared with an incident light basis (Fig. 3). In the SHADE treatment 620-640nm produced the highest α . In the

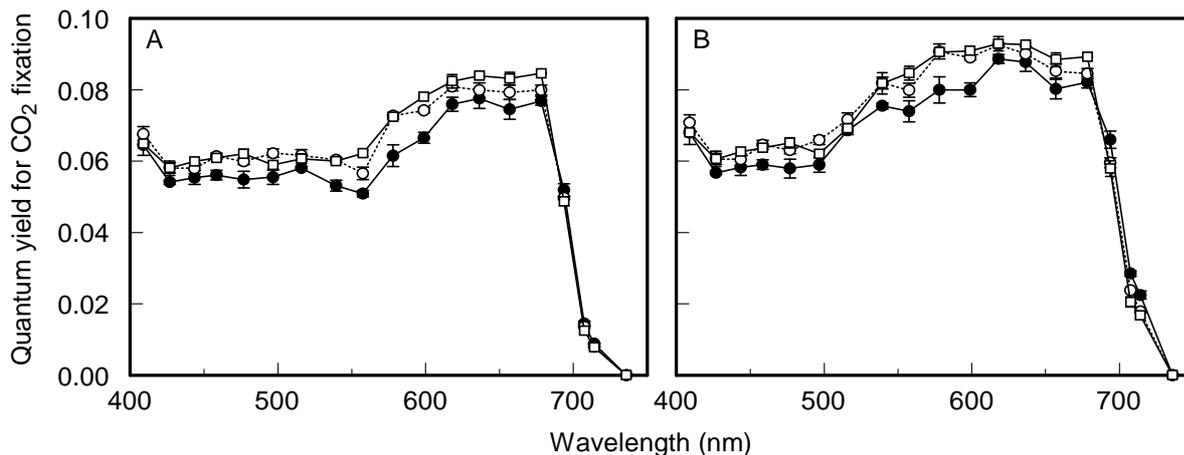


Fig. 3. The quantum yield for CO₂ fixation for 19 different wavelengths on an incident light (A) and absorbed light (B) basis of *Cucumis sativus* leaves developed under SUN (open circles), SHADE (closed circles) and BLUE light (open squares). Error bars represent the s.e.m. (n=3).

green region (520-580 nm) and FR region (≥ 700 nm) the differences between α on an incident and absorbed light basis are greatest due to the relatively low absorption of these wavelength ranges (Fig. 2).

The growth environment spectrum had a considerable influence on α . At wavelengths >680 nm, which are usually considered as 'PSI-light', the SHADE grown leaves had a higher α than the SUN and BLUE grown leaves, e.g. as much as 28% at 710 nm (Fig. 3B). Below 680 nm, which is usually considered to be 'PSII-light' the SUN and BLUE leaves had the highest α at most wavelengths. In more detail, at 580 nm α of the SHADE leaves was as much as 13% lower than α of the SUN and BLUE leaves, while at 520 nm and <460 nm no differences in α were measured for the different growth-light treatments. The difference between the SUN and BLUE light leaves was not significant at most wavelengths, although overall the BLUE leaves tended to have a slightly higher α for 'PSII light' and a slightly lower α for 'PSI-light' than the SUN leaves.

Quantum yield for electron transport through photosystem I and II

The quantum yield for CO₂ fixation remained stable for the actinic light (AL) intensity range used, implying that AL was strictly light-limited. However, the quantum yield for electron transport through PSII (Φ_{PSII}) decreased with increasing irradiance at those wavelengths where Φ_{PSII} was lower than its maximum value (i.e. about 0.8; Fig. 4). For the calculation of the photosystem efficiency balance *in vivo* it appeared therefore most reasonable to use the Φ_{PSI} and Φ_{PSII} values associated with the highest light-limited irradiance. The relative quantum yield for electron transport if all PSII traps were to be open (F_v'/F_m') was always close to 0.8 (i.e. no non-photochemical quenching developed) and the probability of excitation encountering an open PSII (q_p) and the fraction of oxidized Q_A (q_L) followed, respectively, a linear and a curvilinear relationship with Φ_{PSII} (Fig. 5). With decreasing Φ_{PSII} values, q_L decreased significantly more than q_p (Figs. 4 and 5).

The wavelength dependence of the Φ_{PSI} and Φ_{PSII} measured at the highest AL used for each AL wavelength is shown in Fig. 6A. Overall Φ_{PSI} is close to 1.0 at wavelengths <680

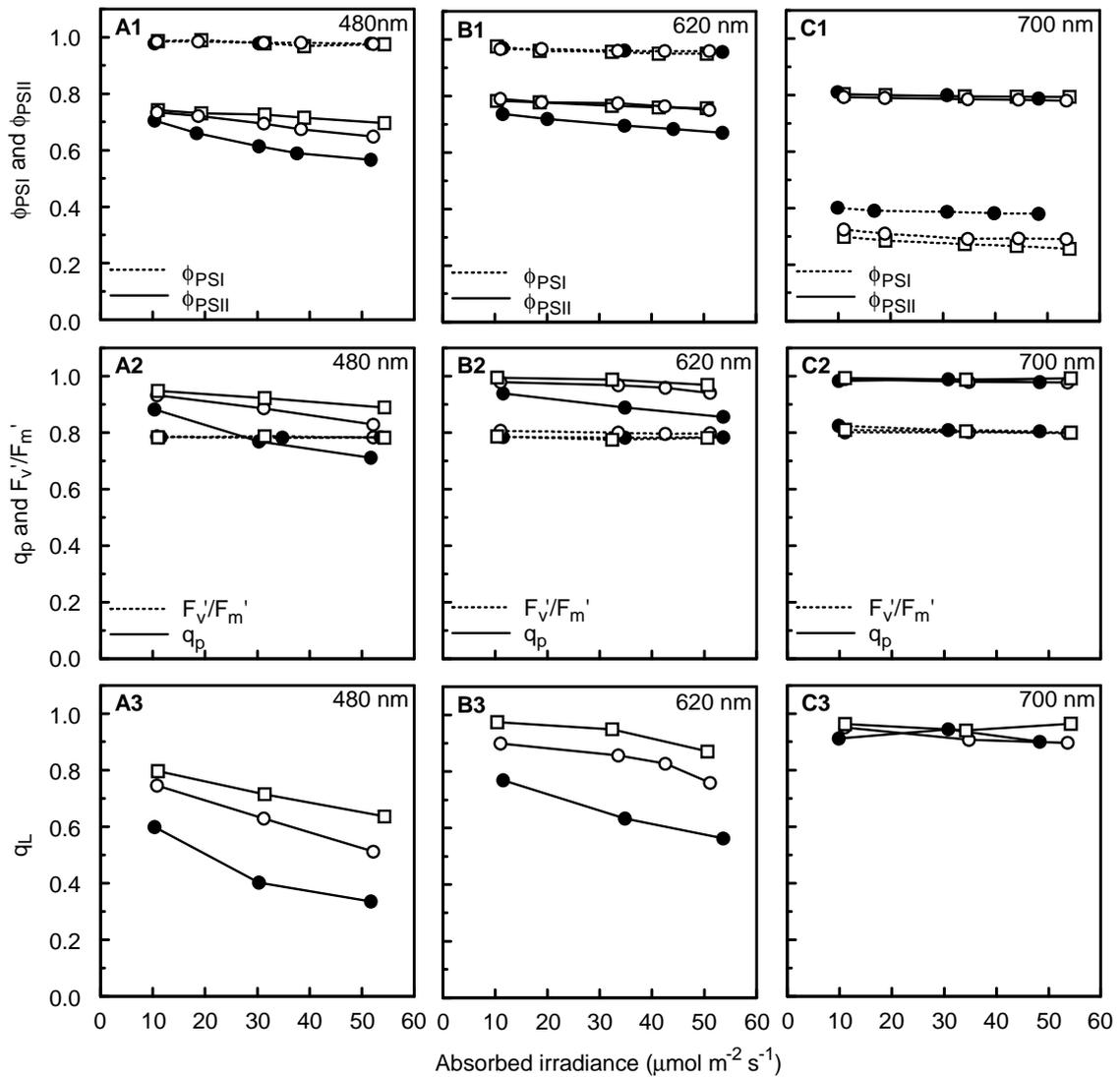


Fig. 4. Response of Φ_{PSI} and Φ_{PSII} (upper row), q_p and F_v'/F_m' (middle row) and q_L (lower row) of cucumber leaves grown under SUN (open circles), SHADE (closed circles) and BLUE (squares) light to absorbed, light-limited actinic irradiance of 480 nm (A), 620 nm (B) and 700 nm (C).

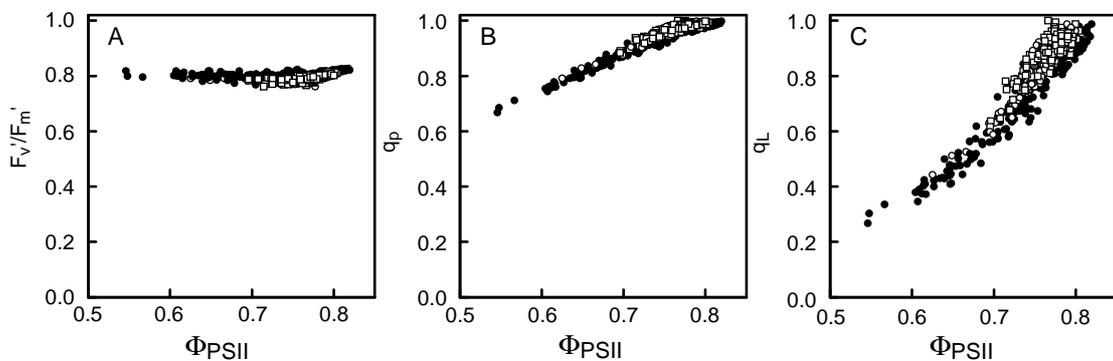


Fig. 5. Relationship of F_v'/F_m' (A), q_p (B) and q_L (C) with Φ_{PSII} . The data corresponding with all actinic light wavelengths (380-740 nm) and all different light intensities used per wavelength are plotted for the three different growth light treatments (SUN: open circles, SHADE: closed circles, BLUE: squares).

nm, whereas it drops progressively ≥ 680 nm, which indicates that the longer wavelengths overexcited PSI. An exception was found at 380nm, for some measurements in the range 400-450 nm and at 520 nm (SUN and BLUE grown leaves); in these cases Φ_{PSI} was slightly less than 1.0. Maximum values for Φ_{PSII} (~ 0.8) were measured >680 nm for all growth-light

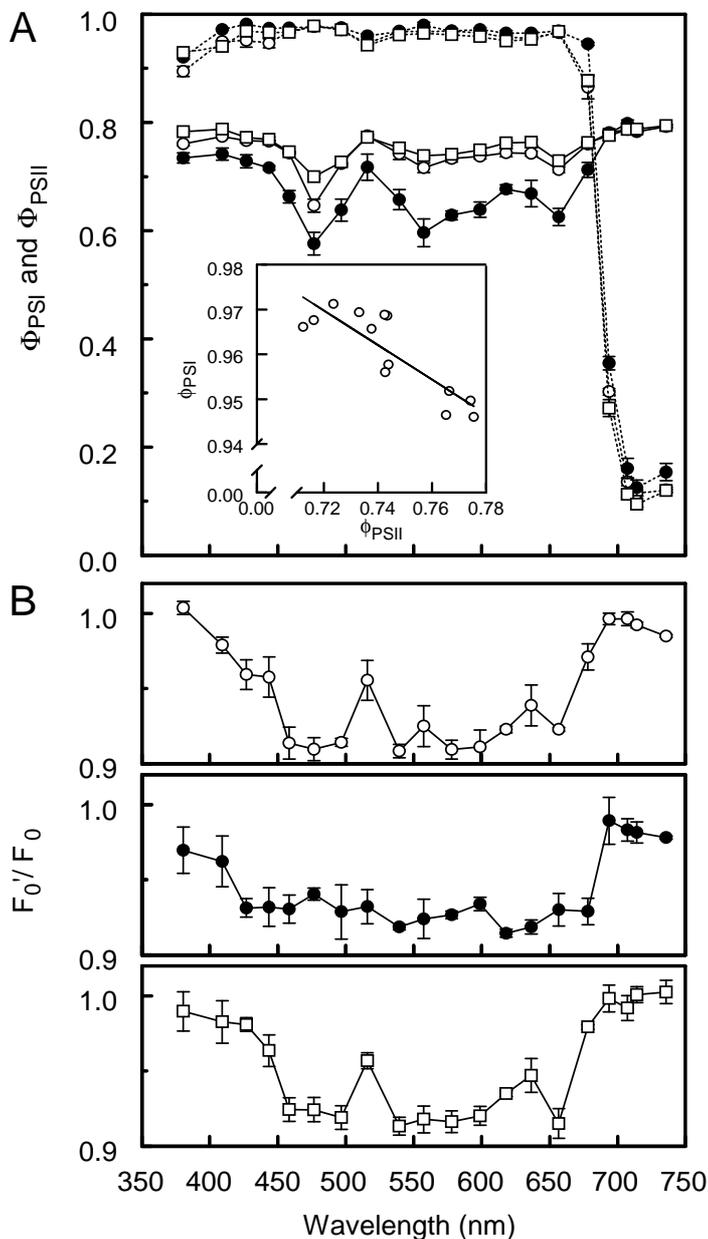


Fig. 6. A: Wavelength dependence of Φ_{PSI} (dashed lines) and Φ_{PSII} (solid lines) of leaves exposed to an irradiance just below an intensity high enough to be no longer light-limited (in the range 50-55 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for most wavelengths. The open circles, closed circles and squares correspond with SUN, SHADE and BLUE grown cucumber leaves, respectively. The inset shows the relationship between Φ_{PSI} and Φ_{PSII} found for SUN-grown leaves for the wavelengths which produced values of Φ_{PSI} within the narrow efficiency range of 0.94-0.98. B: Wavelength dependence of the ratio of light adapted minimum fluorescence (F_0') and dark-adapted minimum fluorescence (F_0), as an indicator of state transitions induced by the actinic measuring-light ($F_0'/F_0=1$ indicates state 1; symbols as in A).

treatments and for the SUN and BLUE grown leaves in the range 380-450 nm and at 520nm. The lowest values were measured around 480 nm, 560 nm and at 660 nm, indicating significant overexcitation of PSII. Overall Φ_{PSII} of the SHADE grown leaves was lower at wavelengths $\leq 680\text{nm}$, and Φ_{PSI} was higher $\geq 680\text{ nm}$, compared with the SUN and BLUE grown leaves (Fig. 6A), which is similar to the pattern found for α (Fig. 3B). Remarkably, though Φ_{PSI} is close to 1.0 when Φ_{PSII} is below its maximum of about 0.8, Φ_{PSI} does show very subtle changes which are anti-parallel to those of Φ_{PSII} (Fig. 6A, inset).

The measurements of Φ_{PSII} (Fig. 6A) were taken after the leaf was exposed to the AL for sufficient time to allow for possible state transitions (≥ 15 mins). The ratio of F_0' and F_0 can be used as an indicator for state-transitions in the absence of non-photochemical quenching (Allen, 1992; Samson and Bruce, 1995). At those wavelengths where Φ_{PSII} is below its maximum of 0.8 and therefore PSII is excited more than PSI, F_0' was almost 10% lower than F_0 , whereas at wavelengths overexciting PSI F_0' and F_0 were similar (Fig. 6B), indicating that wavelengths overexciting PSII indeed induced state transitions. Notably the $F_0':F_0$ ratio is at its lowest value over a broader range of wavelengths in the SHADE leaves than in the SUN and BLUE leaves, which is consistent with the broader range of wavelengths overexciting PSII (i.e. Φ_{PSII} is well below 0.8) in the SHADE leaves (Fig. 6A).

The Φ_{PSI} and Φ_{PSII} measured at the highest AL used at each wavelength were used to estimate photosynthetic efficiency losses due to an imbalance of excitation energy distribution between the two photosystems (i.e. the photosystem efficiency balance). The resulting estimates of α (α_{est}) are shown together with α calculated from the gas exchange measurements (Fig. 7). The two approaches produce similar values at wavelengths ≥ 580 nm, except at 680 nm for the SHADE leaves (Fig. 7B) and at 700 nm for all growth-light treatments. At wavelengths $\leq 560\text{nm}$, where carotenoids and non-photosynthetic pigments absorb, α_{est} overestimates α considerably (up to 50% in the range 420-460 nm).

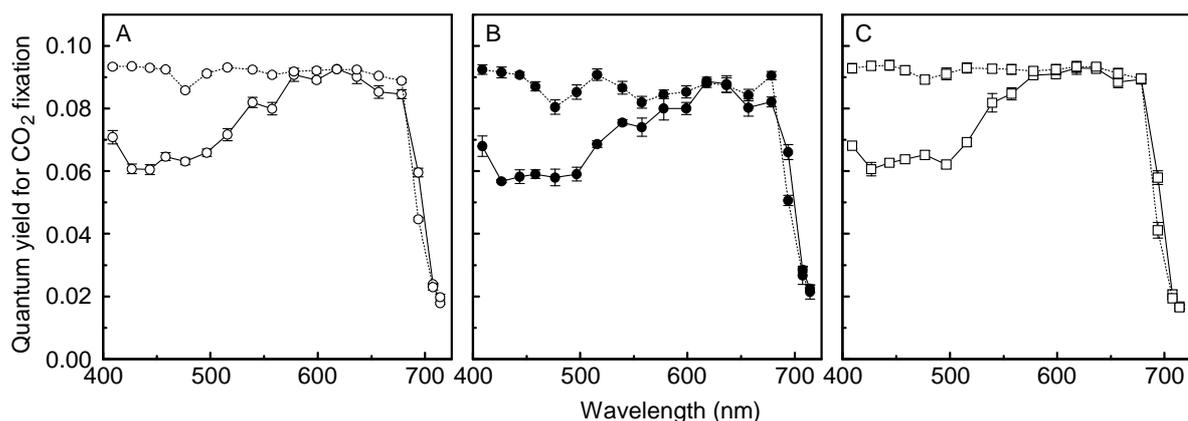


Fig. 7. Quantum yield for CO₂ fixation for 18 different wavelengths (400-720 nm) calculated from gas-exchange measurements (α ; solid lines, as in Fig. 3B) and from the excitation balance of the two photosystems using the measurements of Φ_{PSI} and Φ_{PSII} (α_{est} ; dotted lines). Graphs A, B and C correspond with leaves grown under SUN, SHADE and BLUE irradiance, respectively. Error bars indicate the s.e.m. (n=3).

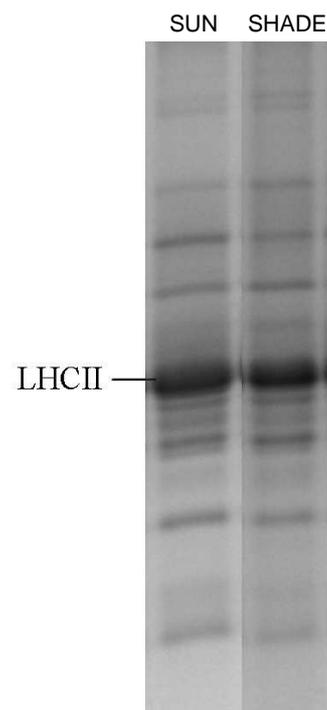
Photosystem composition and excitation balance

The Chl a:b ratio was lowest in the SHADE leaves and slightly lower in the SUN leaves compared with the BLUE leaves (Table 2). At the protein level, SUN and SHADE leaves showed a virtually identical PSII antenna size (Table 2; Fig. 8), while the PSI:PSII ratio was significantly higher for SUN. In the BLUE leaves a reduced amount of LHCII was observed, while the PSI:PSII ratio was identical to that of the SUN leaves. The stoichiometry between the pigment-protein complexes was used to scale their absorption spectra thus allowing calculation of a proxy for the wavelength dependence of the excitation balance of the two photosystems. A comparison of the estimate of photosystem excitation balance with the photosystem efficiency balance (i.e. derived from combined CF and ΔA_{820} measurements *in vivo*) measured at each wavelength revealed a strong linear relationship (Fig. 9). Note that the relationship between the two approaches as in Fig. 9B is improved if the data corresponding with the weighted mean wavelengths 458 nm and 497 nm, where carotenoids have absorption maxima, are not taken into account (Fig. 9C). *In vitro*, where no allowance for the effect of state-transitions was made, excitation appears to be more imbalanced than *in vivo* at those wavelengths overexciting PSII (i.e. PSII-light). For PSI-light (>680 nm) the two approaches produce similar results. The apparently stronger overexcitation of PSII *in vitro* is illustrated by the different slopes of the relationship between the two approaches for the data points corresponding with PSI-light and PSII-light (Fig. 9B).

Table 2. Chlorophyll a:b ratio, number of LHCII trimers per PSII core and the reaction center ratio of the two photosystems of cucumber leaves grown under SUN, SHADE and BLUE growth irradiance. Different letters indicate significant differences (Fisher's LSD; $P \leq 0.05$; $n=3$).

Growth-light:	SUN	SHADE	BLUE
Chl a:b	2.98 ^b	2.67 ^c	3.10 ^a
LHCII per PSII core	3.61 ^{ab}	3.72 ^a	3.29 ^b
RC ratio PSII/(PSII+PSI)	0.53 ^b	0.61 ^a	0.53 ^b

Fig. 8. Analysis of LHCII content in SUN and SHADE grown leaves. SDS-PAGE was followed by Coomassie staining and densitometric analysis of the polypeptide, was used to quantify LHCII levels relative to CP29 (Lhcb4). On each lane 12 μ g of thylakoids were loaded.



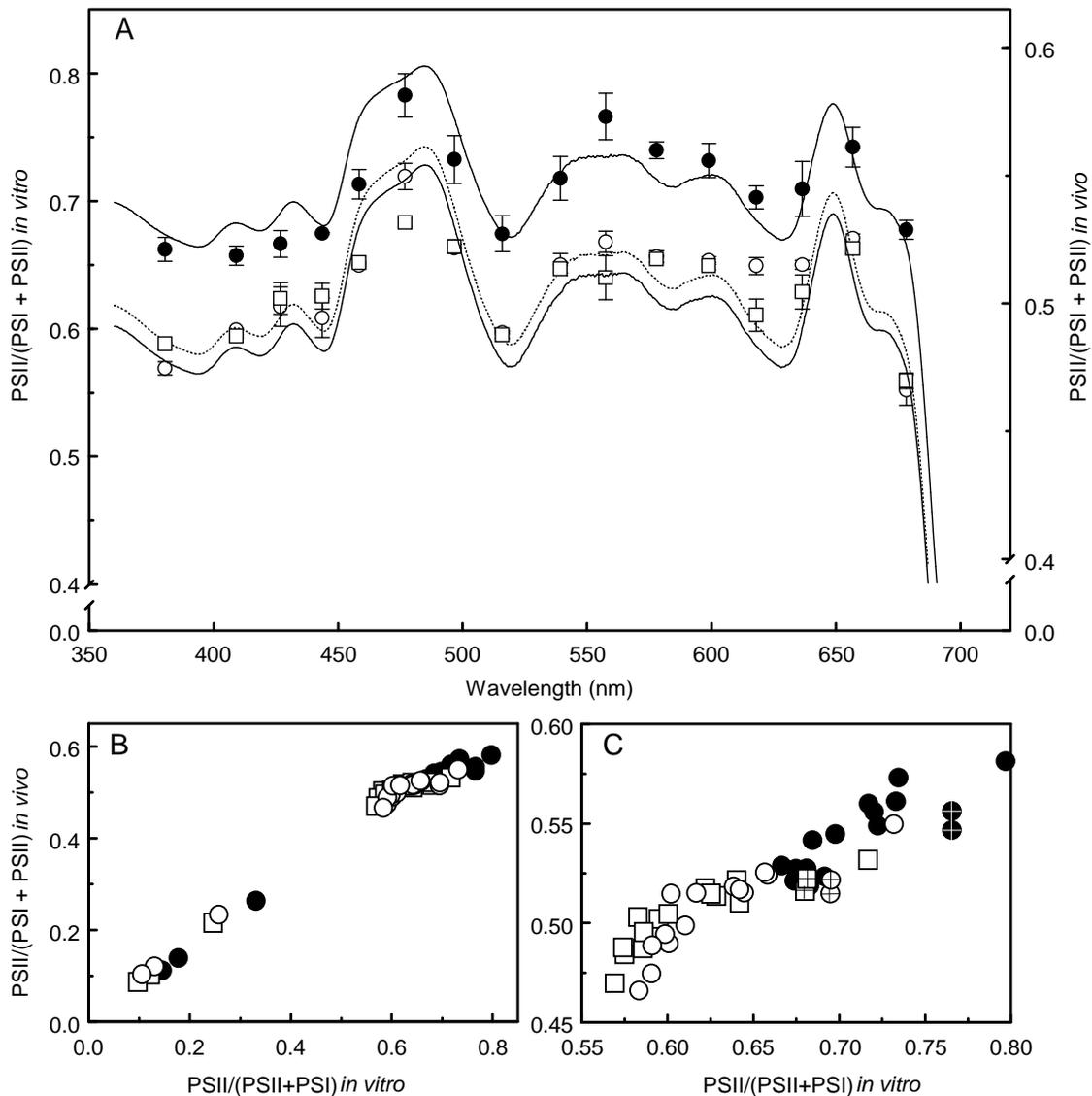


Fig. 9. A: Wavelength dependence of the excitation balance of the two photosystems calculated as absorption by PSII divided by absorption of both PSI and PSII using an *in vitro* (connected lines) and *in vivo* (unconnected data points) approach. The left y-axis corresponds with the *in vitro* approach (SUN: dashed line, SHADE: upper solid line, BLUE: lower solid line) and the right y-axis with the *in vivo* approach (SUN: open circles, SHADE: closed circles, BLUE: squares). Note that the scale is different for the two y-axes. B: Relationship between the excitation balances obtained *via* the *in vitro* and *in vivo* approach. Note that for values of $\text{PSII}/(\text{PSII} + \text{PSI}) < 0.4$, which correspond with wavelengths preferentially exciting PSI, both the *in vitro* and *in vivo* methods produce similar results. At values of $\text{PSII}/(\text{PSII} + \text{PSI}) > 0.5$, which correspond with wavelengths preferentially exciting PSII, the overexcitation of PSII appears to be stronger *in vitro*. C: The data in the upper right corner of plot B, which correspond with wavelengths preferentially exciting PSII, are presented on a more detailed scale in C. The six crossed data points correspond with the absorption peaks of carotenoids (458 and 497 nm weighted mean wavelengths). If these data points are not taken into account, the linearity of the relationship between the *in vitro* and *in vivo* approach improves significantly.

Discussion

Origin of the wavelength dependence of quantum yield

In this study the underlying causes of the wavelength dependence of quantum yield for CO₂ fixation (α) and its adaptation capacity to the growth-light spectrum have been clarified, while the overall wavelength response pattern of α is comparable to that found in earlier studies (e.g. McCree, 1972a; Inada, 1976). Quantitatively, our maximum α of 0.093 CO₂ fixed per absorbed photon at 620-640 nm (Fig. 3) is in line with Singsaas *et al.* (2001), who concluded that the intrinsic maximum α varies little from the average of 0.093 reported by Long *et al.* (1993) for a range of C₃ plants of diverse life form, taxa and habitat. Within the light-limited irradiance range, which was the case for our measurements, Φ_{PSI} and Φ_{PSII} are expected to remain unchanged in the absence of any alternative electron sinks beside photochemistry or back reactions between electron acceptors and donors, even in case of an imbalanced photosystem excitation. However, even at wavelengths strongly over-exciting PSII (e.g. 480 nm; Fig. 6A) reflected by a relatively low Φ_{PSII} at the highest irradiance used (i.e. about 50 $\mu\text{m}^{-2}\text{s}^{-1}$), Φ_{PSII} was relatively high at very low irradiances (i.e. about 10 $\mu\text{m}^{-2}\text{s}^{-1}$) and decreased with increasing irradiance (Fig. 4A1). This phenomenon suggests that an alternative electron acceptor, such as O₂ (Pospíšil, 2009), or possibly back reactions (Quigg *et al.*, 2006) are maintaining the Q_A pool in a relatively oxidized state at low irradiances despite the insufficiency of electron transport through PSI. Though these alternative routes for oxidation of the Q_A pool increase Φ_{PSII} at low irradiances, they do not result in higher rates of linear electron transport and therefore do not increase CO₂ fixation.

In the wavelength range 580-720 nm α_{est} calculated from efficiency losses due to imbalances in excitation of the two photosystems generally matches well with α calculated from gas-exchange (Fig. 7). This proves that non-destructive spectroscopic measurements (CF, ΔA_{820}) are quantitatively useful in order to determine quantum yield losses from photosystem excitation imbalances. From 400-460 nm and at 520 nm the photosystem excitation is balanced (Fig. 6A), but α is up to 35% below α_{est} (Fig. 7). Energy losses at wavelengths ≤ 560 nm are to be expected due to the presence of carotenoids and non-photosynthetic pigments in the leaf (Terashima, 2009). The energy transfer efficiency from carotenoids to Chls *in vivo* has still not been fully elucidated. For photosystem I (PSI), a transfer efficiency of 70% was reported for the core (de Weerd *et al.*, 2003) and approximately 85% for the light harvesting complex (E. Wientjes unpublished, as cited in van Oort *et al.*, 2008). Croce *et al.* (2001) reported an energy transfer efficiency of 85 - 90% from lutein, the most abundant carotenoid in LHCII, to Chls, while violaxanthin was shown not to transfer energy to Chls (Caffarri *et al.*, 2001). In addition to carotenoids which absorb predominantly in the wavelength range 400-520 nm, quantum yield is lowered at wavelengths below 560 nm due to absorption by non-photosynthetic pigments, such as anthocyanins and flavonoids (Inada 1976, Evans 1986). UV and blue light are reported to stimulate the transcription of flavonoid synthesis genes in order to protect the plant against photodamage (e.g. Kubasek *et al.*, 1992, Jackson and Jenkins 1995). However, no notable difference between α of the SUN and the BLUE leaves was found (Fig. 3B). The absorption spectrum of the albino cucumber leaves (Fig. 2) at least qualitatively supports that the lower value of α than that of α_{est} in the blue region (Fig. 7) is due to the presence of non-photosynthetic pigments. A relatively low reflectance, and therefore a significant

absorption, was also found below 520 nm for *Arabidopsis* leaves that were made largely Chl and carotenoid deficient by knocking out a gene encoding a key enzyme in carotenoid biosynthesis (Zheng *et al.*, 2010). Quantitatively the blue light absorption by non-photosynthetic pigments in these albino leaves cannot be related to the relative absorption by such pigments in the green cucumber leaves. It would be valuable to disentangle the contributions of non-photosynthetic pigments and carotenoids to quantum yield losses quantitatively. Yields of food or fuel production crops with a relatively large photosynthetic efficiency loss due to non-photosynthetic pigments may be improved by breeding varieties with a lower non-photosynthetic pigment content.

Quantum yield and photosystem stoichiometry acclimation to growth spectrum

At those wavelengths where the SHADE leaves had a lower α than the SUN or the BLUE leaves (Fig. 3B), Φ_{PSII} was also lower for the SHADE leaves (Fig. 6A). In contrast, where SHADE leaves had a higher α (>680nm) their Φ_{PSI} was also higher. So the SHADE leaves, grown under a spectrum with a large proportion of wavelengths overexciting PSI (Fig. 1), utilize PSI-light more efficiently than SUN and BLUE leaves, whereas SUN and BLUE leaves utilize PSII-light more efficiently than SHADE leaves. The differences in the wavelength dependence of α , Φ_{PSI} and Φ_{PSII} between the SHADE leaves on the one hand and the SUN and BLUE leaves on the other are consistent with the relatively greater number of PSII reaction centers found in the SHADE leaves (Table 2). However, the antenna size of PSII did not differ for SUN and SHADE leaves. These results are in line with those of Chow *et al.* (1990). We further show that across the spectrum of wavelengths used the excitation of PSII is higher than that of PSI in the SHADE leaves compared with the SUN and BLUE leaves, whether this is derived from *in vitro* or *in vivo* measurements (Fig. 9A). This clearly shows the extent and consequences of photosystem acclimation to growth-light spectra exciting PSI and PSII in different proportions. Despite the possible discrepancies between photosystem efficiency balance *in vivo* and excitation balance *in vitro* due to inefficiencies in excitation energy transfer and charge separation, and cyclic electron transport, back-reactions or transfer to O_2 , the relationship between the two approaches is highly linear (Fig. 9B). This indicates that the relevance of these processes for the physiological photosynthetic operation *in vivo* was small under our conditions. The outliers in the relationship between the two approaches (458 nm and 497 nm; Fig. 9C) are likely due to the strong absorption of these wavelengths by carotenoids, which are associated more with PSII than PSI and which on average have a lower excitation energy transfer yield when associated with PSII than PSI. The *in vitro* approach using absorption does not incorporate a correction for excitation transfer efficiency and thus overestimates the relative excitation of PSII at wavelengths where carotenoids absorb strongly. The greater imbalance found for the *in vitro* approach than for the *in vivo* approach at those wavelengths preferentially exciting PSII (Fig. 9) is possibly due to state-transitions, which partly re-balanced photosystem excitation during the *in vivo* measurements; the *in vitro* derived calculation of excitation balance does not make any allowance for the effect of state transitions. The lower F_0' found for PSII light than for PSI-light, which produced an F_0' equal to dark-adapted F_0 , and the broader wavelength range producing minimal F_0'/F_0 values for the SHADE leaves (Fig. 6B), support the proposition that state-transitions diminished the overexcitation of PSII. However, apparently state-transitions are not capable of restoring imbalances in photosystem excitation completely in case of strong

imbalances, as *in vivo* the PSII acceptor side is still reduced substantially over a broad range of wavelengths (Figs 5A and 7).

Notably we found no significant, or only small differences in the wavelength dependence of quantum yield for the SUN or BLUE leaves, despite blue being widely used to preferentially excite PSII. Closer inspection of our data reveals that for both the *in vitro* and *in vivo* derived calculations of photosystem excitation balance (Fig. 9A), only the part of the blue region in the range 460-500 nm strongly overexcites PSII. Thus the light source in our BLUE treatment (447 nm LEDS) would have overexcited PSII to only a small extent, which is in line with the small differences sometimes found between our SUN and BLUE treatment.

Our study shows that photosystem efficiency balance determines the wavelength dependence of leaf photosynthetic quantum yield where absorption by carotenoids and non-photosynthetic pigments is insignificant. State transitions partly counteracted imbalances resulting from illumination with PSII-light. Where the plant's growth-light spectrum excited the two photosystems to different degrees, the PSI:PSII ratio in the thylakoids was adjusted in order to increase the light use efficiency of the growth-light for electron transport. The remarkable match between the *in vitro* and *in vivo* approach used to determine photosystem excitation balance proves for the first time that measurements on isolated thylakoids can be used reliably to make a quantitative estimate of the *in vivo* wavelength dependence of the quantum yield for CO₂ fixation.

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Appendix

Tables S1. The wavelength dependence of the quantum yield for CO₂ fixation on an absorbed light basis for *Cucumis sativus* leaves grown under artificial solar light (SUN), artificial shadelight (SHADE) and blue light (BLUE). The quantum yields are presented on an absolute (i.e. CO₂ molecules fixed per absorbed photon; left table) and relative basis (right table). The spectra of the three different growth-light sources are shown in Fig. 1.

Wavelength (nm)	SUN	SHADE	BLUE	Wavelength (nm)	SUN	SHADE	BLUE
409	0.071	0.068	0.068	409	0.77	0.77	0.73
427	0.061	0.057	0.061	427	0.66	0.64	0.65
444	0.060	0.058	0.063	444	0.65	0.66	0.67
458	0.065	0.059	0.064	458	0.70	0.67	0.69
477	0.063	0.058	0.065	477	0.68	0.65	0.70
497	0.066	0.059	0.062	497	0.71	0.67	0.67
516	0.072	0.069	0.069	516	0.77	0.77	0.74
539	0.082	0.075	0.082	539	0.88	0.85	0.88
558	0.080	0.074	0.085	558	0.86	0.83	0.91
578	0.091	0.080	0.091	578	0.98	0.90	0.97
599	0.089	0.080	0.091	599	0.96	0.90	0.98
618	0.093	0.089	0.093	618	1.00	1.00	1.00
636	0.090	0.088	0.093	636	0.97	0.99	1.00
657	0.085	0.080	0.088	657	0.92	0.90	0.95
678	0.085	0.082	0.089	678	0.91	0.93	0.96
694	0.060	0.066	0.058	694	0.64	0.74	0.62
707	0.024	0.029	0.021	707	0.26	0.32	0.22
714	0.018	0.022	0.017	714	0.19	0.25	0.18
736	0.000	0.000	0.000	736	0.00	0.00	0.00

Tables S2. The wavelength dependence of the quantum yield for CO₂ fixation on an incident light basis (i.e. ‘action spectrum’) for *Cucumis sativus* leaves grown under artificial solar light (SUN), artificial shadelight (SHADE) and blue light (BLUE). The quantum yields are presented on an absolute (i.e. CO₂ molecules fixed per incident photon; left table) and relative basis (right table). The spectra of the three different growth-light sources are shown in Fig. 1.

Wavelength (nm)	SUN	SHADE	BLUE	Wavelength (nm)	SUN	SHADE	BLUE
409	0.068	0.065	0.065	409	0.84	0.83	0.77
427	0.058	0.054	0.058	427	0.72	0.70	0.69
444	0.058	0.055	0.060	444	0.72	0.71	0.71
458	0.061	0.056	0.061	458	0.76	0.72	0.72
477	0.060	0.055	0.062	477	0.74	0.71	0.73
497	0.062	0.055	0.059	497	0.77	0.72	0.70
516	0.062	0.058	0.061	516	0.76	0.75	0.72
539	0.060	0.053	0.060	539	0.75	0.69	0.71
558	0.057	0.051	0.062	558	0.70	0.66	0.73
578	0.073	0.062	0.072	578	0.90	0.79	0.85
599	0.074	0.067	0.078	599	0.92	0.86	0.92
618	0.081	0.076	0.082	618	1.00	0.98	0.97
636	0.080	0.078	0.084	636	0.99	1.00	0.99
657	0.079	0.075	0.083	657	0.98	0.96	0.98
678	0.080	0.077	0.085	678	0.99	0.99	1.00
694	0.050	0.052	0.049	694	0.61	0.67	0.58
707	0.014	0.014	0.012	707	0.17	0.19	0.15
714	0.008	0.009	0.008	714	0.10	0.11	0.09
736	0.000	0.000	0.000	736	0.00	0.00	0.00

CHAPTER 4

The influence of measuring-light spectrum on estimates of photosynthesis *in situ* and its consequences for prediction of biomass production

Abstract

- *Background and Aims* The measuring-light spectrum used during photosynthesis measurements is often different from the irradiance-spectrum that plants are exposed to *in situ*. We investigated the effect of measuring-light (ML) spectrum on the reliability of estimates of photosynthesis *in situ* by gas-exchange and chlorophyll fluorescence measurements. Additionally, we explored the consequences of errors in the estimate of photosynthetic rates *in situ* for the outcome of plant growth models.
- *Methods* *Cucumis sativus* was grown under different combinations of red and blue LEDs and under white fluorescent tubes, which are, respectively, modern and more traditional irradiance sources for growth chambers. CO₂ fixation was measured with low and saturating irradiance provided by different combinations of red and blue LEDs, which are widely used as photosynthesis measuring-light source. Electron transport rates (ETR) were calculated using measurements of chlorophyll fluorescence and leaf light absorptance.
- *Key Results* The effect of ML spectrum on light-limited quantum yield for CO₂ fixation (α) was up to 25% and was independent of the growth-light spectrum. In contrast, at saturating irradiance assimilation was affected significantly by the growth-light spectrum, however, only weakly by the ML spectrum. Therefore ML spectrum mainly has consequences for the validity of α . In contrast to CO₂ fixation, the measured photosystem II electron transport efficiency was similar for the different ML spectra used. Therefore the calculated ETR-CO₂ fixation relationship changed considerably with changes in measuring-light spectrum, which has consequences for the practical use of ETR calculations for estimating photosynthesis.
- *Conclusions* A photosynthesis measuring-light spectrum deviating from the plant growth-light spectrum can lead to significant errors in estimates of CO₂ fixation rates *in situ*. The use of such erroneous estimates as input for crop models can have disproportionately large consequences for predictions of plant growth.

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Introduction

The rate of photosynthesis is a major determinant of plant productivity and accurate measurements of CO₂ fixation are important not only in understanding crop growth, but also as input for plant productivity models. Photosynthesis is known to be influenced by many conditions during plant growth and measurement, and as a result the environment in which plants are grown and in which photosynthesis is measured is usually specified in some detail. Whereas factors such as temperature, CO₂ concentration and the intensity of irradiance have well understood effects on the measured rate of CO₂ fixation, the effect of irradiance spectrum used as growth light (GL) or measuring-light (ML) is less well understood. The most usual light sources in growth-cabinets are fluorescence tubes or gas discharge lamps, which have strong emission lines distributed throughout the photosynthetically active spectrum that are often superimposed upon a weaker semi-continuous spectrum. Recently, however, growth cabinets have been produced that use light-emitting diodes (LEDs) as the radiation source and these often make extensive use of mixtures of red and blue LEDs. In greenhouse horticulture, the use of LEDs as sources of supplementary irradiance is being widely explored (e.g. Hogewoning *et al.*, 2007; Trouwborst *et al.*, 2010). Similar changes can be seen in the evolution of radiation sources used to provide the irradiance for photosynthesis measurements; LEDs are increasingly supplanting quartz-halogen and gas discharge lamps. In this application red/ blue mixtures of LEDs are used in many modern gas analysis systems, whereas older gas analysis systems and systems for more biophysical research into photosynthesis largely use red LEDs alone.

In contrast to the spectrally diverse composition of the light-sources traditionally used for plant growth and the measurement of photosynthesis, LEDs have a relatively narrow emission spectrum. Although a clear effect of light spectrum on the light limited-quantum yield for CO₂ fixation (α) has been demonstrated (e.g. Bulley *et al.*, 1969, Balegh and Biddulph 1970, McCree 1972a, Inada 1976, Evans 1987), the discrepancy between the spectral composition of the GL and the ML is generally not taken into account. The ML spectrum used to measure photosynthesis in studies using relatively narrow band radiation sources for plant growth was either different from the GL spectrum, or the ML spectrum used was not described (e.g. Saebo *et al.*, 1995, Kim *et al.*, 2004, Matsuda *et al.*, 2004). While still being of physiological interest, the interpretation of such data in relation to potential biomass production under spectrally different GL regimes is difficult.

In research where photosynthesis *in situ* under different GL spectra is compared, or when measurements of photosynthesis are used to parameterize models for crop growth, accurate measurements of photosynthetic rates are a requirement for reliable results. Several studies have shown that red light produces the highest α compared with other single colours or white light (e.g. McCree 1972a, Inada 1976, Evans 1987). Thus gas-analysis systems using red LEDs as ML are expected to overestimate photosynthesis under broad-band GL conditions, especially under light-limited irradiance. Under light-saturated conditions spectral effects on assimilation are less well defined, but the ML light spectrum may trigger alterations in the stomatal conductance (e.g. Sharkey and Raschke 1981, Willmer and Fricker 1996) and therefore also in the internal CO₂ concentration (C_i), which would directly alter the light-saturated photosynthesis rate.

The ML spectrum may not only have consequences for the reliability of photosynthetic rates *in situ* estimated by gas analysis, but also for the reliability of calculations of the linear electron transport rate (ETR; Baker *et al.*, 2007). ETR is considered to be linearly correlated with CO₂ fixation in the absence of photorespiration (e.g. Genty *et al.*, 1989). The calculated ETR often depends on the relative quantum yield for photosystem II (PSII) electron transport (Φ_{PSII}) measured by chlorophyll (Chl) fluorescence, as fixed values are often used for the fraction of absorbed light (a_{leaf}) and the excitation balance of the two photosystems ($a_{\text{PSII}}/(a_{\text{PSI}} + a_{\text{PSII}})$). Generally, an a_{leaf} of 0.84 (e.g. Goudriaan and Van Laar 1994), which is reasonable for a daylight spectrum, and a balanced photosystem excitation are assumed. However, the ML-light spectrum can affect these parameters. For example, red or combined red and blue ML results in a higher a_{leaf} than that for daylight, and possibly an overexcitation of photosystem II when measuring on a daylight grown leaf (e.g. Evans 1986, Chow *et al.*, 1990). The blue-green region of the visible part of the spectrum gives rise to additional complications, as these wavelengths are not only absorbed by Chls, but also by carotenoids and non-photosynthetic pigments. The transfer of excitation energy between Chls is highly efficient, whereas the efficiency of transfer from carotenoids to Chls is lower and non-photosynthetic pigments (e.g. flavonoids) do not contribute to electron transport at all (Terashima *et al.*, 2009). Absorbed excitation energy which is not transferred to Chls does not contribute to CO₂ fixation and thus decreases α . However, such a decrease in α is not reflected by a decrease in Φ_{PSII} , resulting in overestimation of ETR.

In this paper we describe how the use of LEDs during measurement affects photosynthesis and its quantification of leaves developed under different growth-light spectra. We used combinations of red and blue LEDs as growth- and measuring-light as these are the wavelengths typically used in modern growth cabinets and photosynthesis measuring systems as well as being increasingly deployed as assimilation lighting in greenhouses. We investigated the effects of growth and measurement spectrum on the measurement of CO₂ fixation and explored the impact of erroneous quantum yields for CO₂ fixation on the outcome of plant growth models. Additionally we studied growth and measurement spectrum effects on measurements of Φ_{PSII} and the use of these data as a proxy for CO₂ fixation measurements.

Materials and methods

Plant material and growth conditions

Cucumber plants (*Cucumis sativus* cv. Hoffmann's Giganta) were cultivated in a climate chamber under similar conditions as in Hogewoning *et al.* (2010c), except for the growth-irradiance spectra. All plants were subjected to $100 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$ irradiance and the light- treatments consisted of 100% blue light (B), 30% blue and 70% red light (BR), 100% red light (R) or cool white fluorescent light (TLD 50W 840 HF, Philips, The Netherlands). Red and blue irradiance was provided by LEDs (details and irradiance measurement routine are described in Hogewoning *et al.*, 2010c). The lamp spectra are shown in Fig. 1A, together with a standard solar spectrum (ASTM, 2003). Leaves of different plants did not overlap and if necessary the second leaf was supported in a horizontal position to ensure that it received the specified irradiance.

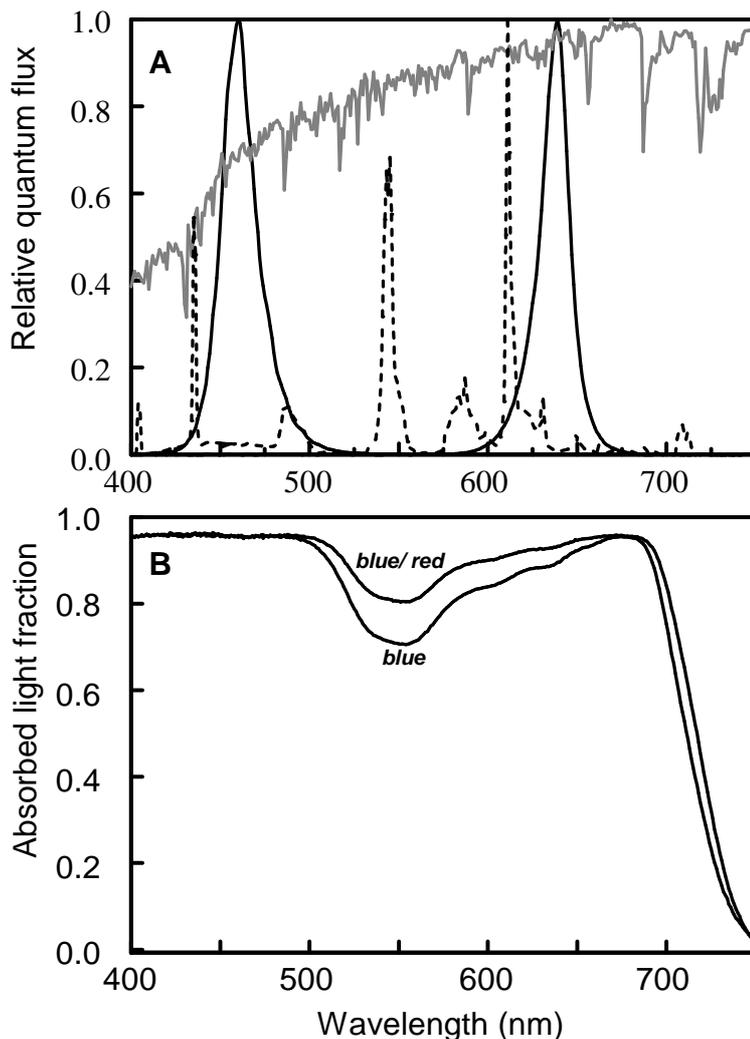


Fig. 1. A. Relative spectra red and blue LED-light (solid lines), white fluorescent light (dotted line) and a solar spectrum (ASTM, 2003; dim line). B: Absorbance spectra of cucumber leaves grown under mixed 30% blue/ 70% red and 100% blue light.

Measurements of gas exchange and chlorophyll fluorescence

Gas exchange and chlorophyll fluorescence were measured on mature second leaves (17–22 days after planting the seedlings) using the system described in Hogewoning *et al.* (2010c). The gas mix was kept constant at $381 \mu\text{mol mol}^{-1}$ (CO_2), $210 \text{ mmol mol}^{-1}$ (O_2) and $20.8 \pm 0.4 \text{ mmol mol}^{-1}$ (H_2O) at a flow rate of at least 300 ml min^{-1} . The CO_2 depletion was kept at approximately $10 \mu\text{mol mol}^{-1}$ at an irradiance of $100 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and never exceeded $15 \mu\text{mol mol}^{-1}$. The leaf temperature was $26.2 \pm 0.6 \text{ }^\circ\text{C}$.

Prior to the gas exchange measurements the leaf was dark-adapted for at least 30 minutes and dark-respiration (R_{dark}) and the dark-adapted F_v/F_m were measured. Leaves with an $F_v/F_m < 0.795$ were rejected. As all red-light grown leaves had an F_v/F_m well below 0.795, none were measured. Measurements with actinic light, provided by independently controlled red (peak wavelength 636 nm) and blue LEDs (peak wavelength 459 nm) of a similar type as used during plant growth (Fig. 1A; Hogewoning *et al.*, 2010c), started using a spectrum equal to GL, followed by a range of different spectra. For each measuring-light

(ML) spectrum the actinic incident irradiance on the leaf surface was $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ which was still strictly light-limiting for all leaves. Gas exchange of leaves grown under blue light was measured, respectively, using blue (B), 30% Blue/ 70%red (BR), B, red (R) and B actinic light. For plants grown under BR, measurements were done, respectively, using BR, R, BR, B and BR. Alternating the ML spectrum in this way was done in order to limit any acclimatory responses of the leaves. For each ML spectrum Φ_{PSII} was measured when gas exchange was in steady-state. After recovery from the saturating light pulse, the leaf was darkened for five minutes in order to measure R_{dark} . This measurement procedure was performed on two plants per GL treatment (block 1). The procedure was repeated on 3 plants per GL treatment, omitting R_{dark} measurements between changes in ML spectrum (block 2). The measurement procedure for a single leaf was performed approximately between 10.00h and 18.00h, allowing ample time for photosynthesis to stabilise under each ML spectrum the leaf was subjected to.

Beside gas-exchange measurements at light-limiting irradiance, measurements were also made on B and BR grown plants using a ML irradiance close to light-saturation. Initially, a complete light-response curve was measured using a spectrum equal to GL, after which irradiance was lowered to $1100 \mu\text{mol m}^{-2} \text{s}^{-1}$ in order to limit light-stress. This irradiance was sufficient to maintain photosynthetic rates $> 90\%$ of light saturated photosynthesis (A_{max}). Gas exchange of B grown leaves was subsequently measured with, respectively, B, BR, B and R, and for plants grown under BR with, respectively, BR, R and BR. The capacity of the blue ML was insufficient to reach photosynthetic rates $>90\%$ of A_{max} in the BR-grown leaves.

An additional set of data was produced using a LI-6400 photosynthesis system with a leaf chamber fluorometer (LiCor Inc., Lincoln Nebraska, USA). The leaf chamber is equipped with 27 red and 3 blue LEDs with peak wavelengths of, respectively, 640 and 464 nm. All other measuring conditions were similar to those described above. Photosynthetic parameters were measured on leaves from plants cultivated under blue LED light and cool white fluorescent tubes. The ML used consisted of $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ of a range of different blue/ red combinations. Measurements on blue light grown plants started with 100% B, whereas the white light grown plants started with 20% B. Each measurement cycle started and ended with the same percentage of blue and the photosynthetic rates did not differ between the start and end of the measurement, implying that there was no change in the photosynthetic properties of the leaf during the measurement regime. Although the blue LEDs did not produce an even light distribution over the leaf surface, no light distribution effects are expected on the measured photosynthesis as we worked with a limiting irradiance (Hogewoning *et al.*, 2010b).

The gas exchange parameters R_{dark} , A_{max} , A_{net} , stomatal conductance (g_{sw}) and internal CO_2 concentration (C_i) were calculated using the equations of von Caemmerer and Farquhar (1981). Gross assimilation (A_{gross}) was calculated as $A_{\text{net}} + R_{\text{dark}}$, which assumes that R_{dark} is a reasonable estimate of respiration in the light as is commonly done and that the ratio between R_{dark} and respiration in the light was not altered by the ML spectrum used. The boundary layer resistance of both sides of the leaf in the leaf chamber during gas exchange measurements was estimated using the method of Jarvis (1971). Fluorescence parameters F_v/F_m and Φ_{PSII} were calculated as described by Baker *et al.* (2007) and electron transport rate estimated according to Krall and Edwards (1992):

$$\text{ETR} = \Phi_{\text{PSII}} \cdot \text{PPFD} \cdot a_{\text{leaf}} \cdot a_{\text{PSII}} / (a_{\text{PSI}} + a_{\text{PSII}}) \quad \text{Eqn 1}$$

Converting Φ_{PSII} measurements to ETR is non-trivial (e.g. Baker *et al.*, 2007, Yin *et al.*, 2009), depending as it does upon several factors (principally, a_{leaf} , the actual quantum yield of PSII rather than Φ_{PSII} measured by fluorescence, $a_{\text{PSII}}/(a_{\text{PSI}} + a_{\text{PSII}})$, and the presence of non-photosynthetic pigments), some of which are difficult to quantify. Often ETR is estimated more simply using generic factors; 0.84 for a_{leaf} , 0.5 (or higher) for $a_{\text{PSII}}/(a_{\text{PSI}} + a_{\text{PSII}})$, and assuming that Φ_{PSII} obtained by means of fluorescence is as an absolute measure of Φ_{PSII} . We largely employed this simpler approach here, using a fixed factor of 0.5 for $a_{\text{PSII}}/(a_{\text{PSI}} + a_{\text{PSII}})$ while employing actual measurements of a_{leaf} , because we wished to explore the ML/GL differences in the relationship between calculations of ETR and CO₂ fixation in a way that reflects the routine use of this technique.

Calculation of the absorbed light fraction

Six leaves of the same age and stage of development as those used for the photosynthesis measurements were selected for each GL treatment, and their light absorbance spectra were calculated in one nm steps (400-800 nm) from transmittance and reflectance spectra measured at 12 different positions per leaf, using the procedure and measuring system described by Hogewoning *et al.* (2010a). Subsequently the a_{leaf} corresponding with the different ML spectra used during photosynthesis measurements was calculated for the different leaves by multiplying the relative leaf absorbance spectrum with the ML spectra (Fig. 1A).

Model simulation

To explore the consequences of changes in the light-limited quantum yield for CO₂ fixation (α) on predictions of crop yield we used a modified version of the model for greenhouse crop biomass production used in TOMSIM (Heuvelink 1995). In the version we used, the leaf photosynthesis module was upgraded by a module based on the leaf photosynthesis model described by Farquhar *et al.* (1980). Growth simulations were run for a period of one year (1 January until 31 December), using a fixed temperature, CO₂ concentration and relative humidity. For the light intensity, the annual radiation profile of SELYEAR (Breuer and van de Braak 1989) was used as an input. This dataset uses the 30-year average irradiance measured at De Bilt (52 °N 5 °E, The Netherlands) combined with realistic day-to-day variation to produce a realistic yearly cycle of irradiance for the Netherlands. The distinct seasonal changes in natural irradiance in the Netherlands allow the model to show the consequences of changes in α under different natural irradiance intensities. The model was run with α under non-photorespiratory conditions set to 0.0840 (the default value for the model) and 0.0966 (a 15% increase).

The 15% higher α represents the overestimation of α when measuring photosynthesis with red light, or red mixed with 10% blue light, compared with natural sunlight. This is a realistic scenario in research using commercially available photosynthesis measuring equipment. The α increase used is based on the calculated α on an absorbed light basis for a standard solar spectrum (400-700 nm; ASTM, 2003; Fig. 1A). For this calculation, we used the absorbance spectrum and the wavelength dependence of the quantum yield for CO₂ fixation on an absorbed light basis of leaves of the same cucumber cultivar as in this study that developed under an artificial sunlight spectrum

(S.W Hogewoning, unpublished; Figs 2, 3 and Tables S1 of chapter 3.2 in this thesis). The calculated α value of the solar spectrum was found to be 17% lower than that of red wavelengths (average 620-640 nm) and 14% lower than that of red mixed with 10% blue (450-460 nm) light. Note that the calculation of the α for the standard solar spectrum does not take the contribution of wavelengths >700 nm and possible broadband enhancement effects (McCree, 1972b) into account. For the simulation, we wished to focus on the effect of errors in α , so we assumed no changes in the gross photosynthetic rate at light-saturation and did not account for changes in the curvature of the light-response curve.

We simulated growth of a vegetative crop with indeterminate growth, planted with a leaf-area index (LAI) of 0.8. In such a simulation, differences in the simulated consequences of a change in α on the productivity of a relatively open canopy transforming into a closed canopy can be observed. Additionally, we simulated daily A_{gross} of an indeterminate crop keeping biomass and LAI constant (LAI= 3) and with an infinite carbohydrate sink to avoid any sink-limitation effects. Such a situation applies for crops which are kept in a relatively constant condition by the regular removal of leaves and fruits, as is typical of many greenhouse grown crops, such as tomato.

Results

Photosynthetic rates under different measuring-light spectra

The net assimilation (A_{net}) measured at a low, light-limiting irradiance and using the same spectrum as during growth was 25% higher for the mixed blue/red (BR) grown plants than it was for the 100% blue (B) grown plants (Table 1). In both growth-light (GL) treatments (BR and B) A_{net} measured under the three different measuring-light (ML) treatments was higher for ML spectra containing relatively more red. For each of the three ML spectra used A_{net} was, however, independent of the GL treatment (student t-test: $P>0.52$; for BR ML in the BR grown leaves and for B ML in the B grown leaves the average of three ML treatments per leaf was used in the t-test). A similar pattern of results was found for gross assimilation (A_{gross} ; Fig. 2A), so there was no significant dependency of dark respiration (R_{dark}) on GL or ML (Table 1). R_{dark} tended to decrease steadily over the day (Table 1), explaining the small rise in A_{net} over time in the B light treatment measured with B, whereas A_{gross} (Fig. 2A) slightly decreased over time. The small decrease in A_{gross} over the day was associated with a decrease in stomatal conductance (g_{sw}) over the day (Table 1), especially towards the end of the day and may be related to the circadian rhythm of these cucumber leaves. The different A_{net} and A_{gross} rates associated with the different ML spectra used on a leaf were not dependent on g_{sw} or C_i (Table 1).

The leaves grown under 100% red irradiance had an average F_v/F_m value of 0.76 and had a slightly chlorotic appearance. A dark-adapted F_v/F_m below 0.8 is normally associated with damage or long-term down-regulation of PSII in response to stress (e.g. Baker 2008, Hogewoning and Harbinson 2007). These leaves were not used for any further measurements.

The leaves grown under blue light and under white fluorescent light measured with the LI-6400 photosynthesis system (Fig. 3) showed rates of A_{net} comparable to those measured with the lab-built system. Net assimilation was 25-30% lower for 100% blue ML than it was for 100% red ML, regardless the GL spectrum or measuring system (Fig. 3).

Table 1. Photosynthetic parameters measured using different measuring-light (ML) spectra over the day on mixed 30% blue/ 70% red (BR) and 100% blue (B) grown leaves at light-limited irradiance ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$). The repeated ML treatments show the changes in the leaf response over the day. Tukey's HSD was used to make post hoc multiple comparisons among ML treatment means within growth-light treatments from significant one way ANOVA tests ($P < 0.01$).

ML	n	BR grown					B grown				
		BR	R	BR	B	BR	B	BR	B	R	B
A_{net} ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	5	3.9 ^c	4.2 ^a	4.0 ^b	3.2 ^d	3.9 ^{bc}	3.0 ^d	4.0 ^b	3.2 ^{cd}	4.3 ^a	3.3 ^c
R_{dark} ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	2	1.13 ^a	0.98 ^a	0.86 ^{ab}	0.82 ^{ab}	0.79 ^b	1.10 ^a	0.92 ^a	0.78 ^a	0.70 ^a	0.67 ^a
g_{sw} ($\text{mol m}^{-2} \text{ s}^{-1}$)	5	0.30 ^a	0.29 ^a	0.27 ^{ab}	0.25 ^{ab}	0.22 ^b	0.33 ^a	0.31 ^{ab}	0.29 ^{abc}	0.23 ^{bc}	0.22 ^c
C_i ($\mu\text{mol mol}^{-1}$)	5	341 ^a	336 ^{ab}	335 ^{ab}	342 ^a	331 ^b	349 ^a	340 ^b	346 ^a	330 ^c	340 ^b

Table 2. Photosynthetic parameters measured using different measuring-light (ML) spectra over the day on mixed 30% blue/ 70% red (BR) and 100% blue (B) grown leaves at near-saturating irradiance ($1100 \mu\text{mol m}^{-2} \text{s}^{-1}$). The repeated ML treatments show the changes in the leaf response over the day. Tukey's HSD was used to make post hoc multiple comparisons among ML treatment means within growth-light treatments from significant one way ANOVA tests ($P < 0.01$); $n=3$ and $n=5$ for BR and B growth-light, respectively).

ML	BR-grown			B-grown			
	BR	R	BR	B	BR	B	R
A_{max} ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	19.6 ^a	19.2 ^a	18.6 ^b	15.5 ^b	17.6 ^a	15.3 ^b	17.5 ^a
g_{sw} ($\text{mol m}^{-2} \text{ s}^{-1}$)	0.51 ^a	0.41 ^b	0.43 ^b	0.57 ^a	0.53 ^b	0.55 ^{ab}	0.48 ^c
C_i ($\mu\text{mol mol}^{-1}$)	276 ^a	264 ^b	269 ^{ab}	297 ^a	284 ^b	297 ^a	279 ^c

The decrease in A_{net} when increasing the percentage blue of the ML was similar for each of the five steps of 20% increase in blue, except for 80% to 100% blue on the leaves grown under the white fluorescent light.

Photosynthesis at a near-saturating irradiance (A_{max}) showed a pattern which was different from the measurements at low light (Table 2). The different GL treatments resulted in a 24% higher A_{max} for the leaves grown under and measured with BR than for the leaves grown under and measured with B. However, ML had no significant or a much smaller (13%) effect on photosynthesis than at low ML irradiance for BR and B grown leaves, respectively. Blue ML was associated with the highest g_{sw} and red ML with the lowest g_{sw} . The same pattern was found for C_i .

Light absorbance, Φ_{PSII} and ETR calculation

The absorbance in the red part of the spectrum was slightly higher for the leaves grown under BR than for those grown under B (Fig. 1B). The fraction of absorbed light (a_{leaf}) for the ML spectra B, red (R) and BR were, respectively, 0.96, 0.93 and 0.94 for the BR grown

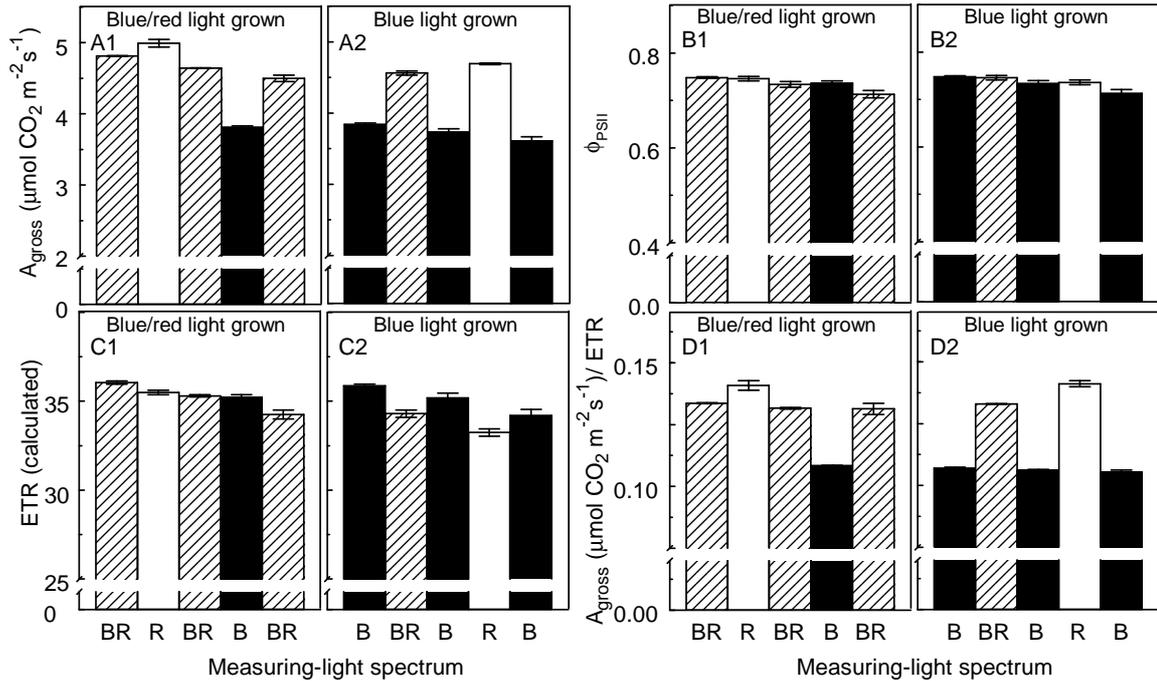


Fig. 2. Measuring-light effect on (A) gross assimilation (A_{gross}), (B) relative quantum yield of photosystem II electron transport (Φ_{PSII}), (C) calculated electron transport rate (ETR) and (D) A_{gross} per unit calculated ETR of cucumber leaves grown under two different spectra. The leaves were subjected to different measuring-light spectra over the day: 100% blue (B), 30% blue/ 70% red (BR) and 100% red (R). The repeated measuring-light treatments show the changes in the leaf response over the day (about eight measuring hours). A_{gross} was calculated as $A_{\text{net}} + \text{dark respiration}$. ETR was calculated using the commonly used expression $\text{ETR} = \Phi_{\text{PSII}} * \text{PPFD} * a_{\text{leaf}} * \text{apsII} / (\text{apsI} + \text{apsII})$. PPFD was $100 \mu\text{mol m}^{-2} \text{s}^{-1}$, Φ_{PSII} was measured (B), light absorbance (a_{leaf}) was measured (Fig. 1B) and the excitation balance $\text{apsII} / (\text{apsI} + \text{apsII})$ was assumed to be 0.5 for all measuring-light spectra. Error bars indicate the se ($n=2$, block 1). Measurements on additional leaves showed similar results for Φ_{PSII} and ETR ($n=3$; block 2, not shown) and the response pattern of A_{net} ($n=5$, Table 1) was similar to that of A_{gross} .

leaves and 0.96, 0.90 and 0.92 for B grown leaves and all standard errors were small (<0.0042).

The quantum yield for PSII electron transport (Φ_{PSII}) decreased slightly over the day, and in contrast to the CO_2 fixation responses (Table 1, Figs 2A) was not affected significantly by the ML used (Fig 2B). The LI-6400 system produced similar results for the leaves grown under blue and under white fluorescent light, measured with different ratios blue to red light (not shown).

The calculated ETR values (Eqn. 1) are shown in Fig. 2C. For the BR grown plants measured with BR the calculated ETR decreased slightly over the day due to the decrease in Φ_{PSII} . For the B grown plants measured with B, calculated ETR decreased likewise over the day. The lower calculated ETR for R and BR than that for B ML can be attributed to the lower absorbance in the red part of the spectrum for the B grown plants (Fig. 1B). The A_{gross} per unit ETR as calculated according to Eqn. 1 decreases with an increasing blue to

red ratio in the ML (Fig. 2D). Note that the difference in A_{gross} per unit calculated ETR for the different ML spectra would be even bigger than that in Fig. 2D if we would have assumed a higher relative excitation of PSII for blue ML than for red ML, instead of using a $a_{\text{PSII}}/(a_{\text{PSI}} + a_{\text{PSII}})$ value of 0.5 for all ML spectra.

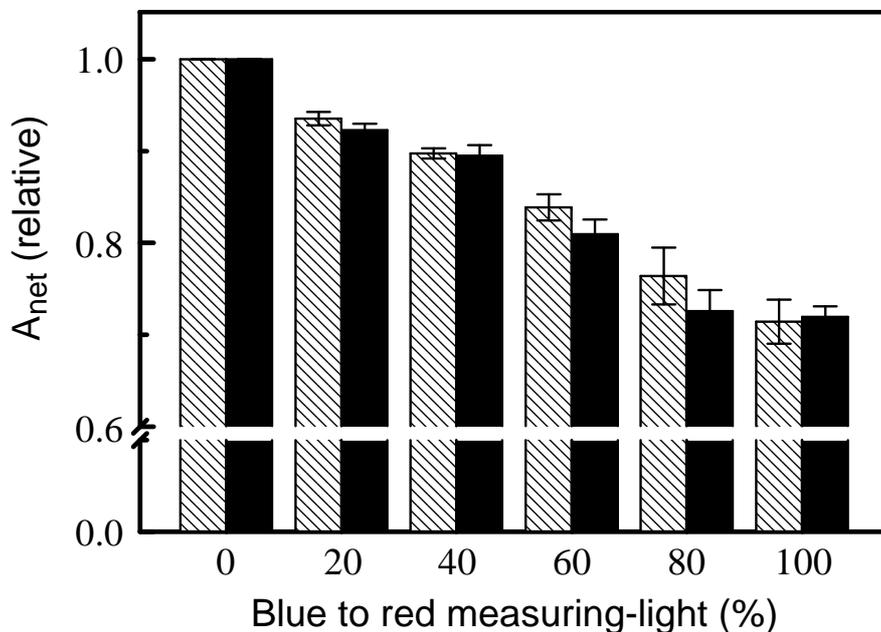


Fig. 3. Measuring-light effect on normalized net assimilation (A_{net}) of cucumber leaves grown under two different spectra. The leaves were subjected to different ratios red and blue LED light by using the LI-6400 at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$; 0 % blue (=100% red) is set as 1. Line-filled and black bars represent leaves grown under, respectively, cool white fluorescent tubes ($n=6$) and under 100% blue light ($n=7$). Error bars indicate the se.

Crop model simulation

When the simulation started with young plants a 15% increase in the light-limited quantum yield for CO_2 fixation (α) resulted in a day-upon-day increase in the daily total A_{gross} by the crop (Fig 4A) which by day 50 was 20% higher than that for the default α . From day 50 to 175 the increase fell to 9%, after which it stabilized. The pattern of initial increase in A_{gross} , followed by a decrease and stabilization can largely be attributed to the effect of crop growth on total light interception. Initially growth of the young plants leads to an exponential increase in light-interception and thus A_{gross} , due to a greater leaf area index (LAI, $\text{m}^2 \text{ leaf} / \text{m}^2 \text{ ground area}$). When the crop becomes denser, the increase in light interception with increasing LAI decreases due to shading, up to the point that extra leaf layers do not intercept any additional irradiance. When the same data were corrected for the total leaf area, A_{gross} per $\text{m}^2 \text{ leaf}$ was highest at the start of the simulation and decreased progressively during the first 80 days of crop growth. After day 80 A_{gross} per $\text{m}^2 \text{ leaf}$ was slightly (1%) lower for the simulation with the higher α and sTable. This can be explained by a greater LAI in the simulation with 15% higher α , hence a lower irradiance interception per square meter leaf. The dry matter accumulation for the simulated crop

with a 15% higher α (Fig. 4B) was 10% greater at the end of the growing season. From day 250, respiration was in balance with assimilation and therefore crop growth stopped. Following a similar pattern to total crop A_{gross} per day, dry matter initially accumulated more rapidly in the simulation with an increased value for α , then the difference in accumulation slowly decreased and finally stabilized.

For the simulation with a constant biomass and a LAI of three, increasing α by 15% resulted in a 10-15% increase of total A_{gross} by the crop per day, dependent on the irradiance intensity (Fig. 5). The enhancement in A_{gross} per day associated with the 15% increase in α was greater in seasons with a low natural irradiance than in seasons with higher irradiance.

Discussion

Consequences of measuring-light spectrum for CO₂ fixation measurements

The light-limited quantum yield for CO₂ fixation (α) of leaves grown under blue (B) or mixed blue/ red (BR) LEDs or white fluorescent tubes was equally affected when measured under different combinations of red and blue. In fact for each measuring-light (ML) used, none of the parameters derived from gas-exchange measurements differed for the growth-light (GL) environments used (Table 1). So at least within the limited range of growth spectra employed in this study differences in GL have no effect on ML induced differences in α . Red ML results in the highest α , and when used in combination with B, the greater the relative amount of R the higher the α . This red-light induced increase in yield occurs even though C_i is lower in the R ML treatments compared with those with B ML (Table 1).

The different GL treatments did affect A_{max} , but had no discernible effect on α . However, the spectrum of the ML used for gas-exchange measurements has significant consequences for the value of α that will be obtained, whereas A_{max} is less sensitive (Tables 1, 2). Measurements of the spectral dependency of α indicate that red light has the highest α on both an absorbed and incident flux basis (Inada 1976). In the case of the cucumber leaves used here adding some blue light, which is routinely done to induce stomatal behaviour that is more typical of leaves subjected to 'sunlight' (e.g. Niinemets *et al.*, 2005, Loreto *et al.*, 2009), does result in a higher C_i , but at the expense of a decreased α . As daylight contains blue light in substantial amounts then a measurement of CO₂ fixation using a ML that is predominantly red will overestimate the natural value of α . In the solar spectrum shown in Fig. 1A, 32% of the photon flux in the range 400-700nm has a wavelength shorter than 520nm (i.e. blue; our BR treatment contained 30% blue).

Notably, the higher α associated with red ML cannot be interpreted as red light being the most effective in terms of plant production. There is a clear distinction between instantaneous effects of spectrum on photosynthesis and spectral effects on plant development. In fact, the leaves developed under pure red light were stressed in our experiment ($F_v/F_m < 0.8$) and biomass production has been reported to be lower under red GL than under a combination of red and blue for several species (Brown *et al.*, 1995, Goins *et al.*, 1997, Yorio *et al.*, 2001, Ohashi *et al.*, 2006). The physiological disorders associated with growth under red light alone have been discussed in detail by Hogewoning *et al.* (2010c).

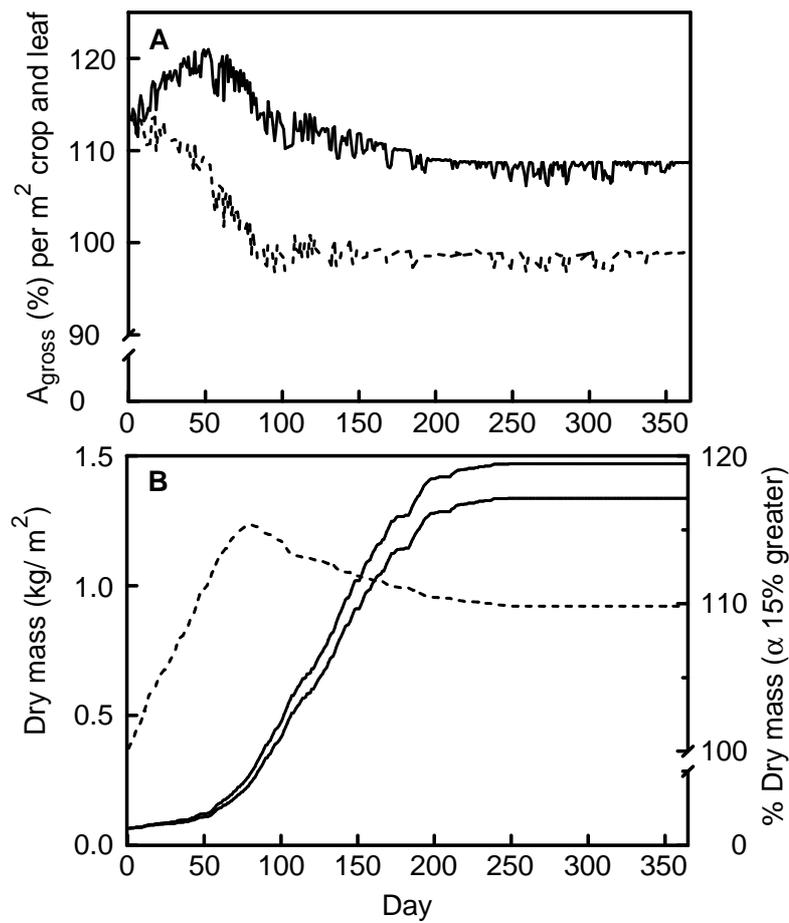


Fig. 4. Simulation of a crop planted as young plants (LAI=0.8) on day 0 using a 15% higher light-limited slope ($\alpha=0.0966$) than the default white-light based model input ($\alpha=0.084$). A. Gross assimilation (A_{gross}) per day (%) compared with $\alpha=0.084$. Solid line: $A_{\text{gross}} / \text{m}^2$ ground surface; dashed line: $A_{\text{gross}} / \text{m}^2$ leaf. B. Cumulative dry mass. Lower solid line: $\alpha=0.084$; upper solid line: $\alpha=0.0966$; dashed line: % increase in dry mass for $\alpha=0.0966$ compared with the $\alpha=0.084$ default input.

Consequences of measuring-light spectrum for biomass production estimates

As leaf photosynthesis measurements are used as calibration inputs for plant growth models (e.g. Hikosaka and Hirose 1998, Medlyn *et al.*, 1999, Bernacchi *et al.*, 2001, Kim and Lieth 2003), the effect of mismeasures of α on the outcome of these models is relevant. In the crop growth model that we used to simulate the potential effect that ML spectrally different from GL can have on biomass production estimates, α was increased by 15% representing the difference in the calculated α of a standard solar spectrum (ASTM, Fig. 1A) and α of red (or a mix of 90% red and 10% blue) light. In the calculation of the α for the standard solar spectrum a possible enhancement effect of broadband light compared with the sum of the separate wavelengths was not taken into account, based upon the absence of a significant enhancement effect of broadband light reported by McCree (1972b). The calculated difference in α for the solar spectrum and red light would be greater than 15% if the calculations had been based on the quantum yields of woody plants (Inada 1976) and or field grown plants (McCree 1972a) instead of the herbaceous

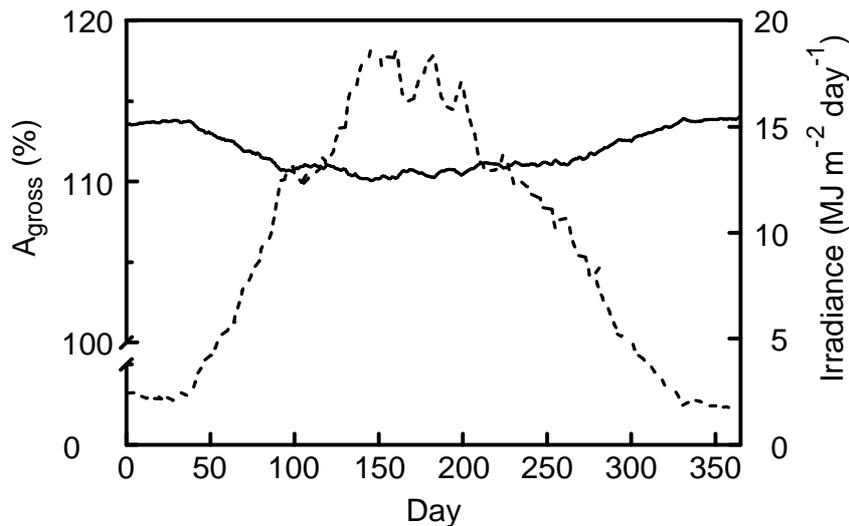


Fig. 5. Simulation of crop with a fixed biomass and LAI of three m² leaf per m² ground area using a 15% higher light-limited slope ($\alpha=0.0966$) than the default white-light based model input ($\alpha= 0.084$). Solid line: Total gross assimilation (A_{gross}) by the crop per day (%) compared with $\alpha= 0.084$; dashed line: the SELYEAR-based irradiance over time. Data are presented as moving averages over 30 days, to eliminate large day-to-day fluctuations to be visible in the figure.

plants grown in a growth chamber (i.e. similar to the plants used in this study). However, if enhancement effects were to be significant, which may be expected from studies on the importance of photosystem excitation balances *in vivo* (Chow et al., 1990; Walter and Horton, 1995), the difference in α for the solar spectrum and red light would be reduced. Nonetheless, this modelling exercise illustrates the potential errors that can be made when the value of α corresponding with the measuring-light differs from the value of α *in situ*.

The simulation using young plants with a low LAI at the beginning of the simulation and allowing the crop to develop resulting in increased LAI (Fig. 4) resembles the situation for an even-aged crop, which is typical of most agricultural or silvicultural crops, though it is also similar to natural vegetation recovering from a clearance episode. An increased α led to a greater dry mass accumulation and total A_{gross} , but in the young canopy with low LAI the increase in A_{gross} was greater than the increase in α , which is due to exponential crop growth in the open canopy. This implies that the simulation of a growing crop can be disproportionately sensitive to errors in α . Independent of α , the crop eventually reached a balance between respiration and assimilation and growth ceased, at which point the total crop biomass was 10% greater for the increased α simulation.

The simulation using a fixed LAI (Fig.5) resembles the situation for a crop which remains relatively constant from a certain growth stage due to crop handling and regular harvest (e.g. a tomato crop in a greenhouse). In this case the estimated increase in α using photosynthesis data obtained using red ML as a model input resulted in an overestimation of assimilate production and thus biomass production in the order of 10-15%, which is approximately proportionate to the mismeasure of α . The impact of a change in α is clearly greater in periods with lower irradiance than in periods with higher irradiance (Fig.5). This is to be expected when irradiance levels increase beyond the light-limited range, as photon flux becomes non-linearly related to ETR and thus A_{gross} . Therefore the potential error in estimates on biomass production using photosynthesis data measured with ML

deviating from GL will generally be greater at higher latitudes and in winter, compared with lower latitudes and summer. In general, these simulations show that a mismeasure of α can have considerable implications for the predictive quality of crop and canopy production models.

Consequences of measuring-light spectrum for the ETR-photosynthesis relationship

Measurements of Φ_{PSII} are increasingly being used to estimate rates of CO_2 fixation. This procedure is based upon converting Φ_{PSII} into an ETR and then converting the ETR into CO_2 fixation, ideally using simultaneous measurements of CO_2 and Φ_{PSII} in a gas analysis system to provide a calibration (e.g. Genty *et al.*, 1990, Evans 2009). It has already been pointed out that the use of a fixed a_{leaf} (typically 0.84) in this procedure can be a source of error as not all leaves have this absorbance and the a_{leaf} for the growth and measurement irradiances will generally be different (Baker *et al.*, 2007, Baker 2008). In our experiments using red and blue light combinations, assuming 0.84 instead of using measured a_{leaf} values would have caused an error in the order of 10%.

In addition to this error we have now identified another error that arises from the use of a ML that differs from the GL. Unlike α , Φ_{PSII} under light-limited irradiances was relatively unaffected by the ML spectra used in this investigation (Fig. 2B). This implies that a calibration of ETR values in terms of CO_2 fixation rates using one type of irradiance spectrum may be in error when used to estimate CO_2 fixation rates under a different irradiance spectrum (Fig. 2D). This error has its origins in the lower values of α obtained under shorter wavelengths of PAR (≤ 560 nm; e.g. Inada 1976) compared with those measured under longer wavelengths. Several factors affect the wavelength dependency of α , such as the energy transfer efficiency of carotenoids to Chls, absorption by non-photosynthetic pigments and the excitation balance of the two photosystems (Terashima, 2009). Energy transfer from carotenoids to Chls has an efficiency of 70-90%, whereas the energy transfer efficiency from Chls to Chls is 100% (Croce *et al.*, 2001; de Weerd *et al.*, 2003; van Oort *et al.*, 2008). Carotenoids have absorption maxima for blue wavelengths. Non-photosynthetic pigments, such as flavonoids and anthocyanins, serve to protect plants against photodamage by excess radiation (e.g. Kubasek *et al.*, 1992, Jackson and Jenkins 1995; Havaux and Kloppstech, 2001; Edreva, 2005) and herbivores (Treutter, 2006), but also absorb light in the UV/blue/green part of the spectrum, and therefore there is a trade-off between plant defense and photosynthetic efficiency. Consequently it is expected that the ETR calibration error will be greater for woody plants than herbaceous plants as the former have a relatively higher content of non-photosynthetic pigments and lower values of α at short wavelength PAR. Finally, wavelength dependent imbalances in the efficiency of the two photosystems result in inefficiencies of photosynthesis (Evans 1986, Evans 1987, Chow *et al.*, 1990, Melis 1991, Walters and Horton 1995, Pfannschmidt 2005), for which the $a_{\text{PSII}}/(a_{\text{PSI}} + a_{\text{PSII}})$ term is in Eqn 1. In normal use, however, this term is kept fixed, despite it being expected to change with wavelength. The ML-spectra that we used produced values of Φ_{PSII} close to the maximum (i.e. around 0.8; Fig. 2B) and therefore PSII was not strongly overexcited. The wavelengths of our blue ML (459 nm peak wavelength) were slightly shorter than the blue region which is associated with strong PSII overexcitation (around 480 nm; Evans and Anderson, 1987).

We conclude that a photosynthesis measuring-light spectrum deviating from the plant growth-light spectrum can lead to significant errors in estimates of the light-limited

quantum yield for CO₂ fixation *in situ*. The use of ETR calculated using chlorophyll fluorescence measurements as a proxy for CO₂ fixation rates *in situ* requires calibration in terms of CO₂ fixation rates with measuring-light spectrally similar to the conditions *in situ*. Erroneous estimates of light-limited quantum yield for CO₂ fixation can have disproportionately large consequences for predictions of plant growth when used as an input for crop models.

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CHAPTER 5

An artificial solar spectrum substantially alters plant development compared with usual climate room irradiance spectra

Abstract

Plant responses to the light spectrum under which plants are grown affect their developmental characteristics in a complicated manner. Lamps widely used to provide growth irradiance emit spectra which are very different from natural daylight spectra. Whereas specific responses of plants to a spectrum differing from natural daylight may sometimes be predictable, the overall plant response is generally difficult to predict due to the complicated interaction of the many different responses. So far studies on plant responses to spectra either use no daylight control or if a natural daylight control is used it will fluctuate in intensity and spectrum. We have engineered an artificial solar (AS) spectrum which closely resembles a sunlight spectrum and compared growth, morphogenesis and photosynthetic characteristics of cucumber plants grown for 13 days under this spectrum with their performance under fluorescent tubes (FT) and a high pressure sodium lamp (HPS). The total dry weight of the AS-grown plants was 2.3 and 1.6 times greater than that of the FT and HPS plants, respectively, and the height of the AS plants was four to five times greater. This striking difference appeared to be related to a more efficient light interception by the AS plants, characterized by longer petioles, a greater leaf unfolding rate and a lower investment in leaf mass relative to leaf area. Photosynthesis per leaf area was not greater for the AS plants. The extreme differences in plant response to the AS spectrum compared with the widely used protected cultivation light sources tested highlights the importance of a more natural spectrum, such as the AS spectrum, if the aim is to produce plants representative of field conditions.

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Introduction

The irradiance spectrum to which plants are exposed during growth has specific effects on different types of plant responses such as photosynthesis, photomorphogenesis, phototropism and photonasty. In plant research and greenhouse horticulture lamps (growth lamps) with different spectral outputs are widely used to provide the growth irradiance. The most commonly used lamp-types are fluorescent tubes and gas-discharge lamps, which emit a spectrum with pronounced emission lines which are characteristic for the different lamp-types. More recently light emitting diodes (LEDs), which are characterized by relatively narrow-band spectra, have become increasingly used in growth-cabinets, on an experimental basis in greenhouse horticulture and in research on growing plants in space (e.g. Hogewoning *et al.*, 2007; Massa *et al.*, 2008; Trouwborst *et al.*, 2010). A common feature of these light-sources is that their spectrum does not resemble that of natural daylight, which has a continuous (i.e. without strong emission lines) spectrum in the PAR region (400-700 nm), even though some lamp emissions appear “white”.

Plants have evolved under broad-band spectra and are exposed to spectral differences under natural conditions dependent on weather conditions, time of the day, season and their growth environment. For example, when the sky is clouded daylight contains relatively more blue and less far-red (FR) between 700 and 750 nm than in full sunlight (Holmes and Smith, 1977). A low sun angle is associated with a low red to FR (R:FR) ratio (Franklin and Whitelam, 2007). Other factors that affect the natural spectrum are altitude, depth for aquatic plants and most obviously, shading by neighboring vegetation. Inherently, leaves exposed to a shade or a sun-spectrum are also exposed to a relatively low and a high irradiance, respectively, so irradiance and spectrum are often linked.

Specific parts of the spectrum are involved in plants’ sun and shade-light responses. Blue light and high R:FR ratios are known to induce the development of sun-type chloroplasts (e.g. Lichtenthaler, 1980; Kasperbauer and Hamilton, 1984). A low R:FR ratio is a text-book example of a spectrum inducing an overall shade-type morphology in a wide range of species, typically characterized by etiolation so that plants can reach above neighboring plants (e.g. Grime, 1981). Other spectral responses do not overtly parallel a shade or sun-spectrum response. Such responses include blue-light induced stomatal opening (e.g. Zeiger, 1990; Willmer & Fricker, 1996), which can be reversed by adding sufficient green light to the spectrum (Frechilla *et al.*, 2000; Talbott *et al.*, 2002), or reduced growth and photosynthesis when plants are grown under red light alone (e.g. Brown *et al.*, 1995; Goins *et al.*, 1997; Yorio *et al.*, 2001; Matsuda *et al.*, 2004). Many spectral responses of plants are regulated via photoreceptors, such as phytochromes, cryptochromes and phototropins, which alter the expression of a large number of genes (Whitelam and Halliday, 2007). These numerous and complicated spectrum-regulated plant responses have been, and remain, the subject of extensive study.

Research on spectral responses of plants normally involves adding irradiance from growth lamps to daylight, modifying daylight using spectral filters, using solely growth lamps, or a combination of these methods. Whereas the specific responses of plants to a spectrum deviating from natural light may sometimes be predictable based on published research, the overall plant response is generally difficult to predict due to the complicated

interaction of the many different responses. For instance, spectra enhancing the photosynthetic capacity of leaves per unit leaf area do not necessarily enhance a whole plant morphology which is favorable for light-interception and therefore also not necessarily enhance plant production.

The lack of a practical source for an irradiance whose spectrum resembles that of any kind of natural daylight means that it is difficult, or impossible, to have a controlled environment in which natural daylight adapted plants can be grown. Plant studies using a daylight spectrum are always under conditions of natural daylight which fluctuates in intensity and spectrum. This makes a clear distinction between plant responses to the intensity or the spectrum of the irradiance difficult. In the past the main criterion for an optimal growth-chamber spectral irradiance was a natural plant appearance with high a production yield (e.g. Deutch and Rasmussen, 1973), rather than producing a spectrum that is inherently like that of sunlight. So though mixtures of fluorescent and incandescent lamps have been used to allow more normal plant growth and development, this spectrum is very dissimilar to that of sunlight. We have engineered a spectrum which closely resembles a sunlight spectrum. We compared growth, morphogenesis and photosynthetic characteristics of young cucumber plants grown for two weeks under this artificial sunlight spectrum with their performance under lamp-types widely used in growth-chambers or glasshouses. We used a growth irradiance in which assimilation was light-limited (or nearly so) to minimize possible effects of different assimilation rates per leaf area, caused by differences in the irradiance response of assimilation, on plant growth and development. The plants grown under the artificial sunlight developed in a strikingly different way than the plants grown under the other lamps tested. An artificial solar spectrum offers the opportunity to grow plants under controlled conditions which are far more representative of field conditions than plants grown under the current growth-chamber irradiance sources.

Materials and Methods

Plant material and growth conditions

Cucumber plants (*Cucumis sativus* cv. Hoffmann's Giganta) were sown in vermiculite and germinated under $100 \mu\text{mol m}^{-2}\text{s}^{-1}$ cool white fluorescent lamps (TLD 50W 840 HF, Philips, The Netherlands) in a climate chamber. After one week, when the cotyledons had just opened, the seedlings were transplanted to a hydroponic system (Hoagland's solution, $\text{pH} = 5.9 \pm 0.2$; $\text{EC} = 1.2 \text{ mS cm}^{-1}$) in a climate chamber. The day/ night-temperature was $25^\circ\text{C} / 23^\circ\text{C}$, the relative humidity was 70% and the CO_2 concentration was ambient.

The light treatments consisted of an irradiance provided by cool white fluorescent tubes (FT; 50 Watts TLD 84 /HF electronic, Philips, the Netherlands), a high pressure sodium lamp (HPS; 400 Watts SON-T agro 400, Philips, the Netherlands) and a continuous broad-band spectrum, referred to as the "artificial solar"-spectrum (AS, see below). The percentage blue photons (i.e. in the range 400- 500 nm) of the PAR (i.e. in the range 400-700 nm) was 23%, 5% and 18% for the FT, HPS and AS spectra, respectively. All plants were subjected to $100 \pm 5 \mu\text{mol m}^{-2}\text{s}^{-1}$ PAR and the photoperiod was 16 hours. Leaf temperature during the photoperiod, which was routinely measured using an infrared thermometer

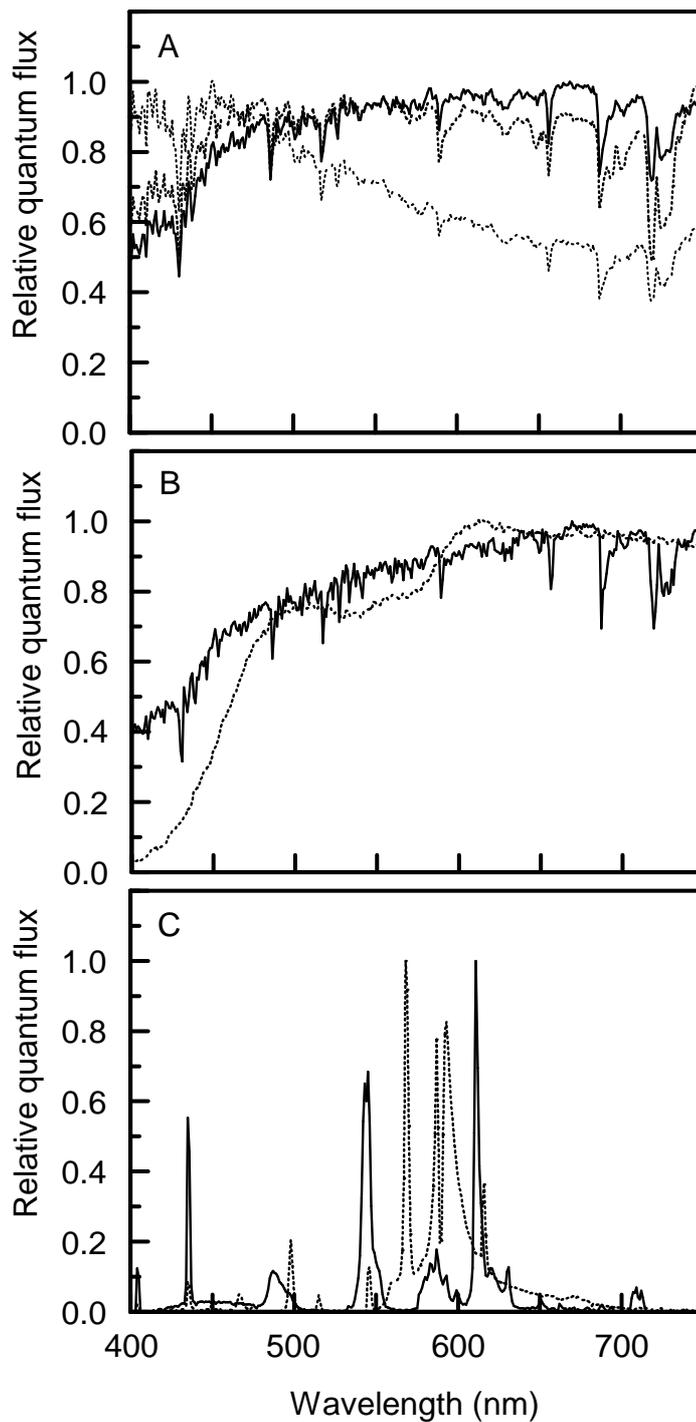


Fig. 1. A: Relative spectra of direct sunlight (solid line), cloud-light (thick dotted line) and skylight (thin dotted line) measured around the autumn equinox (2009) at noon in Wageningen, the Netherlands. B: Relative spectra of the artificial solar spectrum (dotted line) and a standard solar spectrum (solid line; ASTM, 2003). C: Relative spectra of the high pressure sodium lamp (dotted line) and the fluorescent tubes (solid line).

(Raytek ST series, Raytek Corporation, Santa Cruz, USA), was 24 ± 0.5 °C, 25 ± 0.5 °C and 26 ± 1 °C for FT, HPS and AS-grown leaves, respectively.

Artificial solar spectrum

We have been able to construct a light-source which, except for a deficiency in the blue, produces a spectrum that closely resembles that of a standard sunlight spectrum (Fig. 1B). Our reference spectrum for the purposes of this exercise was the ASTM G173-03 direct and circumsolar spectrum, thus it excludes skylight and takes no account of cloud-light. This is a calculated, representative direct and circumsolar irradiance spectrum for 48 contiguous states of the United States, which is available for download in a tabular form (ASTM, 2003). Cloud-light spectra are not very different from direct sunlight spectra, whereas skylight spectra are conspicuously different (e.g. Endler, 1993). The total solar irradiance is comprised of skylight, direct sunlight and cloudlight in various proportions depending on, amongst others, the height of the sun above the horizon and weather conditions. In the absence of clouds, the total irradiance is largely dominated by direct sunlight and under these conditions plants will experience a predominantly direct sunlight spectrum, except under a low sun angle or when the direct sunlight is filtered by other leaves. To the best of our knowledge no comparable typical spectrum exists for other regions and therefore the ASTM spectrum is a reasonable model to use, until a better catalogue of natural spectral irradiances becomes available.

The AS spectrum was provided using a 1300 W microwave driven sulfur plasma lamp (PI-VL1, Plasma International GmbH, Offenbach am Main, Germany), which was filtered using a color correction filter (Gamcolor filter 1581, Los Angeles, Ca, USA) in order to reduce the intensity of the green wavelengths. The resulting irradiance spectrum, lacking sufficient near-infrared wavelengths, was projected onto the plants via reflection by aluminum foil on the ceiling of the climate chamber, so that the light was well distributed over the plants. Additional quartz halogen lamps were used to provide more near-infrared irradiance. The light output of both the plasmalamp and the quartz halogen lamps could be adjusted without any large changes in spectral output. The desired spectrum was obtained by adjusting the light output such that 72% of the PAR was provided by the filtered plasmalamp and 28% by the quartz halogen lamps. The spectrum and intensity of the three light-sources used as growth-treatment were measured using a spectroradiometer (USB2000 spectrometer, Ocean Optics, Duiven, The Netherlands, calibrated against a standard light-source; Fig. 1B, C) and the spectra are also provided as supplementary material at *Journal of Experimental Botany (JXB)* online. Light intensity was routinely measured using a quantum sensor (LI-COR Lincoln, Nebraska USA). The two devices produced comparable results. Additionally the natural spectrum of cloudlight in fully overcast conditions, direct sunlight, and skylight was measured at midday in Wageningen (52 °N 5.5 °E, The Netherlands) around the autumn equinox 2009 on the roof of a tall building (Fig. 1A and in tabular form as supplementary material at *JXB* online).

Growth and morphology analysis

For growth and morphology analysis 10 plants per light treatment were grown for 13 days, at which point plants started shading each other. The height of the table the plants were growing on was adjusted such that the apices of the plants received 100 $\mu\text{mol m}^{-2}\text{s}^{-1}$ irradiance throughout the experiment. The plants were dissected into different parts: leaves plus petioles, cotyledons, hypocotyls, internodes, roots and the remainder (apex and tendrils). The different plant parts, except the roots, were imaged together with a ruler using a digital photo camera in order to determine the area of the leaves and cotyledons

and the length of the petioles of the first two leaves, the hypocotyls and the internodes. Image analysis was carried out using the imaging software ImageJ (<http://rsbweb.nih.gov/ij/>). Leaves with a length of one centimeter or greater were counted for the determination of the leaf number per plant.

After imaging, 10 leaf discs (1.28 cm²) were cut from each first leaf in order to determine the leaf mass per area leaf (LMA, g m⁻²). The leaves plus petioles, cotyledons, hypocotyl, roots, discs to determine LMA and internodes plus the remainder were oven dried at 70 °C for the first 16 hours, 105 °C for the next 22 hours and held at 70 °C until weighing.

The experiment was performed in duplo, the plants were treated as independent experimental units and the repetitions as blocks.

Leaf light absorptance

Leaf light absorptance was calculated from reflectance and transmittance measurements on 12 leaf discs per light treatment, cut randomly from three first leaves per light treatment. An improved version of the system described in Soares *et al.* (2008) was used, consisting of two integrating spheres, each connected to a spectrometer and a custom-made light source. The USB-2000 spectrometers were replaced by USB-4000 spectrometers (Ocean Optics, Dunedin, FL, USA) with a custom enlarged slit width of 100 µm to increase the signal. The spectrometers were cooled to 5 °C in order to further increase the signal/noise ratio and decrease baseline drift. Light sources consisting of two blue LEDs (405nm and 435nm peak wavelength) and a quartz-halogen lamp driven by a stabilized power supply were used to provide the measuring-light for the reflectance and transmittance measurements. The blue LEDs were necessary to increase the intensity of the measuring-light in the blue region of the spectrum. Absorptance was calculated in one nanometer steps in the wavelength range 400- 800 nm. The integrated absorptance of the growth-light was calculated by multiplying the relative leaf absorptance spectrum with the spectrum of the growth-light (spectra of the growth-light are shown in Fig. 1).

Leaf photosynthesis measurements

An additional set of plants was grown under the three spectra for photosynthesis measurements. The plants were grown until the second leaf, which received 100 µmol m⁻² s⁻¹ throughout its growth period, was fully expanded (17-22 days after planting the seedlings) and could be used for photosynthesis measurements. Leaves of different plants did not overlap and if necessary the second leaf was supported in a horizontal position to ensure that it received the specified irradiance.

Light-response curves were measured on six leaves per treatment using a LI-6400 photosynthesis system with a leaf chamber fluorometer (LiCor Inc., Lincoln Nebraska, USA). The leaf chamber is equipped with red and blue LEDs with peak wavelengths of, respectively, 640 and 464 nm. Gas-exchange was measured using a gas-mix containing ambient O₂ and N₂, 22.1 ± 1 mmol mol⁻¹ H₂O and 380 µmol mol⁻¹ CO₂. The flow rate used was 250 µmol s⁻¹. After insertion into the leaf chamber, the leaf was dark-adapted for 15 minutes and then subjected to a far-red pulse (6 µmol m⁻² s⁻¹ for 2 s) to oxidize the Q_A pool of photosystem II after which F_v/F_m was measured. The blue light percentage of the measuring-light was set at 20%. At an irradiance of 1200 µmol m⁻² s⁻¹ and higher the blue light percentage was lower as the capacity for the irradiance intensity of the blue LEDs

was limited to $267 \mu\text{mol m}^{-2} \text{s}^{-1}$. At each light intensity step the rate of photosynthesis was calculated as the mean of the last 40 seconds after a steady-state gas exchange was reached, which was within 10 minutes.

Curve fitting and statistics

The photosynthesis data measured to obtain light-response curves were fitted to a non-rectangular hyperbola (Thornley, 1976) using the non-linear fitting procedure NLIN in SAS (SAS institute Inc. 9.1, Cary, NC, USA) in order to determine the light saturated gross assimilation (A_{max}).

Fisher's LSD was used to make post-hoc multiple comparisons among spectral treatment means from significant one way ANOVA tests ($P < 0.05$; test with blocks for the growth and morphology analysis; without blocks for the photosynthesis data).

Results

Plant Morphology

The difference in visual appearance of the plants growing under the three different spectra was striking (Fig. 2). The plants grown under HPS had a slightly bigger appearance than the plants grown under FT. The AS-grown plants, however, developed considerably faster than those grown under HPS and FT.

The differences in plant morphology are shown quantitatively in Table 1. Leaf one, which was fully expanded on all plants when harvested, was smaller in the FT treatment than in the HPS and AS treatments. Leaf two of the AS-grown plants had twice the area of that grown under HPS and four times the area of that grown under FT. This leaf was, however, not completely expanded on all plants at the time of harvest. The number of leaves also was significantly greater for the AS plants compared with the other two treatments, and the HPS plants had a slightly, but significantly greater number of leaves than the FT plants. Leaf number, therefore, also contributed to the significant differences in total leaf area between the treatments; the AS-grown plants had a total leaf area which was 2.5 and 1.7 times greater than that of FT and HPS plants, respectively. The petioles of leaf one and two were approximately three times longer for the AS plants than those of the other two treatments, whereas the petioles of HPS plants were slightly, but significantly longer than those of FT plants. Due to their long petioles the leaves of individual AS-grown plants did not shade each other, whereas from the third leaf of plants in the other treatments there was leaf shading in individual plants. Also leaf one and two of the FT and HPS plants partially shaded the cotyledons, whereas the cotyledons of the AS plants were not shaded (Fig 2). Leaves of the FT and HPS plants were not completely horizontal and also not oriented towards the incident irradiance such that light interception would be optimal. The leaves of the AS plants were fully horizontal and better orientated for light interception. The hypocotyl was over three times longer for the AS plants than it was for the other treatments. A similar trend was found for total plant length, which was four and five times greater for the AS-grown plants than, respectively, the HPS and FT plants. The total plant length was only slightly greater than the hypocotyl length for HPS and FT plants, whereas the total length of the AS plants was much greater than that of the hypocotyl. This is due to differences in internode-length between the treatments. The

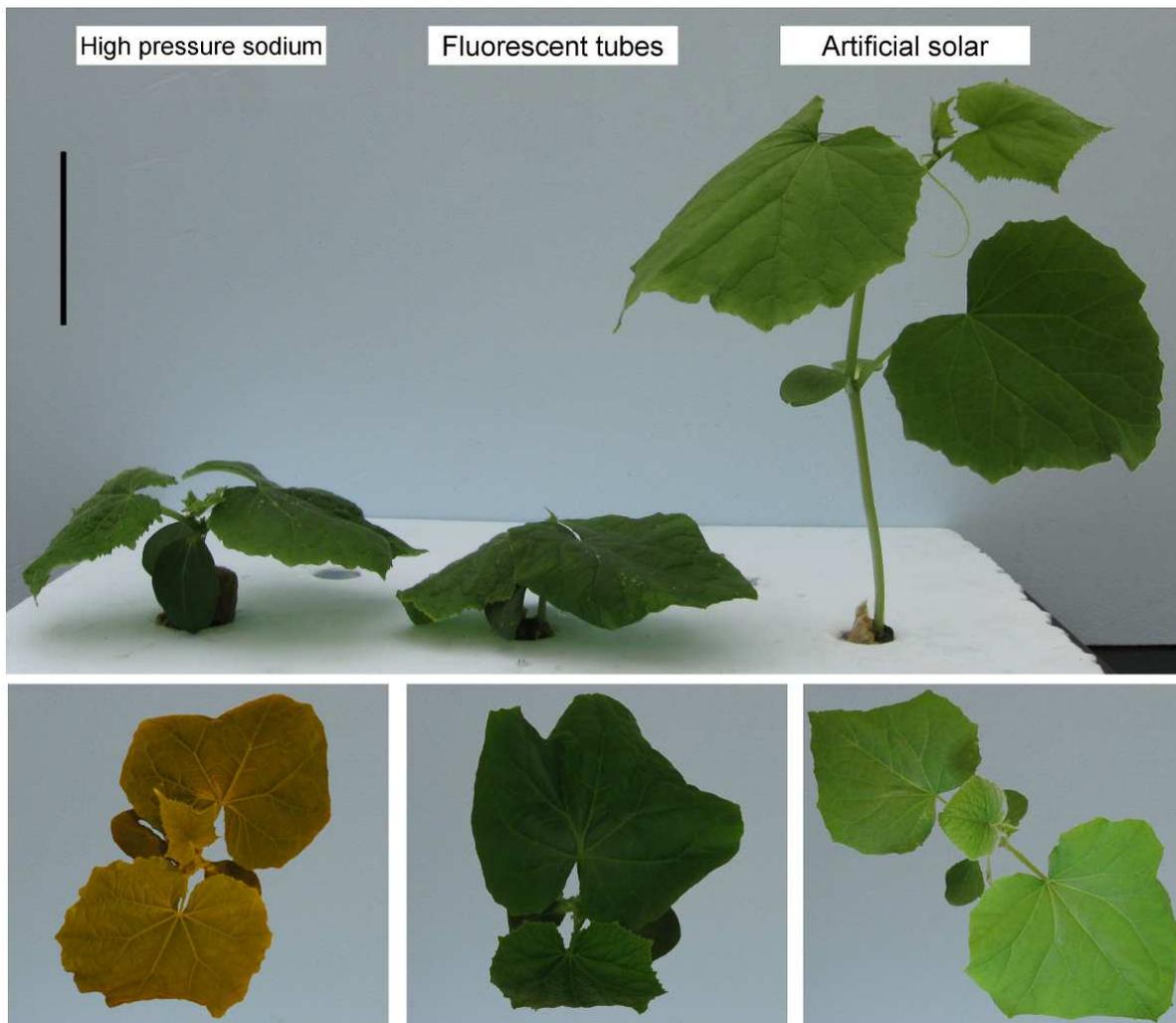


Fig. 2. Cucumber plants grown under a high pressure sodium lamp (left), fluorescent tubes (middle) and an artificial solar spectrum (right) 13 days after planting the seedlings. The upper image was made before the plants were dissected for growth and morphology analysis (bar=10cm). The lower three images were made before harvest and are of different plants than those on the upper image. These three images are not scaled; the leaf colour appears unnatural due to the growth-light environment.

cotyledon area of the FT plants was smaller than that of the HPS and the AS plants, despite having already been partly developed when the plants were transferred to the spectrally different irradiances, implying that the cotyledons were affected by the growth-light treatment.

Plant dry weight and partitioning

Overall the trends observed for the lengths and areas (Table 1) of the different plant parts of plants grown under different spectra also apply for the dry weights (DW; Table 2). The DW differences between spectral treatments for the hypocotyl are even greater than the differences in length as the longer hypocotyls were also thicker and therefore heavier per length unit. The LMA was, in contrast to the general trend for the length, area and DW of

Table 1. Length (cm) and area (cm²) of different plant organs of cucumber plants grown under a high pressure sodium lamp (HPS), fluorescent tubes (FT) and an artificial solar spectrum (AS). Different letters indicate significantly different means (P< 0.05).

	HPS	FT	AS
Leaf 1 (cm ²)	129 ^a	102 ^b	131 ^a
Leaf 2 (cm ²)	98 ^b	55 ^c	207 ^a
All leaves (cm ²)	236 ^b	159 ^c	397 ^a
Cotyledons (cm ²)	27 ^a	23 ^b	27 ^a
Petiole 1 (cm)	3.4 ^b	2.5 ^c	9.3 ^a
Petiole 2 (cm)	3.0 ^b	2.2 ^c	7.0 ^a
Hypocotyl (cm)	4.4 ^b	3.9 ^b	14.0 ^a
Plant length (cm)	5.8 ^b	4.7 ^b	25.8 ^a
Number of leaves	3.4 ^b	3.0 ^c	4.4 ^a

Table 2. Dry weight (DW, in mg) of plants, different plant parts and leaf mass per area of the first leaf (LMA, in g m⁻²) of cucumber plants grown under a high pressure sodium lamp (HPS), fluorescent tubes (FT) and an artificial solar spectrum (AS). Different letters indicate significantly different means (P< 0.05).

	HPS	FT	AS
DW leaf 1	221 ^a	190 ^b	209 ^{ab}
DW all leaves	420 ^b	295 ^c	627 ^a
LMA	17.1 ^b	18.8 ^a	15.9 ^c
DW cotyledons	71 ^a	67 ^a	66 ^a
DW hypocotyl	27 ^b	17 ^c	123 ^a
DW roots	79 ^b	52 ^c	100 ^a
DW remainder	15 ^b	9 ^b	84 ^a
DW plant	611 ^b	440 ^c	1001 ^a

Table 3. Dry weight partitioning (%) to different plant organs of cucumber plants grown under a high pressure sodium lamp (HPS), fluorescent tubes (FT) and an artificial solar spectrum (AS). Different letters indicate significantly different means (P< 0.05).

	HPS	FT	AS
Leaf 1	37 ^b	44 ^a	22 ^c
All leaves	68 ^a	67 ^a	62 ^b
Cotyledons	12 ^b	15 ^a	7 ^c
Hypocotyl	4 ^b	4 ^b	13 ^a
Roots	13 ^a	12 ^a	10 ^b
Remainder	2 ^b	2 ^b	8 ^a

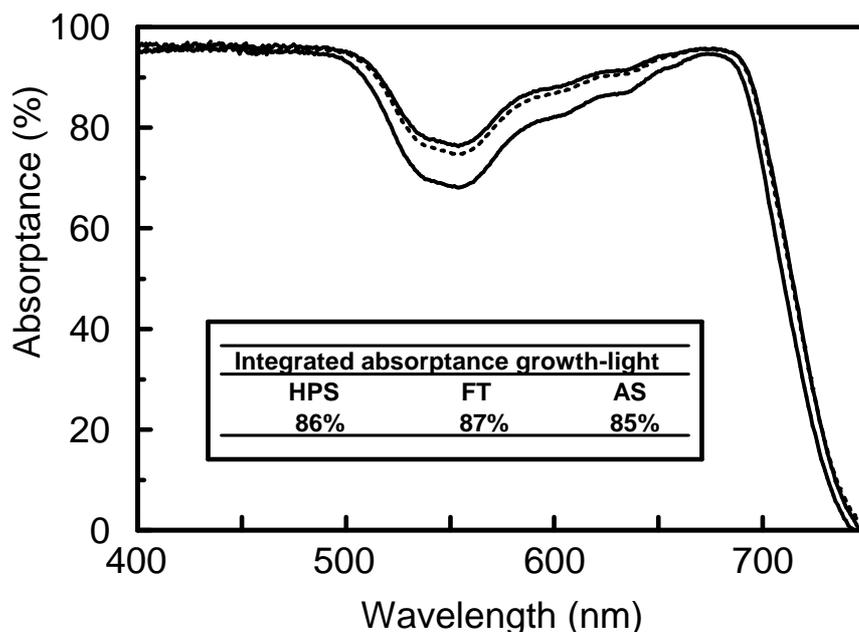


Fig. 3. Absorbance spectra for cucumber leaves grown under $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ incident irradiance provided by fluorescent tubes (FT; upper solid line), a high pressure sodium lamp (HPS; dashed line) and an artificial solar spectrum (AS; lower solid line). The table indicates the integrated absorbance (%) of the three different growth-light sources, the relative spectra of which are given in Fig. 1.

the plant parts, smallest for AS-grown plants and greatest for FT grown plants. This also explains why there are no significant differences in DW of leaf one between AS and FT grown plants, whereas the differences in leaf area are significant. The DW of the cotyledons is also lower for the AS plants than for the HPS plants, whereas the area was identical. The DW of the roots and remainder (mainly internodes) was again greatest for AS-grown plants and smallest for the FT plants. The total DW of the AS plants was 2.3 and 1.6 times greater than that of the FT and HPS plants, respectively.

The DW partitioning to the stem (hypocotyl, remainder) was three to four-times greater for the AS-grown plants compared with the other two treatments, at the expense of partitioning to other plant parts (Table 3). Partitioning to leaf one and the cotyledons is lowest in the AS plants and highest in the FT plants. This result is influenced by the differences in the number of leaves per plant (Table 1). Partitioning to the roots did not differ much between the treatments and was slightly smaller for the AS-grown plants.

Light absorbance

The absorbance spectra were similar for the leaves grown under FT and HPS, whereas the absorbance of the AS-grown leaves was lower (Fig. 3). The difference in absorbed PAR between the treatments was greatest at 554nm where FT, HPS and AS-grown leaves absorbed 76%, 75% and 68% of the incident irradiance, respectively. The integrated absorbance of the growth-light was comparable for the three different spectra: 87%, 86% and 85% for FT, HPS and AS-grown leaves, respectively.

Photosynthesis

All measured leaves had a dark-adapted F_v/F_m of 0.8 or higher. Leaves grown under different spectra had different light-response curves (Fig. 4). The fitted light saturated gross assimilation rate per area leaf (A_{max}) was significantly higher for the FT grown leaves, compared with the two other treatments (Table 4). At growth irradiance ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$) measured net assimilation per leaf area was lowest for the AS-grown leaves and identical for the FT and HPS leaves (Table 4).

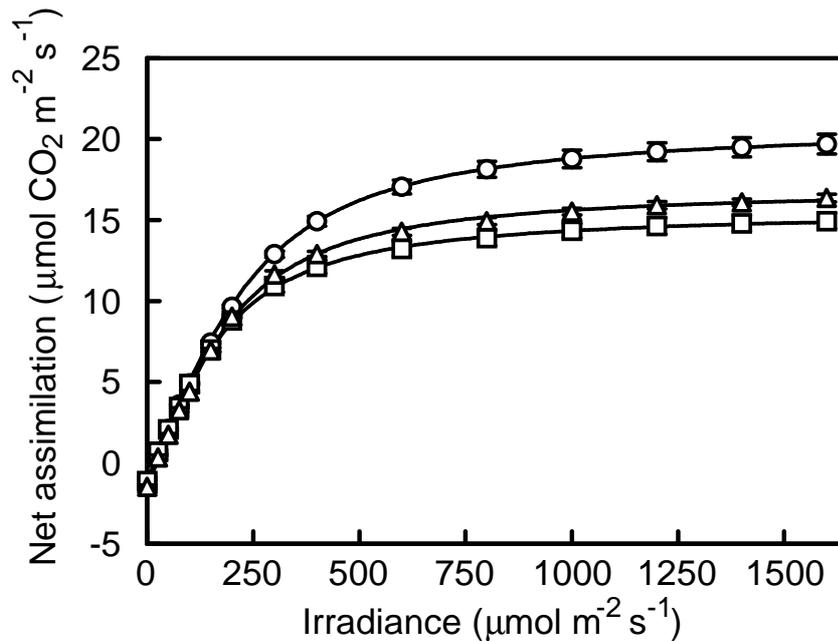


Fig. 4. Irradiance- CO_2 fixation response curves for leaves grown under $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ incident irradiance provided by fluorescent tubes (circles), a high pressure sodium lamp (squares) and an artificial solar spectrum (triangles). Lines through the data points represent the fit to the non rectangular hyperbola. Error bars represent the s.e.m.

Table 4. Net assimilation at growth irradiance (A_{net} at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$) and fitted light saturated gross assimilation (A_{max}) of cucumber plants grown under a high pressure sodium lamp (HPS), fluorescent tubes (FT) and an artificial solar spectrum (AS). Different letters indicate significantly different means ($P < 0.05$).

	HPS	FT	AS
A_{net} at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$	4.9 ^a	4.9 ^a	4.5 ^b
A_{max} (fitted)	16.9 ^b	22.5 ^a	18.7 ^b

Discussion

Plant growth and morphology

The conspicuously greater size and biomass accumulation of the plants grown under an artificial solar (AS) spectrum compared with plants grown under a high pressure sodium lamp (HPS) or fluorescent tubes (FT) appears to be related to the development by the AS plants of an architecture more favorable for light interception. The properties of the AS plants advantageous for light interception were characterized by an optimal leaf orientation (Fig. 2), long petioles preventing self-shading (Table 1), a larger total area (Table 1) and a lower LMA (Table 2). Compared with the FT plants, the HPS plants also displayed many of the features leading to improved whole plant light interception as shown by the AS plants, but in this case the extent of the differences was much smaller.

Light spectrum is known to have a strong influence on plant morphogenesis (e.g. Whitelam and Halliday, 2007). The three growth-light spectra used (Fig. 1) are different in many respects and therefore it is difficult to attribute the differences in morphological responses to specific physiological processes mediated by the spectral environment. However, two conspicuous spectral differences between the growth-light environments have been subject to extensive study. First, the AS-spectrum contains a considerable amount of far-red (FR) wavelengths (> 700nm), whereas FR is almost absent in the two other spectra. Second, the HPS-spectrum contains little blue (5%), whereas AS (17%) and FT (23%) contain substantially more blue.

Studies on the effects R:FR ratios have on plant morphogenesis (e.g. Child *et al.*, 1981; Morgan and Smith, 1981) show a general trend of taller plants, longer petioles and a relatively greater DW partitioning to the stem at the expense of partitioning to the leaves associated with lower R:FR ratios. The R:FR ratio induced responses are regulated via the phytochrome photostationary state (PSS) which is used as an indicator for the relative amount of active phytochrome. Sager *et al.* (1988) developed a method to estimate PSS using the complete spectrum of the plants' light environment instead of simply calculating the R:FR ratio. According to this method the PSS of the plants in our experiment was 0.85, 0.89 and 0.72 for, respectively, FT, HPS and AS. The lower calculated PSS for the AS treatment may partly explain the four to five-times greater height of the AS-grown plants and greater DW partitioning to the stem compared with the two other treatments.

A greater blue light fraction, or a higher absolute amount of blue light, is generally associated with the development of "sun-type" leaves, which are characterized by leaves with a high LMA and a high photosynthetic capacity (e.g. Buschmann *et al.*, 1978; Lichtenthaler *et al.*, 1980; Matsuda *et al.*, 2008). Also, hypocotyl elongation is inhibited by blue light via a cryptochrome-mediated response (Ahmad *et al.*, 2002). Regarding the two lamp types containing very little FR (FT and HPS), the greater blue light fraction may explain the greater LMA and shorter stem and petioles of FT-grown plants compared with HPS grown plants. However, the interaction of blue light fraction, R:FR ratio and other differences in spectrum makes it impossible to draw reliable conclusions on the mechanisms underlying the wavelength-dependency of the responses of the plants grown under the three spectra used in this study. Note that the growth irradiance of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in our experiment is relatively low for a tropical crop plant like cucumber. Therefore despite the differences in spectral output of the three lamp-types used, the leaves of none of the treatments can be regarded as true "sun-type" leaves. Cucumber leaves developing

under much higher irradiances of natural sunlight usually have a considerably greater LMA than the range we found (e.g. Papadopoulos and Hao, 1997). Nonetheless the overtly greater biomass production by the plants grown under the AS-spectrum, compared with the two spectra widely used in protected cultivation, shows the importance of a balanced spectral composition of growth light. The use of a growth irradiance beyond the light-limited range (e.g. $\geq 300 \mu\text{mol m}^{-2} \text{s}^{-1}$) may well result in different assimilation rates per unit leaf area due to different irradiance-photosynthesis response curves for the different treatments (as at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$, Fig. 4). In that case plant assimilation would be determined by both the acclimation of morphology and photosynthesis, further complicating the interpretation of the results. The AS irradiance used is in the range of intensities used in climate chambers and also, both in terms of spectral composition and intensity, representative for cloudy days in, for example, a Dutch greenhouse from autumn to spring.

Beside the morphological responses leading to better light interception by the AS-grown plants, the leaf unfolding rate (LUR, leaves per day) was also greatest for these plants, enhancing light interception even further by increasing leaf number per plant. Both assimilate supply and temperature have been identified as factors affecting LUR (Kiniry *et al.*, 1991; Marcelis, 1993). Although the AS plants had the best light interception and would therefore be expected to produce the most assimilates, leaf temperature of the AS leaves was also slightly higher. In some species, e.g. tomato and sweet pepper, LUR is mainly dependent upon temperature with assimilate supply having little effect (Heuvelink and Marcelis, 1996). However, in cucumber assimilate supply has been reported to have a strong effect on LUR (Marcelis, 1993). Challa and van de Vooren (1980) developed a mathematical model describing the dependency of the leaf development rate per week on light intensity and temperature for cucumber. According to that model, the influence of the differences in leaf temperature ($< 3 \text{ }^\circ\text{C}$) between our treatments on LUR was negligible at the light intensity and temperature used in our experiment, suggesting that the differences in LUR were mainly dependent on assimilate supply. Nonetheless effects on LUR mediated via spectrum-induced signals can not be excluded.

Leaf light absorptance and photosynthesis

The lower light absorptance per leaf area of AS-grown leaves (Fig. 3) may be attributed to the lower LMA (Table 2) of these leaves. Nevertheless, despite the different absorptance spectra, the integrated absorptance of the growth-light was only 2% and 1% greater for FT and HPS, respectively, compared with AS.

The A_{max} values were higher for leaves grown under spectra containing more blue light (Table 4). Blue light has been reported to increase the photosynthetic capacity of leaves (e.g. Buschmann *et al.*, 1978; Lichtenthaler *et al.*, 1980) and leaves developed under blue or mixed red/blue light have a greater A_{max} than leaves grown under red light alone (e.g. Bukhov *et al.*, 1995; Matsuda *et al.*, 2004). In studies on leaf responses to irradiance, a higher irradiance was usually reported to lead to both a higher LMA and A_{max} , as recently reviewed by Poorter *et al.* (2009). Blue light deficiency was associated with a lower LMA in soybean (Britz and Sager, 1990) and the LMA of cucumber leaves grown under a range of different red/blue ratios correlated positively with A_{max} (Hogewoning *et al.*, 2010c). Though we found a trend of increasing A_{max} with increasing blue fraction of the growth irradiance, LMA showed no clear dependency on the blue light fraction during growth. Notably, the

AS (18% blue) grown leaves had a (not significantly) greater A_{\max} , but a smaller LMA, than the HPS (5% blue) grown leaves (Tables 2 and 4). Red to FR ratios do not have a strong effect on LMA (Poorter *et al.*, 2009). It is significant that the generally reported relationship between LMA and A_{\max} can be broken, presumably due to effects of wavelengths in the broad-band AS-spectrum other than the relatively well-studied blue, red and far-red effects on plant development. The change in the relationship between LMA and A_{\max} also indicates that the large differences in morphology between the AS plants and the HPS and FT plants can not be simply attributed to the considerable presence of far-red wavelengths in the AS spectrum whereas the HPS and FT spectra contain very little far-red (Fig. 1).

Measured net assimilation per area (A_{net}) at $100 \mu\text{mol m}^{-2}\text{s}^{-1}$ irradiance was slightly lower for the AS-grown leaves, compared with that of the two other treatments (Table 4). This measured difference may be due to the spectrum of the measuring-light, instead of a real *in situ* difference in A_{net} . The AS-leaves developed under a spectrum containing both wavelengths exciting preferentially photosystem I (>680 nm) and photosystem II (PSII; <680nm), whereas the HPS and FT grown leaves developed under a spectrum preferentially exciting PSII (Evans, 1986, 1987). The measuring-light spectrum, provided by red and blue LEDs, slightly over-excites PSII. Leaves have been shown to be able to tune their photosystem stoichiometry to the growth-light spectrum in order to optimize the excitation balance between the photosystems (Chow *et al.*, 1990; Walters and Horton, 1995). Therefore the PSII antennae of the AS-grown leaves may have been relatively greater than those in the leaves grown under FT and HPS, which would lead to a decrease in light use efficiency of the measuring-light spectrum. Nonetheless, a possible relative decrease in light use efficiency of red and blue wavelengths due to acclimation to the AS-spectrum is not expected to be so large that it could outweigh the 10% lower A_{net} measured on the AS-leaves at $100 \mu\text{mol m}^{-2}\text{s}^{-1}$ irradiance.

Implications of the plant responses to an artificial solar spectrum

Whereas photosynthesis per leaf area at growth irradiance was not markedly different for the leaves grown under the different spectra, plant development and biomass accumulation was. The differences are attributed to spectrum-induced differences in morphogenesis, which led to a DW of the AS-grown plants which was as much as 2.3 times greater than that of FT grown plants after only 13 days growing at a light-limiting irradiance. The use of an artificial solar spectrum is the only method allowing a reliable comparison between a “natural spectrum” and the spectrum of different lamp types or combinations, as under real daylight conditions the light intensity cannot be kept stable or be caused to reliably change in a predictable fashion. So far, to the best of our knowledge, no plant research studies have been published using an artificial solar spectrum resembling a realistic solar spectrum as closely as ours. Fujiwara and Sawada (2006) described a prototype of an LED-based solar lamp which seems promising and Krizek *et al.* (1998) have compared the performance of cucumber grown for 14 days under a microwave-powered sulfur lamp and a metal halide lamp. Although the spectrum of the sulfur lamp was not adjusted in that study so that it fitted a solar-spectrum more closely and the plants were allowed to shade each other during growth, the sulfur-lamp grown plants showed a greater DW, total leaf area, petiole length and total height than the metal halide lamp grown plants, as did the AS-grown plants compared with the FT and HPS grown plants in our experiment.

Even in the 1950s it was recognized that fluorescent tubes alone resulted in ‘short plants’ (Wassink and Stolwijk, 1956). Growth-cabinet lighting was therefore sometimes adjusted (e.g. fluorescent tubes in combination with incandescent lamps). The aim of such lighting modifications was to produce morphologically normal appearing plants rather than to produce plants using a normal (i.e. similar to sunlight) spectral irradiance (see e.g. Deutch and Rasmussen, 1973). Despite the importance of these earlier observations, it is currently uncommon for plants to be grown with the addition of far-red light from incandescent lamps. Even then the extent to which plants grown under these conditions resemble field-grown plants in ways other than their appearance is unclear. A light-source spectrally resembling natural sunlight should allow the production of plants under controlled environment conditions that more closely resemble their field-grown counterparts, or at least to discover for which purposes conventional light-sources are unsuitable. Further, the extra productivity of the AS grown plants in comparison to the HPS plants (1.6 times greater) points to the strong possibility that assimilation lighting in glasshouses could be made more productive. Especially in winter at northern latitudes when the natural photoperiod is short and the natural irradiance intensity is low, a considerable part of the daily irradiance is supplied by HPS lamps. Early in the production cycle when plants are small, crops could be made more productive by developing light sources that stimulate better the development of leaf-area at the expense of LMA to increase light interception, and longer internodes and petioles to reduce self-shading.

Supplementary data

The relative spectra of cloud-light in fully overcast conditions, direct sunlight and skylight at midday in Wageningen (The Netherlands) around the autumn equinox 2009, and the spectra of the three light sources used (artificial solar, high pressure sodium and fluorescent tube light) are available in a tabular form (Table S1) as supplementary material at *Journal of Experimental Botany* online.

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CHAPTER 6

General Discussion

The main emphasis of this thesis is on the physiological functioning and acclimation of *Cucumis sativus* in response to the spectral environment: Intrinsic leaf photosynthetic functioning and photosynthetic capacity, changes in the wavelength dependence of photosynthetic quantum yield, adaptations of plant morphology versus photosynthesis per unit leaf area in relation to biomass production, and the underlying mechanisms of these plant responses have been described in the previous chapters. In the general introduction (Fig. 1) a schematic theoretical framework on how the interacting different plant responses to the irradiance-spectrum relate to biomass production is shown. In this general discussion the consequences of these different classes of plant responses to irradiance-spectrum are set in a broader (eco)-physiological perspective with an emphasis on biomass production and adaptation to different natural environmental conditions. Although responses of plants to unnatural irradiance spectra have been studied in this thesis, the results do provide new insights into the physiology and eco-physiology of plants. Additionally, some consequences of this study for the measurement of photosynthesis are briefly discussed. As a concluding paragraph, the new insights gained here are discussed in relation to implications and opportunities for plant cultivation under controlled conditions.

6.1 Plant responses to the spectral environment: (Eco)-Physiological implications

6.1.1 Long-term adaptation and rapid responses of leaf photosynthesis to the irradiance-spectrum

We clearly show that there is a fundamental difference between long-term adaptation to a particular growth-light spectrum, and rapid responses of leaf photosynthesis to the incident irradiance spectrum. Short term illumination with red light produced the highest photosynthetic rates within the photosynthetically active wavelength range (350-730 nm; chapter 3.2). This result is in line with older work (e.g. McCree, 1972a; Inada, 1976; Evans, 1987). However, when applied as a growth-light, red light (638 nm, 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$) alone resulted in leaves with a dysfunctional photosynthetic operation, characterized by a heterogeneous distribution of F_v/F_m that was below 0.8 between the veins, stomata unresponsive to light intensity and spectrum, and a chlorotic visual appearance (chapter 2). The photosynthetic capacity of these leaves was also low. Although photosynthetic rates of leaves illuminated with blue light (447 nm) for a short period (minutes) were

relatively low, the photosynthetic operation was normal if plants were grown under blue light alone or a combination of red and blue, and photosynthetic capacity was considerably higher than for growth under red light alone. This “red light syndrome” has also been found for tomato (unpublished data).

Lower photosynthetic rates or biomass production for a range of species grown under red alone, compared to a combination of red and blue, were reported by e.g. Goins *et al.*, (1997), Matsuda *et al.* (2004) and Ohashi *et al.* (2006). However, *Spirodela polyrhiza* did produce more biomass grown under 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ red (638 nm), compared to a range of other colors including white (Hogewoning *et al.*, 2007) and Yorio *et al.* (2001) found lower photosynthetic rates for red (660 nm) grown radish, compared to combined red/blue growth, but not for spinach and lettuce. These different responses of photosynthetic functioning may indicate some degree of genetic variation in sensitivity for “red light syndrome” symptoms. However, the wavelength range of the red light used and the growth irradiance may also have a significant influence on the varying responses found. Nonetheless, it is an important observation that a wavelength range producing high photosynthetic rates in the short term can produce a dysfunctional photosynthetic apparatus if used for plant growth.

6.1.2 Spectral effects on photosynthetic capacity

Despite the extreme deviation from any natural irradiance spectrum (chapter 5), a combination of red (638 nm) and blue (447 nm) did produce leaves with a normal photosynthetic functioning, and higher blue to red ratios produced leaf characteristics comparable to those associated with high irradiance (chapter 2). Interpretation of the eco-physiological relevance of these quantitative blue light effects is difficult. At least in case leaves are shaded by other leaves, blue light is relatively weak compared to other wavelengths, due to the efficient blue absorption by other leaf layers (e.g. Dietzel *et al.* 2005). In shade, leaf characteristics associated with a high photosynthetic capacity decrease net assimilation per unit leaf area, as such characteristics increase respiration (e.g. Pons and Pearcy, 1994; Wright *et al.*, 2004). Shade spectra are also characterized pronouncedly by a relatively low red:far red (R:FR) ratio (e.g. Fig.1 in chapter 3.2) and R:FR ratios have a strong effect on phytochrome mediated responses (e.g. Smith, 2000), whereas cryptochromes and phototropins are mainly sensitive to blue light (e.g. Whitelam and Halliday, 2007). Therefore the question arises whether blue light and phytochrome mediated responses might interact in the regulation of photosynthetic capacity. However, phytochrome activity does not regulate LMA, which generally correlates with photosynthetic capacity (e.g. Evans and Poorter, 2001), as R:FR ratios do not affect LMA (Poorter *et al.*, 2009). LMA did increase significantly with an increasing blue light fraction (Fig. 6 in chapter 2) within a range of growth-spectra with a comparable phytochrome equilibrium calculated according to Sager *et al.* (1988), suggesting the possibility of the involvement of cryptochrome and/or phototropin in the regulation of LMA. Schuerger *et al.* (1997) also reported a correlation of changes in the anatomy of the stem and leaves of pepper plants with the amount of blue in the spectrum of the different lamp types the plants were grown under. However, a higher LMA was found for leaves developed under high pressure sodium lamps than for leaves grown under artificial solar light containing considerably more blue (chapter 5). Also, the LMA of *Spirodela polyrhiza* grown under eight different narrow-band wavelength ranges provided by LEDs (peak wavelengths in the

range 460-670 nm) did not show a significant response, whereas it responded strongly to irradiance intensity provided by white fluorescent tubes (Hogewoning *et al.*, 2007).

The question arises whether the responses of photosynthetic capacity to blue light at a constant, low irradiance, are controlled via the same mechanism as the responses to irradiance intensity. The redox state of the photosynthetic machinery controls the expression of genes involved in acclimation responses of photosynthesis (Pfannschmidt *et al.*, 2001). Similar to a high irradiance, a light-limited irradiance with a spectral composition over-exciting PSII will result in a more reduced state of the acceptor side of PSII, although state transitions (in the order of minutes) and photosystem stoichiometry changes (hours to days) will at least partly compensate for imbalances in photosystem excitation (e.g. Pfannschmidt, 2005; chapter 3.2). The extent to which blue light overexcites PSII is highly dependent on the specific wavelength range. Around 475 nm over-excitation of PSII is strong, whereas photosynthetic capacity increased with an increasing ratio of blue (weighted mean wavelength of 447 nm) to red (weighted mean wavelength of 638 nm) light (chapter 2) and both wavelength regions overexcite PSII only slightly (Fig. 6 in chapter 3.2.). Blue light is usually defined as the wavelength range between 400 and 500nm, however, a more nuanced approach to 'blue light effects' is required. It is concluded that during leaf development at a low irradiance, 'blue light' is important in the regulation of photosynthetic capacity, possibly via the action of cryptochromes or phototropins, and that more mechanisms may be involved, but not the regulation of LMA via the action of phytochromes .

6.1.3 The significance of enhancement effects for photosynthetic quantum yield

The absolute maximum quantum yield for CO₂ fixation or O₂ evolution has been a subject of debate for a long time (Govindjee, 1999). Based on an extensive literature survey in combination with measurements, Singaas *et al.* (2001) concluded that the large variation in the maximum quantum yield of C₃ species found over the last few decades is due to variations in the measurement procedure and not due to intrinsic differences between species. We found a maximum quantum yield of 0.093 CO₂ molecules fixed per absorbed photon for red measuring-light (620-640 nm; Table S1 in chapter 3.2) on cucumber leaves. Within the range 400-700 nm, red light generally produces the highest quantum yields in the short term, whereas blue-green light is least efficient (McCree, 1972a, Inada, 1976, Evans, 1987, chapter 3.2). Long *et al.* (1993) found a mean quantum yield for CO₂ fixation in a range of C₃ species which was also 0.093, measured with a quartz-iodine lamp ('white' light). The analysis by Singaas *et al.* (2001) supports that the intrinsic quantum yield for CO₂ fixation varies little from the mean value reported by Long *et al.* (1993). Our maximum quantum yield is therefore consistent with the mean yield by Long *et al.* (1993), but while our maximum was measured with a narrow range of wavelengths, Long *et al.* (1993) used 'white' light, which naturally raises the question of how the maximum quantum yield is influenced by the range of measuring wavelengths used. Not much attention has been given to the issue of enhancement effects, i.e. the quantum yield of a combination of wavelengths is higher than the sum of the parts (Emerson *et al.*, 1957), since McCree (1972b) concluded that enhancement effects are insignificantly small for 'white' light.

Broad-band quartz-iodine light is enriched in the red part of the spectrum compared to sunlight (Evans, 1987), but is nevertheless for a substantial part comprised of less efficient wavelengths. If the quantum yield for quartz-iodine light can be calculated

from the quantum yields of the different wavelengths that it is comprised of, it must be significantly lower than the yield for red light (620-640 nm). The similar quantum yields measured by Long *et al.* (1993) and us (chapter 3.2) leaves three options: our maximum quantum yield for CO₂ fixation of 0.093 measured on cucumber might be exceptionally low, or the mean yield of 0.093 by Long *et al.* (1993) is exceptionally high, or the broad-band spectrum used by Long *et al.* (1993) produced a considerable enhancement.

In addition to these physiological issues, the measurement of irradiance can also contribute to discrepancies in quantum yields. Quantum sensors (e.g. from LI-COR) are predominantly sensitive to wavelengths in the range 400-700 nm, often referred to as photosynthetically active radiation (PAR). This wavelength range is reasonable for estimating the incident PAR on a leaf, for though wavelengths in the range 700-730 nm contribute to photosynthesis, they are absorbed relatively poorly by leaves. However, when determining the maximum quantum yield on an absorbed light basis, these wavelengths do contribute significantly to the quantum yield and therefore a spectroradiometer should be used to determine the incident quantum flux. In some reports of the maximum quantum yield (Björkmann and Demmig, 1987; Long *et al.*, 1993) a quantum sensor was used to calibrate the quantum flux of broad-band measuring-light containing wavelengths > 700 nm. When this wavelength range is not taken into account, quantum yields are overestimated because the absorbed photon flux is underestimated, and, perhaps more importantly, this wavelength range is the most relevant for enhancement effects.

The theoretical basis of enhancement effects is that individual wavelengths can overexcite one of the two photosystems, while a combination of wavelengths overexciting PSI and wavelengths overexciting PSII can produce a balanced photosystem excitation, resulting in a higher quantum yield (e.g. Chow *et al.*, 1990; Walters and Horton, 1995). Earlier estimates of the wavelength dependence of photosystem excitation balance (Evans, 1986; Evans and Anderson, 1987) and our more functional efficiency balance measured *in vivo* (Fig. 9 in chapter 3.2) suggest that enhancement effects can be significant for broad-band light, when the spectrum is substantially comprised of wavelengths overexciting PSI (685-730 nm). Most wavelengths in the range 400-670 nm slightly overexcite PSII, while wavelengths in the range 685-730 nm strongly overexcite PSI (Figs 6 and 9 in chapter 3.2), and therefore a relatively small amount of light in this range of wavelengths may produce a considerable enhancement effect. Nevertheless, even in recent studies the consequences of an enhancement effect are generally ignored (e.g. Zhu *et al.*, 2010). The enhancement calculated for illumination of our artificial-sunlight grown cucumber leaves with sunlight (400-720 nm, spectrum as in chapter 5, Fig. 1A) was 12 % (S.W. Hogewoning, unpublished)¹. A quartz-iodine spectrum contains wavelengths that will over-excite both PSII and PSI and therefore a significant enhancement effect is expected in the work of

¹ For the calculation of a sunlight enhancement effect, the efficiency losses of quantum yield for CO₂ fixation due to an imbalanced photosystem efficiency were determined for each of 18 measuring wavelengths (400-720 nm), using the wavelength dependence of the photosystem efficiency balance of our artificial-sunlight grown cucumber leaves (Fig. 9 in chapter 3.2). The efficiency losses and the quantum yields for CO₂ fixation (Fig. 3 in chapter 3.2) of the 18 wavelengths were calculated per nm by interpolation, allowing calculation of the quantum yield per nm for the case that there were no losses of efficiency due to imbalances (i.e. the enhanced quantum yield). Multiplication of both the measured and enhanced quantum yield with the relative fraction of absorbed sunlight per nm allowed calculation of the weighted mean quantum yield of the sunlight spectrum with and without enhancement effect.

Long *et al.* (1993). It is concluded that enhancement effects of broad-band light can contribute significantly to the maximum quantum yield for CO₂ fixation.

Ideally, the maximum quantum yield is determined using a combination of wavelengths that are not absorbed by carotenoids and non-photosynthetic pigments (i.e. $\geq 580\text{nm}$) and produce a balanced photosystem efficiency, which can be monitored spectroscopically *in vivo* (Baker *et al.*, 2007; chapter 3.2). The measuring-light spectrum should not induce state transitions, as it is uncertain if they contribute to enhancement of the quantum yield for CO₂ fixation (Andrews *et al.*, 1993; see 6.1.4). A correction for losses due to imbalances in photosystem efficiency resulted in a calculated mean maximum quantum yield for CO₂ fixation of 0.0955 ± 0.0004 for red (620-640 nm) measuring wavelengths on our artificial sunlight, artificial shadelight, and blue light grown cucumber leaves (S.W. Hogewoning, unpublished).

6.1.4 Acclimation of quantum yield to spectral changes

Photosynthetic acclimation to growth-light spectrum has been shown to change the wavelength dependence of the light-limited quantum yield for CO₂ fixation in parallel with the wavelength dependence of electron transport efficiency balance and photosystem stoichiometry (chapter 3.2). The quantum yield for CO₂ fixation of the leaves grown under SHADE-light, which is relatively abundant in wavelengths preferentially exciting PSI (PSI-light), was higher for PSI-light compared with SUN and BLUE grown leaves. The SUN and BLUE grown leaves had a higher efficiency for PSII-light compared with SHADE. This confirms the findings by Chow *et al.* (1990) and Walters and Horton (1995) that leaves tune their photosynthetic apparatus such that the photosynthetic quantum yield remains high under different growth-light spectra. Although both state transitions and changes in photosystem stoichiometry are widely believed to re-distribute excitation energy between the two photosystems (Haldrup *et al.*, 2001; Pfannschmidt, 2005), Andrews *et al.* (1993) found no increase in quantum yield for CO₂ fixation in wheat leaves following a state transition, while the effective antenna size of PSII did decrease. Some older studies also reported that there was no increase in the PSI absorption cross section following a state transition (Haworth and Melis, 1983; Deng and Melis, 1986), whereas others did find an increase (Bennett *et al.*, 1980; Horton and Black, 1981). More recently, phosphorylated LHCII of *Arabidopsis* was reported to transfer excitation energy to PSI by Lunde *et al.* (2000), who also speculated that dissociation of LHCII from PSII depends on its coupling to PSI. In contrast to these contradictory results in leaves, results from the green alga *Chlorella* clearly show that state transitions result in a higher O₂ evolution (Bonaventura and Myers, 1969).

So while it is evident that changes in photosystem stoichiometry retain a high quantum yield after long term changes in the spectral environment of leaves, contradictory evidence has been reported for state transitions. Our data suggest that the absorption cross section of PSII is smaller at those wavelengths overexciting PSII (Fig. 6B in chapter 3.2). The photosystem excitation imbalance appears to be considerably greater when measured *in vitro* (state transitions not taken into account), compared with *in vivo* measurements where it concerns PSII-light (Fig. 9 in chapter 3.2), whereas for PSI-light no difference in balance between the two approaches was found. This would be consistent with state transitions. It must be noted that the difference between the *in vitro* and *in vivo* approach could be somewhat smaller than suggested (Fig. 9 in chapter 3.2), as the quantum yield for electron transport through PSII (Φ_{PSII}) decreased with increasing light-limited irradiance

(Fig. 4 in chapter 3.2) and therefore the *in vivo* imbalance may have been underestimated slightly. However, as the photosystem efficiency balance *in vivo* allowed the calculation of the quantum yield for CO₂ fixation at those wavelengths where absorption by carotenoids and non-photosynthetic pigments was insignificant (≥ 580 nm; Fig 7 in chapter 3.2), it appears that state transitions did re-distribute excitation energy and enhanced the quantum yield for CO₂ fixation. Nevertheless, this study did not aim to investigate the effect of state transitions on the quantum yield for CO₂ fixation comprehensively and therefore more detailed research is required to be conclusive in this respect.

The eco-physiological relevance of state-transitions for terrestrial plants can be questioned. Under shade conditions where irradiance is low, it is important for the survival chances of plants to maximize their photosynthetic quantum yield. Long term acclimation responses are expected to enable an efficient use of the shadelight spectrum (e.g. Chow *et al.*, 1990). In the case of a transient spectral change increasing the excitation of PSII (e.g. a sun fleck), a state transition may occur. However, such a spectral change is paralleled by a large increase in irradiance and shade leaves are characterized by low photosynthetic capacities (e.g. Harbinson and Woodward, 1984). Therefore a sun fleck is expected to light-saturate photosynthesis and even if state transitions were to have an effect on light use efficiency, this would only occur at light-limiting (or partially so) conditions. A more sophisticated eco-physiological approach to the issue on the relevance of state transitions is therefore required.

6.1.5 Implications of the similar wavelength dependence of photosystem excitation balance determined *in vivo* and *in vitro*

Both the wavelength dependence of the photosystem efficiency balance obtained spectroscopically *in vivo* and that of the photosystem absorption balance obtained from measurements on isolated protein-pigment complexes *in vitro* were strikingly similar for all three growth-spectra cucumber was grown under (Fig. 9 in chapter 3.2). This indicates that *in vivo* the effects of inefficiencies in excitation energy transfer, charge separation, cyclic electron transport (Baker *et al.*, 2007), back-reactions (Quigg *et al.*, 2006) or transfer to O₂ (Pospíšil, 2009) on the quantum yield for CO₂ fixation is small, at least under our experimental conditions. For the first time we have shown that *in vitro* measurements on isolated thylakoids can provide useful information about the photosystem efficiency balance *in vivo*.

6.1.6 Photosynthetic rates per unit leaf area versus photomorphogenesis in relation to whole-plant-photosynthesis

The growth-light spectrum was shown to have larger implications for the biomass production of young cucumber plants through photomorphogenetic adaptations affecting light interception than through photosynthesis per unit leaf area (chapter 5). Plants grown under artificial sunlight (AS) produced a considerably larger biomass in only 13 days after planting the seedlings than plants grown under an equal quantum flux emitted by fluorescent tubes (FT) or high pressure sodium (HPS) lamps. It is quite striking that the plants grown under artificial sunlight were so much more productive than the plants grown under the other lamp spectra. Although the presence of FR wavelengths in the AS spectrum (Fig. 1 in chapter 5,) may partly explain the morphological properties advantageous for light interception (e.g. Child *et al.*, 1981; Morgan and Smith, 1981),

compared with the plants grown under the largely FR deficient FT and HPS light, not all differences could be attributed simply to FR. Plant responses to other wavelength ranges than blue, red and far-red have been poorly studied. Notably, the AS grown plants had a relatively low LMA and a large individual leaf area, which is beneficial for light interception, whereas the AS spectrum was for a considerable part comprised of blue wavelengths, which enhance LMA (chapter 2). Red to FR ratios do not have a strong effect on LMA (Poorter *et al.*, 2009), suggesting that other wavelength regions were important for the regulation of the morphological properties of the AS-plants as well.

The relative importance of the three factors determining canopy productivity discussed in this thesis, i.e. light-limited quantum yield, light-saturated photosynthetic capacity and morphological properties affecting light interception, is dependent on the growth conditions. In a closed canopy where nearly all incident irradiance is intercepted, a high photosynthetic capacity is the main determinant for productivity where it concerns the leaf layer at the top. For the lower leaf layers, the ability to retain a high light-limited quantum yield is most important, whereas a high photosynthetic capacity can be counterproductive as it requires a relatively large investment of resources (Poorter *et al.*, 1990; Poorter and Nagel, 2000) and higher maintenance respiration rates (e.g. Sims and Pearcy, 1991; Pons and Pearcy, 1994; Wright *et al.*, 2004). Where the morphological properties affecting light interception of an individual plant are less important for the productivity of a closed canopy, they are highly relevant when it concerns an open canopy, likewise photosynthetic rates per unit leaf area. The effect of the spectral environment on leaf orientation is not discussed in this thesis, however, it is also an important plant property in relation to the productivity of a canopy, as it affects light interception and vertical distribution through the canopy (e.g. Long *et al.*, 2006).

6.2 Practical implications for photosynthesis estimates *in situ* and biomass production modeling

Natural daylight spectra contain a larger proportion of wavelengths > 700 nm (i.e. 'PSI-light') than most growth lamps emitting 'white light' (e.g. Fig. 1 in chapter 5). As leaves tune their photosystem stoichiometry to the spectrum of their environment (Chow *et al.*, 1990; chapter 3.2), a considerable enhancement of the measured light-limited quantum yield of sunlight grown leaves may be expected when measuring-light overexciting PSII (e.g. red light) is combined with PSI-light (wavelengths > 685 nm). The biomass production modeling exercise in chapter 4 stresses the importance of the measuring-light spectrum during photosynthesis measurements for an accurate estimate of biomass production *in situ*. The overestimation of *in situ* photosynthetic rates of daylight grown plants when measuring photosynthesis with actinic light largely comprised of red wavelengths, as shown in the example in chapter 4, may be partially compensated by an enhancement effect under natural light conditions. An improved quantitative understanding of enhancement effects under different broad-band light conditions is useful for the development of quantitative correction modules for errors in estimates of quantum yield *in situ* due to discrepancies between growth-spectrum and measuring-light spectrum.

In chapter 4 the error in the calculation of electron transport rate (ETR) via the frequently used equation of Krall and Edwards (1992) is discussed, in the case of a

measuring-light spectrum during chlorophyll fluorescence measurements containing wavelengths that are absorbed partially by carotenoids and non-photosynthetic pigments. The efficiency losses in the quantum yield for CO₂ fixation associated with absorption by these pigments (Terashima, 2009) are not reflected in a lower measured quantum yield for electron transport through PSII (Φ_{PSII}) and therefore ETR is overestimated. A similar error may occur at low measuring-light intensities: As shown in chapter 3.2 (Fig. 4), at very low irradiances with a spectral composition overexciting PSII, Φ_{PSII} was higher than it was at higher irradiances which were still within the light-limited range. This phenomenon was attributed to alternative routes for oxidation of the Q_A pool that increase Φ_{PSII} at low irradiances, but do not result in higher rates of linear electron transport and therefore do not increase CO₂ fixation (see discussion chapter 3.2). These two examples of potential errors introduced in estimates of ETR illustrate the limited quantitative reliability of such tools occasionally used to estimate photosynthesis *in situ*, instead of more laborious, but more reliable gas-exchange measurements, as Baker (2008) has also pointed out.

6.3 Plant responses to the spectral environment: Implications for protected cultivation

Beside the (eco)-physiological insights that this thesis contributes to the understanding of the responses of photosynthesis and plant development to the irradiance spectrum, our results have implications for protected plant cultivation.

6.3.1 Implications for growth-chamber lighting

Plant cultivation in growth chambers is characterized by controlled environmental conditions with illumination by means of growth-lamps emitting spectra dissimilar to those under natural conditions. Frequently used light sources in growth chambers are white fluorescent tubes (FTs), high pressure sodium (HPS) lamps, mercury vapor lamps, or a combination of the latter two. The spectra of these lamps are characterized by pronounced emission lines which are characteristic for the different lamp-types. Sometimes incandescent lamps are added as an additional FR-light source, in order to produce plants with a more 'natural' appearance (e.g. Deutsch and Rasmussen, 1974). Recently light emitting diodes (LEDs), emitting narrow band spectra, have become increasingly used in growth-cabinets, on an experimental basis in greenhouse horticulture and in research on growing plants in space (e.g. Hogewoning *et al.*, 2007; Massa *et al.*, 2008).

Intrinsic photosynthetic functioning and photosynthetic capacity: Inherently, red LEDs have the potential of producing the highest photon flux per Watt energy input within the photosynthetically active spectral bandwidth, as energy of photons depends linearly on the reciprocal of wavelength (Planck, 1901). However, beside the better plant productivity and higher photosynthetic rates associated with a combination of red and blue light that has already been shown in numerous studies (e.g. Goins *et al.*, 1997; Matsuda *et al.*, 2004; Yorio *et al.*, 2006), illumination with red LEDs alone may result in a dysfunctional photosynthetic apparatus (chapter 2). It was also shown in chapter 2 that a higher blue to red light ratio resulted in leaves with properties normally associated with growth under

high irradiance. This can be a useful tool for nurseries to produce ‘high-light acclimated plants’ under a relatively low irradiance. A low irradiance reduces energy costs and overheating, whereas seedlings with a high photosynthetic capacity are less vulnerable to light stress after being transplanted to the field or the greenhouse.

Photosystem excitation balance: As the spectral composition of common growth-chamber light sources (HPS, FTs) is largely deficient in wavelengths > 700 nm, the plants are in fact grown under ‘PSII-light’, compared with plants grown in sunlight. Plants tune their photosystem stoichiometry to retain a high photosynthetic quantum yield (Chow *et al.*, 1990; see 6.1.4). Therefore the organization of the photosynthetic apparatus in growth-chamber plants is usually not representative for field conditions, where plants are either grown in full sunlight or in FR enriched shadelight, which is ‘PSI-light’. An error of such kind is discussed by Fan *et al.* (2007), who postulate that the PSI/PSII ratios of spinach leaves grown under cool white fluorescent tubes reported by Danielsson *et al.* (2004) are unrepresentatively low due to the growth-light spectrum. The different photosystem stoichiometry will also affect measurements of photosynthetic quantum yield. Notably, enrichment of growth-chamber light with the appropriate dose of FR, e.g. by incandescent lamps, mitigates these ‘problems’.

Growth-chamber lighting versus natural light: Despite plants looking more ‘natural’ when grown under usual growth-chamber lamps combined with lamps providing additional FR wavelengths, they are no good substitutes for field grown plants (chapter 5; 6.1.6). The light source producing a spectrum within the photosynthetically active wavelength range that is close to that of natural sunlight (chapter 5) is a considerable step forward in this respect. It would also be a useful step to develop technology allowing natural changes in irradiance intensity and spectrum to be simulated in a growth-chamber. A growth-chamber facility with artificial daylight is an interesting tool for a range of plant sciences disciplines benefiting from the possibility to grow ‘natural plants’ under controlled conditions, including research on greenhouse supplemental lighting. In greenhouses supplemental growth-light is always in combination with natural daylight and therefore it has so far been difficult to investigate greenhouse crop responses to the different supplemental lighting sources reliably in a climate room. The development of artificial sunlight allows the simultaneous simulation of a greenhouse situation over different seasons in growth chambers.

6.3.2 Implications for supplemental lighting in greenhouse cultivation

In greenhouse cultivation at latitudes with short natural photoperiods and low irradiance levels in winter, the natural daylight is often supplemented with light from growth-lamps (Heuvelink *et al.*, 2006). High pressure sodium lamps are the most frequently used lamp-type. Recently, LEDs have been proposed as a possible replacement (Hogewoning *et al.*, 2007) and are already being used on an experimental basis. The diversity of available spectra, low weight, lack of radiative heat and ease of dimming are properties that make LEDs attractive to apply in greenhouses (Hogewoning *et al.*, 2007). The lack of radiative heat also allows LEDs to be applied within the canopy instead of from above (i.e. interlighting; Hovi *et al.*, 2004; Hogewoning *et al.*, 2007; Hovi and Tahvonen, 2008; Trouwborst *et al.*, 2010). Interlighting has the advantage of a better vertical light

distribution through the canopy, which is expected to increase the light use efficiency of the crop in case irradiance at the top of the canopy is beyond the light-limited range (van Ieperen and Trouwborst, 2008).

Intrinsic photosynthetic functioning: The spectral diversity of available LEDs allows plant properties to be manipulated. As discussed above, red LEDs are inherently most energy efficient, but growth under red light alone results in physiological disorders (chapter 2). It is unclear whether such disorders would also develop if red LEDs were to be used as supplemental lighting in a greenhouse, because natural light provides blue wavelengths. In seasons with high levels of natural irradiance and long days it is expected that daylight provides sufficient blue wavelengths to prevent disorders associated with the use of red LEDs, as in cucumber grown in a growth chamber under combined red and blue light, only 7 $\mu\text{mol m}^{-2} \text{s}^{-1}$ blue (447 nm) was sufficient to prevent any overt dysfunctional photosynthesis (chapter 2). However, supplemental lighting is especially beneficial for production in winter, when the natural photoperiod is short and levels of natural irradiance are low. In winter supplemental lighting is often applied for a substantially longer period per day than the natural photoperiod. In winter, therefore, red LEDs might cause disorders in plant functioning and additional blue light (e.g. blue LEDs) may be required.

In a high-wire cropping system (e.g. tomato cultivation; Heuvelink, 2005) leaves develop at the top of the canopy where daylight is relatively abundant, and gradually move deeper into the canopy where progressively less daylight is available. If interlighting with red LEDs is applied in such a cropping system, healthy leaves become exposed to increasingly less daylight and relatively more red light. It would be interesting to investigate if, and to which extent and time-scale, symptoms of the red light syndrome also develop on healthy, mature leaves. After exposing healthy, mature cucumber leaves that had developed under combined red and blue light to red light alone, half the photosynthetic capacity was progressively lost in 10 days, but no photoinhibition was found (Trouwborst *et al.*, unpublished). These results suggest that the necessity of the addition of blue LEDs to red ones depends on the irradiance intensity supplemented by the interlighting.

Photosynthetic capacity: The higher photosynthetic capacity associated with a greater quantity of blue light (chapter 2) may be exploited in greenhouse production. At times when new leaves develop at a low natural irradiance (e.g. a period with overcast weather conditions) and higher irradiance levels are expected based on yearly irradiance statistics, increasing leaf photosynthetic capacity by supplementing additional blue light may enhance production. Likewise, the leaf photosynthetic capacity of young plants grown in nurseries may be enhanced before transplantation to the greenhouse, as was pointed out above.

Photosystem excitation balance: Natural irradiance fluctuates considerably in intensity over a day and supplemental lighting is usually applied when natural light is absent or low. Therefore plants cultivated in a greenhouse with supplemental lighting are continuously exposed to strong spectral fluctuations and therefore different distributions of excitation energy between the two photosystems (chapter 3.2). Supplemental lighting sources are

largely (HPS lamps) or completely (red and blue LEDs) deficient in FR wavelengths, so they excite PSII relatively more compared with natural spectra. The acclimation of the photosystem stoichiometry to spectral changes operates on a timescale of hours to days (e.g. Pfanschmidt, 2005). Kim *et al.* (1993) reported a half-time as much as 20 hours for the acclimation of photosystem stoichiometry in pea. This is too slow to fully counteract photosynthetic quantum yield losses associated with photosystem excitation imbalances due to spectral changes by supplemental lighting in a greenhouse. State transitions operate faster (in the order of minutes), however, it is not clear yet whether they actually serve to improve photosynthetic quantum yield (see 6.1.4). Partial acclimation to lamp spectra may also result in a lower daylight use efficiency at hours that the lamps are turned off. Therefore it would be worthwhile to investigate if intelligent lighting strategies, aiming to retain a balanced photosystem excitation, will produce an enhancement of photosynthetic quantum yield and therefore production that is large enough to justify the investments required. LEDs with a peak wavelength around 690 nm may be an interesting potential candidate for this purpose, as this is the only photosynthetically active wavelength region that overexcites PSI considerably and is still absorbed efficiently by leaves (Figs 2, 6A and 9 in chapter 3.2).

Non-photosynthetic pigments: Considering the quantum yield losses due to absorption by carotenoids and non-photosynthetic pigments at wavelengths < 580 nm (Fig. 7 in chapter 3.2), breeding for varieties containing less non-photosynthetic pigments is an interesting opportunity to increase greenhouse production. Non-photosynthetic pigments serve to protect plants against photodamage by excess radiation (e.g. Edreva, 2005) and herbivores (Treutter, 2006), but also absorb light, and therefore there is a trade-off between plant defense and photosynthetic efficiency. Where these protective properties are important for plants to survive in the field, they do not seem to be of crucial importance in a modern greenhouse. Excess radiation is normally prevented using screens and pests can be controlled increasingly successful by integrated pest management (Pilkington *et al.*, 2010). Breeding in order to increase the photosynthetic rates of crops without the need for additional energy input and technological investments for the growers is a sustainable strategy to improve production.

Photomorphogenesis: The marked increase in dry weight production of the young cucumber plants grown under artificial sunlight in a growth-chamber, compared with FT- and HPS-grown plants, was due to morphological properties advantageous for light-interception (chapter 5). This principle may also be exploited in greenhouse cultivation and nurseries. In contrast to the growth-chamber experiment, in a greenhouse plants are not exposed to light from growth lamps alone, but also to daylight. However, in winter plants are usually exposed only to the supplemental light spectrum for several hours outside the natural photoperiod and natural irradiance levels are low during the day. Therefore a considerable part of the daily quantum flux is supplied by supplemental light alone. Under such circumstances supplemental light sources emitting a spectrum that stimulates morphological properties advantageous for light-interception better than the HPS-spectrum (e.g. artificial sunlight) may considerably enhance biomass production in an early growth stage. In a later growth stage, when nearly all incident irradiance is intercepted by the crop, the light-interception properties of an individual plant become

less important and the photosynthetic rate per unit leaf area becomes more important for crop production, as discussed above. In that respect, a more sophisticated supplemental lighting strategy would be to tune the growth-lamp spectrum to the growth stage of the crop, together with adjustments to retain a balanced photosystem excitation, as mentioned above. Such technological advances, and the development of varieties with a higher light use efficiency, are potentially great advances for the energy use efficiency in greenhouse production.

REFERENCES

- Ahmad M, Grancher N, Heil M, Black RC, Giovani B, Galland P, Lardemer D. 2002.** Action spectrum for cryptochrome-dependent hypocotyl growth inhibition in *Arabidopsis*. *Plant Physiology*, **129**: 774-785.
- Allen JF. 1992.** Protein-phosphorylation in regulation of photosynthesis. *Biochimica Et Biophysica Acta*, **1098**: 275-335.
- Anderson JM, Chow WS, Park YI. 1995.** The grand design of photosynthesis: Acclimation of the photosynthetic apparatus to environmental cues. *Photosynthesis Research*, **46**: 129-139.
- Andrews JR, Bredenkamp GJ, Baker NR. 1993.** Evaluation of the role of state transitions in determining the efficiency of light utilization for CO₂ assimilation in leaves. *Photosynthesis Research*, **38**: 15-26.
- ASTM. 2003.** Standard Tables for reference solar spectral irradiances: Direct normal and hemispherical on 37° tilted surface. Standard G173-03, American Society for Testing and Materials, West Conshohocken, PA, available from: <http://www.astm.org/Standards/G173.htm>
- Baker NR. 2008.** Chlorophyll fluorescence: a probe of photosynthesis *in vivo*. *Annual Review of Plant Biology*, **59**: 89-113.
- Baker NR, Harbinson J, Kramer DM. 2007.** Determining the limitations and regulation of photosynthetic energy transduction in leaves. *Plant Cell and Environment*, **30**: 1107-1125.
- Balegh SE, Biddulph O. 1970.** The photosynthetic action spectrum of the bean plant. *Plant Physiology*, **46**: 1-5.
- Bassi R, Giacometti GM, Simpson DJ. 1988.** Changes in the organization of stroma membranes induced by *in vivo* state-1-state 2 transition. *Biochimica Et Biophysica Acta*, **935**: 152-165.
- Bate-Smith EC. 1962.** The phenolic constituents of plants and their taxonomic significance: Dicotyledons. *Journal of the Linnean Society of London, Botany*, **58**: 95-173.
- Bennett J, Steinback KE, Arntzen CJ. 1980.** Chloroplast phosphoproteins: Regulation of excitation energy transfer by phosphorylation of thylakoid membrane polypeptides. *Proceedings of the National Academy of Sciences of the United States of America-Biological Sciences*, **77**: 5253-5257.

- Bernacchi CJ, Singaas EL, Pimentel C, Portis AR, Long SP. 2001.** Improved temperature response functions for models of Rubisco-limited photosynthesis. *Plant Cell and Environment*, **24**: 253-259.
- Björkman O, Demmig B. 1987.** Photon yield of O₂ evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. *Planta*, **170**: 489-504.
- Bonaventura C, Myers J. 1969.** Fluorescence and oxygen evolution from *Chlorella pyrenoidosa*. *Biochimica et Biophysica Acta*, **189**: 366-383.
- Breuer JJG, van de Braak NJ. 1989.** Reference year for Dutch greenhouses. *Acta Horticulturae*, **248**: 101-108.
- Britz SJ, Sager JC. 1990.** Photomorphogenesis and photoassimilation in soybean and sorghum grown under broad-spectrum or blue-deficient light-sources. *Plant Physiology*, **94**: 448-454.
- Brown CS, Schuerger AC, Sager JC. 1995.** Growth and photomorphogenesis of pepper plants under red light-emitting diodes with supplemental blue or far-red lighting. *Journal of the American Society for Horticultural Science*, **120**: 808-813.
- Bukhov NG, Drozdova IS, Bondar VV. 1995.** Light response curves of photosynthesis in leaves of sun-type and shade-type plants grown in blue or red light. *Journal of Photochemistry and Photobiology B-Biology*, **30**: 39-41.
- Bulley NR, Nelson CD, Tregunna EB. 1969.** Photosynthesis - Action spectra for leaves in normal and low oxygen. *Plant Physiology*, **44**: 678-684.
- Buschmann C, Meier D, Kleudgen HK, Lichtenthaler HK. 1978.** Regulation of chloroplast development by red and blue light. *Photochemistry and Photobiology*, **27**: 195-198.
- Caffarri S, Croce R, Breton J, Bassi R. 2001.** The major antenna complex of photosystem II has a xanthophyll binding site not involved in light harvesting. *Journal of Biological Chemistry*, **276**: 35924-35933.
- Caffarri S, Kouril R, Kereiche S, Boekema EJ, Croce R. 2009.** Functional architecture of higher plant photosystem II supercomplexes. *Embo Journal*, **28**: 3052-3063.
- Caird MA, Richards JH, Donovan LA. 2007.** Nighttime stomatal conductance and transpiration in C₃ and C₄ plants. *Plant Physiology*, **143**: 4-10.
- Challa H, van de Vooren J. 1980.** A strategy for climate control in greenhouses in early winter production. *Acta Horticulturae (ISHS)*, **106**: 159-164.
- Chen CP, Zhu XG, Long SP. 2008.** The effect of leaf-level spatial variability in photosynthetic capacity on biochemical parameter estimates using the Farquhar model: A theoretical analysis. *Plant Physiology*, **148**: 1139-1147.
- Child R, Morgan DC, Smith H. 1981.** Control of development in *Chenopodium album* L. by shadelight: the effect of light quality (red: far red ratio) on morphogenesis. *New Phytologist*, **89**: 545-555.
- Chow WS, Melis A, Anderson JM. 1990.** Adjustments of photosystem stoichiometry in chloroplasts improve the quantum efficiency of photosynthesis. *Proceedings of the National Academy of Sciences of the United States of America*, **87**: 7502-7506.
- Christopher DA, Mullet JE. 1994.** Separate photosensory pathways co-regulate blue-light/ultraviolet-A-activated *psbD-psbC* transcription and light-induced D2 and CP43 degradation in barley (*Hordeum vulgare*) chloroplasts. *Plant Physiology*, **104**: 1119-1129.

- Croce R, Canino G, Ros F, Bassi R. 2002.** Chromophore organization in the higher-plant photosystem II antenna protein CP26. *Biochemistry*, **41**: 7334-7343.
- Croce R, Muller MG, Bassi R, Holzwarth AR. 2001.** Carotenoid-to-chlorophyll energy transfer in recombinant major light-harvesting complex (LHCII) of higher plants. I. Femtosecond transient absorption measurements. *Biophysical Journal*, **80**: 901-915.
- Danielsson R, Albertsson PA, Mamedov F, Styring S. 2004.** Quantification, of photosystem I and II in different parts of the thylakoid membrane from spinach. *Biochimica Et Biophysica Acta-Bioenergetics*, **1608**: 53-61.
- de Weerd FL, Kennis JTM, Dekker JP, van Grondelle R. 2003.** Beta-carotene to chlorophyll singlet energy transfer in the photosystem I core of *Synechococcus elongatus* proceeds via the beta-carotene S-2 and S-1 states. *Journal of Physical Chemistry B*, **107**: 5995-6002.
- Deng XW, Melis A. 1986.** Phosphorylation of the light-harvesting complex II in higher-plant chloroplasts: effect on photosystem II and photosystem I absorption cross section. *Photobiology and Photobiophysics*, **13**: 41-52.
- Deutch B, Rasmussen O. 1974.** Growth chamber illumination and photomorphogenetic efficacy I. Physiological action of infrared radiation beyond 750 nm. *Physiologia Plantarum*, **30**: 64-71.
- Dietzel L, Brautigam K, Pfannschmidt T. 2008.** Photosynthetic acclimation: State transitions and adjustment of photosystem stoichiometry - functional relationships between short-term and long-term light quality acclimation in plants. *Febs Journal*, **275**: 1080-1088.
- Dubois JJB, Fiscus EL, Booker FL, Flowers MD, Reid CD. 2007.** Optimizing the statistical estimation of the parameters of the Farquhar-von Caemmerer-Berry model of photosynthesis. *New Phytologist*, **176**: 402-414.
- Edreva A. 2005.** The importance of non-photosynthetic pigments and cinnamic acid derivatives in photoprotection. *Agriculture Ecosystems & Environment*, **106**: 135-146.
- Eichelmann H, Laisk A. 2000.** Cooperation of photosystems II and I in leaves as analyzed by simultaneous measurements of chlorophyll fluorescence and transmittance at 800 nm. *Plant and Cell Physiology*, **41**: 138-147.
- Emerson R, Chalmers R, Cederstrand C. 1957.** Some factors influencing the long-wave limit of photosynthesis. *Proceedings of the National Academy of Sciences of the United States of America*, **43**: 133-143.
- Endler JA. 1993.** The color of light in forests and its implications. *Ecological Monographs*, **63**: 1-27.
- Evans JR. 1986.** A quantitative analysis of light distribution between the two photosystems, considering variation in both the relative amounts of the chlorophyll-protein complexes and the spectral quality of light. *Photobiology and Photobiophysics*, **10**: 135-147.
- Evans JR. 1987.** The dependence of quantum yield on wavelength and growth irradiance. *Australian Journal Of Plant Physiology*, **14**: 69-79.
- Evans JR. 2009.** Potential errors in electron transport rates calculated from chlorophyll fluorescence as revealed by a multilayer leaf model. *Plant and Cell Physiology*, **50**: 698-706.

- Evans JR, Anderson JM. 1987.** Absolute absorption and relative fluorescence excitation-spectra of the 5 major chlorophyll-protein complexes from spinach thylakoid membranes. *Biochimica et Biophysica Acta*, **892**: 75-82.
- Evans JR, Poorter H. 2001.** Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant Cell and Environment*, **24**: 755-767.
- Fan DY, Hope AB, Smith PJ, Jia H, Pace RJ, Anderson JM, Chow WS. 2007.** The stoichiometry of the two photosystems in higher plants revisited. *Biochimica Et Biophysica Acta-Bioenergetics*, **1767**: 1064-1072.
- Farquhar GD, Caemmerer SV, Berry JA. 1980.** A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta*, **149**: 78-90.
- Felippe GM, Dale JE, Marriott C. 1975.** Effects of irradiance on uptake and assimilation of nitrate by young barley seedlings. *Annals Of Botany*, **39**: 43-55.
- Fey V, Wagner R, Brautigam K, Wirtz M, Hell R, Dietzmann A, Leister D, Oelmuller R, Pfannschmidt T. 2005.** Retrograde plastid redox signals in the expression of nuclear genes for chloroplast proteins of *Arabidopsis thaliana*. *Journal of Biological Chemistry*, **280**: 5318-5328.
- Flexas J, Diaz-Espejo A, Berry JA, Cifre J, Galmes J, Kaidenhoff R, Medrano H, Ribas-Carbo M. 2007.** Analysis of leakage in IRGA's leaf chambers of open gas exchange systems: quantification and its effects in photosynthesis parameterization. *Journal of Experimental Botany*, **58**: 1533-1543.
- Folta KM, Maruhnich SA. 2007.** Green light: a signal to slow down or stop. *Journal of Experimental Botany*, **58**: 3099-3111.
- Franklin K, Whitelam G. 2007.** Red: far-red ratio perception and shade avoidance. In: Whitelam G, Halliday K eds. *Light and plant development*. Oxford, Blackwell publishing.
- Frechilla S, Talbott LD, Bogomolni RA, Zeiger E. 2000.** Reversal of blue light-stimulated stomatal opening by green light. *Plant And Cell Physiology*, **41**: 171-176.
- Fujita Y. 1997.** A study on the dynamic features of photosystem stoichiometry: Accomplishments and problems for future studies. *Photosynthesis Research*, **53**: 83-93.
- Fujiwara K, Sawada T. 2006.** Design and development of an LED-artificial sunlight source system prototype capable of controlling relative spectral power distribution. *Journal of Light & Visual Environment*, **30**: 170-176.
- Genty B, Briantais JM, Baker NR. 1989.** The relationship between the quantum yield of photosynthetic electron-transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta*, **990**: 87-92.
- Genty B, Harbinson J, Baker NR. 1990.** Relative quantum efficiencies of the two photosystems of leaves in photorespiratory and non-photorespiratory conditions. *Plant Physiology and Biochemistry*, **28**: 1-10.
- Goins GD, Yorio NC, Sanwo MM, Brown CS. 1997.** Photomorphogenesis, photosynthesis, and seed yield of wheat plants grown under red light-emitting diodes (LEDs) with and without supplemental blue lighting. *Journal of Experimental Botany*, **48**: 1407-1413.
- Goudriaan J, Van Laar HH. 1994.** *Modelling potential crop growth processes*, Dordrecht, Kluwer Academic Publishers

- Govindjee. 1999.** On the requirement of minimum number of four versus eight quanta of light for the evolution of one molecule of oxygen in photosynthesis: A historical note. *Photosynthesis Research*, **59**: 249-254.
- Grime JP. 1981.** Plant strategies in shade. In: Smith H ed. *Plants and the daylight spectrum*. London, Academic Press.
- Haldrup A, Jensen PE, Lunde C, Scheller HV. 2001.** Balance of power: a view of the mechanism of photosynthetic state transitions. *Trends in Plant Science*, **6**: 301-305.
- Harbinson J, Genty B, Baker NR. 1990.** The relationship between CO₂ assimilation and electron-transport in leaves. *Photosynthesis Research*, **25**: 213-224.
- Havaux M, Kloppstech K. 2001.** The protective functions of carotenoid and flavonoid pigments against excess visible radiation at chilling temperature investigated in *Arabidopsis npq* and *tt* mutants. *Planta*, **213**: 953-966.
- Haworth P, Melis A. 1983.** Phosphorylation of chloroplast thylakoid membrane-proteins does not increase the absorption cross-section of photosystem I. *Febs Letters*, **160**: 277-280.
- Heuvelink E. 1995.** Dry matter production in a tomato crop: measurements and simulation. *Annals of Botany*, **75**: 369-379.
- Heuvelink E. 2005.** *Tomatoes (Crop production science in horticulture)*, Oxford, CAB International.
- Heuvelink E, Bakker MJ, Hogendonk L, Janse J, Kaarsemaker R, Maaswinkel R. 2006.** Horticultural lighting in the Netherlands: New developments. *Acta Horticulturae (ISHS)*, **711**: 25-34.
- Heuvelink E, Marcelis LFM. 1996.** Influence of assimilate supply on leaf formation in sweet pepper and tomato. *Journal of Horticultural Science*, **71**: 405-414.
- Hikosaka K, Hirose T. 1998.** Leaf and canopy photosynthesis of C₃ plants at elevated CO₂ in relation to optimal partitioning of nitrogen among photosynthetic components: theoretical prediction. *Ecological Modelling*, **106**: 247-259.
- Hogewoning SW, Douwstra P, Trouwborst G, van Ieperen W, Harbinson J. 2010a.** An artificial solar spectrum substantially alters plant development compared with usual climate room irradiance spectra. *Journal of Experimental Botany*, **61**: 1267-1276.
- Hogewoning SW, Harbinson J. 2007.** Insights on the development, kinetics, and variation of photoinhibition using chlorophyll fluorescence imaging of a chilled, variegated leaf. *Journal of Experimental Botany*, **58**: 453-463.
- Hogewoning SW, Trouwborst G, Engbers GJ, Harbinson J, Van Ieperen W, Ruijsch J, Schapendonk AHCM, Pot SC, Van Kooten O. 2007.** Plant physiological acclimation to irradiation by light emitting diodes (LEDs). *Acta Horticulturae (ISHS)*, **761**: 183-191.
- Hogewoning SW, Trouwborst G, Harbinson J, van Ieperen W. 2010b.** Light distribution in leaf chambers and its consequences for photosynthesis measurements. *Photosynthetica*, **48**: 219-226.
- Hogewoning SW, Trouwborst G, Maljaars H, Poorter H, van Ieperen W, Harbinson J. 2010c.** Blue light dose-responses of leaf photosynthesis, morphology and chemical composition of *Cucumis sativus* grown under different combinations of red and blue light. *Journal of Experimental Botany*, **61**: 3107-3117.

- Holmes MG, Smith H. 1977.** The function of phytochrome in the natural environment- I. Characterization of daylight for studies in photomorphogenesis and photoperiodism. *Photochemistry and Photobiology*, **25**: 533-538.
- Hoover WH. 1937.** The dependence of carbon dioxide assimilation in a higher plant on wavelength of radiation. *Smithsonian Institution Misc. Collections*, **95**: 1-13.
- Horton P, Black MT. 1981.** Light-dependent quenching of chlorophyll fluorescence in pea-chloroplasts induced by adenosine 5'-triphosphate. *Biochimica et Biophysica Acta*, **635**: 53-62.
- Hovi-Pekkanen T, Tahvonen R. 2008.** Effects of interlighting on yield and external fruit quality in year-round cultivated cucumber. *Scientia Horticulturae*, **116**: 152-161.
- Hovi T, Nakkila J, Tahvonen R. 2004.** Interlighting improves production of year-round cucumber. *Scientia Horticulturae*, **102**: 283-294.
- Hutzler P, Fischbach R, Heller W, Jungblut TP, Reuber S, Schmitz R, Veit M, Weissenbock G, Schnitzler JP. 1998.** Tissue localization of phenolic compounds in plants by confocal laser scanning microscopy. *Journal of Experimental Botany*, **49**: 953-965.
- Inada K. 1976.** Action spectra for photosynthesis in higher plants. *Plant and Cell Physiology*, **17**: 355-365.
- Jackson JA, Jenkins GI. 1995.** Extension-growth responses and expression of flavonoid biosynthesis genes in the *Arabidopsis hy4* mutant. *Planta*, **197**: 233-239.
- Jarillo JA, Gabrys H, Capel J, Alonso JM, Ecker JR, Cashmore AR. 2001.** Phototropin-related NPL1 controls chloroplast relocation induced by blue light. *Nature*, **410**: 952-954.
- Jarvis PG. 1971.** Plant photosynthetic production: Manual of methods In: Sestak Z, Catsky J, Jarvis PG eds. The Hague, Junk.
- Kasahara M, Kagawa T, Oikawa K, Suetsugu N, Miyao M, Wada M. 2002.** Chloroplast avoidance movement reduces photodamage in plants. *Nature*, **420**: 829-832.
- Kasperbauer MJ, Hamilton JL. 1984.** Chloroplast structure and starch grain accumulation in leaves that received different red and far-red levels during development. *Plant Physiology*, **74**: 967-970.
- Kim HH, Goins GD, Wheeler RM, Sager JC. 2004.** Green-light supplementation for enhanced lettuce growth under red- and blue-light-emitting diodes. *Hortscience*, **39**: 1617-1622.
- Kim JH, Glick RE, Melis A. 1993.** Dynamics of photosystem stoichiometry adjustment by light quality in chloroplasts. *Plant Physiology*, **102**: 181-190.
- Kim SH, Lieth JH. 2003.** A coupled model of photosynthesis, stomatal conductance and transpiration for a rose leaf (*Rosa hybrida* L.). *Annals Of Botany*, **91**: 771-781.
- Kingston-Smith AH, Harbinson J, Foyer CH. 1999.** Acclimation of photosynthesis, H₂O₂ content and antioxidants in maize (*Zea mays*) grown at sub-optimal temperatures. *Plant Cell and Environment*, **22**: 1071-1083.
- Kingston-Smith AH, Harbinson J, Williams J, Foyer CH. 1997.** Effect of chilling on carbon assimilation, enzyme activation, and photosynthetic electron transport in the absence of photoinhibition in maize leaves. *Plant Physiology*, **114**: 1039-1046.
- Kiniry J, Rosenthal W, Jackson B, Hoogenboom G. 1991.** Predicting leaf development in crop plants. In: Hodges T ed. *Predicting crop phenology*. CRC Press, Boca Raton, USA.

- Kok B. 1948.** A critical consideration of the quantum yield of *Chlorella* photosynthesis. *Enzymologia*, **13**: 1-56.
- Krall JP, Edwards GE. 1992.** Relationship between photosystem II activity and CO₂ fixation in leaves. *Physiologia Plantarum*, **86**: 180-187.
- Kramer DM, Johnson G, Kiirats O, Edwards GE. 2004.** New fluorescence parameters for the determination of Q_A redox state and excitation energy fluxes. *Photosynthesis Research*, **79**: 209-218.
- Krizek D, Mirecki R, Bailey W. 1998.** Uniformity of photosynthetic photon flux and growth of 'poinsett' cucumber plants under metal halide and microwave-powered sulfur lamps. *Biotronics*, **27**: 81-92.
- Kubasek WL, Shirley BW, McKillop A, Goodman HM, Briggs W, Ausubel FM. 1992.** Regulation of flavonoid biosynthetic genes in germinating *Arabidopsis* seedlings. *Plant Cell*, **4**: 1229-1236.
- Laisk A, Oja V. 1998.** *Dynamics of leaf photosynthesis: Rapid response measurements and their interpretations.*, Collingwood, Csiro Publications.
- Lambers H, Chapin FS, Pons TL. 2008.** *Plant physiological ecology*, New York, Springer Verlag.
- Lawlor D. 2001.** *Photosynthesis: Molecular, physiological and environmental processes*, Oxford, UK Bios Scientific Publishers.
- Lichtenthaler H, Buschmann C, Rahmsdorf U. 1980.** The importance of blue light for the development of sun-type chloroplasts. In: Senger H ed. *The blue light syndrome*. Berlin, Springer-Verlag.
- Long SP, Postl WF, Bolharnordenkampf HR. 1993.** Quantum yields for uptake of carbon dioxide in C₃ vascular plants of contrasting habitats and taxonomic groupings. *Planta*, **189**: 226-234.
- Long SP, Zhu XG, Naidu SL, Ort DR. 2006.** Can improvement in photosynthesis increase crop yields? *Plant Cell and Environment*, **29**: 315-330.
- Loreto F, Tsonev T, Centritto M. 2009.** The impact of blue light on leaf mesophyll conductance. *Journal of Experimental Botany*, **60**: 2283-2290.
- Lunde C, Jensen PE, Haldrup A, Knoetzel J, Scheller HV. 2000.** The PSI-H subunit of photosystem I is essential for state transitions in plant photosynthesis. *Nature*, **408**: 613-615.
- Marcelis LFM. 1993.** Leaf formation in cucumber (*Cucumis sativus* L.) as influenced by fruit load, light and temperature. *Gartenbauwissenschaft*, **58**: 124-129.
- Massa GD, Kim HH, Wheeler RM, Mitchell CA. 2008.** Plant productivity in response to LED lighting. *Hortscience*, **43**: 1951-1956.
- Matsuda R, Ohashi-Kaneko K, Fujiwara K, Goto E, Kurata K. 2004.** Photosynthetic characteristics of rice leaves grown under red light with or without supplemental blue light. *Plant And Cell Physiology*, **45**: 1870-1874.
- Matsuda R, Ohashi-Kaneko K, Fujiwara K, Kurata K. 2007.** Analysis of the relationship between blue-light photon flux density and the photosynthetic properties of spinach (*Spinacia oleracea* L.) leaves with regard to the acclimation of photosynthesis to growth irradiance. *Soil Science and Plant Nutrition*, **53**: 459-465.
- Matsuda R, Ohashi-Kaneko K, Fujiwara K, Kurata K. 2008.** Effects of blue light deficiency on acclimation of light energy partitioning in PSII and CO₂ assimilation capacity to high irradiance in spinach leaves. *Plant And Cell Physiology*, **49**: 664-670.

- McCree KJ. 1972a.** Action spectrum, absorptance and quantum yield of photosynthesis in crop plants. *Agricultural Meteorology*, **9**: 191-216.
- McCree KJ. 1972b.** Significance of enhancement for calculations based on action spectrum for photosynthesis. *Plant Physiology*, **49**: 704-706.
- Medlyn BE, Badeck FW, De Pury DGG, Barton CVM, Broadmeadow M, Ceulemans R, De Angelis P, Forstreuter M, Jach ME, Kellomaki S, Laitat E, Marek M, Philippot S, Rey A, Strassemeier J, Laitinen K, Liozon R, Portier B, Roberntz P, Wang K, Jarvis PG. 1999.** Effects of elevated [CO₂] on photosynthesis in European forest species: a meta-analysis of model parameters. *Plant Cell and Environment*, **22**: 1475-1495.
- Melis A. 1991.** Dynamics of photosynthetic membrane composition and function. *Biochimica et Biophysica Acta*, **1058**: 87-106.
- Melis A, Murakami A, Nemson JA, Aizawa K, Ohki K, Fujita Y. 1996.** Chromatic regulation in *Chlamydomonas reinhardtii* alters photosystem stoichiometry and improves the quantum efficiency of photosynthesis. *Photosynthesis Research*, **47**: 253-265.
- Mochizuki T, Onda Y, Fujiwara E, Wada M, Toyoshima Y. 2004.** Two independent light signals cooperate in the activation of the plastid *psbD* blue light-responsive promoter in *Arabidopsis*. *Febs Letters*, **571**: 26-30.
- Morgan DC, Smith H. 1981.** Control of development in *Chenopodium album* L. by shadelight: the effect of light quantity (total fluence rate) and light quality (red: far-red ratio). *New Phytologist*, **88**: 239-248.
- Morison JIL, Gallouet E, Lawson T, Cornic G, Herbin R, Baker NR. 2005.** Lateral diffusion of CO₂ in leaves is not sufficient to support photosynthesis. *Plant Physiology*, **139**: 254-266.
- Nejad AR, Harbinson J, van Meeteren U. 2006.** Dynamics of spatial heterogeneity of stomatal closure in *Tradescantia virginiana* altered by growth at high relative air humidity. *Journal of Experimental Botany*, **57**: 3669-3678.
- Nejad AR, van Meeteren U. 2005.** Stomatal response characteristics of *Tradescantia virginiana* grown at high relative air humidity. *Physiologia Plantarum*, **125**: 324-332.
- Niinemets U, Cescatti A, Rodeghiero M, Tosens T. 2005.** Leaf internal diffusion conductance limits photosynthesis more strongly in older leaves of Mediterranean evergreen broad-leaved species. *Plant Cell and Environment*, **28**: 1552-1566.
- Nishio JN. 2000.** Why are higher plants green? Evolution of the higher plant photosynthetic pigment complement. *Plant Cell and Environment*, **23**: 539-548.
- Oguchi R, Hikosaka K, Hirose T. 2003.** Does the photosynthetic light-acclimation need change in leaf anatomy? *Plant Cell and Environment*, **26**: 505-512.
- Ohashi-Kaneko K, Matsuda R, Goto E, Fujiwara K, Kurata K. 2006.** Growth of rice plants under red light with or without supplemental blue light. *Soil Science and Plant Nutrition*, **52**: 444-452.
- Oya V, Laisk A. 1976.** Adaptation of the photosynthesis apparatus to the light profile in the leaf. *Soviet Plant Physiol*, **23**: 381-386.
- Papadopoulos AP, Hao XM. 1997.** Effects of three greenhouse cover materials on tomato growth, productivity, and energy use. *Scientia Horticulturae*, **70**: 165-178.
- Paul MJ, Foyer CH. 2001.** Sink regulation of photosynthesis. *Journal of Experimental Botany*, **52**: 1383-1400.

- Pfannschmidt T. 2005.** Acclimation to varying light qualities: Toward the functional relationship of state transitions and adjustment of photosystem stoichiometry. *Journal of Phycology*, **41**: 723-725.
- Pfannschmidt T, Allen JF, Oelmüller R. 2001.** Principles of redox control in photosynthesis gene expression. *Physiologia Plantarum*, **112**: 1-9.
- Pilkington LJ, Messelink G, van Lenteren JC, Le Mottee K. 2010.** "Protected Biological Control" - Biological pest management in the greenhouse industry. *Biological Control*, **52**: 216-220.
- Planck M. 1901.** Über das gesetz der energieverteilung im normalspektrum. *Annalen der Physik*, **4**: 553-563.
- Pons TL, Pearcy RW. 1994.** Nitrogen reallocation and photosynthetic acclimation in response to partial shading in soybean plants. *Physiologia Plantarum*, **92**: 636-644.
- Pons TL, Welschen RAM. 2002.** Overestimation of respiration rates in commercially available clamp-on leaf chambers. Complications with measurement of net photosynthesis. *Plant Cell and Environment*, **25**: 1367-1372.
- Poorter H, Nagel O. 2000.** The role of biomass allocation in the growth response of plants to different levels of light, CO₂, nutrients and water: a quantitative review. *Australian Journal of Plant Physiology*, **27**: 595-607.
- Poorter H, Niinemets U, Poorter L, Wright IJ, Villar R. 2009.** Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist*, **182**: 565-588.
- Poorter H, Niinemets U, Walter A, Fiorani F, Schurr U. 2010.** A method to construct dose-response curves for a wide range of environmental factors and plant traits by means of a meta-analysis of phenotypic data. *Journal of Experimental Botany*, **61**: 2043-2055.
- Poorter H, Remkes C, Lambers H. 1990.** Carbon and nitrogen economy of 24 wild species differing in relative growth rate. *Plant Physiology*, **94**: 621-627.
- Pospíšil P. 2009.** Production of reactive oxygen species by photosystem II. *Biochimica Et Biophysica Acta-Bioenergetics*, **1787**: 1151-1160.
- Quigg A, Kevekordes K, Raven JA, Beardall J. 2006.** Limitations on microalgal growth at very low photon fluence rates: the role of energy slippage. *Photosynthesis Research*, **88**: 299-310.
- Rodeghiero M, Niinemets U, Cescatti A. 2007.** Major diffusion leaks of clamp-on leaf cuvettes still unaccounted: how erroneous are the estimates of Farquhar et al. model parameters? *Plant Cell and Environment*, **30**: 1006-1022.
- Saebo A, Krekling T, Appelgren M. 1995.** Light quality affects photosynthesis and leaf anatomy of birch plantlets *in vitro*. *Plant Cell Tissue and Organ Culture*, **41**: 177-185.
- Sager JC, Smith WO, Edwards JL, Cyr KL. 1988.** Photosynthetic efficiency and phytochrome photoequilibria determination using spectral data. *Transactions of the Asae*, **31**: 1882-1889.
- Samson G, Bruce D. 1995.** Complementary changes in absorption cross-sections of photosystems I and II due to phosphorylation and Mg²⁺-depletion in spinach thylakoids. *Biochimica Et Biophysica Acta-Bioenergetics*, **1232**: 21-26.
- Schmid R, Fromme R, Renger G. 1990a.** The photosynthetic apparatus of *Acetabularia mediterranea* grown under red or blue light. Biophysical quantification and

References

- characterization of photosystem II and its core components. *Photochemistry and Photobiology*, **52**: 103-109.
- Schmid R, Wennicke R, Fleischhauer S. 1990b.** Quantitative correlation of peripheral and intrinsic core polypeptides of photosystem II with photosynthetic electron-transport activity of *Acetabularia mediterranea* in red and blue light. *Planta*, **182**: 391-398.
- Schuenger AC, Brown CS, Stryjewski EC. 1997.** Anatomical features of pepper plants (*Capsicum annuum* L.) grown under red light-emitting diodes supplemented with blue or far-red light. *Annals of Botany*, **79**: 273-282.
- Sharkey TD, Raschke K. 1981.** Effect of light quality on stomatal opening in leaves of *Xanthium strumarium* L. *Plant Physiology*, **68**: 1170-1174.
- Sharp RE, Matthews MA, Boyer JS. 1984.** Kok effect and the quantum yield of photosynthesis - Light partially inhibits dark respiration. *Plant Physiology*, **75**: 95-101.
- Sims DA, Pearcy RW. 1991.** Photosynthesis and respiration in *Alocasia macrorrhiza* following transfers to high and low light. *Oecologia*, **86**: 447-453.
- Singsaas EL, Ort DR, DeLucia EH. 2001.** Variation in measured values of photosynthetic quantum yield in ecophysiological studies. *Oecologia*, **128**: 15-23.
- Smith H. 2000.** Phytochromes and light signal perception by plants - an emerging synthesis. *Nature*, **407**: 585-591.
- Smith S, Weyers JDB, Berry WG. 1989.** Variation in stomatal characteristics over the lower surface of *Commelina communis* leaves. *Plant Cell and Environment*, **12**: 653-659.
- Snyder KA, Richards JH, Donovan LA. 2003.** Night-time conductance in C₃ and C₄ species: do plants lose water at night? *Journal of Experimental Botany*, **54**: 861-865.
- Soares AS, Driscoll SP, Olmos E, Harbinson J, Arrabaca MC, Foyer CH. 2008.** Adaxial/abaxial specification in the regulation of photosynthesis and stomatal opening with respect to light orientation and growth with CO₂ enrichment in the C₄ species *Paspalum dilatatum*. *New Phytologist*, **177**: 186-198.
- Sonneveld PJ, Swinkels GLAM, Bot GPA. 2009.** Design of a solar greenhouse with energy delivery by the conversion of near infrared radiation - Part 1 Optics and PV-cells. *Acta Horticulturae (ISHS)*, **807**: 47-54.
- Sood S, Tyagi AK, Tripathy BC. 2004.** Inhibition of Photosystem I and Photosystem II in wheat seedlings with their root-shoot transition zones exposed to red light. *Photosynthesis Research*, **81**: 31-40.
- Stitt M. 1991.** Rising CO₂ levels and their potential significance for carbon flow in photosynthetic cells. *Plant Cell and Environment*, **14**: 741-762.
- Talbott LD, Nikolova G, Ortiz A, Shmayevich I, Zeiger E. 2002.** Green light reversal of blue-light-stimulated stomatal opening is found in a diversity of plant species. *American Journal of Botany*, **89**: 366-368.
- Telfer A, Bottin H, Barber J, Mathis P. 1984.** The effect of magnesium and phosphorylation of light-harvesting chlorophyll a/b-protein on the yield of P-700-photooxidation in pea chloroplasts. *Biochimica et Biophysica Acta*, **764**: 324-330.
- Tennessen DJ, Singaas EL, Sharkey TD. 1994.** Light-emitting diodes as a light-source for photosynthesis research. *Photosynthesis Research*, **39**: 85-92.

- Terashima I.** 1986. Dorsoventrality in photosynthetic light response curves of a leaf. *Journal of Experimental Botany*, **37**: 399-405.
- Terashima I, Fujita T, Inoue T, Chow WS, Oguchi R.** 2009. Green light drives leaf photosynthesis more efficiently than red light in strong white light: Revisiting the enigmatic question of why leaves are green. *Plant and Cell Physiology*, **50**: 684-697.
- Thornley JHM.** 1976. *Mathematical models in plant physiology : a quantitative approach to problems in plant and crop physiology*, London, Academic press.
- Treutter D.** 2006. Significance of flavonoids in plant resistance: a review. *Environmental Chemistry Letters*, **4**: 147-157.
- Trouwborst G, Oosterkamp J, Hogewoning SW, Harbinson J, van Ieperen W.** 2010. The responses of light interception, photosynthesis and fruit yield of cucumber to LED-lighting within the canopy. *Physiologia Plantarum*, **138**: 289-300.
- Turnbull MH, Doley D, Yates DJ.** 1993. The dynamics of photosynthetic acclimation to changes in light quantity and quality in three Australian rain forest tree species. *Oecologia*, **94**: 218-228.
- van Amerongen H, van Grondelle R.** 2001. Understanding the energy transfer function of LHCII, the major light-harvesting complex of green plants. *Journal of Physical Chemistry B*, **105**: 604-617.
- van Ieperen W, Trouwborst G.** 2008. The application of leds as assimilation light source in greenhouse horticulture: A simulation study. *Acta Horticulturae (ISHS)*, **801**: 1407-1414.
- van Kooten O, Snel JFH.** 1990. The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynthesis Research*, **25**: 147-150.
- van Oort B, Amunts A, Borst JW, van Hoek A, Nelson N, van Amerongen H, Croce R.** 2008. Picosecond fluorescence of intact and dissolved PSI-LHCI crystals. *Biophysical Journal*, **95**: 5851-5861.
- von Caemmerer S, Farquhar GD.** 1981. Some relationships between the biochemistry of photosynthesis and the gas-exchange of leaves. *Planta*, **153**: 376-387.
- Voskresenskaya NP, Drozdova IS, Krendeleva TE.** 1977. Effect of light quality on organization of photosynthetic electron transport chain of pea-seedlings. *Plant Physiology*, **59**: 151-154.
- Wagner R, Dietzel L, Brautigam K, Fischer W, Pfannschmidt T.** 2008. The long-term response to fluctuating light quality is an important and distinct light acclimation mechanism that supports survival of *Arabidopsis thaliana* under low light conditions. *Planta*, **228**: 573-587.
- Walters RG, Horton P.** 1995. Acclimation of *Arabidopsis thaliana* to the light environment: changes in photosynthetic function. *Planta*, **197**: 306-312.
- Walters RG, Rogers JJM, Shephard F, Horton P.** 1999. Acclimation of *Arabidopsis thaliana* to the light environment: the role of photoreceptors. *Planta*, **209**: 517-527.
- Wassink EC, Stolwijk JAJ.** 1956. Effects of light quality on plant growth. *Annual Review of Plant Physiology and Plant Molecular Biology*, **7**: 373-400.
- Wellburn AR.** 1994. The spectral determination of chlorophyll a and chlorophyll b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *Journal of Plant Physiology*, **144**: 307-313.

- Wennicke H, Schmid R. 1987.** Control of the photosynthetic apparatus of *Acetabularia mediterranea* by blue light. Analysis by light-saturation curves. *Plant Physiology*, **84**: 1252-1256.
- Whitelam G, Halliday K. 2007.** *Light and plant development*, Oxford, Blackwell Publishing.
- Willmer C, Fricker M. 1996.** *Stomata*, London, UK, Chapman and Hall.
- Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Navas ML, Niinemets U, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R. 2004.** The worldwide leaf economics spectrum. *Nature*, **428**: 821-827.
- Yakushevskaya AE, Jensen PE, Keegstra W, van Roon H, Scheller HV, Boekema EJ, Dekker JP. 2001.** Supermolecular organization of photosystem II and its associated light-harvesting antenna in *Arabidopsis thaliana*. *European Journal of Biochemistry*, **268**: 6020-6028.
- Yin XY, Struik PC, Romero P, Harbinson J, Evers JB, Van Der Putten PEL, Vos J. 2009.** Using combined measurements of gas exchange and chlorophyll fluorescence to estimate parameters of a biochemical C₃ photosynthesis model: a critical appraisal and a new integrated approach applied to leaves in a wheat (*Triticum aestivum*) canopy. *Plant Cell and Environment*, **32**: 448-464.
- Yorio NC, Goins GD, Kagie HR, Wheeler RM, Sager JC. 2001.** Improving spinach, radish, and lettuce growth under red light-emitting diodes (LEDs) with blue light supplementation. *Hortscience*, **36**: 380-383.
- Young AJ. 1991.** The photoprotective role of carotenoids in higher-plants. *Physiologia Plantarum*, **83**: 702-708.
- Zeiger E. 1990.** Light perception in guard cells. *Plant Cell and Environment*, **13**: 739-744.
- Zheng S-J, Snoeren TAL, Hogewoning SW, van Loon JJA, Dicke M. 2010.** Disruption of plant carotenoid biosynthesis through virus-induced gene silencing affects oviposition behaviour of the butterfly *Pieris rapae*. *New Phytologist*, **186**: 733-745.
- Zhu XG, Long SP, Ort DR. 2010.** Improving photosynthetic efficiency for greater yield. *Annual Review of Plant Biology*, **61**: 235-261.

SUMMARY

A wide range of plant properties respond to the spectral composition of irradiance, such as photosynthesis, photomorphogenesis, phototropism and photonastic movements. These responses have an effect on plant productivity, mainly via changes in the photosynthetic rate per unit leaf area, light interception, and irradiance distribution through the canopy. The spectral environment of plants under natural conditions is dependent on location (e.g. latitude), changes over time (e.g. Sun-angle and cloud cover) and shading of leaves by neighboring vegetation or self-shading by younger leaves. In protected cultivation growth lamps supply unnatural irradiance-spectra, combined with (greenhouses) or without (growth-chambers) natural irradiance. Therefore, not only the acclimation of developing leaves to the irradiance-spectrum of their growth environment is important for plant productivity and survival, but also the capability of mature leaves to respond to changes in spectrum.

A range of different leaf properties that respond to the irradiance-spectrum during leaf development can affect the photosynthetic rate per unit leaf area, such as leaf anatomy, the content and composition of proteins, pigment-protein complexes, and other essential biochemicals. The light-limited quantum yield on an incident light basis, i.e. the maximum incident light use efficiency for photosynthesis, is linearly related to the fraction of light absorbed by the leaf, and, on an absorbed light basis, predominately influenced by the pigment composition. While the transfer of excitation energy between chlorophylls is highly efficient, the efficiency of transfer from carotenoids to chlorophylls is only 70-90% efficient. In addition, the presence of non-photosynthetic pigments within the leaf will result in a loss of photosynthetic light use efficiency. The relative absorption by these different types of pigments, and thus the photosynthetic quantum yield, is wavelength dependent. Quantum yield losses related to absorption by carotenoids or non-photosynthetic pigments become significant for wavelengths smaller than approximately 580 nm. The photosynthetic rate under light-saturating conditions acclimates to the spectral environment via responses of leaf anatomy, the content of leaf substances, many of which require nitrogen (e.g. Rubisco), and stomatal conductance.

When a mature leaf is exposed to changes in the irradiance-spectrum, the light-limited quantum can change rapidly due to changes in the relative absorption by the different pigments and changes in the distribution of excitation energy between the two photosystems. Subsequently, the leaf can acclimate to the new spectrum by altering its pigment composition, and by restoring the photosystem excitation balance via state

transitions (in the order of minutes) and photosystem stoichiometry changes (in the order of hours to days). The relative importance of the photosynthetic rate per unit leaf area for plant productivity, compared with that of other plant properties affecting light-interception and light-distribution, is dependent on the density and type of canopy.

This thesis focuses on the acclimation of photosynthesis per unit leaf area to the growth-light spectrum, the consequences of spectral acclimation for the wavelength dependence of photosynthetic quantum yield, and photomorphogenetic versus leaf photosynthetic acclimation in relation to biomass production. *Cucumis sativus* is used as a model plant. Additionally, the consequences of the photosynthesis measuring-light spectrum for the reliability of estimates of photosynthetic rates *in situ* and the use of such measurements as input in plant productivity models are explored.

Chapter 2 focuses on the effect blue light has on intrinsic photosynthetic functioning and high-light acclimation responses. Blue light dose-response curves were made for the photosynthetic properties and related developmental characteristics of cucumber leaves that were grown at an equal irradiance under seven different combinations of red and blue light provided by light emitting diodes. Only the leaves developed under red light alone (0% blue) displayed a dysfunctional photosynthetic operation, characterized by a sub-optimal and heterogeneously distributed dark-adapted F_v/F_m , a stomatal conductance unresponsive to irradiance and a relatively low light-limited quantum yield for CO₂ fixation. Only 7% blue light was sufficient to prevent any overt dysfunctional photosynthesis, which can be considered a qualitatively blue light effect. The photosynthetic capacity (A_{max}) was two times higher for leaves grown at 7% blue compared with 0% blue and continued to increase with increasing blue percentages during growth measured up to 50% blue. At 100% blue A_{max} was lower but photosynthetic functioning was normal. The increase in A_{max} with blue percentage (0-50%) was associated with an increase in leaf mass per unit leaf area (LMA), N content per unit leaf area, chlorophyll (Chl) content per unit leaf area and stomatal conductance. Above 15% blue the parameters A_{max} , LMA, Chl content, photosynthetic N use efficiency and the Chl:N ratio had a comparable relationship as reported for leaf responses to irradiance intensity. It is concluded that blue light during growth is qualitatively required for normal photosynthetic functioning and quantitatively mediates leaf responses resembling those to irradiance intensity.

Chapter 3 shows the effect of acclimation to different growth-light spectra on the wavelength dependence of light-limited quantum yield for CO₂ fixation and its underlying mechanisms. First, a method for the quantification of the light distribution within leaf chambers using a simple digital camera is described, and an analysis of the consequences of a heterogeneous light distribution for the validity of photosynthesis measurements is made (chapter 3.1). The impact of the light distribution measured within a commercial clamp-on leaf chamber and a lab-built chamber on the photosynthesis-irradiance response curve was calculated for two realistic scenarios. When the average light intensity over the leaf chamber area was estimated accurately, heterogeneity had minor effects on the photosynthesis-irradiance response curve. However, when the irradiance was measured in the chamber centre, which is common practice, and assumed to be homogeneous, in both leaf chambers the photosynthesis-irradiance response curve was subject to considerable error and led to serious underestimation of the light-limited quantum yield of photosynthesis.

This rather technical sub-chapter (3.1) supports the materials and methods section of chapter 3.2, which focuses on the photosystem excitation balance in relation to the wavelength dependency of the quantum yield for CO₂ fixation of plants grown in different spectral environments (artificial SUN, SHADE and BLUE). The quantum yield for PSI and PSII electron transport and CO₂ fixation measured on leaves (*in vivo*) were related to the content, composition and spectroscopic properties of the photosystem supercomplexes from these leaves (*in vitro*). Leaves grown in SHADE, which tends to overexcite PSI, had a higher quantum yield for CO₂ fixation at wavelengths overexciting PSI (≥ 690 nm) and a lower PSI:PSII ratio compared with the SUN and BLUE grown leaves. At wavelengths overexciting PSII, the quantum yield for CO₂ fixation of the SUN and BLUE grown leaves was higher. In the spectral region where absorption by pigments other than chlorophyll is insignificant (≥ 580 nm), the quantum yield for CO₂ fixation could be estimated from the photosystem excitation balance, whereas at lower wavelengths it was overestimated. The wavelength dependence of the photosystem excitation balance calculated via *in vitro* and *in vivo* approaches were substantially in agreement with each other. Where they were not, carotenoid absorption and state transitions are likely to play a role. In this chapter we show for the first time how the wavelength dependence of the quantum yield for CO₂ fixation relates quantitatively to photosystem composition and excitation balance, and how these properties acclimate to the plant's growth-light spectrum.

Chapter 4 deals with the effect of spectral discrepancies between growth-light and measuring-light on the reliability of estimates of photosynthesis *in situ* by gas-exchange and chlorophyll fluorescence measurements. Additionally, the consequences of errors in the estimate of photosynthetic rates *in situ* for the outcome of plant growth models were explored. *Cucumis sativus* was grown under different combinations of red and blue LEDs and under white fluorescent tubes, which are, respectively, modern and more traditional irradiance sources for growth chambers. CO₂ fixation was measured with low and saturating irradiance provided by different combinations of red and blue LEDs, which are widely used as photosynthesis measuring-light source. Electron transport rates (ETR) were calculated using measurements of chlorophyll fluorescence and leaf light absorption. The effect of measuring-light spectrum on light-limited quantum yield for CO₂ fixation (α) was up to 25% and was independent of the growth-light spectrum. In contrast, at saturating irradiance assimilation was affected significantly by the growth-light spectrum, however, only weakly by the measuring-light spectrum. Therefore measuring-light spectrum mainly has consequences for the validity of α . In contrast to CO₂ fixation, the measured photosystem II electron transport efficiency was similar for the different measuring-light spectra used. Therefore the calculated ETR-CO₂ fixation relationship changed considerably with changes in measuring-light spectrum, which has consequences for the practical use of ETR calculations for estimating photosynthesis. It is concluded that a photosynthesis measuring-light spectrum deviating from the plant growth-light spectrum can lead to significant errors in estimates of CO₂ fixation rates *in situ*. The use of such erroneous estimates as input for crop models can have disproportionately large consequences for predictions of plant growth.

Chapter 5 shows the importance of photomorphogenetic responses to the growth-light spectrum for plant productivity. Lamps widely used to provide growth-irradiance produce light with a spectral composition which is very different from natural daylight spectra. Whereas specific responses of plants to a spectrum differing from natural daylight

may sometimes be predictable, the overall plant response is generally difficult to predict due to the complicated interaction of the many different responses. So far studies on plant responses to different irradiance-spectra either use no daylight control, or if a natural daylight control is used, it will fluctuate in intensity and spectrum. We have engineered an artificial solar (AS) spectrum which closely resembles a sunlight spectrum, and compared growth, morphogenesis and photosynthetic characteristics of cucumber plants grown for 13 days under this spectrum with their performance under fluorescent tubes (FTs) and a high pressure sodium lamp (HPS). The total dry weight of the AS-grown plants was 2.3 and 1.6 times greater than that of the FT and HPS plants, respectively, and the height of the AS plants was four to five times greater. This striking difference appeared to be related to a more efficient light interception by the AS plants, characterized by longer petioles, a greater leaf unfolding rate and a lower investment in leaf mass relative to leaf area. Photosynthesis per leaf area was not greater for the AS plants. The extreme differences in plant response to the AS spectrum, compared with the widely used protected cultivation light sources tested, highlights the importance of a more natural spectrum, such as the AS spectrum, if the aim is to produce plants representative of field conditions.

In **chapter 6** the consequences of the different plant responses to their spectral environment are discussed in a broader (eco)-physiological perspective. Additionally, some consequences of this study for measuring photosynthesis are discussed. The chapter is concluded with a discussion on the practical implications of the new insights provided by this study for plant cultivation in climate chambers and greenhouses.

SAMENVATTING

Een verscheidenheid aan planteneigenschappen reageert op de spectrale samenstelling van licht, zoals fotosynthese, fotomorfogenese, fototropisme en nastische bewegingen. Deze reacties beïnvloeden de productiviteit van planten voornamelijk door veranderingen in de fotosynthesesnelheid per eenheid bladoppervlakte, lichtonderschepping, en lichtverdeling binnen het gewas. De spectrale samenstelling van de natuurlijke omgeving van planten hangt af van plaats (bv. breedtegraad), veranderingen in de tijd (bv. hoogte van de zon en bewolgingsgraad) en beschaduwing door bladeren van omringende vegetatie of zelfbeschaduwing door jongere bladeren. In beschermde teelten stralen groeilampen onnatuurlijke lichtspectra uit, in combinatie met (kassen) of zonder (klimaatkamers) natuurlijk licht. Daarom is niet alleen de aanpassing van ontwikkelende bladeren aan het lichtspectrum van hun omgeving van belang voor de plant productiviteit en overlevingskansen, maar ook de capaciteit van volwassen bladeren om te reageren op veranderingen in het spectrum.

De fotosynthesesnelheid per eenheid bladoppervlakte wordt beïnvloed door een verscheidenheid aan bladeigenschappen die reageren op het lichtspectrum, zoals bladanatomie, de inhoud en samenstelling van proteïnen, pigment-proteïne complexen en andere essentiële inhoudsstoffen. De strikt lichtgelimiteerde kwantumefficiëntie op basis van invallend licht, d.w.z. de maximum benuttingsefficiëntie van invallend licht voor fotosynthese, is lineair gerelateerd aan de fractie door het blad geabsorbeerd licht, en wordt op basis van geabsorbeerd licht voornamelijk beïnvloed door de pigmentsamenstelling van het blad. De overdracht van excitatie-energie tussen chlorofylmoleculen is zeer efficiënt (bijna 100%), echter, de overdrachtefficiëntie van carotenoïden naar chlorofyl is slechts 70-90%. Daarnaast resulteert de aanwezigheid van niet-fotosynthetische pigmenten in bladeren in een verlies aan benuttingsefficiëntie van licht voor de fotosynthese. De relatieve absorptie door de verschillende pigmenttypen hangt af van de golflengte van licht en zo ook de kwantumefficiëntie voor fotosynthese. Verliezen van kwantumefficiëntie gerelateerd aan absorptie door carotenoïden en niet-fotosynthetische pigmenten gaan tellen voor golflengten kleiner dan ongeveer 580 nm. De fotosynthesesnelheid bij lichtverzadiging past zich aan het lichtspectrum van de omgeving aan door veranderingen van bladanatomie, hoeveelheid inhoudsstoffen, voor welke veelal stikstof nodig is (bv. Rubisco), en stomataire geleidbaarheid.

Wanneer een volwassen blad blootgesteld wordt aan veranderingen in het lichtspectrum kan de lichtgelimiteerde kwantumefficiëntie snel veranderen vanwege een

verandering in de relatieve absorptie door de verschillende pigmenten en verandering in de excitatie-energie verdeling tussen de twee fotosystemen. Daaropvolgend kan het blad zich aan het nieuwe spectrum aanpassen door zijn pigmentsamenstelling bij te stellen, door de fotosysteem excitatiebalans (deels) te herstellen via 'state transitions' (verschuiving van antenne pigment van het ene naar het andere fotosysteem; tijdschaal van minuten) en door de stoichiometrie van de fotosystemen (verhouding fotosysteem I :fotosysteem II) te veranderen (tijdschaal van uren tot dagen). Het relatieve belang van de fotosynthesesnelheid per eenheid bladoppervlakte voor de productiviteit van planten vergeleken met het belang van andere planteigenschappen die lichtonderschepping en lichtverdeling binnen het gewas beïnvloeden, hangt af van de dichtheid en het soort gewas.

Deze thesis richt zich op de aanpassing van fotosynthesesnelheid per eenheid bladoppervlakte aan het groeilichtspectrum, op de consequenties van aanpassing aan het groeilichtspectrum voor de golflengteafhankelijkheid van de kwantumefficiëntie voor de fotosynthese, en op aanpassing van plantmorfologie t.o.v. bladfotosynthese in relatie tot biomassa productie. *Cucumis sativus* is hierbij gebruikt als modelplant. Daarnaast zijn de gevolgen van het fotosynthese-meetlichtspectrum voor de betrouwbaarheid van schattingen van de fotosynthesesnelheid *in situ*, en het gebruik van zulke metingen als input in groei modellen, onderzocht.

Hoofdstuk 2 richt zich op het effect dat blauw licht heeft op het intrinsiek fotosynthetisch functioneren en op hoog-licht aanpassingsreacties. Blauw licht dosis-respons curven werden gemaakt voor de fotosynthetische eigenschappen en daaraan gerelateerde ontwikkelingskenmerken van *Cucumis sativus* bladeren die onder een gelijke lichtintensiteit opgegroeiden bij zeven verschillende combinaties rood en blauw LED-licht. Alleen de bladeren die onder puur rood licht (0% blauw) ontwikkeld waren vertoonden een disfunctioneel fotosyntheseprocess, gekenmerkt door een suboptimale en heterogeen over het blad verdeelde donker geadapteerde F_v/F_m , een stomataire geleidbaarheid die niet reageerde op lichtintensiteit en spectrum, en een relatief lage lichtgelimiteerde kwantumefficiëntie voor CO₂ fixatie. Slechts 7% blauw licht was voldoende om duidelijke symptomen van een disfunctionele fotosynthese te voorkomen, hetgeen beschouwd kan worden als een kwalitatief effect van blauw licht. De fotosynthesecapaciteit (A_{max}) was twee maal zo hoog voor de bladeren opgegroeid onder 7% blauw, t.o.v. 0% blauw, en nam toe met een toenemend percentage blauw licht tot aan 50%. Bij 100% blauw licht was A_{max} lager, maar het fotosyntheseprocess functioneerde normaal. De toename van A_{max} met het blauw licht percentage (0-50%) ging gepaard met een toename in bladmassa per eenheid bladoppervlakte (LMA), N gehalte per eenheid bladoppervlakte, chlorofyl (Chl) gehalte per eenheid bladoppervlakte en stomataire geleidbaarheid. Boven 15% blauw licht vertoonden de parameters A_{max} , LMA, Chl gehalte, benuttingefficiëntie van N voor fotosynthese, en de Chl:N ratio een vergelijkbare relatie met elkaar als gerapporteerd voor reacties van bladeren op toenemende lichtintensiteit. Er kan geconcludeerd worden dat blauw licht gedurende de groei kwalitatief vereist is voor een normaal functioneren van het fotosyntheseprocess en dat het kwantitatief een rol speelt met betrekking tot reacties van bladeren die vergelijkbaar zijn met reacties op lichtintensiteit.

Hoofdstuk 3 gaat in op het effect van aanpassing aan verschillende groeilichtspectra op de golflengteafhankelijkheid van de lichtgelimiteerde kwantumopbrengst t.b.v. CO₂ fixatie en de mechanismen die daaraan ten grondslag

liggen. Eerst is een methode voor het kwantificeren van de lichtverdeling in een bladkamer m.b.v. een eenvoudige digitale camera beschreven en zijn gevolgen van een heterogene lichtverdeling voor de deugdelijkheid van fotosynthesemetingen geanalyseerd (hoofdstukdeel 3.1). De gevolgen van de gemeten lichtverdeling in de bladkamer van een commercieel verkrijgbare fotosynthesemeter en in een speciaal ontworpen laboratorium bladkamer op de fotosynthese-lichtrespons curve werd berekend voor twee realistische scenario's. Indien de gemiddelde lichtintensiteit over de bladkameroppervlakte nauwkeurig bepaald werd, had een heterogene lichtverdeling slechts kleine effecten op de fotosynthese-lichtrespons curve. Echter, wanneer de lichtintensiteit in het midden van de bladkameroppervlakte werd gemeten, zoals vaak gedaan wordt, en als homogeen verdeeld werd beschouwd, werden voor beide bladkamers aanzienlijke fouten gemaakt in de bepaling van de fotosynthese-lichtrespons curve, hetgeen o.a. leidde tot een flinke onderschatting van de lichtgelimiteerde kwantumefficiëntie voor fotosynthese.

Dit nogal technische hoofdstukdeel (3.1) ondersteunt de materiaal en methoden sectie van hoofdstukdeel 3.2, dat zich richt op de excitatie balans van de twee fotosystemen in relatie tot de golflengteafhankelijkheid van de lichtgelimiteerde kwantumopbrengst t.b.v. CO₂ fixatie van bladeren die onder verschillende lichtspectra opgegroeid zijn (kunstmatig zonlicht, kunstmatig schaduwlicht en blauw licht). De kwantumefficiëntie voor elektrontransport door fotosysteem I en fotosysteem II en voor CO₂ fixatie van intacte bladeren (*in vivo*) werd gerelateerd aan de samenstelling en spectroscopische eigenschappen van de fotosysteem supercomplexen van deze bladeren (*in vitro*). Bladeren opgegroeid onder schaduwlicht, dat fotosysteem I meer neigt te exciteren dan fotosysteem II, hadden een hogere kwantumopbrengst voor CO₂ fixatie bij golflengten die fotosysteem I sterk exciteren (≥ 690 nm) en een lagere fotosysteem I:fotosysteem II ratio, vergeleken met bladeren opgegroeid onder zon- of blauw licht. Bij golflengten die fotosysteem II sterk exciteren was de kwantumopbrengst voor CO₂ fixatie van de zon- en blauw licht bladeren juist hoger. In het spectrale gebied waar absorptie door pigmenten anders dan Chl verwaarloosbaar is (≥ 580 nm), kon de kwantumopbrengst voor CO₂ fixatie goed geschat worden via de excitatiebalans van de fotosystemen, terwijl bij lagere golflengten de kwantumopbrengst werd overschat. De golflengteafhankelijkheid van de excitatiebalans van de fotosystemen, berekend met zowel een *in vivo* als met een *in vitro* benadering, stemde in grote mate overeen. Waar de twee benaderingen niet overeen stemden, speelden absorptie door carotenoïden en state transitions waarschijnlijk een rol. In dit hoofdstuk hebben we voor het eerst laten zien hoe de golflengteafhankelijkheid van de kwantumopbrengst voor CO₂ fixatie zich kwantitatief verhoudt tot de fotosysteem excitatie balans en samenstelling, en hoe deze eigenschappen zich aanpassen aan het groeilichtspectrum van de plant.

Hoofdstuk 4 behandelt het effect van spectrale discrepanties tussen groeilicht en meetlicht op de betrouwbaarheid van *in situ* fotosynthese schattingen m.b.v. gasuitwisseling en chlorofyl fluorescentie metingen. Daarnaast zijn de gevolgen van foutieve schattingen van fotosynthesesnelheden *in situ* voor de uitkomst van groei modellen onderzocht. *Cucumis sativus* groeide op bij verschillende combinaties rood en blauw LED-licht en bij wit TL licht, dat respectievelijk moderne en meer traditionele lichtbronnen voor klimaatkamers zijn. CO₂ fixatie werd gemeten met een lage en een verzadigende lichtintensiteit bij verschillende combinaties rood en blauw licht. De meetlichtbron bestond uit LED's, welke veelvuldig gebruikt worden als lichtbron voor

fotosynthesemetingen. Elektronentransportsnelheid (ETR) werd berekend met gebruik van chlorofyl fluorescentie en licht absorptie metingen. De invloed van het meetlichtspectrum op de gemeten lichtgelimiteerde kwantum-efficiëntie voor CO₂ fixatie (α) was maximaal 25% en was onafhankelijk van het groeilichtspectrum. Daartegenover had het groeilichtspectrum een aanzienlijke effect op de fotosynthesesnelheid bij verzadigend licht, terwijl het meetlichtspectrum in dit geval weinig invloed had. Daaruit volgt dat het meetlichtspectrum voornamelijk consequenties heeft voor de deugdelijkheid van de bepaling van α . In tegenstelling tot CO₂ fixatie was de gemeten elektrontransport efficiëntie door fotosysteem II vergelijkbaar bij de verschillende meetlichtspectra. Daarom veranderende de ETR-CO₂ fixatie verhouding aanzienlijk door veranderingen in meetlichtspectrum, hetgeen gevolgen heeft voor het gebruik van ETR berekeningen om fotosynthesesnelheid te schatten. Er wordt geconcludeerd dat een fotosynthese meetlichtspectrum dat afwijkt van het groeilichtspectrum van planten kan leiden tot aanzienlijke fouten in schattingen van fotosynthesesnelheden *in situ*. Het gebruik van zulke foutieve schattingen als input in groei modellen kan disproportioneel grote gevolgen hebben voor voorspellingen van de gewasproductie.

Hoofdstuk 5 toont het belang van fotomorfo-genetische reacties op lichtspectrum voor plantproductiviteit aan. Algemeen gebruikte groeilampen stralen licht uit met een spectrale samenstelling die sterk verschilt van natuurlijke daglichtspectra. Specifieke reacties van planten op een spectrum dat verschilt van daglicht zijn soms voorspelbaar, echter, de algemene reactie van een plant is moeilijk te voorspellen vanwege de gecompliceerde interacties van verschillende specifieke reacties. Tot dusverre wordt in onderzoek naar plantreacties op lichtspectra ofwel geen daglicht als controle gebruikt, ofwel natuurlijk daglicht. Echter, natuurlijk daglicht fluctueert in intensiteit en spectrum. Wij hebben een kunstmatig zonlichtspectrum (AS) gerealiseerd dat nauw overeenkomt met een natuurlijk zonlichtspectrum, en hebben groei, morfogenese en fotosynthese van komkommerplanten die gedurende 13 dagen onder dit spectrum groeiden vergeleken met de prestaties bij groei onder TL en hogedruk natrium (HPS) licht. Het totale drooggewicht van de AS planten was respectievelijk 2,3 en 1,6 keer zo groot als dat van de TL en HPS planten, en de AS planten waren 4 tot 5 keer langer. Dit opvallende verschil leek gerelateerd te zijn aan een efficiëntere lichtonderschepping door de AS planten, gekenmerkt door langere bladstelen, een hogere bladafsplittingsnelheid en een lagere investering in blad massa t.o.v. bladoppervlakte. De fotosynthesesnelheid per eenheid bladoppervlakte was niet hoger voor de AS bladeren. Het extreme verschil in plantreactie op het kunstzonlicht spectrum, vergeleken met de reactie op de algemeen gebruikte lichtbronnen in beschermde teelten, onderstreept het belang van een meer natuurlijk spectrum, zoals dat van ons kunstzonlicht, als het doel is planten te produceren die representatief zijn voor planten die onder natuurlijk licht opgroeien.

In **hoofdstuk 6** worden de consequenties van de verschillende plantreacties op het lichtspectrum van hun omgeving bediscussieerd in een breder (eco)-fysiologisch perspectief. Daarnaast worden een aantal gevolgen van dit onderzoek voor het meten van fotosynthese bediscussieerd. Dit hoofdstuk sluit af met een discussie over de praktische implicaties van de nieuwe inzichten die dit onderzoek oplevert voor het kweken van planten in klimaatkamers en kassen.

DANKWOORD

Dit proefschrift is het resultaat van meer dan vier jaar onderzoek naar de wijze waarop planten licht omzetten in energie en groei. Ik ben blij dat ik, in alle vrijheid en door nieuwsgierigheid gedreven, heb kunnen werken aan het verder ontrafelen van dit mysterie, dat mij al mijn hele leven fascineert. Er wordt vaak gezegd dat het doen van een promotieonderzoek een eenzaam gebeuren is. Voor een gedeelte is dit waar, maar gelukkig, voor een groot gedeelte ook niet. Om nieuwe creatieve ideeën te kunnen ontwikkelen is een goede onderzoeksomgeving niet alleen van belang, maar zeker ook een plezierig leven daarbuiten. Daarom ben ik dank verschuldigd aan iedereen die bijgedragen heeft aan het tot stand komen van dit proefschrift, zowel direct als indirect in de privésfeer.

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Naast het bestuderen van fysiologische en fysische processen in planten, heb ik ook mijn knutselvaardigheden bij kunnen schaven tijdens dit promotieonderzoek, omdat vrijwel ieder plantengroei- en meetsysteem van de grond af opgebouwd moest worden. Joost Ruijsch was hierbij van onmisbare waarde. Joost, bedankt voor al je ideeën en bijdragen om apparatuur toch weer op een handigere manier operationeel te krijgen dan ik zelf had kunnen bedenken. Bedankt ook voor de gezellige eet en borrel avonden. Jeremy en Wim, ook jullie ben ik dank verschuldigd voor jullie technische bijdragen en adviezen, zodat mijn pogingen tot instrumentenmakerij uiteindelijk resulteerden in iets bruikbaar. Zonder de inspanningen van Jeremy om op hete middagen in augustus de 'lab-built' fluorescentieapparatuur draaiende te krijgen, was hoofdstuk 3.2 er niet gekomen. Theo Damen, bedankt voor het assembleren van de LED-rekken. Verder was de metaalwerkplaats onmisbaar voor mijn meetopstellingen, Evert Janssen en Gradus Leenders, jullie zijn kunstenaars!

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ik daar niet de meest actieve deelnemer aan was) en uitjes. Ep Heuvelink, fijn dat je altijd bereid was vragen te beantwoorden en mee te denken. Ik heb er bewondering voor hoe je zoveel verschillende taken ogenschijnlijk zonder stress kan combineren. Menno Bakker, dank voor je hulp met het modelleergedeelte van hoofdstuk 4. Maaïke Wubs, je promoveerde een maand voor mij, heel fijn dat ik jou als 'proefschrift-expert' met allerlei praktische vragen kon lastig vallen. Uulke van Meeteren, dank voor het uitzoeken van alle 'gedoe' rondom budgetten en klimaatkamer reserveringen. Rob Schouten, Ernst Woltering, Pol Tijskens en andere stafleden, dank voor jullie input tijdens de research-seminars. Pauline Wien, dank voor veel gezelligheid en het uitzoeken van een mooie nieuwe outfit uit je kledingwinkel voor Yvette. Iza, I always enjoy the 'fresh air' breaks with you, and the dinner and beer-drinking nights even better. And how can I start my day somewhere else without your coffee-service? Some more fresh air people: Dimitrios Fanourakis, Andreas Savvides and Kang-Mo Lee, being exposed to the elements is always a good moment to develop new insights, I'm looking forward to the completion of the amazing apex-cooling device! Thanks also to my former and current fellow PhD-candidates, Anke van der Ploeg, Hossein Rezaei Nejad, Peter Twumasi, Cecilia Onyango, Lilian Campos Mota, Aparna Tiwari, Aaron Velez Ramirez, Didi Qian, Brian Farnetti, Fleur Sterk, Vaia Sarlikioti, Julianne Fanwoua, Benno Burema and Sander van Delden, for your input during the FLOP meetings, conversations and friendship.

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Sander

Curriculum Vitae

Sander Willem Hogewoning was born on May 19th 1977 in Rijnsburg, the Netherlands. He attended the Visser 't Hooft Lyceum in Leiden for secondary education, where he obtained a Gymnasium diploma in 1995. He studied Crop Science at Wageningen University, after having switched from Agricultural Economics during the first study year. For his first MSc thesis he investigated chilling injury effects on the photosynthetic functioning of a tropical plant species. During a six months internship at the Centro Internacional de la Papa in Lima (Peru) he studied post-harvest and processing techniques for the local yacon root and investigated marketing opportunities for yacon syrup. Additionally, he was sent to a remote part in the Andes to set-up a pilot-plant to produce this syrup, after which a period of extensive travelling through South-America followed. The research for his second MSc thesis was on the international membership issue at the Dutch flower auctions under supervision of the NCR-endowed chair Theory and Practice of Co-operatives, highlighting his combined interest in plant physiology and economics and management issues. A third thesis extended the work of his first thesis, and finally resulted in publication of this work in *Journal of Experimental Botany*. He graduated and started his PhD research in 2005, resulting in this thesis. From November 2009 he has worked as a post-doc on the exploitation of light spectrum to manipulate horticultural crops and the further development of plant growth facilities equipped with artificial solar lighting. In November 2010 he continued as a post-doc, employed temporarily to further develop his scientific ideas and write project proposals.

List of Publications

Papers published in refereed journals

- Hogewoning SW, Harbinson J. 2007. Insights on the development, kinetics, and variation of photoinhibition using chlorophyll fluorescence imaging of a chilled, variegated leaf. *Journal of Experimental Botany*, 58: 453-463.
- Hogewoning SW, Douwstra P, Trouwborst G, van Ieperen W, Harbinson J. 2010. An artificial solar spectrum substantially alters plant development compared with usual climate room irradiance spectra. *Journal of Experimental Botany*, 61: 1267-1276.
- Hogewoning SW, Trouwborst G, Harbinson J, van Ieperen W. 2010. Light distribution in leaf chambers and its consequences for photosynthesis measurements. *Photosynthetica*, 48: 219-226.
- Hogewoning SW, Trouwborst G, Maljaars H, Poorter H, van Ieperen W, Harbinson J. 2010. Blue light dose-responses of leaf photosynthesis, morphology and chemical composition of *Cucumis sativus* grown under different combinations of red and blue light. *Journal of Experimental Botany*, 61: 3107-3117.
- Paradiso R, Meinen E, Snel JFH, de Visser P, van Ieperen W, Hogewoning SW, Marcelis LFM. Spectral dependence of photosynthesis and light absorptance in single leaves and canopy in *Rosa hybrida*. *Scientia Horticulturae*, in press.
- Trouwborst G, Oosterkamp J, Hogewoning SW, Harbinson J, van Ieperen W. 2010. The responses of light interception, photosynthesis and fruit yield of cucumber to LED-lighting within the canopy. *Physiologia Plantarum*, 138: 289-300.
- Zheng S-J, Snoeren TAL, Hogewoning SW, van Loon JJA, Dicke M. 2010. Disruption of plant carotenoid biosynthesis through virus-induced gene silencing affects oviposition behaviour of the butterfly *Pieris rapae*. *New Phytologist*, 186: 733-745.

Papers to be published in refereed journals

- Hogewoning SW, Wientjes E, Douwstra P, Trouwborst G, van Ieperen W, Croce R, Harbinson J. A quantitative molecular and physiological analysis of photosynthetic quantum yield dynamics (submitted)
- Hogewoning SW, Trouwborst G, van Ieperen W, Bakker MJ, Harbinson J. The influence of measuring-light spectrum on estimates of photosynthesis *in situ* and its consequences for prediction of biomass production (to be submitted)
- Trouwborst G, Hogewoning SW, Harbinson J, van Ieperen W. Photosynthetic acclimation in relation to nitrogen allocation in cucumber leaves in response to changes in irradiance (submitted)
- Trouwborst G, Schapendonk AHCM, Rappoldt K, Pot CS, Hogewoning SW, van Ieperen W. The effect of interlighting on cucumber fruit yield-analysis and simulation (submitted)

Trouwborst G, Hogewoning SW, Harbinson J, van Ieperen W. Effect of the interaction of leaf age and irradiance level on the photosynthetic capacity of tomato leaves (submitted)

Trouwborst G, Hogewoning SW, Savvides A, Harbinson J, van Kooten O, van Ieperen W. Plasticity of photosynthesis after the red light syndrome (submitted)

Conference proceedings

Hogewoning SW, Trouwborst G, Engbers GJ, Harbinson J, Van Ieperen W, Ruijsch J, Schapendonk AHCM, Pot SC, Van Kooten O. 2007. Plant physiological acclimation to irradiation by light emitting diodes (LEDs). *Acta Horticulturae (ISHS)*, 761: 183-191.

Paradiso R, Meinen E, Snel JFH, van Ieperen W, Hogewoning SW, Marcelis LFM. Light Use Efficiency at Different Wavelengths in Rose Plants. *Acta Horticulturae (ISHS)*, in press.

Papers in professional journals

Hogewoning SW, Trouwborst G, van Ieperen W. 2008. Licht op LED's: Effecten van lichtkleur op de fotosynthese. *Onder glas* 5 (4): 43-45 (in Dutch).

Hogewoning SW, van Ieperen W, Kierkels, T. 2009. Eerste proeven met kunstmatig daglicht geven verrassend effect. *Onder Glas* 6 (9): 60-61 (in Dutch).

Abstracts

Hogewoning SW, Harbinson J. 2006. Insights into the effects of chilling on photoinhibition. *Comparative Biochemistry and Physiology: Annual main meeting of the Society for Experimental Biology, Canterbury (UK)*, pp. S152.

Hogewoning SW, Harbinson J. 2006. Plant physiological acclimation to irradiation by light-emitting diodes (LEDs)- implications for use in protected plant cultivation systems. In: *Book of abstracts, 27th International Horticultural Congress & Exhibition, Seoul, (South-Korea)*, pp. 168.

Hogewoning SW, Maljaars H, Harbinson J. 2007. The acclimation of photosynthesis in cucumber leaves to different ratios of red and blue light. In: *Photosynthesis Research 91 (2-3): 14th International Congress of Photosynthesis, Glasgow (UK)*, pp. 287-288.

Hogewoning SW, Trouwborst G, Harbinson J. 2008. Photosynthesis rates *in situ* may significantly deviate from measured rates of photosynthesis due to the measuring-light spectrum used. In: *Book of abstracts, Joint Annual Meeting of the American Society of Plant Biologists and Sociedad Mexicana de Bioquímica Rama, Merida, (Mexico)*, pp. 100.

Trouwborst G, Oosterkamp, J, van Ieperen W, Hogewoning SW. 2009. Inter-lighting with LEDs in greenhouse grown cucumber. In: *Book of abstracts, 6th International Symposium in Light in Horticulture, Tsukuba (Japan)*, pp. 59

Marcelis LFM, Snel JFH, de Visser PHB, Meinen E, Driever SM, van Ieperen W, Hogewoning SW, Paradiso, R. Spectral dependence of photosynthesis at the leaf

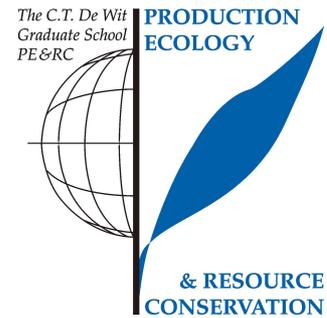
List of publications

and canopy level. In: Book of abstracts, 6th International Symposium in Light in Horticulture, Tsukuba (Japan), pp. 98.

Hogewoning SW, Douwstra P, Trouwborst G, van Ieperen W, Snel JHF, Harbinson J. 2009. Unraveling light spectrum effects on leaf photosynthesis and plant morphology. In: Book of abstracts, 6th International Symposium in Light in Horticulture, Tsukuba (Japan), pp. 101.

PE&RC PhD Education Certificate

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)



Review of literature (6 ECTS)

- On the photosynthetic and developmental responses of leaves to the spectral composition of light (2005)

Writing of project proposal (7.5 ECTS)

- Photosynthetic efficiency under different wavelengths (PT/ LNV; 2007)
- Proposal core projects TTI GREEN GENETICS (confidential; 2008)
- Proposal small cluster projects TTI GREEN GENETICS (confidential; 2009)
- Growth-light provided by solar-spectrum lamps (PT/ LNV; 2009)
- Flowering of Chrysanthemum under a long photoperiod using LEDs (PT/ LNV; 2009)

Post-graduate courses (4.5 ECTS)

- Basic statistics; PE&RC (2006)
- Advanced statistics; PE&RC (2006)
- Protein analysis using anti-bodies; Agrisera (2007)
- Photosystem analysis *in vitro*; RUG (2009)

Laboratory training and working visits (0.5 ECTS)

- Photosynthesis laboratory Prof. Dr. Terashima; University of Tokyo (2009)

Invited review of (unpublished) journal manuscripts (1.5 ECTS)

- Acta Horticulturae: LEDs in relation to photosynthesis (2006)
- Scientia Horticulturae: LEDs in relation to photosynthesis (2010)

Deficiency, refresh, brush-up courses (4 ECTS)

- Electronics and metal working (2007)
- Photosynthesis measurement *via* gas-exchange and spectroscopy (2007)

Competence strengthening / skills courses (1.5 ECTS)

- Career orientation; PE&RC (2009)

PE&RC Annual meetings, seminars and the PE&RC weekend (2.5 ECTS)

- PE&RC 10 Year anniversary (2005)
- Organisation excursion (closed greenhouse and orchid grower; 2006)
- PE&RC Annual meetings (2005, 2006)
- Symposium 'Spectroscopy and remote sensing ' (2008)
- Symposium 'Photosynthesis: from Femto to Peta and from Nano to Global'; SENSE (2009)

Discussion groups / local seminars / other scientific meetings (7.5 ECTS)

- Frontier literature in plant physiology (FLOP) (2005-2009)
- Round table discussion 'Biomass for Energy' (inauguration Prof. Steven de Bie) (2007)
- Several excursions to companies involved in greenhouse production (2007-2009)

International symposia, workshops and conferences (12 ECTS)

- Annual main meeting of the Society for Experimental Biology; Canterbury, UK (poster) (2006)
- 27th International Horticultural Congress; Seoul, South-Korea (oral) (2006)
- 14th International Congress of Photosynthesis; Glasgow, UK (poster) (2007)
- Annual Meeting of the American Society of Plant Biologists; Merida, Mexico (poster) (2008)
- 6th International Symposium on Light in Horticulture; Tsukuba, Japan (oral) (2009)

Lecturing / supervision of practical 's / tutorials (7.5 ECTS)

- Supervision practical 'Physiology and Development of Plants in Horticulture; 10 days (2005-2008)
- Lectures for groups of national and international visitors (Dutch secondary school students, university students and scientists from e.g. the USA, South-Korea, Germany, and representatives from industry); 10 days (2005-2020)
- Lectures and practical on Photosynthesis for students HAS-Den Bosch; 5 days (2010)

Supervision of MSc students (3 students; 20 days)

- Blue light responses of leaf photosynthesis
- Spectral effects on photosynthetic quantum yield
- Light signalling effects on continuous light injury

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