On the photosynthetic responses of crops to intracanopy lighting with light emitting diodes

Govert Trouwborst

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ABBREVIATIONS

A_{gross}	gross photosynthetic rate
A_{max}	net photosynthetic capacity at saturating irradiance
A_{mg}	gross photosynthetic capacity at saturating irradiance
A_{net}	net photosynthetic rate
C_B	ratio of leaf chlorophyll to leaf nitrogen in light harvesting components
C_C	leaf chlorophyll concentration
C_i	internal CO_2 concentration
ETR	electron transport rate
F_v/F_m	maximum quantum efficiency of PSII photochemistry of a dark adapted leaf
F_v'/F_m'	maximum quantum efficiency of PSII photochemistry in the light
g_s	stomatal conductance
J_{max}	maximum electron transport rate at saturating irradiance
J_{mc}	potential rate of photosynthetic electron transport per unit cytochrome f
k	light extinction coefficient
LAI	leaf area index
LMA	leaf mass per area
LUE	light-use efficiency
M_D	dry mass
M_F	fresh mass
N_n	nitrate content
N_{org}	organic nitrogen content
N_{phot}	nitrogen content in the photosynthetic apparatus
NPQ	nonphotochemical quenching
N_t	total nitrogen within a leaf
PAR	photosynthetic active radiation
P_B	fraction of leaf nitrogen allocated to bioenergetics
P_L	fraction of leaf nitrogen allocated to light harvesting components
P_{phot}	fraction of leaf nitrogen allocated to the components of the photosynthetic apparatus
$P_{light\ acquisition}$	fraction of P_{phot} invested in light harvesting
$P_{light\ utilisation}$	fraction of P_{phot} invested in light utilisation
PNUE	photosynthetic nitrogen use efficiency
PPFD	photosynthetic photon flux density
P_R	fraction of leaf nitrogen allocated to carboxylation
Q_A	primary quinone acceptor of PSII
q_P	PSII efficiency factor
R_D	dark respiration rate
V_{Cmax}	maximum carboxylation rate
V_{cr}	specific activity of Rubisco
α	light-limited quantum efficiency for CO_2 fixation
Φ_{PSII}	PSII operating efficiency
Φ_{NPQ}	regulated energy dissipation
Φ_{NO}	non regulated energy dissipation including fluorescence emission
θ	scaling constant for curvature

CHAPTER 1

General Introduction

Assimilation lighting in Dutch greenhouse horticulture

Dutch greenhouse horticultural industry is one of the world's largest. It has a production value of 4.8 billion euro (Tuinbouwcijfers 2009). It plays a leading role in implementing innovative technologies in greenhouses. The land use covers 10.000 ha which consists of about 45% greenhouse vegetables, 30% cut flowers, 15% pot plants and a remaining 10%.

Assimilation lighting is a production factor of increasing importance in Dutch greenhouse horticulture. Assimilation lighting increases production levels and improves product quality and opens possibilities for year round production (Heuvelink, *et al* 2006). In 2004, about 23% of the glasshouse area was equipped with assimilation lighting (Van der Knijff *et al*., 2006), which is almost exclusively used in ornamental production and very little in vegetable production. In the Netherlands, the use of assimilation lighting for crops like tomato and cucumber started in 2001.

As a drawback, this use of assimilation lighting increases energy inputs and CO_2-emission and causes light pollution due to stray light illuminating the night sky (Morrow, 2008). These consequences are in conflict with agreements between the Dutch government and the horticultural sector about sustainability and impose societal disapproval on greenhouse horticulture (Convenant schone en zuinige agrosectoren, 2008). These agreements and the high percentage of energy costs (up to 25% mainly for heating and lighting; Van der Velden, 2008) of the total production costs are a drive to optimise the energy efficiency in greenhouses.

Today, High Pressure Sodium (HPS) lamps are the most commonly used light sources for supplemental assimilation lighting in greenhouse horticulture. Presently, HPS lamps are still one of the most energy efficient assimilation lighting sources available for commercial plant production, but they have certain characteristics that may limit their application in future.

A potentially more efficient light source based on light emitting diodes (LEDs) is under development (Morrow, 2008). LEDs are semi-conductors that emit light by electro luminescence. Recent developments in LED technology resulted in very bright LEDs in colours throughout the visible spectrum. This opens the possibility to use them as assimilation lamps as well (De Ruijter, 2004). LEDs have several advantages:

emittance of irradiance in a narrow band of the spectrum, low voltage operation, low heat emission owing to conduction, a compact and light weight design, solid state construction, superior safety and longevity, lack of noise and an easy control (Bula *et al.*, 1991, Barta *et al.*, 1992, Bourget, 2008). Decreasing costs, increasing efficiency and brightness make these LEDs promising candidates for assimilation lighting in horticulture (Morrow, 2008).

The energy efficiency of assimilation lighting can be improved by (1) increasing the energy conversion efficiency of the light sources (increasing photon output per Watt electricity input), (2) greater system efficiency by effectively using the heat produced by the lamps, and (3) optimisation of the growth system (greater light use efficiency *i.e.* plant productivity per photon input). The first two options are outside the scope of this thesis. The growth system might be optimised due to the use of lamps within, instead of from above the canopy (intracanopy lighting) or by making efficient use of the light spectrum of the used lamps.

Knowledge about photosynthetic acclimation to intracanopy lighting and to specific narrow band light spectra (LED lighting) is scarce. Research conducted in this thesis is mainly limited to effects on photosynthetic acclimation while other processes which can be influenced by light spectrum like photomorphogenesis or phototropism are not actively investigated.

Acclimation to irradiance on different levels

Acclimation of plants to their light environment can occur at several levels. Firstly, plants can change the fraction of biomass invested in leaves, stems and roots. Secondly, plants can modulate the leaf area per unit biomass invested in leaves by altering their anatomy. Thirdly, plant leaves can change the relative investment of nitrogen between photosynthetic components (Evans and Poorter, 2001). A general distinction has been made between sunlit and shade plants or leaves. Generally speaking, shade leaves invest more of their leaf proteins into light capturing while sun leaves invest more proteins into light processing. Table 1. presents a brief overview of differences between both types of leaves.

The plasticity of acclimation to changes in irradiance is different for different phases in leaf development. Morphological and anatomical properties such as number of cell layers, the size of cells and the thickness of cell walls are relatively fixed after the leaf expansion phase has finished (Sims and Pearcy, 1992, Oguchi *et al.*, 2003). Chloroplasts however, are also able to re-acclimate to decreases or increases in irradiances compared to the irradiances they were exposed to during leaf expansion (Pons and Pearcy, 1994, Oguchi *et al.*, 2003).

Table 1. Differences in acclimation to high or low irradiance at different integration levels.

High irradiance	Low Irradiance
Cells	
Large cells	Small cells
Small chloroplasts	Large chloroplasts
Low chlorophyll/rubisco ratio	High chlorophyll/rubisco ratio
High Chl a/b ratio	Low Chl a/b ratio
Leaves	
Small thick leaves (high leaf mass per	Large thin leaves (low leaf mass per
High stomatal conductance	Low stomatal conductance
High photosynthetic capacity	Low photosynthetic capacity
Plants	
Low leaf area ratio	High leaf area ratio
High root / shoot ratio	Low root / shoot ratio
Vertical leaf orientation	Horizontal leaf orientation
High photosynthetic capacity	Low photosynthetic capacity
High compensation irradiance	Low compensation irradiance

(source: Atwell *et al.*, 1999)

Photosynthetic acclimation in crop systems

In crop systems in greenhouses plant growth and production in northern latitudes is mainly limited by the irradiance level, while other limiting factors like water and nutrient supply are presumed to be optimised. Irradiance affects the assimilation process of the plants in two ways: 1) the photosynthetic rate is determined by the *in situ* irradiance and 2) preceding irradiance levels affect the photosynthetic acclimation process which determines the photosynthetic system. Newly developing leaves at the top of the plants in upright growing herbaceous crops (like egg plants, sweet pepper, tomato or cucumber) receive a relatively high irradiance compared to leaves deeper in the canopy. Internal shading results in an exponential decrease in irradiance from top to bottom in the canopy (Monsi and Saeki, 2005).

Besides the exponential decrease in irradiance level within the canopy the spectral distribution of the radiation also changes over canopy depth. Within the region of photosynthetic active radiation (PAR; 400-700 nm) leaf absorption differs over wavelength. However, due to the small transmittance of the leaves in the PAR-region (less than 10%), the contribution of transmitted irradiance to the total irradiance

within the canopy is minor (Terashima and Hikosaka, 1995), though dense canopies lead to a relative depletion in blue and red wavelengths at the bottom of canopies (Endler, 1993). In contrast to the small transmittance in the PAR region, there is a sharp increase in leaf transmission around 700 nm—the distinction between the PAR and the Far-Red region (Goudriaan and Van Laar, 1994). As a consequence, the Red/Far-Red ratio decreases markedly with depth in the canopy (Terashima and Hikosaka, 1995, Grant, 1997).

Together with the external stimuli (decrease in irradiance and spectral changes), leaves deeper in the canopy are inherently older, and a number of leaf characteristics decline with canopy depth: stomatal conductance, photosynthetic capacity, dark respiration, chlorophyll content and a/b ratios, nitrogen, Rubisco and RUBP-regenerating enzymes (Evans, 1993a, Evans, 1993b, Xu *et al.*, 1997, Schapendonk *et al.*, 1999, Walters, 2005, Boonman *et al.*, 2006, Niinemets, 2007). All these responses can be regarded to what is called acclimation to shade. Several mechanisms for this process have been reviewed by Ono *et al.* (2001), Walters (2005) and Niinemets (2007). These acclimation patterns from the top in a downward direction in the canopy are believed to be optimal for plant photosynthesis and growth (Hikosaka, 2005, Hirose, 2005, Terashima *et al.*, 2005). Thus, the introduction of intracanopy lighting with LEDs will have a great influence on irradiance level and spectrum within the canopy and so might have great impact on the described acclimation patterns.

Intracanopy lighting with LEDs?

Intracanopy lighting is based on two important hypotheses: Firstly, it would reduce loss of assimilation lighting due to crop reflection towards the sky. This corresponds to approximately 6-7% of the incident irradiance (Goudriaan and Van Laar, 1994, Marcelis *et al.*, 1998). It would also reduces loss of assimilation lighting due to crop transmittance towards the floor which can also be in the order of 5-10% (LAI of 3 to 4 with an extinction coefficient of 0.75). Secondly, with intracanopy lighting irradiance is more evenly distributed within the crop. This favours the efficiency of the photon flux used in the photosynthetic process. When leaves receive radiation exceeding the linear light-limited phase of the photosynthetic irradiance-response, this irradiance can better be transmitted to leaves deeper in the canopy which are still within their linear phase (Terashima *et al.*, 2005; Long *et al.*, 2006).

Intracanopy lighting with HPS lamps has already been tested experimentally with success in Finland and Norway (Hovi *et al.*, 2004, Hovi *et al.*, 2006, Hovi-Pekkanen and Tahvonen, 2008, Pettersen *et al.*, 2010a) where increases in production in the order of 10-20% were found. Due to smaller aisle widths in the Netherlands and due to the high operating temperature of HPS lamps (>200°C) resulting in a significant near infra red (NIR) heat radiation towards their direct environment, the application of these lamps as light source for intracanopy lighting is limited. LEDs operate at room temperature while the emittance of NIR radiation can be absent (narrow band

lighting). Hence, these lamps might be suitable as light source for intracanopy lighting. LEDs lose their heat due to conduction which affords the opportunity to cool LED lighting systems and to reuse the (low caloric) heat on another time and place, increasing overall system efficiency.

Using LEDs as light source for intracanopy lighting opens questions about the use of the optimal light spectrum and light intensity within the crop. Growing plants under these narrow band light sources might open possibilities for lighting with colours which are inherently more energy efficient due to less energy per photon (*i.e.* red light, thus increasing lamp efficiency) and taking efficient light colours for photosynthesis and plant development (increasing the efficiency of the growth system). Though for red light the highest quantum efficiencies are reported (McCree, 1972a, Inada, 1976, Evans 1987), research at Kennedy Space Centre has shown that supplemental blue light on red light enhanced plant production (Kim *et al*., 2006). Also specific problems with the use of pure red light are reported: Cowpea plants showed intumescence (Massa *et al*., 2008), while red light can block chlorophyll synthesis in wheat seedlings (Tripathy and Brown, 1995, Sood *et al*., 2004, Sood *et al*., 2005). However, when upscaling these results to greenhouse crop systems, specific spectral effects of LED-lighting could be of minor importance due to the presence of natural irradiance.

The used light intensity for intracanopy lighting might be limited by the natural irradiance level and the capacity for photosynthetic re-acclimation in plants. Oguchi and co-workers (Oguchi *et al*., 2003, Oguchi *et al*., 2005) have shown that photosynthetic acclimation to an increase in light intensity after leaf development is limited. During winter, the light intensity applied by intracanopy lighting can easily exceed natural light intensities. Hence, the efficiency of intracanopy lighting might reduce if leaves develop under low natural irradiances while being exposed to higher light intensities (by intracanopy lighting) later on in their life span.

Scope of research and thesis outline

The aim of this study was to obtain insights in photosynthetic acclimation in response to irradiance level and irradiance spectrum in the framework of the applicability of LEDs as light source for intracanopy lighting. Intracanopy lighting with narrow band spectra will act in a complex system with many factors involved. In Fig. 1. a schematic representation of the outline of this thesis has been made: Natural irradiance from above and supplemental irradiance from above or within the canopy (left en right blocks in upper corners) together with the canopy characteristics influence the irradiance (level and spectrum) on leaf level. From top to bottom the irradiance level decreases, the light spectrum changes but also the leaves are inherently older. These factors might influence photosynthetic acclimation. **Chapter 2** treats the question whether or not leaf age intrinsically affects the photosynthetic capacity of tomato leaves over the usual leaf life span on a plant in commercial growth

systems. In **Chapter 3** we questioned if it is still beneficial to apply intracanopy lighting when the irradiance level applied deep in the canopy is higher than at the top of the canopy which could occur in winter. We examined this question in a climate room experiment using young cucumber plants as a model to test the plasticity of leaves to acclimate to an increase in irradiance after leaf development. In **Chapter 4** we addressed the question how plants acclimate to different percentages red and blue LED-light (4.1) and if and how young cucumber plants re-acclimate if the LED spectrum changes after leaf development (4.2). Again we took young cucumber plants as a model.

Irradiance (spectrum and level) and photosynthetic acclimation determine the *in situ* photosynthetic rate, which in turn determines growth, while both photosynthetic acclimation and growth determine the canopy characteristics (Fig. 1). In **Chapter 5** we tried to upscale the results from former experiments to crop level. In Chapter 5.1, we conducted research to the effects of intracanopy lighting on canopy scale. Conventional top-lighting with HPS lamps was compared with a combination of top-lighting with HPS lamps and intracanopy lighting with LED-lamps (partial intracanopy lighting). Based on the obtained photosynthetic measurements, we modelled crop development and production with intracanopy lighting and conducted some scenario studies in Chapter 5.2. In **Chapter 6**, preceding chapters are summarised and discussed in the framework of the applicability of LEDs as light source for intracanopy lighting.

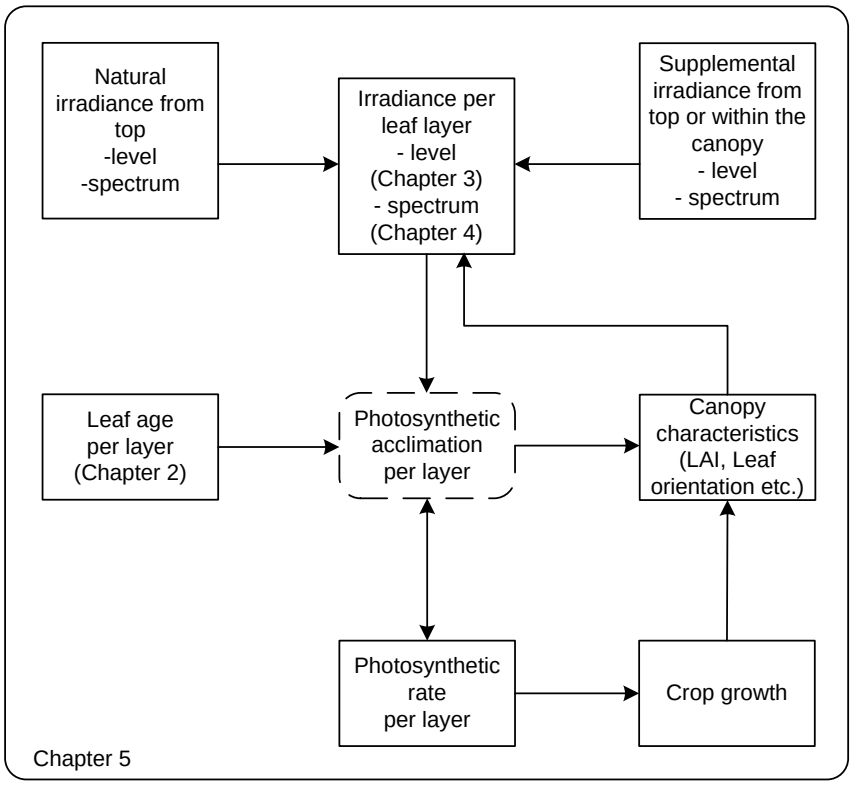

Fig. 1. Framework and scope of the research.

CHAPTER 2

The influence of light intensity and leaf age on the photosynthetic capacity of leaves within a tomato canopy

Abstract

In dense crop stands, the decrease in leaf photosynthetic capacity (A_{max}) is paralleled by a decrease in photosynthetic photon flux density (PPFD) and an increase in leaf age. In greenhouse horticulture, assimilation lighting is traditionally applied from above the canopy. Recently a new lighting technique has been developed in which assimilation lighting is applied within the canopy: intracanopy lighting. This development raises the question whether the decrease in the A_{max} of lower, thus older and shaded, leaves in a crop is solely due to the lower PPFD, or also partly due to ageing of these leaves. We investigated whether leaf ageing influenced changes in the A_{max} of tomato leaves during their usual life-span during cultivation in commercial crop systems (*i.e.*, up to 70 d). To uncouple leaf age from PPFD level, tomato plants were grown horizontally, so that the PPFD was similar for all leaves. To investigate the effect of PPFD during leaf development ($PPFD_{LD}$), A_{max} - leaf age profiles were determined for the leaves of plants grown under conditions with distinctly different natural patterns of PPFD (*i.e.*, Winter, early Spring and late Spring). In addition, from half of the number of plants per experiment, all fully-developed leaves were shaded to 25% of the normal PPFD in the greenhouse using a neutral density filter. Photosynthetic capacity and chlorophyll contents were higher in late Spring than in Winter, but were hardly affected by leaf age. In early Spring the A_{max} and chlorophyll contents were higher in younger leaves than in older leaves. This was to a large extent due to the differences in $PPFD_{LD}$, and hardly due to leaf ageing. Shading fully-developed leaves dramatically decreased their A_{max} and chlorophyll contents within a few days. We conclude that during the normal life-span of tomato leaves in commercial cultivation, the decrease in PPFD within the canopy, and not leaf-ageing, is the most important factor causing changes in A_{max} with canopy depth.

Trouwborst, G, Hogewoning SW, Harbinson J, and Van Ieperen W. 2011, The influence of light intensity and leaf age on the photosynthetic capacity of leaves within a tomato canopy, *provisionally accepted*.

Introduction

In regions of the world with strong seasonal changes in natural PPFD, supplementary lighting in greenhouses is essential to achieve year-round production of tomato fruit (Heuvelink *et al.*, 2006). Therefore, supplementary lighting is increasingly important for the commercial production of tomato fruit, despite the increased energy use and cost (Heuvelink *et al.*, 2006). Higher energy use conflicts with the need to reduce the use of fossil energy in greenhouse horticulture and is driving the search for more energy efficient sources of supplementary light than the commonly used HPS lamps (Morrow, 2008), as well as for more efficient strategies for applying supplementary lighting in existing growing systems to enhance crop production. An example of the latter is the introduction of intracanopy lighting in fruit- and vegetable crops, where the supplementary lighting is provided by light sources suspended within the canopy (Hovi-Pekkanen and Tahvonen, 2008; Hovi *et al.*, 2004; Pettersen *et al.*, 2010a). This reduces light losses by reflection and transmission from the crop, and delivers a more even distribution of vertical light intensity within the canopy, which is believed to have a positive effect on crop photosynthesis due to the non-linear response of leaf photosynthesis to PPFD (Terashima *et al.*, 2005).

In most commercial greenhouses, tomato plants are trained using a high-wire system in which new leaves appear and develop continuously at the top of the canopy, in full light, while mature leaves are gradually lowered into the canopy, where self-shading progressively decreases the light intensity (Van Henten *et al.*, 2002). Consequently, in such growth systems, light intensity, leaf age, and leaf position are interlinked properties. Finally, at the bottom of the canopy, older leaves near the harvest-ripe tomato fruit are removed, generally before visible signs of senescence (*i.e.*, leaf yellowing) are observed. For tomato plants grown in such systems the process from just-visible leaf to leaf removal takes up to 70 d.

The contribution of individual leaves to overall crop photosynthesis depends on the extent of light interception by each leaf, as well as its intrinsic photosynthetic properties. The latter are not constant, but change during and after leaf development due to changes in light intensity, nutrition, or ageing (Hikosaka, 1996; Oguchi *et al.*, 2003; Sassenrath-Cole *et al.*, 1996; Sims and Pearcy, 1989). The simultaneous increase in leaf age and decrease in light intensity in a high-wire grown crop is known to be associated with a decrease in A_{max} (Trouwborst *et al.*, 2010; Xu *et al.*, 1997). However, leaf age and light intensity may become uncoupled when intracanopy lighting is applied. Under such circumstances, the light intensity may even increase while leaf age increases. It is therefore important to know whether leaf ageing has a significant influence on the intrinsic photosynthetic properties of a leaf during its normal 70 d life-span in cultivation, and to what extent any ageing effects interact with light intensity during this time. Recently, Pettersen *et al.* (2010b) showed that, for horizontally-grown cucumber plants under low PPFD, the A_{max} of individual leaves

did not decline with increasing leaf age. The appearance rate of leaves of cucumber plants however, is generally twice as fast as for tomato plants, so the maximum lifetime of a leaf on a cucumber plant is much shorter.

We have investigated whether leaf ageing has a significant influence on the decrease in A_{max} of a tomato leaf during its normal life-span in cultivation. In these experiments, leaf age and light intensity were uncoupled by forcing tomato plants to grow horizontally instead of vertically, so that all leaves received the same intensity of light. The experiments were repeated in different seasons with different natural light intensities (*i.e.*, Winter, early Spring and late Spring) in order to investigate the effect of PPFD level during leaf development on A_{max}-leaf age profiles. In 50% of the plants, fully-expanded leaves were shaded to investigate a possible interaction between light intensity and the A_{max}-leaf age profile. A_{max}-age profiles were also investigated under conditions of different natural light intensities in a vertically grown tomato crop.

Materials and methods

Plant material and growth conditions

Three experiments conducted during consecutive periods (Winter, early Spring, and late Spring 2005/2006) in which tomato plants (*Lycopersicon esculentum* 'Pronto') were forced to grow horizontally in a greenhouse compartment (64 m^2) at Wageningen University, Wageningen, The Netherlands. In each experiment, six plants were grown in 10 l pots, filled with perlite with a constant substrate water content 70% (v/v) controlled by a combination of an ECH_2O dielectric aquameter (Decagon Devices Inc., Pullman, WA, USA) and an automatic drip-irrigation system. A standard nutrient solution for tomato was used (Sonneveld and Bloemhard, 1994; EC = 2.7, pH = 5). Minimum day and night temperatures were maintained at 20°C and 18°C, respectively, and the RH was approx. 70%. Plants were able to grow to a length of approx. 3.5 m. Three plants were exposed to full natural light, while all mature leaves on the remaining three plants were shaded to 25% of full natural light using a neutral shade filter (210.06ND; Lee Filters, Andover, UK). Leaves > 21 d old were fully-expanded and defined as mature. Leaf age was defined as the number of days since the newly emerged leaf was 1-2 cm in length. The rate of leaf appearance in all these plants was approx. three leaves per week.

Measurements

Photosynthetic capacity (A_{max}), chlorophyll content and the PPFD-integral during the first 21 d of leaf development ($PPFD_{LD}$) were determined on five-to-six leaves per plant ranging in age between 20 - 70 d in non-shaded plants and between 20 - 110 d in shaded plants. All leaves per individual plant were measured within a period of two days.

The natural PPFD outside the greenhouse was measured continuously using a solarimeter (Kipp & Zonen, Delft, The Netherlands). PPFD inside the greenhouse was calculated as in Trouwborst *et al.* (2010).

A_{max} was measured using a Li-6400 portable photosynthesis system with fluorescence head (Licor Inc., Lincoln, NE, USA). The level of CO_2 was 400 μmol mol^{-1}, the rate of air flow 250 μmol s^{-1}, and the leaf chamber temperature was 25°C. The RH in the leaf chamber was equal to ambient RH (approx. 70%). The measuring light source consisted of mixed red and blue LEDs and was set at 10% blue. The photosynthesis light response curve of each leaf was measured by increasing the PPFD stepwise until the increment in net CO_2-assimilation was < 0.3 μmol m^{-2} s^{-1}; this was considered to be A_{max}. This procedure was checked by measuring the operating efficiency of PSII (F_q'/F_m' using a saturating light pulse of >7000 μmol m^{-2} s^{-1}), which was less than 0.25 at light saturation (Baker, 2008).

The chlorophyll contents of leaves from the horizontally-grown plants were determined according to Porra *et al.* (1989) using dimethylformamide as solvent. Additional measurements were conducted in a normal, vertically-growing high-wire grown tomato crop (64 m^2 compartment with a stem density of 2.5 stems m^{-2}) to investigate the effects of natural changes in light intensity on leaf A_{max} profiles throughout the canopy. All other conditions were similar to those above except the cultivar used (*Lycopersicon esculentum* 'Belissimo'). A_{max} profiles were determined at approx. month intervals between June - September 2006 in a selected plot of four plants. For each profile, A_{max} was measured on a set of six leaves, ranging in age and position from recently fully-grown (> 21 d-old) at the top of the canopy to the lowest leaves of the canopy, which were approx. 70 d-old. The Leaf Area Index (LAI; m^2 Leaf Area per m^2 ground surface) above each leaf, whose A_{max} was measured, was estimated by marking all leaves within the plot and later measuring their areas (Li-3100; Licor Inc, Lincoln, NE, USA).

The possible effects of leaf age and $PPFD_{LD}$ on A_{max} and on chlorophyll content were analysed using single and multiple regression analysis (Genstat statistical package, release 13.2; Rothamsted Experimental Station, Harpenden, UK). *P*-values ≥ 0.05 were regarded as non-significant (ns).

Results

During the experimental periods, leaves developed and functioned as mature leaves at different levels of daily PPFD integrals (Figure 1). During Winter the daily PPFD integrals were low and stable. In early Spring daily PPFD integrals increased, while in late Spring the daily PPFD integrals fluctuated around a level that was approx. six times higher than in Winter.

Multiple linear regression analysis on the data from all seasons together shows a small, but significant effect of leaf age and a large significant effect of $PPFD_{LD}$ on the

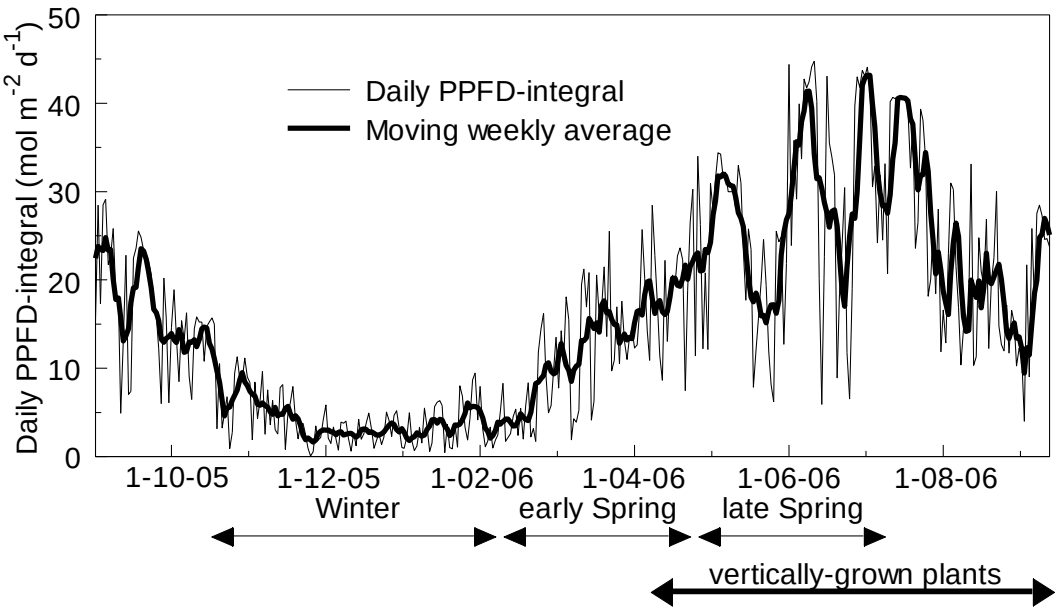

Fig. 1. Daily PPFD integral inside the greenhouse during the experiments with horizontally- and vertically-grown tomatoes (thin solid line). Heavy solid line shows the moving average over 7 d. Three thin arrows on the x-axis show the growth periods of horizontally-grown plants in 'Winter', 'early Spring', and 'late Spring'. Leaves were measured at the end of each period. The thick arrow shows the growth period for the vertically-grown plants.

A_{max} of the leaves of non-shaded plants. There is no significant interaction between leaf age and the $PPFD_{LD}$, and the model accounts for 64.5% of the variance (with $P = 0.003$ and $P < 0.001$ for the variables leaf age and the $PPFD_{LD}$, respectively), of which approx. 80% is due $PPFD_{LD}$. However, when the data from the different seasons is analysed separately, multiple linear regression does not show any significant effect of $PPFD_{LD}$ in Winter and late Spring, while only in late Spring a significant effect of leaf age ($P = 0.024$) is found. In early Spring the model accounts for 76% of the variance but the estimates of leaf age and $PPFD_{LD}$ in the model were not significant, which indicates that the explanatory variables in the model are confounded. Simple regression analysis on the relationships between A_{max} and leaf age and between A_{max} and $PPFD_{LD}$ show more straightforward results per season. In both Winter and late Spring, leaf photosynthetic capacity (A_{max}) was similar in all mature leaves on horizontally growing non-shaded plants, irrespective of leaf age (Figure 2A; P = ns). In late Spring, A_{max} values were significantly higher than in Winter ($P < 0.008$). In early Spring, the A_{max} decreased with increasing leaf age ($P < 0.001$) and, in contrast to Winter and late Spring, a positive correlation was found between the $PPFD_{LD}$ and A_{max} (Figure 2B; $P < 0.001$). In late Spring, the A_{max} was remarkably constant over a wide range of $PPFD_{LD}$ values. Chlorophyll contents per leaf area were independent of leaf age in all seasons, but differed between seasons. Chlorophyll contents were significantly lower in Winter than in early and late Spring ($P < 0.001$). A small but positive effect of $PPFD_{LD}$ on

chlorophyll content was found in early and late Spring, but not in Winter (Figure 2D; $P = 0.004$). These results imply that the decreasing effect that leaf age has on A_{max} is much smaller than the effect of $PPFD_{LD}$ on A_{max}, because variations in $PPFD_{LD}$ can be large (between 50 and 800 mol m^{-2}) while the life-time of a leaf on a tomato plant in commercial tomato production is relatively short (approx. 50 days after leaf expansion).

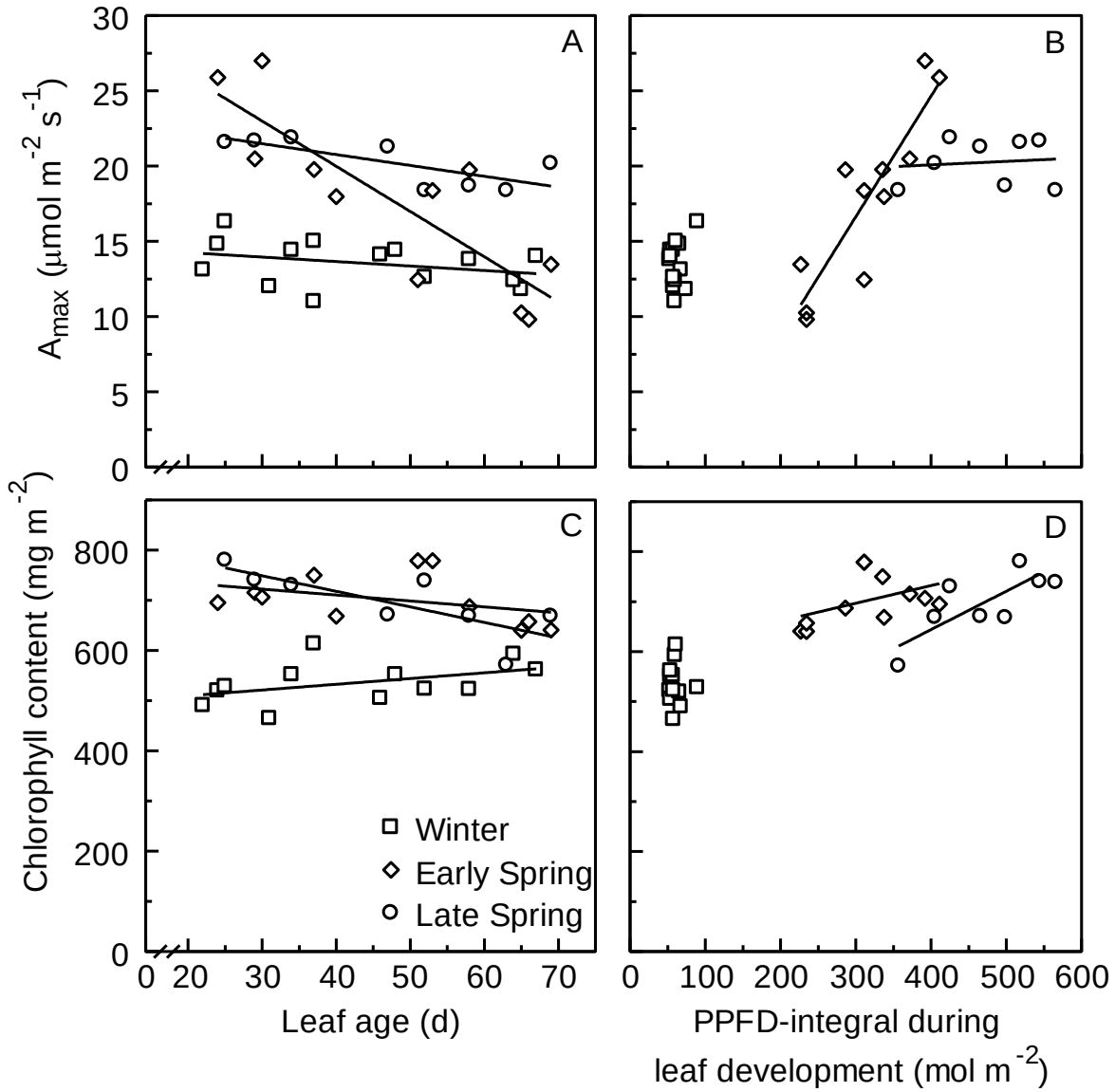

Fig. 2. A_{max}-values and chlorophyll contents of leaves on horizontally-grown tomato plants, measured in Winter, early Spring, and late Spring vs. leaf age (A, C) or vs. the PPFD integral received during the first 21 d of leaf development (B, D).

Shading of mature leaves resulted in decreases in both A_{max} and chlorophyll content in all seasons (Figure 3A, C). In early and late Spring, but not in Winter, A_{max} in fully expanded leaves decreased with leaf age and increased with $PPFD_{LD}$ (Figure 3A, B; $P < 0.001$ and $P < 0.007$, respectively), but similar as in the non-shaded leaves,

the effect of PPFD-integral during leaf development was much larger than the effect of leaf ageing. Chlorophyll content was independent of leaf age and $PPFD_{LD}$ (Figure 3C, D; P = ns). In shaded leaves, the chlorophyll contents in late Spring were greater than in early Spring, which was also greater than in Winter ($P < 0.007$; $P < 0.001$, respectively).

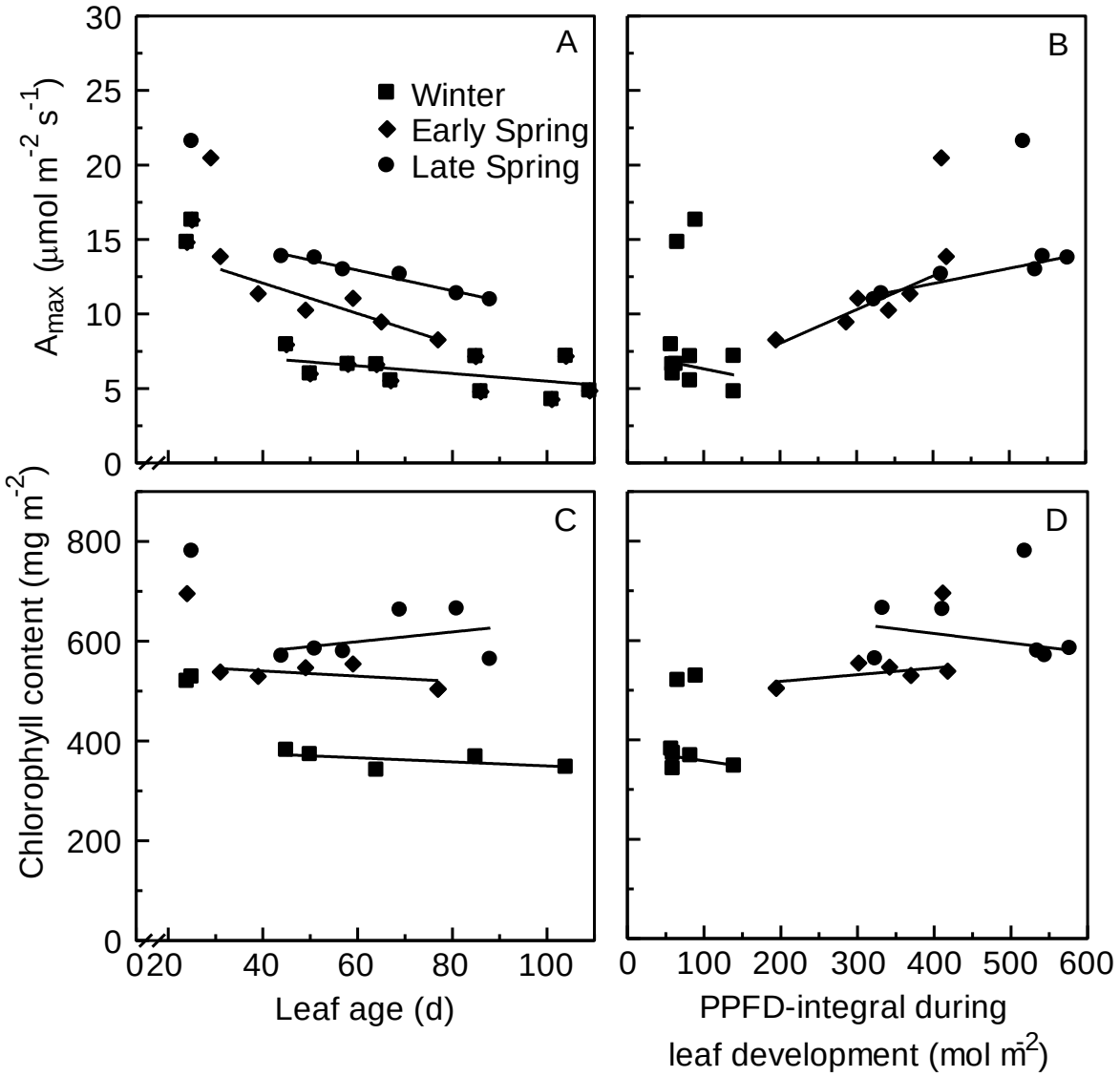

Fig. 3. A_{max}-values and chlorophyll contents of leaves on shaded horizontally-grown tomato plants, measured in Winter, early Spring, and late Spring vs. leaf age (A, C) or vs. the PPFD integral received during the first 21 days of leaf development (B, D). Leaves were shaded after approx. 25 d of leaf development. Youngest non-shaded leaves were excluded from the regression analysis.

Figure 4A shows the decrease in A_{max} from the top to the bottom of the canopy in a vertically-growing crop, measured at four dates in the Summer. Large differences in A_{max} between the young, fully-expanded, upper non-shaded leaves were found at different dates. Also in the vertically-grown crop the $PPFD_{LD}$ correlated well with A_{max}

(Figure 4B), whereas deeper in the canopy almost no differences in A_{max} were found (Figure 4A). The vertical A_{max} profiles measured in mid-Summer (July 25th) showed a sharper decrease in A_{max} with depth in the canopy, than the vertical A_{max} profiles measured in late Spring (June 7th) and late Summer (September 6th).

Discussion

Effects of leaf age, total $PPFD_{LD}$, and daily PPFD-integrals in the recent past on photosynthetic capacity.

In this research we wished to determine if leaf age could negatively affect A_{max} during the usual life-span of leaves on tomato plants cultivated in a commercial greenhouse. It is generally assumed that A_{max} decreases with leaf age, but most studies examining leaf photosynthesis in response to leaf age did not consider the interaction between PPFD and leaf age (Hikosaka, 1996; Hikosaka *et al.*, 1994). Reports that took this interaction explicitly into account are scarce. Sassenrath-Cole *et al.* (1996) found that the A_{max} of cotton plants decreased with leaf age (between 24 - 58 d) independent of the PPFD. Hikosaka (1996) reported reductions in chlorophyll- and rubisco-contents in the oldest leaves (between 42 -46 d) of horizontally growing *Ipomoea* vines without any external induction (light intensity and nitrogen supply) and proposed a genetically determined upper limit to longevity of leaves. Recently, Pettersen *et al.* (2010b) showed that the A_{max} of individual leaves did not decline with leaf age during the first 30 d in horizontally grown cucumber plants under low PPFD. Our results show that in Winter and late Spring, leaf age (up to 70 d) not negatively influences A_{max} of tomato leaves (Figure 2A).

The effect of $PPFD_{LD}$ on A_{max} and on the later changes in A_{max} during ageing of the leaves in present experiments with horizontally-grown plants is complex. In contrast to the Winter and late Spring experiments, in the early Spring experiment A_{max} seems to decrease with leaf age. However, in this experiment the older leaves on the plants developed under significantly lower $PPFD_{LD}$ than the younger leaves (Figure 1). Consequently, the A_{max}-values before ageing of leaves started were lower in the older leaves than in the younger leaves, and a lower measured A_{max} in older leaves than in younger leaves could have been caused simply by a lower initial A_{max} instead of by a longer period of ageing. Especially in the early spring experiment, with a rather steep increase in daily PPFD-integrals, A_{max} significantly correlated well with the $PPFD_{LD}$ (Figure 2B). The multiple regression analysis on the complete data set revealed that both leaf age and $PPFD_{LD}$ influenced A_{max}, but that the effect of light intensity during leaf development, probably on initial A_{max}, was much larger than the effect of leaf ageing. In the vertically-grown plants, the A_{max} of just fully-grown, upper leaves (approx. 25 d old) also strongly correlated with the PPFD integral during leaf development (Figure 4B), as was previously observed in many other species (*e.g.* Evans, 1993; Oguchi *et al.*, 2003; Sims and Pearcy, 1989; Sims and Pearcy, 1992).

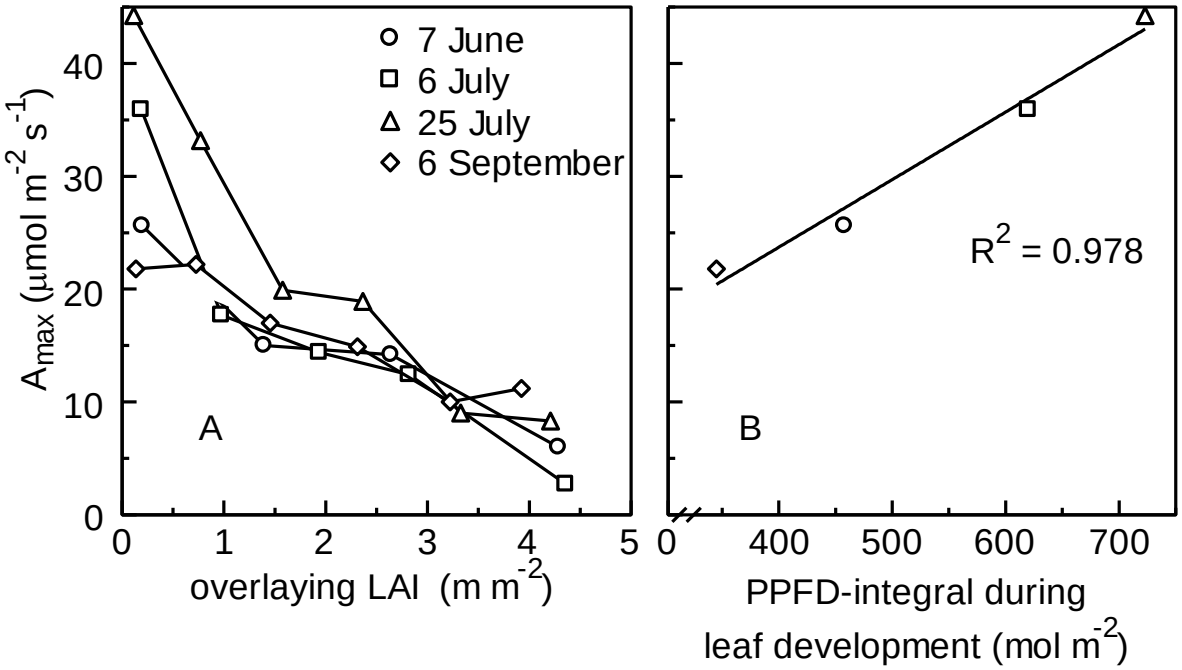

Fig. 4. The effect of overlaying LAI on the A_{max} of leaves in vertically-grown tomato plants measured on four dates in Summer (A) and the effect of the PPFD integral received during 21 d of leaf development on the A_{max} of the youngest mature leaves (B).

Oguchi *et al.* (2003) showed that leaf anatomy influences A_{max}, and that cell size is fixed after a period of leaf development in response to PPFD. A further increase in A_{max} after the leaf expansion phase is physically limited by cell size. Chlorophyll contents increased with increasing $PPFD_{LD}$'s (Figure 2D), which is commonly found when plants are grown at higher PPFDs (Boardman, 1977).

The effect of the $PPFD_{LD}$ on A_{max} and on later changes in A_{max} imply that measurements of vertical A_{max}-profiles in a canopy must be carefully interpreted. Seasonal patterns in light intensity can influence the outcome of these measurements. During increasing daily PPFD-integrals, such as occurs in Spring, a very steep vertical profile of A_{max} can be found in a canopy, only because the A_{max}-values of the older leaves deeper in the canopy were initially lower than the A_{max} of the younger upper leaves that developed later (Figure 4A).

The A_{max} of late Spring leaves does not clearly show an increase with increasing $PPFD_{LD}$, while the daily PPFD-integrals were relatively high. This suggests that for cv 'Pronto' at a certain PPFD integral a maximal A_{max} is established. This maximal A_{max} was lower than the A_{max} measured on the uppermost leaves of the vertically-grown tomato plants (cv Belissimo; Figure 2A and Figure 4A).

We also investigated if shading of mature leaves interacted with the A_{max}-leaf age profiles in the plants. Shading of mature leaves caused a sharp drop in A_{max} and chlorophyll contents (Figure 3A, C). In Winter, at very low light intensity, no age dependent decrease in A_{max} was found. In early and late Spring, however, a small

statistically significant effect of leaf age on A_{max} was found. The decrease of A_{max} with increasing leaf age under shade might also be caused by the changes in the $PPFD_{LD}$. The A_{max}-values of shaded leaves positively correlated with an increase in $PPFD_{LD}$ (Figure 3B). However, the A_{max}-values of non-shaded leaves with approx. similar $PPFD_{LD}$'s were higher (Figure 2B and 3B). It is therefore not likely that the $PPFD_{LD}$ limited the A_{max} after shading. Weaver and Amasino (2001) found that older leaves senesce earlier if they are in a lower light environment than the younger leaves, while shading of whole plants delayed the senescence of older leaves. In our experiments with some non-shaded young leaves and many shaded older leaves on one plant, leaf senescence seems unlikely, because after the immediate drop in chlorophyll content of just fully-grown leaves after shading, chlorophyll contents remained constant with leaf age (Figure 3C). A decrease in chlorophyll content due to shading (Figure 3B) was also found by Pons and Pearcy (1994). Nonetheless, the shading treatments show that A_{max} of leaves can be strongly influenced by the recent incident PPFD level.

Implications for the use of intracanopy lighting

The efficiency of intracanopy lighting as assimilation lighting strategy in horticulture would be limited if leaf age negatively influenced A_{max}. Our results show that leaf age did not constrain A_{max} within the life-span (up to 70 d) of tomato leaves in a commercial growth system. This implies that for the application of intracanopy lighting in a commercial tomato growth system the photosynthetic capacity of older leaves is not an intrinsically limiting factor that restricts crop photosynthesis. However, intracanopy lighting, which is applied deep in the canopy, might be more beneficial in autumn than in Spring, because in Winter or early Spring, leaves deep in the canopy will have developed under low PPFDs while in autumn, leaves deep in the canopy have developed under high PPFDs.

Acknowledgements

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CHAPTER 3

Photosynthetic acclimation in relation to nitrogen allocation in cucumber leaves in response to changes in irradiance

Abstract

Leaves deep in canopies can suddenly be exposed to increased irradiances following *e.g.* gap formation in forests or pruning in crops. Studies on the acclimation of photosynthesis to increased irradiance have mainly focussed on changes in photosynthetic capacity (A_{max}), although actual irradiance often remains below saturating level. We investigated the effect of changes in irradiance on the photosynthesis irradiance-response and on nitrogen allocation in fully grown leaves of *Cucumis sativus*. Leaves that fully developed under low (50 μmol m^{-2} s^{-1}) or moderate (200 μmol m^{-2} s^{-1}) irradiance were subsequently exposed to, respectively, moderate (LM-leaves) or low (ML-leaves) irradiance or kept at constant irradiance level (LL- and MM-leaves). *A*cclimation of photosynthesis occurred within seven days with final A_{max} highest in MM-leaves, lowest in LL-leaves, and intermediate in ML- and LM-leaves, whereas full acclimation of thylakoid processes underlying PSII efficiency and non-photochemical quenching occurred in ML- and LM-leaves. Dark respiration correlated with irradiance level, but not with A_{max}. Light-limited quantum efficiency was similar in all leaves. The increase in photosynthesis at moderate irradiance in LM-leaves was primarily driven by nitrogen import, and nitrogen remained allocated in a similar ratio to Rubisco and bioenergetics, while allocation to light harvesting relatively decreased. A contrary response of nitrogen was associated with the decrease in photosynthesis in ML-leaves. Net assimilation of LM-leaves under moderate irradiance remained lower than in MM-leaves, revealing the importance of photosynthetic acclimation during the leaf developmental phase for crop productivity in scenarios with realistic, moderate fluctuations in irradiance that leaves can be exposed to.

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Introduction

It is well known that plants vary the composition and organization of the photosynthetic apparatus in response to changes in incident irradiance. This acclimation phenomenon is expected to retain efficient photosynthesis and utilisation of resources such as nitrogen (Walters 2005). In erect natural and agricultural plant stands, irradiance decreases exponentially with canopy depth (Monsi and Saeki 2005) and therefore young leaves are exposed to a progressively decreasing irradiance due to shading by newly developing leaves. Conversely, these shade acclimated leaves can suddenly be exposed to higher irradiance, *e.g.* due to gap formation in forests (Naidu and DeLucia 1997ab, 1998; Oguchi *et al*. 2006, 2008; Yamashita *et al*. 2000) or due to pruning or intermediate harvests in crops (Calatayud *et al*. 2007). Acclimation of low-light acclimated leaves to higher irradiance levels can also occur in leaves of greenhouse crops under low natural irradiance conditions. For example, seedlings may be exposed to supplementary assimilation lighting after transplantation, or when leaves of high-wire grown crops are exposed to intracanopy lighting, *i.e.* assimilation lamps positioned within the canopy instead of above the canopy (*e.g.* Heuvelink *et al*. 2006; Hovi *et al*. 2004, 2006; Hovi-Pekkanen and Tahvonen 2008; Trouwborst *et al*. 2010).

It has been shown that the light-limited quantum efficiency does not differ between leaves which developed under either sun or shade conditions, but dark respiration is generally lower in shade leaves, which allows for a higher net photosynthesis at low irradiances (Boardman 1977; Björkman and Demmig 1987). The strictly linear light-limited range of the photosynthetic irradiance-response, however, is smaller for shade leaves than for sun leaves (Boardman 1977). Although several studies reported small to big increases in A_{max} after low-light acclimated leaves were exposed to a higher irradiance (Naidu and DeLucia 1997ab, 1998; Oguchi *et al.* 2003, 2005, 2006, 2008), no details have been published on how these changes affect the light-limited range and the curvature of the photosynthetic irradiance-response curve of fully expanded leaves (Niinemets 2007). However, for crops at higher latitudes, which are often growing under non-saturating irradiance conditions, these features are more important for productivity than A_{max}.

The organic nitrogen content (N_{org}) of leaves is closely linked to the size of the photosynthetic apparatus, the proteins of which contain more than half of the leaf N_{org} (Evans and Seemann 1989; Makino and Osmond 1991). The photosynthetic apparatus has two important functions that require nitrogen: light acquisition and light utilisation (Hikosaka 2005; Evans and Seemann 1989). Light acquisition is due to the chlorophyll-protein complexes of the light harvesting complexes (including in this case the antenna complexes of PSII) of both photosystems. For light utilization a distinction is normally made between electron transport and metabolism. Different approaches

for making this distinction have been used. Evans (1996), for example, subdivided the components associated with light utilisation into thylakoid (light reaction, non-soluble) and stromal (dark reaction, soluble) protein pools. Some studies using an analysis based upon the properties of A-C_i curves subdivided the components into those associated with Rubisco limitation and limitation by RuBP-regeneration (*e.g.* Feng *et al*. 2007; Katahata *et al*. 2007; Pons and Pearcy 1994). Originally the latter approach could be viewed analogously to the thylakoid electron transport and stromal dark reaction division (*e.g.* Evans, 1996), but the realisation that SBPase, an enzyme of the Calvin cycle, may limit RuBP-regeneration (Harrison *et al*. 1998; Harrison *et al*. 2001; Raines 2003) blurs the simple association of RuBP-regeneration with limitation by thylakoid electron transport processes and Rubisco limitation with the Calvin cycle. As both methods have their limitations we chose to use the allocation model of Niinemets and Tenhunen (1997), which is based on A-C_i curves, as it is a well described and functional approach. In this model, the fraction of N_{org} in the RuBP regeneration process is expressed as N_{org} in bioenergetics (P_B), and the fraction of N_{org} in carboxylation, which is mainly Rubisco-limited, is expressed as N_{org} in Rubisco (P_R). The fraction of N_{org} involved in light harvesting is expressed here as P_L.

The absorption of the photosynthetically active wavelength range of incident daylight radiation by green leaves is curvilinearly related to the chlorophyll content (Evans and Poorter 2001). When the absorption is over 85% (for most crop plants) this is nearly independent of chlorophyll concentration, thus light harvesting is approximately saturating in most cases (De Groot *et al*. 2003; Evans 1993). Assuming that light harvesting is non-limiting, an increase in the light-limited range of the photosynthetic irradiance-response curve and A_{max} would require increases in the rate limiting processes (linear electron transport and Rubisco limited processes) and likewise their nitrogen content. Both redistribution of nitrogen within the leaf or the photosynthetic apparatus, and nitrogen import into the leaf, could contribute to an increase in the light-limited range and A_{max}, which is also likely to be associated with changes in the fractions of nitrogen invested in the different pools, P_L, P_B and P_R. However, so far, limited data on the consequences of an increase in irradiance on nitrogen allocation within the photosynthetic apparatus have been published. Frak *et al*. (2001) found that in leaves exposed to an increase in irradiance, both the amount of nitrogen increased and the allocation of nitrogen between the photosynthetic functions was altered. Oguchi *et al*. (2003) reported that in leaves exposed to an increase in irradiance both the amount of nitrogen and the photosynthetic nitrogen use efficiency (PNUE; A_{max} divided by N_{org}) increased, implying a change in the allocation of nitrogen to the different photosynthetic functions.

We investigated acclimation of the leaves of a high-light crop plant (cucumber) developed under a low or a moderate irradiance that were subsequently exposed to moderate or low irradiance. Although the irradiance levels used are relatively low, they are similar to common natural and supplemental irradiances in greenhouses in

the Netherlands in winter (Heuvelink *et al*. 2006). We focussed not only on A_{max}, but on the complete photosynthesis irradiance response curve, including chlorophyll fluorescence parameters, dark respiration, light-limited quantum efficiency and curvature and related the responses of photosynthesis to changes in irradiance to nitrogen allocation within the photosynthetic apparatus using the model of Niinemets and Tenhunen (1997).

Materials and methods

Plant growth

Cucumber plants (*Cucumis sativus* cv. Hoffmann's Giganta) were cultivated in a climate chamber (conditions as in Hogewoning *et al*., 2010b, except that here we used an electrical conductivity of 2.0±0.1 mS cm^{-1}). Half of the plants were exposed to moderate irradiance (200 μmol m^{-2} s^{-1}) and the other half to low irradiance (50 μmol m^{-2} s^{-1}) provided by cool white fluorescent tubes (12h photoperiod; TLD 50W 840HF master, Philips, The Netherlands). Low irradiance was achieved by using a neutral density filter with a transmittance of 25% (Lee filters 210.06ND, Andover, England). Irradiance was routinely measured with a Li-190 quantum sensor (Li-Cor Inc., Lincoln, Nebraska, USA). The plants were trained horizontally to avoid shading of older leaves by newly developing leaves. Measurements were made on the second leaf of each plant and the experiments started when these leaves were fully grown. At the start of an experiment, plants were exposed to either a decrease in irradiance (ML-leaves) or an increase in irradiance (LM-leaves). As controls, plants were grown under a constant moderate (MM-leaves) or a low irradiance (LL-leaves).

Measurements

Photosynthesis irradiance-response curves were determined on day 0, 2, 4 and 7 and 10 after the beginning of the trial. The experiments ended when A_{max} was stable over two subsequent measuring days, which was for the LM-leaves on days 4 and 7 and for ML-leaves on days 7 and 10. On day 0 and 7 the photosynthetic response to internal CO_2 (A-C_i curve) was measured and samples for leaf mass per area (LMA), chlorophyll and organic nitrogen content were collected.

Photosynthetic rates (A_{net}) were measured with a portable gas analysis system (LI-6400 equipped with a leaf chamber fluorometer; Li-Cor inc., Lincoln, NE, USA). The response of A_{net} to irradiance was determined by increasing the irradiance from zero to saturation and the A-C_i curve was measured just after reaching the saturating irradiance level. The leaf chamber temperature was set at 25°C, the air flow at 250 μmol s^{-1}, the CO_2 concentration during the irradiance-response measurements at 400 μmol mol^{-1} and the LED light source was set at 10% blue light. Water vapour concentration was similar to that in the ambient air (22.5 mmol mol^{-1}; RH=70%). At

each irradiance level (maximum time to steady-state: 15 minutes) or CO_2 level (time to steady-state: 5 minutes), the A_{net} and C_i were calculated as the mean value during a 40s window following the establishment of a stable CO_2-fixation rate. Measured A_{net} during A-C_i curve determinations were corrected for diffusion leaks as experimentally determined according to the Li-COR manual (2005) and Flexas *et al*. (2007). Dark respiration (R_D) and F_v/F_m (maximum quantum efficiency for PSII photochemistry of dark adapted leaves; Van Kooten and Snel,1990) were measured at the start of each irradiance-response curve after 30 minutes of dark adaptation in the leaf chamber. For the chlorophyll fluorescence measurements the measuring beam intensity was 0.1 μmol m^{-2} s^{-1} and the saturating light pulse was >7000 μmol m^{-2} s^{-1} for 0.8s.

Chlorophyll, LMA, total nitrogen (N_t) and nitrate content (N_n) were determined as described in Trouwborst *et al* (2010). Organic nitrogen (N_{org}) was calculated by subtracting N_n from N_t. The leaf absorptance spectrum was measured in nm steps according to Hogewoning *et al*. 2010ab and by multiplying the absorptance spectrum with the growth-light spectrum (or measuring-light spectrum), leaf absorption was obtained.

Calculations and statistics

Maximum PSII efficiency in light (F_v'/F_m'), PSII operating efficiency (Φ_{PSII}), PSII efficiency factor (q_P) and the electron transport rate (ETR) at growth light level were calculated according to Van Kooten and Snel (1990) and Baker *et al*. (2007) with use of a calculated F_0' according to Oxborough *et al*. (1997). For the calculation of ETR, we assumed an excitation balance of 0.5 and used the measured leaf absorption for the measuring-light spectrum used and Φ_{PSII}. A modified version of the Farquhar, von Caemmerer and Berry (FvCB) model (Farquhar *et al*. 1980) was fitted to the A-C_i data. We estimated J_{max} and V_{Cmax} normalized to 25°C using the non-linear fitting procedure NLIN in SAS (release 9.1.3; SAS institute, Cary, NC, USA). The model equations were adopted from Yin *et al*. (2004) and a parameterisation originally developed by Bernacchi *et al*. (2001) and Medlyn *et al*. (2002) was used. This model simultaneously fits J_{max} and V_{Cmax} without splitting the dataset, a procedure recommended by Dubois *et al*. (2007). Electron transport capacity was fitted using the equation for ATP limitation instead of NADPH limitation because the ATP-limited model includes a correction for pseudo-cyclic electron transport (Yin *et al*. 2006).

A non-rectangular hyperbola (Thornley 1976) was used to fit the irradiance-photosynthesis response data using the non-linear fitting procedure NLIN in SAS to determine dark respiration (R_D), maximum gross photosynthetic rate (A_{mg}), light-limited quantum efficiency (α) and the scaling constant for the curvature (θ):

$$A_{net} = \frac{\alpha \cdot PPF + A_{mg} - \sqrt{(\alpha \cdot PPF + A_{mg})^2 - 4\theta \cdot PPF \cdot A_{mg}}}{2\theta} - R_D \qquad \text{(eq. 1)}$$

The estimated fractions of N_{org} involved in carboxylation (fraction N_{org} in Rubisco; P_R), in the protein component limiting the capacity for photosynthetic

electron transport and photophosphorylation (fraction N_{org} in bioenergetics; P_B), and in the protein component of chlorophyll-complexes associated with light harvesting (fraction N_{org} in light harvesting; P_L) were calculated according to Niinemets and Tenhunen (1997) as:

$$P_R = \frac{V_{Cmax}}{6.25 \cdot V_{cr} \cdot N_{org}} \quad \text{(eq. 2)}$$

$$P_B = \frac{J_{max}}{8.06 \cdot J_{mc} \cdot N_{org}} \quad \text{(eq. 3)}$$

$$P_L = \frac{C_C}{C_B \cdot N_{org}} \quad \text{(eq. 4)}$$

Where C_C is the chlorophyll concentration (mmol m^{-2}). V_{cr}, J_{mc} and C_B are, respectively, the specific activity of rubisco (20.78 μmol CO_2 g^{-1} Rubisco s^{-1}), the specific activity of electron transport (155.65 μmol electrons μmol^{-1} cytochrome f s^{-1}) and the ratio of leaf chlorophyll to organic leaf nitrogen in light harvesting components (2.15 mmol g^{-1}) at 25 °C (Niinemets and Tenhunen 1997; Niinemets *et al*. 1998). The factor 6.25 (g Rubisco g^{-1} nitrogen in Rubisco; Eq. 2) is the conversion coefficient between N_{org} and the protein content of Rubisco, and 8.06 (μmol cytochrome f g^{-1} nitrogen in bioenergetics; Eq. 3) is the conversion coefficient between cytochrome f and nitrogen in the bioenergetics pool (Niinemets and Tenhunen 1997; Niinemets *et al*. 1998).

The fraction of N_{org} invested in the photosynthetic apparatus (P_{phot}) was calculated as the sum of P_R, P_B and P_L, and the absolute amount of N_{org} invested in the photosynthetic apparatus (N_{phot}) as the product of N_{org} and P_{phot}. The sub-fraction of P_{phot} allocated to light acquisition ($P_{light\ acquisition}$) was calculated as $P_{light\ acquisition}=P_L/P_{phot}$, and the sub-fraction of P_{phot} dedicated to light utilization ($P_{light\ utilization}$) was calculated as $P_{light\ utilization}= (P_B+P_R)/P_{phot}$. Photosynthetic nitrogen use efficiencies (PNUE) were calculated as the photosynthetic rates at both growth irradiances and at saturating irradiance divided by N_{org}.

The four treatments together (blocks) were repeated four times in time. All results were analysed with one way ANOVA with time as blocks followed by a post hoc Fisher's LSD multiple comparisons test (P<0.05) using Genstat (release 11.1, Rothamsted Experimental Station, Harpenden UK).

Results

Kinetics and extent of photosynthetic acclimation

The photosynthetic capacity (A_{max}) of MM-leaves was twice as high as the A_{max} of LL-leaves (Fig. 1A and Table 1). Full acclimation, represented by stable values of A_{max}, was reached at day 4 (and verified on day 7) for LM-leaves, and at day 7 (and verified on day 10) for ML-leaves (Fig. 2A; data point at day 10 not shown). The fully acclimated

A_{max} of the LM-leaves remained lower than of the MM-leaves, whereas the final value of A_{max} in the ML-leaves remained significantly higher than that of the LL-leaves (Fig. 2A and Table 1).

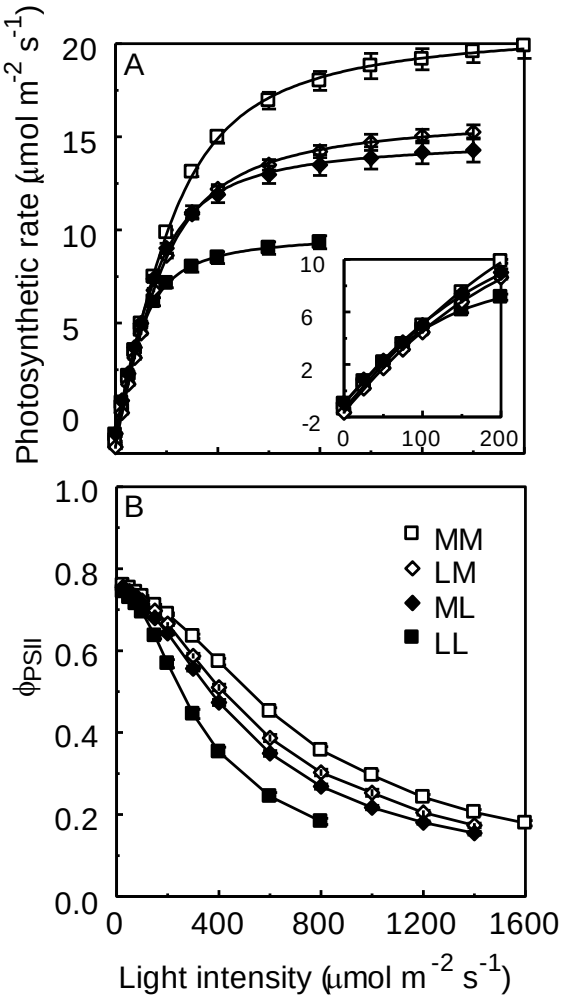

Fig. 1. The effect of a change in growth irradiance on the photosynthetic irradiance-response of fully expanded cucumber leaves grown under low irradiance and exposed to moderate irradiance (LM) and vice versa (ML) after an acclimation period of 7 days. LL and MM respectively, represent the treatments grown under continuously low or moderate irradiance. Lines through data points represent the fit to the non rectangular hyperbola (eq. 1). Each data point represents the mean of 4 repetitions (two plants per replicate in time) and vertical bars represent the SE. Inset shows the light-limited quantum efficiency on a bigger scale.

The assimilation rate, Φ_{PSII}, ETR and g_s at moderate growth irradiance ($A_{net(200)}$, $\Phi_{PSII(200)}$, $ETR_{(200)}$ and $g_{s(200)}$; 200 μmol m^{-2} s^{-1}) also increased significantly in LM-leaves compared to LL-leaves, but did not reach the level of the MM-leaves. For the ML-leaves, $A_{net(200)}$, $\Phi_{PSII(200)}$, $ETR_{(200)}$ decreased significantly compared to the MM-leaves, but did not fall to the level of the LL-leaves (Fig. 2B, Table 1), whereas $C_{i(200)}$ did not differ among all treatments. The net assimilation rate at low growth irradiance ($A_{net(50)}$;

50 μmol m^{-2} s^{-1}) of LM-leaves fell to a value lower than all other treatments (Fig. 2C), due to a fast increase in R_D slightly over the level of MM-leaves (Fig. 2D; Table 1). For ML-leaves the R_D decreased significantly compared to MM-leaves, whereas $A_{net(50)}$ increased only slightly (Fig. 2CD; Table 1).

Table 1. Measured photosynthetic rates and fitted parameters of fully expanded cucumber leaves grown under low irradiance and exposed to moderate irradiance (LM) and vice versa (ML) after an acclimation period of 7 days. LL and MM represent the control treatments grown under, respectively, low or moderate irradiance. Data are means ± SE (n=4). Different letters in a row indicate a significant difference at P<0.05.

	MM	LM	ML	LL
measured parameters				
F_v/F_m	0.80±0.002	0.79±0.003	0.80±0.003	0.80±0.001
R_D (μmol m^{-2} s^{-1})	1.41±0.06^a	1.66±0.09^a	0.94 ±0.05^b	1.00±0.09^b
$A_{net(50)}$ (μmol m^{-2} s^{-1})	2.09±0.07^a	1.72±0.06^b	2.29±0.10^a	2.15±0.10^a
$A_{net(200)}$ (μmol m^{-2} s^{-1})	9.85±0.14^a	8.62±0.13^b	9.01±0.27^b	7.13±0.23^c
A_{max} (μmol m^{-2} s^{-1})	19.9±0.7^a	15.3±0.4^b	14.3±0.7^b	9.33±0.34^c
$\Phi_{PSII(200)}$	0.69±0.002^a	0.67±0.003^b	0.64±0.008^c	0.57±0.01^d
$ETR_{(200)}$	67.5±0.2^a	63.9±0.3^b	62.3±0.8^b	54.6±1.0^c
$g_{s(200)}$	0.27±0.01^a	0.21±0.02^{bc}	0.26±0.03^{ab}	0.17±0.01^c
$C_{i(200)}$	325±1	318±6	326±5	320±4
fitted parameters				
α	0.072±0.003	0.072±0.002	0.071±0.001	0.070±0.003
θ	0.71±0.04	0.68±0.03	0.74±0.02	0.73±0.03
J_{max} (μmol m^{-2} s^{-1})	150.3±7.1^a	121.4±3.0^b	97.6±5.3^c	69.4±5.4^d
V_{Cmax} (μmol m^{-2} s^{-1})	83.7±2.7^a	62.4±1.4^b	53.5±2.5^c	38.6±2.2^d
J_{max}/V_{Cmax} ratio	1.80±0.08	1.95±0.04	1.83±0.03	1.80±0.04

Although F_v/F_m on day 0 did not significantly differ from F_v/F_m on day 7 (Table 1), it significantly changed during the acclimation period of the LM-leaves. In these leaves a decrease in F_v/F_m of 3.0% and 2.1% was found on day 2 and 4, compared with the value of MM-leaves (P<0.002, t-test), after which F_v/F_m progressively increased up to the value of MM-leaves (data not shown). Transient photoinhibition is not unusual when leaves are exposed to a higher irradiance (Naidu and DeLucia 1997a; Oguchi *et al*. 2006; Yamashita *et al*. 2000). J_{max} and V_{Cmax} showed approximately the same patterns as A_{max}. The J_{max} to V_{Cmax} ratio did not differ among the treatments (Table 1).

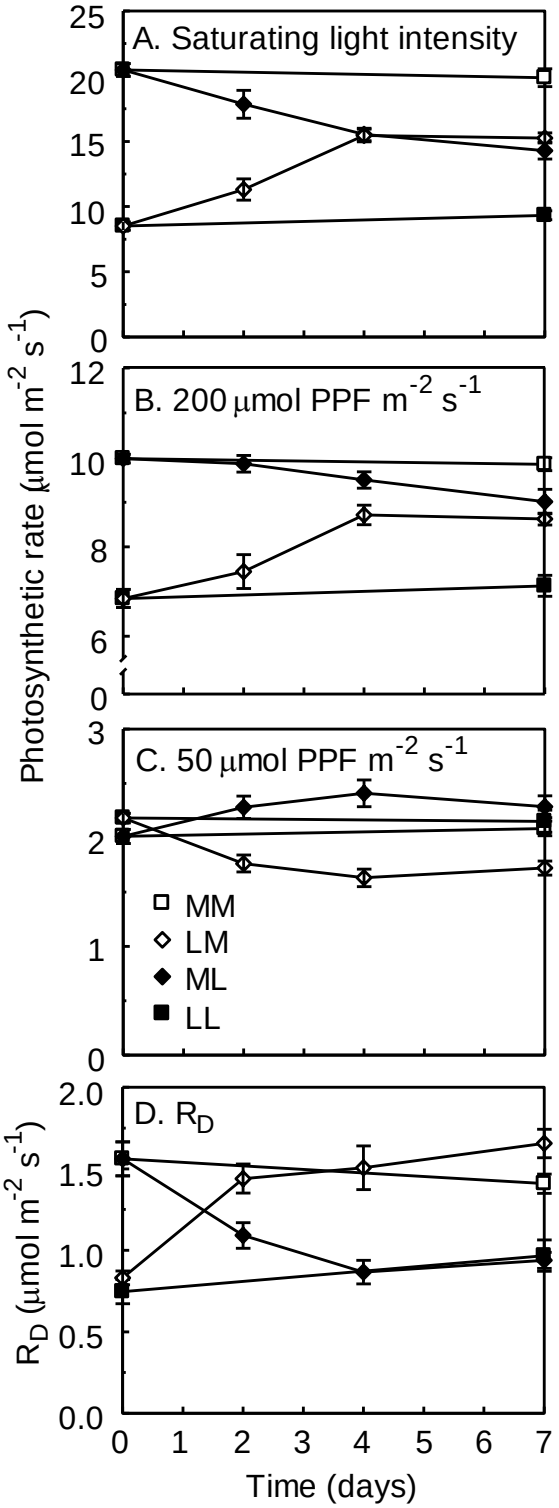

Fig. 2. Dynamics of change of photosynthetic rates at saturating irradiance, 200 and 50 μmol m^{-2} s^{-1} irradiance and the dark respiration (R_D) of fully expanded cucumber leaves grown under low irradiance and exposed to moderate irradiance (LM) and vice versa (ML) during an acclimation period of 7 days. LL and MM respectively, represent the treatments grown under continuously low or moderate irradiance. Each data point represents the mean of 4 repetitions (two plants per replicate in time) and vertical bars represent the SE.

The light-limited quantum efficiency (α) and the curvature parameter (θ) did not differ among treatments (Table 1). The linear light-limited range of the photosynthetic irradiance-response was smallest for the LL-leaves and largest for the MM-leaves (inset Fig. 1A). Similar to the photosynthetic irradiance response curves, Φ_{PSII} versus irradiance (Fig. 1B) showed a gradual response over all treatments: the fastest decrease for LL-leaves and the slowest decrease for MM-leaves, whereas LM- and ML-leaves showed an intermediate response. Numerically, Φ_{PSII} is the product of q_P and F_v'/F_m'. The relationships between Φ_{PSII} and both q_P, and F_v'/F_m' were almost identical for all treatments (Fig. 3A, B). Only at lower values for Φ_{PSII} the relationship between Φ_{PSII} and F_v'/F_m' fell into two classes (Fig. 3B inset): the two treatments ending with moderate irradiance (LM and MM) had, when Φ_{PSII} was low, values for F_v'/F_m' that were 5% lower, than those found in the treatments ending with low irradiance (ML and LL) (the opposite effect occurs for q_P, but is less conspicuous). Though the changes in the regulation of PSII revealed by this study are small, it is clear that there is full acclimation of these thylakoid processes for the LM- and the ML-leaves compared with these in respectively MM- and LL-leaves. The consequence of this acclimation is a slightly greater role for non-photochemical quenching (*i.e.* $1-F_v'/F_m'$ is a proxy for non-photochemical quenching) at low values of Φ_{PSII} in the leaves maintained under, or acclimated to, moderate irradiances, compared to the leaves maintained under, or acclimated to, low irradiances.

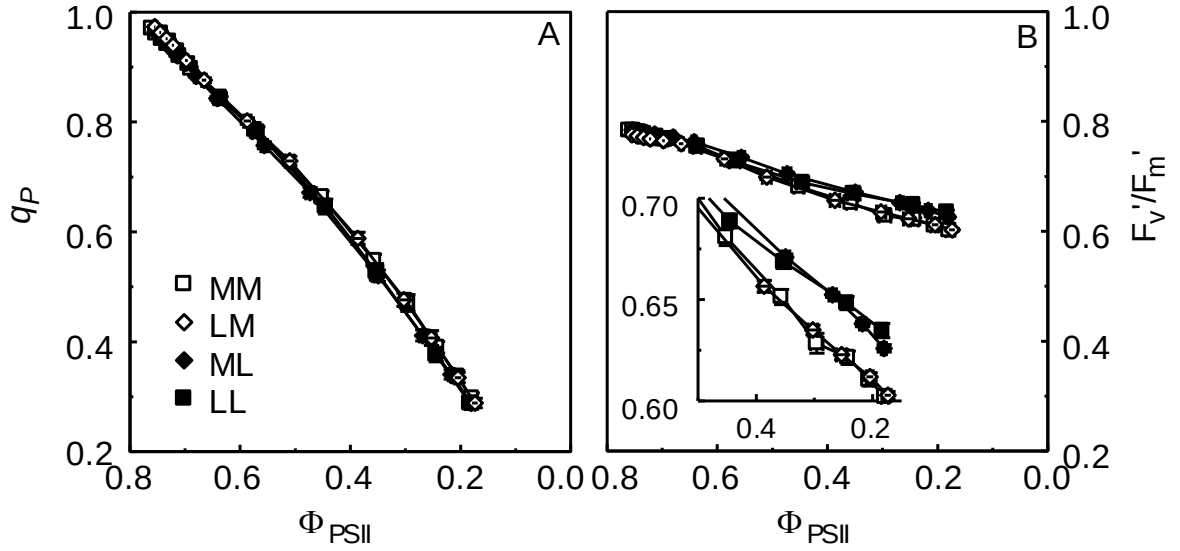

Fig. 3. The effect of a change in growth irradiance on the PSII operating efficiency (Φ_{PSII}) and the maximum PSII efficiency in the light (F_v'/F_m') versus the PSII efficiency factor (q_P) of fully expanded cucumber leaves grown under low irradiance and exposed to moderate irradiance (LM) and vice versa (ML) after an acclimation period of 7 days. LL and MM respectively, represent the treatments grown under continuously low or moderate irradiance. Each data point represents the mean of 4 repetitions (two plants per replicate in time) and vertical bars represent the SE.

Leaf parameters

At the end of the acclimation period, LMA was highest in MM-leaves and lowest in LL-leaves (Table 2). Notably the LMA of LM-leaves increased significantly, but not to the level of MM-leaves, while LMA of ML-leaves decreased significantly but not to the level of the LL-leaves (Table 2). Similar trends were observed for organic nitrogen content (N_{org}), nitrogen involved in the photosynthetic apparatus (N_{phot}), and (although not in all cases to a statistically significant degree) for chlorophyll content and leaf absorption under the growth light spectrum (Table 2). Leaf absorption remained significantly the highest for leaves developed under moderate (MM and ML) compared to under low irradiance (LL and LM). Nitrate content per unit area (N_n) showed a different pattern of response: LL-leaves and MM-leaves did not differ significantly in N_n, while the ML-leaves had an increased N_n and the LM-leaves had a reduced N_n compared with leaves of all other treatments. No significant differences in chlorophyll a/b ratio were observed (Table 2). Differences in light absorption by leaves of different treatments were small with growth light (Table 2) and even smaller with measuring light of photosynthesis (data not shown).

Table 2. Leaf composition of fully expanded cucumber leaves grown under low irradiance and exposed to moderate irradiance (LM) and vice versa (ML) after an acclimation period of 7 days. LL and MM represent the control treatments grown under, respectively, low or moderate irradiance. Data are means ± SE (n=4). Different letters in a row indicate a significant difference at $P<0.05$.

	MM	LM	ML	LL
LMA (g m^{-2})	27.6±1.1^{a}	24.3±1.3^{b}	23.3±1.2^{b}	15.4±0.9^{c}
N_{org} (g m^{-2})	1.37±0.05^{a}	1.20±0.03^{b}	1.17±0.05^{b}	0.70±0.04^{c}
N_n (g m^{-2})	0.06±0.01^{bc}	0.04±0.01^{c}	0.10±0.02^{a}	0.07±0.02^{b}
N_{phot} (g m^{-2})	1.08±0.02^{a}	0.87±0.03^{b}	0.80±0.04^{b}	0.56±0.03^{c}
Chlorophyll (mg m^{-2})	570±32$_{a}$	549±24^{a}	563±26^{a}	400±23^{b}
Chlorophyll a/b ratio	3.26±0.04	3.17±0.02	3.12±0.02	3.16±0.03
Leaf absorption (%)	90.6±0.3^{a}	88.3±0.3^{b}	91.0±0.1^{a}	86.7±0.1^{c}

Nitrogen allocation and photosynthetic nitrogen use efficiency

The fraction of organic leaf nitrogen (N_{org}) invested in the photosynthetic apparatus (P_{phot}) did not differ between both control treatments (LL- and MM-leaves; Table 3). The fraction N_{org} allocated to light harvesting components (P_L) was significantly higher in LL-leaves than in MM-leaves. Although no significant difference was found in the fractions of total N_{org} allocated towards the leaf components associated with light

utilisation (bioenergetics, P_B and carboxylation, P_R), both components tended to be lower in LL-leaves than in MM-leaves. As a result, the fractions of N_{org} within the photosynthetic apparatus allocated to light acquisition ($P_{light\ acquisition}=P_L/P_{phot}$) or light utilisation ($P_{light\ utilisation}=(P_B+P_R)/P_{phot}$) were clearly shifted between low and moderate irradiance: $P_{light\ acquisition}$ was significantly higher and $P_{light\ utilisation}$ significantly lower in LL-leaves compared to MM-leaves (Table 3).

Irrespective of the direction of the change in irradiance (being either an increase in LM-leaves or a decrease in ML-leaves) the fraction of total organic leaf nitrogen allocated to the photosynthetic apparatus decreased (although not statistically significant in LM-leaves). The shift in nitrogen partitioning between light utilisation ($P_{light\ utilisation}$) and light acquisition ($P_{light\ acquisition}$) within the experimental period was complete in ML-leaves and partial in LM-leaves (Table 3).

At the low measuring irradiance, $PNUE_{Anet(50)}$ was significantly higher for LL-leaves than for MM-leaves (Table 3). This difference disappeared with increasing measuring irradiance ($PNUE_{Anet(50)}$, $PNUE_{Anet(200)}$ and $PNUE_{Amax}$) during the photosynthesis measurements (Table 3). Leaves that acclimated to increased or decreased growth irradiance (respectively LM and ML) exhibited a lower PNUE than the leaves that were kept under steady growth irradiance (LL and MM), though for LM not at all measuring light intensities significant.

Table 3. Apparent nitrogen allocation within the photosynthetic apparatus and PNUE at different irradiances of fully expanded cucumber leaves grown under low irradiance and exposed to moderate irradiance (LM) and vice versa (ML). LL and MM represent the control treatments grown under, respectively, low or moderate irradiance. Data are means ± SE (n=4). Different letters in a row indicate a significant difference at $P<0.05$.

	MM	LM	ML	LL
P_B	0.092 ± 0.004^{a}	0.085 ± 0.002^{a}	0.069 ± 0.002^{b}	0.082 ± 0.004^{a}
P_R	0.48 ± 0.02^{a}	0.41 ± 0.01^{b}	0.36 ± 0.02^{c}	0.44 ± 0.02^{ab}
P_L	0.22 ± 0.01^{b}	0.24 ± 0.01^{b}	0.25 ± 0.01^{b}	0.30 ± 0.01^{a}
P_{phot}	0.79 ± 0.02^{a}	0.73 ± 0.02^{ab}	0.68 ± 0.02^{b}	0.82 ± 0.03^{a}
$P_{light\ acquisition}$	0.28 ± 0.01^{c}	0.33 ± 0.01^{b}	0.37 ± 0.01^{a}	0.37 ± 0.01^{a}
$P_{light\ utilisation}$	0.72 ± 0.01^{a}	0.67 ± 0.01^{b}	0.63 ± 0.01^{c}	0.63 ± 0.01^{c}
$PNUE_{Anet(50)}$ ($\mu mol\ s^{-1}\ g^{-1}\ N_{org}$)	1.52 ± 0.10^{bc}	1.38 ± 0.11^{c}	1.78 ± 0.07^{b}	3.17 ± 0.17^{a}
$PNUE_{Anet(200)}$ ($\mu mol\ s^{-1}\ g^{-1}\ N_{org}$)	7.21 ± 0.22^{b}	7.11 ± 0.31^{b}	7.52 ± 0.19^{b}	10.41 ± 0.55^{a}
$PNUE_{Amax}$ ($\mu mol\ s^{-1}\ g^{-1}\ N_{org}$)	14.5 ± 0.5^{a}	12.8 ± 0.4^{ab}	11.8 ± 0.4^{b}	14.0 ± 0.7^{a}

Discussion

Extent of acclimation

In agreement with, and in addition to, Boardman (1977), all light-limited quantum efficiencies (α) were equal, irrespective whether leaves were grown under steady low or moderate irradiance, or exposed to a change in irradiance (Fig. 1A; Table 1).

Although the net photosynthetic rate (A_{net}) at moderate growth irradiance (*i.e.* 200 μmol m^{-2} s^{-1} actinic light) was higher for LM-leaves than for LL-leaves, A_{net} of MM-leaves was still significantly higher (gross and net assimilation of respectively 10 and 14% higher). As the R_D in the MM- and LM-leaves was similar, this implies that the strict light-limited part of the photosynthesis-irradiance response extended to a higher irradiance in the MM-leaves than in the LM-leaves and the other treatments (inset Fig. 1A). This more rapid loss of strict light-limitation with increasing irradiance for LM-leaves was interrelated with the lower Φ_{PSII} and reduced leaf absorption, producing a 5% lower calculated ETR (Table 1). The cause of the remaining 5% difference in gross assimilation between LM- and MM-leaves is hard to explain from present data: the shape of the relationships between ETR and A_{gross} between dark and saturation (Fig. 4) do not show remarkable differences and C_i did not differ among the treatments (Table 1). Limitation by Rubisco activity/content at an irradiance level well below light-saturation is unlikely (Farquhar *et al*. 2001) and limitation by mesophyll conductance (Terashima *et al* 2006) is unexpected in a species like cucumber (Warren 2008), although they cannot be fully excluded as they were not measured.

Though on leaf level the photosynthesis-irradiance curves showed gradual responses from MM- to LL-leaves (Fig. 1A), on thylakoid level, the extent by which decreases in Φ_{PSII} produced by increasing irradiance originated in decreases in q_P (the PSII efficiency factor (Baker 2008)) or maximum quantum yield in the light (F_v'/F_m') were almost identical for all treatments (Fig. 3AB). Only at lower values for Φ_{PSII} did the relationship between F_v'/F_m' and Φ_{PSII} fall into two classes (Fig. 3B inset), one for leaves grown or acclimated to low irradiance, and one for leaves grown or acclimated to moderate irradiance, which implies full acclimation of these parameters to the growth irradiance. None of the leaves investigated in this study displayed the extent of light-induced decrease in F_v'/F_m' in relation to decreases in Φ_{PSII} reported in other studies (Bilger and Björkman 1990; Genty *et al*. 1990; Demmig-Adams *et al.* 1996), implying that these leaves had a relatively small ability for non-photochemical quenching (Baker, 2008). This implies that the differences in Φ_{PSII} among all treatments at moderate measuring irradiance ($\Phi_{PSII(200)}$; Table 1) are primarily attributable to differences in q_P and not in F_v'/F_m'. Hence, the lower the $\Phi_{PSII(200)}$ (LL<ML<LM<MM; Table 1) the more the ability to oxidise Q_A was limited (Baker 2008). Presumably, this limitation was due to a decreased amount of electron carriers in the electron transport chain per unit leaf area and / or down-regulation of electron transport rate as a result

of lower carboxylation, respectively reflected in less nitrogen in bioenergetics per unit area (P_B*N_{org}) and nitrogen in Rubisco per unit area (P_R*N_{org}; Table 2 and 3).

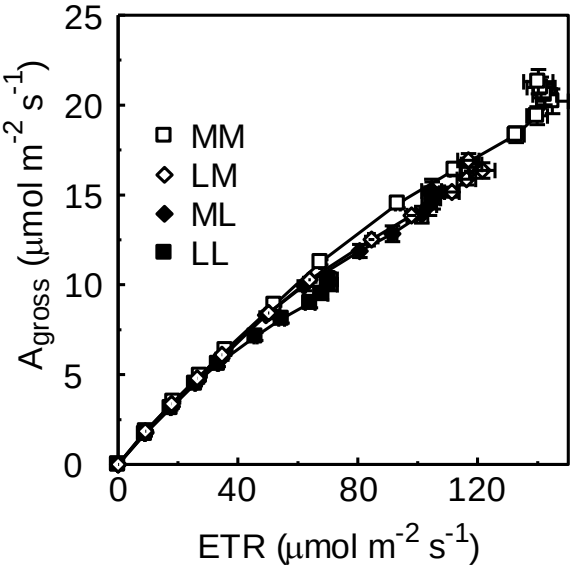

Fig. 4. The effect of a change in growth irradiance on the relationships between linear electron transport through PSII (ETR) and gross assimilation rate (A_{gross}) of fully expanded cucumber leaves grown under low irradiance and exposed to moderate irradiance (LM) and vice versa (ML) after an acclimation period of 7 days. LL and MM respectively, represent the treatments grown under continuously low or moderate irradiance. Each data point represents the mean of 4 repetitions (two plants per replicate in time) and bars represent the SE.

The photosynthetic capacity (A_{max}) of fully expanded leaves increased by more than 50% after a change from low to moderate irradiance, but did not reach the A_{max} level of leaves that developed under moderate irradiance. The present result in cucumber is in agreement with the work of Oguchi and co-workers on other non-woody species (Oguchi *et al*. 2003, 2005, 2006). They clearly showed that anatomical constraints were involved in the limitation for fully expanded leaves to increase their A_{max}. After full expansion little plasticity in mesophyll cell size remains and the increase in A_{max} can be restricted by the amount of unoccupied space for chloroplast expansion along the intercellular membrane surfaces of the mesophyll. Remarkably, the LMA of LM-leaves increased by almost 60% after exposure to moderate irradiance (Table 2). Oguchi *et al*. (2003) observed an increase in LMA of the same order of magnitude in fully expanded leaves of *Chenopodium album* that were exposed to an increase in irradiance, while leaf thickness only slightly increased. This increase in LMA may be due to an increase in protein content, as the increase in LMA was in the same order of magnitude as N_{org} (around 60%). A similar correlation was found for ML-leaves, though in this case both LMA and N_{org} decreased by 15% compared to MM-leaves.

Kinetics of acclimation

The stable A_{max} after 4-7 days (Fig. 2A) showed that leaves of *Cucumis sativus* acclimated to an increase in irradiance within only a few days. Full acclimation of processes underlying Φ_{PSII} (*i.e.* q_P and F_v'/F_m') also applied on thylakoid level (Fig. 3AB). Such a fast acclimation upon an increase in irradiance has also been found in other herbaceous plants like *Alocasia macrorrhiza* (Sims and Pearcy 1991), *Pisum sativum* (Chow and Anderson 1987) and *Chenopodium album* (Oguchi *et al*. 2003). Acclimation to a change in irradiance usually takes as much as 2 to 5 weeks in woody plants (Frak *et al*. 2001; Naidu and DeLucia 1997a; Oguchi *et al*. 2005; Oguchi *et al*. 2006; Yamashita *et al*. 2000).

Dark respiration (R_D) has often been correlated with the nitrogen content in the leaf or with A_{max} (Hirose and Werger 1987a; Hirose and Werger 1987b; Niinemets and Tenhunen 1997; Posada *et al*. 2009; Raulier *et al*. 1999; Yin *et al*. 2004). We did not find such a correlation. For example, R_D of ML-leaves was equal to that of LL-leaves, while N_{org} and A_{max} were considerably higher for ML (Table 1). Our data (Fig 2A and 2D) show that following the change from low to moderate irradiance and vice versa, R_D acclimated faster than A_{max}. These data are in agreement with Sims and Pearcy (1991), who showed that irradiance itself is the major determinant of R_D, while the size of the photosynthetic apparatus is of minor importance.

Nitrogen allocation and Photosynthetic Nitrogen Use Efficiency (PNUE)

Coupled to an increased A_{max} for LM-leaves, both J_{max} and V_{Cmax} increased (Table 1). The increases in J_{max} and V_{Cmax} could be the result of redistribution of nitrogen within the leaf in favour of the photosynthetic apparatus, re-allocation within the photosynthetic apparatus itself in favour of light utilisation, import of nitrogen into the leaf, or a combination of these. Frak *et al*. (2001) and Oguchi *et al*. (2003) have shown that both nitrogen import and (re)allocation can play a role in the photosynthetic acclimation of leaves exposed to an increase in irradiance. Although we found a large increase in A_{max} for LM-leaves, we found a lack of any changes in photosynthetic nitrogen use efficiency at A_{max} ($PNUE_{Amax}$), P_{phot} and in the allocation of nitrogen to Rubisco and bioenergetics, though the fractional allocation to light utilisation ($(P_R+P_B)/P_{phot}$) increased. Most importantly, both N_{org} and the calculated nitrogen content in the photosynthetic apparatus (N_{phot}) increased in LM-leaves by about 60-70% (Table 2) which was in the same order of magnitude as the increase in A_{max}. This suggests that the increase in A_{max} during acclimation to moderate irradiance was primarily due to nitrogen import of the leaf, only slightly due to re-allocation within the photosynthetic apparatus and not to re-allocation of nitrogen within the leaf.

Shade leaves are known to invest relatively more nitrogen in light acquisition than in light utilization (Evans and Seemann 1989; Walters 2005). This trend was evident in the LL- and ML-leaves (highest $P_{light\ acquisition}$; Table 3). Acclimation resulted

in a significantly higher fraction of nitrogen invested in light acquisition in ML-leaves, compared with MM-leaves, because the chlorophyll content of ML-leaves did not decrease, while N_{org} decreased significantly. As a consequence, the fractions of nitrogen invested in bioenergetics and Rubisco was lowest in the ML-leaves (Table 3). Thus acclimation to shade was partly due to a decrease in N_{org}, which mostly occurred at the expense of nitrogen invested in bioenergetics (P_B)and Rubisco (P_R). The preservation of chlorophyll during the first seven days after a decrease in irradiance was also found by Pons and Pearcy (1994) in soybean plants, though subsequently there was a breakdown of chlorophyll. If such a delayed breakdown of chlorophyll were to develop in our ML-leaves it would be expected to result in the pattern of allocation of N_{org} more closely resembling that of the LL-leaves.

Nitrogen allocation model

The allocation pattern of N_{org} within the photosynthetic apparatus between bioenergetics, chlorophyll and Rubisco (Table 3) was similar to the fractions reported by Evans and Seemann (1989) and Makino and Osmond (1991). However, they measured a P_{phot} of approximately 55% for C_3 plants, while we determined a P_{phot} of around 75%, which is within the range recently reported by Feng and co-workers (Feng, 2008ab; Feng *et al*. 2007, 2008; Feng and Fu 2008), who also used the model of Niinemets and Tenhunen (1997). Hikosaka and Shigeno (2009) reported a potential underestimation of Rubisco content by this model if Rubisco is estimated from A-C_i curves without taking into account the effect of mesophyll conductance. However, the mesophyll conductance for a plant like cucumber is likely to be high (Warren, 2008). The value for the specific activity of Rubisco by Niinemets and Tenhunen (1997) may be too conservative considering the more recent published values, which are at least a factor of two higher (Eichelmann *et al*. 2009; Sage 2002). Though this value is still subject of debate, and might be dependent on the environment (Sage 2002), or could be under control of ETR (Eichelmann *et al*. 2009), this does not affect the allocation patterns found. Nonetheless, given the recent increase in interest in mesophyll conductance and the consequences of mesophyll conductance limitations for the determination of V_{Cmax} and J_{max} (Dubois *et al*. 2007; Flexas *et al*. 2008; Niinemets *et al*. 2009a, b; Pons *et al*. 2009; Yin and Struik 2009), it is likely that questions about the accuracy of calculations of the kind employed here will be revisited.

Consequences of acclimation for crop photosynthesis

For upright growing plant stands with a steep extinction of irradiance penetrating the canopy from above, fast acclimation of shaded leaves to a lower irradiance is beneficial. The decrease in dark respiration rate (R_D), which is primarily dependent on prevailing irradiance and not on photosynthetic capacity in leaves, will result to an increased contribution of the lower leaves to net crop photosynthesis. The preservation of a part of the higher photosynthetic capacity during acclimation to low irradiance

retains the capacity to make better use of transient periods of higher irradiance (*e.g.* sunflecks; Bukhov 2004; Pearcy 1990).

The opposite gradient of acclimation (from low to high irradiance) has been shown to be important during gap formation in forests (Naidu and DeLucia 1997ab, 1998; Oguchi *et al*. 2006, 2008; Yamashita *et al*. 2000), but will certainly also be important in agronomic production systems. Production crops can be exposed to increases in irradiance after *e.g.* pruning, partial harvest, transplantation of young plants from a nursery to the field, or intracanopy lighting in greenhouses (*i.e.* the use of supplemental growth-light within the canopy instead of above; Heuvelink *et al*. 2006; Hovi *et al*. 2004, 2006; Hovi-Pekkanen and Tahvonen 2008; Trouwborst *et al*. 2010). Our data show the importance for crop productivity of acclimation of developing leaves to an irradiance sufficiently high to retain an efficient net assimilation when exposed to an increase in irradiance.

Acknowledgements

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CHAPTER 4

Effect of red and blue LED lighting on the photosynthetic acclimation of cucumber leaves

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

4.2 Plasticity of photosynthesis after the "red light syndrome"

CHAPTER 4.1

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 CO_2 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.

Hogewoning SW, Trouwborst G, Maljaars H, Poorter H, van Ieperen W and 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.

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 (Tennessen *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 μmol m^{-2} s^{-1} mixed red/blue irradiance containing 30 μmol m^{-2} 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 μmol m^{-2} 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 μmol m^{-2} 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 CO_2 concentration was ambient. All plants were subjected to 100 ± 5 μmol m^{-2} 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≥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 cm^2 of leaf surface was illuminated. A LI-7000 CO_2/H_2O gas analyzer (LI-COR, Lincoln, Nebraska USA) measured the CO_2 and H_2O 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} CO_2, 20.8 ± 0.4 mmol mol^{-1} H_2O and either 210 or 20 mmol mol^{-1} O_2 (ambient O_2 or low O_2), dependent on the type of measurement. A flow rate of 200-700 ml min^{-1} was used, depending on the CO_2 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 CO_2 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 O_2 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_D) 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 CO_2 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 μmol m^{-2} 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_D. Gross assimilation (A_{gross}) was calculated as net assimilation (A_{net}) plus R_D, which assumes, as is commonly done, that R_D is a reasonable estimate of respiration in the light. Light absorptance (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 absorptance

Leaf light-absorptance 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 absorptance of the actinic measuring irradiance used during gas exchange measurements was subsequently calculated by multiplying the relative leaf absorptance 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 °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 °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 CO_2-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_{gross}-Φ_{PSII}*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 CO_2-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}.

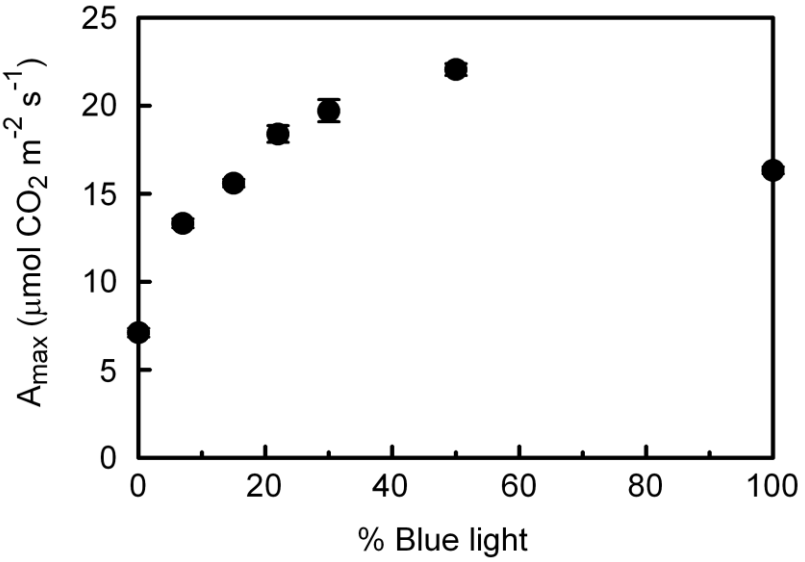

Fig. 1. The effect of light quality (the proportion of total PAR that is from the blue rather than from the red part of the spectrum) during growth on the photosynthetic capacity (A_{max}) of cucumber leaves. Error bars indicate the SE (n=6).

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 µmol m^{-2} 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 µmol m^{-2} s^{-1} incident irradiance on 0B and 30B grown leaves, gross assimilation (A_{gross}) was markedly higher for the low O_2 measurements than it was for the ambient O_2 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 O_2 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 O_2 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).

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-Φ_{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 (α)	0.045^{c}	0.052^{ab}	0.053^{a}	0.053^{a}	0.053^{a}	0.048^{bc}	0.045^{c}
R_{dark} (µmol m^{-2} 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 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 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.

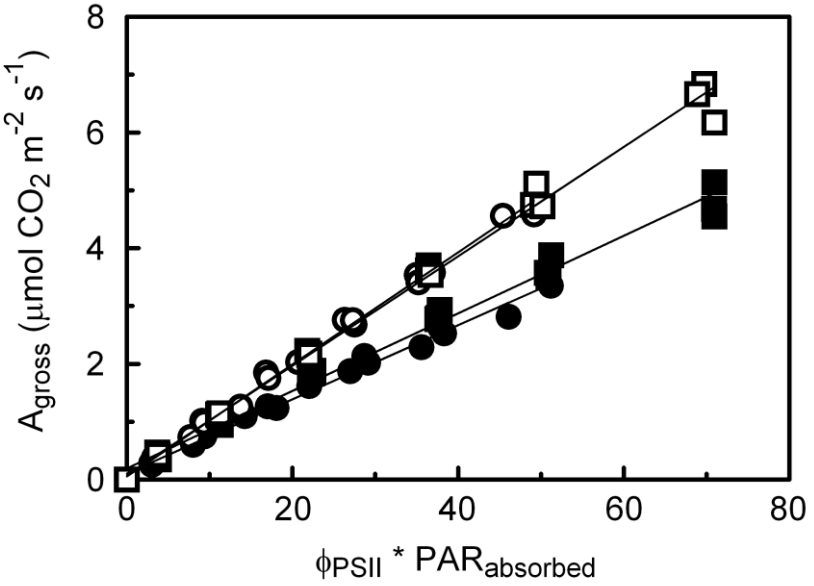

Fig. 2. Relationship between gross CO_2 assimilation (A_{gross}) and the product of Φ_{PSII} and the actinic measuring-light absorbed by the leaves, which serves as an index of electron transport (*e.g.* Kingston-Smith *et al.*, 1997), at an incident irradiance ≤100 µmol m^{-2} s^{-1}. The cucumber leaves 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 O_2 (closed symbols). Gross assimilation was calculated as dark respiration plus net assimilation. The slopes of the regression lines are significantly different for the two O_2 levels ($P< 0.001$), but not for the spectral treatments ($P\geq0.23$).

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 μmol m^{-2} 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 μmol m^{-2} 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).

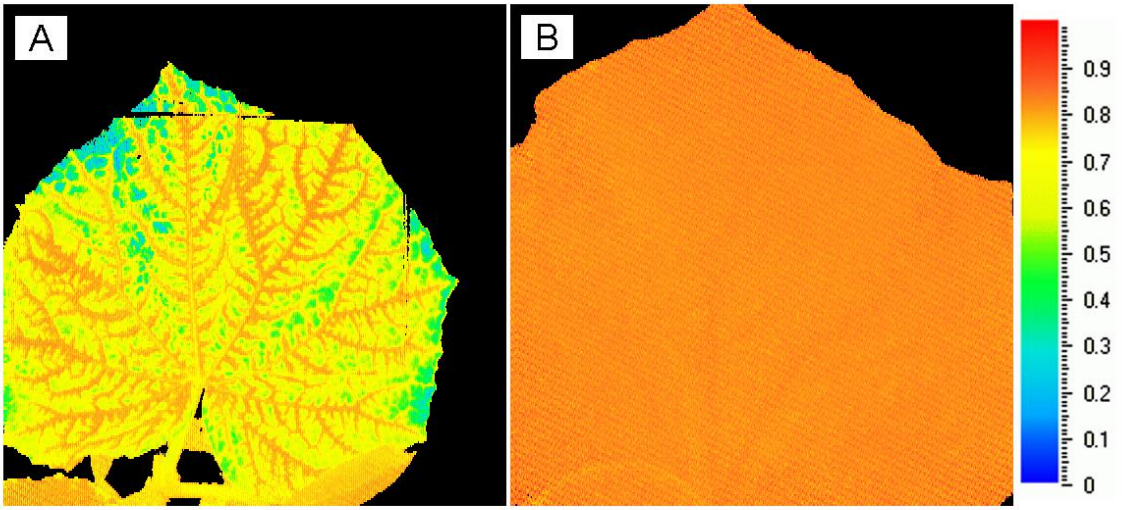

Fig. 3. Image of the dark-adapted F_v/F_m distribution over a 0B (=100% red; A) and 30B (B) irradiance grown cucumber leaf. The mixed blue-red grown leaf (B) has a homogeneous F_v/F_m distribution centered around an F_v/F_m of 0.82, whereas the 0B grown leaf (A) has a heterogeneous distribution with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

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).

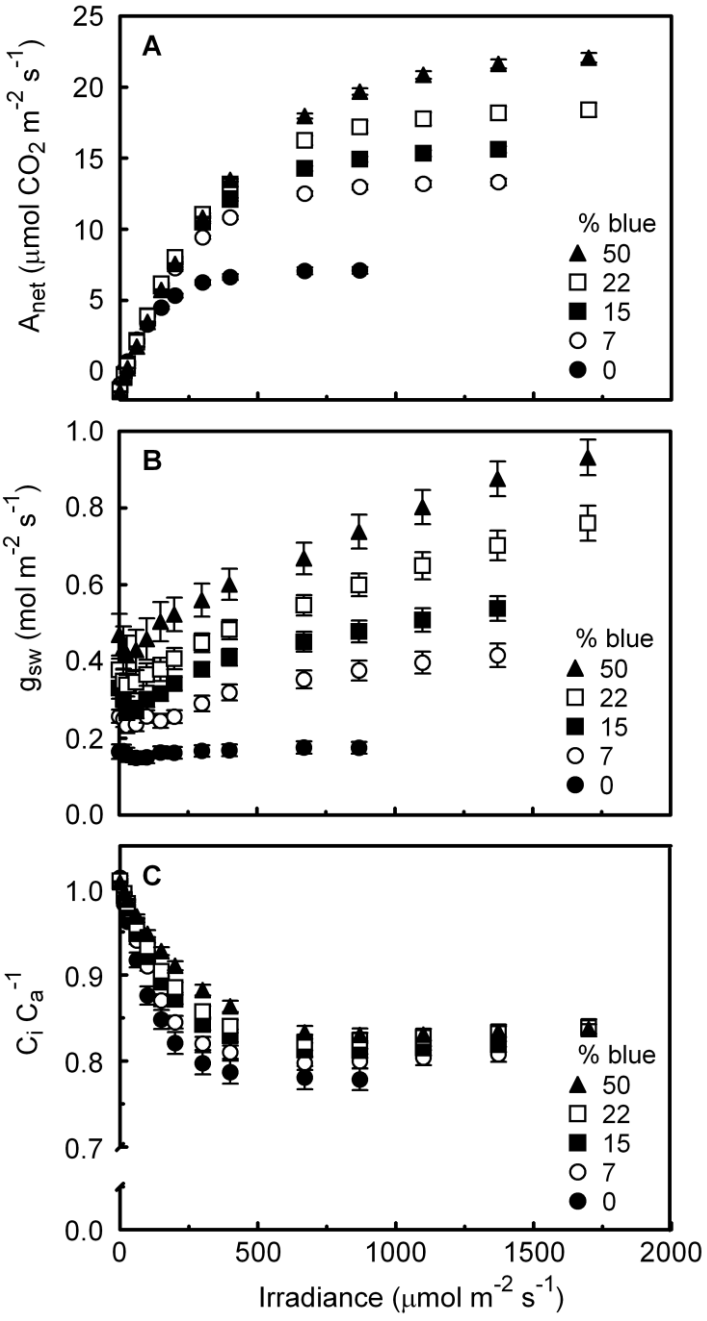

Fig. 4. Response of net assimilation (A_{net}; A), stomatal conductance (g_{sw}; B) and leaf internal CO_2 concentration relative to that of the leaf chamber air (C_i C_A^{-1}; C) to irradiance for cucumber leaves grown under different light qualities (the proportion of total PAR that is from the blue rather than from the red part of the spectrum). The actinic-light quality was identical to that during growth. Error bars indicate the SE (n=6).

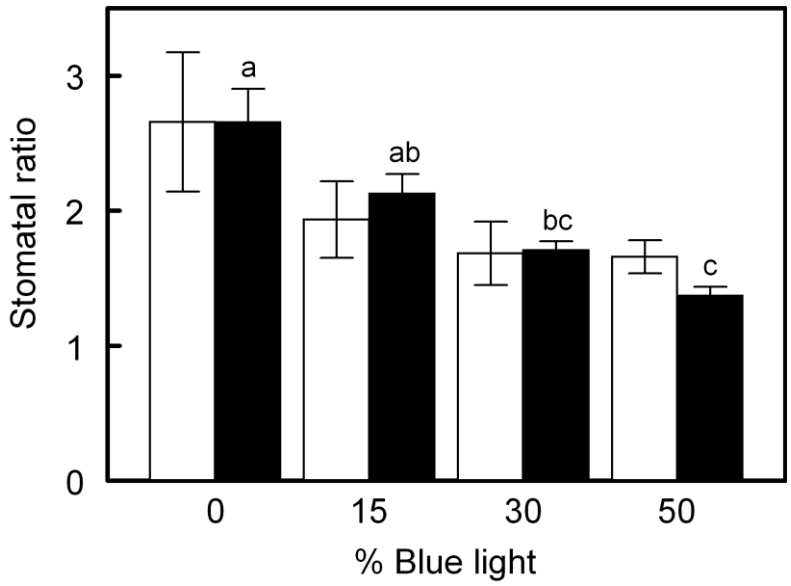

Fig. 5. Ratio of stomatal density (open bars; n≥3) and stomatal conductance measured with a porometer (filled bars; n=6) for the abaxial and adaxial leaf surface of cucumber leaves grown under different light qualities (the proportion of total PAR that is from the blue rather than from the red part of the spectrum; both parameters are labeled 'stomatal ratio' in the plot). Error bars indicate the SE 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.

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).

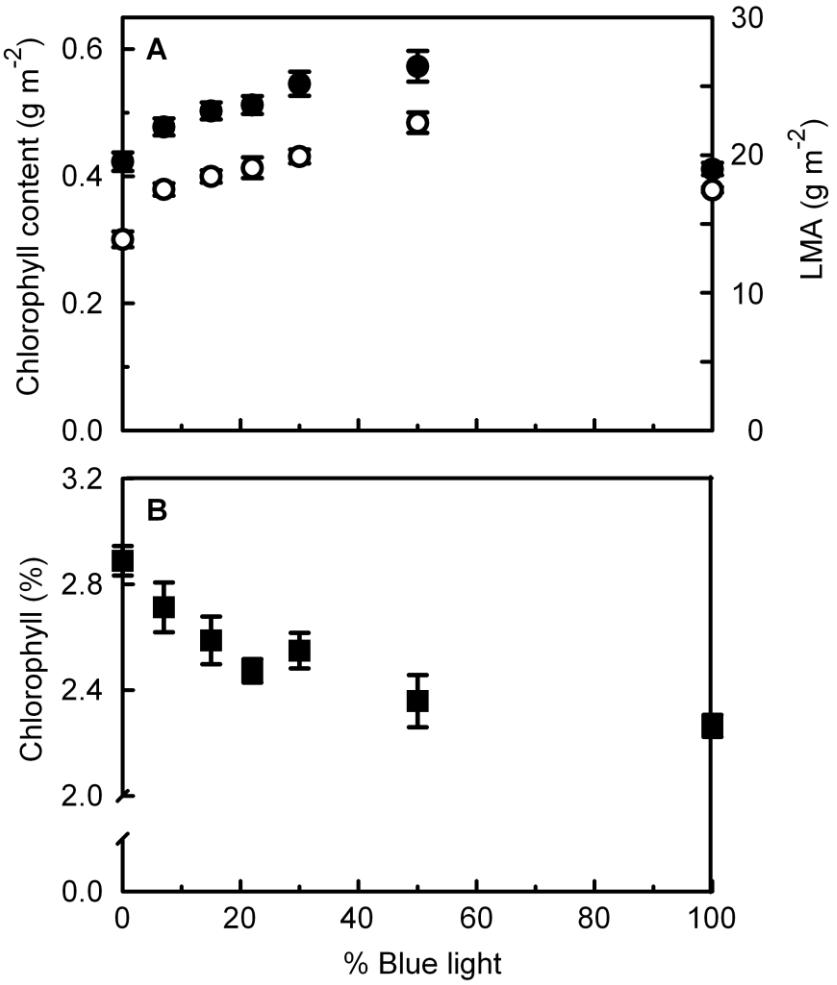

Fig. 6. The effect of light quality (the proportion of total PAR that is from the blue rather than from the red part of the spectrum) during growth on the chlorophyll content per unit leaf area (A, closed symbols, left axis), leaf mass per unit leaf area (LMA; A, open symbols, right axis) and the percentage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

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.

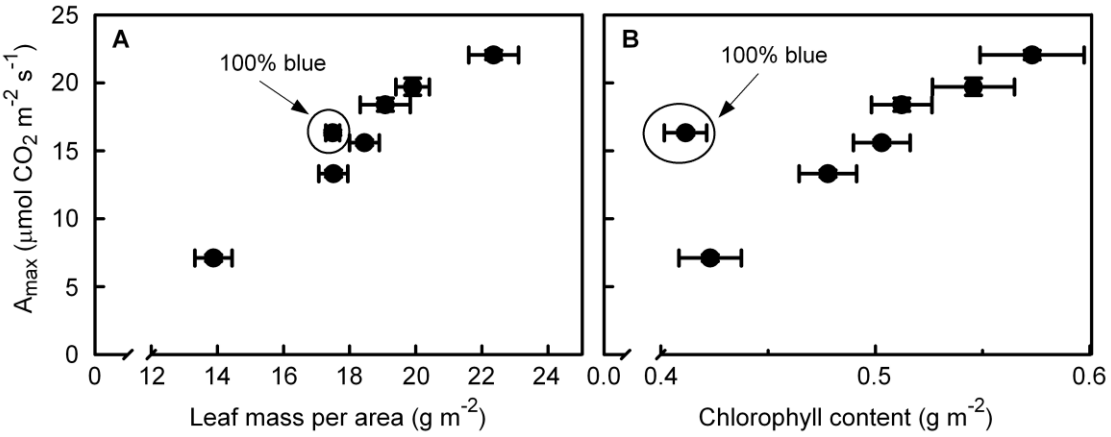

Fig. 7. Relationship of leaf photosynthetic capacity (A_{max}) with leaf mass per unit leaf area (A) and chlorophyll content per unit leaf area (B) of cucumber grown under different combinations of red and blue light at an equal irradiance. The order of the values related to the data-points correspond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

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).

	End dark period			End photoperiod		
Blue %	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).

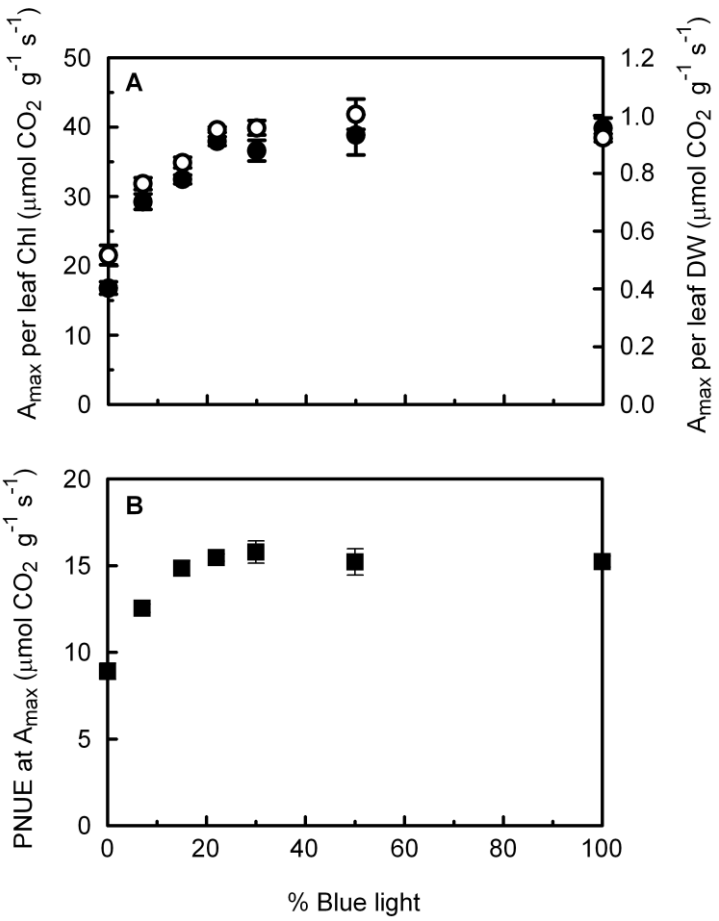

Fig. 8. The effect of light quality (the proportion of total PAR that is from the blue rather than from the red part of the spectrum) during growth of cucumber on leaf photosynthetic capacity (A_{max}) reached per unit chlorophyll (A, closed symbols, left axis), per unit leaf dry weight (A, open symbols,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

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.* Felippe, *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 CO_2 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 O_2 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 O_2 but not at low O_2 (*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 μmol m^{-2} 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.* 1990*a*, *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 μmol m^{-2} 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 μmol m^{-2} 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 ≥ 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 ≥ 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 ≤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 ≤15B are subject to limitations which may be related to the disorders associated with 0B leaves as discussed above, whereas ≥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 4.2

Plasticity of photosynthesis after the "red light syndrome"

Abstract

It is well established that the quantum efficiency of photosynthesis in leaves is wavelength dependent and highest around 620-670 nm (red light). However when Cucumber plants are grown under red LED-light alone photosynthesis was impaired. This "red light syndrome" is characterised by a low F_v/F_m, unresponsive stomatal conductance (g_s), a low photosynthetic capacity (A_{max}) and a low photosynthetic nitrogen use efficiency. Little is known about physiological causes and consequences of this impairment. This study investigated the plasticity of the leaf and photosynthetic apparatus after inducing or releasing the "red light syndrome" in fully developed leaves under low light conditions. Fully expanded leaves which were developed under red (R) or mixed red/blue (RB) LED-light were exposed to respectively RB (R/RB) and R (RB/R) or remained unchanged (R/R and RB/RB). Photosynthetic acclimation was monitored with gas exchange and chlorophyll fluorescence. Chlorophyll fluorescence was also used to analyse the energy dissipation pathways in PSII. R/RB-leaves completely recovered from the low F_v/F_m within 4 days after exposure to RB-light. A_{max}, g_s, leaf mass per area and leaf nitrogen content also increased, but in this case did not reach the level of the RB/RB-leaves, showing limitations in plasticity due to constraints arising from the prior leaf development. RB/R-leaves showed decreases in A_{max}, g_s, leaf nitrogen content and F_v/F_m. R/R- and RB/R-leaves revealed an increased dissipation of the absorbed light into non-regulated energy dissipation, which implies a lower capacity, or weaker activation of nonphotochemical quenching (NPQ) in comparison to RB/RB- and R/RB-leaves. Consequently the leaves developed under RB also revealed the "red light syndrome" within 7 days of red illumination.

Trouwborst G, Hogewoning SW, Savvides A, Van Kooten O, Harbinson J, Van Ieperen W. 2011, Plasticity of photosynthesis after the "red light syndrome", (*in preparation).*

Introduction

Light is an indispensible energy source for plant growth which is usually supplied by the Sun. Artificial light is, however, used as an energy source for plants in certain situations, such as growth cabinets, or in greenhouse horticulture in high latitudes where natural sunlight is severely limits plant growth in the late autumn – early spring period (Trouwborst *et al.,* 2010). Many different light sources are used to generate artificial light for plant growth (*e.g.* incandescent lamps, fluorescent tubes, gas discharge lamps), and all the lamps in common use emit a broad light spectrum within the PAR range, though often also with distinct emission lines in the case of gas discharge lamps or fluorescent tubes (McCree, 1972b). In the last decade improvements in the light output and electrical energy to light conversion efficiency of light emitting diodes (LED) have made them viable sources of plant growth light (Hogewoning *et al.*, 2007; Massa *et al.*, 2006; Massa *et al.*, 2008; Trouwborst *et al.*, 2010). In contrast to the broad spectrum light sources that have been conventionally used to, LEDs emit light in a narrow wavelength band (typically 25-50 nm half-power bandwidth). This allows the development of artificial light sources with a better and more flexible control over their spectrum, and in principle will allow the production of a more optimal irradiance for photosynthesis and growth.

Of particular interest is the influence of wavelength on leaf photosynthesis. Early work on effects of light spectrum on photosynthesis of leaves has shown that the instantaneous photosynthetic quantum yield of leaves is highest in the red region of the spectrum (Evans, 1987; Inada, 1976; McCree, 1972a). However, highest instantaneous photosynthesis does not necessarily result in optimal photosynthesis and growth in the long term. Leaves of cucumber plants that were grown under pure red LED-light (100 μmol m^{-2} s^{-1}; 640 nm; R-grown leaves) developed a low F_v/F_m (Hogewoning *et al.*, 2010b). This "red light syndrome" was further characterised by unresponsive stomata, a low photosynthetic capacity, low photosynthetic nitrogen use efficiency, a low leaf mass per area and impaired growth (Hogewoning *et al.*, 2010b). Similar results were observed with Tomato (unpublished results), but none of these effects occurred in leaves that were grown under mixed red (640 nm) and blue (450 nm) light (RB-grown leaves). It appears therefore that exposure to red light alone during leaf development influences photosynthesis at different functional levels extending from the thylakoid to the whole leaf level. It is unknown if the adverse effects of red light during leaf development are structural and persist at all integration levels after a change in light spectrum or can be partially or completely overcome. Based on how photosynthesis acclimates to changes in light intensity, different extents of acclimation due to changes in light spectrum might be expected at thylakoid and whole leaf level. Sims and Pearcy (1992) have shown that the capacity to acclimate to an increase in irradiance interacted with the leaf developmental phase, while recently

Oguchi *et al.*, (2003) observed that the increase in leaf photosynthetic capacity after an increase in irradiance was limited by leaf thickness.

The objective of this study was to investigate the plasticity of photosynthesis at different functional levels (thylakoid to whole leaf) in response to the induction as well as the release of the "red light syndrome". This was done by changing the spectrum of incident light from pure red (640nm) to mixed red and blue light (640 & 450nm) on leaves that previously developed the "red light syndrome", and vice versa on healthy leaves (without the "red light syndrome").

At the thylakoid level we investigated changes in fate of excitation energy in PSII using chlorophyll fluorescence (Cailly *et al.*, 1996; Genty *et al.*, 1996; Hendrickson *et al.*, 2004) before, during and after changes in light spectrum to assess changes in energy dissipation between photosynthetic electron transport (Φ_{PSII}) and regulated (Φ_{NPQ}) and constitutive energy dissipation processes (Φ_{NO}). Increased Φ_{NO} at the expense of Φ_{NPQ} is thought to be associated with damage of PSII (Klughammer and Schreiber, 2008) or a reduced capacity to activate nonphotochemical quenching (NPQ). At the leaf level we used gas exchange measurements to determine changes in photosynthetic light- and CO_2 response curves before, during and after changes in light spectrum, and we measured several leaf anatomical parameters.

Materials and methods

Plant material and growth conditions

One week old seedlings (*Cucumis sativus* "Hoffmann's Giganta") were transplanted to a continuously aerated hydroponic system in a climate chamber as described in Hogewoning *et al*. (2010b),and subsequently grown horizontally to avoid shading of older leaves by younger leaves. Immediately after planting, the plants were subjected to the following light treatments: 100% red LED (640 nm dominant wavelength) light (R) or a mixture of 70% red and 30% blue LED (450 nm dominant wavelength) light (RB) to allow full leaf development under distinct different light spectra. After three weeks, when the second leaves were fully expanded, half of the plants per light treatment were changed to the other light spectrum, resulting in 4 light treatments: 2 with a distinct change in spectral composition (RB/R and R/RB) and two controls (R/R or RB/RB). Photosynthetic photon flux density (PPFD) and duration of photoperiod were the same for all light treatments: 100±5 μmol m^{-2} s^{-1} for 16h a day and was regularly verified (Li-190, Li-Cor inc., Lincoln, NE, USA). Temperature and relative humidity inside the climate room were respectively 25°C and 70%.

Gas exchange and chlorophyll fluorescence measurements

All measurements were made on the second fully expanded leaf of the plant. Measurements started on the day that half of the plants were subjected to the change in light treatment (day zero). Photosynthetic irradiance-response curves were

repeatedly measured with a portable gas analyzer (LI-6400 with fluorescence head and standard LED-irradiance light source; Li-Cor Inc., Lincoln, NE, USA) system from day zero to ten. Photosynthetic CO_2-response (A-C_i) curves were measured on the first and the 7th or 8th day of this period. The blue to red ratio of the actinic light in the clamp-on leaf chamber was always set equal to the growth irradiance of the particular leaf subjected to measurements. However above an intensity of 900 μmol m^{-2} s^{-1} the blue light fraction gradually decreased from 30% to 18% at 1600 μmol m^{-2} s^{-1} due to limitations of the blue light source in the leaf chamber. Leaf chamber temperature, air flow speed and the CO_2-concentration were set at 25 °C, 250 μmol s^{-1}, and 380 μmol mol^{-1} respectively. Air humidity in the leaf chamber was kept similar as during growth, approximately 70%. For the chlorophyll fluorescence measurements, the measuring beam intensity was set at 0.1 μmol m^{-2} s^{-1}, and the saturating light pulse had an intensity of >7000 μmol m^{-2} s^{-1} and a duration of 0.8s.

Dark respiration (R_D) and maximum quantum yield for PSII photochemistry for dark adapted leaves (F_v/F_m; for fluorescence terminology see Van Kooten and Snel, 1990 and Baker *et al.*, 2007) were measured before each photosynthetic irradiance-response curve following a 30 minute period of dark adaptation in the leaf cuvette. At each irradiance or CO_2 level the assimilation rate (A), leaf internal CO_2-concentration (C_i) and stomatal conductance (g_s) were measured as the mean value during a 40s period after steady state gas exchange was achieved. The measured photosynthetic rates during A-C_i curve determinations were corrected for diffusion leaks as determined according to the LI-COR manual (2005) and by Flexas *et al.* (2007).

Determinations of leaf parameters

At the start and the 7th and 8th day of the experiment, samples were taken to measure chlorophyll, LMA and organic nitrogen (N_{org}). To determine the dynamics in chlorophyll a/b ratio an additional time series was measured in R-leaves exposed to RB or R-light at sampling intervals of hours to days. Samples were collected and chlorophyll, LMA and N_{org} measured as described in Trouwborst *et al.* (2010).

The leaf absorptance spectrum was measured in single nanometer steps according to Hogewoning *et al.* (2010ab), and the quantum flux absorbed by the leaf was calculated by multiplying the absorptance spectrum by the growth-light spectrum.

To determine stomatal densities, indexes (stomatal density divided by the total amount of epidermis cells including the stomatal cells) and apertures, silicon rubber impressions were made (Smith *et al.*, 1989) on both the ad- and abaxial side of the leaves. Stomatal densities, indexes and apertures were determined from digitized images according to Nejad and van Meeteren (2005).

Calculations and statistics

Maximum PSII efficiency in light (F_v'/F_m'), PSII operating efficiency (Φ_{PSII}), PSII efficiency factor (q_P) and the electron transport rate (ETR) at growth light level were calculated according to Baker *et al.* (2007) and F_0' was caculated according to Oxborough *et al.* (1997). For the calculation of ETR, we assumed an excitation balance of 0.5 and used the measured leaf absorption and Φ_{PSII}. The energy dissipation in PSII was calculated according to Genty *et al.* (1996) and Cailly *et al.*, (1996), which is equal to the approach of Kramer *et al*. (2004) when a calculated F_0' is used (Klughammer and Schreiber, 2008). The quantum yield of PSII electron transport is Φ_{PSII}, the quantum yield of regulated heat dissipation, Φ_{NPQ}, is calculated as $F_s/F_m'-F_s/F_m$ and the quantum yield of non-regulated energy dissipation, Φ_{NO}, is calculated as F_s/F_m.

A modified version of the Farquhar, Von Caemmerer and Berry (FvCB) model (Farquhar *et al.*, 1980) was fitted to the A-Ci response data. We estimated J_{max} and V_{Cmax} (the latter believed to be linearly related to the active Rubisco content; *e.g.* Niinemets and Tenhunen (1997)) normalized to 25°C using the non-linear fitting procedure NLIN in SAS (release 9.1.3; SAS institute, Cary, NC, USA). The model equations were adopted from Yin *et al.* (2004) and the parameterisation from Bernacchi *et al.* (2001) and Medlyn *et al.* (2002). This model simultaneously fits J_{max} and V_{Cmax} without splitting the dataset, a procedure recommended by Dubois *et al.* (2007). Electron transport capacity was fitted using the equation for ATP limitation instead of NADPH limitation because the ATP-limited model includes a correction for pseudo-cyclic electron transport (Yin *et al.*, 2006).

A non rectangular hyperbola (Thornley, 1976) was fitted to the photosynthesis irradiance-response data using the non-linear fitting procedure NLIN in SAS to determine dark respiration (R_D), maximum gross photosynthetic rate (A_{mg}), light-limited quantum efficiency (α) and the scaling constant for the curvature (θ) of the leaves in the different treatments:

$$A_{net} = \frac{\alpha \cdot PPF + A_{mg} - \sqrt{(\alpha \cdot PPF + A_{mg})^2 - 4\theta \cdot PPF \cdot A_{mg}}}{2\theta} - R_D \qquad \text{(eq.1)}$$

A_{max} based on net assimilation was calculated as A_{mg} minus R_D.

All treatments were repeated four times (2-4 plants per replicate) and data were analysed by using one way ANOVA. When *P*-values of the ANOVA were lower than 0.05 then a post hoc multiple comparison test with Fisher's LSD was conducted.

Results

Photosynthetic responses at the leaf level

The assimilation capacity (A_{max}) of leaves that were continuously grown under an RB-spectrum (RB/RB-leaves) was more than 3 times higher than A_{max} of leaves that were continuously grown under an injurious R-spectrum (R/R-leaves; Fig 1A). After a change in light spectrum, A_{max} of both R- and RB-grown leaves started to acclimate for

approximately 7-8 days to new intermediate values for A_{max}. Release of injured leaves from the R-spectrum (R/RB-leaves) resulted in an approximately 2-fold rise of A_{max}, while exposure of non-injured mature leaves to an R-spectrum (RB/R-leaves) caused a halving of A_{max}.

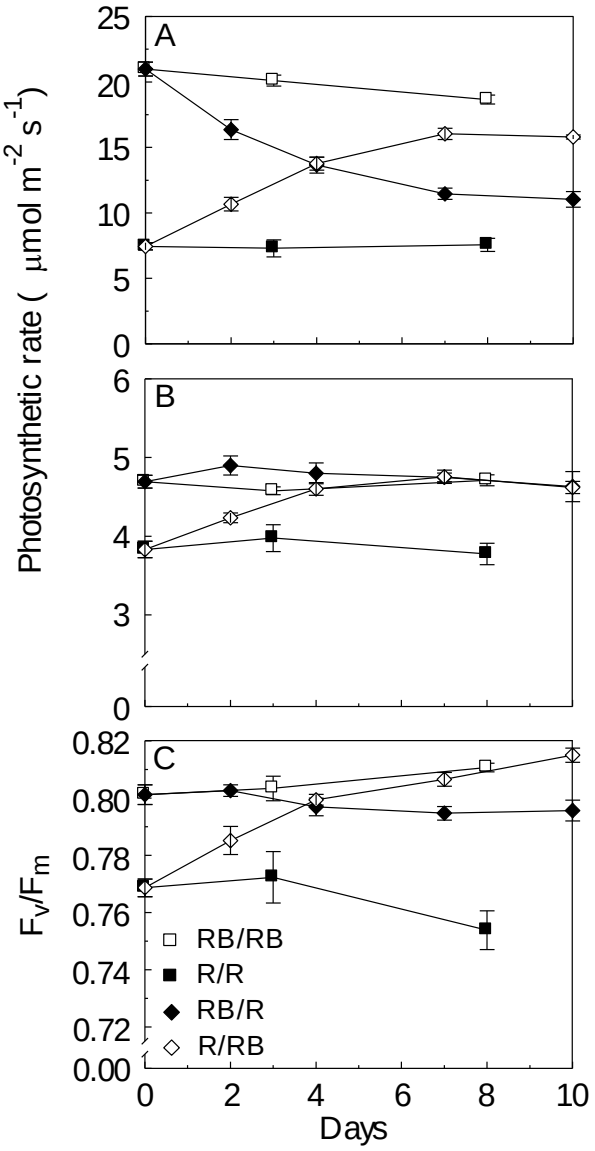

Fig. 1. Time course of the effect of a change in irradiance spectrum on photosynthetic rates at saturating (A) and at growth (B) irradiance, and the F_v/F_m during the acclimation period (days). For symbols see legend. Each data point represents the mean of 4 repetitions (>2 plants per replicate) and vertical bars represent the SE.

Initial quantum efficiency (α) was reduced in R-grown leaves but completely recovered after a change to an RB-spectrum (Table 1). The exposure of RB-grown leaves to an R-spectrum did not induce a reduction in α. The curvature (θ) of the photosynthesis-light response curve did not differ between the light treatments (Table 1). Dark respiration (R_D) was higher in RB/RB-leaves than in R/R-leaves, and leaves

that were exposed to a change from RB to R-spectrum had a significantly lower R_D than RB/RB-leaves. In contrast, leaves that were exposed to a change from R to RB increased their R_D to a value that was not significantly different to that in the RB/RB leaves (Table 1). The maximum carboxylation rate allowed by Rubisco (V_{Cmax}) and maximum rate of linear electron transport, *i.e.* that through PSII, (J_{max}) showed a similar pattern across the light treatments as A_{max}. The balance between the capacity for the light and dark reaction (J_{max}/V_{Cmax}) tended to shift to the dark reaction for the treatments ending with an R-spectrum though this was not significant (P=0.063; Table 1).

Table 1. The effect of a change in growth light spectrum on photosynthetic parameters of fully expanded cucumber leaves developed under red (R) or a combination of red and blue light (RB) and exposed to a different spectrum for a period of 7-8 days (n=4; >2 plants per replicate). Different letters indicate statistical significant differences (P<0.05).

	R/R	RB/R	R/RB	RB/RB
F_v/F_m	0.759^{c}	0.795^{b}	0.808^{a}	0.810^{a}
F_0	202^{a}	188^{ab}	165^{c}	173^{bc}
F_m	842	919	864	914
R_D (μmol m^{-2} s^{-1})	0.97^{c}	1.00^{bc}	1.25^{ab}	1.31^{a}
α	0.060^{b}	0.066^{a}	0.070^{a}	0.066a
θ	0.78	0.78	0.7	0.77
A_{max} (μmol m^{-2} s^{-1})	8.47^{d}	12.08^{c}	17.11^{b}	19.67^{a}
V_{Cmax} (μmol m^{-2} s^{-1})	32.2^{d}	48.9^{c}	66.2^{b}	75.0^{a}
J_{max} (μmol m^{-2} s^{-1})	58.6^{d}	90.6^{c}	131.2^{b}	153.1^{a}
J_{max}/V_{Cmax}	1.83	1.86	1.99	2.05
Growth irradiance				
A_{100} (μmol m^{-2} s^{-1})	3.84^{b}	4.69^{a}	4.82^{a}	4.73^{a}
Φ_{PSII}	0.60^{c}	0.71^{b}	0.73^{ab}	0.74^{a}
Φ_{NPQ}	0.051^{a}	0.032^{c}	0.036^{b}	0.027^{d}
Φ_{NO}	0.35^{a}	0.26^{b}	0.23^{c}	0.23^{c}
F_v'/F_m'	0.732^{d}	0.776^{c}	0.785^{b}	0.793^{a}
q_P	0.827^{d}	0.911^{c}	0.924^{b}	0.932^{a}
ETR (μmol m^{-2} s^{-1})	27.8^{c}	33.2^{b}	34.1^{ab}	35.0^{a}
g_s (mol m^{-2} s^{-1})	0.129^{c}	0.162^{c}	0.241^{b}	0.304^{a}
C_i (μmol mol^{-1})	320^{b}	322^{b}	339^{a}	346^{a}

At growth irradiance, the photosynthetic rate was significantly higher in RB/RB leaves compared to R/R leaves. By the fourth day of acclimation in the RB-spectrum, R-spectrum grown leaves had increased their photosynthetic rates at growth irradiance to the same values found in RB/RB. while exposure of RB grown leaves to the R-spectrum did not result in a lowered photosynthetic rates at growth irradiance (Fig. 1B). After exposure of R-grown leaves to the RB-spectrum, stomatal conductance (g_s) at growth irradiance almost doubled, but did not reach the g_s measured in RB/RB-leaves. The g_s of RB-grown leaves, on the other hand, decreased to the level of R-grown leaves after exposure to R-light. These g_s values resulted in lower C_i values (at growth irradiance) in leaves grown under, or subsequently acclimated to, R-light compared to those grown under or acclimated to RB-light (Table 1).

Photosynthetic responses at the thylakoid membrane level

The depressed F_v/F_m of R-grown leaves recovered within four days of exposure to a RB-spectrum to a normal F_v/F_m level, while RB-grown leaves exposed to R-light only showed a slight decrease in F_v/F_m (Fig. 1C; Table 1). The lower F_v/F_m of R-leaves was due to a significantly higher F_0, while no significant differences in F_m were measured (Table 1).

The PSII operating efficiency (Φ_{PSII}) and ETR of R/R-leaves at growth irradiance, were the lowest of all treatments (Table 1), but these increased to the level displayed by RB-grown leaves during acclimation to the RB-spectrum. While RB-grown leaves that were subjected to an R-spectrum showed a significant decrease in both ETR and Φ_{PSII}, they did not decrease to the levels observed in R-grown leaves (Table 1).

At growth irradiance, the regulated thermal dissipation (Φ_{NPQ}) was small though significantly different in all treatments. The highest Φ_{NPQ} was observed in R-grown leaves and was almost twice as high as Φ_{NPQ} observed in RB-grown leaves. Constitutive, non-regulated energy dissipation (Φ_{NO}) was also 50% greater in R-grown leaves compared to RB-grown leaves. Acclimation to RB light resulted in the Φ_{NO} of R leaves decreasing to that of the RB leaves, while acclimation to R light resulted in a small increase in the Φ_{NO} of the RB leaves. The absolute difference in Φ_{NO} between RB/R- and RB/RB-leaves was larger than the difference in Φ_{NPQ} indicating an enhanced proportion of non-photochemical energy dissipation via non-regulated mechanisms for RB/R-leaves.

Irradiance response curves after the acclimation period revealed that PSII operating efficiency (Φ_{PSII}) versus irradiance showed, like A_{max}, a graded response between the treatments (Fig. 2B): with increasing irradiances the RB/RB-leaves had the highest values, the R/R-leaves the lowest values, while the RB/R- and R/RB-leaves showed intermediate patterns with the R/RB having higher values than the RB/R. These patterns were mirrored in the patterns of qP versus irradiance (Fig. 2D) but not in the irradiance responses of F_v'/F_m' (Fig. 2C), which revealed that with increasing irradiance F_v'/F_m' decreased only slightly with only minor differences in the response

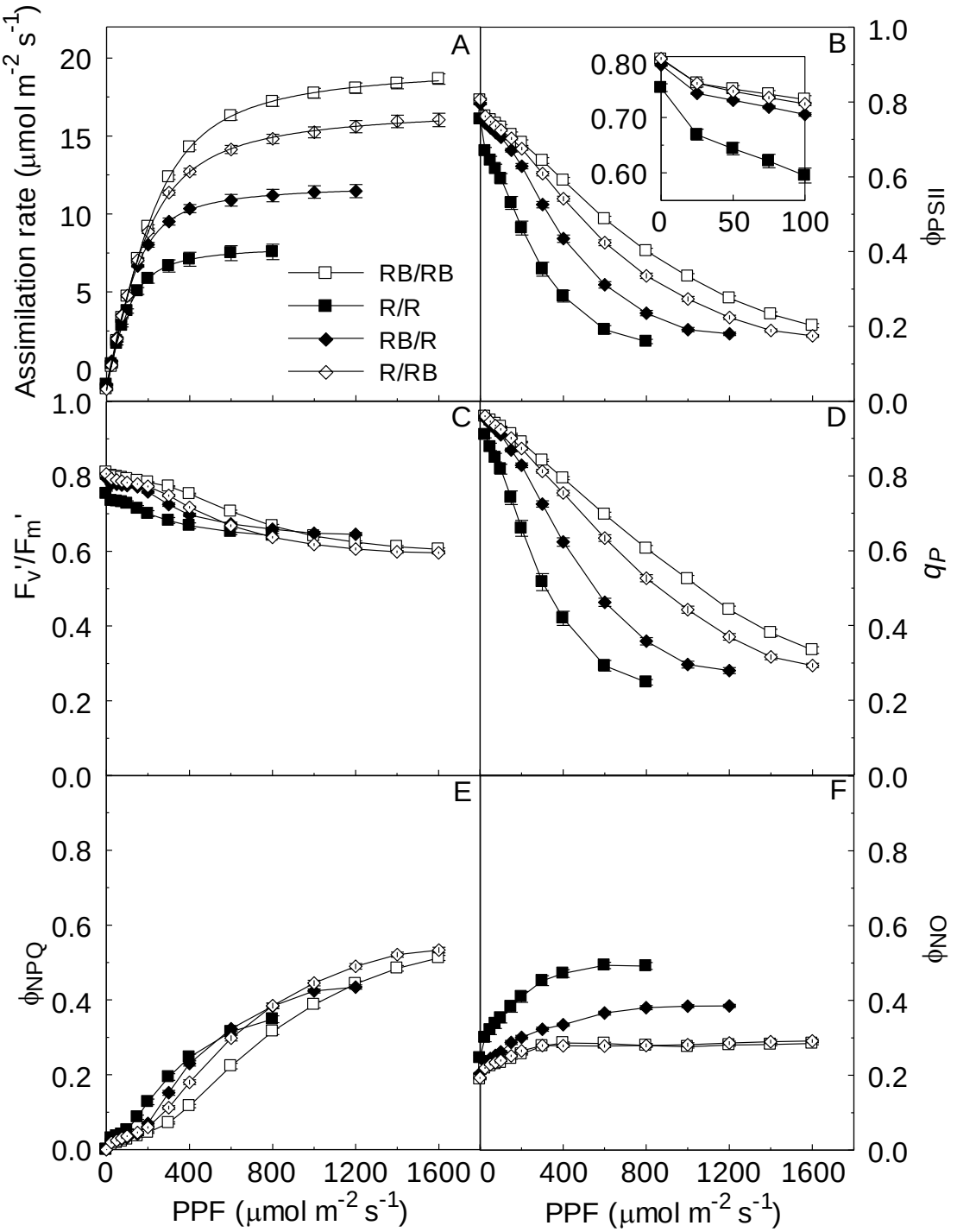

Fig. 2. The effect of a change in growth light spectrum on the irradiance-response of CO_2 exchange (2A), the maximum PSII efficiency in the light (F_v'/F_m'; 2B), the PSII efficiency factor (q_P; 2C), the PSII operating efficiency (2D), the regulated energy dissipation (Φ_{NPQ}; 2E) and the non regulated energy dissipation (Φ_{NO}; 2F) in cucumber leaves developed under red (R) or a combination of red and blue (RB) irradiance after an acclimation period of 7-8 days. For symbols see legend. Lines through data points of the irradiance response curves represent the fit to the non rectangular hyperbola (eq. 1). For each data point n=4 (>2 plants per replicate) and vertical bars represent the SE.

of the different leaf types. At low irradiances R/R-leaves have a distinctly lower F_v'/F_m', and for both the R/R and RB/R treatments the minimum values of F_v'/F_m' are greater than those from the R/RB and RB/RB leaves. As a consequence of this limited development of F_v'/F_m', the decrease in Φ_{PSII} with increasing irradiance can be largely attributed to the decrease in q_P (Fig. 3A). The decrease in F_v'/F_m' with increasing irradiance is paralleled by an increase in Φ_{NPQ} that differs between the treatments; at high irradiances the Φ_{NPQ} from the R/RB and RB/RB leaves converge and are higher than the Φ_{NPQ} from the R/R and RB/R leaves, both of which saturate at lower values of

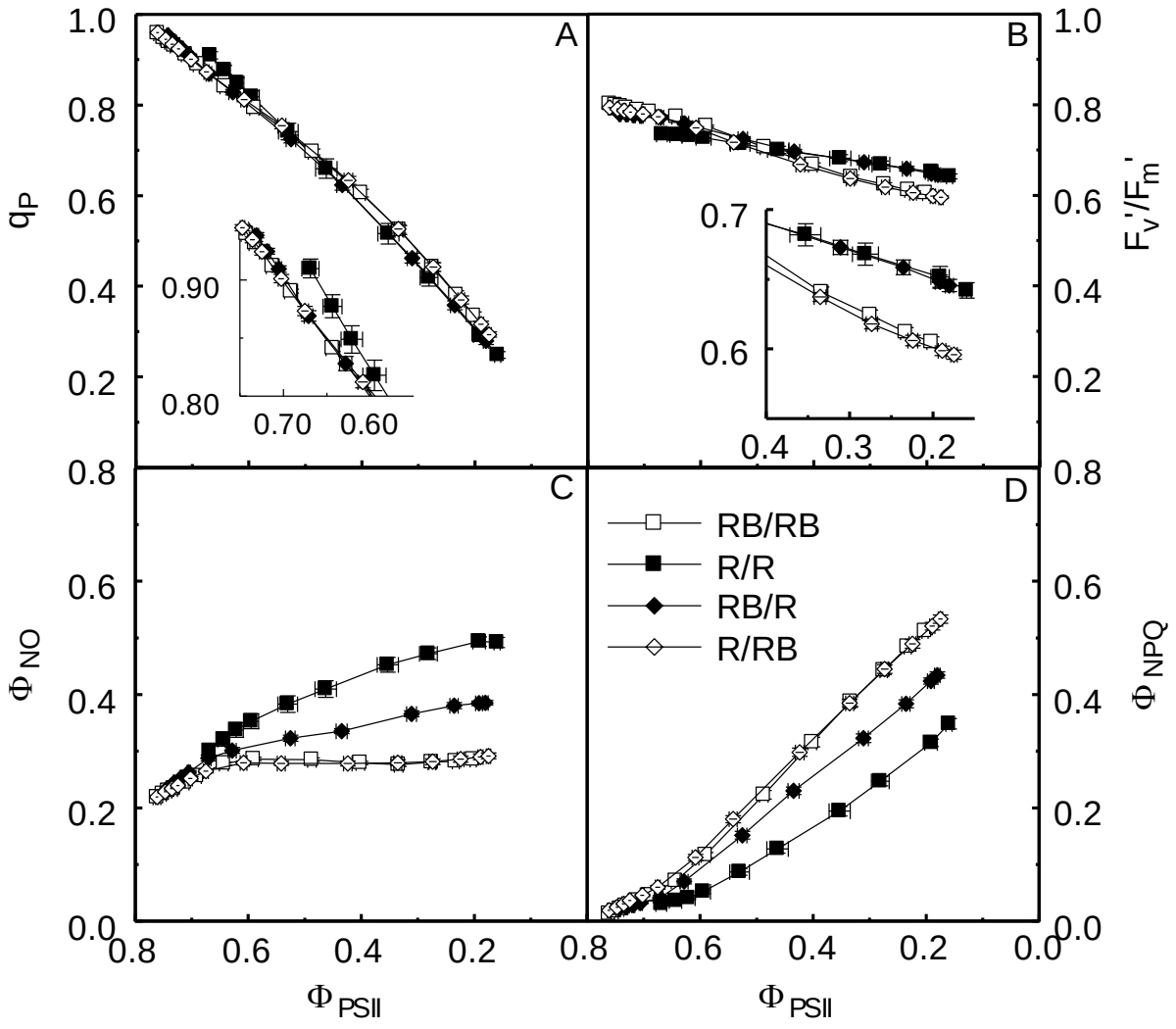

Fig. 3. The effect of a change in growth light spectrum on (A) the PSII operating efficiency (Φ_{PSII}) and on (B) the regulated energy dissipation (Φ_{NPQ}), and on (C) the non regulated energy dissipation (Φ_{NO}) and on (D) the non photochemical quenching (1 - F_v'/F_m') versus the PSII efficiency factor (q_P) in fully expanded cucumber leaves either developed under red (R) or a combination of red and blue (RB) irradiance after an acclimation period of 7-8 days. Each data point represents the mean of 4 repetitions (>2 plants per replicate) and vertical bars and horizontal bars represent the SE. For symbols see legend.

Φ_{NPQ}, with the R/R leaves having a lower maximum value of Φ_{NPQ} than the RB/R leaves (Fig. 2CE). The RB/RB- and R/RB-leaves showed a similar low response for non-regulated energy dissipation (Φ_{NO}) with increasing irradiance (Fig. 2F) while Φ_{NO} increased most with increasing irradiance for R/R-leaves with the RB/R-leaves showing an intermediate response (Fig. 2F).

RB/RB and R/RB leaves had the same relationship between regulated energy dissipation and Φ_{PSII} and also between non-regulated energy dissipation and Φ_{PSII} (Fig. 3CD). In the case of R/R leaves the increase of Φ_{NPQ} with decreasing Φ_{PSII} was the weakest of all the treatments and, correspondingly, the increase in Φ_{NO} with decreasing Φ_{PSII} was greatest for this treatment. The responses of the Φ_{NPQ} and Φ_{NO} with decreasing Φ_{PSII} in RB/R leaves were intermediate between those of the R/R leaves on the one hand and the RB/RB and R/RB leaves on the other (Fig. 3CD). F_v'/F_m' versus Φ_{PSII} divided the data into two classes for the treatments ending on either RB- or R-light: with decreasing Φ_{PSII} the treatments ending under RB-light had lower maximum quantum yields in the light than the treatments ending under R-light (Fig. 3B) showing their greater ability for non-photochemical quenching.

Table 2. The effect of a change in growth light spectrum on leaf parameters of fully expanded cucumber leaves developed under red (R) or a combination of red and blue (RB) light and exposed to a different spectrum for a period of 7-8 days (n=4; >2 plants per replicate). Different letters indicate statistical significant differences (P<0.05).

	R/R	RB/R	R/RB	RB/RB
LMA (g m^{-2})	19.7^{c}	23.9^{b}	23.7^{b}	28.1^{a}
N_{org} (g m^{-2})	0.81^{c}	0.99^{b}	1.07^{ab}	1.24^{a}
$\%N_{org}$	4.11	4.15	4.51	4.42
Chlorophyll (mg m^{-2})	405.1^{b}	481.7^{a}	516.3^{a}	550.2^{a}
Chlorophyll a/b ratio	3.40^{b}	3.43^{b}	3.49^{ab}	3.54^{a}
PNUE	10.06^{b}	12.34^{b}	16.14^{a}	16.15^{a}
A_{max}/LMA	0.40^{b}	0.51^{b}	0.72^{a}	0.71^{a}
A_{max}/chlor	19.91^{c}	25.40^{b}	33.78^{a}	35.69^{a}
Leaf absorption (%)	91.7^{c}	94.1^{b}	94.1^{b}	95.7^{a}
Stomatal density	475^{b}			722^{a}
Stomatal index	16.6^{b}			20.0^{a}
Stomatal apertures	0.82			1.01

Acclimation of leaf parameters

LMA, N_{org}, chlorophyll content and leaf absorption were lowest for R/R-leaves and highest for RB/RB-leaves. Acclimation to the other spectrum resulted in intermediate values (Table 2). The chlorophyll a/b ratio was significantly higher in RB/RB-leaves than in R/R- and RB/R-leaves. The $\%N_{org}$ did not differ between treatments. Stomatal density and index were significantly higher in RB/RB leaves than in R/R leaves, while stomatal apertures did not differ. Photosynthetic Nitrogen Use Efficiency (PNUE) and A_{max}/LMA were the lowest for R/R- and RB/R-leaves. A_{max}/chl was lowest for R/R leaves and highest for RB/RB- and R/RB-leaves, while RB/R-leaves showed intermediate values.

Discussion

The "red light syndrome" during leaf development

Recently the "red light syndrome" was characterised as leaves having, on the leaf level, a low A_{max}, LMA and PNUE, (Hogewoning *et al*. (2010b). On the thylakoid level the leaves have a decreased dark adapted F_v/F_m, suggesting the presence of either net photodamage to PSII (*i.e.* photoinhibition) or slowly reversible down-regulation of PSII (Demmig-Adams and Adams, 2006). The reason for the decrease in F_v/F_m is unclear as the usual causes for this response did not apply: the light level used was low (100 μmol m^{-2} s^{-1}) and no sink limitation occurred as no starch accumulation was found (data not shown). Notably, that while some features of the red-light syndrome are consistent with acclimation low-light (*e.g.* the low A_{max} and LMA) others, such as the low PNUE and F_v/F_m, are not. Here we analyse the effect of the "red light syndrome" on both the leaf and the thylakoid level.

Leaf level—In agreement with Hogewoning *et al.* (2010b), the smaller gross assimilation rate of R/R-leaves at growth irradiance compared to RB/RB-leaves was interrelated with a smaller ETR of R/R leaves. Both were 26% lower (Table 1). The 50% lower g_s of R/R-leaves resulted in only an 8% lower C_i compared to RB/RB leaves, indicating that stomata play a minor role. The smaller ETR is mainly due to a decreased Φ_{PSII} (23% lower) as the decrease in leaf absorption was only minor (4% lower; Table 1&2). Differences in other leaf parameters between R/R- and RB/RB-leaves as LMA, N_{org}, PNUE and others (Table 2) have already been discussed in detail by Hogewoning *et al* (2010b).

Though it is known that a lower g_s is obtained when using a red actinic light during the measurement of gas exchange compared to mixed red-blue light (Sharkey and Raschke, 1981; Goins *et al*., 1997), here we show that also the stomatal density and more importantly the stomatal index was negatively affected by the red light (Table 2). Until now information about spectral effects on stomatal index and density is sparse (Casson and Gray, 2008). A possible explanation for the lower stomatal index is a

direct effect of the red light (or lack of blue light) on developing leaves, but we cannot rule out an influence from signals originating from the older leaves (cotyledons and the first true leaf) of the R/R-plants, which would be expected to have had a decreased g_s under red light (Sharkey and Raschke, 1981; Goins *et al*., 1997), that is believed to reduce the stomatal index of later developing leaves (Lake *et al*., 2001; Lake and Woodward, 2008; Miyazawa *et al*., 2006; Schoch *et al*., 1980).

We conclude that the limitation of assimilation rate at growth irradiance in R/R-leaves is mostly due to a disturbed light reaction or photosynthetic metabolism and limitations due to stomatal effects or leaf light absorption are of minor importance.

Thylakoid level—Further analysis of chlorophyll fluorescence parameters revealed that the extent by which decreases in Φ_{PSII} produced by increasing irradiance originated in decreases in q_P (the PSII efficiency factor) or maximum quantum yield in the light (F_v'/F_m') were similar for R/R- and RB/RB-leaves (Fig. 3AB) (n.b. numerically, Φ_{PSII} is the product of q_P and F_v'/F_m'). None of the leaves investigated in this study displayed the extent of light-induced decrease in F_v'/F_m' reported in other studies (Bilger and Björkman, 1990; Genty *et al*., 1990; Demmig-Adams *et al*., 1996), implying that these leaves had a relatively small ability for non-photochemical quenching (Baker, 2008), which is a common feature for short-lived, fast-growing species (Demmig-Adams and Adams 2006) like cucumber. The decrease in F_v'/F_m' in R/R leaves with increasing irradiance or decreasing Φ_{PSII} was less than that developed by RB/RB (Fig. 2C and 3B), Though this effect is small, it implies that leaves developing without blue light show a greater extent of 'shade acclimation', as reported earlier (Hogewoning *et al*., 2010b; Lichtenthaler *et al*., 1980; Matsuda *et al*., 2004; Matsuda *et al*., 2008; Voskresenskaya, 1979).

At growth irradiance, the non regulated energy dissipation (Φ_{NO}) of R/R-leaves was 50% greater than that of RB/RB-leaves (Table 1) and even greater than the value of RB/RB-leaves at light saturation (Fig. 2F). This increased Φ_{NO} in the R/R leaves compared to RB/RB is due to lesser development of NPQ (F_v'/F_m' does not decrease much with increasing irradiance) combined with the greater decrease of Φ_{PSII} with increasing irradiance in the R/R leaves. With increasing irradiance, Φ_{NO} increased in the R/R leaves to a level which was 60% higher compared with RB/RB-leaves (Fig. 2F). In relation to decreasing Φ_{PSII}, the Φ_{NO} of all leaves increased reflecting the increased dissipation of excitation energy via the basal NPQ pathway to compensate for the loss of photochemical quenching. Until Φ_{PSII} had decreased to about 0.65 the increase in Φ_{NO} was similar for all leaves (Fig. 3C), but below this the responses of the leaves differed due to the different extents of development of Φ_{NPQ} (Fig. 3D). The weaker development of dissipation by the regulated NPQ in the R/R leaves (a consequence of the small decrease in F_v'/F_m' in this leaf (Fig. 3B)) results in a greater dissipation by the basal NPQ – a greater Φ_{NO} – when Φ_{PSII} was less than 0.65. In contrast, in the RB/RB leaves

Φ_{NO} only increased slightly once Φ_{PSII} has fallen below 0.65 as a result of the stronger development of inducible NPQ (a reflection of the lower F_v'/F_m') at Φ_{PSII} below 0.65.

The occurrence of the "red light syndrome" after the completion of leaf development

In general, leaves developed under red/blue light and which were then exposed to red light (RB/R-leaves) displayed decreases in A_{max}, V_{Cmax}, J_{max}, chlorophyll a/b ratio, LMA and N_{org}. These responses are similar to shade acclimation responses (*e.g.* Pons and De Jong-van Berkel, 2004; Pons and Pearcy, 1994; Table 1&2). However, the RB/R-leaves also showed "red light syndrome" symptoms.

Leaf level—At growth irradiance, the α and net assimilation rate (A_{100}) did not decrease during the acclimation period (Table 1). Gross assimilation rate ($A_{100}+R_D$), ETR, leaf absorption and C_i decreased slightly. However, PNUE and A_{max}/LMA decreased to the level of R/R-leaves (Table 2) suggesting symptoms of the "red light syndrome" on this level.

Thylakoid level— Chlorophyll fluorescence measurements at growth irradiance also revealed changes in the RB-leaves due to red light exposure: Φ_{PSII} was lower and both q_P and Φ_{NO} were respectively lower and higher compared to RB/RB-leaves, though Φ_{NO} did not reach the level of R/R-leaves.

At saturating irradiances we see an evenly spaced decrease in A_{max} between the four treatments (Fig. 2A). However, at the higher irradiances, the F_v'/F_m' versus Φ_{PSII} (Fig. 3B) revealed only two responses for the four treatments. The F_v'/F_m' versus Φ_{PSII} of RB/R leaves followed the same pattern as R/R-leaves which was distinctly lower than for the leaves grown under, or acclimated to RB-light. This is paralleled by the nearly identical relationships of Φ_{NPQ} and Φ_{NO} to decreasing Φ_{PSII} shown by these leaves (Fig. 3C and 3D). The responses of Φ_{NPQ} and Φ_{NO} to decreasing Φ_{PSII} shown by leaves grown under, or acclimated to, red light reveals differences between the R/R and RB/R leaves, with the R/R leaves having a greater development of Φ_{NO} and less development of Φ_{NPQ} than the RB/R leaves. This is due to the R/R leaves having a lower dark-adapted F_v/F_m than is developed by the RB/R leaves (Table 1). The result of this is that F_v'/F_m' is also lower in relation to Φ_{PSII} in the R/R leaves compared to the RB/R leaves until Φ_{PSII} has decreased to 0.5 (Fig 3B). As a result Φ_{NO} is greater at higher Φ_{PSII} values in the R/R leaves than in RB/R leaves, and Φ_{NPQ} is correspondingly lower. This reveals on thylakoid level that RB/R-leaves only partially developed the full red-light syndrome with the duration of the experiment. Thus apart from shade acclimation the "red light syndrome" also occurs after normal leaf development.

Plasticity after the "red light syndrome"

Thylakoid level—The low F_v/F_m of the red grown leaves recovered rapidly during exposure to RB-light (R/RB treatment) (Fig. 1C). After the acclimation period, Φ_{PSII} at

growth irradiance was the same for the RB/RB and the R/RB plants (Table 1), and this adaptability in thylakoid functionality is further illustrated by the identical responses shown by F_v'/F_m', Φ_{NO} and Φ_{NPQ} to Φ_{PSII} (Fig. 3B, 3C and 3D) in the RB/RB and R/RB leaves, which differ considerably from those shown by the R/R leaves

Leaf level—The α, gross and net A_{100} and ETR also recovered to the level of RB/RB-leaves, whereas leaf absorption, LMA, N_{org}, g_s and A_{max} increased compared with R/R-leaves but remained lower than in RB/RB-leaves, revealing limitations on the plasticity for acclimation on this level.

The lower g_s both at growth (Table 1) and saturating irradiance (data not shown) is likely due to the lower stomatal index of red developed leaves (Table 2). However the consequences for C_i at these irradiance levels were slight (Table 1 and data not shown). Thus although the plasticity of g_s in R/RB-leaves compared to RB/RB leaves was limited due to the stomatal anatomy, g_s did not limit A_{100} and A_{max} in R/RB-leaves compared to RB/RB leaves.

Oguchi and co-workers (2003) concluded that A_{max} of low light developed leaves which are exposed to high light are physically restricted by the cell size of the leaves and the unoccupied cell surface along which the chloroplasts can expand. Presumably the leaves expanding under red light developed smaller cell sizes which limited full acclimation of these leaves to RB-light resulting in a smaller LMA than RB/RB-leaves (Table 2). The ratios A_{max}/LMA and PNUE did not differ between R/RB and RB/RB leaves (Table 2), supporting the suggestion that cell or leaf structure is limiting for A_{max}. As boundaries in cell size and maximal stomatal conductance are set during the leaf developmental phase (Schoch *et al.*, 1980; Sims and Pearcy, 1992), the implication for later plasticity in acclimation is evident.

Conclusions

We conclude that chlorophyll fluorescence analysis revealed some further symptoms of the "red light syndrome" (on which the increased Φ_{NO} is the most pronounced). The process behind this "red light syndrome" has not been clarified yet. Normal developed leaves exposed to red light showed a strong shade-acclimation-like response, but also the occurrence of the "red light syndrome" (decreased F_v/F_m, a decreased Φ_{PSII} and an increased Φ_{NO}). Leaves after releasing the "red light syndrome" could recover fully at the thylakoid level, while photosynthesis at increasing irradiances was limited at the leaf level possibly due to constraints imposed by morphology.

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CHAPTER 5

Intracanopy lighting with LEDs in cucumber

5.1 The responses of light interception, photosynthesis and fruit yield of cucumber to LED-lighting within the canopy

5.2 The effect of intracanopy lighting on cucumber fruit yield — model analysis

CHAPTER 5.1

The responses of light interception, photosynthesis and fruit yield of cucumber to LED-lighting within the canopy

Abstract

Mathematical models of light attenuation and canopy photosynthesis suggest that crop photosynthesis increases by more uniform vertical irradiance within crops. This would result when a larger proportion of total irradiance is applied within canopies (intracanopy lighting) instead of from above (top lighting). These irradiance profiles can be generated by Light Emitting Diodes (LEDs). We investigated the effects of intracanopy lighting with LEDs on light interception, on vertical gradients of leaf photosynthetic characteristics and on crop production and development of a greenhouse-grown *Cucumis sativus* 'Samona' crop and analysed the interaction between them. Plants were grown in a greenhouse under low natural irradiance (winter) with supplemental irradiance of 221 μmol photosynthetic photon flux m^{-2} s^{-1} (20 h per day). In the intracanopy lighting treatment, LEDs (80% Red, 20% Blue) supplied 38% of the supplemental irradiance within the canopy with 62% as top lighting by High-Pressure Sodium (HPS)-lamps. The control was 100% top lighting (HPS lamps). We measured horizontal and vertical light extinction as well as leaf photosynthetic characteristics at different leaf layers, and determined Total plant production. Leaf mass per area and dry mass allocation to leaves were significantly greater but leaf appearance rate and plant length were smaller in the intracanopy lighting treatment. Although leaf photosynthetic characteristics were significantly increased in the lower leaf layers, intracanopy lighting did not increase total biomass or fruit production, partly because of a significantly reduced vertical and horizontal light interception caused by extreme leaf curling, likely because of the LED-light spectrum used, and partly because of the relatively low irradiances from above.

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Introduction

The photosynthetic rate of a leaf strongly depends on its position in a canopy because of climatic (irradiance, temperature) and physiological factors (photosynthetic properties, stomatal conductance). In closed canopies irradiance strongly decreases with canopy depth. Under natural sunlight and in cases where supplemental irradiance is applied at the top of the canopy (*e.g.* in intensive greenhouse horticulture systems at northern latitudes), the vertical irradiance profile follows an exponential decay with canopy depth that can be described by a modified form of the Lambert-Beer law (Monsi and Saeki 2005):

$$I_d = I_0 e^{-k*LAI} \qquad \text{(eq. 1)}$$

in which I_d is the incident radiation at a depth d from the top of the canopy, I_0 is the incident irradiance just above the canopy, LAI is the leaf area index between the top of the canopy and depth d and k the light extinction coefficient, k depends on the spatial distribution of the incident radiation and leaf position and inclination (Marcelis *et al*. 1998). This relationship has been widely tested for many different types of crops and k-values are typically found to lie in the range of 0.3-1.0 (Monsi and Saeki 2005). Grass-type crops, with vertically inclined leaves have k-values in the range 0.3-0.5. These low k -values result in a more homogeneous vertical irradiance distribution compared with broad-leaf type crops which have more horizontally inclined leaves and k -values of 0.7-1.0 (Monsi and Saeki 2005, Thornley and France 2007). The response of a leaf to any environmental factor, such as irradiance, will be strongly determined by the photosynthetic properties of the leaf. These develop during leaf expansion but can subsequently acclimate even in mature leaves in response to the ambient irradiance, and other conditions. Within an upright plant stand with an exponentially decaying irradiance profile this acclimatory response usually results in a progressive decrease in photosynthetic capacity of leaves (A_{max}) with increasing depth in the canopy (Boonman *et al*. 2006, Xu *et al*. 1997). The amount of nitrogen per unit leaf area is strongly correlated with A_{max} and the adjustment of A_{max} of leaves within the canopy in response to the developing irradiance gradient can be understood as a mechanism for optimising the photosynthetic nitrogen use efficiency of plants within the canopy (Hikosaka 2005, Hirose 2005, Hirose and Werger 1987, Terashima *et al*. 2005). Crop productivity and growth depends on net crop photosynthesis. The Monsi-Saeki approach for calculating vertical irradiance profiles within a crop has been widely used to up-scale photosynthesis from the leaf to the canopy level and forms the basis of many models used to calculate productivity of agricultural and horticultural crops (Marcelis *et al*. 1998, Van Ittersum *et al*. 2003).

Advanced production systems in horticulture at northern latitudes increasingly rely on the addition of artificial assimilation lighting, which is usually applied by a gas discharge lamp-type (High-Pressure Sodium, HPS). Traditionally these lamps are positioned above the canopy because their high operating temperature (>1400 K in the arc tube) precludes positioning them within dense canopies with small aisle widths like in the Netherlands, though in countries around Scandinavia where wider aisle widths are common, these lamps are used within canopies (Gunnlaugsson and Adalsteinsson 2006, Hovi *et al*. 2004, Hovi-Pekkanen and Tahvonen 2008, Pettersen *et al*. 2010a). Because of their position above the canopy the irradiance produced by these lamps follows a similar exponential decay with depth in the canopy as does natural irradiance. It has been realized for some time that placing the artificial light source within the canopy (intracanopy lighting) would generate a more homogeneous vertical irradiance profile within a canopy and that this might increase the light-use efficiency of the supplemental lighting by two routes. Firstly, it would eliminate the loss of some supplemental irradiance by reflection from the upper canopy layer toward the sky, whereas reflected light within the canopy can be absorbed by other leaves (this corresponds to approximately 6-7% of the incident irradiance; Goudriaan and Van Laar 1994, Marcelis *et al*. 1998). Secondly, light intensities (natural + supplementary irradiance) that exceed the linear, light-limited phase of the photosynthetic irradiance-response of leaves in the canopy can be more easily avoided by supplying the supplemental irradiance to the lower rather than the upper leaves. On the other hand, not all responses to intracanopy lighting need be positive: leaf inclination, for example, might change in response to intracanopy lighting and decrease the absorption of natural irradiance that enters the canopy from above.

During the last decade, light emitting diodes (LEDs) have attracted interest as a light source for assimilation lighting. LEDs emit radiation within a narrow band of the spectrum. In particular their low operating temperature (approximately 25-35°C), low operating voltage and physical robustness make LEDs uniquely suitable for use in intracanopy lighting applications. Using LED arrays as supplemental light sources, we investigated the effects of a combination of intracanopy lighting and conventional supplementary irradiance on leaf photosynthetic characteristics, crop productivity and yield in a cucumber (*Cucumis sativus* 'Samona') crop over a period of approximately 3 months under low natural irradiances (winter). Crop production, leaf photosynthetic characteristics along the vertical crop axis and both vertical and horizontal light interception were measured. Partial intracanopy lighting was chosen to avoid complications because of limits in the acclimatory responses of the leaves arising from their development under the low natural irradiances that prevailed during the experiment. (Sims and Pearcy 1989, 1992).

Materials and methods

Plant material and growth conditions

Cucumber plants (*Cucumis sativus* 'Samona') were planted on 30 September 2008 and grown for a total period of 18 weeks in two greenhouse compartments (144 m^2) in Wageningen, the Netherlands (52°N, 5.5°E). All plants were grown on rockwool substrate, in a double row 'high wire' system (Van Henten *et al*. 2002) at a relatively high density of 3.4 plants m^{-2}, which was previously shown to improve cucumber yield over lower planting densities (Janse *et al*. 2004, 2005). Average day and night temperatures, RH and CO_2 concentration of the greenhouse air were, respectively, 20.8 and 18.1°C, 81% and 915 μmol mol^{-1}. A standard nutrient solution for cucumber growth was used (Sonneveld 1996).

All plants were grown for 5 weeks under the same light conditions until they reached the 'high wire' (plant length >2.1 m): natural daylight plus supplemental assimilation lighting (221 μmol photosynthetic photon flux (PPF) m^{-2} s^{-1}) from 0:00 to 20:00 h supplied by HPS lamps (600 W, 400 V, Philips Master Greenpower CG, Philips, Eindhoven, The Netherlands) positioned above the canopy. Then the treatments (control and partial intracanopy lighting; see below) were started, resulting in an experimental period of 13 weeks. According to normal cultivation practise, all plants were lowered twice a week to keep their apices at a constant distance from the assimilation lamps above the canopy, old leaves were removed from the bottom of the canopy, and from every second axil one flower bud was removed, leaving two leaves per fruit in each treatment.

Lighting treatments

After the initial growth phase, each greenhouse compartment was divided in two halves with different supplemental top lighting irradiance levels: one-half was kept at 221-μmol PPF m^{-2} s^{-1} (as described above; control treatment), in the other half the top lighting supplemental irradiance level was reduced to 139 μmol PPF m^{-2} s^{-1} (400 W HPS lamps, 230 V, Philips Master Greenpower CG, Philips, Eindhoven, The Netherlands). Experimental plots in the latter half were supplemented with 82 μmol PPF m^{-2} s^{-1} intracanopy lighting supplied by LEDs (partial intracanopy lighting treatment) to reach the same 221 μmol PPF m^{-2} s^{-1} as in the control treatment. The lamp spectra used are shown in Fig. 1. The input of supplemental photosynthetic active radiation (PAR) on energy basis was, respectively, 44.8 and 44.3 W m^{-2} for control and partial intracanopy lighting. The LED arrays were positioned in the aisles between the rows and illuminated the plants from aside (Fig. 2). The LED arrays consisted of the same number of independently dimmable red (peak wavelength at 667 nm) and blue (peak wavelength at 465 nm) LED modules (Philips, Greenpower LED modules HF, Philips, Eindhoven, The Netherlands) mounted on a 2 m × 1 m aluminium frame with a perfectly mixed red/blue ratio (20% blue on quantum basis and 26% blue on energy

basis) at >35 cm distance from the frames. The top of the LED frames were placed at 70 cm below the top of the canopy at the level of the first fully grown leaf. In each half of the two compartments, two lighting plots were situated. Each plot consisted of eight plants surrounded by a large number of border plants.

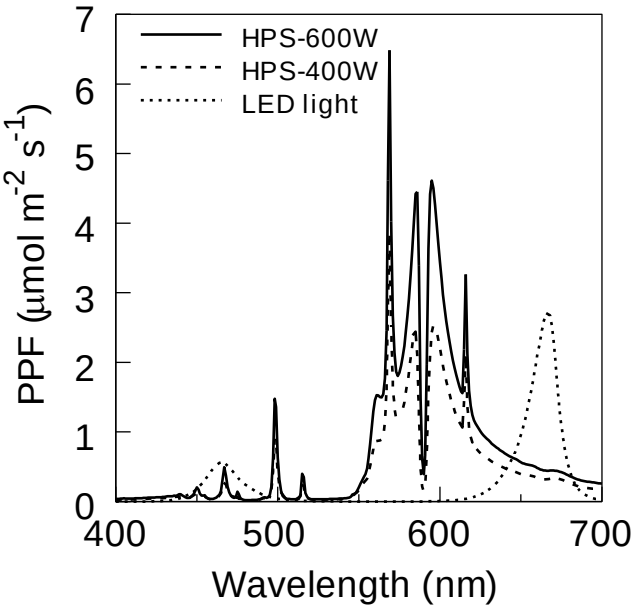

Fig. 1. Irradiance spectra of the lamps used in the experiment. Solid line represents the 600-W HPS lamp, the dashed line the 400-W HPS lamp and the dotted line the LED lamps.

At the start of the experiment the light intensity of HPS assimilation lighting from above was checked above all plots using a line quantum sensor (LI-191SA, Li-Cor Inc., Lincoln, NE). To ensure a light absorption of 82 µmol PPF m^{-2} s^{-1} in the intracanopy lighting plots (to mimic an 'infinite' crop in horizontal direction) horizontal transmission of LED-light was checked weekly with the line quantum sensor (see procedure below) and, if necessary, irradiance output of the LED arrays adjusted. Horizontal transmission in the intracanopy lighting plots (8 ± 1%) was approximately constant over the experimental period. Horizontal reflectance losses in the intracanopy lighting plots were measured once during the night (1.7 ± 0.2%; see procedure below) and were not corrected for. In each greenhouse compartment the plots were situated in such a way that pair-wise comparisons could be made between the control and partial intracanopy lighting treatment in the plots on the south and the north side of the greenhouse compartments.

Irradiance profile measurements

Vertical irradiance profiles were measured in the absence of natural daylight and intracanopy lighting with the line quantum sensor perpendicular to the path direction at intervals of 30 cm from top to bottom. Per plot, three measurements were made at

each height. Horizontal irradiance profiles were measured in the absence of natural daylight and supplemental top lighting. Measurements were taken at a height corresponding to the middle of the LED arrays, just before the first plant row next to the LEDs, between the rows and after the second plant row (Fig. 2). For measurements of the horizontal irradiance profile in the control plots, LED frames were temporarily placed within the canopy so that any difference in horizontal light extinction between both treatments could be attributed to differences in leaf inclination or other plant factors.

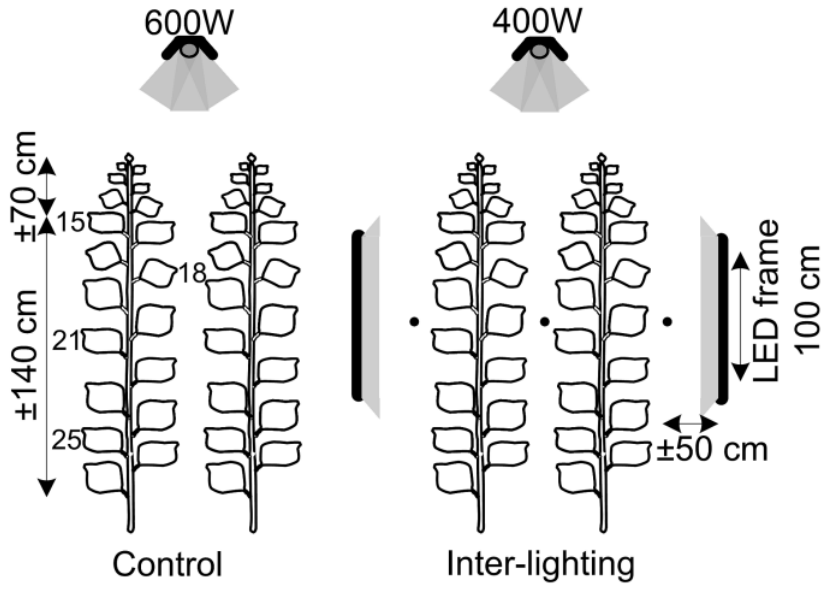

Fig. 2. Schematic representation of the control and partial intracanopy lighting treatment. The control consisted of 100% supplemental top lighting (600-W HPS lamps), partial intracanopy lighting consisted of 62% top light (400-W HPS lamps) and 38% intracanopy lighting by mixed red and blue LEDs (80 and 20%, respectively). Numbers next to the leaves of the left plant indicate the leaf number counted from top downwards which indicate, respectively, layer one to four. Black dots in the partial intracanopy lighting treatment indicate the locations where the horizontal irradiance profile was measured.

The PPF of natural irradiance inside the greenhouse just above the crop was calculated from the irradiance measured on top of the greenhouse with a solarimeter (Kipp en zonen, Delft, The Netherlands) assuming that 45% of the global radiation is PAR (Jacovides *et al*. 2003) and that the conversion factor from energy flux to quantum flux for natural sunlight in the PAR region is 4.57 μmol J^{-1} (McCree 1972b). These factors were verified by placing a quantum sensor (Li190, Li-Cor Inc.) next to the solarimeter for a few days during the experimental period. PPF transmission through the greenhouse was determined to be 62% by comparing the output of the quantum sensor at crop level with the PPF outside the greenhouse.

Crop measurements

Fruits were harvested biweekly. Fresh and dry weight of harvested fruits and removed leaves were recorded, as well as was the leaf area of the removed leaves (Li-3100, Li-Cor Inc.). Dry weight was determined after drying leaves for one night, and harvested fruits for two nights at 105°C. At the end of the experiment, all plants within each plot were harvested and fresh and dry weights of leaves, fruits and stems were determined as was stem length. Total dry mass (M_D) production over the experimental period was calculated from the intermediate and end harvest. Leaf appearance rate was determined on two plants per plot by monitoring leaf appearance three times a week. Temperature of the apex of the plants in each of the plots was measured with a handheld infrared thermometer (Raytek Raynger ST Temperature Device, Santa Cruz, CA) on a sunny day and on a cloudy day.

Measurements at leaf level

Four leaf layers were defined: the first layer started at the first fully expanded leaf (approximately the 15th leaf counted from the first developing leaf >2 cm length), the subsequent layers, respectively, at the 18th, the 21st and the 25th leaf (Fig. 2). The distance from the top of the plant was. respectively, approximately 70, 105, 140 and 185 cm for these layers. Photosynthetic irradiance-response curves, leaf mass per area (LMA), organic nitrogen and chlorophyll content were determined for each leaf layer. These measurements were done in December and January on a representative plant in each plot, so eight measurements were made per treatment per layer. All measurements at leaf layer level were made simultaneously in a control and an intracanopy lighting plot positioned in the south or the north part of a compartment to enable pair-wise comparisons.

Leaf photosynthesis was determined using a Li-6400 portable photosynthesis system equipped with a leaf chamber fluorometer (Li-Cor Inc.). During all measurements, CO_2 concentration in the leaf chamber was 1000 μmol mol^{-1}, the airflow was 250 μmol s^{-1}, the leaf chamber temperature 22°C, the humidity was approximately 80% (similar to the humidity in the greenhouse), and the percentage blue light in the leaf chamber was set at 10%. Irradiance-response curves were determined from zero to saturating irradiance. At each irradiance level the rate of photosynthesis was calculated as the mean of the last 40 s after steady state gas exchange was reached, and photo system II operating efficiency (F_q'/F_m' ; Baker *et al*. 2007) was determined by recording F_s at steady state and F_m' after applying a saturating light pulse (>7000 $\mu molm^{-2}$ s^{-1} for 0.8 s). The measurement of the irradiance-response was stopped when the measured F_q'/F_m' was lower than 0.14. The measurement of a full irradiance-response curve took about 3 hours.

Late in the afternoon, after the photosynthesis measurements, 12 leaf discs of 1 cm in diameter were removed randomly from over the measured leaf. LMA was determined by freeze drying these leaf discs. Organic nitrogen was defined as the total

nitrogen measured with an elemental C/N analyzer (model EA 1108, FISONS Instruments, Milan, Italy) minus nitrate. Nitrate was measured using an automatic inorganic nitrogen analyzer (Auto Analyzer II System; Technicon Industrial Systems, Technicon Instruments, St. Denis, France). Fifteen leaf discs of 5.5 mm diameter were cut randomly from the same leaf for chlorophyll measurements. Dimethyl formamide was used as solvent and the absorbance of the extracts were measured using a Cary 4000 photospectrometer (Varian instruments, Walnut Creek, CA) and chlorophyll concentrations were calculated using the equations derived by Porra *et al*. (1989).

Shortly after the photosynthesis measurements in January we took two other sets of leaf samples. The first set was taken just before the end of the photoperiod and we determined LMA, organic nitrogen (as described above), starch and soluble sugar (glucose, fructose and sucrose) content, and structural carbon content, the latter being defined as total carbon minus the carbon content of starch and soluble sugars. We randomly cut 12 leaf discs of 1 cm diameter from each layer at both sides of each plot and we, in addition, defined a fifth layer, which were bottom leaves to be removed the next morning. Starch and other sugars were measured using the method described in Hogewoning and Harbinson (2007). The second set of leaf samples were taken to determine leaf absorptance (1-reflectance-transmittance) between 400 and 700 nm. We randomly cut five leaf discs from each layer of each plot and transmittance and reflectance was measured as described in Soares *et al*. (2008).

Calculations and statistics

The measured photosynthetic irradiance-response data were fitted with a non-rectangular hyperbola (Eq. 2; Thornley 1976) using the non-linear fitting procedure NLIN in SAS (SAS institute Inc. 9.1, Cary, NC) to determine dark respiration (R_D), the maximum gross photosynthetic rate (A_{mg}), light-limited quantum efficiency (α) and the scaling constant for curvature (θ) of leaves in the different leaf layers and light treatments:

$$A_{net} = \frac{\alpha \cdot PPF + A_{mg} - \sqrt{(\alpha \cdot PPF + A_{mg})^2 - 4\theta \cdot PPF \cdot A_{mg}}}{2\theta} - R_D \qquad \text{(eq. 2)}$$

The extinction coefficient (k; Eq. 1) was calculated by using the non-linear fitting procedure NLIN in SAS by combining the vertical light measurements and the measured LAI at different plant heights at the final harvest.

Overall light-use efficiency (LUE) was defined as the ratio between the total M_D produced and the total sum of absorbed light during the experimental period. Though a small part of this M_D was produced before the start of the experiment this was not treated differently in the analysis. The sum of the PPF absorbed by the canopy from different sources in the different light treatments was calculated using the measurements of reflection and transmission (fraction absorbed PPF minus fractions of reflected and transmitted PPF) of all irradiance types (natural PPF, HPS-assimilation

lighting from above and (only in the partial intracanopy lighting treatment) lateral LED assimilation lighting).

Data are presented as the average of four plots per treatment, and each plot is based on the average of eight plants. Paired Students t -tests (two-tailed) were used to test for statistical significant differences between the control and intracanopy lighting treatment. *P*-values smaller than 0.05 were regarded as significantly different.

Results

The fraction of PPF because of natural irradiance that reached the canopy in both light treatments (control and partial intracanopy lighting) over the whole experimental period was only 18% (Table 1) of the total amount of incident PPF. The transmittance of PPF to the greenhouse floor was significantly higher in the partial intracanopy lighting treatment compared with the control (respectively, 6.0 ± 0.9 vs 2.6 ± 0.2%; P = 0.044; Fig. 3A). Vertical entering PPF attenuated faster with increasing depth (and overlaying LAI) in the control compared with the partial intracanopy lighting treatment (Fig. 3A, B). The fitted k (Eq. 1) was significantly lower in the partial intracanopy lighting treatment than in the control (0.57 vs 0.87). So, contrary to the

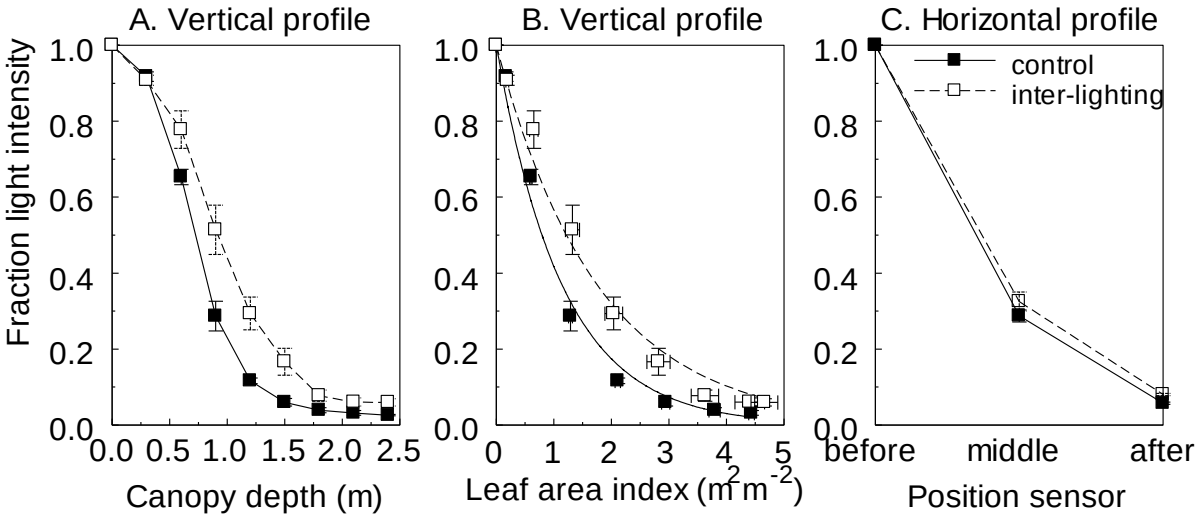

Fig. 3. The effect of partial intracanopy lighting on vertical and horizontal fraction profiles of light intensity within a cucumber crop. Vertical fraction profiles as a function of canopy depth (A) and overlaying LAI (B). Horizontal fraction profiles of light intensity as determined just before, in between (middle) and after the double plant rows (C; see for places black dots in Fig. 1). The control is represented by solid symbols and the partial intracanopy lighting treatment by open symbols. Vertical and horizontal bars indicate SE (n = 4). Lines in B are the result of fitting the fraction light intensity against overlaying LAI to the Monsi-Saeki equation (Eq. 1). Estimated values for k from the control and intracanopy lighting fits were, respectively, 0.87 and 0.57.

Table 1. Incident and absorbed PAR integrals over the experimental period (expressed in mol photons per m^2 ground surface) for the control and partial intracanopy lighting treatment.

	Control		Intracanopy lighting	
	incident light	absorbed light[1]	incident light	absorbed light[1]
Natural irradiance	312 (17.9%)	286 (17.9%)	312 (17.9%)	275 (17.3%)
Top-lighting	1433 (82.1%)	1314 (82.1%)	904 (51.8%)	798 (50.1%)
Inter-lighting			529 (30.3%)	520 (32.6%)
Total	1745 (100%)	1600 (100%)	1745 (100%)	1593 (100%)

[1] Absorbed PAR integrals were calculated from incident PAR integrals using measured reflectance and transmittance factors of vertical (control and intracanopy lighting) and horizontal irradiance profiles (intracanopy lighting only).

expected increase in the fraction of PPF absorbed in the partial intracanopy lighting treatment because of a reduced reflection loss from the upper leaf layer, the absorbed PPF was approximately similar in the two light treatments (Table 1) and consequently the total absorbed PPF was the same in the partial intracanopy lighting treatment as in the HPS treatment. The horizontal transmittance was also significantly higher in the partial intracanopy lighting treatment than it was in the control treatment (respectively, 5.8 ± 0.3 vs 8.0 ± 0.3; P = 0.0005; Fig. 3C). Leaf morphology greatly differed between control and intracanopy lighting plots: control leaves had a normal appearance (Fig. 4A, C), whereas extreme leaf curling was observed in the partial intracanopy lighting plots (Fig. 4B, C). Time lapse photography (an image per 10 min) during several 24h periods showed that this curling was a permanent feature. Likely, the decrease in the k in the partial intracanopy lighting treatment could be because of this morphological effect. The total amount of M_D produced during the experimental period was not altered by the partial intracanopy lighting treatment (Table 2). The lack of difference in both absorbed PPF and M_D production resulted in a similar LUE of the absorbed PPF (expressed as g M_D per mol PPF) in the partial intracanopy lighting treatment and the control (Table 2).

However, partial intracanopy lighting caused some conspicuous effects on development and morphology of the plants and on the M_D distribution between the organs leaf, stem and fruits. Partial intracanopy lighting reduced total stem length as well as the total number of leaves that emerged over the experimental period, and on cumulative leaf area (Table 3). The difference in the total number of leaves per plant was in agreement with the separately measured rate of leaf appearance (Table 3). Temperature of the plant apex did not differ significantly between the control and partial intracanopy lighting plots on sunny days (respectively, 23.1 ± 0.2 and 22.7 ±

0.3°C; *P* = ns) and was about 1°C higher in the control treatment on cloudy days (respectively, 21.1 ± 0.4 and 20.0 ± 0.4; *P* = 0.0014). Despite the lower number of leaves, more M_D was allocated to the leaves at the expense of the fruits in the partial intracanopy lighting treatment. The difference was small but statistically significant (Table 2). This, when taken together with the reduction in amount of leaf area produced, resulted in a considerably larger LMA in the partial intracanopy lighting treatment (Table 3). Neither the Total amount of M_D allocated to the fruits (Table 2) nor the M_D allocated to all harvestable fruits (Table 4) differed significantly between the control and partial intracanopy lighting treatments. As the fruit yield (fresh mass, M_F) was significantly lower in the partial intracanopy lighting treatment the % M_D in fruits was significantly higher in the partial intracanopy lighting treatment (Table 4). In agreement with the lower number of leaves in the partial intracanopy lighting treatment the number of fruits (harvestable and total) was also significantly lower in this treatment. The percentage aborted fruits was for both treatments around 40% (Table 4). Intriguingly, the % M_D in leaves and stems was also, respectively, 14 and 9% higher for the partial intracanopy lighting plants compared with the control plants (respectively, *P* = 0.0004 and *P* = 0.042; data not shown).

Fig. 4. The effect of intracanopy lighting on the leaf inclination of a cucumber crop, control (A) and partial intracanopy lighting (B). C and D show, respectively, one representative leaf for the control and the partial intracanopy lighting treatment.

Table 2. Crop M_D production, proportioned over leaves, fruits and stem in grams M_D per m^2, % partitioning of M_D to the different organs and calculated overall LUE (in grams M_D per mol absorbed PAR) in the control and partial intracanopy lighting treatments (n = 4). *P*-values > 0.05 were regarded as non-significant (ns).

	Control±SE	Intracanopy lighting±SE	*P*-value
Leaves (g m^{-2})	723±9.2	769±7.1	0.004
Fruits (g m^{-2})	795±19.4	763±18.1	ns
Stem (g m^{-2})	216±1.3	226±3.7	ns
Total (g m^{-2})	1734±20.4	1758±13.8	ns
Leaves (%)	41.7±0.6	43.8±0.5	0.016
Fruits (%)	45.8±0.7	43.4±0.8	0.017
Stem (%)	12.4±0.1	12.9±0.3	ns
LUE (g M_D mol^{-1})	1.084±0.012	1.104±0.015	ns

Table 3. The effect of partial intracanopy lighting on developmental and morphological characteristics of leaves and whole plants in a cucumber crop. *P*-values > 0.05 were regarded as non-significant (ns).

	Control±SE	Intracanopy lighting±SE	*P*-value
LAI (at final harvest; m^2 m^{-2})	4.44±0.05	4.66±0.23	ns
Total leaf area produced (m^2 m^{-2})	25.3±0.3	21.8±0.28	0.005
Leaf mass per area (LMA; g m^{-2})	27.8±0.4	34.3±0.21	0.001
Average area of leaves (cm^2 $leaf^{-1}$)	785±7	750±9.65	ns
Leaf appearance rate (d^{-1})	0.9±0.02	0.78±0.01	0.021
Number of leaves per plant	94.2±1.9	84.8±0.6	0.03
Plant length (at final harvest; m)	11.9±0.08	10.7±0.1	0.005
Average internode length (cm)	11.6±0.2	11.5±0.1	ns

Partial intracanopy lighting significantly changed the photosynthetic properties of leaves at different canopy depths compared with the control (Fig. 5). In both light treatments, photosynthetic capacity (A_{mg}) was higher in the second than in the first leaf layer (Table 5). A similar trend was observed for chlorophyll content (Fig. 6A). It is possible that the leaves in the first leaf layer were not completely mature despite being

fully expanded. In the highest two leaf layers, A_{mg} was not different for both light treatments, which indicated a comparable start position for all leaves between the light treatments when they start their path downwards in the canopy. In the third and fourth leaf layers, however, A_{mg} was significantly higher in the partial intracanopy lighting treatment compared with the control (Table 5, Fig. 5). The light-limited quantum efficiencies did not notably differ between the two light treatments for each of the four leaf layers, but was reduced to a similar extent in both light treatments in the fourth leaf layer compared with all leaf layers above (P = 0.025). In all leaf layers except the uppermost, significantly higher chlorophyll contents and chlorophyll a/b ratios were observed in the partial intracanopy lighting treatment compared with the control (P < 0.05; Fig. 6A, B). The average leaf absorptance per leaf layer did not significantly differ between both treatments (Fig. 6C). LMA, structural carbon content and organic nitrogen content per leaf layer decreased gradually from top to bottom in the control treatment, while they were approximately constant in the partial intracanopy lighting treatment and significantly higher than the control from layer three downwards (P < 0.05; Fig. 7A, C, D). Starch accumulated most in the highest leaves in the control treatment, while in the partial intracanopy lighting treatment the greatest starch accumulation was found in the lower leaf layers (except the 5th layer) (P < 0.05; Fig. 7B).

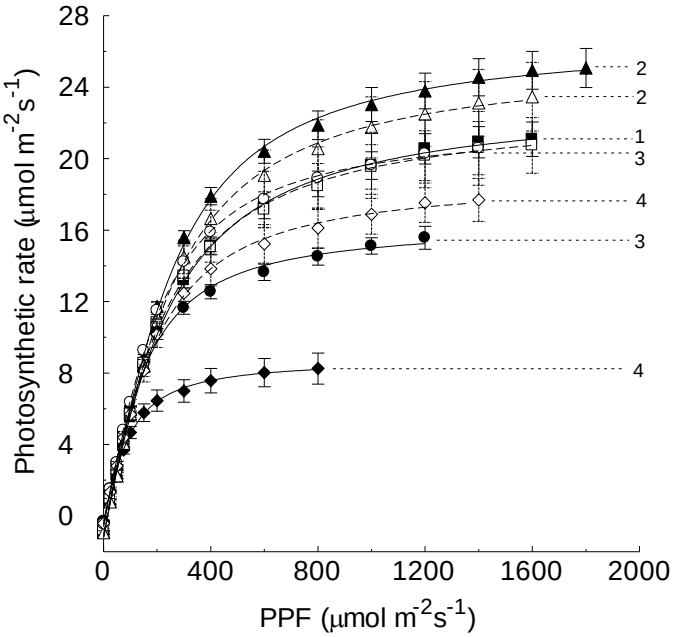

Fig. 5. The effect of partial intracanopy lighting on photosynthetic irradiance response curves of leaves at different depths in the canopy. Partial intracanopy lighting (open symbols and dashed lines), control (solid symbols and lines). Squares, triangles, circles and diamonds indicate, respectively, leaf layer 1, 2, 3 and 4 counted from top to bottom in the canopy. Bars indicate SE (n = 4). Lines through data points represent the fit of the non-rectangular hyperbola (Eq. 2).

Table 4. Total cucumber fruit yield of harvestable and aborted fruits of the control and partial intracanopy lighting treatment (n = 4). *P*-values >0.05 were regarded as non-significant (ns).

	Control±SE	Intracanopy lighting±SE	*P*-value
Harvestable fruits			
M_F (kg/m^2)	26.3±0.6	24.9±0.5	0.030
M_D (g/m^2)	688±15.7	680±17.8	ns
% M_D	2.61±0.01	2.73±0.02	0.024
Number (m^{-2})	79.6±2.8	74.3±2.6	0.032
Aborted fruits			
M_F (kg m^{-2})	1.26±0.05	1.13±0.05	0.041
M_D (g m^{-2})	47.0±2.4	44.8±2.16	ns
% M_D	3.72±0.07	3.98±0.16	ns
Number (m^{-2})	50.9±0.9	50.8±1.5	ns

Discussion

Partial intracanopy lighting did not increase crop productivity

Applying part of the supplemental irradiance within the canopy (intracanopy lighting) of a fully grown glasshouse cucumber crop instead of wholly from above did not result in a statistically significant increase in produced M_D over the 3-months experimental period (Table 2). Nonetheless, partial intracanopy lighting resulted in significant changes in light interception profiles, leaf photosynthetic characteristics, crop development and dry-matter partitioning among the different organs in the crop. These other changes brought about by intracanopy lighting account for the absence of any yield increase.

Absorption of natural and supplemental irradiance from above is altered by partial intracanopy lighting

In contrast to what was expected, the total absorbed PAR (on quantum basis) in the partial intracanopy lighting treatment was not higher than in the control (Table 1). As the intracanopy lighting is applied within the canopy it would be expected that the total absorption of radiation would be higher than if all irradiance was applied from above as losses because of reflection from the upper surface of the canopy to the sky are avoided by intracanopy lighting because of absorption of reflected light by other leaves. There are two explanations for this discrepancy. The first accounts for only a

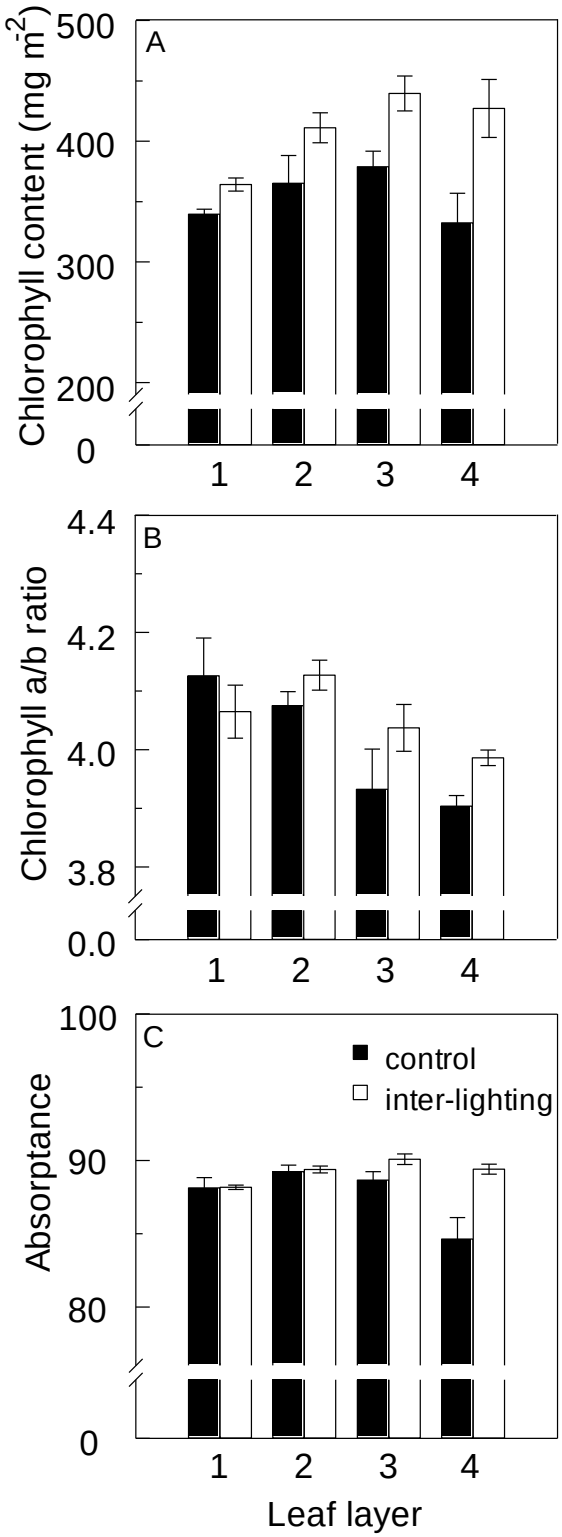

Fig. 6. The effect of partial intracanopy lighting on chlorophyll content (A), the chlorophyll *a*/*b* ratio (B) and the average leaf absorptance between 400 and 700 nm (C) in leaves at different depths in a cucumber canopy. Control (solid bars), partial intracanopy lighting treatment (open bars). Bars indicate SE (n = 4).

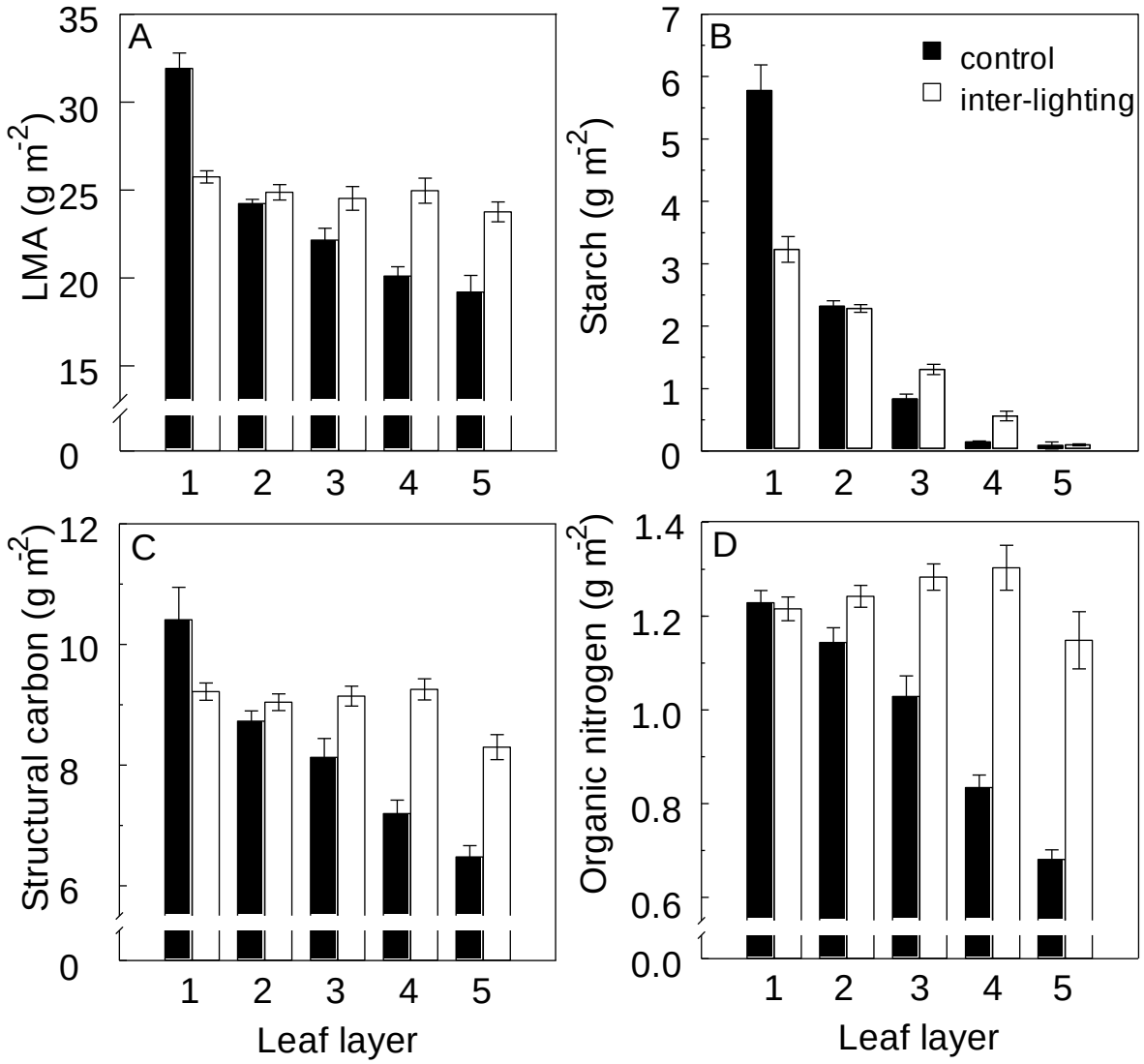

Fig. 7. The effect of partial intracanopy lighting on LMA (A), starch content (B), structural carbon content (total carbon minus carbon in soluble sugars and starch; C) and organic nitrogen content (D) in leaves at different depths in a cucumber canopy. Control (solid bars), partial intracanopy lighting treatment (open bars). The additional fifth layer represent leaves which were picked the next day. Bars indicate SE (n = 4).

small part of the error, approximately 0.5%, and is because of the omission of a correction for horizontal reflection of the supplemental intracanopy lighting. The more important explanation, however, is that intracanopy lighting negatively influenced the crop absorption of PPF from above (*i.e.* natural PPF and the supplemental PPF provided by HPS lamps mounted above the canopy): The fraction of PPF from above that was absorbed by the canopy was 3-4% lower in the partial intracanopy lighting treatment than in the control (Fig. 3A). As a result an important part of the expected increase in crop carbon gain because of increased light absorption in the partial intracanopy lighting treatment was lost. The geometry of PPF entering the crops from above was the same in the control and intracanopy lighting treatments. Compared

Table 5. The effect of partial intracanopy lighting in a cucumber crop on the R_D, A_{mg} and light-limited quantum efficiency (α) of individual leaves at different depths in the canopy (1 = high and 4 = low in the canopy; n = 4). *P*-values >0.05 were regarded as non-significant (ns).

	A_{max} (µmol $m^{-2}s^{-1}$)			α			R_D (µmol $m^{-2}s^{-1}$)		
Leaf layer	Control ± SE	Intracanopy lighting ± SE	*P*-value	Control ± SE	Intracanopy lighting ± SE	*P*-value	Control ± SE	Intracanopy lighting ± SE	*P*-value
1	24.8 ± 1.4	24.1 ± 1.8	ns	0.084 ± 0.004	0.085 ± 0.003	ns	1.28 ± 0.08	1.08 ± 0.15	ns
2	27.7 ± 1.6	27.0 ± 1.9	ns	0.084 ± 0.001	0.087 ± 0.005	ns	1.21 ± 0.11	1.35 ± 0.18	ns
3	16.9 ± 0.5	23.0 ± 1.4	0.018	0.081 ± 0.003	0.085 ± 0.002	ns	0.44 ± 0.08	0.66 ± 0.05	ns
4	9.3 ± 0.9	19.7 ± 0.7	0.005	0.077 ± 0.002	0.076 ± 0.005	ns	0.33 ± 0.08	0.63 ± 0.05	0.039

with the control crop, light penetration in the partial intracanopy lighting crop started to differ at the top of the light field produced by the LED arrays and this difference in penetration persisted through the remaining depth of the canopy (Fig. 3A). Measurements on canopy structure (*i.e.* area of individual leaves, internode length, leaf area per leaf layer and individual leaf absorptance properties; Table 3 Figs 3B and 5C) did not show any significant difference between the light treatments, and thus cannot explain the altered light penetration in the intracanopy lighting canopy. The deeper light penetration measured seems largely in accordance with the estimated lower value for the extinction coefficient *k* in the Monsi-Saeki approach for vertical light extinction in the canopy (Fig. 2B). A more vertical inclination of leaves, such as commonly observed in grasses (Monsi and Saeki 2005), facilitates a deeper penetration of irradiance in the canopy. However, light transmission in horizontal direction (Fig. 3C) was also increased in the partial intracanopy lighting treatment, which is not consistent with a more vertical leaf inclination. So the most likely explanation for the difference in vertical and horizontal light attenuation in the canopy is the occurrence of extreme leaf curling in the partial intracanopy lighting treatment (Fig. 4B, D) which decreased the effective light intercepting leaf area in both the vertical and horizontal direction.

This leaf curling seems a kind of light-avoidance response. Leaf light-avoidance responses have been described previously as a consequence of water stress (Shackel and Hall 1979, Wainwright 1977) and possibly as a means for avoiding photo-inhibition (Berg and Hsiao 1986, Powles and Bjorkman 1982). Both explanations are unlikely. At first, the significantly higher % M_D of all measured plant organs within the partial intracanopy lighting treatment implies a structurally higher evaporative demand in the partial intracanopy lighting treatment, which could have been caused by specific spectral (enhanced % blue in intracanopy lighting) and intensity effects of light on stomatal conductance in the lower leaf layers (Zeiger *et al*. 1981). However, though we expect higher evaporation in the partial intracanopy lighting treatment we do not feel that this would lead to water stress: air humidity and water availability to the crop were both very high, while the evaporative demand induced by the natural and supplemental irradiance was rather low during the whole experimental period and even reduced in the upper leaf layers of the partial intracanopy lighting treatment because of the lower intensity of supplemental irradiance from above. At second, photo protective leaf curling also seems unnecessary because actual light intensities employed were far from saturation, though light-avoidance responses provoked at high irradiances are also regulated by the spectral composition of incident irradiance (Koller 1990). Though in our experiment we cannot separate between a light intensity effect or a spectral effect on leaf curling, because for such a comparison we miss a treatment of intracanopy lighting with HPS lamps (which was impossible as explained in the introduction), comparable experiments with intracanopy lighting in cucumber while using HPS lamps (low % blue light) did improve fruit yield while effects of

intracanopy lighting on leaf curling were not reported (Hovi *et al*. 2004, Hovi-Pekkanen and Tahvonen 2008, Pettersen *et al*. 2010a). So it is possible that the leaf curling that developed in the mature leaves subjected to the partial intracanopy lighting treatment is a photomorphogenetic effect provoked by the relatively high blue content of the irradiance used.

Partial intracanopy lighting influences leaf photosynthetic properties

Incident irradiance during leaf formation was inevitably higher in the control than in the partial intracanopy lighting treatment because of the 38% higher level of supplemental HPS-light from above. The higher light intensity during leaf development in the control was clearly reflected in a higher LMA and a higher structural carbon content (Fig. 7A, C), which is in accordance with the view that an higher LMA can be seen as an acclimatory response of developing leaves to high light intensity (Evans and Poorter 2001). However, this higher LMA was not accompanied by higher chlorophyll and organic nitrogen contents (Figs 6A and 7D), nor by a higher photosynthetic capacity (A_{mg}; Fig. 2). Further, a large part of the difference in LMA in the upper leaves can be attributed to non-structural starch (Fig. 7B). An higher irradiance on mature leaves can increase the thickness of leaves developing on the same plant, as was shown in *Chenopodium* plants by shading the developing leaves and subjecting older leaves to high irradiance (Yano and Terashima 2001, 2004). Whether the intracanopy lighting can have triggered such effects is not clear in our data, but an effect of this kind could explain the absence of any difference in certain parameters between young leaves in the two treatments. In both light treatments used here the upper leaf layer was not fully mature, as can be deduced from the higher A_{mg} in layer two than in layer one. This might also explain why the higher LMA in the first layer of the control is not accompanied by an higher organic nitrogen and chlorophyll content. The reduction in photosynthetic capacity (A_{mg}) from leaf layer two downwards in the control treatment (*i.e.* with increasing leaf age and decreasing light intensity) was accompanied by a decrease in organic nitrogen per area and the chlorophyll a/b ratio (Figs 6B and 7D) which is in line with other results (Boonman *et al*. 2006, Xu *et al*. 1997), but not with chlorophyll per unit area (Fig. 6A). In the partial intracanopy lighting treatment, there was a smaller decrease in A_{mg} with canopy depth, while chlorophyll content and organic nitrogen content did not decrease with canopy depth (Figs 6A and 7D).

In crop production in relatively low irradiance levels, the light-limited quantum efficiency and the R_D are the major limiting factors in crop performance and not A_{max}. Changes in R_D were small although the higher R_D in the lower leaf layers of the partial intracanopy lighting treatment compared with the control may have reduced net crop photosynthesis (Table 5). Present experiment was conducted in winter at a relatively low natural PPF and short days, so the total irradiance from above was in both treatments rather low (<300-µmol PPF m^{-2} s^{-1}). The photosynthetic irradiance-response

curves (Fig. 5) of the upper leaf layers illustrate that even in the control treatment the maximum PPF on the upper leaves because of both natural and supplemental irradiance during the experimental period was still in the largely light-limited part of the irradiance-response curve (Fig. 5). Under these conditions redistribution of irradiance from the upper leaves toward the lower leaves in order to increase crop photosynthesis would hardly have had any advantage unless the photosynthetic efficiency was higher in the lower leaf layers. The latter was not the case (Table 5).

Partial intracanopy lighting influences crop development and M_D partitioning

Leaf appearance rate was approximately 15% lower in the partial intracanopy lighting treatment (Table 3), which resulted in an approximately 1-m shorter stem at the end of the experiment. In cucumber, leaf appearance rate is influenced by temperature, integrated light intensity (or assimilate availability) and sink strength (Marcelis 1993). Especially on cloudy days and presumably also during the relative long daily periods without natural sunlight, the temperature of the plant apex was approximately 1°C lower in the partial intracanopy lighting treatment than in the control. This temperature difference might cause a difference in leaf appearance rate in cucumber of approximately 9% (Eq. 7 in Marcelis 1994). The difference in temperature of the plant apex between the lighting treatments was most likely caused by different output of infrared radiation from the different types of high-pressure sodium lamps used for the two lighting treatments. Although total fruit production on fresh weight basis was slightly reduced in the partial intracanopy lighting treatment, fruit production hardly differed on dry weight basis. Over the whole growth period partial intracanopy lighting plants allocated relatively fewer assimilates to fruits and more to leaves (Table 2). Leaf area per leaf layer was not influenced by partial intracanopy lighting but the higher partitioning of assimilates toward leaves was clearly visible in the LMA, which was over all significantly higher in the partial intracanopy lighting treatment (except layer one). In leaf layer one, the difference in LMA between control and partial intracanopy lighting treatment reflected the higher LMA in the control (because of an higher irradiance level, as discussed above). This was followed by a fast decrease in LMA and structural carbon with increasing canopy depth in the control, which is in line with results of Pons and Pearcy (1994) and of Boonman *et al*. (2006). This decrease might be because of a decrease in the carbon involved in photosynthetic proteins, because the decrease of organic nitrogen between layer one and layer four is in the same order of magnitude as the decrease in structural carbon (around 31%). In addition, this decrease in LMA with canopy depth did not occur in the partial intracanopy lighting treatment, presumably because of partial maintenance of photosynthetic capacity and the organic nitrogen content with depth in the canopy in this treatment.

Conclusions

Our results showed that in a cucumber crop, a more homogeneous vertical irradiance profile, because of the application of intracanopy lighting by LEDs within the canopy during winter, did not lead to higher net crop photosynthesis and production in a greenhouse cucumber crop. Though photosynthetic properties significantly increased, we suggest that partial intracanopy lighting in winter did not improve net crop photosynthesis partly because of the reduced light interception and partly because of the relatively low light intensities from above.

Acknowledgements

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CHAPTER 5.2

The effect of intracanopy lighting on cucumber fruit yield — model analysis

Abstract

Intracanopy lighting is a recently developed supplementary lighting technique for high-wire grown vegetable production in greenhouses where a part of the lamps is mounted within instead of above the canopy. A potentially higher yield using intracanopy lighting compared with top-lighting, is based on three assumptions; (1) increased light-absorption by the crop; (2) a higher photosynthetic light use efficiency due to a more homogeneous vertical light distribution (3) a preserved photosynthetic capacity of leaves deeper in the canopy. We used an explanatory crop model to quantify the relative importance of these assumptions for a cucumber crop during an experiment in Winter in the Netherlands (Trouwborst *et al*., 2010). Photosynthesis and yield data of this intracanopy lighting experiment with light-emitting diodes (34% of supplemental PAR) in combination with top-lighting (66% of supplemental PAR) were used to parameterise our model. In that study intracanopy lighting did not result in an increased yield compared with 100% top-lighting due to extreme leaf curling and a lower dry matter partitioning to the fruits. Our model predicted an 8% increase in fruit yield for the intracanopy lighting treatment if there were to be no leaf curling and no lower dry matter partitioning. This increase can be largely explained by the change in light distribution and light absorption. The model further revealed unexpectedly large consequences of the lower dry matter partitioning to the fruits whereas the negative effect of leaf curling was small. The direct effect of a greater A_{max} at deeper canopy layers was slightly positive. The last however might have indirectly caused the greater partitioning to the leaves as the greater A_{max} was associated with a preserved leaf mass per area. Solutions for this problem are discussed. Our explanatory model allowed us to disentangle the interacting effects of intracanopy lighting on fruit yield. Overall, intracanopy lighting has been shown here to potentially increase the assimilation light use efficiency.

Trouwborst G, Schapendonk AHCM, Rappoldt C, Pot CS, Hogewoning SW, Van Ieperen W. 2011, The effect of intracanopy lighting on cucumber fruit yield — model analysis, (*provisionally accepted*).

Introduction

Intracanopy lighting is a recently developed supplementary lighting technique for greenhouse vegetable production where a part of the lamps are applied within instead of above the canopy. Although intracanopy lighting has been shown to increase fruit yield of cucumber, tomato and sweet pepper up to 15% (Gunnlaugsson and Adalsteinsson, 2006; Hovi-Pekkanen and Tahvonen, 2008; Hovi *et al*., 2006; Hovi *et al*., 2004; Pettersen *et al*., 2010) in some studies no increase in yield was found (Gunnlaugsson and Adalsteinsson, 2006; Heuvelink *et al*., 2006; Trouwborst *et al*., 2010). Explanatory modelling is a useful tool to unravel the underlying causes for different experimental results and to explore the consequences of intracanopy lighting for production under different circumstances (*e.g.* season, latitude, greenhouse climate).

Trouwborst *et al*. (2010) used red and blue LEDs as light source for intracanopy lighting in cucumber because the high operating temperature of HPS lamps, precludes positioning them within the canopy due to the small aisle widths used in the Netherlands. The low response of fruit yield to intracanopy lighting by means of LEDs was suggested to be due to extreme leaf curling and a lower dry matter partitioning to the fruits. On the other hand, intracanopy lighting preserved a high photosynthetic capacity in the lower leaf layers *i.e.* intracanopy lighting prevented shade acclimation (Trouwborst *et al*. 2010).

The potential increase in fruit yield for inter-lit crops has been explained by three mechanistic factors (Trouwborst *et al*., 2010). First, intracanopy lighting reduces light loss due to a decrease in reflection losses at the top of the canopy. Also transmission losses are reduced if light is directed horizontally. In traditional top-lit systems approximately 6-7% of the incident irradiance is lost by reflection (Goudriaan and Van Laar, 1994; Marcelis *et al*., 1998), whereas transmission losses can vary between 5 and 10%. Second, intracanopy lighting facilitates a more homogeneous vertical light distribution within the canopy. This enhances the efficiency of crop photosynthesis compared with a less homogeneous light distribution in case irradiance levels at the top of the canopy are beyond the linear phase of the photosynthetic response to irradiance (Terashima *et al*., 2005), which is usually the case in practise. Third, a more homogeneous vertical light distribution within the crop will also change the photosynthetic acclimation pattern of leaves from top to bottom (Trouwborst *et al*., 2010), which may further increase the efficiency of intracanopy lighting. The relative importance of these factors for crop photosynthesis and thus fruit yield by intracanopy lighting has not been quantified yet. A dynamic explanatory crop model which can deal with intracanopy lighting would be a valuable addition to the static crop model to evaluate the economic benefits of intracanopy lighting published recently (Koivisto and Hovi, 2008).

The aim of this study was to quantify the relative importance of (1) the change in light distribution within the crop, (2) increase in light absorption on crop level and (3) the effect of a preservation of photosynthetic capacity deeper in the crop on the fruit yield of a cucumber crop exposed to intracanopy lighting with use of an explanatory crop model. We used the results of the experiment partly reported by Trouwborst *et al*. (2010) and reported here to assess our mechanistic approach. We also quantified the negative side effects (extreme leaf curling and a lower dry matter partitioning to the fruits) in the experiment as reported by Trouwborst *et al*. (2010).

Materials and methods

Growth conditions and experimental set-up

The experimental set-up has been described in detail in Trouwborst *et al*. (2010). In short: In October 2008 cucumber plants (*Cucumis sativus* 'Samona') were planted in a greenhouse in the Netherlands. Plants were grown according to the high wire system (Van Henten *et al*., 2002) at a density of 3.4 stems m^{-2}. Lamps (Greenpower 400V/600W SON-T, Philips, The Netherlands) producing 220 μmol m^{-2} s^{-1} top lighting incident on the canopy were used. Two treatments were defined: Top lighting (TL): same settings as above; intracanopy lighting (IL): top lighting was reduced to 140 μmol m^{-2} s^{-1} and 80 μmol m^{-2} s^{-1} was applied as intracanopy lighting by the use of LEDs (20% blue with a peak wavelength of 465 nm and 80% red with a peak wavelength of 667 nm). The photoperiod was 20 hours a day. The experiment started in November, when the plants reached the high wire. During the whole experimental period, plant growth and production was monitored and climate data, *i.e.* outside radiation, inside temperature and CO_2 concentration, was logged. The experiment was conducted in two greenhouse compartments. In each compartment each treatment was replicated twice. For statistical details, see Trouwborst *et al*. (2010).

Explanatory crop model

a. Model description

For the analyses we used a model out of the Explorer series (description below) which has been tested successfully in horticultural practice for tomato, sweet pepper and roses (Nederhoff *et al*., 2010a; Nederhoff *et al*., 2010b; Schapendonk *et al*., 2009, 2010). This model, presently adapted for cucumber, enables calculations of greenhouse production in association with a total energy balance of the greenhouse, including the contribution of the standing canopy. The model consist of modules for (1) radiation interception by the crop, (2) leaf and canopy photosynthesis and transpiration, and (3) dry-matter production and dry-matter partitioning among plant organs (roots, stem, leaves and fruits). The modules were written in FST (Rappoldt and Van Kraalingen, 1996). The module for radiation interception is based on SUCROS (Goudriaan and van Laar, 1994). Leaf gross photosynthesis is calculated with the biochemical FvCB-model

(Farquhar *et al*., 1980) with parameterisation according to Bernacchi *et al.* (2001). Net assimilate production is calculated as the difference between canopy gross photosynthesis and maintenance respiration. Leaf and crop transpiration are calculated by the empirical model of Leuning (1995). Maintenance respiration is calculated as a function of dry weights of the different plant organs and their temperature according to Schapendonk and Challa (1981). Dry matter partitioning between vegetative parts and individual fruits is simulated on the basis of source-sink interactions as based on Schapendonk and Brouwer (1984). Fruit sink strength is simulated by the beta growth function of Yin *et al*. (2003). Leaf area evolves from the leaf appearance rate which is dependent on the temperature of the growing point, the amount of assimilates available, and the amount of assimilates required to attain potential growth. Interception of radiation, and canopy gross photosynthesis is calculated for individual canopy layers. The input parameters per canopy layer for IL- and TL-crop were measured in the experiment by Trouwborst *et al*. (2010) as described below.

b. Measurements for the parameterisation of the light extinction, dry matter partitioning and photosynthetic characteristics over different leaf layers within the crop

To quantify the effect of leaf curling on the vertical irradiance profile we measured the light intensity at different heights within both treatments and determined the light extinction coefficients (Monsi and Saeki, 2005; Trouwborst *et al*., 2010); Table 1).

We also quantified the dry matter partitioning between fruit, stem and leaves by determining the dry mass of these organs during the whole experimental period (Table 1; Trouwborst *et al*., 2010).

The photosynthetic acclimation within the crop was modelled assuming 6 conceptual leaf layers in the crop: layer zero consisted of the unfolding top leaves, layer one started at the first fully expanded leaf (approximately the 15th leaf counted from the first developing leaf >2 cm length), layer two at the 18th leaf, layer three at the 21st leaf, layer four at the 25th leaf and the last layer consisted of the oldest leaves which were picked twice a week. The distance from the top of the plant was approximately 70, 105, 140, 185 and 210 cm for the layers 1 to 5 respectively. At the layers 1-4, photosynthetic CO_2-response curves (A-C_i curves) at light saturation were determined for the determination of electron transport capacity (J_{max}) and maximal Rubisco carboxylation rate (V_{Cmax}) as input for the photosynthesis module. The measurements were done in December and January on a representative plant in each plot, so in total 8 measurements were made per treatment per layer. Measurements of leaf photosynthesis at different CO_2 levels and subsequent parameter fitting were done according to the procedure as described in Trouwborst *et al*. (2011).

c. Model input and validation

For both treatments we used the radiation data during the experimental period (Fig. 1). Daily global radiation, supplemental lighting, greenhouse temperature and CO_2 concentration were model input (5 minute values). Model parameter values for dry matter partitioning, light extinction and photosynthetic acclimation over the leaf layers as measured for both treatments are given in Table 1. Simulated fruit yields were expressed in fresh weight per meter square based on a dry matter fraction of 2.61% (value of TL-fruits; Trouwborst *et al*., 2010). Weekly obtained cucumber fruit yield were used to validate the model.

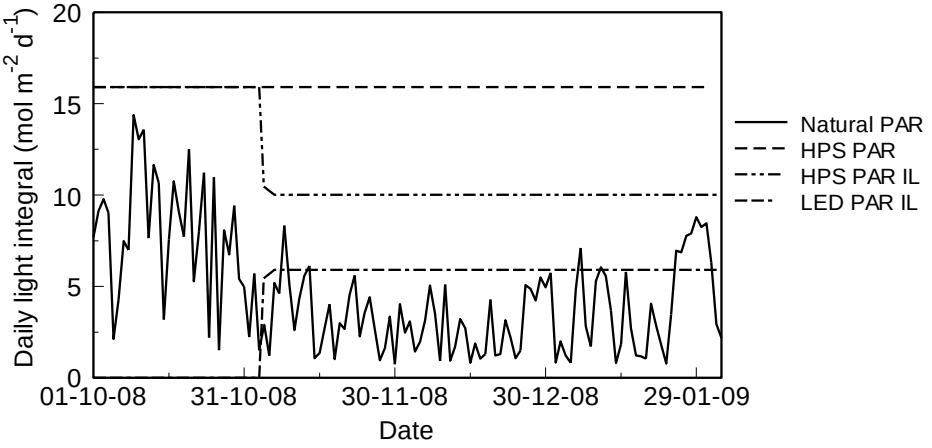

Fig. 1. Natural irradiance and supplemental PAR lighting from high pressure sodium (HPS) lamps from above the canopy (top lighting: TL) and LED lamps within the canopy (intracanopy lighting: IL) during the experiment.

Model-analysis of the experiment

The modular set up (modules of light interception, photosynthesis over different leaf layers, and dry matter partitioning) of the crop model opens the possibility to exchange parameter values from the TL-crop to the IL-crop. First we quantified the negative side effects of leaf curling and decrease in dry matter partitioning towards the fruits and the effect of photosynthetic acclimation for the IL-crop. To simulate an IL-crop without leaf curling, we used the light extinction coefficient of the TL-crop (Table 1). To simulate an IL-crop without a decrease in dry matter partitioning into the fruits we changed the dry matter partitioning-values from IL to TL (Table 1). The effect of photosynthetic acclimation on the fruit production in an IL-crop was quantified by running the model with IL-crop parameters except those related to photosynthetic acclimation which were taken from the TL-crop (Fig. 2).

We additionally quantified the main effects owing to intracanopy lighting: (1) an increase in light absorption on canopy level, (2) a more homogeneous light distribution within the crop, and (3) the change in photosynthetic acclimation within the crop. These factors were quantified excluding the adverse side effects (curled leaves and lower dry matter partitioning to the fruits) by simulating an IL-crop using

the TL-parameters for light extinction and dry matter partitioning. The effect of light distribution *per se* was quantified by simulating the same reflection and transmission losses for intracanopy lighting as for top lighting. This resulted in an equal light absorption for the IL-crop and the TL-crop so leaving the light distribution as the only difference between the simulations. The effect of an increase in light absorption is always interrelated with the light distribution, therefore these factors were simulated together. Again, the effect of photosynthetic acclimation was quantified by running the IL-crop with the photosynthetic acclimation pattern of the TL-crop (Fig. 2).

Table 1. Fruit yield and parameter settings for the top-lit and the inter-lit-crop: light extinction coefficient, % partitioning to the different organs and the photosynthetic acclimation over different crop-layers. Values partly published in Trouwborst *et al*. (2010).

	Top lighting[1]	Intracanopy lighting
Cumulative harvest (g DW m^{-2})	688[a]	680[a]
Leaf curling (used in table 2)		
Light extinction coefficient	0.87 [a]	0.57 [b]
Partitioning (used in table 2)		
Stem (%)	12.4 [a]	12.9 [a]
Fruits (%)	45.8 [a]	43.4 [b]
Leaves (%)	41.7 [b]	43.8 [a]
Photosynthetic acclimation (used in table 2-5)		
J_{max} / V_{Cmax} layer 1	113 [a] / 62 [a]	112 [a] / 59 [a]
J_{max} / V_{Cmax} layer 2	122 [a] / 74 [a]	122 [a] / 69 [a]
J_{max} / V_{Cmax} layer 3	84 [b] / 47 [b]	111 [a] / 47 [a]
J_{max} / V_{Cmax} layer 4	59 [b] / 35 [b]	97 [a] / 59 [a]

[1] Mean separation within rows by t-test, $P<0.05$.

Results

Experimental results

Applying additional assimilation lighting within instead of above the canopy significantly changed the photosynthetic properties of cucumber leaves deeper in the canopy: J_{max} and V_{Cmax} were significantly higher for IL-leaves deeper in the canopy compared to TL-leaves at the same vertical position ($P<0.021$ and $P<0.035$ for layer three and four respectively; Fig. 2 and Table 1).

Model validation

The only differences for the TL- and IL-crop simulation were 1) difference in photosynthetic properties of the leaves (Fig. 2), 2) difference in leaf curling as simulated by a different light extinction coefficient (Table 1), 3) and difference in dry matter partitioning (Table 1). In both the TL- and the IL-crop, the model accurately simulated the total fruit yields respectively with an R^2 of 0.982 and 0.987 (Fig. 3).

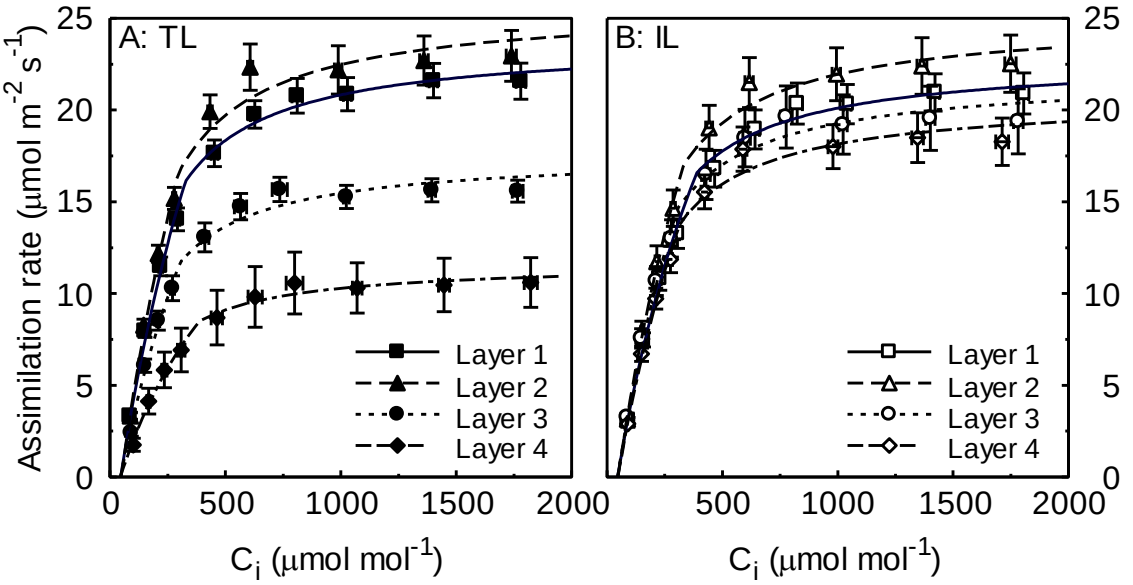

Fig. 2. The assimilation response to the intercellular CO_2 concentration (C_i) for leaves at different depths in the canopy of a crop exposed to top lighting (A) or intracanopy lighting (B). The fitted values of J_{max} and V_{Cmax}.for these curves are presented in Table 1. Horizontal and vertical bars show the SE (n=8).

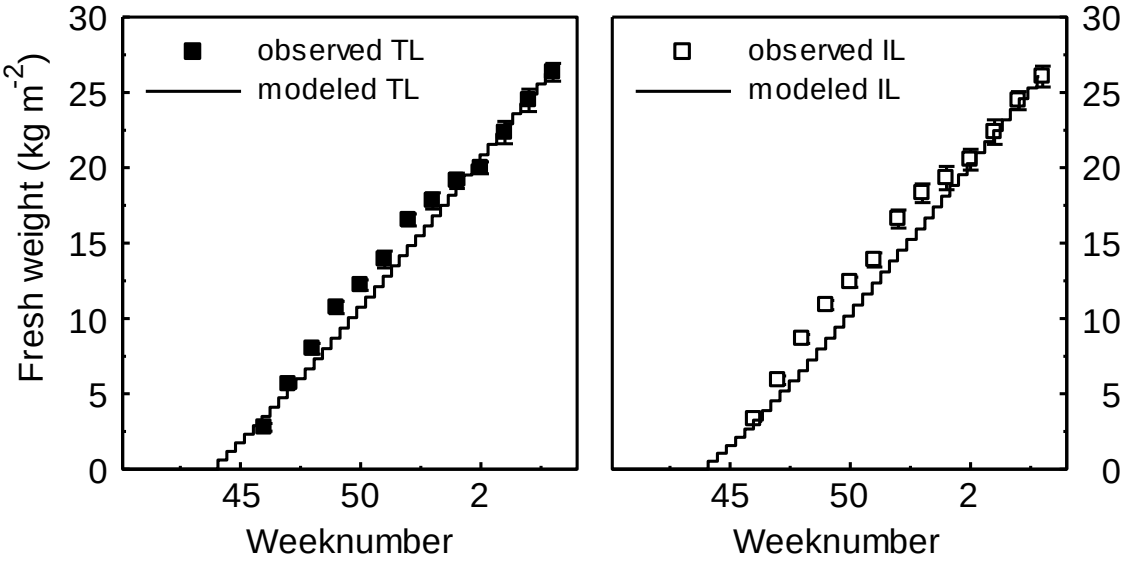

Fig. 3. Cumulative fresh weight of observed (symbols) and simulated cucumber fruit yield (lines) for the top lighting (TL) and the intracanopy lighting (IL) treatment. Vertical bars show the SE (n=4).

Model-analysis of the experiment

The validated crop model was used to analyse the extent to which the difference in expected and obtained production in the IL-crop compared to the TL-crop was due to 1) changes in photosynthetic properties of leaves (photosynthetic acclimation), 2) changes in light absorption by the crop (leaf curling in IL-crop), and 3) changes in dry matter partitioning within the crop. Using the photosynthetic properties (J_{max} and V_{Cmax}) from the TL-crop in the IL-simulation (Fig. 2) decreased fruit yield with 1.3% (Table 2). Using the light extinction coefficient from the TL-crop in the IL-simulation, thus simulating an IL-crop without curled leaves, increased fruit yield with 2.3% (Table 2). Applying an dry matter partitioning in the IL-simulation as observed in the TL-crop increased fruit yield with 5.7%. The interaction of these three factors together resulted in a potential increase in fruit yield of 8.5% for the IL-treatment (Table 2) and an increase of 7.6% compared to the TL-treatment (Table 3).

Table 2. Simulated effect of a change in parameter values of photosynthetic acclimation, leaf curling and dry matter partitioning from Intracanopy lighting (IL) to Top lighting (TL) on the fruit yield of an IL-crop.

	Parameter Settings[1]			Yield (kg m^{-2})	% increase / decrease
	Leaf curling	Dry matter partitioning	Photosynthetic acclimation		
Intracanopy lighting (IL)	IL	IL	IL	26.0	0.0%
- Effect of photosynthetic acclimation	IL	IL	TL	25.7	-1.3%
- IL-crop without leaf curling	TL	IL	IL	26.6	+2.3%
- IL-crop with TL-partitioning	IL	TL	IL	27.5	+5.7%
- Potential effect of IL	TL	TL	IL	28.3	+8.5%

[1] parameters values are shown in Table 1 and Fig. 2.

We also tested the most important processes hypothesised enhancing fruit yield under IL conditions: (1) an increase in light absorption, (2) a more homogeneous light distribution within the crop and (3) preservation of photosynthetic capacity deeper in the crop. Calculation of the effect of a more homogeneous light distribution within the crop resulted in 4.4% increase in fruit yield. The effect of an increase in light absorption is always interrelated with the light distribution, therefore these factors

were simulated together. This combined effect resulted in an increase in production of 7.2% (Table 3). The difference between this combined effect and the more homogeneous light distribution *per se* can be attributed to the increase in light absorption and its interaction with the more homogeneous light distribution. A change in the light distribution within the crop changes the photosynthetic acclimation pattern over canopy depth. However, this only slightly increased fruit yield with 0.4% (Table 3). The interaction of these three factors increased the production with 7.6% (Table 3).

Table 3. Simulated effect of a more homogeneous light distribution, increased light absorption and changed photosynthetic acclimation on the fruit yield of an IL-crop.

	Parameter Settings[1]		Yield (kg m^{-2})	% increase
	Photosynthetic acclimation	Light absorption		
Top lighting (TL)	TL	TL	26.2	0.0%
Intracanopy lighting (IL)				
- More homogeneous light distribution	TL	TL	27.4	4.4%
- Increased light absorption and more homogeneous light distribution[2]	TL	IL	28.1	7.2%
- Photosynthetic acclimation	IL	IL	28.3	7.6%

[1] TL stands for top lighting and IL for intracanopy lighting parameters, values are shown in Table 2 and Fig. 2. The same light absorption for both treatments was reached by simulating the same reflection and transmission losses for intracanopy lighting as for top lighting. All simulations were done with TL-parameters for leaf curling and dry matter partitioning.

[2] The effect of an increase in light absorption is always interrelated with the light distribution, therefore these factors were simulated together.

Discussion

Photosynthetic acclimation within the crop

The pattern of J_{max} and V_{Cmax} over the different leaf layers (Figure 2 and Table 1) was consistent with the observed pattern in photosynthetic capacity (A_{max}) as found in

Trouwborst *et al*. (2010). The pattern of photosynthetic acclimation over canopy depth of the TL-treatment (Fig. 2A) is comparable with that of tomato and tobacco crops irradiated from above (Boonman *et al*., 2006; Xu *et al*., 1997). The IL-crop showed a markedly smaller decrease in J_{max} and V_{Cmax} with canopy depth compared to a TL-crop (Table 2), indicating that intracanopy lighting preserves A_{max} of leaves at deeper leaf layers in the canopy. The second leaf layer had higher A_{max} than the first leaf layer as the first leaves were presumably not fully mature yet (Trouwborst *et al*., 2010).

Difference in expected and obtained yield

Surprisingly, the lower dry matter partitioning to the fruits and thus greater partitioning to the leaves was by far the most important cause of the difference between expected and obtained fruit yield of the IL-crop in the experiment, whereas the effect of the curled leaves was of less importance (Table 2). The greater dry matter partitioning to the leaves was due to an about 25% greater leaf mass per area (LMA) of the lowest IL-leaves than that of the lowest TL-leaves. The lower leaf appearance rate in the IL-treatment reduced the impact of this phenomenon to a 6% difference in total measured leaf dry mass over the whole experimental period (Trouwborst *et al*. 2010). The greater LMA of these lower IL-leaves was presumably due to the preservation of photosynthetic proteins in these leaves (Trouwborst *et al*. 2010). LMA and A_{max} often show a tight relationship (Poorter *et al*. 2009). This suggests that although the direct effect of photosynthetic acclimation was slightly positive, this factor indirectly had a great negative impact on dry matter partitioning via the preservation of A_{max} in the lower part of the canopy. This seems inherently connected to the use of intracanopy lighting, however this does not need to be the case: The smaller partitioning to the fruits was also due a slight but just not significant increase in total stem weight of the IL-treatment whereas the stem length of IL-plants was 1.2 m (10%) shorter than TL-plants after the experimental period. The reason for this phenomenon is unknown. More importantly, the LMA of upper canopy layers was similar for both treatments but decreased dramatically for the TL-treatment resulting in the about 25% difference for the lowest leaf layer, suggesting that reallocation of proteins from older leaves is an important process for a cucumber crop. In this experiment, intracanopy lighting was projected on the upper three canopy layers 1-3 (approx. 1m) whereas below the fourth layer the leaves were picked. Thus after 'passing' the intracanopy lighting the leaves were picked within four days. The greater dry matter partitioning to the leaves might be simply reduced by (1) keeping the leaves longer on the plant or (2) by, in this case, reducing the area of intracanopy lighting or mounting the lamps not too deep in the canopy. Both methods will give the lowest leaves more time to naturally acclimate to shade and reallocate their resources before these leaves are picked. (3) Leaves in the lower layers of the IL-crop had an 'overcapacity' of A_{max} compared to the incident light levels in these positions thus preserving a too high LMA. Besides that this resulted in higher dark respiration rates (Trouwborst *et al*. 2010), this high A_{max} might be partly

induced by the LED spectrum used (20% blue light). Hogewoning *et al*. (2010) showed that with increasing % blue light cucumber leaves had increasing LMA's and A_{max}'s. Intracanopy lighting with LEDs with a lower % of blue light might thus be beneficial. (4) Lastly, growers are able to influence the dry matter partitioning to fruits by *e.g.* manipulating fruit and crop temperature which might partly decrease partitioning to leaves and stem.

Relative importance of assumptions underlying intracanopy lighting

An expected increase in yield for intracanopy lighting was mainly due to the more homogeneous light distribution and the increased light absorption while photosynthetic acclimation was of less importance (Table 3). The relative importance of the first two would likely change depending on the ratio of IL to TL. A more homogeneous light distribution would have the greatest impact on fruit yield in summer, when the irradiance on top of the crop is high. Economically, intracanopy lighting is probably unfeasible in summer because of the already high fruit production levels and low product prices, whereas electricity costs are relatively high. However methods to improve the light distribution in summer can be beneficial for fruit production, as shown by making direct sun light diffuse (Hemming *et al*., 2008; Dueck *et al*. 2009).

Multi-layer or two-layer models are often preferred above big leaf models because the last overestimate crop-photosynthesis due to a lack of photosynthetic acclimation within such models (De Pury and Farquhar, 1997, 1999; Leuning *et al*., 1998). Van Ieperen and Trouwborst (2008) already showed with a simple static multi-layer model that crop photosynthesis in a TL-crop is only slightly overestimated when the decrease in photosynthetic parameter values over the leaf layers is not taken into account. Our results, with two types of photosynthetic acclimation with a multi-layer model, confirm this conclusion for a dynamic situation (Table 3).

Conclusions

Using a explanatory crop model, we showed that an increase in yield by using IL instead of TL can be mainly explained by an increase in light absorption and a more homogeneous light distribution. The direct effect of photosynthetic acclimation over the vertical axis was slightly positive. However this factor presumably negatively influenced the dry matter partitioning during the experiment. The smaller partitioning to the fruits mainly reduced the fruit yield in our IL-experiment while leaf curling was of less importance. We conclude that the explanatory model allowed us to disentangle the interacting effects of intracanopy lighting on fruit yield. Overall, intracanopy lighting has been shown here to potentially increase the assimilation light use efficiency.

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CHAPTER 6

Summarising Discussion

6.1 Introduction

Intracanopy lighting with LEDs in greenhouse horticulture is a promising new technique to enhance the efficiency of supplemental lighting systems. The efficiency of supplemental lighting systems can be improved by either an increase in the energy conversion efficiency of the light sources or an increase in the light use efficiency of crops. Intracanopy lighting is an approach to increase the light use efficiency by changing the position of (a part of) the lamps from above to within the canopy of the crops. Intracanopy lighting would firstly reduce reflection and transmission losses of the supplemental lighting on crop level. These losses are high in traditional top-lighting systems, hence intracanopy lighting yields a higher light absorption on crop level. Secondly, intracanopy lighting creates a more homogenous vertical light distribution which can result in higher light use efficiencies. LEDs have characteristics which make them suitable light sources for intracanopy lighting (Chapter 1).

The aim of the present study was to obtain insights in photosynthetic acclimation in response to irradiance level and spectrum in the framework of the applicability of LEDs as light sources for intracanopy lighting in indeterminate growing vegetable crops. Intracanopy lighting may vary in (1) position within the crop, in (2) irradiance level and in (3) spectrum. These points correspond with questions dealt in the Chapters 2-4, respectively about photosynthetic acclimation in relation to leaf age, which is inherently related to leaf position within crops (Chapter 2), photosynthetic acclimation to different irradiance levels during and after leaf development (Chapter 3), and photosynthetic acclimation to light spectrum during and after leaf development (Chapter 4). Aspects of these chapters come together in Chapter 5. In that chapter we tested intracanopy lighting with LEDs on crop scale. The preceding division in three points served to structure this discussion. We further discuss some practical points related to intracanopy lighting and we end with future perspectives for intracanopy lighting with LEDs.

6.2 LEDs as light source for intracanopy lighting

6.2.1 Positioning of intracanopy lighting

In dense crop stands, the decrease in leaf photosynthetic capacity (A_{max}) from top to bottom is paralleled by a decrease in irradiance and an increase in leaf age. The development of intracanopy lighting for greenhouse horticulture gives rise to the question whether the decrease in A_{max} of lower, thus older and shaded, leaves in a crop is partly due to leaf age, or solely due to the lower irradiance. If leaf age is involved in the decrease in A_{max}, then the potential lamp positions of intracanopy lighting would be reduced (*i.e.* the lamps cannot be placed to low if leaf age is a limiting factor). We investigated in **Chapter 2** whether leaf age decreased A_{max} of tomato leaves during their usual cultivation life-span in commercial crop systems (up to 70 days). To separate an effect of leaf age from an effect of irradiance level, tomato plants were grown horizontally, so that irradiance was similar for all leaves from 0-70 days old. To investigate the effect of irradiance during leaf development, A_{max}–leaf age profiles were determined for leaves of plants grown under conditions with a distinctly different natural irradiance pattern (winter, early spring and late spring). Additionally, the effect of irradiance on A_{max}–leaf age profiles of fully developed leaves was investigated by shading all fully expanded leaves of half of the plants to 25% of initial irradiance. We observed that A_{max} was higher in late spring than in winter, but was not affected by leaf age. In early spring, however, A_{max} was higher in younger leaves than in older leaves which correlated well with the irradiance integral during the developmental period of those leaves. Shading fully developed leaves strongly decreased A_{max} (30%) within a few days. We concluded that during the normal life-span of tomato leaves in cultivation, irradiance and not ageing is the most important factor affecting A_{max}. Similar results have been reported for horizontally grown cucumber by Pettersen *et al.* (2010b). They found that A_{max}, V_{Cmax} and J_{max} did not significantly differ for leaves of different ages within the usual cultivation life span of cucumber leaves within a canopy. For cucumber leaves, this cultivation life span is around 30 days, which is much shorter than for tomato leaves. These aforementioned observations suggest that lamp positioning within the canopy is not constrained by leaf age effects on A_{max}.

The irradiance levels common for supplemental lighting (up to 250 μmol m^{-2} s^{-1}; Heuvelink *et al.*, 2006) are usually above the strictly linear light-limited phase of the leaf photosynthesis-irradiance response of greenhouse grown crops. In practice, irradiance in greenhouses (natural + supplemental irradiance) is often below saturating levels, hence assimilation rates *in situ* are influenced by the light-limited quantum efficiency (α), the light-limited irradiance range and the curvature (θ) , rather than by A_{max}. In **Chapter 5.1** leaf age apparently decreased the light-limited quantum efficiency (more details below). In this chapter, we investigated the effects of intracanopy lighting with LEDs on light interception, on vertical gradients of leaf

photosynthetic characteristics and on production and development of a high-wire grown cucumber crop. We also analysed the interaction between those parameters. Plants were grown in a greenhouse under low natural irradiance (winter) with supplemental irradiance of 221 μmol m^{-2} s^{-1} (20 h per day). In the intracanopy lighting treatment, LEDs (80% Red, 20% Blue) supplied 38% of the supplemental irradiance within the canopy; the remaining 62% was supplied as top lighting by High-Pressure Sodium (HPS) lamps. The control was 100% top lighting (HPS lamps). We measured horizontal and vertical light extinction as well as leaf photosynthetic characteristics at four different canopy layers ranging from top to bottom in the canopy (Fig. 5.1.2), and determined total plant production. Each canopy layer consisted of 3 leaves. We found that deep in the canopy, leaves exposed to intracanopy lighting had a higher A_{max} and the linear, light-limited range of the photosynthesis-irradiance response extended to a higher irradiance than that in leaves grown with top lighting. The light-limited quantum efficiencies however, did not notably differ between the two light treatments for each of the four canopy layers. Only in the lowest layer, the light limited quantum efficiency was reduced, suggesting an age effect. Compared to the control, the intracanopy lighting not only changed the irradiance gradient within the canopy but also the leaf age gradient due to a lower leaf appearance rate in the intracanopy lighting treatment (Table 5.1.1). This resulted in more than four days older leaves in the lowest canopy layer of the intracanopy lighting treatment compared to the control. Consequently, the sudden decrease in light-limited quantum efficiency in the lowest canopy layer, which was found in both light treatments, cannot simply be attributed to either ageing of the leaves or incident irradiance. Probably the leaves of the lowest layer of both treatments acclimated to shade light (enriched in far-red). The spectrum of the measuring light of the Li-6400 portable photosynthesis meter, however, is distinctly different. A mismatch between the spectrum of growth and measuring light could cause appreciably lower light-limited quantum efficiencies (Chow *et al*., 1990; Walters and Horton, 1995; Walters, 2005; Hogewoning 2010). From the above we might conclude that lamp positioning within the canopy is not constrained by leaf age effects on the light-limited quantum efficiency.

Another important consideration in positioning the lamps rises from our intracanopy lighting experiment (as described in Chapter 5.1). Although leaf photosynthetic characteristics (*i.e.* greater A_{max} and organic nitrogen) were significantly increased in the lower canopy layers of the intracanopy lighting treatment compared to the control treatment, intracanopy lighting did not increase total biomass or fruit production. This was partly due to a significantly reduced vertical and horizontal light interception caused by extreme leaf curling, which partly counteracted the expected higher light absorption by the crop compared with top-lighting, and partly due to a lower dry matter partitioning to the fruits, and thus a greater dry matter partitioning

to the leaves compared to the control (Table 5.1.2)[1]. In **Chapter 5.2** we quantified the influence of these factors on fruit yield using an explanatory crop model. The model calculations revealed an unexpectedly large consequence on the fruit yield caused by the lower dry matter partitioning to the fruits (greater partitioning to the leaves) whereas the negative effect of leaf curling was small. The effect of a greater A_{max} at deeper canopy layers was slightly positive. The last however might have indirectly caused the greater partitioning to the leaves as the greater A_{max} was associated with preserved LMA's in these lowest leaves (Fig. 5.1.7), which is often a tight relationship (Poorter *et al*. 2009). This suggests that the preservation of A_{max} indirectly had a great negative effect on the yield.

In this experiment, intracanopy lighting was projected on the upper three canopy layers 1-3 (Fig. 5.1.2) whereas below the fourth layer the leaves were picked. Thus after 'passing' the intracanopy lighting the leaves were picked within four days. The greater dry matter partitioning to the leaves might be simply reduced by keeping the leaves longer on the plant or by reducing the area of intracanopy lighting in such a way that these leaves have time to naturally acclimate to shade and reallocate their resources before these leaves are picked.

6.2.2 Light intensity aspects in relation to the use of intracanopy lighting

A potentially higher yield using intracanopy lighting compared with top-lighting, was hypothesized based on two assumptions; (1) larger light-absorption by the crop; (2) a greater light use efficiency due to a more homogeneous vertical light distribution. But also (3) a preserved A_{max} of leaves deeper in the canopy could play a role. Again, the crop model was used to quantify the relative importance of these three factors during the intracanopy lighting experiment. Model calculations predicted an 8% increase in fruit yield for the intracanopy lighting treatment without leaf curling and no decreased dry matter partitioning to the fruits. This 8% increase in fruit yield can be largely explained by the change in light distribution and light absorption and only slightly by the increased leaf photosynthetic characteristics in the lower canopy layers of the intracanopy lighting treatment (Table 5.2.3)

Considering an increase in light absorption and more homogeneous light distribution owing to intracanopy lighting, it seems that assimilation lighting wholly applied as intracanopy lighting is beneficial (*i.e.* no supplemental top lighting). However, two arguments against 100% intracanopy lighting will be discussed: (1) photosynthetic acclimation of young developing leaves and (2) the leaf appearance rate of the plants.

[1] The dry matter partitioning to fruits, leaves or stem was calculated as the sum of respectively all fruits, all leaves and whole stem divided by the total biomass of the above ground parts of the plants. Hence, the dry matter partitioning is an integral over the whole experimental period.

Nowadays the irradiance levels of supplemental assimilation lighting in greenhouses go up to 250 μmol m^{-2} s^{-1} (Heuvelink *et al.*, 2006), whereas natural irradiances in greenhouses at northern latitudes in winter can vary between 50-100 μmol m^{-2} s^{-1}. Thus in the case of 100% intracanopy lighting, leaves might develop at low irradiances at the top of the canopy, whereas these leaves are exposed to substantially higher irradiances later on due to the intracanopy lighting lamps. The limited number of studies on the acclimation of photosynthesis to increased irradiance have mainly focussed on changes in A_{max}, whereas in practice irradiance in greenhouses (natural + supplemental irradiance) is often below saturating levels. We therefore investigated the effect of changes in irradiance on the photosynthesis irradiance-response in fully grown leaves of cucumber (**Chapter 3)**. Leaves fully developed under a low (L; 50 μmol m^{-2} s^{-1}) or a moderate (M; 200 μmol m^{-2} s^{-1}) irradiance were subsequently exposed to, respectively, moderate (LM-leaves) or low (ML-leaves) irradiance. As controls the irradiance levels remained unchanged (LL and MM). Acclimation of photosynthesis occurred within four *(*in the LM-leaves*)* to seven days (in the ML-leaves). Final A_{max} was the highest in MM-leaves and lowest in LL-leaves, and reached intermediate values in ML- and LM-leaves. However, chlorophyll fluorescence parameters underlying Φ_{PSII} (*i.e.* the maximum PSII quantum efficiency in the light (F_v'/F_m') and the PSII efficiency factor (q_P)) revealed full acclimation on thylakoid level to either low or moderate irradiance. Dark respiration correlated with irradiance level, but not with A_{max}. The light-limited quantum efficiency was similar in all leaves, though the linear light-limited range was shorter in LM-leaves than in MM-leaves. This shorter light-limited range resulted in a net assimilation of LM-leaves under moderate irradiance which remained 14% lower than that of leaves developed under moderate irradiance (MM-leaves). This reveals the importance of photosynthetic acclimation to specific environmental conditions during the leaf developmental phase for crop productivity in scenarios with realistic, moderate fluctuations in irradiance that leaves can be exposed to. In addition this acclimation period took around 4 days. For tomato leaves, where the life time of a fully expanded leaf may go up to 40-50 days, this acclimation time seems not that long. For cucumber however, with a life time in the order of 14-20 days this time is substantial. It can be concluded that leaf expansion under light intensities lower than those which leaves are to be exposed to in later stadium of the cultivation cycle must be avoided for crops used in greenhouses. This indicates that the application of intracanopy lighting up to an irradiance of 250 μmol m^{-2} s^{-1} without the use of top-lighting in winter will therefore be less optimal to increase crop photosynthesis than a combined use of top and intracanopy lighting in such a way that the level of intracanopy lighting never exceeds the irradiance level received at the top of the canopy.

An optimal ratio of supplemental top to intracanopy lighting is not only influenced by the photosynthetic acclimation but also by the leaf appearance rate. The latter is influenced by fruit load, light but primarily by the temperature of the apex

(Marcelis, 1993). In the intracanopy lighting experiment the leaf appearance rate was significantly reduced (Chapter 5.1). Most likely this was due to a decreased level of top lighting, which also reduced the near infra red (NIR) radiation from the HPS lamps. This lower NIR radiation reduced the apex temperature compared to the control treatment. A different leaf appearance rate can influence the source to sink ratio of plants, because together with new leaves, new fruits develop. Hence, the leaf appearance rate determines the potential fruit load of the plant. Regulatory factors on this fruit load are fruit abortion or a change in the ripening period of older fruits. This fruit load is an important parameter in the dry matter partitioning between different plant parts and subsequently in yield (Schapendonk and Brouwer, 1984). In the intracanopy lighting treatment, the leaf appearance rate was reduced, while the total amount of harvested fruits was similar compared to the control. Thus the ratio between harvested fruits and leaves was greater for the intracanopy lighting treatment. Hence, if re-allocation of leaf constituents would have occurred to a similar extent as in the control it might be that the dry matter partitioning to the fruits would have increased for the intracanopy lighting treatment.

6.2.3 Spectral aspects in relation to the use of intracanopy lighting

The blue part of the light spectrum has been associated with leaf characteristics which also develop under high irradiances (Lichtenthaler *et al.*, 1980; Matsuda *et al.*, 2004; Matsuda *et al.*, 2008; Voskresenskaya, 1979). In **Chapter 4.1.** blue light dose-response curves were made for the photosynthetic properties and related developmental characteristics of cucumber leaves. Those leaves were grown at an equal irradiance under seven different combinations of red and blue light, provided by LEDs. Only the leaves which developed under red light alone (0% blue light) 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 efficiency for CO_2 fixation ("red light syndrome"). Only 7% blue light was sufficient to prevent any overt dysfunctional photosynthesis, which can be considered as a qualitative blue light effect. The A_{max} was two times higher for leaves grown at 7% blue light compared with 0% blue light and continued to increase with increasing blue percentages during growth measured up to 50% blue light. At 100% blue light A_{max} was lower but photosynthetic functioning was normal. The increase in A_{max} with a blue light percentage ranging from 0-50% was associated with an increase in LMA, nitrogen (N) content per area, Chlorophyll (Chl) content per area and stomatal conductance. Above 15% blue light the parameters A_{max}, LMA, Chl content, photosynthetic N use efficiency and the Chl:N ratio showed a relationship that is comparable 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, *e.g.* sun adaptation.

In Chapter 6.1.2 we concluded that it might be beneficial to apply intracanopy lighting in combination with supplemental top lighting, so that leaves during their life span are never exposed to higher irradiances than during leaf expansion. However, a higher A_{max} associated with a greater percentage of blue light may be exploited for the spectrum of top-lighting lamps. If the spectrum of this top lighting favours a high A_{max} of the young developing leaves, then the percentage supplemental top lighting might be reduced in favour of the percentage intracanopy lighting.

It is well established that the light-limited quantum efficiency of photosynthesis in leaves is wavelength dependent and highest around 620-670 nm red light (Balegh and Biddulph, 1970; Evans, 1987; Hogewoning, 2010; Inada, 1976; McCree, 1972a). However, for cucumber plants grown under 100% red LED-light photosynthesis was impaired (*i.e.* the "red light syndrome"). Little is known about physiological causes and consequences of this impairment. In **Chapter 4.2** we investigated the plasticity of leaf characteristics and the photosynthetic apparatus in relation to the "red light syndrome" in fully developed leaves under low light conditions. Fully expanded leaves which were developed under red (R) or mixed red/blue (RB) LED light were exposed to respectively RB (R/RB) and R (RB/R) or remained unchanged (R/R and RB/RB). Photosynthetic acclimation was monitored with gas exchange and chlorophyll fluorescence measurements. Chlorophyll fluorescence was also used to analyse the energy dissipation pathways in PSII. It was shown that R/RB-leaves completely recovered from the low F_v/F_m within 4 days after exposure to RB-light. A_{max}, g_s, leaf mass per area and leaf nitrogen content also increased, but in this case did not reach the level of the RB/RB-leaves, showing limitations in plasticity due to constraints arising from the prior leaf development. RB/R-leaves showed decreases in A_{max}, g_s and nitrogen and in F_v/F_m. R/R- and RB/R-leaves revealed an increased dissipation of the absorbed light into non-regulated energy dissipation, which implies a lower capacity, or weaker activation of non photochemical quenching (NPQ) in comparison to RB/RB- and R/RB-leaves. Consequently the leaves developed under RB also revealed the "red light syndrome" within 7 days of red illumination.

Described results are based on climate room research without natural daylight. It may be questioned if the problems of plants growing under or exposed to red light occur in greenhouses when supplemental lighting is added to the natural irradiance. Only during specific parts of the total photoperiod (before and/or after the natural photoperiod) plants are wholly exposed to supplemental lighting. Especially in winter with short natural days and low natural irradiance levels, this time per day can be substantial. So, is it still an interesting question if intracanopy lighting with 100% red supplemental light is possible without the occurrence of the "red light syndrome". Tomato leaves, when illuminated with red supplemental intracanopy lighting for 16 hours a day still showed chlorotic effects (G. Trouwborst, unpublished results.) Presumably the occurrence of adverse responses to 100% red intracanopy lighting

depends on the supplemental irradiance intensity used and the level of natural irradiance.

In the intracanopy lighting experiment described in Chapter 5, the difference between expected and obtained production for the intracanopy lighting treatment was partly explained by the additive effect of extreme leaf curling of the intracanopy lighted plants (Chapter 5.2), which created a greater loss of natural and supplemental top lighting. This curling was likely dependent on the light spectrum. In our experiment we cannot separate between a light intensity effect or a spectral effect on leaf curling, because for such a comparison we miss a treatment of intracanopy lighting with HPS lamps. Comparable experiments with intracanopy lighting in cucumber by HPS lamps (low % blue light) did improve fruit yield while effects of intracanopy lighting on leaf curling were not reported (Hovi *et al*. 2004, Hovi-Pekkanen and Tahvonen 2008, Pettersen *et al*. 2010a). Mild leaf curling was also observed on cucumber leaves grown in a climate room under HPS lamps and fluorescent tubes. Leaves under the first two lamp types curled slightly, whereas when grown under an artificial solar lamp the leaves were extremely flat (Hogewoning *et al*., 2010). A genetic component in this curling might be involved as cucumber seedlings with a different genetic background growing under 100% red LED light showed different responses from mild-curling to extreme curling (SW. Hogewoning unpublished data). However, the process behind this curling, remains still unknown.

Earlier, we reported that the absence of reallocation of constituents of older leaves was a reason for a greater dry matter partitioning to the leaves. Whereupon we proposed to enhance reallocation by keeping these older leaves longer on the plant, but also the light spectrum might offer a potential solution. As we compare the balance of efficiency loss of Φ_{PSII} between the maximum PSII quantum efficiency in the light (F_v'/F_m') and PSII efficiency factor (q_P) (numerically, Φ_{PSII} is the product of of F_v'/F_m' and q_P) of RB/R-leaves with that of the treatments in Chapter 3, we observe that red light after leaf development results in extreme low light acclimation on thylakoid level (Fig. 1): exposing developed leaves to 100 μmol m^{-2} s^{-1} red light shows at low values for q_P (instantaneous high light levels) even a higher F_v'/F_m' and so a lower ability for nonphotochemical quenching (*i.e.* 1- F_v'/F_m' is a proxy for nonphotochemical quenching; Baker *et al*., 2007) than the F_v'/F_m' of leaves grown at 50 μmol m^{-2} s^{-1} fluorescent tube light (Fig. 1). In our intracanopy lighting experiment we used 20% blue light. Installing a diminishing percentage of blue light going from the top down into the canopy, might be beneficial and may give a more natural acclimation to shade which might induce sufficient re-allocation of resources.

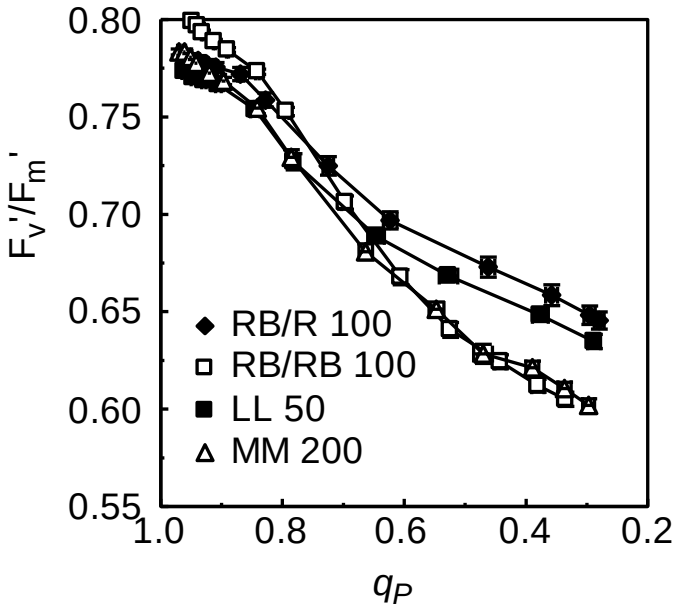

Fig. 1. The balance of efficiency loss of Φ_{PSII} between F_v'/F_m' and q_P (Φ_{PSII} is the product of F_v'/F_m' and q_P) of leaves acclimated to different light qualities and intensities: RB/RB and RB/R leaves developed under constant red/blue light with an intensity of 100 μmol m^{-2} s^{-1} and were respectively exposed to red/blue and 100% red light (Chapter 4), LL and MM leaves developed under constant fluorescent tube light with an intensity of respectively 50 μmol m^{-2} s^{-1} and 200 μmol m^{-2} s^{-1} (Chapter 3). The value of q_P=1 at PAR=0 has been omitted and around q_P=0.3 leaves were light saturated.

6.3 Future perspectives of intracanopy lighting with LEDs

LEDs have several potential advantages over HPS lamps as a growth-light source: emittance of irradiance in a narrow spectral bandwidth, low voltage operation (safety), heat emission via conduction instead of NIR-radiation, a compact and light weight design, solid state construction, longevity, lack of noise and an easy control (Bula *et al.*, 1991, Barta *et al.*, 1992, Bourget, 2008,). These characteristics also make LEDs suitable for use as intracanopy lighting (Hogewoning *et al.* 2007). Due to their small size it might be possible to implement LEDs in the standing greenhouse structure so that blockage of natural and supplemental irradiance from the top down into the canopy by the armatures can be minimised. Directing the light beam of intracanopy lighting lamps horizontally, so resulting in an infinite LAI in the horizontal direction, will minimise transmission and reflection losses on crop scale. This will also reduce 'light pollution' (*i.e.* public agitation about stray light from greenhouses illuminating the night sky). In a rose crop the percentage of intracanopy lighting leaking towards the sky was less than 0.5%, whereas the reflected light of the HPS lamps was over 4% (Trouwborst *et al.*, 2010b). This percentage of stray light from LED-lighting might be further reduced when the heat of the lamps is removed by *e.g.* water cooling, allowing the energy screen (which blocks stray light) to be fully closed. Though LEDs can be

placed closer to the crop compared to HPS-lamps due to their lack of NIR radiation, at very small distances (0-5 cm) the irradiance rises to such a high level that severe leaf photodamage and even necrosis develops (Pot and Schapendonk, 2009; Schapendonk *et al.*, 2010b; Trouwborst *et al*., 2010b). The use of intracanopy lighting with mainly red LEDs can also drastically change the red to far-red ratio deep within the canopy (Trouwborst *et al.* 2010b), which might provoke (un)desirable photo-morphogenetic effects.

In this thesis it was shown that: (1) The positioning of the lighting within the canopy does not seem to be constrained by leaf age effects on quantum yield and A_{max} but on re-allocation of constituents of old leaves. (2) Exposure of leaves to a substantial increase in irradiance later on in their life span must be prevented. (3) The direct positive effect of the preservation of photosynthetic characteristics such as A_{max} and N-content in deeper canopy layers has only a small positive impact on crop photosynthesis, though it presumably also affected crop yield negatively due to a decreased dry matter partitioning to the fruits. (4) Exposing leaves to 100% red LED light resulted a dysfunctional photosynthetic apparatus ("red light syndrome"), which was reversible by blue light addition. Some additional observations relevant for supplemental lighting in greenhouses deserve a more in depth analysis: First, the occurrence of leaf curling in response to intracanopy lighting with LEDs (Chapter 5.1); Second, the issue of reallocation of leaf constituents of older leaves (Chapter 5.2); Third, the relevance of the "red light syndrome" (Chapter 4.2) for greenhouse production, where a crop is also exposed to natural daylight.

In 2010 the state of the art concerning the energy conversion of LED-lighting systems was comparable to that of the 1000W electronic HPS-systems (Pot *et al.*, 2010; Trouwborst *et al*., 2010b). The investment costs of LED-systems however, were still about 5 times higher than those of the HPS-systems (Schapendonk *et al.*, 2010a). Real breakthrough's for using LED lighting as supplemental (intracanopy)lighting in horticultural greenhouse production can be expected when (1) the efficiency of the light source will rise above that of HPS lamps, decreasing the running costs; (2) the investment costs decrease; and (3) when above mentioned physiological oriented problems can be prevented.

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SAMENVATTING

In de Nederlandse glastuinbouw is het toepassen van assimilatiebelichting van toenemend belang. Door assimilatiebelichting neemt de productie toe, verbetert de productkwaliteit en worden perspectieven geopend voor jaarrond-productie. De keerzijde van assimilatiebelichting is een toename van het energieverbruik en de CO_2-uitstoot. Tussenbelichting (met LEDs) is een techniek om de efficiëntie van het lichtgebruik van gewassen toe te laten nemen door (een deel van) de lampen tussen het gewas te hangen in plaats van boven het gewas. Dit heeft twee effecten: Ten eerste verkleint tussenbelichting op gewasschaal de reflectie- en transmissieverliezen van de assimilatiebelichting. Deze verliezen zijn hoog (rond de 15%) in standaard topbelichtingssystemen, zodat tussenbelichting zal resulteren in een verhoogde lichtabsorptie op gewasschaal. Ten tweede resulteert tussenbelichting in een homogenere verticale lichtverdeling wat kan resulteren in hogere lichtbenuttings-efficiënties van bladeren. Het doel van deze studie was het verkrijgen van inzicht in de fotosynthese-acclimatie van bladeren in reactie op lichtniveau en lichtspectrum in het kader van het toepassen van LEDs als lichtbronnen voor tussenbelichting in vruchtgroente gewassen als tomaat en komkommer. De toepassing van tussenbelichting met LEDs kan variëren in: (1) positie in het gewas, (2) lichtniveau, en (3) lichtspectrum.

In dichte gewassen gaat de afname in de fotosynthesecapaciteit van bladeren van boven naar beneden in een gewas samen met een afname in lichtintensiteit en een toename in bladleeftijd. Het concept tussenbelichting werpt de vraag op of de afname in fotosynthesecapaciteit van bladeren dieper in het gewas (die dus ouder en meer beschaduwd zijn) veroorzaakt wordt door de toename in bladleeftijd of door het lagere lichtniveau. In **hoofdstuk 2** is onderzocht of bladleeftijd de verandering in fotosynthesecapaciteit van tomatenbladeren beïnvloed gedurende hun levensduur in commerciële kassen (max 70 dagen). Om bladleeftijd en lichtintensiteit te ontkoppelen, werden tomatenplanten horizontaal gekweekt zodat, ongeacht de bladleeftijd, de daglichtintensiteit gelijk was. Om het effect van lichtintensiteit gedurende bladontwikkeling te onderzoeken, werd over een range van bladleeftijden de fotosynthesecapaciteit gemeten aan bladeren van planten die onder onderscheiden lichtomstandigheden werden gekweekt (winter, vroege voorjaar, late voorjaar). Bovendien werd het effect van lichtintensiteit op volgroeide bladeren onderzocht door van een gedeelte van de planten de volgroeide bladeren te beschaduwen met een neutraal filter tot 25% van de oorspronkelijke lichtintensiteit; ook hier werd de fotosynthesecapaciteit bij bladeren met een verschillende bladleeftijd bepaald. De

fotosynthesecapaciteit en het chlorofylgehalte van de bladeren waren hoger in het late voorjaar dan in de winter maar werden niet beïnvloed door bladleeftijd. In het vroege voorjaar echter waren de fotosynthesecapaciteit en het chlorofylgehalte hoger in de jongere bladeren dan in de oudere bladeren. Dit correleerde met de lichtsom die betreffende bladeren gedurende hun ontwikkeling gehad hadden. Het beschaduwen van volgroeide bladeren deed de fotosynthesecapaciteit en het chlorofylgehalte binnen enkele dagen dramatisch dalen. Er is geconcludeerd dat gedurende de gangbare levensduur van tomatenbladeren in een kasteelt de lichtintensiteit (en niet bladleeftijd) de meest bepalende factor is die leidt tot veranderingen in de fotosynthesecapaciteit.

Beschaduwde bladeren diep in een gewas kunnen plotseling worden blootgesteld aan een toename in lichtintensiteit door bijvoorbeeld snoei of oogsten van takken. Doordat in de winter de natuurlijke lichtintensiteit laag is ontwikkelen bladeren zich als 'laag-licht-bladeren'. Deze bladeren zouden bij implementatie van 100% tussenbelichting na enige tijd worden blootgesteld aan veel hogere lichtintensiteiten dan bij de bladontwikkeling. In **hoofdstuk 3** is het effect van een toe- of afname in lichtintensiteit op de fotosynthese-lichtrespons en op de stikstofverdeling in het fotosynthese-apparaat in volgroeide komkommerbladeren onderzocht. Bladeren die ontwikkelden onder een lager lichtniveau (L: 50 $\mu molm^{-2}s^{-1}$) of onder een gemiddeld lichtniveau (M: 200 $\mu molm^{-2}s^{-1}$) werden respectievelijk blootgesteld aan een gemiddeld (LM) en een laag lichtniveau (ML). Als controles werden planten gekweekt waarbij het lichtniveau niet veranderde (LL en MM). Acclimatie van fotosynthese trad op in 4 tot 7 dagen. De uiteindelijke fotosynthesecapaciteit was het hoogste in MM-bladeren en het laagste in LL-bladeren. ML- en LM- bladeren zaten hier tussenin. Echter, chlorofylfluorescentie parameters lieten volledige acclimatie zien op thylakoid niveau naar enerzijds de lage of anderzijds de gemiddelde lichtintensiteit. De donkerademhaling correleerde met het lichtniveau en niet met de fotosynthesecapaciteit. De licht gelimiteerde kwantumefficiëntie was voor alle behandelingen hetzelfde. De toename in fotosynthese op het gemiddelde lichtniveau in LM-bladeren werd primair gedreven door stikstofimport. Stikstofallocatie bleef in een gelijke ratio tussen Rubisco en bio-energetica, terwijl allocatie naar het 'oogsten' van licht (light harvesting) relatief gezien afnam. Een tegengestelde respons van stikstof ging samen met een afname van de fotosynthese in ML-bladeren. Netto fotosynthese van LM-bladeren bleef gelimiteerd. Dit toont het belang aan van de fotosynthese-acclimatie gedurende de bladontwikkelingsfase voor gewasproductiviteit in scenario's met realistische fluctuaties in lichtintensiteit waaraan bladeren kunnen worden blootgesteld.

Hoofdstuk 4.1 richt zich op het effect dat blauw licht heeft op het intrinsiek fotosynthetisch functioneren en op hoog-licht aanpassingsreacties. Blauw licht dosis-respons curves werden gemaakt voor de fotosynthetische eigenschappen en daaraan gerelateerde ontwikkelingskenmerken van *Cucumis sativus* bladeren die onder een gelijke lichtintensiteit opgroeiden bij zeven verschillende combinaties rood en blauw

LED-licht. Alleen de bladeren die onder puur rood licht (0% blauw) ontwikkeld waren vertoonden een disfunctioneel fotosyntheseproces gekenmerkt door een suboptimale en heterogeen over het blad verdeelde donker geadapteerde F_v/F_m, een huidmondjesgeleidbaarheid die niet reageerde op lichtintensiteit en spectrum, en een relatief lage lichtgelimiteerde kwantumefficiëntie voor CO_2-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 licht (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 fotosyntheseproces 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 huidmondjesgeleidbaarheid. Boven 15% blauw licht vertoonden de parameters A_{max}, LMA, Chl gehalte, benuttingsefficiëntie 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 fotosyntheseproces en dat het kwantitatief een rol speelt met betrekking tot reacties van bladeren die vergelijkbaar zijn met reacties op lichtintensiteit.

Het is reeds lang bekend dat de kwantumefficiëntie van de fotosynthese golflengtegevoelig is en het hoogste is rond 620-670 nm (rood licht). Als komkommerplantjes echter alleen onder rood licht opgroeiden, vertoonde het fotosynthesesysteem schade (verlaagde F_v/F_m). Dit zogeheten "rood-licht-syndroom" wordt gekarakteriseerd door een verlaagde F_v/F_m, een niet- responsieve huidmondjesgeleidbaarheid, vergezeld met een lage fotosynthesecapaciteit en een lage fotosynthese-stikstof gebruiksefficiëntie. Er is weinig bekend over de fysiologische oorzaken en consequenties. In **hoofstuk 4.2** is de plasticiteit van bladeren en het fotosynthese-apparaat na inductie of opheffing van het "rood-licht-syndroom" in ontwikkelde bladeren onder lage lichtniveaus onderzocht. Volledig ontwikkelde bladeren die ontwikkeld waren onder rood (R) of gemengd rood/blauw (RB) LED-licht werden blootgesteld aan respectievelijk RB (R/RB) en R (RB/R) licht of een onveranderd lichtspectrum (R/R en RB/RB). Fotosynthese acclimatie werd gemeten aan de hand van gasuitwisseling en chlorofyl fluorescentie. Chlorofyl fluorescentie werd gebruikt om de energie verdeling in fotosysteem II (PSII) te analyseren. R/RB-bladeren herstelden in vier dagen volledig van hun verlaagde F_v/F_m. De fotosynthesecapaciteit, het geleidingsvermogen van de huidmondjes, bladgewicht per oppervlak en stikstof namen toe, maar bereikten niet het niveau van de RB/RB bladeren. Dit laat beperkingen in plasticiteit zien die gerelateerd zijn aan bladontwikkeling. RB/R bladeren lieten een verlaagde fotosynthese capaciteit,

geleidingsvermogen en stikstof zien, maar ook een kleine verlaging in F_v/F_m. R/R en RB/R bladeren lieten een toegenomen verdeling van het geabsorbeerde licht in niet-gereguleerde energie dissipatie zien. Dit impliceert een lagere plasticiteit voor dissipatie van te veel lichtenergie in vergelijking met RB/RB en R/RB bladeren. Bladeren die ontwikkelden onder RB-licht lieten dus symptomen van het rood-licht-syndroom zien binnen 7 dagen belichting met rood licht.

Wiskundige modellen van lichtuitdoving in een gewas en van gewasfotosynthese suggereren dat gewasfotosynthese toeneemt bij een uniformere verticale lichtverdeling in het gewas. Dit kan gedeeltelijk worden bereikt wanneer een gedeelte van het licht wordt toegepast in het gewas (tussenbelichting) in plaats van vanaf de top van het gewas (topbelichting). Deze tussenbelichting kan worden gerealiseerd met LED-belichting. In **hoofdstuk 5.1** is het effect van tussenbelichting met LEDs op de lichtinterceptie, de fotosynthese en gewasproductie en gewasontwikkeling onderzocht bij een komkommergewas. De planten werden in de winter gekweekt in een kas. De intensiteit van de assimilatiebelichting was 220 μmol m^{-2} s^{-1} (20 uur per dag). In de tussenbelichtingsbehandeling werd 38% assimilatielicht gegeven door middel van rood/blauwe LEDs in het gewas. De andere 62% was SON-t topbelichting. De controle bestond uit 100% SON-t topbelichting. Zowel de horizontale en verticale lichtverdeling als bladfotosynthese karakteristieken werden gemeten. Tevens werd de totale plantproductie bepaald. Bladgewicht per oppervlak en de drogestofverdeling naar de bladeren waren significant groter maar de blad-afsplitsingssnelheid en de plantlengte waren kleiner voor de tussen-belichtingsbehandeling vergeleken met de controle. Hoewel de fotosynthese-parameters van bladeren dieper in het gewas significant toegenomen waren, leidde tussenbelichting niet tot een grotere komkommerproductie. Gedeeltelijk kan dit verklaard worden door de verlaagde lichtabsorptie vanwege een extreme bladkrulling en gedeeltelijk door een verlaagde drogestofverdeling naar de vruchten bij de tussenbelichtingsbehandeling.

In **hoofdstuk 5.2** is een verklarend gewassimulatiemodel gebruikt om de relatieve effecten van de factoren die aan het concept tussenbelichting ten grondslag liggen te kwantificeren voor bovengenoemd tussenbelichtingsexperiment. Dit zijn (1) verhoogde lichtabsorptie, (2) een verhoogde lichtbenuttingsefficiëntie door een homogenere verticale lichtverdeling en (3) een hogere fotosynthesecapaciteit van bladeren dieper in het gewas. Tevens werd het effect van de opgetreden bladkrulling en de verlaagde drogestofverdeling naar de vruchten gekwantificeerd. Het model voorspelde voor de tussenbelichtingsbehandeling een toename in de productie van 8% als er geen bladkrulling en verlaagde drogestofverdeling naar de vruchten was opgetreden. Deze 8% wordt vooral verklaard door de verandering in lichtverdeling en lichtabsorptie en maar weinig door de verhoogde fotosynthesecapaciteit dieper in het gewas. Het model liet verder een onverwacht groot negatief effect zien van de verlaagde drogestofverdeling naar de vruchten en een relatief klein effect door de

bladkrulling. De verhoogde fotosynthesecapaciteit dieper in het gewas had een licht positief effect op de productie maar doordat de verhoogde fotosynthesecapaciteit van deze bladeren gekoppeld is aan een verhoogde bladgewicht per oppervlak veroorzaakte deze factor waarschijnlijk indirect de verhoogde drogestofverdeling naar de bladeren.

In **Hoofdstuk 6** worden alle deelhoofdstukken bediscussieerd in het licht van de hoofdvraag en er wordt afgesloten met het bespreken van de implicaties van het toepassen van tussenbelichting in de praktijk.

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Tot besluit. "*Wat er in de wereld van God blijkt, duidt noch op een volkomen uitsluiting, noch op een duidelijke tegenwoordigheid van de godheid, maar op de aanwezigheid van een God die zich verbergt. Alles draagt daarvan het stempel.*" (Pascal, Gedachten, fragment 449). In deze gedachte verwoordt de 17e-eeuwse filosoof Pascal dat de keuze tussen naturalisme of theïsme niet uit de natuur te maken is. Het wetenschappelijk onderzoek van de natuur wekt verwondering. Nog meer verwondering wordt gewekt door wat zich niet laat vangen door de wetenschap. Namelijk dat deze zich verbergende God niet de "Onbewogen Beweger" is van Aristoteles, maar zich in Christus geopenbaard heeft als het mens geworden Woord dat onder ons heeft gewoond (naar Johannes 1:14). Hij is het Die boven alle dankwoorden uit alle eer, lof en aanbidding toekomt!

CURRICULUM VITAE

Govert Trouwborst was born on February 5th in Gouda, the Netherlands. In 1999 he obtained his VWO-diploma at Driestar college in Gouda. In that year he started his study Crop Science at Wageningen University. The main subjects of his study were horticulture and plant physiology. In september 2004 he graduated (cum laude). From April 2005 till October 2009 he was employed as PhD-student at the Horticurtural Supply Chains Group of Wageningen University and did research to effects of LEDs on plant growth and photosynthesis which resulted in this thesis. Since January 2010 he works as researcher at Plant Dynamics BV in Wageningen. Govert is married with Ingeborg and is a proud father of three daughters.

LIST OF PUBLICATIONS

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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)

- Optimisation of canopy photosynthesis by the application of supplemental narrow band intracanopy lighting in greenhouse production systems? (2005)

Post-graduate courses (5.8 ECTS)

- Basic statistics; PE&RC (2006)
- Advanced statistics; PE&RC (2006)
- The art of modelling; PE&RC (2006)

Laboratory training and working visits (0.3 ECTS)

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

Invited review of (unpublished) journal manuscript (0.5 ECTS)

- Lighting of a tomato crop; journal: Hortscience (2006)

Competence strengthening / skills courses (5.6 ECTS)

- The art of writing; WGS (2005)
- Scientific writing; WGS (2007)
- Afstudeervak organiseren en begeleiden; Docentondersteuning (2006)
- Scientific publishing; PE&RC (2005)
- PhD Competence assessment; WGS (2005)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.5 ECTS)

- PE&RC 10 Year anniversary (2005)
- PE&RC Annual meetings (2006, 2007)
- 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 (9 ECTS)

- Frontier literature in plant physiology (FLOP) (2005-2009)
- Workshop PRI (own presentation) + excursion day greenhouse climate (2009)
- Excursion Themato (closed greenhouse) and Pannekoek Orchideeën (2006)
- Excursion Dekker Glascultures (2009)

International symposia, workshops and conferences (4.8 ECTS)

- Minisymposium Utrecht University-Tohoku University on plant canopies (2006)
- 14th International Congress of Photosynthesis; Glasgow, UK (poster) (2007)
- 6th International Symposium on Light in Horticulture; Tsukuba, Japan (2009)

Lecturing / Supervision of practical’s / tutorials (13.5 ECTS)

- Hortonomy (2006-2008)
- Biophysics (2005)
- Consult light and photosynthesis for students HAS-Den Bosch (2008)

Supervision of MSc 3 students (50 days)

- Intracanopy lighting in a tomato crop
- Acclimation of leaf photosynthesis deep in a tomato canopy
- Midday depression by Anthurium

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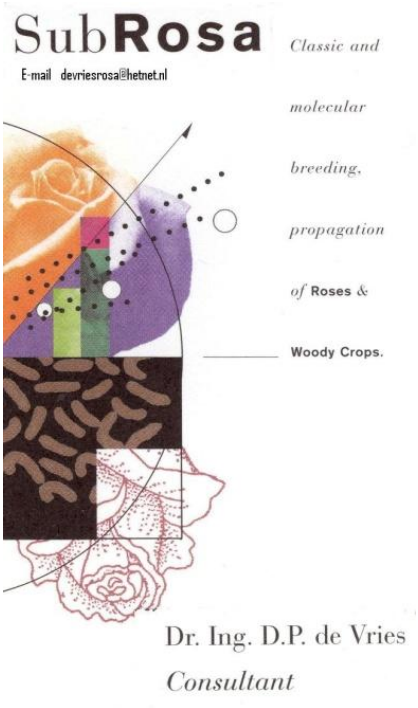

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