Improving Radiation Use Efficiency in Greenhouse Production Systems

Tao Li

Thesis committee

Promotor

Prof. Dr L.F.M Marcelis

Professor of Horticulture and Product Physiology Group

Wageningen University

Co-promotor

Dr E. Heuvelink

Associate professor, Horticulture and Product Physiology Group

Wageningen University

Other members

Prof. Dr P.C. Struik, Wageningen University

Prof. Dr M. Boehme, Humboldt University, Berlin, Germany

Dr A.G.T. Schut, Wageningen University

Dr S.W. Hogewoning, Plant Lighting B.V. , Bunnik

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CONTENTS

CHAPTER 1

General introduction

T. Li

Horticulture and Product Physiology Group, Wageningen University,
P.O. Box 630, 6700AP, Wageningen, the Netherlands

High yields in greenhouse production systems

Within the emergent international policy arena of 'food security', the imperative to double global food production by 2050 has become ubiquitous. Although the exact number can be debated (Tomlinson, 2013), it is clear that a large increase in production is needed to feed the increasing world population with their increasing demand per capita (Godfray *et al.*, 2010). However, growing competition for arable land, water, energy, and the degradation of the environment impose challenges to improve the crop production (Godfray *et al.*, 2010; Wegner and Zwart, 2011). In this context, improving agricultural production efficiency is imperative. Greenhouses provide the possibility to create optimal growth conditions for crops, thereby improving production and product quality.

In greenhouses in the Netherlands, an annual tomato fresh yield of 65 kg m^{-2} and a sweet pepper yield of 36 kg m^{-2} are common (Vermeulen, 2012). This represents 36 and 29 tons of dry matter per ha, as dry matter content is about 5.5% and 8% for tomato and sweet pepper fruit, respectively. These yields are 5 to 10 times higher than the annual yield for field production of tomato and sweet pepper in Spain or the USA (Heuvelink, 2005; Bosland and Votova, 2012; Stanghellini, 2014). Production of fruit vegetables per unit greenhouse area in the Netherlands has doubled over the last 25 years (De Gelder *et al.*, 2012). This increase in yield is caused by many factors, e.g. improvements in greenhouse transmissivity and developments in cultivation techniques such as extension of the growing season, soilless culture, high wire training systems, CO_2 enrichment, and environmental control by computers (Heuvelink *et al.*, 2003). Also breeding has contributed to this increase. Higashide and Heuvelink (2009) concluded that due to breeding Dutch tomato yield has increased on average about 0.9 % per year over the period 1950-2000. Although many innovations have been developed during the past decade that improve yield (Marcelis *et al.*, 2013), greenhouse industry is still in search for further improvements of crop production.

Light is the most limiting factor in greenhouse production systems

Crop growth largely depends on light, as light is the driving force for photosynthesis. Therefore, a better use of light [i.e. improving radiation use efficiency (RUE)] in greenhouse production systems is important. RUE is a multi-definitional term. At the leaf level, RUE is often defined as the ratio between CO_2 assimilation and absorbed photons. At the crop level, RUE can be defined as the ratio of dry matter production of the crop to absorbed, intercepted or incident radiation. Finally, RUE can also be described as the relationship between crop

production and the available radiation for the production system (e.g. greenhouse). Improving RUE at both leaf level and crop level contribute to RUE at the level of the production system.

RUE can be improved by cultivation methods as well as plant breeding. In fact improved RUE (expressed as the ratio between total dry matter production and intercepted radiation) is the main factor explaining why modern cultivars have higher production rates than older cultivars (Higashide and Heuvelink, 2009).

The light environment varies substantially during the year. High light levels in summer are not always efficiently utilised in greenhouses. For crops which form a large canopy such as tomato, cucumber and pepper, leaves at the top of the canopy may receive too much light while leaves deeper in the canopy receive too little light. This unequal distribution of light may also reduce the quality of produce. In particular in summer when a large fraction of solar light is direct light, the light distribution in the canopy is very heterogeneous. Studies have shown that plants use diffuse light more efficiently than direct light (Gu *et al.*, 2002; Mercado *et al.*, 2009; Urban *et al.*, 2012). This suggests that improving crop RUE in greenhouses could be realised through diffusing the incident light in the greenhouse. Furthermore, in summer a large amount of solar light is not used for production of shade-tolerant pot plants such as anthurium, orchids and bromeliads, because growers regularly apply shading screens to prevent damage of leaves and flowers. Growers often shade that much that not more than about 5 mol m^{-2} d^{-1} PAR (Photosynthetically Active Radiation) is realised in the greenhouse (in summer this is about 10-20% of the PAR outside the greenhouse). This severe shading potentially limits crop photosynthesis. This brings the question whether or not we can apply less shading for cultivation of shade-tolerant pot plants without negatively influencing their quality.

In winter when solar light levels are low, supplementary light is widely applied in greenhouses at northern latitudes to improve assimilation rate of the crop. Growers apply large quantities of supplementary light, which costs a lot of energy. The question can be raised if these high levels of supplementary light are always efficient. More light may not lead to more production if the crop is sink limited, i.e. the source strength (assimilate production) is larger than the sink strength (ability to utilise assimilates). In these cases less supplementary light will not reduce crop growth and consequently it will increase crop RUE. For crops such as tomato, the source-sink balance may vary during plant development and between cultivars, which often differ in sink size of the fruits, while fruits are the major sink organs in tomato (Heuvelink, 1996). Nevertheless, growers often apply similar lighting regimes for different cultivars and developmental stages. Therefore, quantitative understanding of the source-sink

balance of plants may provide knowledge for reducing the use of supplementary light without reducing production and therefore improving crop RUE.

It is obvious that there is room for improvement of RUE in greenhouse production systems. In this thesis, we focus on improving RUE in greenhouse production systems through improving the uniformity of light distribution in the canopy, applying less shading in summer and applying supplementary lighting in winter in relation to the source-sink balance.

Improving RUE by improving light distribution

Light interception as well as light distribution in the canopy play a pivotal role for crop photosynthesis and growth (Wilson *et al.*, 1992; Sarlikioti *et al.*, 2011a; Sarlikioti *et al.*, 2011b; Duursma *et al.*, 2012). In a canopy internal shading results in an exponential decrease in light intensity from top to bottom in the canopy (Monsi and Saeki, 2005). The heterogeneous light distribution in the canopy may occur particularly when leaf area index (LAI) is high, as this results in shaded spots as well as sunflecks on other leaves, where sunflecks are defined as a brief increase in natural sunlight intensity above a certain threshold (Kaiser et al., 2014). Due to the curvilinear response of leaf photosynthesis to light intensity, a more homogeneous light distribution in the canopy could be advantageous for crop photosynthesis (Marshall and Biscoe, 1980; Farquhar and Roderick, 2003). Solar light is composed of a direct and a diffuse component. Direct light arrives in a straight line from the sun, which usually leads to sunflecks within the canopy because upper leaves lead to shaded areas. Diffuse light arises from the scattering of light by molecules or larger particles in the atmosphere; consequently it arrives at an object from many directions simultaneously. When the light is diffuse, there is less shading and less variation of light intensity within a canopy. Some studies indicated that at given climate conditions, an increase in the fraction of diffuse light increases plant photosynthesis (Healey *et al.*, 1998; Gu *et al.*, 2002; Cavazzoni, 2006; Hemming *et al.*, 2007; Mercado *et al.*, 2009). Diffuse light penetrates deeper into the crop canopy as indicated by a lower light extinction coefficient (Urban *et al.*, 2012). Therefore, lower positioned leaves in the canopy may receive a higher average light intensity in diffuse compared to direct light conditions. As a consequence, in diffuse light conditions these leaves may have a higher photosynthetic capacity due to acclimation to higher average light intensities. This may correlate with changes in leaf nitrogen content which is affected by the prevailing light intensity at the position of the leaf within the canopy (Anten *et al.*, 1998; Drouet and Bonhomme, 2004). Acclimation to light intensity also includes acclimation of

crop morphological properties such as LAI and leaf angle (Mc Millen and Mc Clendon, 1979). This acclimation could further contribute to increased crop photosynthesis.

Apart from spatial light distribution, crop photosynthesis is also influenced by the transient light environment (temporal light distribution), in particular at the leaf level (Pearcy, 1990). Stomata regulate carbon uptake of a leaf. In response to fluctuating light, stomata exhibit a dynamic response that is slower than the response of fluctuating light itself, which may limit the CO_2 assimilation under fluctuating light conditions (Pearcy *et al.*, 2004). Stomatal responses to dynamic light vary dramatically among species, from virtually no response to rapid stomatal responses (Knapp and Smith, 1990; Vico *et al.*, 2011), thereby resulting in different consequences for instantaneous leaf photosynthesis. In greenhouses, the shadow and sunflecks generated by overstory leaves, leaf movement, greenhouse construction parts as well as equipment may exacerbate the variation of temporal light distribution. This may substantially limit crop photosynthesis compared to constant light intensities (Pearcy, 1990; Way and Pearcy, 2012). This variation in light intensity could be minimized under diffuse light, which would consequently lead to less limitation on leaf photosynthesis.

Recently, new greenhouse cover materials have become available, which scatter the incident light without affecting light transmission (Hemming *et al.*, 2008; 2014). Preliminary studies reported that greenhouses covered by diffuse glass could increase production by 6-10 % in fruit vegetables compared to greenhouses covered by clear glass (Dueck *et al.*, 2009). However, detailed studies, in which the vertical and horizontal distribution of light in a canopy and its consequences for crop physiological and morphological acclimation are determined, are lacking. Neither have the consequences of diffusing the light for crop photosynthesis been quantified in detail. Furthermore, no studies have investigated the effect of increasing the ratio of diffuse to direct light at the same total light level on instantaneous leaf photosynthesis and plant growth. A part of this thesis will be dedicated to filling these knowledge gaps.

Improving RUE by reducing shading losses

Even in northern countries, there are periods in summer with too high light levels for many shade-tolerant pot plants such as anthurium, bromeliads and orchids. When excessive light energy is being absorbed by the light harvesting antennae at a rate which surpasses the capacity for photochemical and non-photochemical energy dissipation, this may lead to photoinhibition or photo-damage (Long *et al.*, 1994). In the long term, this may result in discolouring of leaves or even necrosis. Light damage occurs mostly as a result of prolonged

exposure to excessive peaks in light intensity (Asada, 1999; Niyogi, 1999; Kasahara *et al.,* 2002). Consequently, growers regularly apply shading in commercial production of many shade-tolerant pot plants in summer by closing a screen or having white wash on the greenhouse cover. However, shading may also reduce plant growth, as it substantially reduces the light incident on the canopy. In this respect, exploring possibilities to allow more light into the greenhouses is a potential way of increasing crop photosynthesis and yield.

When a greenhouse is covered by diffuse glass, the incident light in the greenhouse is more homogeneously distributed with less sunflecks. The more homogeneous light distribution might prevent photoinhibition and decrease local peaks in leaf temperature when global radiation is high. Therefore, the problem of discolouring of leaves or necrosis in shade-tolerant pot plants under relatively high light might be less when cultivated under diffuse glass cover. Moreover, according to Trouwborst *et al.* (2011) most pot plants have a relatively low stomatal conductance, which reduces further under high light levels when grown in a greenhouse. High light levels in greenhouses usually correlate with low air humidity and high temperatures. This may result in a drop in stomatal conductance due to excessive transpiration (Aphalo and Jarvis, 1991), which consequently limits plant photosynthesis and growth because stomata regulate CO_2 exchange between greenhouse air and leaf intercellular cavities. Therefore, allowing more light in the greenhouses must be done in combination with increasing air humidity. In an experiment with *Phalaenopsis*, in which air humidity was kept constant at a high level, photosynthesis was more than doubled, and growth rate was increased when daily light integral was increased from 3 to 5 mol m^{-2} d^{-1} PAR (Dueck *et al.*, 2010a). In this context, allowing more light under diffuse glass cover in combination with high air humidity may boost plant growth without negatively influencing ornamental quality. This hypothesis will be tested in this thesis.

Improving RUE by balancing source and sink

Plant growth closely correlates with source and sink strength and the balance between them (Gifford and Evans, 1981; Wardlaw, 1990; Paul and Foyer, 2001; Dingkuhn *et al.*, 2007; Smith and Stitt, 2007; Gent and Seginer, 2012). The measures to be taken to improve crop growth and production are different for a source or a sink limited crop. In a source limited crop, a plant can usually benefit from more assimilates produced. Therefore, growth of a source limited crop might be improved by treatments that are likely to stimulate photosynthesis such as optimizing the light distribution in the canopy, allowing more solar light in the greenhouse, applying CO_2 enrichment as well as supplementary light. In a sink

limited crop, an improvement in source strength would not result in an improved plant growth. In fully producing tomato crops under greenhouse conditions plant growth is source limited (De Koning, 1994; Heuvelink, 1996; Matsuda *et al.*, 2011; Qian *et al.*, 2012). Therefore, CO_2 enrichment as well as supplementary light are often applied in commercial greenhouse tomato production in northern latitudes in order to improve productivity (Ayari *et al.*, 2000). Although tomato plants are source limited during fruiting stage, Yelle *et al.* (1989) have shown that in a young tomato crop (grown up to 14 weeks after sowing) an increase in atmospheric CO_2 results in carbohydrate accumulation in the leaves, which suggests that sink limitation occurs. This phenomenon is probably due to the limited number of fruits in young plants, in which case there are not sufficient sinks to utilize all the produced assimilates. The surplus assimilates are usually stored as carbohydrates in the plant. If large amounts of carbohydrates are stored in the source leaves, feedback inhibition on leaf photosynthesis may occur (Layne and Flore, 1995; Franck *et al.*, 2006). Source-sink balance may also differ between cultivars which differ in fruit load and potential fruit growth rate (i.e. fruit growth under non-limiting assimilate supply) which is a measure for sink strength (Marcelis, 1996). Dueck *et al.* (2010b) observed that under commercial crop management tomato cultivars with small fruits (cherry tomato) benefited less from the use of supplementary light than cultivars with larger fruits, and they argued that this was mainly due to the limited sink demand in cherry tomato.

The source-sink balance is important for crop growth and development in greenhouses. Although there are many studies on source-sink balance in tomato plants (Hocking and Steer, 1994; Cockshull and Ho, 1995; Heuvelink and Buiskool, 1995; Matsuda *et al*., 2011), a detailed analysis of the source-sink balance from early growth stage to fully fruiting stage for cultivars with different potential fruit size is still lacking. This gap will be filled in this thesis, which might provide knowledge for optimizing the lighting strategy.

Scope of research and thesis outline

The overall objective of this thesis is to obtain insights in improving RUE in greenhouse production systems through better understanding of crop physiology. Improving RUE in greenhouse production systems is a broad and complex topic in which many factors are involved. In Fig. 1 a schematic representation of the outline of this thesis is given. The high light levels in summer are not efficiently utilised due to the heterogeneous light distribution in the canopy as well as the use of shading screens in the production of shade-tolerant pot plants. In winter, supplementary light is widely applied in greenhouses to improve assimilation rate

of the crop. However, for a sink limited crop, supplying extra light might not improve the crop growth, consequently reducing RUE. Therefore, in this thesis we focus on improving light distribution in the canopy by diffusing the incident light, allowing more light in the greenhouse in summer, and identifying the plant source-sink balance. This results in three specific objectives:

a) To analyse in a quantitative way the factors which contribute to an increase in photosynthesis of tomato crops grown in diffuse light.

b) To improve the use of solar light in summer by diffusing the light and applying less shading in the shade-tolerant pot plant anthurium.

c) To quantify the source-sink balance of tomato cultivars with different potential fruit size during crop development.

In this thesis, we study tomato (*Solanum lycopersicum*) and anthurium (*Anthurium andreanum*) pot plants. Tomatoes are one of the most widely produced and consumed vegetables in the world; consequently, tomato production has high economic importance (Heuvelink, 2005). Furthermore, tomato is used as a model crop in many studies (in particular for fleshy fruit species), e.g. plant genetic, physiological, morphological, pathological studies (Kimura and Sinha, 2008). Protected cultivation of pot plants represents an important industry in the Netherlands, of which anthurium is one of the most important representatives (Kromdijk *et al.*, 2012). It is typically cultivated at low light conditions; therefore, a large amount of solar light is not utilized in commercial production.

This thesis consists of six chapters. **Chapter 1** (this chapter) introduces the high yields obtained in greenhouse horticulture and the options for improving RUE in greenhouse production systems. This chapter also identifies knowledge gaps and lists objectives for the research conducted in this thesis. **Chapter 2** deals with the effect of scattering of incident light on vertical and horizontal light distribution within the canopy of a fully-grown tomato crop, and their consequences for plant morphological and physiological acclimation. These factors together influence crop photosynthesis. The effect of each of these factors on crop photosynthesis is quantified. In **Chapter 3**, we explore the possibility of allowing more light into the greenhouse under diffuse glass cover to speed up growth of shade tolerant pot-plants in summer. For this, we use two anthurium (*Anthurium andreanum*) cultivars and analyse plant growth parameters and ornamental quality. In **Chapter 4**, we address the question (as raised in Chapter 3) why the stimulating effect of diffuse light on crop RUE in anthurium pot-plants is cultivar specific. We answer this question based on the response of instantaneous leaf

photosynthesis to the temporal distribution of incident light under diffuse glass cover. In **Chapter 5**, we quantitatively investigate the source-sink balance of of tomoto plants from the early growth stage to the fully fruiting stage under commercial crop management. This investigation is performed for three types of tomatoes with different potential fruit sizes. In **Chapter 6**, the preceding chapters are summarised and discussed, together with some practical points and perspectives for further research are mentioned.
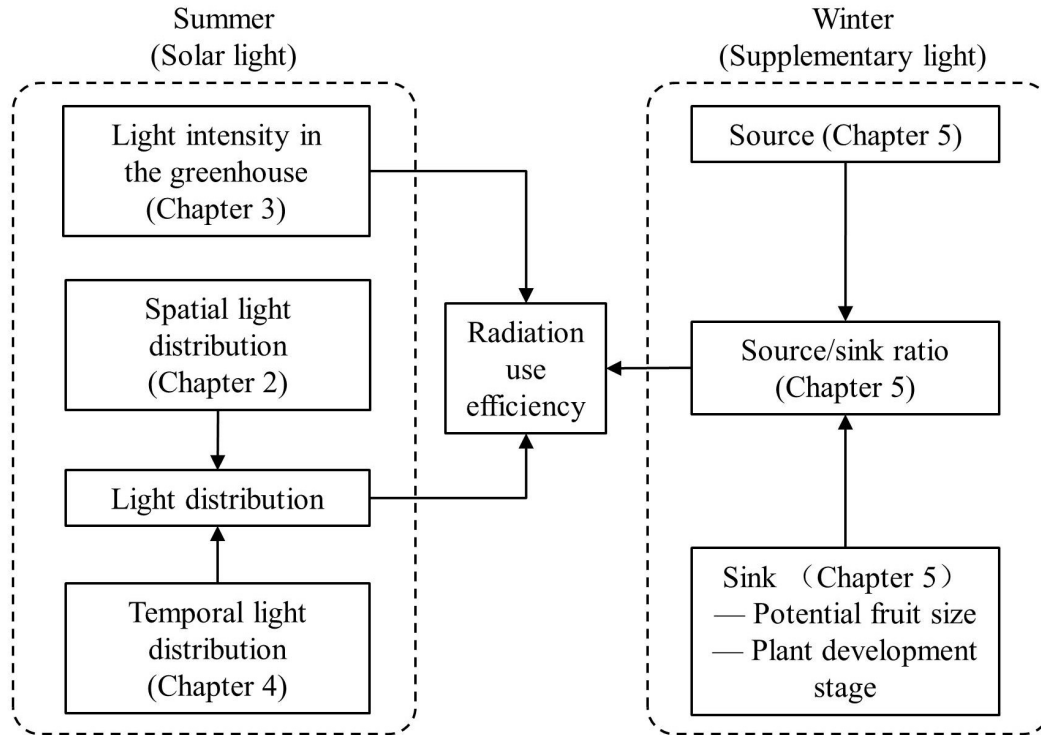
Fig. 1. Framework and scope of the research reported in this thesis.

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CHAPTER **2**

Enhancement of crop photosynthesis by diffuse light: quantifying the contributing factors

T. Li[1], E. Heuvelink[1], T.A. Dueck[2], J. Janse[2], G. Gort[3], L.F.M. Marcelis[1]

[1]Horticulture and Product Physiology Group, Wageningen University,
P.O. Box 630, 6700AP, Wageningen, the Netherlands
[2]Wageningen UR Greenhouse Horticulture, Wageningen University and Research Centre,
P.O. Box 644, 6700AP, Wageningen, the Netherlands
[3]Biometris, Wageningen University and Research Centre,
6700AC, Wageningen, The Netherlands

ABSTRACT

Plants use diffuse light more efficiently than direct light. However, until now all experimental comparisons between diffuse and direct light are obscured by concurring differences in climate conditions (e.g. light intensity). This study aims at analysing the factors which contribute to an increase in crop photosynthesis in diffuse light and to quantify their relative contribution under different levels of diffuseness at similar light intensities. The hypothesis is that the enhancement of crop photosynthesis in diffuse light not only results from the direct effects of more uniform vertical and horizontal light distribution in the crop canopy, but also from crop physiological and morphological acclimation. Tomato crops were grown in three greenhouse compartments which were covered by glass with different light diffuseness (0 %, 45 % and 71 % of the direct light being converted into diffuse light), while maintaining a similar light transmission. Measurements of horizontal and vertical photosynthetic photon flux density (PPFD) distribution in the crop, leaf photosynthesis light response curves and leaf area index (LAI) were used to quantify their contribution to an increase in crop photosynthesis in diffuse light. Furthermore, leaf temperature, photoinhibition, leaf biochemical and anatomical properties were studied. The highest degree of light diffuseness (71 %) increased calculated crop photosynthesis by 7.2 %. This effect was mainly attributed to a more uniform horizontal (33 % of the total effect) and vertical PPFD distribution (21 %) in the crop. Furthermore, plants acclimated to the high diffuseness by a higher photosynthetic capacity of leaves in the middle of the crop and higher LAI, which contributed 23 % and 13 % to the total increase in crop photosynthesis in diffuse light, respectively. Moreover, diffuse light resulted in lower leaf temperatures and less photoinhibition at the top of the canopy when global irradiance was high. We conclude that diffuse light enhanced crop photosynthesis; more uniform horizontal PPFD distribution played the most important role in this enhancement, and a more uniform vertical PPFD distribution and higher leaf photosynthetic capacity contributed more to the crop photosynthesis enhancement than a higher LAI.

INTRODUCTION

Light in a crop canopy is heterogeneously distributed in the horizontal and the vertical plane. Due to the curvilinear response of leaf photosynthesis to light intensity, a more homogenous light distribution in the canopy could be advantageous. Solar light is composed of a diffuse and a direct component. Diffuse light arises from the scattering of light by molecules or larger particles in the atmosphere and comes from many directions simultaneously. Direct light arrives in a straight line from the sun without being scattered. Many studies suggest that plants use diffuse light more efficiently than direct light and this is mainly attributed to a more uniform light distribution in the canopy (Cohan *et al.*, 2002; Farquhar and Roderick, 2003; Gu *et al.*, 2003; Alton *et al.*, 2007; Mercado *et al.*, 2009).

Light direction plays a pivotal role for the distribution of light in a canopy (Goudriaan and Van Laar, 1994). Light intensity usually decreases exponentially from the top to the bottom in a canopy, as described by the Lambert-Beer law (Monsi and Saeki, 2005). Diffuse light usually exhibits a lower extinction coefficient than direct solar light (Urban *et al.*, 2007; Urban *et al.*, 2012), although the effect depends on solar position (Allen, 1974; Morris, 1989). In many crop photosynthesis models horizontal light distribution in the canopy is determined by the sunlit and shaded leaf area (Spitters, 1986; De Pury and Farquhar, 1997; Wang and Leuning, 1998; Johnson *et al.*, 2010). At a certain canopy depth some leaves are shaded and thus do not receive direct light, while others do (sunlit leaves).

Light distribution in the canopy is not only influenced by the direction of the light or the fraction diffuse or direct light incident on the canopy, but also depends on crop architecture. Sarlikioti *et al.* (2011*a*, *b*) indicated that plant architecture has a large impact on crop light distribution and photosynthesis.

Diffuse light penetrates deeper into the canopy; thus, the lower positioned leaves will receive on average a higher light intensity when light is diffusing. This higher light intensity leads to an acclimation of the nitrogen distribution in the canopy (Johnson *et al.*, 2010). Related to changes in nitrogen distribution, leaf photosynthetic capacity might differ in diffuse and direct light. In many plant species, the photosynthetic capacity and leaf nitrogen content depend on the prevailing light intensity within the canopy (Anten *et al.*, 1998; Drouet and Bonhomme, 2004). Acclimation to diffuse or direct light also includes acclimation of leaf morphology and anatomy which affect light absorption, and consequently, photosynthesis (Hemming *et al.*, 2007; Brodersen *et al.*, 2008; Markvart *et al.*, 2010). Furthermore, diffuse

light might also decrease leaf temperature and the extent of photoinhibition as less local peaks in light intensity occur.

Many studies on diffuse light have been carried out by comparing plant responses on cloudy and clear days which implies not only a difference in the fraction of diffuse light, but also large differences in light intensity, and the subsequent changes in microclimatic parameters such as air and soil temperature, and vapour pressure deficit (VPD) (Gu *et al.*, 2002; Farquhar and Roderick, 2003; Alton *et al.*, 2007; Urban *et al.*, 2007; Knohl and Baldocchi, 2008; Urban *et al.*, 2012). These changes directly or indirectly influence canopy photosynthesis. Due to such large differences in microclimate, a quantitative exploration of the specific effects of light distribution on canopy photosynthesis in diffuse and direct light conditions is hardly possible in the open field.

The relative importance of the different effects of diffuse light on crop photosynthesis mentioned above has never been quantified. Many studies relevant to light distribution are based on model simulations and give a good estimation of light distribution in a canopy (Johnson and Thornley, 1984; Thornley, 2002; Johnson *et al.*, 2010; Suwa, 2011). However, to the best of our knowledge, detailed analyses based on measurements of both the vertical and horizontal light distribution in a canopy and their consequences for crop photosynthesis are lacking. In particular, no studies have been performed in crops grown at different levels of diffuseness with similar incident light intensity on the top of the crop.

This study aims at analysing the factors which contribute to an increase in crop photosynthesis in diffuse light and to quantify their relative contribution under different levels of diffuseness at similar light intensities. The hypothesis is that the enhancement of crop photosynthesis in diffuse light not only depends on the direct effects of a more uniform vertical and horizontal light distribution, but also on crop physiological and morphological acclimation. To test this hypothesis, a study was conducted in glasshouses covered with diffuse glass, converting a portion of the direct solar light into diffuse to different degrees of diffuseness, without affecting light transmission. Tomato, often seen as a model plant (Kimura and Sinha, 2008), was used in this study.

MATERIALS AND METHODS

Plant material and growth condition

Tomato (*Solanum lycopersicum*, cv. 'Komeett'; Monsanto, Creve Coeur, USA) plants, grafted on the rootstock Maxifort (Paramount, Quinter, USA), were planted on Rockwool on 16 Dec.

2010 in three adjacent east-west oriented Venlo-type glasshouse compartments. Each compartment had an area of 144 m^2 with a gutter height of 5.5 m, and was located in Bleiswijk, the Netherlands (52° N, 4.5° E). The compartments were covered by glass (Guardian Agro, Dudelange, Luxembourg) with 0 % (control), 45 % and 71 % haze factor for the three compartments, respectively. The haze factor indicates the fraction of direct light converted into diffuse light. The hemispherical transmission for PPFD of the glass was 83 % for the three glass types. The haze factor and hemispherical transmission of the glass were determined in an optical sphere according to ASTM international (2007). The photostationary state of phytochrome (PSS), R/FR ratio (the ratio between red light and far-red light) and the B/R ratio (the ratio between blue light and red light) of the penetrating light were estimated from the spectral distribution of hemispherical glass transmission and solar photon flux, based on 1 nm intervals from 400 to 800 nm (Sager *et al.*, 1988). PSS of the penetrating light in 0 %, 45 % and 71 % haze treatment were 0.718, 0.718, 0.713, respectively; R/FR ratios were 1.25, 1.25, 1.22, respectively; B/R ratios were 0.44, 0.44 and 0.42, respectively. An overview of the spectral transmittance of the glass is provided as supplementary material (Fig. S1). The greenhouse transmissivity was 66 %, measured in March 2011 on a cloudy day and no significant difference ($P = 0.07$) was found among the three compartments.

Stem density was initially 2.55 stem m^{-2} and increased to 3.4 stem m^{-2} ten weeks after planting. Plants were grown on Rockwool with drip irrigation. Mean pH of the irrigation water was 6.1 and mean EC (Electrical conductivity) was 5.1 dS m^{-1}. Plant rows were in east to west orientation. The distance between rows was alternating between 60 and 90 cm, resulting in a double row followed by a path. A standard horticultural computer (Hogendoorn-Economic, Hogendoorn, Vlaardingen, The Netherlands) was used to control the same climate (temperature, air humidity and CO_2 concentration) in the three compartments. Solar radiation was continuously measured outside the greenhouse with a weather station system (WS-GP2 Weather station, Delta-T, Cambridge, UK) throughout the growing period. PPFD was estimated from solar radiation, assuming that half the global radiation is PPFD (Jacovides *et al.*, 2003). Relevant measurements were mainly performed from June to September 2011. During this period, average daily outside global radiation was 15 MJ m^{-2} d^{-1}; average day/night temperature inside the greenhouses was 22/17 °C, average daytime CO_2 concentration was 526 µmol mol^{-1} and relative humidity was 72 %. During the growing period, 53 % of the global radiation was direct radiation, which was estimated from hourly values of global radiation according to Spitters *et al.* (1986).

Fruit production and crop morphological properties

Three double rows in the middle of the greenhouse were selected for production determination; ripe fruits were harvested weekly, starting from 13 weeks after planting. Fruit fresh weight was determined at each harvest. Crop specific leaf area (SLA) was measured four times (in April, June, July and September) during the growing season. Leaves (20-25) were randomly picked from top to bottom in the crop to estimate the leaf area of one plant (one replicate); four replicates were selected in each treatment at each time. Leaf area was measured with a leaf area meter (LI-3100C, Li-Cor inc., Lincoln, USA); leaves were dried for 48 h at 80 °C in a ventilated oven to determine their dry weights. SLA was calculated by dividing leaf area by leaf dry weight. Crop leaf area index (LAI) was estimated non-destructively from measuring length and width at the widest point of each leaf of the six randomly selected plants, the relationship between leaf area and leaf width and length was estimated from destructive measurements of 56 leaves of comparable plants. The angle of all leaves of six plants were manually measured with a protractor in each treatment at the beginning of August, these measurements were mainly made during the morning hours (0900-1300 h). Leaf angle was determined as the angle of the leaf rachis in relation to the horizontal plane at the leaf insertion point on the stem.

PPFD distribution in the canopy

PPFD distribution within the canopy was measured with a 75 cm long light probe, in relation to a reference sensor above the crop (Sunscan, Delta-T, Cambridge, UK). The light probe was positioned perpendicularly to the row. For characterisation of the vertical PPFD distribution within the canopy, six spots in each treatment were randomly selected; PPFD was measured from the top to the bottom of the canopy at 25 cm height intervals at each spot, at each height level four measurements were taken at equal distance (15 cm) from each other. The LAI at each height was estimated from measurements of length and width of leaves. Under clear sky conditions, the measurements were repeated on six days in the 0 % and 71 % haze treatments, and on three days in the 45 % haze treatment. During the measurements, solar elevation ranged from 29° to 56° and the fraction of diffuse global radiation ranged from 12 % to 28 %. Measurements under fully overcast sky conditions were repeated on four days.

The horizontal PPFD distribution was measured at 50 cm below the top of the crop in three double rows. Measurements were taken at 25 cm intervals over 5 m length in each double row in the 0 % and 71 % haze treatments. Measurements were repeated on two days under clear sky conditions. During these measurements, the solar elevation ranged from 40°

to 46° and the fraction of diffuse global radiation ranged from 25 % to 32 %. Measurements under fully overcast sky conditions were repeated on four days.

Leaf photosynthesis light response curve

Leaf photosynthesis light response curves were measured with a portable gas exchange device equipped with a leaf chamber fluorometer (LI-6400; LI-COR, Lincoln, USA) at three canopy depths in June. Canopy depths were defined as leaf number five (uppermost fully expanded leaf; leaf number one was the uppermost leaf longer than 5 cm), leaf ten and 15 (this definition of canopy depth is used for all measurements in this paper). Measurements were carried out between 0900 and 1500 h. At each canopy depth, six leaves from different plants were randomly selected for six light response curves. On one day, one leaf from each treatment was measured at one specific canopy depth before 1200 h, similar measurements, but at another canopy depth, was done after 1200 h. Each canopy depth was measured on three days in the morning and three days in the afternoon. The adaxial side of the leaf was illuminated by the light source (10 % blue, 90 % red). The starting PPFD was 500 μmol m^{-2} s^{-1}, followed by 250, 100, 50, 25, 0, 750, 1000, 1250 and 1500 μmol m^{-2} s^{-1}, at each PPFD, the measurements were taken when the photosynthesis rate reached steady state (after about 10 min). The highest PPFD was applied at the end of measurements to avoid photoinhibition (Leverenz *et al.*, 1990). VPD in the leaf chamber was maintained below 1 kPa; leaf temperature and CO_2 concentration in the measurement chamber were maintained at 25 °C, and 700 μmol mol^{-1}, respectively.

Leaf temperature

Leaf temperature was measured with K-type fine-wire thermocouples at three canopy depths with three replicates in each treatment, and continuously recorded for 19 days. The thermocouples were positioned in the centre of the abaxial side of leaflets. The contact between the thermocouples and leaf surface was checked three times per day. Air temperature was measured with one thermocouple positioned in the middle of a ventilated measuring box (20 × 20 × 10 cm) per treatment per canopy depth. The measurement positions were adjusted weekly as the plants grew.

Chlorophyll fluorescence

The maximum PSII efficiency (Fv/Fm) was measured on four fully expanded sun-exposed leaves at each of the three canopy depths with a portable chlorophyll fluorometer (PAM-2000, Walz, Germany) at five time points (0900, 1300, 1400, 1500, 1600, 1700 h) on clear days.

Red light was used as measuring light (2 μmol m^{-2} s^{-1}) and saturating flashes (8000 μmol m^{-2} s^{-1}). A leaf clip holder (DLC-8) was used for dark adaptation for 30 min prior to the measurements.

Leaf biochemical and anatomical

Leaf samples were collected at the beginning of July for biochemical and anatomical measurements. Six leaf discs, 1.6 cm in diameter, were punched out of six leaves at each canopy depth in the morning; they were used to determine chlorophyll *a* and *b* content. Dimethylformamide was used as solvent and the absorbance of the extracts was measured using a Cary 4000 spectrophotometer (Agilent Technologies, Santa Clara, USA). The chlorophyll concentrations were calculated using the equations derived by Wellburn (1994). Total nitrogen content was determined with an elemental C/N analyser (model EA 1108, FISONS Instruments, Milan, Italy). Stomata density was estimated by using a silicone rubber impression technique (Fanourakis *et al.*, 2011). For leaf anatomical observations, six leaf samples (0.5 × 1 cm) from six plants were cut at each canopy depth and immediately fixed in 4% neutralized formalin-acetic acid-alcohol solutions. Before being embedded in Technovit 7100 plastics, leaf samples were dehydrated in a graded ethanol series. Sections of each leaf sample, 7 μm thick, were cut with an ultra-microtome (Leica 2030, Biocut, USA) and stained with Schiff's reagent and Toluidine blue for light microscopy. Leaf thickness, palisade tissue and spongy tissue thickness were measured from microscopic photographs taken from the sections at 100 × magnification.

Statistical analysis

Treatment effects on measured plants and light characteristics were evaluated by analysis of variance (ANOVA), using GenStat 14th edition, assuming replications in the same greenhouse compartment as being independent.

A nonlinear mixed effects model (Qian *et al.*, 2012), using the non-rectangular hyperbola function (Thornley, 1976) was fitted to the measured photosynthetic light response data. The statistical software program R, version 2.14.2, package nlme was used to estimate the parameters P_{max}, a, Θ and R_d in the non-rectangular hyperbola function

$$P_n = \frac{aI_a + P_{max} - \sqrt{(aI_a + P_{max})^2 - 4aI_a\Theta P_{max}}}{2\Theta} - R_d \qquad (1)$$

where dependent variable P_n is the net leaf photosynthesis rate (μmol m^{-2} s^{-1}) and regressor I_a is the PPFD absorbed by the leaf (μmol m^{-2} s^{-1}); I_a was estimated from the incident PPFD

multiplied by the absorption coefficient of single leaves (0.88, measurements on reflectance and transmittance of single leaves is described in APPENDIX). Parameter P_{max} represents the light saturated net leaf photosynthesis rate (µmol m^{-2} s^{-1}), a is the light-limited quantum efficiency (µmol CO_2 µmol^{-1} photons), Θ is the curvature parameter, and R_d is the photosynthesis rate at light intensity $I_a = 0$ (dark respiration, µmol m^{-2} s^{-1}). A non-linear random coefficients model was used to accommodate the repeated photosynthesis measurements per leaf. The random part of the model specified that the four parameters have random effects per plant. In the fixed part of the non-linear mixed effect model we allowed the four parameters to be different for the nine treatment × canopy layer combinations, splitting effects in main effects and interaction. Special interest was in the comparison between treatments at the three individual canopy depths.

Crop photosynthesis calculation

An overview of the crop gross photosynthesis (from here onwards called crop photosynthesis) calculation is presented in this section, while more details are presented in the APPENDIX. Crop photosynthesis was calculated from the photosynthesis-light response curve of single leaves, the incoming radiation, the canopy light profile and LAI. Integrating instantaneous crop photosynthesis over time yielded the cumulative crop photosynthesis. The variables and parameters, with default values, are listed in Tables A 1-A 3 of APPENDIX.

Leaf gross photosynthesis

The rate of leaf gross photosynthesis (P_g, µmol CO_2 m^{-2} leaf s^{-1}) was calculated according to eqn 2, which was derived from the fitted light response curves [eqn (1)]

$$P_g = \frac{aI_a + P_{max} - \sqrt{(aI_a + P_{max})^2 - 4aI_a\Theta P_{max}}}{2\Theta} \tag{2}$$

Canopy light profile and crop photosynthesis

PPFD decreases exponentially with canopy depth according to the Lambert-Beer law (Monsi and Saeki, 2005)

$$I(L) = I_0 \, e^{-KL} \tag{3}$$

where I_o is incident PPFD at the top of the canopy (µmol m^{-2} s^{-1}), K is extinction coefficient, L is cumulative LAI from top of canopy to a given depth in the canopy (m^2 leaf m^{-2} ground), $I(L)$ is PPFD (µmol m^{-2} s^{-1}) at canopy depth L.

Subscripts '*df*' and '*dr*' denote the diffuse and direct components of PPFD. Hence, incident PPFD at the top of the canopy (I_o) is

$$I_0 = I_{0,df} + I_{0,dr} \tag{4}$$

Subscript '*a*' denotes the absorption of PPFD, thus the absorbed PPFD at canopy depth *L* is

$$I(L)_a = I(L)_{a,df} + I(L)_{a,dr} \tag{5}$$

For calculating canopy photosynthesis, the crop canopy was divided into three canopy depths which were selected by Gaussian integration (Goudriaan, 1986). The rate of leaf photosynthesis at each selected Gaussian canopy depth L_p [$P_g(L_p)$, µmol CO_2 m^{-2} leaf s^{-1}] is calculated according to

$$P_g(L_p) = P_g[I(L_p)_a] \tag{6}$$

The rate of whole canopy photosynthesis [$P_{g,c}$, µmol CO_2 m^{-2} ground s^{-1}] is calculated by integrating the weighted average of the leaf photosynthesis rate at the three canopy depths, using eqn (6) gives

$$P_{g,c} = L_T(P_{g,-1} + 1.6P_{g,0} + P_{g,1})/3.6 \tag{7}$$

where L_T is total LAI from top to the bottom of the canopy, $P_{g,-1}$, $P_{g,0}$ and $P_{g,1}$ are the leaf photosynthesis rate at the three Gaussian canopy depths (top, middle and bottom of the canopy, respectively), for more discussion of the Gaussian integration used for canopy photosynthesis calculation see Spitters (1986) and Goudriaan (1986).

Canopy photosynthesis rate was calculated at 126 spots in each treatment where the horizontal PPFD distribution measurements were taken, canopy PPFD absorption at these spots was estimated according to the relative variation of PPFD in the horizontal direction. Average canopy photosynthesis rate over these spots represents instantaneous canopy photosynthesis, which was integrated from 1 Apr to 1 Oct. 2011 to obtain cumulative crop photosynthesis.

Quantifying the contributing factors to crop photosynthesis in diffuse light treatment
Cumulative crop photosynthesis was calculated in 0 % and 71 % haze treatments, respectively. Afterwards, four factors (vertical PPFD distribution, horizontal PPFD distribution, photosynthesis light response curves and LAI) were separately considered in each of the treatments for calculation of the cumulative crop photosynthesis, this aims to

quantify their relative contribution for crop photosynthesis enhancement in 71 % haze treatment. When quantifying each of the four factors, the other three factors were assumed to be constant in the two treatments, using the measured values of the 0 % haze treatment. Constant LAI (Fig. 1) and leaf photosynthetic traits (Fig. 8) in each treatment were applied for cumulative crop photosynthesis calculation over the growing period from 1 Apr to 1 Oct, as based on measurements during the summer months. Furthermore, an assumption was that the horizontal variability of PPFD at the three canopy depths was similar.

RESULTS

Fruit Production and crop morphological properties

The cumulative fresh weight of harvested fruits was 59.1 kg m^{-2} at the end of the growing season in the control treatment. It was 8.4 % higher in the 45 % haze and 10.4 % higher in the 71 % haze treatment. Detailed information about fruit production see Dueck *et al*. (2012).

In all treatments, the upper leaves of a plant showed a positive leaf angle with respect to the horizontal plane, ranging from + 50° to 0°, while the lower leaves showed a negative leaf angle, ranging from 0° to - 15°. No treatment effect on leaf angle was observed (Supplemental Fig. S2). Furthermore, haze treatments resulted in a higher LAI ($P < 0.01$) (Fig. 1) and a lower crop SLA ($P < 0.01$) (Fig. 2) compared to the control.
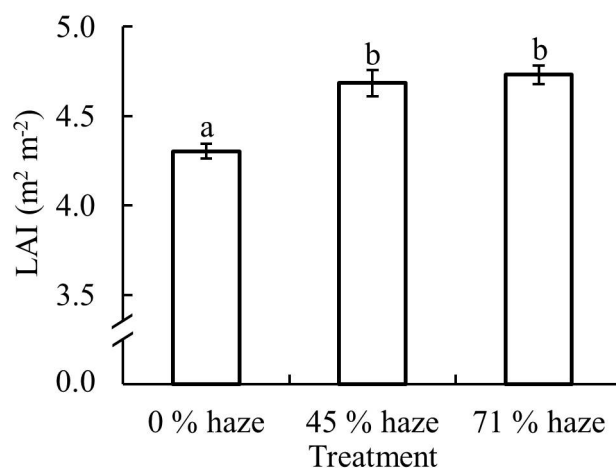
Fig. 1. Average leaf area index (LAI) in summer months. Measurements were done on 25 May, 05 and 27 Jul. Six plants were measured in each treatment each time. Error bars show ± SE (n = 18). Letters show statistical significant differences ($P < 0.05$).
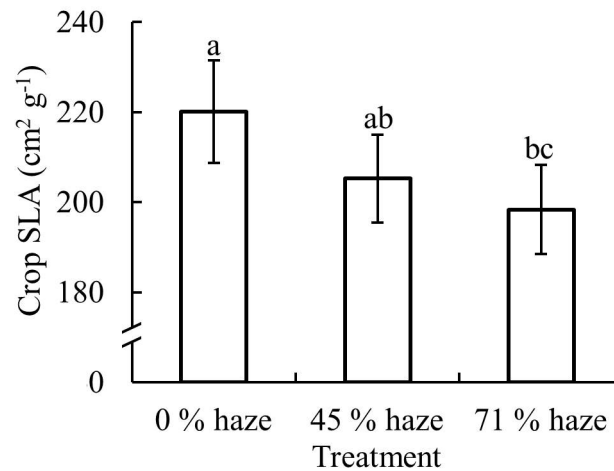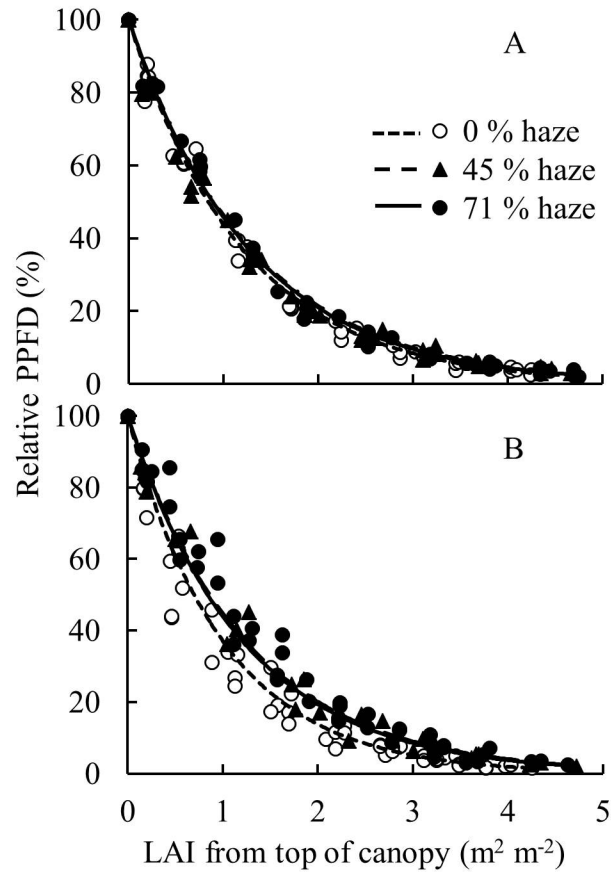
Fig. 2. The effect of haze treatments on crop specific leaf area (SLA). Leaf samples were averaged down the canopy and over two sunny (7 Apr, 4 Jul) and two cloudy days (8 Jun, 1 Sep). On each date four replicate plants were sampled per treatment. Error bars show ± SE (n = 16). Letters show statistical significant differences ($P < 0.05$).

PPFD distribution in the crop canopy

PPFD decayed exponentially with increasing LAI according to the Lambert-Beer law (Fig. 3). On cloudy days when PPFD was fully diffuse, the vertical distribution of PPFD in the canopy was similar in all three treatments (Fig. 3A). On clear days, however, PPFD decayed more rapidly in the control in comparison to the haze treatments (Fig. 3B). The PPFD inside the canopy was up to 9 % higher at LAI 1, 6 % higher at LAI 2 and 1 % higher at LAI 3 in the haze treatments compared to the control. Consequently, the extinction coefficient on clear days [K; eqn (3)] was significantly lower ($P < 0.01$) in both haze treatments ($K = 0.81$) than in the control ($K = 0.99$). The extinction coefficient in both haze treatments on clear days was similar to that on cloudy days.

In the horizontal plane, PPFD was homogeneously distributed in the upper part of the canopy on cloudy days in 0 % and 71 % haze treatment (Fig. 4A). On clear days, however, a large variation in PPFD in the 0 % haze treatment was observed compared with the 71 % haze treatment as indicated by the coefficient of variation (42 % and 14 %, respectively) (Fig. 4B).

Fig. 3. The effect of haze treatments on the vertical PPFD distribution within a tomato crop on cloudy days (A; 13, 14, 29 Jul, and 12 Sep) and clear days (B; 1, 4 Aug, 2, 3, 29, 30 Sep for 0 % and 71 % haze treatment; 1, 4 Aug and 3 Sep for 45 % haze treatment). Each symbol represents the average of six replicates measured in one day. Lines are fitted curves based on the Lambert-Beer law [eqn (3)].

Leaf temperature

Leaf temperature differed greatly between the control and haze treatments on clear days; this difference in leaf temperature was observed only at the top of the canopy where leaves were exposed to the sun (Fig. 5), but not in the middle and bottom leaves (Supplemental Fig. S3). The difference between top leaf and air temperature (ΔT) at midday on clear days was 3 to 5 °C higher in the control than in haze treatments. Moreover, top leaf temperature in haze treatments remained close to the air temperature on clear days.
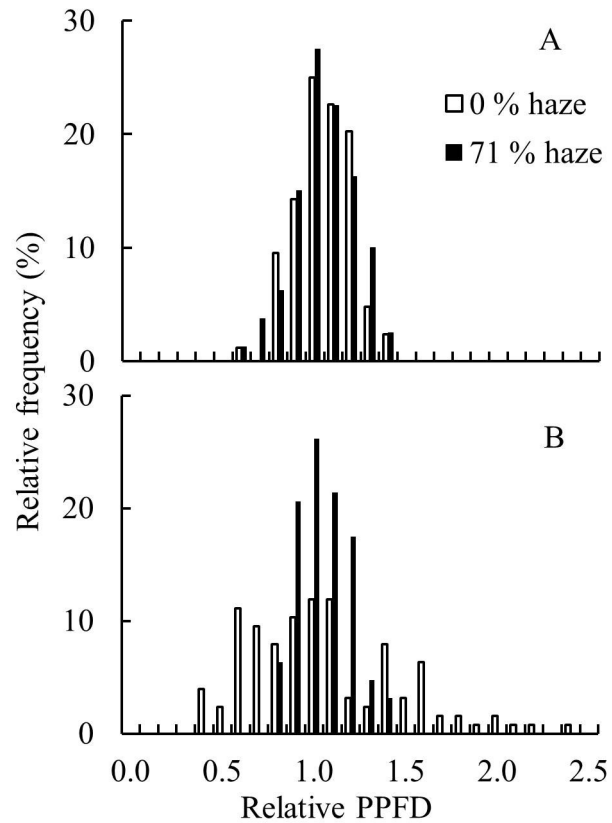
Fig. 4. The effect of haze treatments on the horizontal PPFD distribution within a tomato crop canopy on cloudy days (A; 13, 14, 29 Jul, and 31 Aug) and clear days (B; 2 and 3 Sep). All measurements were performed at 50 cm below the canopy top. In both figures the Y-axis indicates frequency distribution of PPFD in a horizontal plane. The X-axis indicates the relative PPFD; 1.0 represents the average PPFD on the horizontal plane.
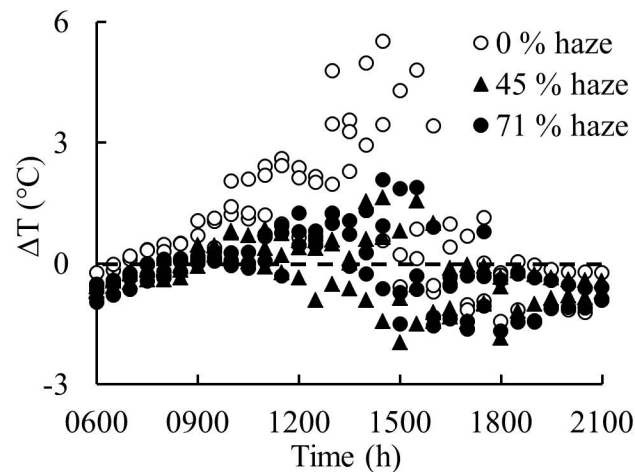

Fig. 5. Diurnal pattern of the temperature difference between leaf and air (ΔT) at the canopy top layer on three clear days (2, 4 and 5 Aug; average global radiation was 18.8 ± 1.3 MJ m^{-2} day^{-1}). Each symbol represents the average of measurements over 30 minutes on three leaves.

Photoinhibition

When top leaves were exposed to the full radiation (midday), maximum PSII efficiency (Fv/Fm) was reduced compared to measurements in the morning, suggesting photoinhibition. This reduction was more pronounced in the control than in haze treatments (Fig. 6). At 1400 h, when global radiation was more than 800 W m^{-2}, Fv/Fm was reduced by 21 % in the control and 12 % in haze treatments, while this difference disappeared later in the afternoon when the global radiation was low. Although Fv/Fm in the middle and bottom leaves also showed a diurnal trend with small differences between morning and midday, treatment effects were not found in these leaves (Supplemental Fig. S4).
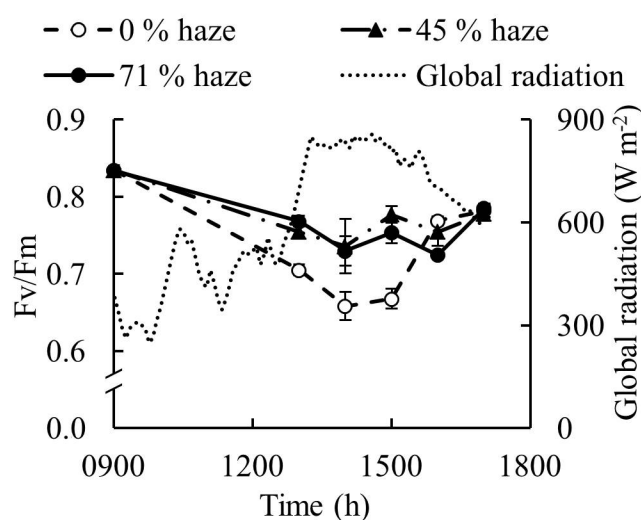
Fig. 6. Maximum PSII efficiency (Fv/Fm) of top leaves on a clear day (15 Aug). Error bars represent ± SE (n = 4).

Leaf photosynthesis, biochemical and anatomical properties

For the parameters in the leaf photosynthesis-light response curves, a significant difference in P_{max} in the middle canopy layer ($P = 0.01$) was found between treatments, but not in the top and bottom layers ($P = 0.97$ and 0.45, respectively) (Table 1). P_{max} decreased from top to bottom canopy layer in all treatments (Table 1). For parameters a, Θ, and R_d, significant differences were found between canopy layers but not between treatments (Table A3). At the crop level, significantly higher chlorophyll contents, chlorophyll a/b ratios and total nitrogen concentrations were observed in the haze treatments compared with the control treatment (Table 2). Stomatal density was not influenced by haze treatments (Supplemental Fig. S5). Haze treatments resulted in thicker mesophyll, palisade and spongy tissue in the bottom leaves (Fig. 7), while these differences did not occur in top and middle leaves (Supplemental Fig. S6).
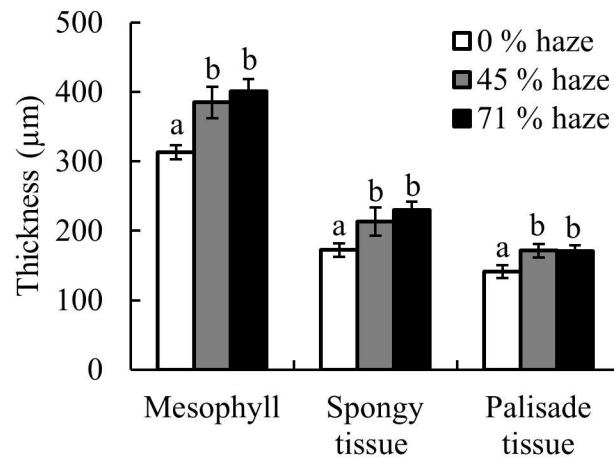
Fig. 7. Thickness of leaf mesophyll, spongy tissue and palisade tissue in bottom leaves. Leaf samples were taken on 5 Jul. Error bars show ± SE (n = 6). Letters within each parameter show statistical significant differences (*P* < 0.05).

Table 1. Maximum net photosynthesis rate (P_{max}, µmol m^{-2} s^{-1}) in three canopy layers in response to haze treatments (n = 6). P_{max} was fitted from measured light response curves [eqn (1)].

reatment	Top	Middle	Bottom
0 % haze	52.9	41.7	35.1
45 % haze	53.3	45.2	37.4
71 % haze	53.5	49.5	38.1
P-value	0.97	0.01	0.45

P-values > 0.05 were regarded as non-significant.

Table 2. Leaf chemical components in a crop in response to haze treatments (n = 6).

Treatment	Total Nitrogen (g m^{-2})	*Chl (a+b)* (mg m^{-2})	*Chl a/b* ratio
0 % haze	2.05 a	348 a	3.28 a
45 % haze	2.12 b	379 b	3.42 b
71 % haze	2.23 c	368 b	3.47 b
LSD	0.04	17	0.07

Means followed by different letters within one column differ significantly (*P* < 0.05) as established by the least significant difference (*LSD*) test. All values are averaged values of three (Top, middle and bottom) canopy layers. *Chl* represents chlorophyll.
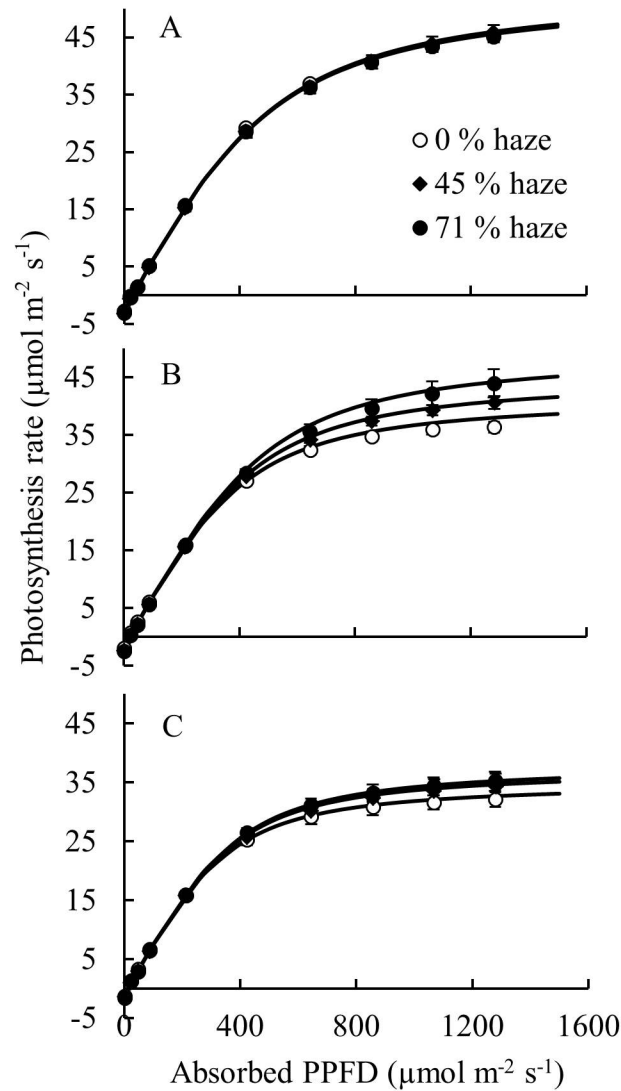
Fig. 8. Photosynthetic light response curves of leaves at top (A), middle (B) and bottom (C) of the canopy. These measurements were taken in June. During the measurements, leaf temperature and CO_2 concentration in the measurement chamber were maintained at 25 °C, and 700 µmol mol^{-1}, respectively. Error bars show ± SE (n = 6).

Quantifying contributing factors to crop photosynthesis

The cumulative crop photosynthesis calculated over a growing period (1 Apr to 1 Oct) increased by 7.2 % in the 71 % haze treatment compared to the control treatment. This enhancement mainly resulted from four factors: more uniform vertical and horizontal PPFD distribution (Figs 3 and 4), greater leaf photosynthetic capacity in the middle part of the crop (Fig. 8) and larger LAI (Fig. 1). Among them, horizontal PPFD distribution accounted for 33 % of this enhancement, while 23 %, 21 % and 13 % for leaf photosynthetic capacity, vertical PPFD distribution and LAI factors, respectively, the remaining 10 % was attributed to the interaction effect of the four factors (Fig. 9). Similar calculations were performed over the

whole growing season (1 Jan to 1 Nov). In that case cumulative crop photosynthesis increased by 6.6 % in the 71 % haze treatment compared to the control treatment, the relative contribution of the four contributing factors was similar as the calculations during 1 Apr to 1 Oct.
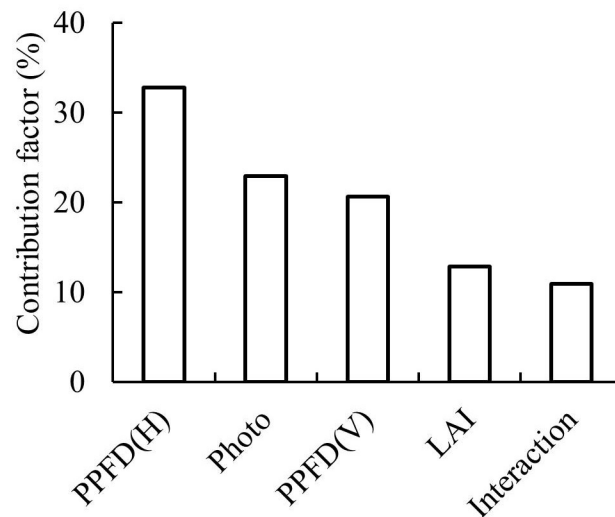
Fig. 9. Quantification of the contributing factors to crop photosynthesis enhancement over a designated growing period (1 Apr to 1 Oct. 2011) in the 71 % haze treatment. The X-axis represents the influence factors: PPFD(H): horizontal PPFD distribution; Photo: leaf photosynthesis light response curve; PPFD(V): vertical PPFD distribution; LAI: leaf area index; Interaction: interaction effect of the four factors.

DISCUSSION

Plants use diffuse light more efficiently than direct light (Farquhar and Roderick, 2003; Gu *et al.*, 2003; Mercado *et al.*, 2009). This study is the first to show experimentally the effects of diffuse light on plant growth, while all other growth conditions remained similar. By conducting a controlled experiment in a greenhouse, this study avoided the side-effects of other studies conducted on cloudy versus clear days, such as the increased proportion of diffuse light at the expense of total global radiation (Urban *et al.*, 2012). Furthermore, the long-term investigation of the effect of diffuse light on plant growth and acclimation is possible under such conditions and is necessary in order to discriminate the effects of diffuse light from that of other factors in interaction with diffuse light.

Crop photosynthesis is correlated with the light intensity profile within the canopy (Gonzalez-Real *et al.*, 2007; Ninemets, 2007; Sarlikioti *et al.*, 2011*b*). The horizontal PPFD distribution in the crop was directly linked to the proportion of sunlit and shaded leaf area

(Spitters, 1986). Sunlit leaves are usually brightly illuminated, while shaded leaves are illuminated by diffuse light, often with relatively lower intensities. Thus, the photosynthetic light use efficiency per unit leaf area is lower in sunlit leaves than in shaded leaves due to the nonlinearity of leaf photosynthetic light response (Baldocchi, 1997; Gu *et al.*, 2002). Plants grew in treatments with diffuse light under a more uniform PPFD distribution in the horizontal plane than in the control treatment (Fig. 4), this is in consistent with Acock *et al.* (1970) who concluded that the variability of PPFD in the horizontal plane increases with increase in the ratio of direct sunlight to diffuse skylight. We quantified this effect and showed that it was the most important factor for the enhancement of crop photosynthesis in diffuse light (Fig. 9). This is in contrast to Urban *et al.* (2012), who stated that a more uniform vertical light distribution in the canopy is the most important cause of the higher plant photosynthetic efficiency under diffuse light. However, these authors did not compare that to the effect of the horizontal light profile in the canopy. To calculate crop photosynthesis, many models artificially separate leaves into sunlit and shaded groups in the crop horizontal plane (Norman, 1980; De Pury and Farquhar, 1997; Wang and Leuning, 1998; Johnson *et al.*, 2010). However, in this study, we measured and used the real variability of PPFD in a horizontal plane at crop level. Thus, the current calculation of crop photosynthesis might be more close to reality.

To quantify the effect of horizontal variability of PPFD on crop photosynthesis, we assumed that the relative variation of horizontal PPFD was similar at each of the three canopy depths, as we measured the horizontal PPFD distribution only in the upper part of the crop. However, Acock *et al.* (1970) concluded that the variability of PPFD distribution in the horizontal direction increases with increasing depth in the canopy. To test our assumption, we did the calculations based on relative differences in variation coefficient of horizontal PPFD distribution in the three canopy depths in each treatment according to Acock *et al.* (1970, Fig. 2). This resulted in only 0.1 % deviation of cumulative crop photosynthesis compared with the calculation based on our assumption in both treatments. As the change in variation coefficient mainly occurred in the lowest leaf layer, this negligible effect is not surprising because the PPFD in the lower part of the crop is intrinsically low compared with the upper part of the crop. Therefore, we conclude that the assumption of equal horizontal PPFD distribution can be used for our purpose.

Increasing the fraction of diffuse light resulted in a deeper light penetration, which is characterized by a lower extinction coefficient (Fig. 3B); this is in agreement with Urban *et al.* (2012), who reported a lower extinction coefficient on cloudy days. This phenomenon

occurred due to the properties of diffuse light which scatter in many directions and thus cause less shadow, while direct light either concentrates in a beam or casts a shadow in the canopy, which results in the upper leaves brightly illuminated and lower leaves in deep shade, or strong lightflecks at a given canopy depth. We quantified the effect of vertical PPFD distribution on crop photosynthesis and showed that the deeper PPFD penetration in haze treatments also played a substantial role in crop photosynthesis enhancement. However, the deeper penetration of PPFD is only advantageous at a high LAI. At a low LAI more PPFD would reach the floor and would not be absorbed by the crop. Furthermore, Sarlikioti *et al.* (2011*b*) indicated that the advantages of a deeper light penetration in the canopy depends on the seasonal distribution of leaf photosynthetic capacity over the canopy, and the seasonal pattern of light intensity due to the solar elevation changes during the year. Similarly it changes in a diurnal pattern. Therefore, further exploring these effects can improve our understanding of the effect of diffuse light on plant processes.

Although the light profile in a canopy is partly linked to leaf angle (Falster and Westoby, 2003; Sarlikioti *et al.*, 2011*b*), this factor can be ignored in the current study because haze treatments did not affect the leaf angle (Supplemental Fig. S2). This is further supported by a similar vertical light profile among the three treatments on cloudy days (Fig. 3A). Furthermore, Alton (2008) concluded from model simulations that canopy architecture plays only a minor role in the enhancement of radiation use efficiency on cloudy days. In contrast, the simulations by Sarlikioti *et al.* (2011*b*) showed that improving plant architecture leads to an increase of 6-10 % in plant photosynthesis in tomato. In this context, more investigations on plant architecture are needed to further explore the effect of diffuse light on plant photosynthesis.

Physiological and morphological properties of plant organs can be affected by their prevailing microclimate (Ellsworth and Reich, 1993; Niinemets, 2007). A homogeneous light distribution in the canopy will likely result in a higher fraction of leaves grown under relatively higher light intensities in the lower part of the crop, which is reflected by lower crop SLA (Fig. 2) and a thicker bottom leaves (Fig. 7) in haze treatments. This is in accordance with the view that a lower SLA can be seen as an acclimatory response to high light intensity (Evans and Poorter, 2001; Trouwborst *et al.*, 2010). Furthermore, higher leaf total nitrogen, chlorophyll content and chlorophyll *a/b* ratio at crop level (Table 2), and a higher photosynthetic capacity in the middle of the crop (Table 1) were observed in both haze treatments, which will enhance crop photosynthesis (Fig. 9). Another aspect of plant acclimation in haze treatments was the development of a higher LAI (Fig. 1). A higher LAI is

highly relevant for crop photosynthesis, as long as the fraction of light interception is increased as well (Wunsche and Lakso, 2000). The higher LAI in haze treatments was attributed to greater leaf width (data not shown). However, the increased LAI played a minor role in crop photosynthesis enhancement in haze treatments (Fig. 9), because the light interception was already close to saturation in the control treatment (Fig. 3).

When global radiation was high, temperature of top leaves in the haze treatments was lower than in the control treatment (Fig. 5), this correlated with a lower light absorption of the top leaves under diffuse light. Furthermore, a higher maximum PSII efficiency (Fv/Fm) was observed in the haze treatments (Fig. 6) in comparison to the control treatment, suggesting less photoinhibition. This indicated that diffuse light minimized the potential for photodamage or photoprotection arising from the over-excitation of the photosynthetic apparatus. This was corroborated by a lower Fv/Fm ratio in the control treatment. In this study, reversible change in Fv/Fm was found in late afternoon in the control treatment (Fig 6), suggesting photoprotection rather than photodamage. The decreased Fv/Fm at high light intensity is likely caused by the reversible inactivation or downregulation of PSII, rather than photodamage to PSII or loss of D1 protein (Long *et al.*, 1994; Demmig-Adams *et al.*, 1996; Demmig-Adams and Adams, 2000; Huang *et al.*, 2006). In addition, a higher leaf temperature might further intensify the photoinhibition (Bongi and Long, 1987).

To calculate crop photosynthesis, analytical or numerical expression is commonly applied (Thornley, 2002; Johnson, 2010). In this study, we used Gaussian integration for this purpose, because Goudriaan (1986) proved that Gaussian integration is a simple and proper method to integrate photosynthesis over the canopy. Furthermore, we applied non-rectangular hyperbola function in the Gaussian integration procedure instead of rectangular hyperbola function which used by Goudriaan (1986) and Spitters (1986), this is because Marshall and Biscoe (1980) indicated that the non-rectangular hyperbola is a better description of the response of photosynthesis to irradiance compared with rectangular hyperbola. Our objective is to quantify the relative importance of each factor; therefore, the current calculation procedure should be sufficient. For the measurements of the light response curves of leaf photosynthesis, only the adaxial side of the leaf was illuminated by the light source. However, diffuseness of light may affect the fraction of light on the abaxial surface, which surface may have a different response curve (e.g. Paradiso and Marcelis, 2012). This might have led to a minor error in estimating the effects of diffuse light. Measurements of light absorption and photosynthesis light response curves on both the adaxial and abaxial side of leaves in the

canopy in combination with functional structural plant modelling might help to estimate these effects.

Solar radiation on the Earth's surface is the primary driver for plant photosynthesis. However, the increased anthropogenic emissions over the past century have affected solar radiation at the Earth's surface (global dimming). On the other hand, these emissions also increased the diffuse component of solar radiation (Mercado *et al.*, 2009). According to our study and previous studies (Gu *et al.*, 2002; Gu *et al.*, 2003; Hemming *et al.*, 2007; Zhang *et al.*, 2011) indicating the advantages of diffuse radiation for plant photosynthesis, it stands to reason that global dimming does not negatively influence plant photosynthesis if the enhanced plant photosynthesis resulting from the increased fraction of diffuse light can sufficiently offset the reduction in global radiation level. Furthermore, our findings have implications for improving agricultural efficiency. Crop productivity can be improved by increasing the fraction of diffuse light.

CONCLUSIONS

Transforming 71 % of direct PPFD into diffuse PPFD increased cumulative crop photosynthesis by 7.2 % in a tomato crop. Four factors contributed to this increase in crop photosynthesis. The most important factor was a more homogeneous horizontal PPFD distribution; a more uniform vertical PPFD distribution and higher leaf photosynthetic capacity contributed more to the crop photosynthesis enhancement than a higher LAI. Based on these findings, we conclude that the enhancement of crop photosynthesis in diffuse light not only depends on the direct effects of a more uniform vertical and horizontal light distribution, but also on crop physiological and morphological acclimation.

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APPENDIX

The basic set of equations for crop photosynthesis calculation was presented in the main text. The more detailed implementations are presented here. The variables and parameters, with default values, are listed in Tables A1-A3.

The outdoor intensities of direct ($I_{out,dr}$) and diffuse ($I_{out,df}$) PPFD were calculated from the total outdoor PPFD and the fraction of diffuse and direct PPFD, estimated according to Spitters *et al.* (1986). PPFD reaching the top of the canopy consists of a diffuse ($I_{o,df}$) and a direct ($I_{o,dr}$) component [eqn (4)], $I_{o,df}$ and $I_{o,dr}$ in eqn (4) are given by

$$I_{o,df} = \lambda(I_{out,df} + hI_{out,dr}) \quad \text{and} \quad I_{o,dr} = \lambda I_{out,dr}(1 - h) \quad \text{(A 1)}$$

where λ is the greenhouse transmissivity (%), h is the haze factor of the greenhouse cover.

It is assumed that both direct and diffuse components of PPFD exponentially decline through the canopy [eqn (3)]. Thus the total amount of PPFD absorbed at any given level of canopy depth L can be calculated according to eqn (5). $I(L)_{a,dr}$ and $I(L)_{a,df}$ in eqn (5) are given by

$$I(L)_{a,dr} = (1 - \rho)I_{o,dr}K_{dr}e^{-K_{dr}L} \quad \text{and} \quad I(L)_{a,df} = (1 - \rho)I_{o,df}K_{df}e^{-K_{df}L} \quad \text{(A 2)}$$

The extinction coefficient for diffuse PPFD (K_{df}) was derived from fitting eqn (3) to the measured vertical PPFD distribution on overcast days in the 0 % haze treatment, when the global irradiance was fully diffuse (measurements are described in the section 'PPFD distribution measurements in the canopy'). Similarly, an extinction coefficient for clear days (K_c) was determined on clear days in the 0 % haze treatment. On clear days the total outdoor PPFD consists of a diffuse and direct component. Therefore, the extinction coefficient for the direct component PPFD (K_{dr}) was calculated as

$$K_{dr} = (K_c - f_{df}K_{df})/f_{dr} \quad \text{(A 3)}$$

where f_{df} and f_{dr} represent the average fraction of outdoor diffuse and direct PPFD during PPFD distribution measurements on clear days, respectively.

Incoming PPFD is partly reflected by the canopy. According to Spitters [1986, eqn (1)], the reflection coefficient [ρ; in eqn (A 2)] of a closed canopy is

$$\rho = \left[\frac{1 - (1 - \sigma)^{0.5}}{1 + (1 - \sigma)^{0.5}}\right]\left(\frac{2}{1 + 1.6sin\beta}\right) \quad \text{(A 4)}$$

where σ is the scattering coefficient which is the sum of reflectance and transmittance of the visible spectrum of individual leaves. Reflectance and transmittance of leaves was measured

on 108 tomato leaves with a spectrophotometer (Perkin Elmer Inc. Waltham, MA, USA). β is solar elevation above the horizon estimated from time and location (Spitters *et al.*, 1986). Canopy reflection coefficient of diffuse PPFD [the first term in eqn (A 4)] does not change with solar elevation.

For calculating canopy photosynthesis, the crop canopy was divided into three canopy depths which were selected by Gaussian integration (Goudriaan, 1986). Thus, L_p in eqn (6) is

$$L_p = (0.5 + p0.15^{1/2})L_T \quad p = \text{-1, 0, 1} \tag{A 5}$$

where p is a specific factor to define top (-1), middle (0) and bottom (1) canopy depth.

Leaf photosynthesis rate at each of the selected canopy depth was calculated according to eqn (6). Whole canopy photosynthesis was calculated as a weighted average of the leaf photosynthesis rate at three Gaussian canopy depths as described in eqn (7).

According to equations presented in this section and in the main text, instantaneous canopy photosynthesis can be derived. Integrating the instantaneous canopy photosynthesis rates over the designated growing period yielded the cumulative crop photosynthesis.

Note that the canopy depth defined by the Gaussian integration differs from the three canopy depths in all the measurements. Photosynthetic parameters [in eqn (1)] for the three Gaussian canopy depths were obtained by linear interpolation of the parameters derived from photosynthetic light response curve fitting (Tables 1 and A 3) and the LAI where the leaf photosynthesis light response curves were measured.

Table A 1. Model variables, definitions and units

Variable	Definition	Units
I_a	PPFD absorbed by the leaflet surface. [eqn (1)]	μmol m^{-2} s^{-1}
$I_{out,dr}$, $I_{out,df}$	Total outside direct and diffuse PPFD. [eqn (A 1)]	μmol m^{-2}s^{-1}
I_o, $I_{o,dr}$, $I_{o,df}$	Incident total, direct and diffuse PPFD at top of the canopy. [eqn (4)]	μmol m^{-2}s^{-1}
$I(L)_a$, $I(L)_{a,dr}$, $I(L)_{a,df}$	Absorbed total, direct and diffuse PPFD at a given canopy depth. [eqn (5)]	μmol m^{-2}s^{-1}
L	Cumulative leaf area index at a given canopy depth. [eqn (3)]	m^2 leaf m^{-2} ground
L_p	Leaf area index was selected for Gaussian integration. [eqn (6)]	m^2 leaf m^{-2} ground
L_T	Total leaf area index. [eqn (7)]	m^2 leaf m^{-2} ground
P_n	Net leaf photosynthesis rate. [eqn (1)]	μmol CO$_2$ m^{-2} leaf s^{-1}
P_g	Gross leaf photosynthesis rate. [eqn (2)]	μmol CO$_2$ m^{-2} leaf s^{-1}
$P_g(L_p)$	Gross leaf photosynthesis rate at Gaussian selected canopy depth. [eqn (6)]	μmol CO$_2$ m^{-2} leaf s^{-1}
$P_{g,-1}$, $P_{g,0}$, $P_{g,1}$	Gross leaf photosynthesis rate at top, middle and bottom of the canopy. [eqn (7)]	μmol CO$_2$ m^{-2} leaf s^{-1}
$P_{g,c}$	Whole canopy photosynthesis rate. [eqn (7)]	μmol CO$_2$ m^{-2} ground s^{-1}
β	Solar elevation above the horizon. [eqn (A 4)]	$^{\circ}$
ρ	Canopy reflection coefficient. [eqn (A 2)]	-
p (-1,0,1)	Specific factor to define top (-1), middle (0), and bottom (1) canopy depth. [eqn (A 5)]	-

PPFD, photosynthetic photon flux density (μmol m^{-2} s^{-1}). Eqn number follows the definition indicates where the variable first occurs and is introduced.

Table A 2. Model parameters, definitions, units and default values

| Parameter	Definition	Default value
K_{dr}	Direct PPFD extinction coefficient. [eqn (A 2)]	1.06
K_{df}	Diffuse PPFD extinction coefficient. [eqn (A 2)]	0.82
K_c	PPFD extinction coefficient measured on clear days. [eqn (A 3)]	0.99
f_{df}	Average fraction of diffuse PPFD during the PPFD distribution measurements on clear days. [eqn (A 3)]	27.5 %
f_{dr}	Average fraction of direct PPFD during the PPFD distribution measurements on clear days. [eqn (A 3)]	72.5 %
h	Haze factor of the greenhouse cover. [eqn (A 1)]	0 %, 45 %, 71 %
λ	Greenhouse transmissivity. [eqn (A 1)]	66 %
σ	Scattering coefficient of single leaves for visible radiation. [eqn (A 4)]	0.12

PPFD, photosynthetic photon flux density ($\mu mol\ m^{-2}\ s^{-1}$). Eqn number follows the definition indicates where the parameter first occurs and is introduced.

Table A 3. Light response curve parameters at three canopy layers (n = 6). These are fitted from measured light response curves [eqn (1)].

Canopy layer	α ($\mu mol\ CO_2\ \mu mol^{-1}$ photons)	Θ	R_d ($\mu mol\ m^{-2}\ s^{-1}$)
Top	0.097	0.77	3.00
Middle	0.094	0.81	2.06
Bottom	0.092	0.83	1.27
P-value	0.018	0.02	< 0.01

Statistical analysis showed that light response curve parameters were not significantly different between the three treatments except P_{max}, thus these parameter values were used for crop photosynthesis calculation in all the treatments. *P*-values < 0.05 were regarded as significant.

SUPPLEMENTARY DATA
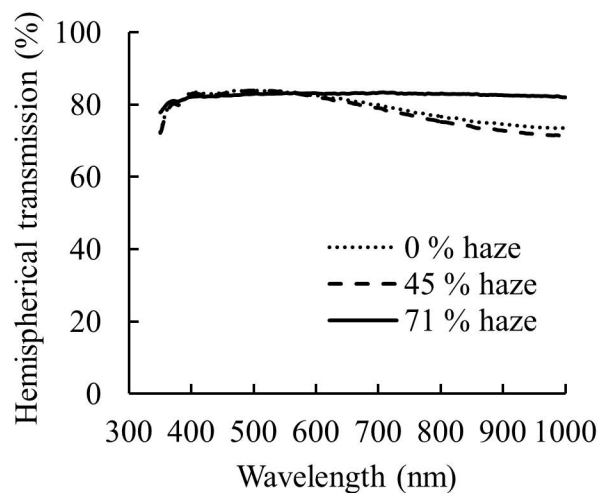
Fig. S1. The hemispherical transmission of PPFD for the three glass types.
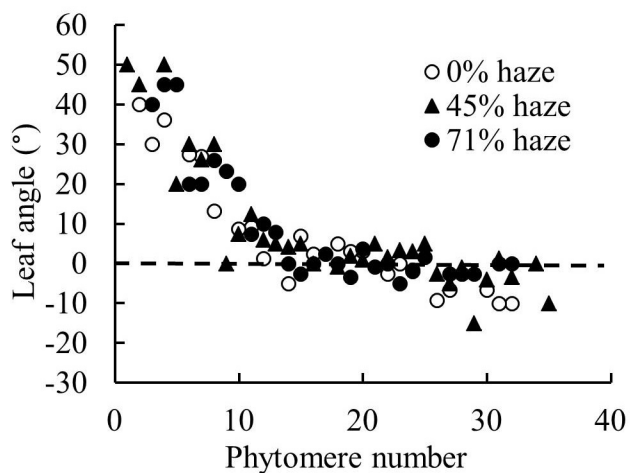
Fig. S2. Relationship between leaf angle to the horizontal plane. Each symbol represents the average of measurements of six leaves. These measurements were taken on 9 and 10 Aug. Leaf angle was determined as the angle of the leaf rachis in relation to the horizontal plane at the leaf insertion point on the stem. 0 in Y-axis indicates the horizontal plane.
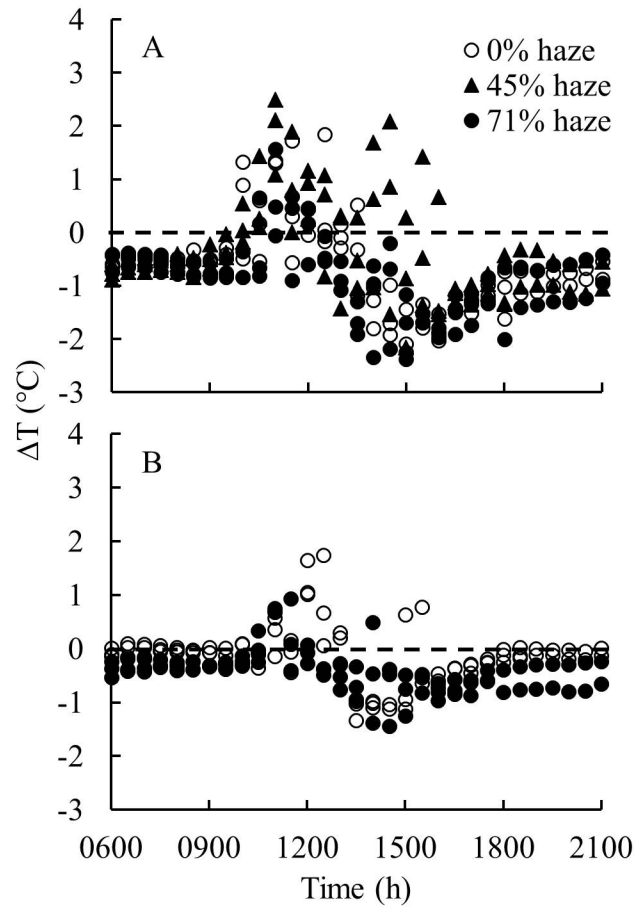
Fig. S3. Diurnal pattern of the temperature difference between leaf and air (ΔT) at the canopy of middle (A) and bottom (B) leaves on three clear days (2, 4 and 5 Aug), average global radiation was 18.8 ± 1.3 MJ m^{-2} day^{-1}). Each symbol represents the average of measurements over 30 minutes on three leaves.
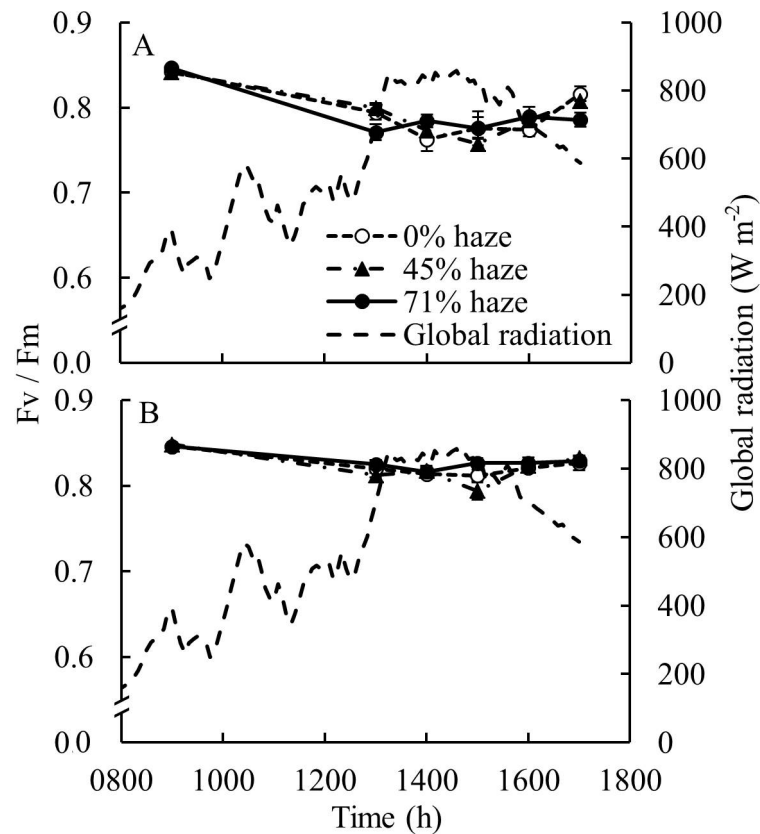
Fig. S4. Maximum PSII efficiency (Fv/Fm) of middle (A) and bottom (B) leaves on a clear day (15 Aug). Error bars represent ± SE (n = 4).
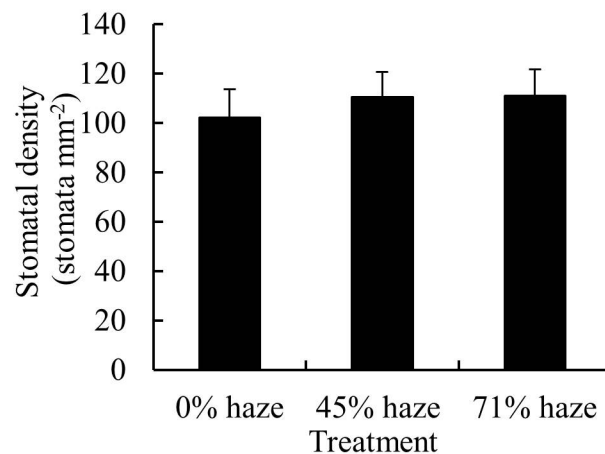
Fig. S5. Stomatal densities on fully expanded leaves of tomato plants grown at three levels of haze treatments. Leaf samples were taken on 2 Jul, six leaves were collected in each treatment per canopy layer (top, middle and bottom). Stomatal densities were averaged down the canopy. Error bars show ± SE (n = 18). No statistical significant differences was found among the three treatments ($P = 0.151$).
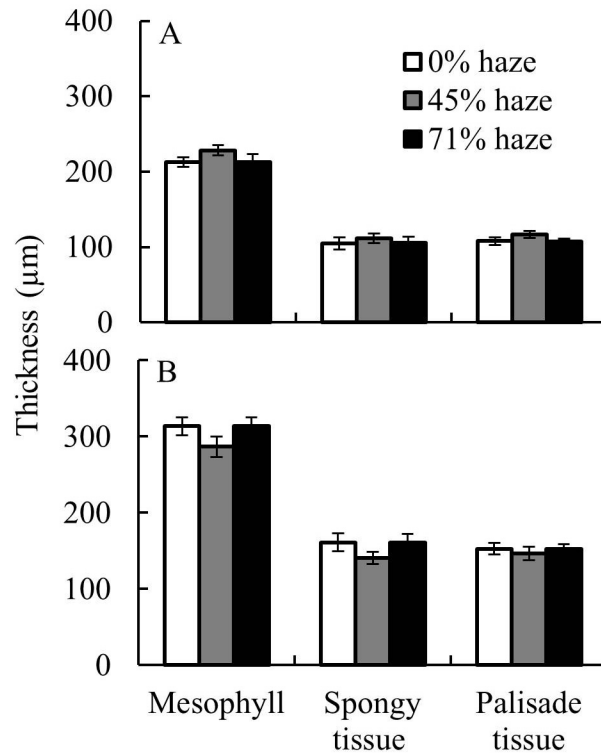
Fig. S6. Thickness of leaf mesophyll, spongy tissue and palisade tissue in top (A) and middle (B) leaves. Leaf samples were taken on 5 Jul. Error bars show ± SE (n = 6). No statistical significant differences was found within each parameter ($P > 0.05$).

Responses of two *Anthurium* cultivars to high daily integrals of diffuse light

T. Li[1], E. Heuvelink[1], F. van Noort[2], J. Kromdijk[3], L.F.M. Marcelis[1]

[1] Horticulture and Product Physiology Group, Wageningen University,
P.O. Box 630, 6700AP, Wageningen, the Netherlands
[2] Wageningen UR Greenhouse Horticulture, Wageningen University and Research Centre,
P.O. Box 644, 6700AP, Wageningen, the Netherlands
[3] Institute for Genomic Biology, University of Illinois,
1206 W, Gregory Drive, Urbana, IL 61801, USA

ABSTRACT

Heavy shading is commonly applied during production of pot-plants in order to avoid damage caused by high light intensities; usually the daily light integral (DLI) is limited to 5-8 mol m^{-2} d^{-1} photosynthetically active radiation (PAR). However, shading carries a production penalty as light is the driving force for photosynthesis. Diffuse glass has been developed to scatter the incident light in greenhouses. This study aims at investigating the effect of diffuse glass cover and high DLI under diffuse glass cover on the growth of pot-plants; furthermore, to systematically identify and quantify the yield components which are influenced by these treatments. Experiments were carried out with two *Anthurium andreanum* cultivars (Royal Champion and Pink Champion) in a conventional modern glasshouse compartment covered by clear glass with DLI limited to 7.5 mol m^{-2} d^{-1} (average realized DLI was 7.2 mol m^{-2} d^{-1}), and another two glasshouse compartments covered by diffuse glass with DLI limited to 7.5 (average realized DLI was 7.5 mol m^{-2} d^{-1}) and 10 mol m^{-2} d^{-1} (average realized DLI was 8.9 mol m^{-2} d^{-1}). Diffuse glass cover resulted in less variation of temporal photosynthetic photon flux density (PPFD) distribution compared with the clear glass cover. Under similar DLI conditions (DLI limited to 7.5 mol m^{-2} d^{-1}), diffuse glass cover stimulated dry mass production per unit intercepted PPFD (RUE) in 'Royal Champion' by 8 %; whilst this stimulating effect did not occur in 'Pink Champion'. Under diffuse glass cover, biomass production was proportional to DLI in both cultivars (within the range 7.5-9 mol m^{-2} d^{-1}). Consequently higher DLI led to more flowers, leaves and stems. Furthermore, high DLI resulted in more compact plants without light damage in leaves or flowers in both cultivars. 'Pink Champion' produced more biomass than 'Royal Champion' in all treatments because of higher RUE which resulted from a more advantageous canopy architecture for light capture and more advantageous leaf photosynthetic properties. We conclude that less shading under diffuse glass cover not only stimulates plant growth but also improves plant ornamental quality (i.e. compactness).

INTRODUCTION

Light is the most important factor in determining plant growth. However, when excessive light energy is absorbed by the light harvesting antennae at a rate which surpasses the capacity for photochemical and non-photochemical energy dissipation, this may lead to photo-damage (Long *et al*., 1994). In the long term, this may result in discolouring of leaves or even necrosis in the most extreme case. Light damage occurs mostly as a result of prolonged exposure to excessive peaks in light intensity (Asada, 1999; Niyogi, 1999; Kasahara *et al*., 2002). Consequently, growers apply shading during summer cultivation of many greenhouse crops by closing a screen or having a white wash on the greenhouse cover in order to prevent damage under conditions of high light.

In greenhouses, the distribution of light over the different leaves of a canopy shows large variations. The greenhouse construction, equipment and overstory leaves cast shade, resulting in shade-spots and lightflecks, of which the position continuously changes depending on solar angle. Light damage may occur particularly in those lightflecks (Way and Pearcy, 2012). It has been shown that diffuse light is more homogeneously distributed over the crop canopy than direct light (Gu *et al*., 2002; Farquhar and Roderick, 2003; Mercado *et al.*, 2009; Li *et al*., 2014). Recently diffuse glass has become available that increases the diffuseness of light without affecting light transmission in the greenhouse (Hemming *et al*., 2008; Baeza and López, 2012). Li *et al*. (2014) observed that diffuse glass cover result in a more homogeneous light distribution not only in the vertical plane, but also in the horizontal plane within a tomato canopy, which compared with clear glass cover, lead to 10 % higher yield (Dueck *et al*., 2012). Additionally, diffuse light also results in lower leaf or flower temperature and less photoinhibition (Kempkes *et al*., 2011; Urban *et al*., 2012; Li *et al*., 2014), because of less severe local peaks in light intensity. Considering these properties, we speculate that diffuse glass cover may help stimulate plant growth at higher daily light integral [DLI, mol m^{-2} d^{-1} photosynthetically active radiation (PAR)] without leading to light damage.

Increasing DLI increases plant growth and development (Marcelis *et al*., 2006; Poorter *et al*., 2013). Fausey *et al*. (2005) reported a linear relationship between the amount of light (5-20 mol m^{-2} d^{-1} PAR) and shoot dry mass in a number of greenhouse grown herbaceous perennial species. Similar findings were reported by Faust *et al*. (2005) in a number of bedding plants. Pot-plants are often grown under very low DLI conditions in commercial greenhouse production. For instance in the Netherlands growers limit the DLI in many pot-

plants to 5-8 mol m^{-2} d^{-1}. However, it is clear that low DLI can carry a production penalty (Scuderi *et al.*, 2012; Scuderi *et al.*, 2013), since potential crop growth is positively related to the amount of light that can be captured. Pot-plants could grow faster when less shading was applied in combination with moderately high air humidity (Kromdijk *et al.*, 2012). Furthermore, less shading could increase plant compactness as indicated by a higher ratio of aboveground dry mass to plant height with increasing DLI in a number of bedding plants (Faust *et al.*, 2005). Therefore, increasing DLI can improve not only plant growth but also plant ornamental quality.

Yield component analysis has been valuable in many crop research programs (Jolliffe *et al.*, 1990; Plénet *et al.*, 2000; Higashide and Heuvelink, 2009). Lawlor (1995) suggested that plant growth and production is determined by component processes integrated over the canopy, e.g. dry mass production per unit intercepted photosynthetic photon flux density (PPFD) (RUE), leaf photosynthesis, canopy architecture, biomass allocation (e.g. shoot/root ratio). These components vary across species and environments (Sinclair and Muchow, 1999; Sultan, 2000; Falster and Westoby, 2003; Barthelemy and Caraglio, 2007; Sarlikioti *et al.*, 2011b), resulting in differences in crop production.

The objective of this study was to investigate the effect of diffuse glass cover and high DLI under diffuse glass cover on the growth in pot-plants. It aims to systematically identify and quantify the yield components which are influenced by diffuse glass cover and high DLI. Our hypothesis is that high daily integral of diffuse light not only stimulates plant growth but also improves plant ornamental quality (i.e. more compact plants without light damage). To test this hypotheses, a study was conducted under diffuse glass cover with two levels of DLI. Two *Anthurium* cultivars (Pink Champion and Royal Champion) were used in this study; these two cultivars differed in light sensitivity based on grower's experience that 'Royal Champion' is more sensitive to light than 'Pink Champion'.

MATERIALS AND METHODS

Plant material and growth conditions

Two *Anthurium andreanum* cultivars (Pink Champion and Royal Champion, Anthura, Bleiswijk, The Netherlands) were grown in three Venlo-type glasshouse compartments of 144 m^2 (15 m × 9.6 m) with a gutter height of 5.5 m at Wageningen UR Greenhouse horticulture in Bleiswijk (The Netherlands, 52° N, 4.5° E). The three compartments were covered by glass (Guardian Agro, Dudelange, Luxembourg) with 0 % haze (clear glass; one compartment) and

71 % haze (diffuse glass; two compartments). Haze is defined as the percentage of transmitted light that is scattered such that it deviates more than 1.5° from the direction of the incident beam. The hemispherical transmission of PPFD of the glass was 84 % for both glass types. The haze factor and hemispherical transmission of the glass was measured in an optical sphere according to ASTM international (2007). The spectral properties of the two glass types are presented in Supplementary Fig. S1 and Table S1. The DLI was limited to 7.5 mol m^{-2} d^{-1} in the clear glass treatment, and to 7.5 and 10 mol m^{-2} d^{-1} in the two diffuse glass treatments. The DLI treatment of 10 mol m^{-2} d^{-1} under clear glass cover was not included in this experiment, because a similar treatment in an earlier experiment resulted in leaf damage (Van Noort *et al*., 2011). The DLI treatments were realized by controlling a white sunscreen (XLS 16 F Revolux, transmission of 37 % and haze factor of 10 %, LudvigSvensson, Kinna, Sweden) and blackout screen (XLS obscural Revolux A/B + B/B, LudvigSvensson, Kinna, Sweden) which were placed in the top of the greenhouse (below gutter height). The white sunscreen was fully closed in the low DLI compartments (7.5 mol m^{-2} d^{-1}) and 50 % closed in the high DLI compartment (10 mol m^{-2} d^{-1}) when global outside radiation reached 250 W m^{-2}; it was fully closed in the high DLI compartment when global outside radiation reached 450 W m^{-2}. The blackout screen was closed when DLI reached the DLI limitation point in the afternoon in all compartments. Three quantum sensors (LI-190, LI-COR, USA) were installed in each of the greenhouse compartments to measure incident PPFD at 5 min intervals. Fogging systems were used to maintain high air humidity (80 %). A standard horticultural computer (Hogendoorn-Economic, Hogendoorn, Vlaardingen, The Netherlands) was used to control the greenhouse temperature, air humidity, CO_2 concentration, as well as opening and closing of the screens.

Plants, propagated *in vitro*, were raised in a greenhouse by a nursery. When the first flowers had appeared, the plants were repotted and moved to the experimental greenhouses on 6 Apr 2012. The experiment ended on 28 Aug 2012. Plants were grown on potting soil (30 % fine peat + 10 % coarse peat + 43 % coco peat + 10 % bark + 7 % perlite) in black plastic pots (12 cm diameter and 11 cm height) on cultivation tables (4 m by 1.8 m) with an automatic ebb/flood irrigation system. In each compartment, six cultivation tables were used and each table was equally divided into two parts for the cultivation of two cultivars. The outer two rows of each plot were considered as border plants. The starting plant density was 30 plants m^{-2}; this was reduced to 20 plants m^{-2} three weeks after the start of the experiment. After each destructive harvest, plants were moved to maintain the same plant density. During the growing season, average daily outside global radiation was 16 MJ m^{-2} d^{-1}. Inside the

greenhouse the average day/night temperature was 25/21°C; relative air humidity was 75/78 %; average daytime CO_2 concentration was 754 µmol mol^{-1}; average realized DLI were 7.2 mol m^{-2} d^{-1} in the compartment of clear glass + low DLI, 7.5 mol m^{-2} d^{-1} in the compartment of diffuse glass + low DLI, and 8.9 mol m^{-2} d^{-1} in the compartment of diffuse glass + high DLI. An overview of DLI during the growing season in the three compartments is provided in Supplementary Fig. S2.

Plant measurements

Plants were destructively measured at 4, 10, 16, 18 and 21 weeks after start of the experiment (at 18 weeks one extra measurement was added in order to improve accuracy of the results in the later growth stages). For each destructive measurement, two plants per cultivar were randomly selected from each cultivation table, which resulted in 12 replicates. Fresh and dry weight of plant organs (leaves, flowers, stems, petioles and roots) were determined. Plant organs were dried for at least 48 h at 80 °C in a ventilated oven. Leaf area was measured with a leaf area meter (LI-3100C, Li-Cor inc., Lincoln, USA). Specific leaf area (SLA) was calculated by dividing the leaf area by leaf dry weight. Number of flowers, leaves and stems, as well as plant height were determined. During the fourth destructive harvest, in all the harvested plants, three to four images were taken of the main stem from the side view of each plant, i.e. after all other leaves were removed (Supplementary Fig. S3), which showed the leaf angle information of a single leaf. These images were used to quantify the leaf angle which was determined as the angle of the leaf surface with the horizontal plane (0°) (Supplementary Fig. S3).

Canopy PPFD interception and light extinction coefficient

Canopy PPFD interception was measured on four overcast days (11 May, 15 Jun, 17 Jul and 24 Aug) and three clear days (23 May, 20 Jun and 25 Jul). These days were close to the period when destructive measurements were taken in order to correlate light interception data with leaf area index (LAI). The measurements were done with a line light probe, in relation to a reference sensor just above the crop (Sunscan, Delta-T, Cambridge, UK). Six measurements were done above as well as below the canopy for each cultivar on each cultivation table. Measurements at the top of the canopy were taken just above the highest leaf, while the bottom measurements were done at pot height.

The light extinction coefficient (K) was calculated according to Lambert-Beer law (Monsi and Saeki, 2005) by combining the PPFD interception measurements on cloudy days and the measured LAI

$$I(L) \ = \ I_0 \, e^{-KL} \tag{1}$$

where I_o is incident PPFD at the top of the canopy (μmol m^{-2} s^{-1}), L is LAI (m^2 leaf m^{-2} ground), $I(L)$ is PPFD (μmol m^{-2} s^{-1}) at L. Differences between treatments and cultivars in K were considered significant when K of one treatment/cultivar was out of the range of 95 % confidence interval of the opposite treatment/cultivar.

Canopy radiation use efficiency (RUE)

Canopy RUE was defined as the ratio between the accumulated total dry mass (TDM) and the sum of intercepted PPFD during the experimental period, which was estimated by the slope of the linear relationship between the accumulated TDM and the sum of intercepted PPFD. For calculating the sum of intercepted PPFD, the time course of fraction of intercepted PPFD [$I(L)/I_o$] was estimated from the four periodic canopy PPFD interception measurements on cloudy days [eqn (2)]. These data can represent $I(L)/I_o$ over the growing season due to $I(L)/I_o$ measured on clear days was similar as on cloudy days (data not shown). In each treatment $I(L)/I_o$ could be well fitted by a negative exponential curve with number of days after start of the experiment and reaching a plateau in the end (r^2 = 0.99 for all treatments)

$$I(L)/I_0 \ = 1 - e^{-ad} \tag{2}$$

where a is saturating coefficient, d is number of days after start of the experiment.

Daily canopy intercepted PPFD was calculated as the product of the interpolated daily $I(L)/I_o$ multiplied by the measured DLI. Integrating the daily canopy intercepted PPFD during the designated growing period yields the sum of intercepted PPFD.

Net leaf photosynthesis

At 17 weeks after start of the experiment, net leaf photosynthesis rates were measured with a portable gas exchange device equipped with a leaf chamber fluorometer (LI-6400; LI-COR, Lincoln, USA). One fully expanded leaf of each cultivar was randomly selected from each cultivation table in each treatment for measuring gas exchange at 500 followed by 100 μmol m^{-2} s^{-1} PPFD, the measurements were taken when the photosynthesis rate reached steady state (after about 10 min). The light source (10 % blue and 90 % red) only illuminated the adaxial side of the leaf. All measurements were carried out between 9:00 and 16:00. In the
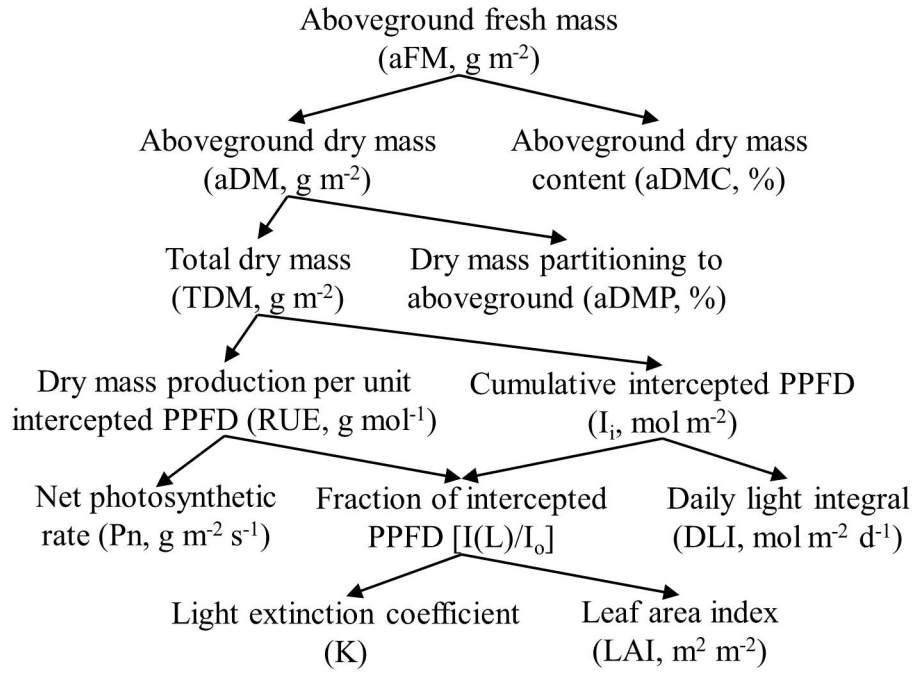
measurement chamber, vapour pressure deficit (VPD) was maintained in the range of 0.5-1 kPa, reference CO_2 concentration was set at 800 μmol mol^{-1}, leaf temperature at 27 °C, these parameters were close to that in the greenhouse compartments.

Plants ornamental quality determination

Plant compactness was determined based on the above ground fresh mass/plant height ratio at each destructive harvest. At 18 weeks after start of the experiment, two plants of each cultivar on each cultivation table were randomly selected from each greenhouse compartment (12 plants) to determine the colour of flowers and leaves, and projected area of flowers and leaves. Top-view image of each plant was recorded with an image acquisition system consisting of a colour CCD-Camera (Hitachi HV C-20). Based on the images, the CIE-L*a*b* colour space was used to quantify the colour of flowers and leaves (Minolta, 1994); the CIE-L*a*b* space is defined as a sphere, it is typified by the lightness parameter L* (lightness: black–white), and the colour co-ordinates a* (-a = green, +a = red) and b* (-b = blue, +b = yellow). Furthermore, the projected area of flowers and leaves were also quantified from these images.

Calculations and statistical analysis

Treatment or cultivar effects on yield of a plant can be analysed by breaking down the effect in different underlying components (Fig. 1). For example, higher aboveground fresh mass (aFM) can be caused by higher aboveground dry mass (aDM) and/or by lower aboveground dry mass content (aDMC, i.e. aboveground dry mass/fresh mass). An increase in aboveground dry mass (aDM) can be explained by an increase in dry mass partitioning to aboveground (aDMP, i.e. aboveground dry mass/total dry mass) and/or an increase in total dry mass (TDM). The latter results from a higher dry mass production per unit intercepted PPFD (RUE) and/or higher cumulative intercepted PPFD (I_i). An increase in dry mass production per unit intercepted PPFD (RUE) is determined by an increase in net photosynthetic rate (Pn) and/or by a decrease in fraction of intercepted PPFD [$I(L)/I_o$]. Higher cumulative intercepted PPFD (I_i) is directly linked with higher daily light integral (DLI) and/or higher fraction of intercepted PPFD [$I(L)/I_o$] which results from higher light extinction coefficient (K) and/or higher leaf area index (LAI). Aboveground fresh mass (aFM), aboveground dry mass (aDM) and total dry mass (TDM) represent the accumulated biomass during the growing season.

Aboveground fresh mass
(aFM, g m^{-2})

Aboveground dry mass
(aDM, g m^{-2})

Aboveground dry mass
content (aDMC, %)

Total dry mass
(TDM, g m^{-2})

Dry mass partitioning to
aboveground (aDMP, %)

Dry mass production per unit
intercepted PPFD (RUE, g mol^{-1})

Cumulative intercepted PPFD
(I$_i$, mol m^{-2})

Net photosynthetic
rate (Pn, g m^{-2} s^{-1})

Fraction of intercepted
PPFD [I(L)/I$_o$]

Daily light integral
(DLI, mol m^{-2} d^{-1})

Light extinction coefficient
(K)

Leaf area index
(LAI, m^2 m^{-2})

Fig. 1. General scheme with a top-down analysis of aboveground fresh mass (aFM) into component variables. Brackets following each component indicates abbreviations and units. Scheme is a modification of Fig. 2 presented by Higashide and Heuvelink (2009).

Parameters determined from destructive harvests (aFM; aDM; aDMC; TDM; aDMP; LAI; SLA; plant height; number of flowers, leaves and stems; plant compactness) were presented as the average of the last two measurements (18 and 21 weeks after start of the experiment) in order to get more reliable information (number of replicates were doubled). The two destructive harvests were considered as two blocks in statistical analysis. Treatment and cultivar effects on measured parameters were evaluated by analysis of variance (ANOVA). Differences between treatments and cultivars in RUE were tested using a multiple linear regression model. Assuming replications in the same greenhouse compartment as being independent. *P*–values smaller than 0.05 were regarded as significantly different.

RESULTS

Light distribution and interception

In the greenhouse with clear glass (0 % haze) incident PPFD at a given spot just above the plants fluctuated much stronger than in the greenhouse with diffuse glass (71 % haze) on clear days (Fig. 2). In all treatments, incident PPFD inside the greenhouse was kept below 500 μmol m^{-2} s^{-1} whereas outside PPFD reached 1800 μmol m^{-2} s^{-1} in the middle of a clear day.

On overcast days, incident PPFD at a given spot showed similar dynamic patterns in the three compartments (data not shown).
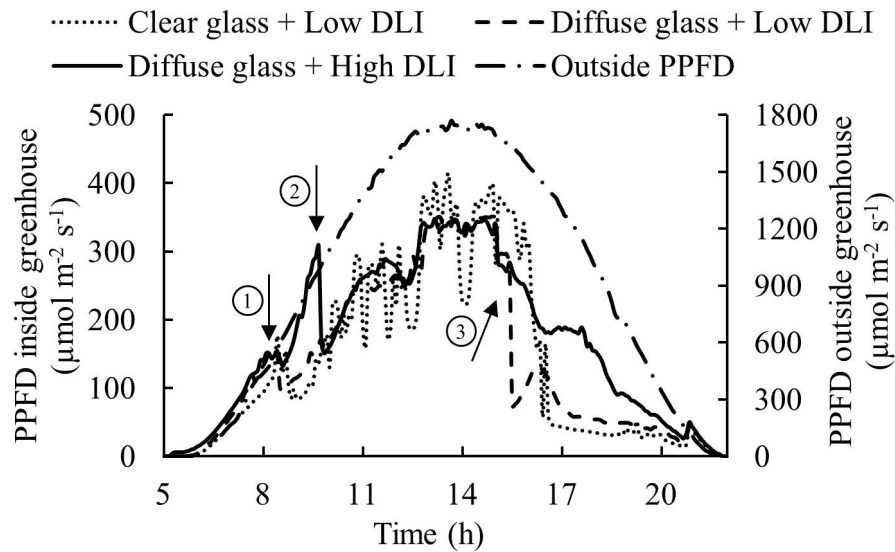
Fig. 2. Photosynthetic photon flux density (PPFD) inside and outside of the greenhouse compartments on a typical clear day (25 Jul 2012) as measured by a point sensor. Clear glass + Low DLI (daily light integral, mol m^{-2} d^{-1} PAR) represents 0 % haze with DLI of 7.2 mol m^{-2} d^{-1}; Diffuse glass + Low DLI represents 71 % haze with DLI of 7.5 mol m^{-2} d^{-1}; Diffuse glass + High DLI represents 71 % haze with DLI of 8.9 mol m^{-2} d^{-1}. ①indicates the white sunscreen was fully closed in the low DLI and 50 % closed in the high DLI treatment; ② indicates the white sunscreen was fully closed in the high DLI treatment; ③ indicates the DLI reached the limitation point and the blackout screen was closed.

Both on clear as well as cloudy days treatments did not affect canopy PPFD interception, which was reflected by similar relative PPFD at the same LAI on clear days (Supplementary Fig. S4) and similar light extinction coefficient on cloudy days (supplementary Table S2). PPFD penetrated deeper into the canopy in 'Pink Champion' than in 'Royal Champion' (Fig. 3), which was reflected by a lower light extinction coefficient (K) in 'Pink Champion' [K = 1.01; 95 % confidence interval (0.99-1.05)] than in 'Royal Champion' [K = 1.08; 95 % confidence interval (1.03-1.14)].
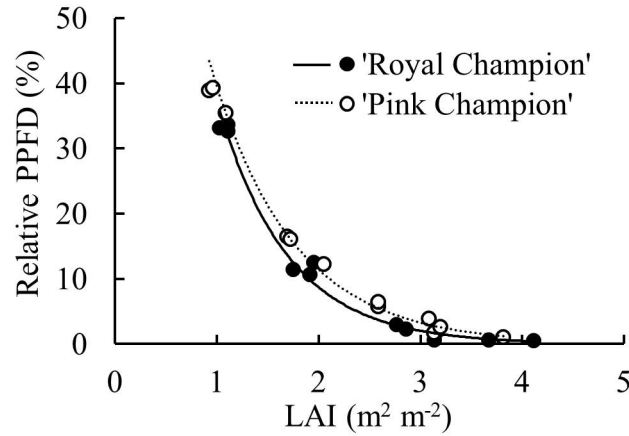
Fig. 3. Relationship between relative PPFD (relative to the top of canopy) and LAI in 'Royal Champion' and 'Pink Champion' on cloudy days. Data presented in each cultivar were measurements from the three compartments. Each symbol represents the average of six replicates measured in one day. Lines are fitted curves based on the Lambert-Beer law [eqn (1)].

Plant measurements

At low DLI, diffuse glass significantly increased the number of leaves and stems in 'Royal Champion' compared with clear glass treatment; while these effects did not occur in 'Pink Champion' (Table 1). Under diffuse glass cover, high DLI significantly increased the number of leaves, flowers and stems in both cultivars compared with the low DLI (Table 1). Furthermore, high DLI increased plant height in 'Pink Champion'; while it decreased plant height in 'Royal Champion' (Table 1).

In terms of plant ornamental quality (Table 2), high DLI resulted in more compact plants in both cultivars compared with low DLI. Furthermore, high DLI resulted in higher projected leaf area and fraction projected area flowers/area leaves in 'Pink Champion'; while in 'Royal Champion', only higher projected leaf area was observed. The colour of leaves and flowers were not affected by the diffuse glass cover as well as high DLI.

SLA and leaf angle were neither influenced by diffuse glass cover nor high DLI. In all treatments, SLA in 'Pink Champion' was lower than in 'Royal Champion' (Supplementary Fig. S5). Moreover, 'Pink Champion' had positive leaf angle compared with the horizontal plane (0°), while 'Royal Champion' had negative leaf angle (Supplementary Fig. S6).

Net leaf photosynthesis rates were not affected by the treatments when measured at the same conditions in leaf measurement chamber. 'Pink Champion' had significantly higher net photosynthesis rates than 'Royal Champion' at 500 µmol m^{-2} s^{-1} PPFD in all treatments; while this effect did not occur at 100 µmol m^{-2} s^{-1} PPFD (Supplementary Fig. S7).

Table 1. The effect of diffuse glass cover and daily light integral (DLI, mol m^{-2} d^{-1} PAR) on plant growth parameters in two *Anthurium* cultivars (Royal Champion and Pink Champion) (n = 24). Data represent the average of the last two harvests (18 and 21 weeks after start of the experiment).

Treatment	Number of leaves (no. plant^{-1})	Number of flowers (no. plant^{-1})	Number of stems (no. plant^{-1})	Plant height (cm)
'Royal Champion'				
[a] Clear glass + Low DLI	29.0 a	9.0 a	2.7 a	29.6 b
[b] Diffuse glass + Low DLI	36.0 b	9.3 a	3.1 b	28.2 b
[c] Diffuse glass + High DLI	41.1 c	10.4 b	3.6 c	25.8 a
P-value	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
'Pink Champion'				
Clear glass + Low DLI	35.6 a	8.7 a	3.9 a	33.7 ab
Diffuse glass + Low DLI	39.0 a	9.3 a	4.0 a	32.2 a
Diffuse glass + High DLI	44.5 b	11.6 b	4.5 b	35.0 b
P-value	*< 0.001*	*< 0.001*	*0.04*	*0.002*

[a] 0 % haze with DLI of 7.2 mol m^{-2} d^{-1}; [b] 71 % haze with DLI of 7.5 mol m^{-2} d^{-1} ; [c] 71 % haze with DLI of 8.9 mol m^{-2} d^{-1}. Means of each cultivar followed by different letters within one column differ significantly.

Yield component analysis

Under low DLI, diffuse glass significantly increased aboveground fresh mass (aFM), aboveground dry mass (aDM), total dry mass (TDM), dry mass production per unit intercepted PPFD (RUE) and leaf area index (LAI) in 'Royal Champion' compared with clear glass treatment. However, these effects did not occur in 'Pink Champion' except aboveground dry mass content (aDMC) which was increased by 5 % in the diffuse glass treatment (Fig. 4). Absolute values of all component variables are presented in the supplementary Table S3.

Under diffuse glass cover, high DLI significantly increased aboveground fresh mass (aFM), aboveground dry mass (aDM), total dry mass (TDM), and leaf area index (LAI) in both cultivars compared with low DLI (Fig. 4). Additionally, high DLI decreased aboveground dry mass content (aDMC) by 3 % and increased aboveground dry mass partitioning (aDMP) by 2 % in 'Pink Champion'.
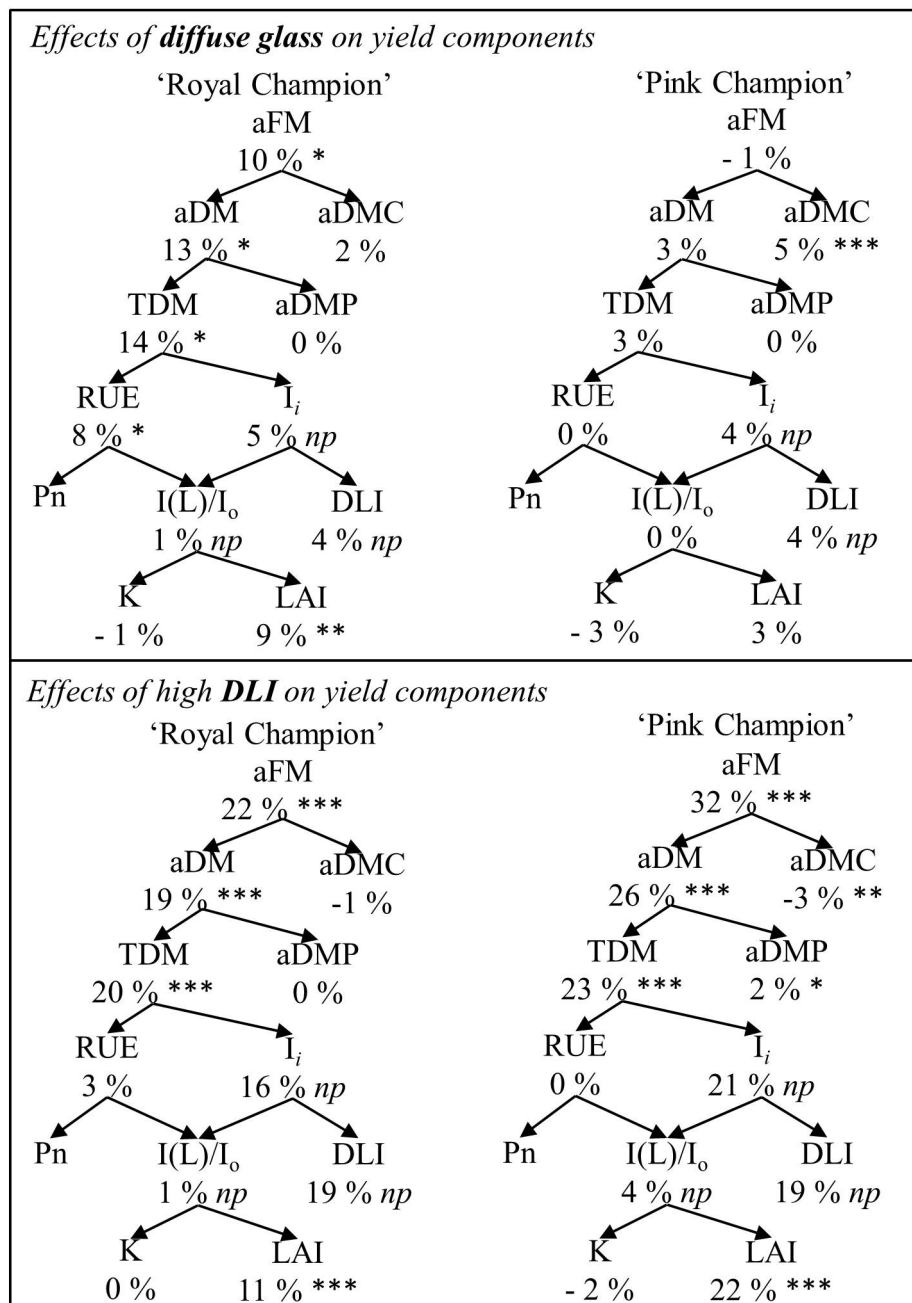
In all treatments, 'Pink Champion' had higher aboveground fresh mass (aFM), aboveground dry mass (aDM), total dry mass (TDM) and dry mass production per unit intercepted PPFD (RUE), and lower aboveground dry mass content (aDMC), aboveground dry mass partitioning (aDMP) and light extinction coefficient (K) in comparison with 'Royal Champion' (Fig. 5).

Table 2. The effect of diffuse glass cover and daily light integral (DLI, mol m^{-2} d^{-1} PAR) on colour values of flowers and leaves in the CIE-L*a*b* colour space and the projected area of leaves and flowers (n = 12), as well as the plant compactness (aboveground fresh mass/plant height ratio) (n = 24). All measurements were done at 18 weeks after start of the experiment except compactness determination which was done based on the last two destructive harvest measurements (18 and 21 weeks after start of the experiment).

Treatment	[a] L* (lightness)		[b] a* (green-red)		[c] b* (blue-yellow)		Projected leaf area (cm^2)	Fraction projected area flowers/area leaves	Compactness (g cm^{-1})
	Flower	Leaf	Flower	Leaf	Flower	Leaf			
'Royal Champion'									
[d] Clear glass + Low DLI	35.7	30.2	20.9	- 5.3	15.7	4.3	694 a	0.26	5.7 a
[e] Diffuse glass + Low DLI	35.1	31.4	19.8	- 4.8	15.4	3.8	710 ab	0.28	6.4 b
[f] Diffuse glass + High DLI	36.1	31.2	19.9	- 4.6	16.0	4.6	766 b	0.28	8.0 c
P-value	*0.38*	*0.08*	*0.26*	*0.07*	*0.59*	*0.26*	*0.04*	*0.68*	*< 0.001*
'Pink Champion'									
Clear glass + Low DLI	50.6	28.3	30.4	- 5.6	17.1	5.8	729 a	0.13 a	5.8 a
Diffuse glass + Low DLI	51.2	28.6	30.2	- 4.9	17.7	5.6	767 a	0.14 ab	6.0 a
Diffuse glass + High DLI	52.3	28.0	30.6	- 5.1	17.8	6.3	828 b	0.16 b	7.3 b
P-value	*0.20*	*0.29*	*0.89*	*0.09*	*0.14*	*0.11*	*0.003*	*0.03*	*< 0.001*

[a] Lightness parameter L* [lightness: black-white]; [b] colour co-ordinates a* (-a = green, + a = red); [c] colour co-ordinates b* (- b = blue, + b = yellow); [d] 0 % haze with DLI of 7.2 mol m^{-2} d^{-1}; [e] 71 % haze with DLI of 7.5 mol m^{-2} d^{-1}; [f] 71 % haze with DLI of 8.9 mol m^{-2} d^{-1}. Means of each cultivar followed by different letters within one column differ significantly.

Effects of **diffuse glass** on yield components

'Royal Champion'
aFM
10 % *
aDM   aDMC
13 % *   2 %
TDM   aDMP
14 % *   0 %
RUE I_i
8 % *   5 % *np*
Pn   I(L)/I_o DLI
         1 % *np*   4 % *np*
      K   LAI
    - 1 %   9 % **

'Pink Champion'
aFM
- 1 %
aDM   aDMC
3 %   5 % ***
TDM   aDMP
3 %   0 %
RUE I_i
0 %   4 % *np*
Pn   I(L)/I_o DLI
          0 %   4 % *np*
      K   LAI
    - 3 %   3 %

Effects of high **DLI** on yield components

'Royal Champion'
aFM
22 % ***
aDM   aDMC
19 % ***   -1 %
TDM   aDMP
20 % ***   0 %
RUE I_i
3 %   16 % *np*
Pn   I(L)/I_o DLI
          1 % *np*   19 % *np*
      K   LAI
     0 %   11 % ***

'Pink Champion'
aFM
32 % ***
aDM   aDMC
26 % ***   -3 % **
TDM   aDMP
23 % ***   2 % *
RUE I_i
0 %   21 % *np*
Pn   I(L)/I_o DLI
          4 % *np*   19 % *np*
      K   LAI
    - 2 %   22 % ***

Fig. 4. Effects of diffuse glass cover [diffuse glass VS. clear glass, both at low DLI (daily light integral, mol m^{-2} d^{-1} PAR)] or high DLI (low DLI VS. high DLI, both under diffuse glass cover) on the yield components in 'Royal Champion' and 'Pink Champion'. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. *np* indicates statistical analysis was not possible. Relative difference in net photosynthetic rate (Pn) was not determined. For explanation of abbreviations of the variables see Fig. 1.
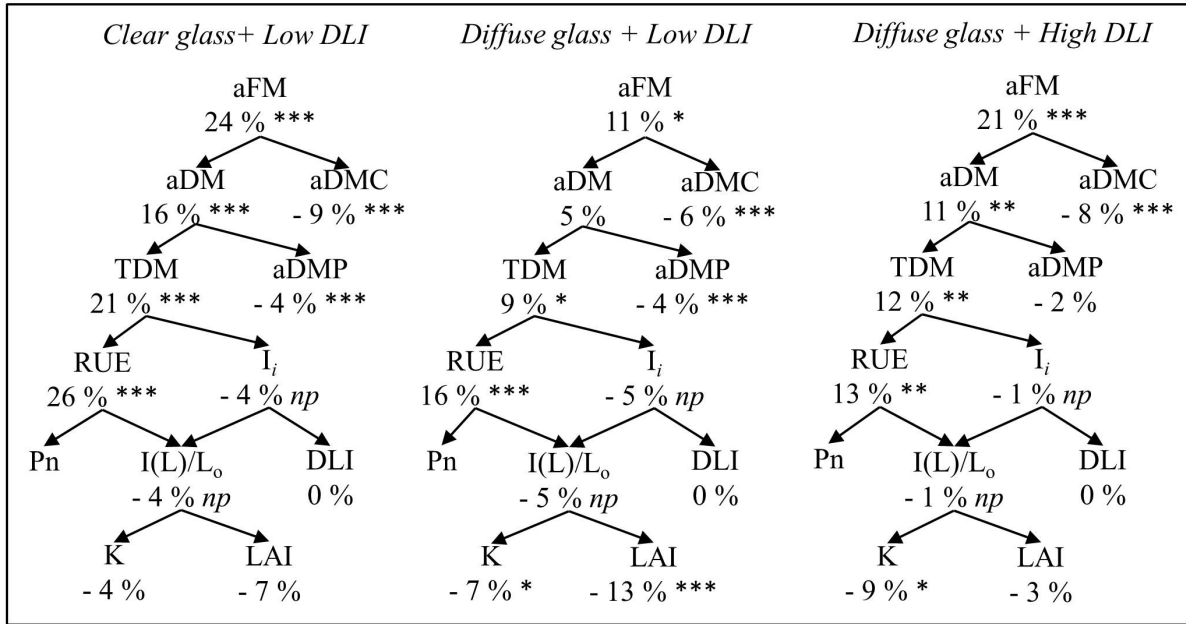
Fig. 5. Cultivar effects on the yield components in 'Pink Champion' and 'Royal Champion'. Expressed relative to 'Royal Champion'. Clear glass + Low DLI (daily light integral, mol m^{-2} d^{-1} PAR) represents 0 % haze with DLI of 7.2 mol m^{-2} d^{-1}; Diffuse glass + Low DLI represents 71 % haze with DLI of 7.5 mol m^{-2} d^{-1}; Diffuse glass + High DLI represents 71 % haze with DLI of 8.9 mol m^{-2} d^{-1}. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. *np* indicates statistical analysis was not possible. Relative difference in net photosynthetic rate (Pn) was not determined. For explanation of abbreviations of the variables see Fig. 1.

DISCUSSION

Diffuse glass cover improves temporal rather than vertical light distribution

Scattering the direct component of solar light in the greenhouse by diffuse glass cover is an interesting way to improve plant growth and production of greenhouse crops (Hemming *et al.*, 2007; García Victoria *et al.*, 2011; Dueck *et al.*, 2012; Li *et al.*, 2014). Diffuse glass cover strongly reduced short term fluctuations in PPFD at a given spot in the greenhouse on clear days (Fig. 2), because the diffuse glass minimized effects of local shade by construction parts, equipment and overstory leaves. In terms of spatial light distribution, we observed a similar relationship of canopy PPFD interception in response to LAI between the clear and diffuse glass treatments on clear days (Supplementary Fig. S4), which suggests diffuse glass treatment had no effect on vertical light distribution in *Anthurium*. This contradicts with the finding in cucumber (Hemming *et al.*, 2007) and tomato (Li *et al.*, 2014) in which diffuse glass cover resulted in deeper PPFD penetration within the canopies on clear days. This phenomenon could be explained by the characteristics of canopy architecture which usually

plays a pivotal role for canopy PPFD interception (Falster and Westoby, 2003; Valladares and Niinemets, 2007; Sarlikioti *et al*., 2011a). *Anthurium* pot-plants are characterised by short and compact canopies with relatively large leaves (Supplementary Fig. S8), resulting in substantial leaf overlap and self-shading especially when LAI is high. Self-shading decreases the net amount of leaf area exposed to light and this leads to poor light distribution even under diffuse light condition (Falster and Westoby, 2003). Therefore, this type of canopy structure is less responsive for scattering the light, and limits the potential effect of diffuse glass cover on canopy light distribution. Furthermore, shading screens were applied in all the greenhouse compartments, which already transformed a portion of direct PPFD into diffuse (10 %). Therefore, we conclude that diffuse glass cover had negligible effect on vertical PPFD distribution within the canopy of *Anthurium* pot-plants mainly because of the short and compact canopy structures, as well as the experimental management practice.

Effect of diffuse glass cover on plant growth is cultivar specific

Total dry mass (TDM) was 14 % higher in the diffuse glass than in the clear glass treatment in 'Royal Champion', which lead to higher aboveground dry mass (aDM). Consequently the aboveground fresh mass (aFM) was also higher as the aboveground dry mass content (aDMC) was not significantly affected by the treatments (Fig. 4). The increased biomass production was attributed to a higher (5 %) cumulative intercepted PPFD (I_i) and a higher (8 %) dry mass production per unit intercepted PPFD (RUE) (Fig. 4). The former resulted mainly from an unexpected higher (4 %) average DLI in the diffuse glass treatment, which may have been caused by a higher (about 3 %) light transmission during condensation under diffuse glass cover (unpublished data) or the side-effects of neighbouring greenhouses. In spite of 5 % more cumulative intercepted PPFD (I_i), diffuse glass still increased RUE by 8 %. This could be explained by leaf photosynthesis because the fraction of intercepted PPFD [$I(L)/I_o$] played a negligible role (1 % difference between treatments) (Fig. 4). However, under steady state measurement conditions, net leaf photosynthetic rates of the fully expanded leaves were similar in both treatments (Supplementary Fig. S7). Maybe there were treatment differences in dynamic leaf photosynthesis, as dynamic photosynthesis can be affected by the dynamic incident light distribution (Tinoco-Ojanguren and Pearcy,1992; Pearcy *et al*., 2004). This needs to be confirmed by further study. In 'Pink Champion', however, diffuse glass treatment had no effect on dry mass production per unit intercepted PPFD (RUE), as well as fresh and dry mass production (Fig. 4). The different response of these two cultivars to diffuse glass treatment needs to be further explored.

Increasing DLI under diffuse glass cover not only stimulates plant growth, but also improves plant ornamental quality

DLI is an important variable to determine plant growth and development (Warner and Erwin, 2003; Marcelis *et al*., 2006). The minimum DLI in this study was 7.2 mol m^{-2} d^{-1} which was already higher than the commercial practice (about 5 mol m^{-2} d^{-1}), this is because air humidity was kept at moderately high level (75-80 %) in all greenhouse compartments which could result in open stomata (Aphalo and Jarvis, 1991), thereby avoiding overheating leaves and maintaining photosynthesis. Furthermore, an increase of DLI may also lead to increase in temperature which could promote plant development and flowering (Oh *et al*., 2010); while in this study greenhouse temperature in all treatments were maintained equal, therefore, the DLI and temperature interaction effect need not to be considered. Additionally, this experiment did not include the treatment of high DLI (10 mol m^{-2} d^{-1}) under clear glass cover, because a similar treatment in an earlier experiment resulted in leaf damage (Van Noort *et al*., 2011).

Increasing DLI by 19 % resulted in 20-23 % higher total dry mass (TDM) (Comparing DLI under diffuse glass cover), this resulted in an increase in aboveground dry mass (aDM) and aboveground fresh mass (aFM) (Fig. 4), as well as more stems, leaves and flowers (Table 1). The effect of high DLI on plant growth mainly resulted from a higher cumulative intercepted PPFD (I_i), while dry mass production per unit intercepted PPFD (RUE) was not affected by high DLI treatment (Fig. 4). In line with the absence of an effect of DLI on RUE, there was also no effect of DLI on the net leaf photosynthesis rates when measured at the same PPFD (Supplementary Fig. S7). Furthermore, the fraction of intercepted PPFD [$I(L)/I_o$] was also not affected by the treatments, although LAI was increased by the high DLI. This is because LAI in both treatments was already high and therefore not limiting light interception.

Plant shape and colour are important parameters determining the quality of pot-plants. Increasing DLI from 7.5 to 8.9 mol m^{-2} d^{-1} resulted in more compact plants in both cultivars as indicated by a higher ratio of plant fresh mass to height (Table 2). In 'Royal Champion', the more compact plants as a result of a lower plant height and higher aboveground fresh mass (aFM). In 'Pink Champion', plants were also more compact even though plant height was higher at high DLI (Table 1). Increasing DLI under diffuse glass cover did not induce damage of flowers and leaves as indicated by the colour values (Table 2). When Van Noort *et al*. (2011) increased DLI under clear glass to a similar level as in our study, mild discolouring of leaves occurred in *Anthurium* 'Baby talk' and severe necrotic spots in leaves of *Guzmania* 'Hilda', even though plant growth was stimulated. The possible reason of this leaf damage

was that their treatments were conducted under a conventional glass type where the plants were exposed to a higher occurrence of more severe local peaks in light intensity.

Differences in plant growth between the two cultivars is mainly due to differences in dry mass production per unit intercepted PPFD (RUE)

In all the treatments, 'Pink Champion' had significantly higher aboveground fresh mass (aFM) than 'Royal Champion' (Fig. 5), which was explained by a lower aboveground dry mass content (aDMC, i.e. aboveground dry mass/fresh mass) and a higher aboveground dry mass (aDM). The latter resulted from higher total dry mass (TDM) which was determined by an increased dry mass production per unit intercepted PPFD (RUE).

RUE can be influenced by plant morphological and physiological properties (Sinclair and Muchow, 1999). 'Pink Champion' had leaves pointing upward (positive leaf angle; Supplementary Fig. S6), while leaves in 'Royal Champion' were pointing downward which might generate more self-shading compared with the upward leaves (Falster and Westoby, 2003). Furthermore, 'Royal Champion' was shorter than 'Pink Champion' (Table 1), this lead to more foliage packing, thereby, self-shading. The differences in leaf angle and plant height resulted in different spatial PPFD distribution within the canopy as indicated by a lower light extinction coefficient (K) in 'Pink Champion' than in 'Royal Champion' (Fig. 3), consequently, lower fraction of intercepted PPFD [$I(L)/I_o$] which contributed to higher RUE in 'Pink Champion'. Furthermore, these two cultivars are characterized by relatively large area of flowers which are standing above the canopy (Supplementary Fig. S8), this could exert a negative effect on RUE because flowers can intercept a portion of PPFD which cannot be utilized for photosynthesis. This negative effect is likely to be smaller in 'Pink Champion' due to less area of flowers (Table 2, Supplementary Fig. S8). Additionally, 'Pink Champion' had higher net leaf photosynthesis rates than 'Royal Champion', though this difference occurred only at high PPFD (Supplementary Fig. S7). All these together resulted in the higher RUE in 'Pink Champion'.

Implications for commercial production

Although shading is indispensable for production of pot-plants in order to avoid light damage, we have shown that allowing more light under diffuse glass cover (71 % haze) in combination with moderately high levels of air humidity (75-80 %) not only stimulates plant growth but also improves plant ornamental quality (i.e. more compact plants without light damage) in *Anthurium* pot-plants. The realized DLI in the reference treatment (clear glass, 7.2 mol m^{-2} d^{-1}) in the present study was already higher than the commercial practice (about 5 mol m^{-2} d^{-1}).

Growers regularly visited the experiment, and estimated that plants even in the reference treatment were growing 25 % faster than their plants. A further increase of DLI by 24 % (8.9 mol m^{-2} d^{-1}) under diffuse glass cover resulted in additional 27-37 % increase in dry mass production. Therefore, it is clear that our strategy can reduce the cultivation time to reach the marketable value.

CONCLUSIONS

Our conclusions are (1) The stimulating effect of diffuse glass cover on dry mass production per unit intercepted PPFD (RUE) in *Anthurium* pot-plants is cultivar specific. (2) Increasing DLI under diffuse glass cover not only stimulates biomass production, but also improves plant ornamental quality (i.e. more compact plants without light damage). (3) Differences in plant growth between the two *Anthurium* cultivars mainly resulted from difference in RUE.

ACKNOWLEDGEMENTS

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SUPPLEMENTARY DATA
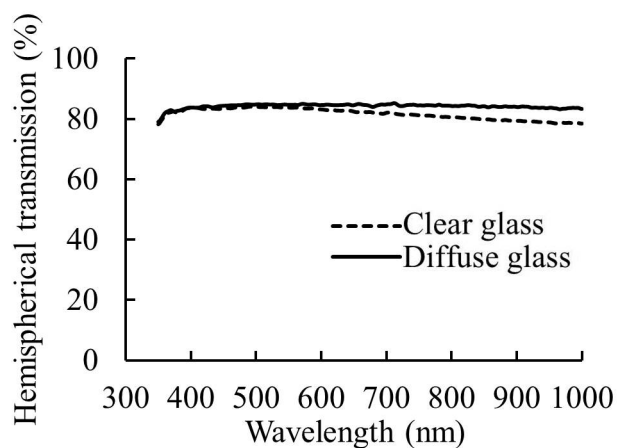
Fig. S1. The hemispherical transmission of PPFD for the clear glass (0 % haze) and diffuse glass (71 % haze).
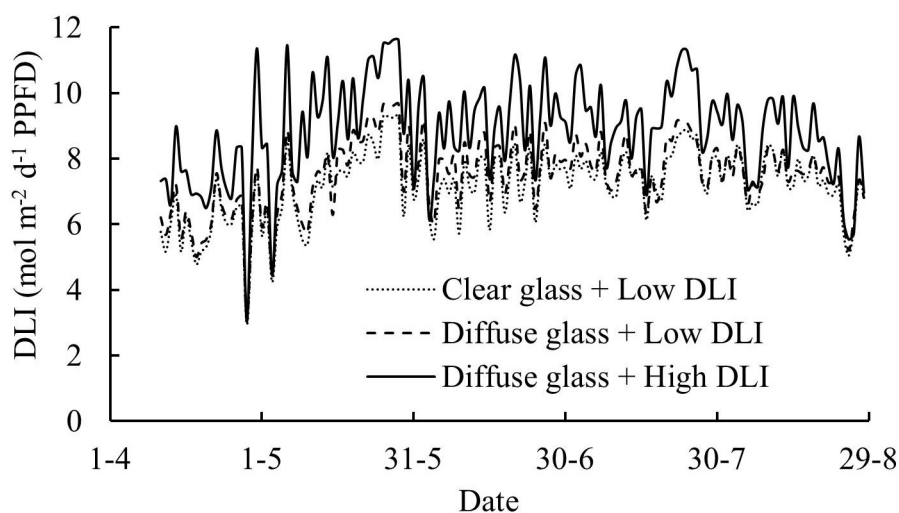
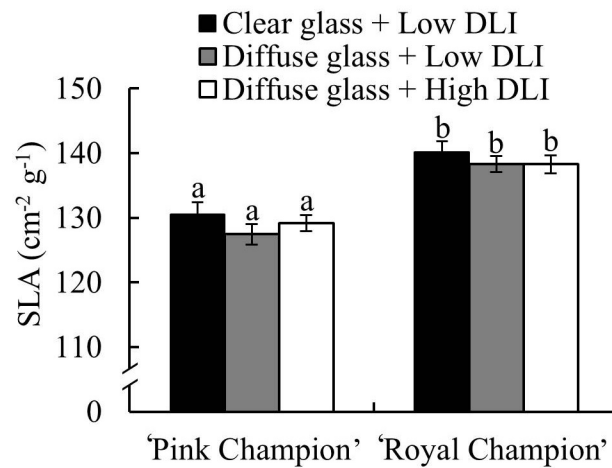**Fig. S2**. Daily light integral (DLI, mol m^{-2} d^{-1} PAR) during the growing season in the three treatments. Clear glass + Low DLI represents 0 % haze with DLI of 7.2 mol m^{-2} d^{-1}; Diffuse glass + Low DLI represents 71 % haze with DLI of 7.5 mol m^{-2} d^{-1}; Diffuse glass + High DLI represents 71 % haze with DLI of 8.9 mol m^{-2} d^{-1}.
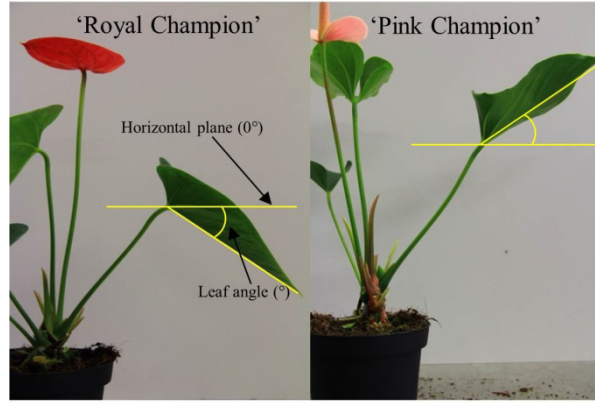
Fig. S3. Schematic diagram of leaf angle measurements. The measurements were taken at 18 weeks after start of the experiment.
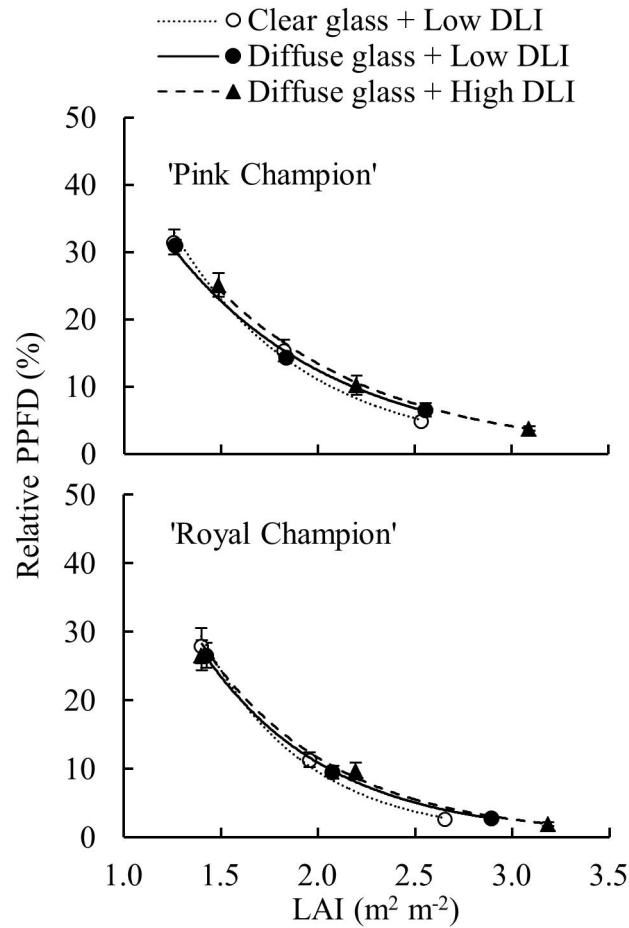
Fig. S4. The effect of diffuse glass cover and daily light integral (DLI, mol m^{-2} d^{-1} PAR) on the canopy PPFD interception in 'Pink Champion' and 'Royal Champion' on clear days. Clear glass + Low DLI represents 0 % haze with DLI of 7.2 mol m^{-2} d^{-1}; Diffuse glass + Low DLI represents 71 % haze with DLI of 7.5 mol m^{-2} d^{-1}; Diffuse glass + High DLI represents 71 % haze with DLI of 8.9 mol m^{-2} d^{-1}. Each symbol represents the average of six replicates measured in one day. Error bars show ± SE (n = 6). Lines are fitted curves based on the Lambert-Beer law [eqn (1)].

Fig. S5. The effect of diffuse glass cover and daily light integral (DLI, mol m^{-2} d^{-1} PAR) on specific leaf area (SLA). Clear glass + Low DLI represents 0 % haze with DLI of 7.2 mol m^{-2} d^{-1}; Diffuse glass + Low DLI represents 71 % haze with DLI of 7.5 mol m^{-2} d^{-1}; Diffuse glass + High DLI represents 71 % haze with DLI of 8.9 mol m^{-2} d^{-1}. Leaf samples were averaged 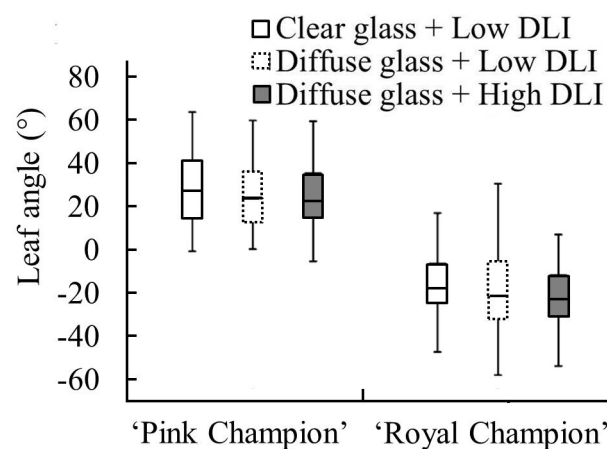over the plants and the last two destructive harvests (18 and 21 weeks after start of the experiment). Error bars represent ± SE (n = 24). Letters show statistical significant differences ($P < 0.05$).

Fig. S6. Box plots of the leaf angle in the three treatments [Clear glass + Low DLI (daily light integral, mol m^{-2} d^{-1} PAR) represents 0 % haze with DLI of 7.2 mol m^{-2} d^{-1}; Diffuse glass + Low DLI represents 71 % haze with DLI of 7.5 mol m^{-2} d^{-1}; Diffuse glass + High DLI represents 71 % haze with DLI of 8.9 mol m^{-2} d^{-1}]. Leaf angle was determined as the angle of the leaf surface in relation to the horizontal plane (0°) at the leaf connection point on the petiole. Data were collected from 40-45 leaves in each cultivar per treatment. Leaf angle was determined at 18 weeks after start of the experiment.
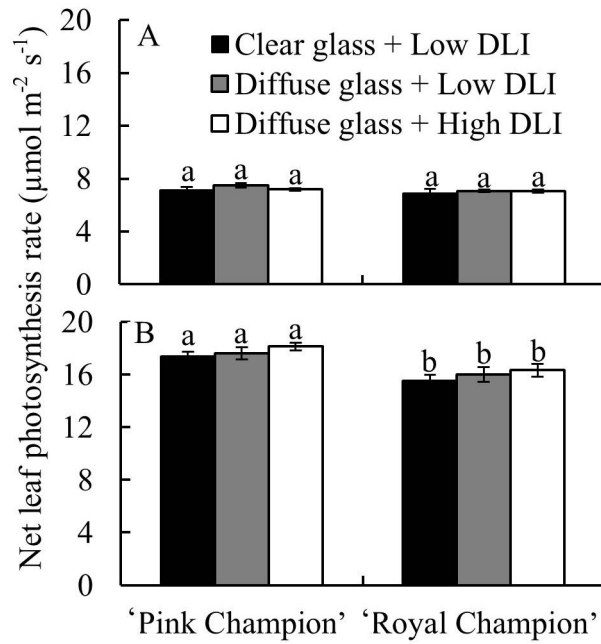
Fig. S7. Net leaf photosynthesis rates at 100 (A) and 500 (B) μmol m^{-2} s^{-1} PPFD in 'Pink Champion' and 'Royal Champion'. Clear glass + Low DLI (daily light integral, mol m^{-2} d^{-1} PAR) represents 0 % haze with DLI of 7.2 mol m^{-2} d^{-1}; Diffuse glass + Low DLI represents 71 % haze with DLI of 7.5 mol m^{-2} d^{-1}; Diffuse glass + High DLI represents 71 % haze with DLI of 8.9 mol m^{-2} d^{-1}. During the measurements, vapour pressure deficit (VPD) was maintained in the range of 0.5-1 kPa, leaf temperature and CO_2 concentration in the measurement chamber were maintained at 27 °C and 800 μmol mol^{-1}, respectively. Error bars show $\pm$ SE (n = 6).

Fig. S8. Top and side view of 'Royal Champion' and 'Pink Champion' at 18 weeks after start of the experiment.

Table S1. Spectral properties of solar light under clear (0 % haze) and diffuse (71 %) glass.

Glass type	[a] PSS	[b] R/FR	[c] B/R	[d] UV transmission (%)
Clear	0.723	1.23	0.43	61
Diffuse	0.722	1.22	0.42	74

[a] The photostationary state of phytochrome (Sager *et al*., 1988); [b] the ratio between red light (655-665nm) and far-red light (725-735 nm); [c] the ratio between blue light (450-495 nm) and red light (620-750 nm). [d] Ultraviolet (300-400 nm). (**Sager JC, Smith WO, Edwards JL, Cyr KL. 1988.** Photosynthetic efficiency and phytochrome photoequilibria determination using spectral data. *Transactions of the ASAE,* **31**: 1882-1889).

Table S2. The effect of diffuse glass cover and daily light integral (DLI, mol m^{-2} d^{-1} PAR) on light extinction coefficient (K) in two *Anthurium* cultivars.

Treatments	Light extinction coefficient (K)	95 % confidence interval
'Royal Champion'		
[a] Clear glass + Low DLI	1.09	0.91 - 1.28
[b] Diffuse glass + Low DLI	1.08	0.99 - 1.21
[c] Diffuse glass + High DLI	1.08	1.03 - 1.13
'Pink Champion'		
Clear glass + Low DLI	1.05	0.98 - 1.12
Diffuse glass + Low DLI	1.01	0.94 - 1.09
Diffuse glass + High DLI	0.99	0.92 - 1.05

[a] 0 % haze with DLI of 7.2 mol m^{-2} d^{-1}; [b] 71 % haze with DLI of 7.5 mol m^{-2} d^{-1}; [c] 71 % haze with DLI of 8.9 mol m^{-2} d^{-1}. K is determined according to Lambert-Beer law [eqn (1)] by combining the PPFD interception measurements on cloudy days and the measured LAI.

Table S3. Absolute value of all component variables in the three treatments [Clear glass + Low DLI (daily light integral, mol m^{-2} d^{-1} PAR) represents 0 % haze with DLI of 7.2 mol m^{-2} d^{-1}; Diffuse glass + Low DLI represents 71 % haze with DLI of 7.5 mol m^{-2} d^{-1}; Diffuse glass + High DLI represents 71 % haze with DLI of 8.9 mol m^{-2} d^{-1}].

Components	'Royal Champion'			'Pink Champion'		
	Clear glass + Low DLI	Diffuse glass + Low DLI	Diffuse glass + High DLI	Clear glass + Low DLI	Diffuse glass + Low DLI	Diffuse glass + High DLI
aFM (g m^{-2})	2449	2704	3288	3028	3003	3984
aDMC (%)	15.6	15.9	15.7	14.3	15.0	14.5
aDM (g m^{-2})	369	418	498	428	440	554
TDM (g m^{-2})	436	495	596	526	542	666
aDMP (%)	81.5	81.6	81.3	78.9	78.5	79.8
RUE (g mol^{-1})	0.62	0.67	0.69	0.78	0.78	0.78
I$_i$ (mol m^{-2})	705	744	865	679	708	856
DLI (mol m^{-2} d^{-1})	7.2	7.5	8.9	7.2	7.5	8.9
LAI (m^2 m^{-2})	3.2	3.5	3.9	3.0	3.1	3.8
K	1.09	1.08	1.08	1.05	1.01	0.99
Pn (g m^{-2} s^{-1})	-	-	-	-	-	-
I(L)/I$_o$	-	-	-	-	-	-

For explanation of abbreviations of the variables see Fig. 1. Absolute value of Pn is not possible to determine. No specific value of I(L)/I$_o$ is presented because the relative difference between treatments or cultivars in I(L)/I$_o$ was calculated as the average difference of daily I(L)/I$_o$ between treatments or cultivars over the whole growing season.

Effects of diffuse light on radiation use efficiency depend on the response of stomatal conductance to dynamic light intensity

T. Li[1], J. Kromdijk[2], E. Heuvelink[1], F. van Noort[3], E. Kaiser[1], L.F.M. Marcelis[1]

[1] Horticulture and Product Physiology Group, Wageningen University,
P.O. Box 630, 6700AP, Wageningen, the Netherlands

[2] Institute for Genomic Biology, University of Illinois,
1206 W Gregory Drive, Urbana, IL 61801, USA

[3] Wageningen UR Greenhouse Horticulture, Wageningen University and Research Centre,
P.O. Box 644, 6700AP, Wageningen, the Netherlands

ABSTRACT

Diffuse light stimulates radiation use efficiency (RUE, the ratio between biomass production and intercepted radiation), which is often explained by the more homogeneous spatial light distribution within the canopy. However, diffuse light can also generate a more uniform temporal light distribution at a specific leaf, which could decrease the fluctuations of stomatal conductance (g_s) and consequently result in a higher integrated net photosynthesis. This study aims to investigate the effect of artificially increased diffuse/direct ratio of incident light on crop RUE, and to explain the observed effects in terms of dynamic responses of leaf photosynthesis to temporal changes in light intensity. Two *Anthurium andreanum* cultivars (Pink Champion and Royal Champion) were grown in two glasshouse compartments covered by glass with 0 % haze (clear glass, control) and 71 % haze (diffuse light treatment), respectively, while maintaining a similar light transmission. On clear days, diffuse light treatment resulted in less temporal fluctuations in incident photosynthetic photon flux density (PPFD) compared with control. Stomatal conductance (g_s) was generally low in both cultivars, and varied strongly in response to transient PPFD in 'Royal Champion', whereas it remained more constant in 'Pink Champion'. Instantaneous net leaf photosynthesis (P_n) in both cultivars approached steady state P_n in diffuse light treatment. However, in the control treatment this only occurred in 'Pink Champion', while in 'Royal Champion' instantaneous P_n showed strong fluctuations. 'Royal Champion' showed significantly higher canopy RUE (8 %) in diffuse light treatment compared with control. In contrast, this did not occur in 'Pink Champion'. We conclude that the stimulating effect of higher fraction of diffuse/direct PPFD on canopy RUE depends on the dynamic response of g_s to transient PPFD. Diffuse light can smooth the temporal variation of PPFD on a leaf and hence the temporal variation of g_s in plants with stomata that show a strong opening and closing response to changes in light intensity. Integrated over time, this results in a higher g_s and consequently less g_s limitation for P_n.

INTRODUCTION

Plants are usually subjected to rapidly alternating periods of sun and shade in nature, which are caused by variable cloud cover, shade from the overstory, leaf flutter, and diurnal rotation of the solar angle (Pearcy, 1990). Consequently, a large fraction of CO_2 assimilation occurs in fluctuating light conditions, and plant growth strongly correlates with their prevailing light environment and ability to photosynthesize efficiently during light transitions.

Solar light includes a diffuse and a direct component. Diffuse light arises from the scattering of light by molecules or larger particles in the atmosphere and comes from many directions simultaneously. Direct light arrives in a straight line from the sun without being scattered, thereby having strong fluctuations in intensity. Leaves of plant canopies experience rapid fluctuations in the photosynthetic photon flux density (PPFD) available for photosynthesis because of the changing patterns of sunflecks (direct light) and shade (diffuse light) (Pearcy *et al.*, 2004). Direct light beams that are partly absorbed by upper leaf-layers result in sharp light fluctuations on leaves in the lower part of the canopy. Fluctuations in light intensity within the canopy become less severe when the incident light above the canopy lacks a strong directional component (high diffuse/direct ratio) (Gu *et al.*, 2002; Alton *et al.*, 2007; Urban *et al.*, 2007a; Knohl and Baldocchi, 2008; Urban *et al.*, 2012; Li *et al.*, 2014a). A homogenous light distribution over the canopy is more efficient for crop photosynthesis, as leaf photosynthesis shows a saturating response to light intensity (Farquhar and Roderick, 2003; Gu *et al.*, 2003; Mercado *et al.*, 2009). Consequently, canopy radiation use efficiency (RUE) which describes the relation between accumulated plant biomass and intercepted light is higher in diffuse than in direct light (Sinclair *et al.*, 1992; Healey *et al.*, 1998; Alton *et al.*, 2007). Furthermore, the extent of leaf photoinhibition can be alleviated by diffuse light as fewer local peaks in light intensity occur (Urban *et al.*, 2012; Li *et al.*, 2014a), which further improves canopy RUE.

Several studies have explained increased crop production (or RUE) in diffuse light by the more homogeneous spatial distribution of diffuse light within the canopies (Hemming *et al.*, 2007; Markvart *et al.*, 2010; Dueck *et al.*, 2012). Additionally, increasing diffuse/direct ratio of incident light also results in less variation of temporal light distribution above the plant canopy (Li *et al.*, 2014b), which might also play a role for the stimulating effect of diffuse light on canopy RUE. This could be related to the dynamic properties of leaf photosynthesis, which, among others, depend on the dynamic properties of stomatal conductance (g_s).

Leaf photosynthetic properties strongly influence canopy RUE (Sinclair and Muchow, 1999). Under dynamic light conditions, loss and gain of photosynthetic induction plays a pivotal role for carbon gain (Urban *et al.*, 2007b), which is regulated by rates of RuBP regeneration, Rubisco activation, and stomatal movement (Pearcy *et al.*, 2004; Kaiser *et al.*, 2014). Of all the combined processes involved in photosynthetic induction, changes in stomatal aperture take most time to reach a new steady state (Pearcy *et al.*, 2004; Way and Pearcy, 2012). Many studies have investigated dynamic stomatal behaviour, i.e. comparing plants from different ecological niches under varying environmental conditions (Pfitsch and Pearcy, 1989; Tinoco-Ojanguren and Pearcy, 1993; Whitehead and Teskey, 1995; Valladares *et al.*, 1997; Zipperlen and Press, 1997). These studies suggest that the behaviour of leaf photosynthetic performance under dynamic light conditions depends on stomatal behaviour to a large extent. Tinoco-Ojanguren and Pearcy (1992) showed that sun and understory species showed identical responses of g_s to light intensity in steady state. However, under transient light conditions, understory species had a larger and more rapid response of g_s to sunflecks than sun species. Strong fluctuations in light intensity might trigger stomatal closure, due to intermittent periods of low light intensity or darkness. Therefore, it can be speculated that stomatal closure that is induced by dynamic light can be alleviated when the incident light above the canopy is made diffuse, because light intensity at a given leaf of the canopy is expected to show smaller temporal fluctuations.

The aim of this study is to investigate the effect of artificially increased diffuse/direct ratio of incident light on canopy RUE, and to explain the observed effects in terms of dynamic responses of leaf photosynthesis to temporal changes in light intensity. The hypothesis is that the magnitude of the beneficial effect of diffuse light on canopy RUE depends on the response of g_s to dynamic light intensity. To test this hypothesis, a study was conducted in glasshouses covered with diffuse glass which converting a portion of direct solar light into diffuse light, without affecting light transmission. Two *Anthurium andreanum* cultivars (Pink Champion and Royal Champion) were used in this study, because these two cultivars differ in light sensitivity based on grower's experience that 'Royal Champion' is more sensitive to light than 'Pink Champion'.

MATERIALS AND METHODS

Plant materials and growth conditions

Two *Anthurium andreanum* cultivars (Pink Champion and Royal Champion, Anthura, Bleiswijk, The Netherlands) were grown in two Venlo-type glasshouse compartments of 144 m^2 (15 m × 9.6 m) with a gutter height of 5.5 m at Wageningen UR Greenhouse horticulture in Bleiswijk (The Netherlands, 52° N, 4.5° E). The two compartments were covered by glass (Guardian Agro, Dudelange, Luxembourg) with 0 % haze (clear glass, control) and 71 % haze (diffuse glass), respectively. Haze is defined as the percentage of transmitted light that is scattered such that it deviates more than 1.5° from the direction of the incident beam. The hemispherical transmission of PPFD of the glass was 84 % for both glass types. The haze factor and hemispherical transmission of the glass were measured in an optical sphere according to ASTM international (2007). The spectral properties of the two glass types were similar in the visible spectrum (400-700 nm) as described by Li *et al*. (2014b). Three quantum sensors (LI-190, LI-COR, USA) were installed in each of the greenhouse compartments to measure incident PPFD at intervals of 5 minutes. A standard greenhouse computer (Hogendoorn-Economic, Hogendoorn, Vlaardingen, The Netherlands) was used to control the glasshouse climate (temperature, air humidity and CO_2 concentration). The experiment included two growing seasons: summer and winter.

Plants, propagated *in vitro*, were raised in a glasshouse by a nursery. When the first flowers had appeared, plants were repotted and moved to experimental glasshouses. The summer growing season started from 6 Apr to 28 Aug 2012. The daily light integral [DLI, mol m^{-2} d^{-1} photosynthetic active radiation (PAR)] was limited to 7.5 mol m^{-2} day^{-1} in both compartments, which was realized by controlling the white sunscreen (LS 16 F Revolux, transmission of 37 % and haze factor of 10 %, LudvigSvensson, Kinna, Sweden) and blackout screen (XLS obscural Revolux A/B + B/B, LudvigSvensson, Kinna, Sweden) which were placed in the top of the glasshouse (below gutter height). The white sunscreen was closed when global radiation reached 250 W m^{-2}. The blackout screen was closed when DLI reached the limitation point in the afternoon (Fig. 1). Opening and closing of screens was controlled by a standard greenhouse computer. Plants were grown on potting soil (30 % fine peat + 10 % coarse peat + 43 % coco peat + 10 % bark + 7 % perlite) in black plastic pots (12 cm diameter and 11 cm height) on cultivation tables (4 × 1.8 m) with an automatic ebb/flood irrigation system which supplied irrigation solution once per week in the beginning of the cultivation, while this increased to three times per week from week 10 after the start of the experiment onwards. In each compartment, six cultivation tables were used and each table was equally divided into two parts for the cultivation of both cultivars. The outer two rows of each plot were considered as border plants. The starting plant density was 30 plants m^{-2}; this was

reduced to 20 plants m^{-2} three weeks after start of the experiment. After each destructive harvest plants were moved to maintain the same plant density. During the growing season, average daily outside global radiation was 16 MJ m^{-2} d^{-1}. Inside the greenhouse the average day/night temperature was 25/21°C; relative air humidity was 75/78 %; average daytime CO_2 concentration was 754 µmol mol^{-1}; average realized DLI was 7.2 mol m^{-2} d^{-1} in the control and 7.5 mol m^{-2} d^{-1} in the diffuse light treatment.

The winter growing season started from 5 Sep 2012 to 25 Apr 2013. The experimental set-up was similar as in the summer growing season except that High Pressure Sodium lamps (Master GreenPower Plus 1000W EL, Philips, Eindhoven, The Netherlands) were applied to supplement the day light when outside global radiation was below 100 W m^{-2}, which led to an average realized DLI of 5.6 mol m^{-2} d^{-1} in the control and 5.7 mol m^{-2} d^{-1} in the diffuse light treatment. Average daily outside global radiation during the winter growing season was 6 MJ m^{-2} d^{-1}; average day/night temperature inside the greenhouses was 22/19 °C; relative air humidity was 75/73 %; average daytime CO_2 concentration was 802 µmol mol^{-1}.

Plant measurements

During the summer growing season, total plant dry weight (including roots) was destructively determined in 4, 10, 16, 18 and 21 weeks after start of the experiment. Roots were cleaned with water. Plant organs were dried for at least 48 h at 80 °C in a ventilated oven. In each destructive harvest, two plants per cultivar were randomly selected from each cultivation table, which resulted in 12 replicates per treatment.

Canopy light interception

Canopy PPFD interception was determined during the summer growing season, which was measured on four overcast days (11 May, 15 Jun, 17 Jul and 24 Aug) and three clear days (23 May, 20 Jun and 25 Jul). These days were close to the period when destructive measurements were taken. The measurements were done with a line light probe, in relation to a reference sensor just above the crop (Sunscan, Delta-T, Cambridge, UK). Six measurements were done above as well as below the canopy for each cultivar on each cultivation table. Measurements at the top of the canopy were taken just above the highest leaf, while the bottom measurements were done at pot height.

Canopy radiation use efficiency (RUE)

Canopy RUE was defined as the ratio between the accumulated total dry mass (TDM) and the sum of intercepted PPFD during the experimental period, which was estimated by the slope of

the linear relationship between the accumulated TDM and the sum of intercepted PPFD. Accumulated TDM was determined by using TDM from each destructive harvest minus TDM determined at the first harvest. For calculating the sum of intercepted PPFD, the time course of fraction of intercepted PPFD was estimated from the four periodic canopy PPFD interception measurements on cloudy days [eqn (1)]. These data can represent the fraction of intercepted PPFD over the growing season because the fraction of intercepted PPFD measured on clear days was similar as on cloudy days (data not shown). In each treatment, the fraction of intercepted PPFD could be well fitted by a negative exponential curve with number of days after start of the experiment and reaching a plateau in the end ($r^2 = 0.99$ for all treatments)

$$I(L)/I_0 = 1 - e^{-ad} \qquad (1)$$

where *I(L)/I$_o$* is daily fraction of intercepted PPFD, in which *I(L)* is PPFD at leaf area index *L*, *I$_o$* is PPFD at top of canopy; *a* is saturating coefficient; *d* is number of days after start of the experiment.

Daily PPFD intercepted by the canopy was calculated as the product of the interpolated daily fraction of intercepted PPFD [*I(L)/I$_o$*] multiplied by the measured DLI. Integrating the daily PPFD intercepted by the canopy during the designated growing period yielded the sum of intercepted PPFD.

Leaf photosynthesis

Leaf photosynthesis was measured with portable gas exchange device (LI-6400XT; LI-COR, Lincoln, USA). During the summer growing season, the transparent leaf chamber (Part No. 6400-08) was used to measure instantaneous leaf photosynthesis rates in the control treatment (clear glass) on three clear days for each cultivar. Incident solar light in the greenhouse was used as light source for this measurement. The leaf chamber was horizontally positioned. Three measurements from three plants were taken for each cultivar. In each measurement, leaf photosynthesis rate, g$_s$ and incident PPFD were recorded at one minute intervals on one fully expanded leaf for two to four hours. Steady state leaf photosynthesis during the summer growing season was measured at 100 and 500 μmol m^{-2} s^{-1} PPFD in both treatments as described by Li *et al.* (2014b).

During the winter growing season, instantaneous leaf photosynthesis rates were measured on four clear days for each cultivar in both treatments. The measurement procedure was similar as during the summer growing season. Furthermore, instantaneous leaf photosynthesis rates of both cultivars were measured on overcast days in the control treatment

to further verify their responses to diffuse light.. Steady state leaf photosynthesis light response curves were measured with the portable gas exchange device equipped with a leaf chamber fluorometer (Part No. 6400-40). Six fully expanded leaves were randomly selected from each cultivar in each treatment for this measurement. The adaxial side of the leaf was illuminated by the light source (10% blue, 90% red). Selected leaves were adapted at 300 μmol m^{-2} s^{-1} PPFD for 10 min before the measurements were taken. PPFD was varied stepwise in the following sequence: 700, 600, 500, 400, 300, 200, 100, 50, 25 and 0 μmol m^{-2} s^{-1}. At each PPFD, the measurements were taken when the photosynthesis rates reached steady state.

All measurements were carried out between 9:00 and 16:00 h. In the measurement chamber, vapour pressure deficit (VPD) was kept between 0.5-1 kPa, reference CO_2 concentration was set at 800 μmol m^{-2} s^{-1}, leaf temperature at 27 and 25°C for the summer and winter growing season, respectively. These parameters were close to those in the greenhouse.

Chlorophyll fluorescence

During the summer growing season, maximum photosystem II (PSII) efficiency (Fv/Fm) was measured in week 16 after start of the experiment. Measurements were taken with a portable chlorophyll fluorometer (PAM-2000, Walz, Germany) at five time points (9:00, 11:00, 13:00, 15:00, 17:00 h) on clear days. Red light was used as measuring light (2 μmol m^{-2} s^{-1}) and saturating flashes (8000 μmol m^{-2} s^{-1}). At each time point, four fully expanded leaves of each cultivar in each treatment were randomly selected. A leaf clip holder (DLC-8) was used for dark adaptation for 30 minutes prior to measurements.

Statistical analysis

The non-rectangular hyperbola function [eqn (2)] (Cannell and Thornley, 1998) was fitted to steady state leaf photosynthetic light response data.

$$P_n = \frac{Ia + P_{max} - \sqrt{(Ia + P_{max})^2 - 4Ia\Theta P_{max}}}{2\Theta} - R_d \qquad (2)$$

where P_n is net leaf photosynthesis rate (μmol m^{-2} s^{-1}), I is incident PPFD (μmol m^{-2} s^{-1}), P_{max} is maximum net leaf photosynthesis rate (μmol m^{-2} s^{-1}), a is the leaf photosynthetic efficiency (μmol CO_2 μmol^{-1} photons), Θ is the curvature parameter, and R_d is dark respiration (μmol m^{-2} s^{-1}).

Eqn (2) was also fitted to the measured instantaneous leaf photosynthetic light response data. The purpose of this fitting was to obtain the standard error of the fit to quantify the

variability of the instantaneous leaf photosynthesis rates in response to incident PPFD. Comparisons of the standard error of the fit and the time course of maximum PSII efficiency (Fv/Fm) between treatments were evaluated by analysis of variance (ANOVA), assuming replications in the same greenhouse compartment as being independent. Differences between treatments in RUE were tested by multiple linear regression.

RESULTS

Incident PPFD

On clear days, the temporal distribution of incident PPFD at a given spot above the canopy showed continuous fluctuations during the day in the control treatment, while it showed less variation in the diffuse light treatment (Fig. 1). In both treatments, incident PPFD was kept below 500 µmol m^{-2} s^{-1} whereas outside PPFD reached 1800 µmol m^{-2} s^{-1} in the middle of a clear day. On overcast days, incident PPFD at a given spot showed similar dynamic patterns in the two treatments (data not shown).
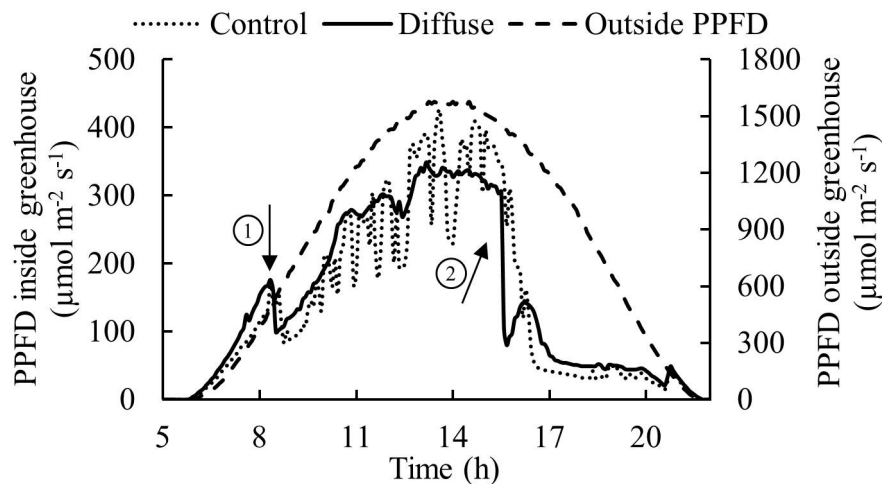
Fig. 1. Incident photosynthetic photon flux density (PPFD) inside and outside of the control and diffuse light treatment on a typical clear day (26 July 2012) as measured by a point sensor. ① indicates the white sunscreen was fully closed; ② indicates the daily light integral (DLI) reached to the limitation point and the blackout screen was closed.

Canopy RUE and biomass production

The diffuse light treatment stimulated canopy RUE by 8 % in 'Royal Champion' compared with control; while this effect did not occur in 'Pink Champion' (Fig. 2). Furthermore, the diffuse light treatment significantly increased biomass production in 'Royal Champion' (*P* =

0.01), but not in 'Pink Champion' ($P = 0.52$). For detailed information about biomass production see Li et $al.$ (2014b).
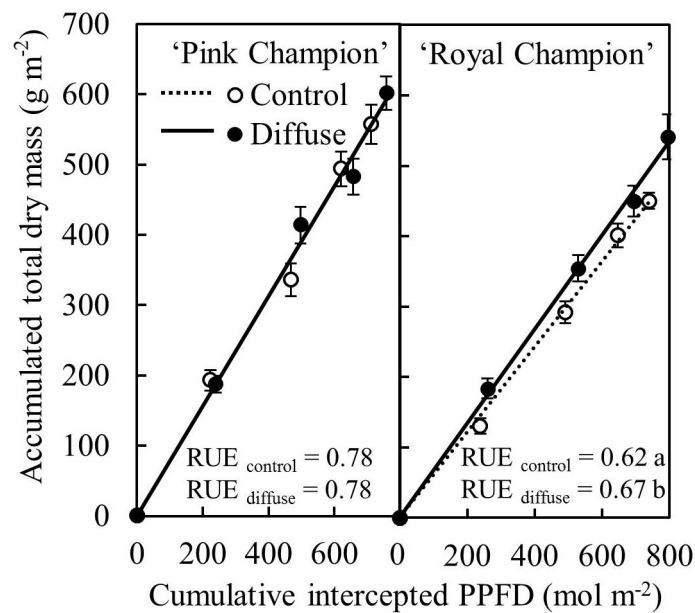
Fig. 2. Relationship between accumulated total dry mass and cumulative intercepted PPFD for 'Pink Champion' and 'Royal Champion' in the control and diffuse light treatments. Solid and dashed lines represent fitted linear relationships for diffuse light and control treatments, respectively. The slope of the fitted linear relationship is the canopy radiation use efficiency of biomass production (RUE, g dry mass mol^{-1} PPFD). Dashed line in 'Pink Champion' is obscured by the solid line. Error bars show ± SE (n = 12). Letters show statistical significant differences (P < 0.05).

Steady state leaf photosynthesis and maximum PSII efficiency

In the winter growing season, steady state leaf photosynthesis light response data could be well fitted with a smooth non-rectangular hyperbolic shape curve (Fig. 3). Photosynthetic light response curve parameters (i.e. P_{max}, a, Θ, R_d) in both cultivars were not influenced by the treatments (Supplementary Table S1). Treatments also had no effect on steady state leaf photosynthesis in the summer growing season (Li et $al.$, 2014b). Leaf photosynthetic capacity in 'Pink Champion' was higher than in 'Royal Champion' in both treatments (Fig. 3).

On clear days, maximum PSII efficiency (Fv/Fm) of the fully expanded leaves in both cultivars showed a diurnal trend with significantly lower values in the early afternoon compared with the early morning (Fig. 4). Treatment effects were not observed in 'Pink Champion'. In 'Royal Champion', the diffuse light treatment resulted in higher Fv/Fm compared with control ($P = 0.008$).
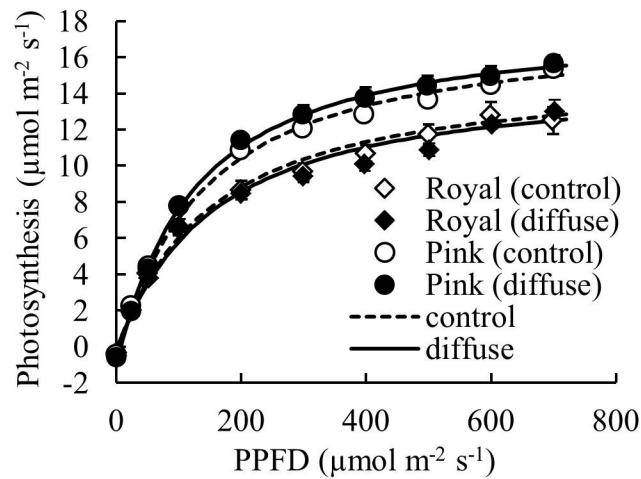
Fig. 3. Steady state net leaf photosynthetic light response curves in 'Pink Champion' and 'Royal Champion' in the control and diffuse light treatments. The measurements were taken in March 2013 (winter growing season). During the measurements, air temperature, CO_2 concentration, VPD in the measurement chamber were maintained at 25 °C, 800 µmol mol^{-1}, and 0.5-1kPa, respectively. Lines represent the fit of the non-rectangular hyperbola function (Eqn 2). Error bars show ± SE (n = 6).
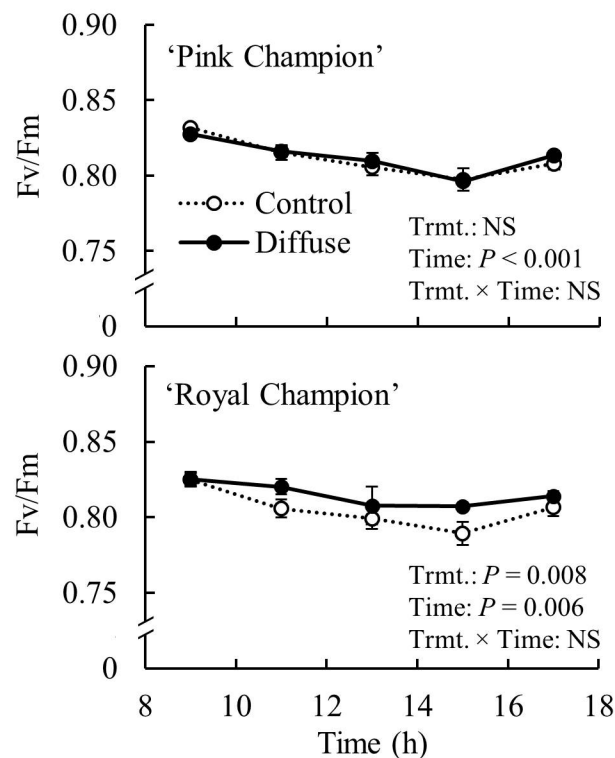
**Fig. 4.** Maximum PSII efficiency (Fv/Fm) in 'Pink Champion' and 'Royal Champion' in the control and diffuse light treatment on a clear day in the summer growing season (26 July 2012: for PPFD see Fig 1). Error bars show ± SE (n = 4). Two-way ANOVA with treatment (Trmt) and time as independent variables and their interaction (Trmt × Time) for each dependent variable is shown in each cultivar. NS: not significant (P ≥ 0.05).

Stomatal conductance (g$_s$) and leaf photosynthesis response to the dynamic light

On clear days, g$_s$ of 'Pink Champion' varied slightly when incident PPFD was temporally fluctuating in the control treatment (Fig. 5A, C); while in 'Royal Champion', g$_s$ strongly responded to the variation of incident PPFD (Fig. 5B, D). In diffuse light treatment, g$_s$ of both cultivars varied slightly when incident PPFD was relatively constant on clear days (Fig.5E, F). Furthermore, g$_s$ was also relatively constant in both cultivars in the control treatment on overcast days when global radiation was relatively stable and fully diffuse (data not shown).

In 'Pink Champion', instantaneous leaf photosynthesis rates followed a non-rectangular hyperbolic relationship with incident PPFD on both overcast and clear days in both treatments (Fig. 6A, C, E, G). Similar response patterns were also observed for 'Royal Champion' in the control treatment on overcast days (Fig. 6F) as well as in the diffuse light treatment on clear days (Fig. 6H). However, in the control treatment instantaneous leaf photosynthesis rates of 'Royal Champion' as a function of incident PPFD showed a highly variable (scattering) response on clear days (Fig. 6B, D); this phenomenon was more obvious in the summer growing season than in the winter growing season (Fig. 6B, D). Differences in the scattering of the instantaneous leaf photosynthesis rates as a function of incident PPFD were quantified by comparing the standard errors of best-fits of the data to non-rectangular hyperbolic curves, which showed that standard errors for 'Royal Champion' were significantly larger than for 'Pink Champion' in the control treatment in both summer and winter growing seasons (Fig. 7). In the diffuse light treatment, standard errors were low and no significant difference between cultivars was found ($P = 0.824$).
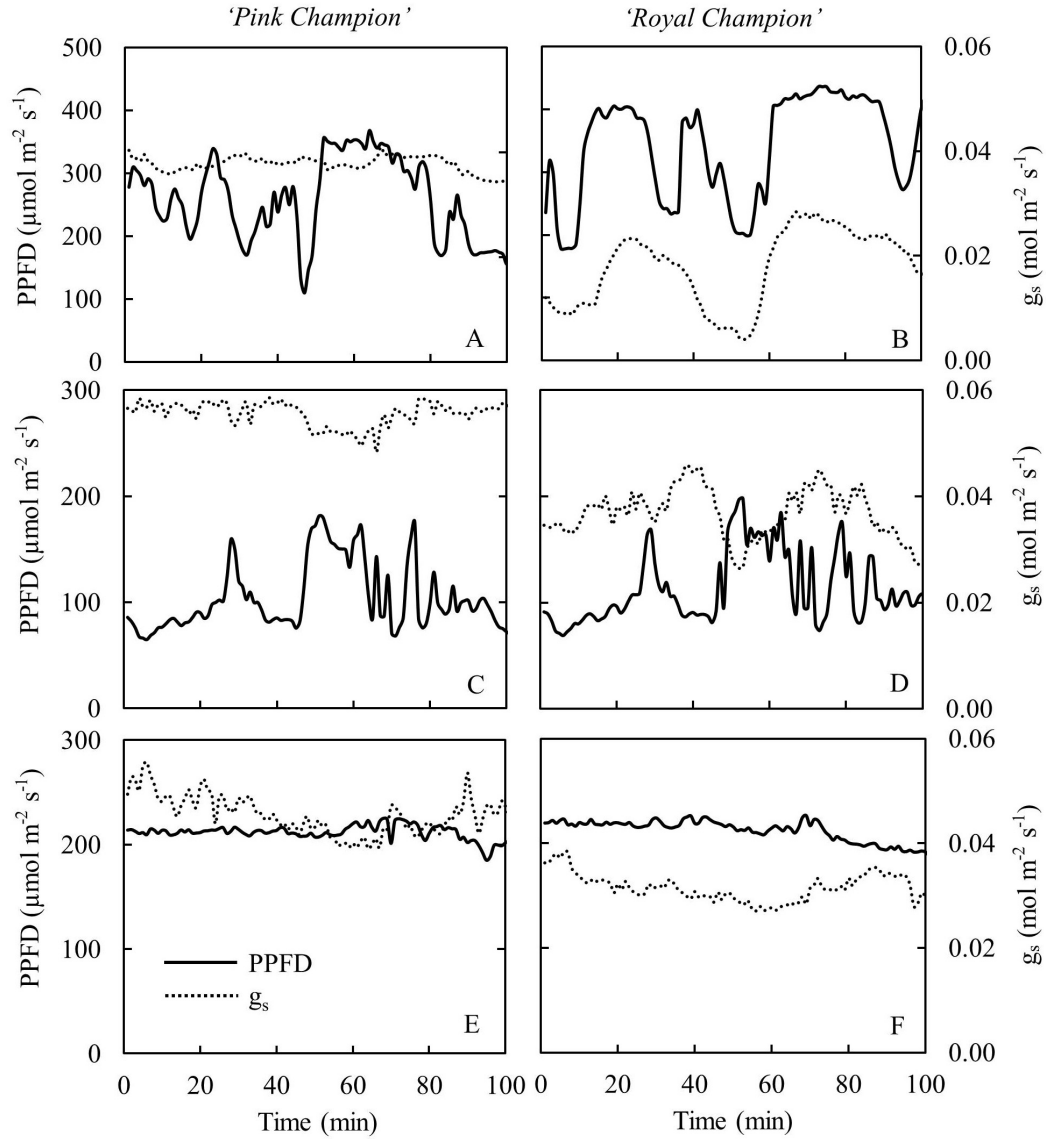
Fig. 5. Response of stomatal conductance (g_s, dashed line) to transient PPFD (solid line) in 'Pink Champion' (A, C, E) and 'Royal Champion' (B, D, F). A and B were measured in the control treatment in July 2012 (summer growing season); C and D were measured in the control treatment in March 2013 (winter growing season); E and F were measured in the diffuse light treatment in March 2013. The measurements were taken at one minute interval.
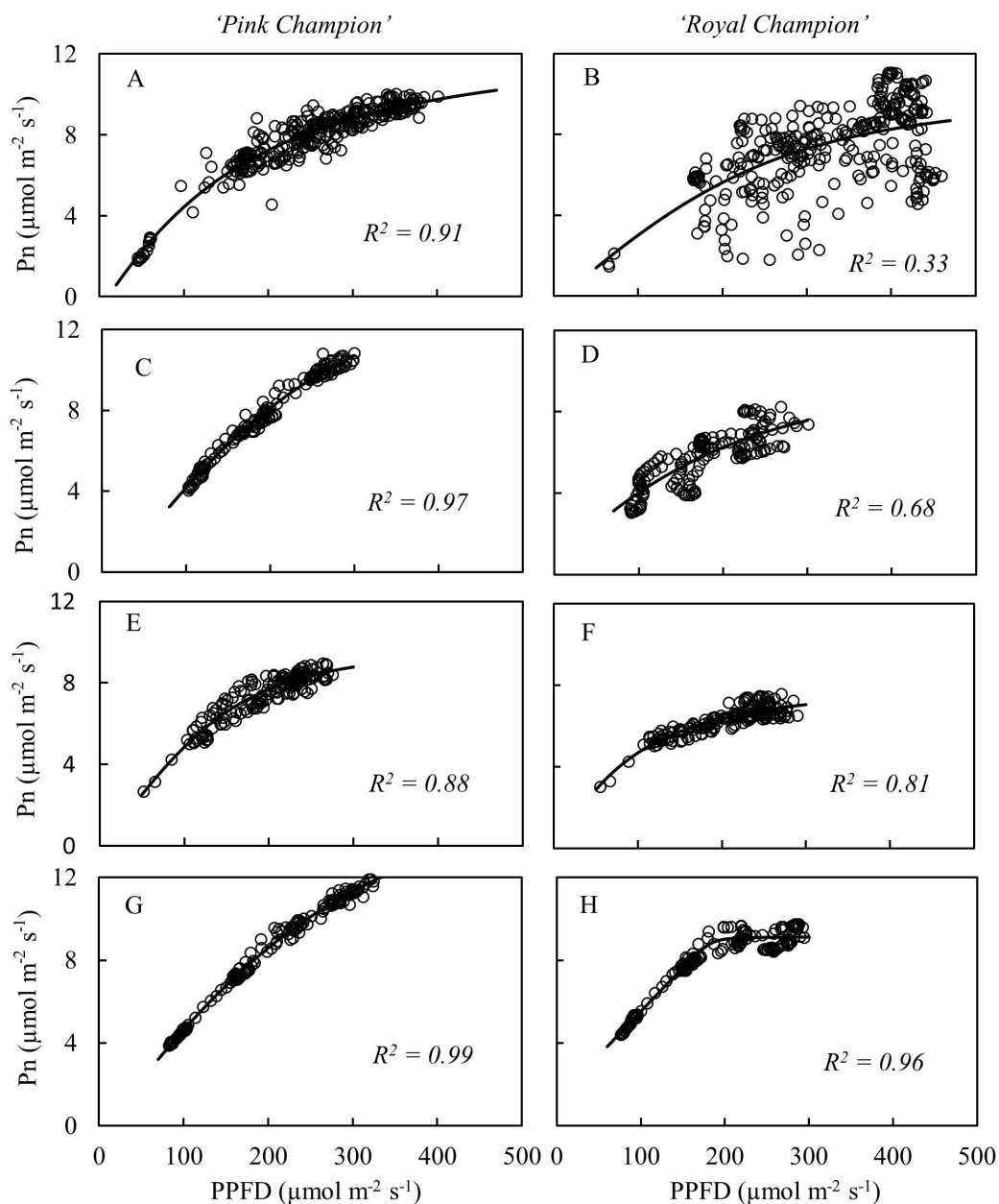
Fig. 6. Relationship between instantaneous net leaf photosynthesis rates (Pn) and transient PPFD in 'Pink Champion' (A, C, E, G) and 'Royal Champion' (B, D, F, H). A and B were measured on clear days in the control treatment in July 2012 (summer growing season); C, D, E, F,G, H were measured in March 2013 (winter growing season; C and D were measured on a clear day in the control treatment; E and F were measured on a cloudy day in the control treatment; G and H were measured on a clear day in the diffuse light treatment. Each symbol represents the instantaneous leaf photosynthesis rate at one minute interval. Lines represent the best fit of a non-rectangular hyperbola [Eqn (2)].
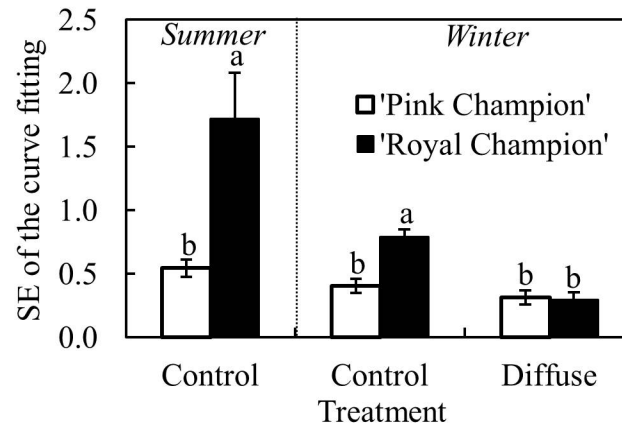
Fig. 7. Standard error (SE) of fitting non-rectangular hyperbola to instantaneous net leaf photosynthesis rates as a function of incident PPFD [Eqn (2)] in summer growing season in the control treatment (n = 3), and in winter growing season in the control and diffuse light treatments (n = 4). Data used for curve fitting in this figure were collected on clear days. Error bars show ± SE. Letters within each growing season show statistical significant differences (P < 0.05).

DISCUSSION

Diffuse light is more homogeneously distributed within the canopy than direct light, which results in an enhanced canopy photosynthesis (Spitters, 1986; Healey *et al.*, 1998; Roderick *et al.*, 2001; Li *et al.*, 2014a). Additionally, at the leaf level, diffusivity of the incident light above the canopy also strongly alleviates the temporal variation in light intensity (Fig. 1). To our knowledge, we are the first to show the consequences of a decrease in temporal variation of light intensity due to an artificially increased diffuse/direct light ratio (diffuse glass cover) for leaf photosynthesis and radiation use efficiency (RUE). We explain how differences in dynamic stomatal and photosynthetic properties between varieties can modulate the response of canopy RUE as a function of diffusivity of incident light.

Diffusing the light results in less temporal variation of incident light on clear days

The effects of the diffuse light treatment on the light environment within the compartments depended on external light conditions. Clear days resulted in an obvious difference in temporal PPFD distribution between the two treatments (Fig. 1). Incident PPFD measured at a given spot above the canopy showed strong fluctuations in the control treatment, which resulted from the shadow cast by the greenhouse structures and equipment due to a continuously changing solar angle. The diffuse light treatment minimized the effects of local shade by construction parts, and produced less variation of temporal PPFD distribution above canopies. Accordingly, we may expect that the temporal variation in PPFD within the canopy

is much less under the diffuse light than the control treatment on clear days. Obviously, these effects did not occur on cloudy days, as the light incident on the greenhouse already had a high diffuse/direct ratio.

What could be the potential explanation for the stimulating effect of diffuse light on RUE?

Radiation use efficiency (RUE) provides the measure that directly reflects the efficiency of a crop to utilize the radiant energy for producing biomass, which is usually determined by an integration of many factors, for instance, leaf photosynthetic rates, plant prevailing light environment and canopy structure (Sinclair and Muchow, 1999). Increasing the diffuse/direct ratio of incident light resulted in 8 % increase in RUE in 'Royal Champion' (Fig. 2). The stimulating effect of diffuse light on RUE has been found in many studies; this was mainly explained by a more homogeneous vertical light distribution within the canopy (Sinclair *et al.*, 1992; Healey *et al.*, 1998; Alton *et al.*, 2007; Zhang *et al.*, 2011). In our study, the relation between fraction of light intercepted by the canopy and LAI was not affected by treatments (Li *et al.*, 2014b), indicating that the vertical light distribution was not affected. The absence of an effect can be explained by the fact that anthurium pot-plants have a short and compact canopy structure, which is less responsive for scattering of the light (Li *et al.*, 2014b). Additionally, shading screens were applied in both treatments, which already transformed a portion of direct into diffuse light (10 %). Li *et al.* (2014a) showed that diffuse light is also more homogeneously distributed in the horizontal plane within a tomato canopy. Although we did not investigate the horizontal light distribution within the canopy in this study, we speculate that its effect on RUE could be negligible, because about 80 % of the time incident PPFD was below 300 μmol m^{-2} s^{-1} in both treatments during the summer growing season, which implies that leaf photosynthesis rates during a large part of the growing season were likely in the linear range of the leaf photosynthesis light response curve (Fig. 3). Therefore, the importance of the homogeneous spatial light distribution within the canopy might not play an important role for improving crop photosynthesis in this study. Furthermore, current treatments also had no effect on the canopy architecture in both cultivars as shown by Li *et al.* (2014b).

Radiation use efficiency (RUE) could also be affected by other environmental factors such as temperature (Andrade *et al.*, 1993), VPD (Stockle and Kiniry, 1990), water availability (Jamieson *et al.*, 1995) and nutrient condition (Allen *et al.*, 2005). However, these

factors were kept similar in both treatments, which eliminated the potential effect of these factors as explanations for the differences in RUE between treatments.

Shade plants are particularly susceptible to photoinhibition when exposed to high light (Long *et al.*, 1994). Previous studies found evidence for photoinhibition in understory plants as a result of exposure to sunflecks, which can be seen as a rapid low-to-high light transition (Powles and Bjorkman, 1981). Increasing diffuse/direct ratio of incident light resulted in a slightly higher maximum PSII efficiency (Fv/Fm) in 'Royal Champion' on clear days (Fig. 4), which could contribute to the higher RUE. This effect could be due to fewer sunflecks under the diffuse light treatment on clear days (Fig. 1). However, the diffuse light treatment had no effect on Fv/Fm in 'Pink Champion' (Fig. 4). The effect of diffuse light on Fv/Fm was rather small in this study compared with Li *et al.* (2014a) in a tomato canopy, because shading screens were applied in the current study where the incident PPFD was three times lower than the global PPFD during the middle of a clear day (Fig. 1).In this case, Fv/Fm was not strongly affected even under the control treatment.

Increasing leaf photosynthesis rate is directly linked to increase in RUE (Sinclair and Muchow, 1999). Steady state leaf photosynthesis rates as a function of PPFD showed similar response patterns between the two treatments in both cultivars with a smooth rectangular hyperbola curve during the winter experiment (Fig. 3; Supplementary Table S1). Similarly, steady state leaf photosynthesis was also not influenced by the treatments in the summer experiment (Li *et al.*, 2014b). Therefore, it is clear that steady state leaf photosynthesis did not contribute to the stimulating effect of diffuse light on canopy RUE. A higher leaf photosynthetic capacity was observed in 'Pink Champion' than in 'Royal Champion' (Fig. 3), which correlates with higher RUE in 'Pink Champion' than in 'Royal Champion'(Fig. 2). Our results suggest that the stimulating effect of diffuse light on RUE in 'Royal Champion' is attributed to dynamic properties of leaf photosynthesis

Effects of diffuse light on RUE depends on the dynamic properties of leaf photosynthesis
Despite the absence of an effect of diffuse light on the light response of steady state leaf photosynthesis in both cultivars, the effect of diffuse light treatment on light response of dynamic leaf photosynthesis differed between cultivars in the control treatment (Fig. 6). Plants usually experience frequent variations in light intensity, which are of particular important because interactions between stomatal and photosynthesis responses may result in prolonged periods where a steady state is not achieved (Knapp and Smith, 1990b).

When temporal incident PPFD varied above the canopy, the two cultivars showed different response behaviour with respect to g_s (Fig. 5). In 'Pink Champion', g_s remained relatively constant irrespective of the short term fluctuations in incident PPFD in both treatments (Fig. 5A, C, E). This is likely due to the slow responses of guard cells to changes in PPFD or the initial g_s was already at its maximum level even at low PPFD (Knapp and Smith, 1990b). In 'Royal Champion', on the other hand, g_s varied considerably with fluctuations in PPFD (Fig. 5B, D), this behaviour may reflect the intrinsic characteristics of stomata that might maximize water use efficiency at the expense of carbon gain (Knapp and Smith, 1990a; Vico *et al.*, 2011). The strong variation in g_s in 'Royal Champion' was not observed in the diffuse light treatment where the temporal incident PPFD was less variable on clear days (Fig. 5E,F).

The response in stomatal movement usually lags behind the response of photosynthesis in fluctuating light (Barradas and Jones, 1996). Therefore, g_s can limit leaf photosynthesis under dynamic light conditions, which was indicated by the strong scattering of the instantaneous leaf photosynthesis rates when plotted as a function of incident PPFD in 'Royal Champion' in the control treatment on clear days (Fig. 6B, D; Fig. 7). In 'Pink Champion', instantaneous leaf photosynthesis rates in many cases approached the values of steady state leaf photosynthesis rates, when compared at similar PPFD (Fig. 3). This indicates that in 'Pink Champion' g_s did not impose a limitation to leaf photosynthesis under dynamic light conditions because of a smaller response of g_s to variations of incident PPFD.

Incident PPFD with less temporal variation may smooth the fluctuations in g_s, especially in those plants with stomata that react strongly to fluctuations in PPFD. Consistent with this hypothesis, the diffuse light treatment reduced the temporal variation in the incident PPFD (Fig. 1), and consequently alleviated stomatal limitation to leaf photosynthesis in 'Royal Champion'. This was illustrated by a less scattering of instantaneous leaf photosynthesis rates as a function of incident PPFD in the diffuse light treatment on clear days (Fig. 6H; Fig. 7); a similar phenomenon was observed in the control treatment on cloudy days when global radiation was fully diffuse (Fig. 6F). Therefore, we conclude that less stomatal limitation in diffuse light treatment is beneficial for crop photosynthesis in 'Royal Champion' and consequently improves RUE. This effect did not occur in 'Pink Champion', mainly because of a smaller response of g_s to the variation of incident PPFD.

The scattering response of instantaneous leaf photosynthesis rates as a function of incident PPFD for 'Royal Champion' in the control treatment was more pronounced in the summer growing season than in the winter growing season (Fig. 6B,D; Fig. 7). This could be

a consequence of the higher fraction of direct light on clear days entering the greenhouse in the summer season compared to the winter season. Therefore, it can be speculated that the stimulating effect of diffuse light on canopy RUE is more pronounced in the summer season than in the winter season in the northern region.

We cultivated *Anthurium* pot-plants at 800 μmol mol^{-1} CO_2 condition which is twice the ambient CO_2 concentration. We proved that plant photosynthesis rates of these two cultivars were still limited by CO_2 concentration as indicated by the continuous strong increase in leaf photosynthesis rates with raising CO_2 concentration in the range of 50-1600 μmol mol^{-1} (supplementary data Fig. S1). A likely explanation for this is the remarkably low g_s that both cultivars showed. This phenomenon further implies that the variation of g_s imposes a limitation on leaf photosynthesis rates.

CONCLUSIONS

Increasing the diffuse/direct ratio of the incident PPFD results in a less temporal variation of incident PPFD at a specific leaf. This allows for higher transient rates of leaf photosynthesis and subsequently RUE in plants with stomata that show a strong response to variation in PPFD. In these plants, g_s is relatively constant and less limiting for leaf photosynthesis under high diffuse/direct ratio PPFD conditions. For plants with less response of stomata to the variation of PPFD, the effect of the homogeneous temporal distribution of PPFD on RUE was limited or non-existed. Therefore, we conclude that the stimulating effect of diffuse light on RUE depends on the dynamic response of g_s to incident PPFD.

ACKNOWLEDGEMENTS

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SUPPLEMENTARY DATA
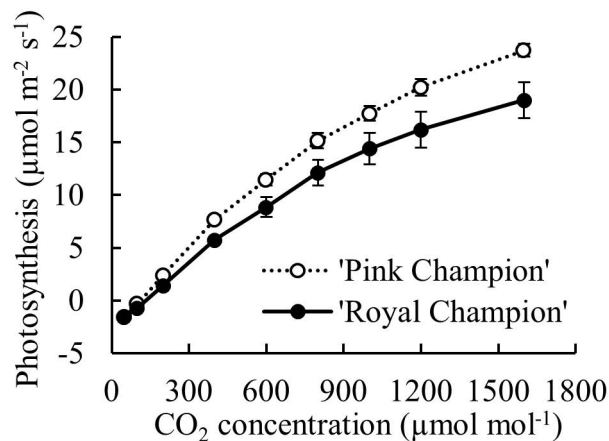
Fig.S1. CO_2 response of photosynthesis of the fully expanded leaves in 'Pink Champion' and 'Royal Champion'. This measurement was carried out with the portable gas exchange device equipped with a leaf chamber fluorometer (LI-6400XT; LI-COR, Lincoln, USA) at CO_2 concentration levels between 50 and 1600 μmol mol^{-1}. Six fully expanded leaves from six plants each were randomly selected for six response curves in each cultivar. The starting CO_2 concentration was 400 μmol mol^{-1}, followed by 200, 100, 50, 400, 600, 800, 1000, 1200, 1600 μmol mol^{-1}; at each CO_2 concentration, the measurements were taken after about 5 min. In the measurement chamber, photosynthetic photon flux density (PPFD, 10% blue, 90% red), leaf temperature and vapour pressure deficit (VPD) were maintained at 500 μmol m^{-2} s^{-1}, 25 °C, and 0.5-1 kPa, respectively. Vertical bars indicate standard error of mean (n=6).

Table S1. Light response curve parameters in 'Pink Champion' and 'Royal Champion' in the control and diffuse light treatments (n = 6). These are fitted from measured light response curves [eqn (2)].

Treatments	$P_{max,}$ (μmol m^{-2} s^{-1})	α (μmol CO_2 μmol^{-1} photons)	θ	R_d (μmol m^{-2} s^{-1})
'Royal Champion'				
Control	16.0	0.10	0.20	-0.16
Diffuse	15.5	0.09	0.20	-0.16
'Pink Champion'				
Control	17.4	0.13	0.20	-0.65
Diffuse	18.0	0.13	0.20	-0.80

Statistical analysis showed that light response curve parameters were not significantly different between the two treatments.

Quantifying the source-sink balance and carbohydrate content in three tomato cultivars

T. Li, E. Heuvelink, L.F.M. Marcelis

Horticulture and Product Physiology Group, Wageningen University,
P.O. Box 630, 6700AP Wageningen, the Netherlands

ABSTRACT

Supplementary assimilation light is frequently applied in winter season for crop production in greenhouses. The effect of supplementary assimilation light on plant growth depends on the balance between assimilate production in source leaves and the overall capacity of the plants to use assimilates. This study aims at quantifying the source-sink balance and carbohydrate content of three tomato cultivars, and to investigate to what extent the source/sink ratio correlates to the potential fruit size. Cultivars Komeett (large size), Capricia (medium size) and Sunstream (small size, cherry tomato) were grown under commercial crop management from 16 Aug to 21 Nov, supplementary assimilation light (162 μmol m^{-2} s^{-1} ; maximum 10 hours per day according to natural irradiance level) was applied from 19 Sep onwards. Source strength was estimated using the crop growth model TOMSIM; Sink strength was estimated from potential fruit growth rate which was determined from non-destructively measuring the fruit growth rate at non-limiting assimilate supply as created by removing all except one fruit on each truss. Carbohydrate content in leaves and stems were periodically determined. During the early growth stage, 'Komeett' and 'Capricia' showed sink limitation and 'Sunstream' was close to sink limitation, during this stage reproductive organs were hardly formed or still small and natural irradiance was relatively high (early Sep.) compared to winter months. All three cultivars were strongly source-limited during the fully fruiting stage as indicated by the extremely low source/sink ratio (average source/sink ratio from 50 days after planting onwards was 0.17, 0.22 and 0.33 for 'Komeett', 'Capricia' and 'Sunstream', respectively). Carbohydrate content in leaves and stems increased linearly with the plant source/sink ratio. We conclude that under ample irradiance tomato plants are sink limited during their early growth stage, the level of sink limitation differs between cultivars but is not correlated with their potential fruit size. During the fully fruiting stage tomato plants are source limited and the extent of source limitation of a cultivar is positively correlated with its potential fruit size.

INTRODUCTION

Plant growth is closely correlated to source and sink strength and the balance between them (Gifford and Evans, 1981; Wardlaw, 1990; Smith and Stitt, 2007). Source strength is defined as the rate at which assimilates are produced (photosynthesis rate); while sink strength is the competitive ability of an organ to attract assimilates (Marcelis, 1996). The source-sink balance of a plant varies significantly during its life span because of the continuous organ initiation and development which controls the growth of sinks as well as the photosynthetic sources (Wardlaw, 1990). During the early growth stage, tomato plants might be prone to sink limitation as there are not sufficient sinks to utilize all the produced assimilates. This might occur particularly at ample irradiance. During the reproductive stage, tomato plants generally bear high fruit load, and assimilate supply might not meet the sink demand. This has been proven in studies where fruit pruning increased fruit size of the remaining fruits without influencing the total plant biomass production (Cockshull and Ho, 1995; Heuvelink, 1996b; Matsuda *et al.*, 2011), which suggests source limitation. Tomato source-sink balance could also differ between cultivars which often differ in fruit load and potential fruit growth rate which is a measure for sink strength (Heuvelink and Marcelis, 1989; Marcelis, 1996). Cultivars may also differ in source strength as leaf photosynthetic properties, leaf area and plant architecture may differ. Dueck *et al.* (2010) observed that under commercial crop management cherry tomato did not benefit that much from the use of artificial lighting compared with the cultivars with large-sized fruits, and they argued that cherry tomato had less sink demand although it has a higher number of fruit load. A detailed analysis of the source-sink balance from early growth stage to fully fruiting stage for cultivars with different potential fruit size has not been done so far. Crop growth models have been applied to quantify the source and sink strength (Heuvelink, 1996b; Wubs *et al.*, 2009; Wubs *et al.*, 2012). The sink strength of a growing organ can be determined by its potential growth rate (i.e. growth under non-limiting assimilate supply), and depends on its developmental stage (Marcelis and Baan Hofman-Eijer, 1995); integrating the sink strength of each growing organ over the whole plant results in total plant sink strength. The source strength is the supply of assimilates, which can be determined by integrating the leaf photosynthesis over the crop canopy (Heuvelink, 1995).

The growth environment also plays a pivotal role in determining source-sink balance, particularly irradiance level as it is the driving force for photosynthesis. The application of supplementary assimilation light in greenhouses is rapidly increasing (Heuvelink *et al.*, 2006;

Moe *et al*., 2005), this considerably increases the source strength and source/sink ratio. The beneficial effect of supplementary assimilation light is determined by the balance between assimilate production in source leaves and the overall capacity of the plants to use these assimilates. This implies that it is necessary to identify the plant source-sink balance in order to efficiently utilize supplementary assimilation light.

Source-sink balance regulates carbohydrate content in plants. Differences in source-sink balance are expected to result in different carbohydrate content in plants (Paul and Foyer, 2001; Dingkuhn *et al*., 2007; Patrick and Colyvas, 2014). In a source-limited situation, carbohydrate content in the plants might be relatively low as plants have sufficient sinks to utilize the produced assimilates. However, in a sink-limited situation plant growth cannot keep pace with assimilate production. The difference between assimilate production and utilisation is generally stored as carbohydrates (starch and soluble sugars) in the vegetative organs, being characterised by a high carbohydrate content in leaves (Yelle *et al*., 1989) as well as stems (Hocking and Steer, 1994; Scofield *et al*., 2009). Limited sink demand could down-regulate the net photosynthetic activity through carbohydrate accumulation in source leaves (Iglesias *et al*., 2002; Franck *et al*., 2006; McCormick *et al*., 2006; Velez-Ramirez *et al*., 2014), which is called feedback regulation.

The objectives of this study are to provide a detailed quantitative analysis of source-sink balance as well as carbohydrate content of tomato plants during their development; and to investigate to what extent the source/sink ratio depends on the potential fruit size. Our hypotheses are 1) under ample irradiance tomato plants are sink limited during their early growth stage; 2) tomato plants are source limited during the fully fruiting stage, and the source-sink ratio negatively correlated with the potential fruit size (when comparing cultivars at their commercial fruit load). To test these hypothesises, three types of tomato plants with different potential fruit size were grown under commercial crop management from mid-August till end of November, the source/sink ratio and carbohydrate content were examined during this period through experimental observation and model simulation.

MATERIALS AND METHODS

Plant materials and growth conditions

Tomato (*Solanum lycopersicum*, cv.'Komeett', 'Capricia', 'Sunstream') plants were planted in a Venlo-type glasshouse compartment on 16 Aug and grown until 21 Nov 2013. Basic information on the three cultivars is given in Table 1. The greenhouse compartment had an

area of 150 m^2 with a gutter height of 5 m, and was located in Wageningen, the Netherlands (52° N, 5° E). Eight growth gutters were evenly arranged in the compartment, each gutter was equally divided into three sections for three cultivars each. All plants grown at each border gutter of each side of the compartment, and two plants at each end of the gutters were considered as border plants. All plants were grown with single shoot. Plant density was initially 3.3 plants m^{-2} and gradually decreased to 2.2 plants m^{-2} at the end of the experiment due to periodical destructive harvests. Plants were grown on Rockwool with drip irrigation. Solar radiation was continuously measured outside the greenhouse with a weather station system (WS-GP2 Weather station, Delta-T, Cambridge, UK) throughout the experimental period. Photosynthetic photon flux density (PPFD) was estimated from solar radiation, assuming half the global radiation is PPFD (Jacovides *et al.*, 2003). Greenhouse transmissivity was 62 %. Supplementary assimilation light (High Pressure Sodium lamps, Hortilux Schreder, HPS600W/400V) was applied from 6:00 to 16:00 since 19 Sep till the end of the experiment. PPFD of the supplementary assimilation light was 162 ± 9 µmol m^{-2} s^{-1} at the top of the canopy. The lamps were automatically turned on when global radiation was below 200 W m^{-2} and turned off when it exceeded 300 W m^{-2}. A standard greenhouse computer (Hogendoorn-Economic, Hogendoorn, Vlaardingen, The Netherlands) was used to control the greenhouse climate as well as supplementary assimilation light. During the experiment, average daily outside global radiation was 9 MJ m^{-2} d^{-1}; inside the greenhouse, average temperature was 21 °C, day time CO_2 concentration was 577 µmol mol^{-1} and relative humidity was 77 %. Daily photosynthetic active radiation (PAR) integral inside the greenhouse is presented in Fig. 1.
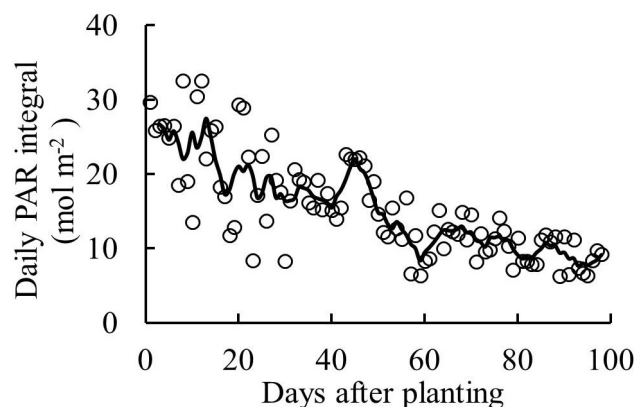
Fig. 1. Daily photosynthetic active radiation (PAR) integral inside greenhouse (sum of natural irradiance and supplementary assimilation light) during the experiment period. Line represents moving average over five days.

Treatments and plant management

All plants of each cultivar were divided into three fruit pruning groups, i.e. plants were pruned with commercial fruit load (standard truss), half commercial fruit load (half pruning truss) and single fruit per truss (Table. 1). Within each gutter of each cultivar, six plants were labeled for six periodic destructive harvests; these plants were maintained with commercial fruit load; one plant was labeled and maintained with half commercial fruit load, this plant was destructively harvested at the end of the experiment. The labeled plants were always surrounded by guard plants. All trusses of border plants and half of the guard plants were maintained with single fruit per truss. Part of these plants were used for determining the potential fruit growth rate. Fruit pruning was done immediately after fruit set for each truss. From 43 days after planting onwards, bottom leaves were regularly removed according to commercial practice. Fruits were picked when they turned red-ripe.

Plant development registration

Observations on flowering and fruit set were taken three times a week on 12 plants of each cultivar which were labeled for the last two destructive harvests (plants with commercial fruit load). Flowering was defined as three fully open flowers on a truss. Similar observations were taken on 15-20 plants of each cultivar which were pruned with single fruit per truss. Furthermore, the length and diameter of the fruits from plants with single fruit per truss were measured with caliper three times a week since fruit set in order to obtain fruit volume over time, number of measured fruits ranged from 34 to 48 fruits per cultivar. These observations were used for estimating the plant sink strength.

Destructive measurements

Six plants of each cultivar were destructively measured before planting (15 Aug) to determine their initial total biomass and leaf area. Thereafter, six labeled plants of each cultivar (one from each gutter) were harvested on 3,18 Sep, 2,16 Oct, 5 and 21 Nov. Fresh and dry weight of leaves, stems and fruit trusses were determined. Plant organs were dried for at least 48 h at 105 °C in a ventilated oven. Leaf area was measured with a leaf area meter (LI-3100C, Li-Cor inc., Lincoln, USA). Specific leaf area (SLA) was calculated by dividing leaf area by leaf dry weight. All plants with half pruning truss were destructively measured at the end of the experiment (21 Nov). The regularly removed leaves and harvested fruits from the labeled destructive harvest plants were dried and dry weight was added to obtain the total plant dry weight; area of the regularly removed bottom leaves from these plants was also determined

for estimating total LAI at different moments which was needed as model input.

For all cultivars, 97 to 148 fruits from the plants with single fruit per truss were randomly sampled during the experiment, and fruit diameter, length, age, fresh and dry weight were recorded. These observations were used to get two relationships: a relationship between fruit volume and fresh weight; and a relationship between fruit age and fruit dry matter content.

Sample collection and carbohydrates analysis

Plants labeled for periodic destructive harvests (plants with commercial fruit load) were also used for collecting leaf and stem samples for carbohydrate analysis. Leaf samples were taken at the beginning of the day (6:00-7:00 AM) at one day before each destructive harvest. The samples were taken at every other leaf from leaf number 5 (uppermost fully expanded leaf; leaf number 1 was the uppermost leaf longer than 5 cm) downward to the bottom canopy. In each selected leaf, one leaflet adjacent to the terminal leaflet was collected. The collected leaflets from one plant were pooled together to represent one canopy leaf sample. Stem samples were taken during each destructive harvest. Stem sections (0.5 cm length) were taken from top to the bottom where the leaf samples were taken, these sections were pooled together to represent one stem sample. Six replicates were taken for each type of sample at each time. Fresh weight of all collected samples were determined and added to the total plant weight.

The samples were inserted in vials and flash-frozen in liquid nitrogen. They were transferred to a freezer (-80 °C) for storage. Starch and soluble sugar content were measured using the method described in Savvides *et al.* (2014); the soluble sugars that were monitored were fructose, glucose and sucrose.

Leaf photosynthesis measurements

Leaf photosynthesis rates were measured with a portable gas exchange device equipped with a leaf chamber fluorometer (Li-6400; LI-COR) at leaf number 6 from top of the canopy. The measurements were taken at the plants with commercial fruit load as well as half commercial fruit load. Measurements were taken at six moments during the experiment (20, 28, 39, 54-55, 64-65 and 75-76 days after planting, respectively. Since 54 days after planting the measurements of the plants with half commercial fruit load were included). For each cultivar at each measurement moment, 6 measurements were taken before noon (12:00) and 6 were taken after noon. In the measurement chamber, light intensity (10% blue, 90% red) was 1000 μmol m^{-2} s^{-1}, CO_2 concentration was 500 μmol mol^{-1}, air temperature was 23 °C and VPD was between 0.5-1 kPa.

Plant source/sink ratio determination

Plant growth rate (g dry mass plant^{-1} day^{-1}) was considered as source strength, which was calculated using the crop growth model TOMSIM (Heuvelink, 1996b) with measured SLA (from planting date to first destructive harvest date) and LAI (from first destructive harvest date onwards), dry matter partitioning to plant parts (leaves, fruits, stems, roots), and the registered climate data (global radiation, intensity and timing of the supplementary assimilation light, greenhouse temperature and CO_2) as input. The SLA, LAI, dry matter partitioning to leaves, stems and fruits were calculated from the destructive measurements, the dry matter partitioning to roots was set according to Heuvelink (1995). A specific factor was determined to calibrate the model simulated plant growth rate. This factor was estimated by minimizing the sum of squares of the residuals between periodical destructive harvests determined and simulated total dry weight.

Fruit sink strength, quantified by the potential fruit growth rates, was obtained by non-destructive measurements on potentially growing fruits (i.e. one fruit per truss). On the basis of the lengths and diameters of the potentially growing fruits, their volume was calculated assuming a deformed sphere

$$v = \frac{4}{3}\pi\left(\frac{d}{2}\right)^2\frac{h}{2}$$ (1)

where v is fruit volume (cm^3), d is fruit diameter (cm), h is fruit length (cm).

Fruit volume was subsequently converted into fresh weight, using a cultivar specific linear regression between fruit volume and fruit fresh weight (r^2 = 0.97-0.99 for three cultivars). A Gompertz function was fitted through fresh weight over time

$$w(t) = w_{max}e^{-e^{-k(t-t_m)}}$$ (2)

where $w(t)$ is the weight at age t (d after anthesis), w_{max} is upper asymptote of fruit weight (g), k represents the weighted mean relative growth rate and t_m the age (d) at maximum growth rate.

The Gompertz function was fitted through the data with non-linear mixed modelling. Non-linear mixed models take into account that the measurements on one fruit are grouped. A lower variation is assumed between the measurements of one fruit than between the measurements of different fruits. The three parameter means (w_{max}, t_m, k) were estimated to describe fruit growth (Wubs *et al.*, 2009).

A fourth-order polynomial function was fitted through the data of fruit age and dry matter content of fruits. The necessity of this relationship was described by Wubs *et al.* (2012).

The single fruit sink strength (representing the potential growth rate in g dry matter per day) was the product of the derivative of the Gompertz function and the fourth-order polynomial function. The sink strength of a set fruit was calculated for each day over the average fruit growth duration (from flowering to harvest). The daily total fruit sink strength of a plant was calculated by accumulating the sink strength of all fruits which were present that day.

Vegetative sink strength was estimated as the integral of sink strengths of each vegetative unit (De Koning, 1994; Heuvelink, 1996b). The development rate of a vegetative unit (stem and three leaves between two trusses) is equal to that of a fruit (De Koning, 1994) and a unit stops growing when its development stage exceeds 1. Therefore, possible growth of (the stem part of) a vegetative unit after this stage has been reached, was ignored.

$$PVGR = ae^{-0.168(T-19)}PFGR \qquad (3)$$

where *PVGR* is the potential growth rate for a vegetative unit (g d^{-1}) and *PFGR* is the potential fruit growth rate (g d^{-1}) for a single fruit, *a* is a specific factor between potential fruit growth rate and growth rate of a vegetative unit, which was estimated by minimizing the sum of squares of the residuals between measured and simulated dry matter partitioning to fruits; this factor is cultivar dependent. *T* is the average greenhouse temperature during the experiment period (°C).

Usually about three vegetative units precede the first truss (Dieleman and Heuvelink, 1992), the sink strength of these three units were estimated by using *PVGR* multiply three specific factors [0.6, 0.75, 0.9, respectively, from the first to the third unit, these factors were derived based on Heuvelink (1996a)], this is because the first few units are relatively small and hence have a low sink strength. The daily total vegetative sink strength of a plant was calculated by accumulating the vegetative sink strength of all units which were present that day. For a more detailed description see De Koning (1994) and Heuvelink (1996a).

Source/sink ratio was calculated based on source strength, total fruit sink strength and vegetative sink strength.

Statistical analysis

The effects of cultivars, days after planting, and fruit pruning treatments on destructive harvest determined parameters, carbohydrate content and leaf photosynthesis were evaluated by Fisher's protected least significant difference test (l.s.d), assuming replications in the same greenhouse compartment as being independent.

RESULTS

Plant growth

Maximum fruit growth rate and fruit growth duration were highest in 'Komeett'; while these parameters were lowest in 'Sunstream' (Fig. 2). These differences together resulted in the largest potential fruit size in 'Komeett' and smallest in 'Sunstream' (Table 1).

'Sunstream' had highest LAI during a large part of the growing period (Fig. 3A), and highest total dry weight except for the initial period after planting (Fig. 3B); while these parameters were not much difference for 'Komeett' and 'Capricia' (Fig. 3). For all cultivars, plant total dry weight was not affected by the half fruit pruning treatments (Table 2). However, half fruit pruning treatments resulted in significantly higher dry mass partitioning to leaves and stems, and lower partitioning to fruits (Table 2).
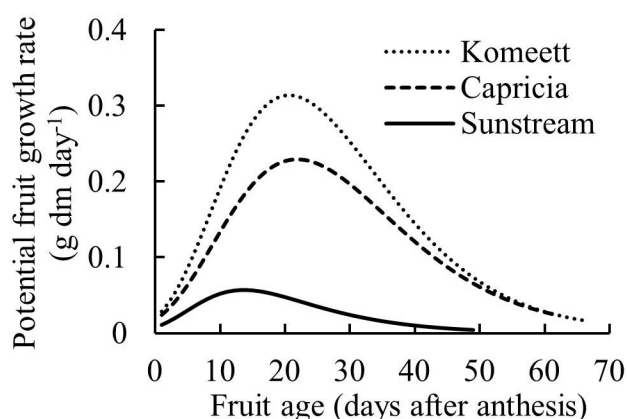
Fig. 2. Potential fruit growth rate curves for three tomato cultivars. Curves end at the average growth duration (time from anthesis until harvest ripe) of each cultivar. Individual curves are derivatives of fitted Gompertz function [Eqn (2)] through fruit fresh weight over time multiplied by fruit dry matter content over time.

Table 1. Potential fruit size, maximum fruit growth rate, fruit growth duration and the fruit number per truss for the three tomato cultivars. Standard error is given for the measured variables within parentheses.

Cultivar	Potential fruit size (g fm fruit^{-1})	Maximum fruit growth rate (g dm d^{-1})	Fruit growth duration (d)	Number of fruits per truss	
				Standard truss	Half pruning truss
'Komeett'	180 (±4.6)	0.31	66 (±0.5)	5	2
'Capricia'	137 (±5.4)	0.23	62 (±0.7)	6	3
'Sunstream'	20 (±3.0)	0.06	49 (±1)	10	5

fm indicates fresh mass; dm indicates dry mass.
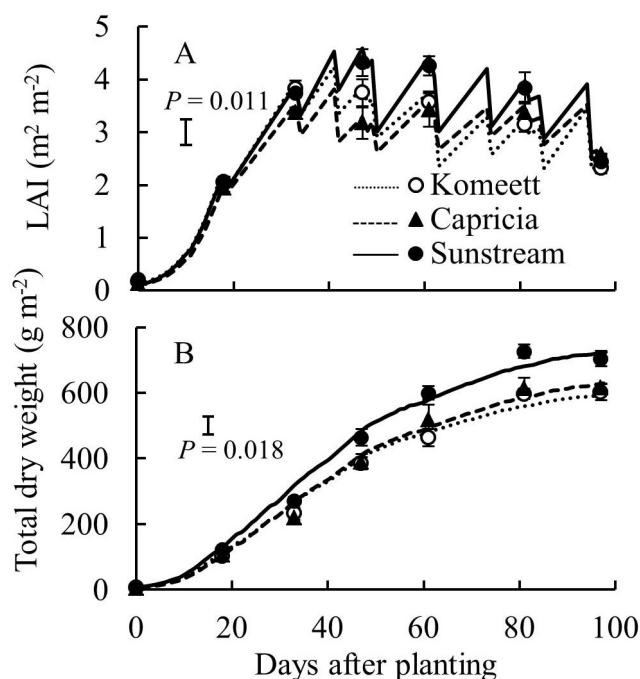
Fig. 3. Measured (symbols) and simulated (lines) leaf area index (LAI) (A) and total dry weight (B) over time for three tomato cultivars. Error bars through data points show ± s.e. (n = 6). Error bar and *P* value in each figure represent least significant difference (l.s.d) and the significance of the interaction between cultivar and days after planting.

Table 2. Plant total dry mass and dry mass partitioning to leaves, stems and fruits of three tomato cultivars in response to fruit pruning treatment (data are collected at the end of the experiment, n = 6).

Treatment	Total dry weight (g plant^{-1})	Dry mass partitioning (%)		
		Leaves	Stems	Fruits
'Komeett'				
Standard truss	271.5 a	37.9 a	16.3 a	45.8 b
Half pruning	275.1 a	42.3 b	20.2 b	37.5 a
'Capricia'				
Standard truss	278.2 a	36.3 a	17.3 a	46.4 b
Half pruning	277.0 a	41.0 b	19.5 b	39.5 a
'Sunstream'				
Standard truss	317.3 a	45.2 a	20.1 a	34.7 b
Half pruning	316.4 a	52.7 b	25.1 b	22.2 a

Means followed by different letters within one column of each cultivar differ significantly (*P* ≤ 0.05) as established by the least significant difference (l.s.d) test.

Carbohydrate content and leaf photosynthesis rate

In tomato stems, starch content was negligible compared to sugar content which was apparently the main carbohydrate in stems (Fig. 4A,B). For all cultivars, soluble sugar content was at a relatively high level until 33 days after planting. Thereafter, it decreased gradually till the end of the experiment (Fig. 4A). This phenomenon was not observed for starch content which reached a peak at 33 days after planting for 'Capricia' and 'Sunstream', and remained relatively constant from 60 days after planting onwards for all three cultivars (Fig. 4B). 'Sunstream' had higher sugar and starch content than the other two cultivars except for at 18 days after planting (Fig. 4A).

Fig. 4. Time course of the soluble sugar (A, C) and starch (B, D) concentration in the stems (A, B) and leaves (C, D) of three tomato cultivars. Soluble sugar is the sum of glucose, fructose and sucrose. Error bars through data points show ± s.e. (n = 6). Error bar and *P* value in each figure represent least significant difference (l.s.d) and the significance of the interaction between cultivar and days after planting.
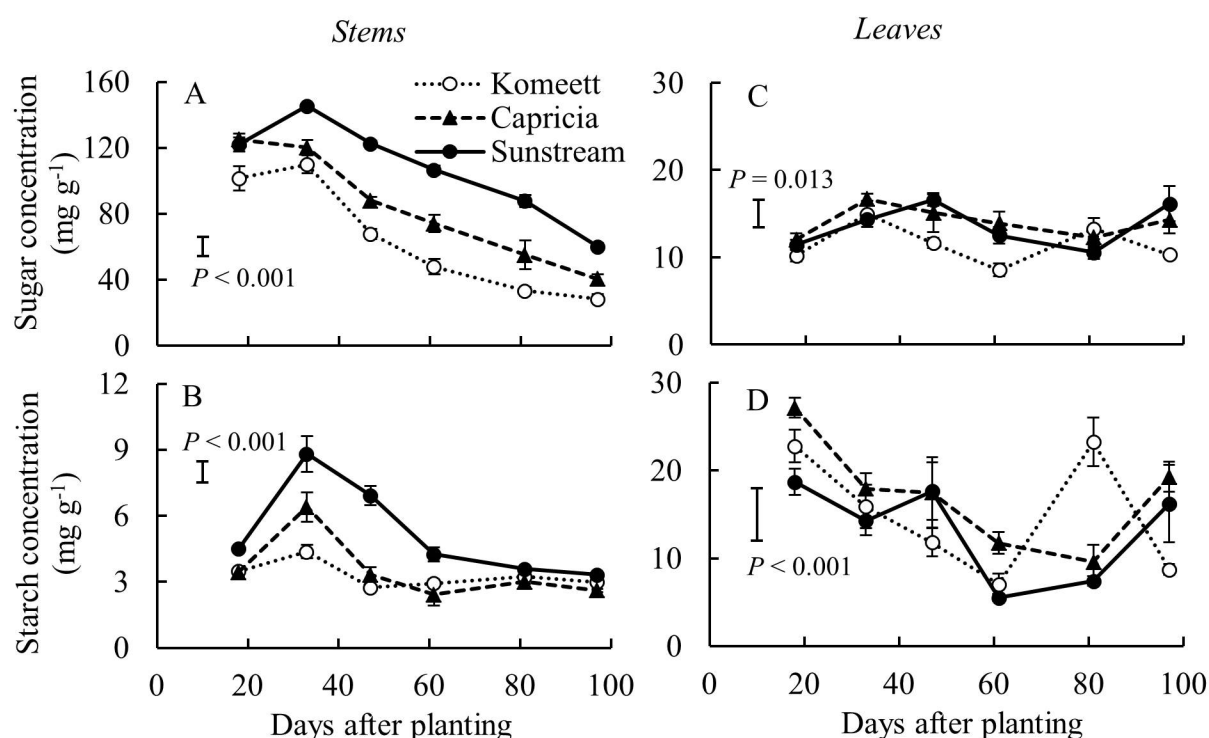
In leaves, soluble sugar content was relatively constant during the growing period compared to starch content (Fig. 4C,D). For all cultivars, starch content was initially (18 days after planting) high and decreased gradually until 60 days after planting. Surprisingly, starch content at 80 days after planting suddenly increased and reached to the level as high as that

observed at 18 days after planting in 'Komeett'. At the end of the experiment, starch content increased in 'Capricia' and 'Sunstream' (Fig. 4D).

For all cultivars, the highest leaf photosynthesis rates were observed at 28 days after planting; thereafter it decreased gradually until the end of the experiment (Fig. 5). Interestingly, leaf photosynthesis rates at 20 days after planting were slightly lower than at 28 days after planting, although this difference was only significant in 'Capricia' (Fig. 5). Furthermore, 'Capricia' had higher leaf photosynthesis rates than the other two cultivars. Half fruit pruning treatments had no effect on leaf photosynthesis rates in all three cultivars (data not shown).
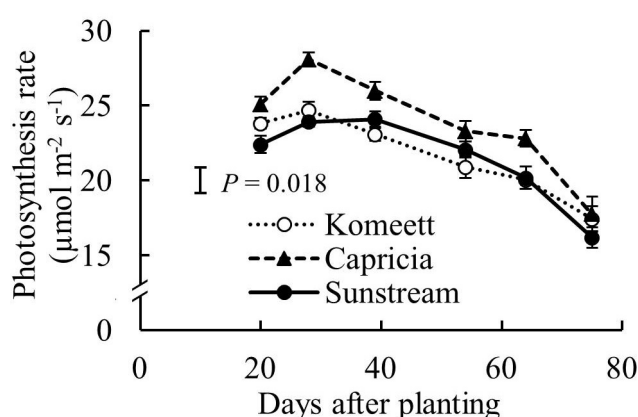
Fig. 5. Time course of the leaf photosynthesis rate of leaf number six from top of the canopy. In the measurement chamber, light intensity, CO_2 concentration, air temperature and VPD were maintained at 1000 μmol m^{-2} s^{-1}, 500 μmol mol^{-1}, 23 °C and 0.5-1 kPa, respectively. Error bars through data points show ± s.e. (n = 12). Error bar and *P* value represent least significant difference (l.s.d) and the significance of the interaction between cultivar and days after planting.

Source-sink balance and its relationship with plant carbohydrate content

The vegetative sink strength differed between cultivars and was highest for 'Sunstream' and lowest for 'Capricia' (Fig. 6A). The generative sink strength was highest for 'Komeett' and lowest for 'Sunstream' (Fig. 6B). Furthermore, the generative sink strength was initially low and soon increased to a plateau and kept constant onwards. 'Sunstream' had highest total plant sink strength before 25 days after planting; thereafter, 'Komeett' had highest and 'Sunstream' had lowest total plant sink strength (Fig. 6C).
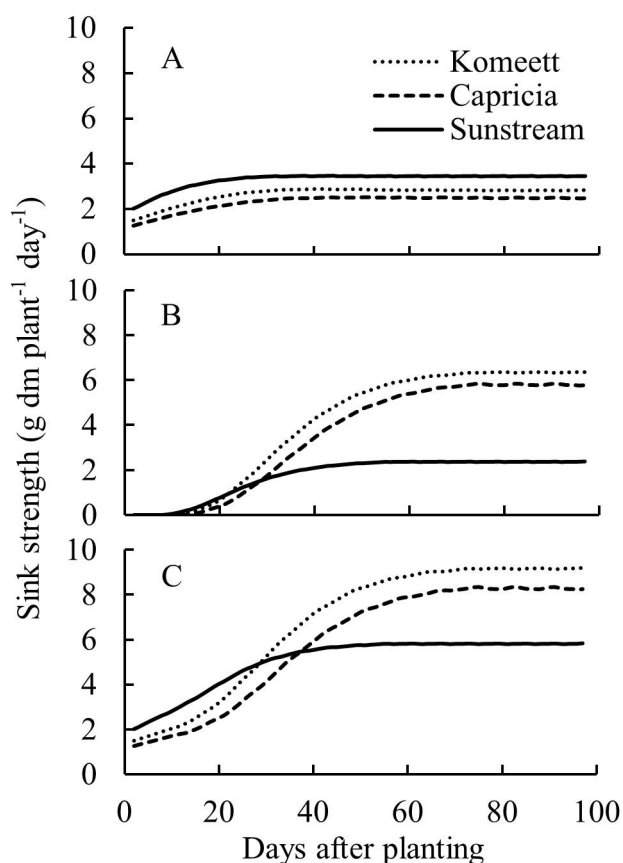
Fig. 6. Simulated vegetative (A), generative (B), and total (C) plant sink strength over time for the three tomato cultivars. Lines are moving averages over five days.

Source strength (crop growth rate) was initially low and drastically increasing until about 30 days after planting (Fig. 7A); it was decreasing from 45 days after planting onwards till the end of the experiment. 'Sunstream' had slightly higher source strength than the other two cultivars during a large part of the growing period (Fig. 7A).

Plant source/sink ratio was initially low (below 1) for all three cultivars, and it soon exceeded 1 in 'Komeett' and 'Capricia', and came close to 1 in 'Sunstream' (Fig. 7B). 'Komeett' had shorter duration of sink limitation than 'Capricia', the source/sink ratio in 'Komeett' was also lower than in 'Capricia'. During the fully-fruiting stage, source/sink ratio was lower than 1 for all three cultivars, 'Sunstream' had the highest and 'Komeett' had lowest source/sink ratio during this stage. Total carbohydrate content in stems and leaves over the three cultivars increased linearly with the source/sink ratio (Fig. 8).
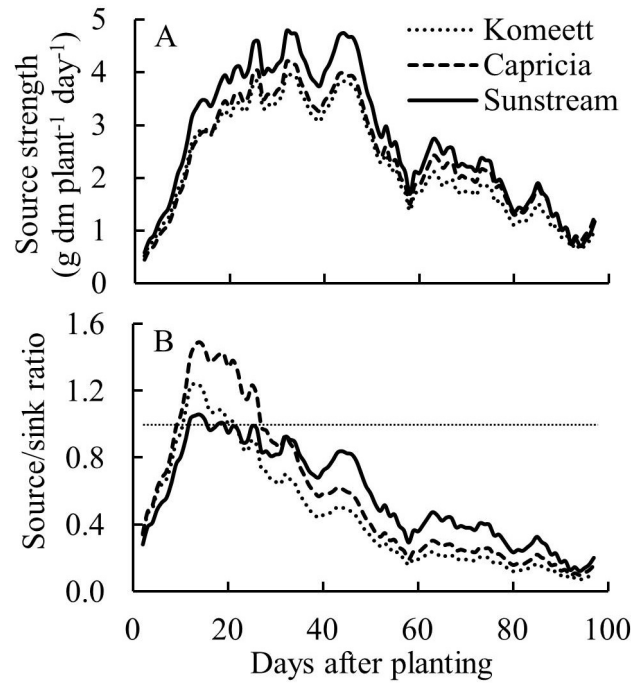
Fig. 7. Simulated source strength (crop growth rate) (A) and source/sink ratio (B) over time for the three tomato cultivars. Lines are moving averages over five days. Dashed horizontal line in B represents a source/sink ratio of 1.
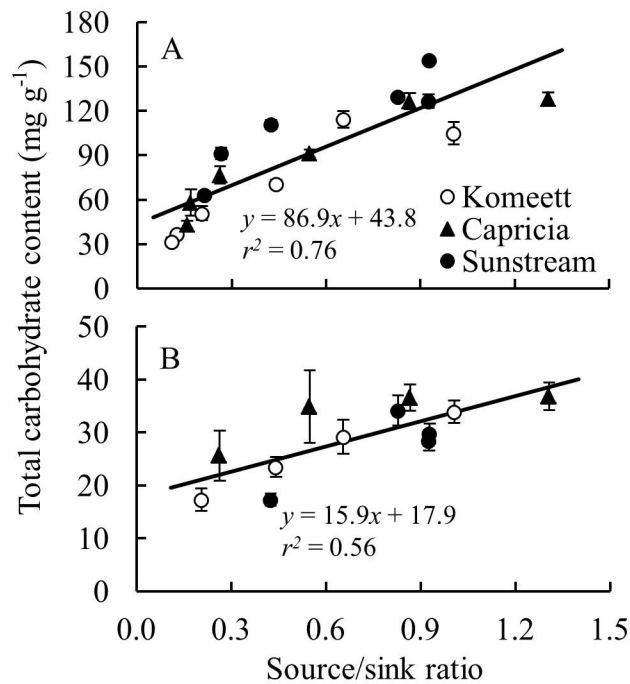
Fig. 8. The relationship between total carbohydrate content (sum of soluble sugar and starch content) and plant source/sink ratio in stems (A) and leaves (B) over three tomato cultivars. Lines represent linear regression line. In B, carbohydrate content determined at 81 and 97 days after planting (Fig. 4D) were not included as these data were unexpected and remain unexplained.

DISCUSSION

Tomato plants are sink-limited during their early growth stage in greenhouses with ample irradiance

Young plants are likely to be sink limited (Ark and Drake, 1991). Indeed, we found in our study that three types of tomato plants experienced a period of sink limitation or came close to sink limitation during their early growth stage (Fig. 7B). Sink limitation during the early growth stage was caused by the relatively low total plant sink strength (Fig. 6C) combined with a fast increase in source strength (Fig. 7A). This increase in source strength resulted from a fast increase in leaf area index (LAI). In addition, irradiance might also have played an important role, because sink limitation was observed during early September during which plants had ample natural irradiance for photosynthesis compared to late autumn and winter months (Fig. 1). The combination of relatively high irradiance and fast increase in LAI with limited reproductive organs during the early growth stage, resulted in plants not being able to use the extra assimilates, so that a relatively high sugar content in stems was observed during this stage (Fig. 4A). Tomato stems have been reported as an important storage organ for assimilates (Hocking and Steer, 1994), this is in line with our study that carbohydrate content in stems was higher than in leaves. In stems, sugar content was significantly higher than starch content (Fig. 4A). Starch is predominantly utilized for diurnal carbon storage in leaves, it degrades to soluble sugar at night for mobilization and utilization (Smith and Stitt, 2007). In leaves the highest starch content during the growing period was observed at 18 days after planting which was during the period of sink limitation (Fig. 4B). Similarly, Nakano *et al*. (2000) and Plaut *et al*. (1987) also reported starch accumulation in leaves when sink limitation occurs.

Photosynthetic capacity often correlates with the source-sink balance (Iglesias *et al*., 2002; McCormick *et al*., 2006). In this study, leaf photosynthesis rates at 20 days after planting were slightly lower than at 28 days after planting when measured at the same conditions, although this difference was only significant for 'Capricia' (Fig. 5). Sink limitation around 20 days after planting in combination with the relatively high starch content in leaves (Fig. 4D) might have led to a slight down-regulation of leaf photosynthesis (Nakano *et al*., 2000; Paul and Foyer, 2001; Iglesias *et al*., 2002). Irradiance induced acclimation could not play a role because the daily light sum was similar during this period (Fig. 1). When young tomato plants not yet producing fruits were grown under elevated CO_2, this resulted in photosynthetic acclimation (Yelle *et al*., 1989; Besford, 1993), which is probably caused by

an imbalance in the supply and demand of assimilates. These studies further indicate that tomato plants are likely sink limited during the early growth stage.

Source-sink balance is cultivar specific (Fig. 7B). During the early growth stage cultivar differences in source/sink ratio were mainly due to differences in vegetative sink strength, as reproductive organs had hardly been formed or were still small and source strength was similar for the different cultivars (Fig. 7A). 'Sunstream' had the highest vegetative sink strength (Fig. 6A), and hence the lowest source/sink ratio during this period (Fig. 7B). Wubs *et al*. (2009) also reported that cultivars with the smallest potential fruit size had the highest vegetative sink strength in sweet pepper. 'Capricia' had the lowest vegetative sink strength and the highest source/sink ratio during the early growth stage (Fig. 7).

Fruiting tomato plants are source limited and source/sink ratio negatively correlates with the potential fruit size when commercial fruit load is maintained

A major change in plant development is the switch from vegetative growth to generative growth. In our experiment this change was followed by a marked change in source-sink balance (Fig. 7B). For all three cultivars, source/sink ratio was below 1 during the fully fruiting stage (Fig. 7B), suggesting source limitation. This is also supported by the observation that pruning half of the fruits did not influence the total plant dry weight (Table 2). This result is in agreement with many previous studies that fruiting tomato plants grown in greenhouses are source limited (De Koning, 1994; Cockshull and Ho, 1995; Heuvelink and Buiskool, 1995; Matsuda *et al*., 2011; Qian *et al*., 2012). Our results contradicts those of Dueck *et al*. (2010) who estimated that cherry tomato is most likely sink limited. The source/sink ratio of fruiting tomato plants in this study (average source/sink ratio was 0.17-0.33 from 50 days after planting onwards for all three cultivars) was lower than the value (about 0.5) which has been reported by De Koning (1994) and Heuvelink (1996b). This is mainly attributed to the low irradiance level (Fig. 1) in combination with the high sink strength (Fig. 6B). Furthermore, De Koning (1994) reported that tomato potential fruit growth rate is positively correlated with the irradiance level. In this study, the potential fruit growth rate used for sink strength estimation was mainly determined from those fruits that developed under relatively high irradiance level. This might have slightly overestimated the sink strength of the plants during the low irradiance period. Additionally, fruit position within a truss also plays a role, i.e. potential growth rate of the first six fruits was higher than the other fruits within a truss (De Koning, 1994). In this study, the potential fruit growth rate was estimated from the first three fruits within a truss, therefore, the sink strength of 'Sunstream' (10 fruits

per truss) might have been overestimated. Although there were several pitfalls for sink strength estimation in this study, the average fresh weight of the individual ripe harvested fruits from the half fruit pruning plants was 1.4, 2.2 and 2.3 times higher than the fruits from plants with standard fruit load in 'Sunstream', 'Capricia' and 'Komeett', respectively. This clearly indicates that for all three cultivars fruiting tomato plants are source-limited.

During the fully fruiting stage, generative sink strength played a pivotal role in determining the source/sink ratio, because differences in source strength and vegetative sink strength between cultivars were relatively small (Fig. 6). 'Sunstream' (cherry tomato) showed the lowest generative sink strength, while 'Komeett' (large-sized fruits) showed the highest generative sink strength (Fig. 6B). Hence, a negative correlation between potential fruit size and source/sink ratio during the fully fruiting stage was observed when commercial fruit load was maintained (Fig. 7B).

Plant carbohydrate content is positively correlated with the source-sink balance (Schnyder, 1993; Iglesias *et al.*, 2002; Li *et al.*, 2002), this is supported by a common linear relationship between plant source/sink ratio and total carbohydrate content in stems (Fig. 8A) as well as in leaves (Fig. 8B) for different cultivars during plant growth. During the fully fruiting stage, source/sink ratio was lower than during the early growth stage except for the first two weeks after planting when LAI was very low (Fig. 7B). Accordingly, carbohydrate content (i.e. sugar content in stems and starch content in leaves) during the fully fruiting stage was generally lower than during the early growth stage (Fig. 4). Among the three cultivars, 'Sunstream' showed the highest source/sink ratio and consequently the highest sugar content in stems during the fully fruiting stage, while 'Komeett' showed the lowest source/sink ratio and sugar content in stems (Fig. 4A). The positive correlation between carbohydrate content in stems and source/sink ratio was also observed by Ho *et al.* (1983) as well as Hall and Milthorpe (1978). In leaves, the sudden increase in starch content at 80 days after planting in 'Komeett' and to a lesser extent at 97 days after planting in the other two cultivars was unexpected as source/sink ratio was very low during this period (Fig. 7B); this remains unexplained.

Implications

Fruiting tomato plants are strongly source limited even for cherry tomato ('Sunstream') as indicated by the low source/sink ratio (average source/sink ratio from 50 days after planting onwards was 0.17-0.33 for three tomato cultivars). Despite the application of supplementary assimilation light (162 μmol m^{-2} s^{-1} PPFD; maximum 10 hours per day), irradiance in the

greenhouse declined due to decreased natural irradiance towards the winter. Therefore, extending the duration or increasing the PPFD of supplementary assimilation light in combination with maintaining lower fruit load could be considered to maintain a balance between source and sink strength. Early growth stage tomato plants showed sink limitation as indicated by a source/sink ratio exceeding 1. For sink-limited plants, giving more light will not increase plant growth as surplus assimilates in leaves could down-regulate leaf photosynthesis.

CONCLUSIONS

Our conclusions are: (1) tomato plants are sink-limited during the early growth stage under ample irradiance, (2) under commercial crop management fully fruiting tomato plants are source-limited, this is also the case for small fruited cherry tomato, (3) during the fully fruiting stage of tomato cultivars, the source/sink ratio is negatively correlated with the potential fruit size when commercial fruit load is maintained, and (4) carbohydrate content in tomato stems and leaves increases linearly with the plant source/sink ratio.

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CHAPTER **6**

General discussion

T. Li

Horticulture and Product Physiology Group, Wageningen University,
P.O. Box 630, 6700AP, Wageningen, the Netherlands

Improving crop production is a major goal in agriculture in order to meet the rapidly growing world food demand (Miflin, 2000). In greenhouse horticulture, production improvement is often a way to reduce production costs. Greenhouses offer the possibility to control growth conditions and therefore fully understanding the response of crop growth and physiology to growth conditions is necessary. In greenhouses, growth conditions such as water and nutrient availability, temperature and CO_2 concentration are already fairly well controlled. Light is the driving force for crop photosynthesis and often limiting growth in greenhouses. Therefore, this thesis aims at improving the radiation use efficiency (RUE) in greenhouse production systems. As explained in Chapter 1, RUE is a multi-definitional term, which can be defined at leaf level, crop level as well as the production system level. In a production system, any factor increases crop production has a direct consequence for increasing RUE. In this chapter, we discuss the source-sink balance, light distribution and light quantity (allowing more light in greenhouses) and relating it to some practical points and perspectives for further research.

Identifying crop source-sink balance provides implications for improving crop production

Plant growth closely correlates with source and sink strength and the balance between them (Gifford and Evans, 1981; Wardlaw, 1990; Smith and Stitt, 2007). Source strength is defined as the rate at which assimilates are produced (photosynthesis rate); while sink strength is the competitive ability of an organ to attract assimilates (Marcelis, 1996). For a source limited crop, crop growth depends on the source strength therefore an increase in light intensity and CO_2 increase photosynthesis and plant growth. For a sink limited crop, however, there are not sufficient sinks to utilize all the produced assimilates, thus increasing light intensity and CO_2 might lead to feedback inhibition of photosynthesis. In this respect, understanding the plant source-sink balance may give implications in the regulation of crop growth.

There is not a straightforward method of measuring the plant source and sink strength. Manipulating source and sink organs (e.g. fruit and leaf pruning) are often applied to investigate plant source-sink balance (Cockshull and Ho, 1995; Matsuda *et al.*, 2011; Iglesias *et al.*, 2002). These studies were usually qualitatively interpreted. Quantitative approaches (e.g. model simulation) for interpreting source-sink balance have also been available to date (De Koning, 1994; Marcelis, 1994; Heuvelink, 1996; Wubs, 2010), which can provide information about the extent of imbalance between source and sink strength. In this thesis (Chapter 5), the source/sink ratio from early growth stage to fully fruiting stage of three tomato cultivars with different fruit size has been quantitatively estimated. Plant source

strength was estimated by the crop growth model TOMSIM (Heuvelink, 1996). Potential fruit growth rate was used as a measure of fruit sink strength (Heuvelink and Marcelis, 1989), which was estimated from non-destructively measuring the fruit growth rate at non-limiting assimilate supply as created by removing all except one fruit on each tomato truss. An important issue regarding the determination of the sink strength is whether the growth of the measured fruits is really potential. Wubs (2010) indicated that the ideal experiment to measure the sink strength should be performed under levels of high irradiance and CO_2 concentration. De Koning (1994) showed that fruit position within a tomato truss also plays a role in determining the potential fruit growth rate. The vegetative sink strength was derived from the estimated fruit sink strength, the simulated source strength and the observed dry matter partitioning. This derivation is based on a number of assumptions which have been reasonably proven so far (De Koning, 1994; Heuvelink, 1996).

Leaf photosynthesis has been demonstrated to depend on the sink demand in various species (Marcelis, 1991; Nakano *et al.*, 2000; Iglesias *et al.*, 2002; McCormick *et al.*, 2006). This might be important for crops, such as tomato, where sink demand varies considerably during its life span. In this thesis (Chapter 5), tomato plants showed a period of sink limitation during the early growth stage (limited reproductive organs existed) as indicated by the source/sink ratio was higher than 1, during which carbohydrate content in leaves and stems were relatively high, and also a slightly lower leaf photosynthesis was observed during this period. In some studies, high CO_2 concentration resulted only in a temporary stimulation of photosynthesis for young plants which had not yet producing fruits (Peet *et al.*, 1986; Yelle *et al.*, 1989; Besford, 1993). One reason for the failure to maintain high photosynthetic inputs over prolonged periods under high CO_2 concentrations may be due to the sink demand for assimilates could not keep up with the photosynthetic source supply. The negative feedback of sink demand on photosynthesis has also been shown during the fully fruiting stage when reducing sink demand by an extreme reduction of fruit number in cucumber (Marcelis, 1991). Our experiment showed that leaf photosynthesis was not influenced by pruning half the number of fruits, which is in line with the fact that during the fully fruiting stage tomato plants were strongly source limited (Chapter 5).

In an optimum situation the sink and source strength should be kept in the right balance. In our study, the source strength appeared to be far less than the sink demand during the fully fruiting stage in all the three tomato cultivars as indicated by the low source/sink ratio (average source/sink ratio was 0.17-0.33 from 50 days after planting onwards) (Chapter 5). Dry matter partitioning is primarily regulated by the sinks (Marcelis, 1996). A relatively large

fraction of dry matter is partitioned into the fruits which are usually considered as the strongest sinks of a plant. Under severely source limited situation, vegetative organs (e.g. leaf and root) may almost stop growing. This further influences plant growth because photosynthetically active leaf area is needed to capture the incident light for photosynthesis. In addition, low assimilates availability can lead to flower/fruit abortion which is an important yield-limiting factor in many greenhouse crops, which is closely correlated with the source/sink ratio (Marcelis *et al.*, 2004; Wubs *et al.*, 2009; Kang *et al.*, 2011). Therefore, reducing the sink strength or increasing the source strength should be considered for maintaining a balance between source and sink strength.

Total fruit sink strength can be reduced by removing flowers or fruits from the plant. Fruit pruning enhances the growth of all remaining parts of the plant (Heuvelink and Buiskool, 1995). This was also observed in our study (Chapter 5). At sufficient sink capacity, fruit pruning may in some cases even result in equal (Hurd *et al.*, 1979) or even higher (De Koning, 1994, simulation results) total fruit yield, because leaf area and hence the source strength of the plant increased after fruit pruning. Choosing a lower plant density in winter than in summer could also be considered to compensate for the seasonal variation in source strength per plant. Cockshull and Ho (1995) showed that decreasing plant density increases the fruit size and produced more uniform size of tomato fruits in greenhouses. The source strength can be increased by increasing the CO_2 concentration and irradiance level. An increase in source strength immediately enhances fruit growth when plants are source limited; in the long term the fraction of dry matter partitioned into the fruits also increases due to the reduction of fruit abortion (Marcelis, 1993). Therefore, greenhouse production in northern latitudes relies typically on CO_2 enrichment as well as supplementary assimilation light to stimulate growth and productivity (Ayari *et al.*, 2000). Source strength could also be stimulated by improving the light distribution in the canopy. In this thesis (Chapter 2 and 4), we have explored the effects of light distribution on source strength (crop photosynthesis).

Improving light distribution improves crop photosynthesis

Crop photosynthesis to a large extent depends on the light distribution and interception in the crop canopy (Sarlikioti *et al.*, 2011a; Sarlikioti *et al.*, 2011b; Duursma *et al.*, 2012). It is obvious that the more homogeneous light distribution in the crop canopy the higher the crop photosynthesis, because the photosynthetic rate of a single leaf shows a curvilinear response to the light flux density (Marshall and Biscoe, 1980). Canopy light distribution and photosynthesis are highly affected by the fraction of diffuse light (Gu *et al.*, 2002; Mercado *et*

al., 2009; Urban *et al.*, 2012). Previous studies have investigated the effect of diffuse light on light distribution and its consequences for canopy photosynthesis by comparing plant responses on cloudy and clear days (Still *et al.*, 2009; Urban *et al.* 2012; Zhang *et al.*, 2011); or by comparing the aftermath of volcanic and anthropogenic emissions (Farquhar and Roderick, 2003; Gu *et al.*, 2003; Mercado *et al.*, 2009). However, in these studies global radiation decreased simultaneously with the increase in the fraction of diffuse light. Therefore, these studies cannot demonstrate the pure effects of diffuse light on plant photosynthesis. Nowadays, diffuse glass is available, which transforms a portion of direct solar light into diffuse without affecting the light transmission (Hemming *et al.*, 2013). In this thesis (Chapter 2, 3 and 4), diffuse glass was applied as greenhouse cover. This provides the opportunity to explicitly explore the effects of diffuse light on light distribution over the canopy and its direct and indirect effects on crop photosynthesis and plant growth.

Effects of diffuse light on spatial light distribution

In the crop canopy, distinct lightflecks make an apparent sharp contrast with the shaded portions of foliage on clear days, this phenomenon is commonly observed in the canopies both in open field and in greenhouses with the commonly used clear glass (Fig. 1, 0 % haze). The variability of light intensity in the horizontal plane of the canopy has rarely been quantified, except for Acock *et al.* (1970). In this thesis (Chapter 2), light intensities in the horizontal plane of the fully developed tomato canopy were measured at many points on clear as well as cloudy days. The horizontal light distribution was quantified by the relative frequency distribution of light intensities. In the vertical direction of the canopy, light intensity under the plant canopy is often very low compared with the top canopy. This reduction of light intensity is a result of light interception by plant organs within the canopy. The particular pattern of light intensity along the vertical profile of the canopy is correlated with the downward cumulative leaf area index (LAI) as well as canopy architecture, which can be well described by a negative exponential equation according to the lambert beer-law (Monsi and Saeki, 2005). In this equation the light extinction coefficient (k) is an important parameter. For determining this parameter, laborious and time consuming measurements are required and this explains why many indirect approaches were developed for its assessment (Sinoquet *et al.*, 2000; Pronk *et al.*, 2003; Wang *et al.*, 2007). We measured light intensity and non-destructively estimated LAI at every 25 cm from top to bottom of the canopy to derive the parameter k. Thus, the current estimation of k can well represent vertical light distribution within the canopy. We showed that increasing the fraction of diffuse light smoothed the

variation of light distribution in the horizontal plane of the canopy and resulted in a deeper light penetration as characterized by a lower *k* on clear days (Fig. 1, 71 % haze). In our study, the comparison of vertical light distribution between treatments was determined during the middle of the day (11-14h). Nevertheless, the vertical light distribution in the canopy could vary during the day, which might be due to diurnal variation of solar position and leaf movement (Kao and Forseth, 1992). This is important for understanding the effect of diffuse light on spatial light distribution within the crop canopy and should therefore be taken into account in further study.

A homogeneous light distribution within the crop canopy under diffuse light gives rise to the question whether plant physiological and morphological acclimation occurs. We showed that diffuse light treatments resulted in a higher leaf photosynthetic capacity in the lower part of the canopy and also a higher LAI compared with the clear glass treatment. These effects were probably due to a relatively higher average light intensity of middle and bottom leaves.
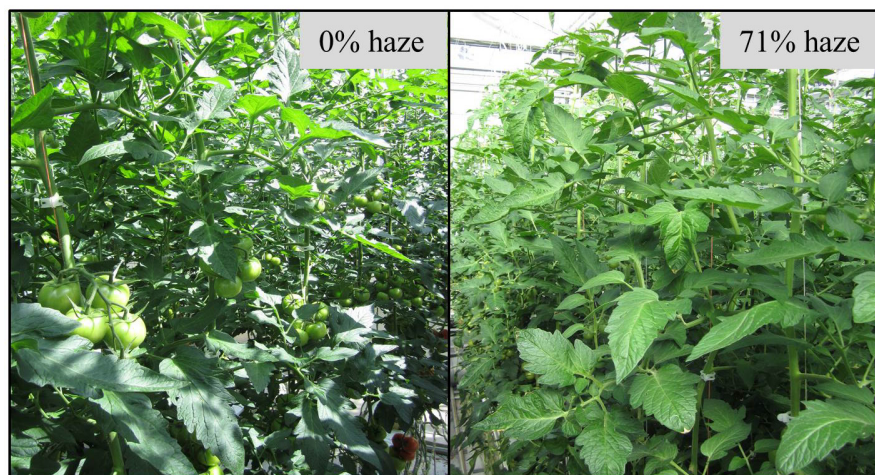
Fig. 1. Light distribution in tomato canopy in the conventional clear (0% haze) and diffuse (71% haze) glasshouse on a clear day. Light is more homogeneously distributed in 71 % haze compared with 0 % haze treatment where many light and shaded spots in the middle and lower of the canopy.

The importance of this work was not confined to explore which factors are affected by diffuse light, but also to quantitatively understand to what extent each of these factors influence the crop photosynthesis. For this, we adapted the crop photosynthesis model (Spitters, 1986; Spitters *et al*., 1986) and combined with the measured vertical and horizontal light distribution, leaf photosynthesis and LAI to calculate crop photosynthesis. We showed that crop photosynthesis was enhanced by 7.2 % under diffuse light (71 % haze) during the specified growing season (1st April- 1st Oct). This enhancement was mainly caused by four

factors (starting with the most important one): more homogeneous horizontal light distribution, higher leaf photosynthetic capacity, more uniform vertical light distribution and higher LAI. Surprisingly, previous studies rarely paid attention to the effect of horizontal light distribution on plant photosynthesis under diffuse light, the possible explanation might be that adding a dimension involves a number of tedious measurements of light distribution in the horizontal plane that are time consuming. Now, it is important to realize that horizontal light distribution plays the most important role for enhancement of crop photosynthesis under diffuse light.

For estimating canopy photosynthesis, firstly measuring leaf photosynthesis is necessary. Sarlikioti *et al*. (2011a) reported that for greenhouse tomato crop leaves situated within the two plant rows received lower amount of light than leaves at the same height situated towards the path, this might result in different leaf photosynthetic rate at the same plant height due to photosynthetic acclimation. However, it is a common experimental approach to measure leaf photosynthesis only on leaves facing the path and not in the middle of the plant row. This approach can lead to errors (overestimation) if these data are used for estimating canopy photosynthesis (Sarlikioti *et al*., 2011a). Under diffuse light, due to the more uniform light distribution within the canopy, differences in light acclimation of leaf photosynthesis at the same plant height might be small. In this respect, canopy photosynthesis as estimated from measurements on individual leaves under diffuse light is more close to reality. Therefore, the calculated potential effect of diffuse light on canopy photosynthesis is likely underestimated in our study.

In our experiment, tomato production was increased by 8-11 % under diffuse glass compared with clear glass treatment during the whole growing season. The most important reason for the increased production was an increase in individual fruit weight by 5-8 g (Dueck *et al*., 2012). This indicates that (fruiting) tomato plants are source limited, which is in agreement with our study (Chapter 5) as well as many previous studies (De Koning, 1994; Heuvelink, 1996; Matsuda *et al*. 2011; Qian *et al*., 2012).

Effects of diffuse light on temporal light distribution

In nature, temporal light distribution in the canopy is characterized by alternating periods of relatively high light followed by periods of background low light at a given point (lightflecks). Under these circumstances, a large fraction of CO_2 assimilation may occur under transient light conditions, in which loss and gain of photosynthetic induction plays a pivotal role for carbon assimilation (Urban *et al*., 2007). Photosynthetic induction is regulated by

rates of RuBP regeneration, Rubisco activation, and stomatal response (Pearcy *et al.*, 2004; Kaiser *et al.*, 2014). Of all the combined processes involved in photosynthetic induction, changes in stomatal aperture take most time to reach a new steady state (Vico *et al.*, 2011; Way and Pearcy, 2012), which consequently limit leaf photosynthesis. Studies have recognized the importance of dynamic light to photosynthesis of understory plants in forest in which the dynamic light is caused by variable cloud cover, wind, shade from overstory and earth rotation (Pearcy, 1990). On clear days, the greenhouse construction and equipment in combination with the earth's rotation could create a series of fluctuations for incident light at a given point in the canopy. This variation of incident light could be minimized when the light would be made diffuse. In this thesis (Chapter 3, 4), we tested two *Anthurium andreanum* cultivars ('Pink Champion' and 'Royal Champion') under diffuse glass cover. Crop RUE (defined as the ratio between total dry mass production and cumulative intercepted light) was increased by 8 % under diffuse glass compared with clear glass cover for 'Royal Champion'. This was mainly due to less temporal variation of incident light under diffuse glass cover on clear days, consequently, the variation of stomatal conductance could be minimized, resulting in less stomatal limitation on leaf photosynthesis (Chapter 4). However, this effect could not be found for 'Pink Champion' because stomata of this cultivar showed small responding to the variation of incident light. Therefore, we conclude that the potential effect of diffuse light on crop photosynthesis to some extent depends on the dynamic response of stomatal conductance to the incident light.

In Chapter 2, we explained the stimulating effect of diffuse light on tomato canopy photosynthesis in terms of spatial light distribution. However, the effect of temporal light distribution on leaf photosynthesis of tomato plants was not investigated. We speculate that this effect was not that important for tomato plants in our study, because stomatal conductance of tomato plants was much higher than anthurium pot-plants. The relatively high stomatal conductance in combination with CO_2 enrichment in the greenhouse could result in leaf photosynthesis close to the saturation phase of the photosynthesis CO_2 response curve. In this case, the variation of stomatal conductance was not likely limit leaf photosynthesis under dynamic light condition.

Other potential aspects about diffuse light

Although we have explored the effects of diffuse light on light distribution and its direct and indirect effects on crop photosynthesis, there are still several aspects that were not considered in our study

a) Diffuse light penetrates deeper into the canopy and its effect on crop photosynthesis reported in this thesis was mainly studied during summer and autumn months. The effects of diffuse light on crop photosynthesis could strongly differ between winter and summer light conditions. Under winter light conditions, photosynthesis of the upper leaves is far from light saturation. With the same light intensity at leaf level, upper leaves have a higher rate of photosynthesis than lower leaves. Therefore, deeper penetration of light may have less effects on crop photosynthesis in winter (Sarlikioti *et al.*, 2011b). Furthermore, light interception follows a seasonal pattern with on average, a lower fraction of light intercepted during summer than during winter because of changes in solar elevation (Sarlikioti *et al.*, 2011a). The higher solar elevation in summer months results in an orientation of light rays more perpendicular to the plant canopy, resulting in a higher light penetration and lower interception. Model simulation in sweet pepper, cucumber and tomato showed that about 55 % of all benefits of diffuse glass cover were obtained in summer months in the Netherlands, because during summer months solar position is high, the quantity of solar radiation and the fraction of direct photosynthetically active radiation (PAR) is the greatest (Jongschaap *et al*., 2006). Therefore, seasonal variation of light intensity, directional light quality as well as solar position should be considered when studying the effect of diffuse light on light distribution and crop photosynthesis.

b) For the measurements of the leaf photosynthesis light response curves, only the adaxial side of the leaf was illuminated by the light source. However, diffuseness of light may affect the fraction of light on the abaxial leaf surface. As the abaxial surface may have a different photosynthesis light response curve than adaxial surface (Paradiso and Marcelis, 2012). This might have led to a minor error in estimating the effects of diffuse light on crop photosynthesis. Measurements of light absorption and photosynthesis light response curves on both the adaxial and abaxial side of leaves in the canopy in combination with functional–structural plant modeling might help to estimate these effects.

c) Brodersen *et al.* (2008) as well as Brodersen and Vogelmann (2010) showed that high-light grown leaves (e.g. top canopy leaves) use direct light more efficiently than diffuse light because direct light can more easily penetrates into the multiple palisade layers of high-light grown leaves than diffuse light do. This indicates that leaf-level and canopy-level photosynthetic processes may respond differently to the directionality of light. This is not taken into account in the present study because the photosynthetic carbon exchange in leaves was measured under conditions of direct light where light comes from the upper

part of the measurement chamber (Long and Bernacchi, 2003). A mismatch between the growth and measuring light could cause small errors for estimating the crop photosynthesis.

d) Row crop systems are commonly used in horticultural and agronomic crops. This system facilitates crop management and allows higher light penetration inside the plant canopy. In this system, a fraction of light reaches the ground floor in the middle of the path (Stewart *et al.*, 2003; Sarlikioti *et al.*, 2011a), the reflection of light by the floor can be reused for photosynthesis. Furthermore, row orientation substantially affects canopy light interception (Palmer, 1989; Borger *et al.*, 2010; Sarlikioti *et al.*, 2011a). These effects may differ between diffuse and direct light conditions.

e) Light distribution and absorption is highly dependent on crop architecture (Falster and Westoby, 2003; Zheng *et al.*, 2008; Sarlikioti *et al.*, 2011b). We showed that diffuse light treatment had limited effect on the fraction of canopy light interception as a function of LAI in anthurium pot-plants on clear days (Chapter 3). This is probably due to the short and compact canopies with relatively large leaves, which may generate substantial leaf overlap and self-shading, therefore decreases the net amount of leaf area exposed to light. Plants also vary widely in leaf angle, leaf orientation, internode length, and leaf length to width ratio, these traits have a direct effect on light absorption and photosynthesis (Falster and Westoby, 2003; Sarlikioti *et al.*, 2011b). Furthermore, LAI is a predominant factor for canopy light interception, at low LAI mutual shading of leaves within the canopy is small, thus light may readily penetrate deeper into the canopy. In forest ecosystems, Letts *et al.*(2005) reported that diffuse light has limited effect on ecosystem CO_2 exchange for short-stature canopies (e.g. shrubs) mainly due to low LAI. In this context, investigating the effect of canopy architecture and LAI on light distribution and photosynthesis under diffuse light might be worthwhile to do.

f) Fruit and vegetable quality is closely correlated with the pre-harvest growth condition. In open field and conventional clear greenhouses, fruit and vegetables often experience diurnal fluctuations or long-term exposure to direct sunlight, with associated high tissue temperatures. This may result in harvest disorders (i.e. sunburn), and heterogeneity of internal quality properties such as sugar content, tissue firmness, mineral content (Woolf and Ferguson, 2000). Fruit with different temperature histories will also respond differently to postharvest low temperatures (i.e. chilling injury) (Ferguson *et al.*, 1999). The quality problems induced by sunlight exposure could be reduced if plants were grown

under diffuse light where less fluctuations in temperature and light intensity occurs.

Further exploration of these potential aspects can improve our understanding about the effect of diffuse light on plant processes.

Allowing more light in the greenhouses improves plant growth

As a rule of thumb for most greenhouse crops a 1 % light increment results in 0.5-1 % increase in harvestable product in the Netherlands when averaged over a prolonged period (Marcelis *et al.*, 2006). Therefore, improving greenhouse light transmissivity and applying supplementary assimilation light are commonly considered. On the other hand, growers usually apply shading screens or white wash on the greenhouse for production of many shade tolerant pot-plants in summer in order to avoid the high light induced leaf or flower damage. It is clear that shading wastes the available solar radiation in greenhouse production systems. Shading often carries a penalty on potential crop growth as it is positively related to the amount of light that can be captured. Considering the advantageous properties of diffuse glass cover, e.g. more homogeneous light distribution, lower leaf temperature and less photoinhibition when global radiation is high, we hypothesized that less shading under diffuse glass cover might be feasible for growing shade tolerant pot-plants without negative influence on plant ornamental quality.

In this thesis (Chapter 3), we suggested that increasing daily light integral (DLI) under diffuse glass cover not only accelerates plant growth but also improves plant ornamental quality (more compact plants, not any damages) in two *Anthurium andreanum* cultivars ('Pink Champion' and 'Royal Champion'). Similar effects were also observed in the same experiment when the solar light was made diffuse by diffuse screens (Marcelis *et al.*, 2014). In another treatment plants were grown in an advanced Fresnel greenhouse where direct solar light was transformed into heat and electricity, while the diffuse part of the solar light was used for crop production (Sonneveld *et al.*, 2011). The maximum DLI inside this greenhouse was 12-15 mol m^{-2} d^{-1} PAR which was higher than we reported in this thesis (10 mol m^{-2} d^{-1} PAR), and plant growth was comparable to that at diffuse glass cover and diffuse screens (Marcelis *et al.*, 2014). Similarly, these effects were observed in two *Bromeliad* cultivars ('Rana' and 'Miranda') which were also grown in these treatments (Marcelis *et al.*, 2014). However, high DLI resulted in undesired side shoots of *Bromeliad*, probably as result of increased assimilates availability or increased light intensity at the level of axillary buds. This suggests the requirement for a higher plant density or higher average temperature when the DLI is increased. From these studies, we conclude that allowing more light and making the

light diffuse can increase the growth of shade tolerant pot-plants without compromising the plant quality.

Apart from allowing more light in the greenhouse via diffuse cover materials, allowing more light under clear glass cover in combination with manipulating other climatic factors could also stimulate growth of shade tolerant pot-plants. Kromdijk *et al*. (2012) summarized a number of experiments where DLI was increased under clear glass cover and plant growth was stimulated in several species (e.g. *Anthurium, Calathea, Ficus, Phalaenopsis, Aechmea*). These authors concluded that the stimulating effect of plant growth mainly resulted from the interactive effect of high air humidity (which keeps the stomata opening), high daytime temperature (speeding up development rate) and associated with high daytime CO_2 concentration. In this thesis (Chapter 3), DLI in the control treatment (Clear glass cover) could be maintained at 7.5 mol m^{-2} d^{-1} PAR which was higher than in commercial practice (5 mol m^{-2} d^{-1} PAR). The absence of damage to leaves or flowers under the high light level was probably due to the air humidity was kept at moderately high level (75-80 %) which could result in open stomata, thereby avoiding overheating of the plants and maintaining photosynthesis. Trouwborst *et al*. (2011) reported that most pot-plants have a relatively low level stomatal conductance, which reduces further under high light levels because high light levels in greenhouses usually correlate with low air humidity and high temperature. The positive effects of increasing air humidity on plant growth has also been reported by Mortensen and Gislerød (1990) as well as Van Noort *et al*. (2011). Dueck *et al* (2010) reported that high DLI in combination with increasing temperature stimulated growth of *Phalaenopsis*. It is also important to realize that increasing the greenhouse temperature set-point also had a positive effect on the CO_2 concentration within the greenhouse, due to less cooling via ventilation. Furthermore, when increasing DLI, monitoring of the leaf status is also important in order to prevent photoinhibition. Monitoring based on chlorophyll fluorescence is a potential technique which could be considered when high DLI is incorporated in cultivation practices (Kromdijk *et al.*, 2012).

Development of diffuse cover materials and practical applications

Properties of diffuse cover materials are usually judged by hemispherical light transmission and haze factor (Humming *et al*., 2008). Haze in this thesis is defined as the percentage of transmitted light that is scattered such that it deviates more than 1.5° from the direction of the incident beam. Making light diffuse (haze) and light loss seem to be connected. Recently, new diffuse glass materials have become available of which the hemispherical light transmission is

similar or even higher than the traditional clear glass. This is mainly attributed to the improvements in glass materials (i.e. low-iron glass) in combination with anti-reflection coating treatments on the glass surface (Hemming *et al.*, 2014). These improvements also resulted in different spectral transmission, i.e. low-iron glass shows higher light transmission at wavelength longer than 600 nm (Hemming *et al*., 2014) and at ultraviolet spectrum (300-400 nm) (Chapter 3). The differences in spectral transmission could influence crop processes and therefore should be taken into account for further research. Recently Hemming *et al*. (2014) showed that two diffuse glass materials may have the same haze but different angular distribution of transmitted light. Therefore, next to hemispherical light transmission and haze, information on spectral light transmission, and spatial distribution of transmitted light are also important for judging the diffuse cover materials.

Under Dutch conditions diffuse glass cover increased year-round production by 8-11 % in tomato (Chapter 2) and 4.3 % in cucumber (Hemming *et al.* 2008). When considering the stimulating effect of diffuse cover materials on crop production, it is important to consider the directional quality of solar radiation (i.e. direct light) at different latitudes. Hemming *et al.* (2008) analyzed the potential of diffuse cover materials for the Netherlands (52° N), South Italy (41° N) and Arizona (34° N). They concluded that the potential of diffuse cover materials for Italy and Arizona is much higher than for the Netherlands, because the direct PAR in Italy is almost three and in Arizona is almost five times higher than in the Netherlands.

In practical applications, the investment cost of diffuse cover materials and their benefits for crop production are important for growers to make a decision. A traditional glass material costs ca. € 4 m^{-2}, the additional costs of diffuse glass are ca. € 4-10 m^{-2} depending on the type of structure and the number and type of coatings added to the glass (Hemming *et al.*, 2014). Apart from diffuse glass, cheaper alternatives for diffusing the light are available such as diffuse plastic films or screens (Magnani *et al.*, 2007; Romero-Gámez *et al.*, 2012), as well as diffuse coatings. However, these alternatives usually also decrease the light transmission simultaneously.

Other options for improving RUE in greenhouse production systems

In this thesis, we discussed three aspects for improving RUE in greenhouse production systems, i.e. improving light distribution in the crop canopy (Chapter 2, 4); increasing the daily light integral in the greenhouse during summer (Chapter 3); and balancing the source sink strength of a crop (Chapter 5). In the last decade, many innovations have been developed in greenhouse horticulture, which could improve plant growth and crop production. For

instance, the (semi-)closed greenhouse can increase annual production by 10-20 %. This increase in production is primarily obtained through increase of photosynthesis rates due to the higher CO_2 concentration in the (semi-)closed greenhouse (De Gelder *et al.*, 2012; Qian *et al.*, 2012). However, the high investment cost hampers a widespread application of this type of greenhouses. Thus, exploring possibilities to make it economically feasible is important. Light emitting diodes (LEDs) open possibilities for improving the yield and quality of plants. The spectrum of LEDs can be modified. LEDs generate less heat radiation than high pressure sodium lamps. Therefore, it can be positioned within the canopy, resulting in a more homogeneous vertical light distribution compared with the top lighting (Trouwborst, 2011). However, the horizontal light distribution may remain a challenge. Interlighting with LEDs affects the plant morphology such as leaf curling which has negative effect on light interception (Trouwborst, 2011). Therefore, finding the best positioning of lamps in the greenhouse and best light spectrum is important for improving the RUE of LEDs. Plant monitoring based on the combination of plant sensors (e.g. fluorescence signals) with models can be used to optimize the plant growth and production, e.g. prevent photoinhibition at high radiation or turn off the assimilation light when plant photosynthetic efficiency is low (Marcelis *et al.*, 2014). For these innovations, the benefits are obvious, while some challenges are also involved. We expect that based on the better understanding of plant processes in combination with the new technologies, the current challenges will be overcame and new innovations will become available, which could improve RUE in the greenhouse production systems and consequently resulting in optimized crop production.

Conclusions

The aim of this thesis was to obtain insights in improving RUE in greenhouse production systems through better understanding of crop physiology. We conclude that making light diffuse in greenhouses via diffuse cover materials improves spatial light distribution in the crop canopy, thereby stimulating crop photosynthesis; the more uniform horizontal light distribution within the canopy plays the most important role for this effect (Chapter 2). Making light diffuse also lessens the variation of the temporal light distribution at any specific point in the canopy, however, its effect on plant growth depends on the dynamic responses of stomatal conductance to the incident light (e.g. *Anthurium* 'Royal Champion') (Chapter 4). A diffuse greenhouse cover makes it possible to allow more light in the greenhouse which strongly stimulates crop growth of shade tolerant pot-plants without compromising plant quality (Chapter 3). We also conclude that tomato plants are sink limited during the early

growth stage under ample irradiance, while fruiting tomato plants are source limited (even for cherry tomato) (Chapter 5); this indicates that supplementary assimilation light during the early growth stage could result in sink limitation, consequently lower RUE for supplementary assimilation light during this stage than during the fully fruiting stage.

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SUMMARY

A large increase in agricultural production is needed to feed the increasing world population with their increasing demand per capita. However, growing competition for arable land, water, energy, and the degradation of the environment impose challenges to improve crop production. Hence agricultural production efficiency needs to increase. Greenhouses provide the possibility to create optimal growth conditions for crops, thereby improving production and product quality. Light is the driving force for plant photosynthesis and in greenhouse horticulture, light is often the most limiting factor for plant growth. Therefore, improving radiation use efficiency (RUE) in greenhouse production systems is imperative in order to improve plant growth and production. The objective of this thesis is to obtain insight in improving RUE in greenhouse production systems through better understanding of crop physiology. Three aspects related to RUE have been studied in this thesis, 1) improving light distribution in the crop canopy; 2) allowing more light in the greenhouse during summer; and 3) balancing the source and sink strength during plant growth.

Light is heterogeneously distributed in the crop canopy. Due to the saturating response of leaf photosynthesis rate to light, a more homogeneous light distribution in the canopy will result in a higher crop photosynthesis. In **Chapter 2**, the effect of diffuse glass on spatial light distribution in a fully developed tomato canopy and its direct and indirect effects on crop photosynthesis were explored. Diffuse glass, which transforms a portion of direct solar light into diffuse light without influencing the light transmissivity of the glass, was applied as greenhouse cover. Under diffuse glass cover, light was more evenly distributed (in both horizontal and vertical direction) within the canopy compared with plants grown under conventional clear glass cover. Besides a more uniform light distribution, diffuse glass also resulted in higher leaf photosynthetic capacity in the middle of the crop canopy and in a higher leaf area index (LAI). The higher leaf photosynthetic capacity was positively correlated with a higher leaf total nitrogen and chlorophyll content. Moreover, lower leaf temperature and less photo-inhibition of top canopy leaves were observed under diffuse glass cover when global radiation was high. Total crop photosynthesis between 1st April and 1st October was enhanced by 7.2 % under diffuse glass. This enhancement mainly resulted from four factors (in order of decreasing importance): a more homogeneous horizontal light distribution, a higher leaf photosynthetic capacity, a more uniform vertical light distribution and a higher LAI.

In summer growers of shade tolerant pot-plants often apply shading screens in the greenhouse or white wash on the greenhouse cover in order to avoid leaf or flower damage caused by high light. Shading carries a penalty on potential crop growth which is positively related to the amount of light that can be captured. Considering the advantageous properties of diffuse glass cover, i.e. a more homogeneous light distribution, a lower leaf temperature and less photo-inhibition when global radiation is high, in **Chapter 3** we tested the feasibility of allowing more light (i.e. less shading) via diffuse glass cover for cultivation of shade tolerant pot-plants during summer. Two *Anthurium andreanum* cultivars (Pink Champion and Royal Champion) were grown in 3 greenhouse compartments. Under similar DLI [7.5 mol m^{-2} d^{-1} PAR (photosynthetic active radiation)], diffuse glass cover resulted in 8 % higher crop RUE (i.e. dry mass production per unit intercepted light) in 'Royal Champion' compared with clear glass cover treatment, which consequently resulted in higher total biomass production. This effect was not observed in 'Pink Champion'. Under diffuse glass cover, high DLI (10 mol m^{-2} d^{-1} PAR) resulted in 20-23 % higher total biomass production in both cultivars compared with low DLI (7.5 mol m^{-2} d^{-1} PAR), this mainly resulted from the higher cumulative intercepted light. No flower or leaf damage was observed in these treatments. High DLI even resulted in more compact plants as indicated by a higher ratio of aboveground fresh mass to plant height.

In **Chapter 4**, we addressed a question resulting from Chapter 3, i.e. why the stimulating effect of diffuse light on crop RUE in anthurium pot-plants is cultivar specific? We excluded the fraction of canopy light interception and steady-state leaf photosynthesis as potential explanations, and explained it from instantaneous leaf photosynthesis which closely correlates with the temporal light distribution. Diffuse glass cover smoothed the variation of temporal light distribution at a given point on a leaf during a clear day, which consequently resulted in less temporal variation of stomatal conductance in 'Royal Champion' which had stomata showing a fast-response to the variation in light intensity. As stomata are the gateway for CO_2 uptake, less variation in stomatal conductance imposed less limitation for leaf photosynthesis under diffuse glass cover, thereby resulting in a higher crop RUE. For 'Pink Champion', however, stomata were less responding to variations in light intensity. Therefore, stomata imposed only a marginal limitation on leaf photosynthesis even under clear glass cover where the temporal incident light intensity varied substantially due to the shadow cast by the greenhouse construction parts and equipment.

Application of supplementary assimilation light in greenhouses is rapidly increasing. The beneficial effect of supplementary assimilation light is determined by the balance between assimilate production in source leaves and the overall capacity of the plant to use

these assimilates. Therefore, it is important to identify the source-sink balance during plant growth. In **Chapter 5**, three tomato cultivars with different potential fruit size ['Komeett' (large size); 'Capricia' (medium size); 'Sunstream' (small size, cherry tomato)] were grown under commercial crop management. We estimated the source-sink ratio from the early growth stage to fully fruiting stage through experimentation and model simulation. Carbohydrate content of leaves and stems were periodically determined. Tomato plants showed a period of sink limitation ('Komeett' and 'Capricia') or came close to sink limitation ('Sunstream') during the early growth stage under ample natural irradiance (early September) as indicated by a source-sink ratio higher than or close to 1. Fruiting tomato plants were source-limited as indicated by an extremely low source-sink ratio (average source-sink ratio from 50 days after planting onwards was 0.17, 0.22 and 0.33 for 'Komeett', 'Capricia' and 'Sunstream', respectively). During the fully fruiting stage, the source-sink ratio was negatively correlated with the potential fruit size when commercial fruit load was maintained. Carbohydrate content in tomato stems and leaves increased linearly with plant source-sink ratio.

The experiments and results described in this thesis provide insights for improving RUE in greenhouse production systems. The main achievements and limitations as well as practical applications are discussed in **Chapter 6**.

SAMENVATTING

Verhoging van de voedselproductie is van groot belang vanwege de snel groeiende wereldbevolking. Deze veel hogere productie zal vooral gerealiseerd moeten worden door een hogere productie per oppervlakte-eenheid landbouwgrond. Teelt in kassen is een intensief productiesysteem met hoge producties per oppervlakte-eenheid. Kassen maken het mogelijk gewassen onder optimale omstandigheden te telen, met als gevolg een hoog productieniveau en een goede productkwaliteit. Licht is de drijvende kracht voor fotosynthese en vaak de limiterende factor voor gewasgroei in kassen. Het is daarom van groot belang om de lichtbenuttingsefficiëntie in kasteelten te verhogen om daarmee gewasgroei en -opbrengst te verbeteren. Het onderzoek beschreven in dit proefschrift is gericht op het verkrijgen van inzicht in de mogelijkheden om de lichtbenuttingsefficiëntie in kassen te verhogen door een beter begrip van de gewasfysiologie. In dit proefschrift worden drie factoren die de lichtbenuttingsefficiëntie van kasteelten beïnvloeden nader bestudeerd: (1) een homogenere verdeling van licht in het gewas, (2) het toelaten van meer licht in kassen (minder gebruik van schermen) in de zomer, en (3) het in balans houden van aanmaak en gebruik van assimilaten van de plant.

In een gewas is licht heterogeen verdeeld. Als gevolg van het kromlijnige verband tussen lichtintensiteit en bladfotosynthesesnelheid zal een meer homogene verdeling van licht in een gewas tot een hogere gewasfotosynthese leiden. In **Hoofdstuk 2** wordt het effect van diffuus licht op de ruimtelijke verdeling van licht in een volgroeid tomatengewas, alsmede de directe en indirecte effecten daarvan op de gewasfotosynthese bestudeerd. Diffuus glas werd gebruikt als kasdekmateriaal; diffuus glas zet een deel van het directe zonlicht om in diffuus licht zonder dat de kasdektransmissie beïnvloed wordt. Onder diffuus glas was het licht in het gewas meer homogeen verdeeld (zowel in horizontale als verticale richting) vergeleken met conventioneel glas. Naast een meer homogene lichtverdeling, resulteerde diffuus glas ook in een hogere bladfotosynthesecapaciteit midden in het gewas en een hogere bladoppervlakte-index (LAI). De hogere bladfotosynthesecapaciteit was positief gecorreleerd met een hoger stikstofgehalte en chlorofylgehalte in het blad. Verder werd bij hoge lichtintensiteiten een lagere bladtemperatuur en minder foto-inhibitie van de bovenste bladeren waargenomen onder diffuus glas ten opzichte van conventioneel glas. Over de periode van 1 april tot 1 oktober was de gewasfotosynthese 7,2% hoger onder diffuus glas. Deze toename was hoofdzakelijk het gevolg van een viertal factoren (in volgorde van afnemende belangrijkheid):

een homogenere horizontale lichtverdeling, een hogere bladfotosynthese-capaciteit, een homogenere verticale lichtverdeling en een hogere LAI.

Telers gebruiken vaak schermen in de kas of krijten het kasdek om de productie van schaduwminnende potplanten in de zomer mogelijk te maken zonder schade aan bladeren of bloemen als gevolg van te hoge instraling. Het wegschermen van licht heeft echter als nadeel dat de potentiële productie negatief beïnvloed wordt. De positieve eigenschappen van een diffuus kasdek in ogenschouw nemend, namelijk een homogenere lichtverdeling, een lagere bladtemperatuur en minder foto-inhibitie onder hoge instraling, worden in **Hoofdstuk 3** de mogelijkheden onderzocht om meer licht (minder gebruik van schermen) in de vorm van diffuus licht toe te laten in de teelt van schaduwminnende potplanten in de zomer. In drie kascompartimenten werden twee rassen van *Anthurium andreanum* (Pink Champion en Royal Champion) geteeld. Onder eenzelfde dagelijkse lichtsom van 7,5 mol m^{-2} d^{-1} resulteerde voor diffuus glas vergeleken met conventioneel glas in een 8% hogere lichtbenuttingsefficiëntie (drogestofproductie per eenheid onderschept licht) bij 'Royal Champignon', met als gevolg een hogere totale biomassaproductie; dit effect was afwezig bij 'Pink Champignon'. Onder diffuus glas gaf voor beide cultivars een lichtsom van 10 mol m^{-2} d^{-1} 20-23% meer biomassaproductie vergeleken met een lichtsom van 7,5 mol m^{-2} d^{-1}. Dit was vooral het gevolg van een grotere hoeveelheid cumulatief onderschept licht. Er werd in deze kascompartimenten geen schade aan bloemen of bladeren waargenomen. Een hogere lichtsom resulteerde in een meer compacte plant, hetgeen blijkt uit een hogere ratio tussen bovengronds versgewicht en planthoogte.

In **Hoofdstuk 4** wordt ingegaan op een vraag volgend uit Hoofdstuk 3, namelijk waarom het stimulerende effect van diffuus glas op de lichtbenuttingsefficiëntie van anthurium potplanten cultivar-specifiek is. De door het gewas onderschepte fractie licht en de steady-state bladfotosynthese konden als mogelijke verklaringen uitgesloten worden. Het rasverschil werd verklaard vanuit de momentane bladfotosynthese, die nauw correleerde met de temporele lichtverdeling in het gewas. Diffuus glas leidde tot een meer homogene temporele lichtverdeling voor een gegeven punt op een blad op een heldere dag. Dit resulteerde in geringere variatie in de huidmondjesgeleidbaarheid over de dag voor 'Royal Champignon'; de huidmondjes van dit ras reageren namelijk snel op veranderingen in lichtintensiteit. Omdat de huidmondjes de poorten zijn voor CO_2 opname, betekent minder variatie in de huidmondjesgeleidbaarheid minder belemmering voor de bladfotosynthese onder diffuus glas, met als gevolg een hogere lichtbenuttingsefficiëntie. Voor 'Pink Champignon' daarentegen reageerden de huidmondjes veel minder op veranderingen in

lichtintensiteit. Daarom spelen voor dit ras ook op een heldere dag de huidmondjes maar een geringe rol als limiterende factor voor bladfotosynthese, terwijl de lichtintensiteit over de dag sterke fluctuaties vertoonde als gevolg van schaduw van kasconstructiedelen en onderdelen van de kasuitrusting zoals motoren en schermpakketten boven in de kas.

Het gebruik van assimilatiebelichting in de glastuinbouw neemt snel toe. Het effect dat assimilatiebelichting heeft op gewasgroei en productie hangt af van de balans tussen aanmaak van assimilaten in de bladeren (source) en de capaciteit van de plant om deze assimilaten te gebruiken (sink). Het is derhalve belangrijk om de source-sink verhouding tijdens de teelt te kennen. In **Hoofdstuk 5** worden proefresultaten gepresenteerd en bediscussieerd van een kasexperiment met drie tomatenrassen met een verschillende potentiele vruchtgrootte ['Komeett' (grote vruchten); 'Capricia' (gemiddelde vruchtgrootte); 'Sunstream' (kleine vruchten, cherrytomaat)] die zoveel mogelijk volgens de praktijk geteeld werden. Op basis van het kasexperiment gecombineerd met een gewasgroeimodel werd de source-sink verhouding geschat vanaf het jonge gewasstadium (rond bloei eerste tros) tot en met circa zeven weken na de oogst van de eerste rijpe vruchten. Ook werd het koolhydraatgehalte van de bladeren en de stengels periodiek gemeten. Het jonge tomatengewas was onder hoge instraling kort na planten (begin september) sink-gelimiteerd ('Komeet' en 'Capricia') of kwam daar dicht bij ('Sunstream'). Dit uitte zich in een source-sink verhouding hoger dan of net onder een waarde 1. Tomatenplanten met uitgroeiende vruchten aan meerdere trossen waren source-gelimiteerd hetgeen zich uitte in een extreem lage source-sink verhouding. De gemiddelde source-sink verhouding vanaf 50 dagen na planten bedroeg 0,17, 0,22 en 0,33 voor respectievelijk 'Komeett', 'Capricia' en 'Sunstream'. In deze gewasfase was de source-sink verhouding negatief gecorreleerd met de potentiele vruchtgrootte, wanneer een plantbelasting (aantal vruchten per tros) zoals in de praktijk gebruikelijk is werd aangehouden. Het koolhydraatgehalte van tomatenstengels en -bladeren nam lineair toe met de source-sink verhouding.

De experimenten en resultaten zoals beschreven in dit proefschrift geven inzicht in de mogelijkheden om de lichtbenuttingsefficiëntie van kasteelten te verhogen. De belangrijkste bevindingen en beperkingen van dit onderzoek, alsmede de consequenties voor de praktijk worden in **Hoofdstuk 6** bediscussieerd.

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Tao Li

Beijing, China,

December, 2014

CURRICULUM VITAE

Tao Li was born on December 15th 1985 in Shangluo, China. In 2003 he finished his secondary education in his home town. In the same year, he commenced his study 'Landscape designing' at Northwest A&F university in Yangling, China. During his study, he was attracted by plant landscaping and ornamental plants. In 2007 he obtained his bachelor degree and consequently started his MSc programme in the same university. The subject of his MSc study was Landscape Plant Ecology and Ornamental Horticulture. In July 2010 he graduated from MSc study and received a grant from the China Scholarship Council (CSC) for his PhD study in Wageningen UR. In October 2010 he arrived in Wageningen and firstly followed half year MSc courses in the subject of Greenhouse Horticulture. In April 2011 he commenced his PhD programme at the Horticultural Product Physiology Group of Wageningen University and did research about improving radiation use efficiency in greenhouse production systems which resulted in this thesis.

LIST OF PUBLICATIONS

Papers published or to be published in refereed journals

Li T, Heuvelink E, Dueck TA, Janse J, Gort G, Marcelis LFM. 2014. Enhancement of crop photosynthesis by diffuse light: quantifying the contributing factors. *Annals of botany,* 114 (1): 145-156

Li T, Heuvelink E, Van Noort F, Kromdijk J, Marcelis LFM. 2014. Responses of two *Anthurium* cultivars to high daily integrals of diffuse light. *Scientia Horticulturae*, 179: 306-313.

Li T, Kromdijk J, Heuvelink E, Van Noort F, Kaiser E, Marcelis LFM. Effects of diffuse light on radiation use efficiency depend on the response of stomatal conductance to dynamic light intensity. *Under review.*

Li T, Heuvelink E, Marcelis LFM. Quantifying the source-sink balance and carbohydrate content in three tomato cultivars. *Under review.*

Other publications

Dueck TA, Janse J, **Li T**, Kempkes F, Eveleens B. 2012. Influence of diffuse glass on the growth and production of tomato. *Acta Horticulturae,* 956: 75-82. (Conference proceeding)

Marcelis LFM, Buwalda F, Dieleman JA, Dueck TA, Elings A, Gelder A de, Hemming S, Kempkes F, **Li T**, Noort FR van, Visser PHB de. 2014. Innovations in crop production: a matter of physiology and technology. *Acta Horticulturae* 1037: 39 - 45. (Conference proceeding)

Li T, Dueck TA, Heuvelink, E, Kierkels T. 2013. Waarom diffuus licht werkt en wat we niet weten. *Onder Glas* 10 (12): 36 - 37. (Professional publication)

Dueck TA, Janse J, Kempkes FLK, **Li T**, Elings A, Hemming S. 2012. Diffuus licht bij tomaat. Wageningen: Wageningen UR Glastuinbouw, Rapporten GTB 1158, 60 p. (Project report)

Noort FR van, Kromwijk J, Snel JFH, Warmenhoven MG, Meinen, E, **Li T**, Kempkes FLK, Marcelis LFM. 2013. 'Grip op licht' bij potanthurium en bromelia: Meer energie besparing bij Het Nieuwe Telen Potplanten met diffuus licht en verbeterde monitoring meer natuurlijk. Wageningen: Wageningen UR Glastuinbouw, Rapporten 1287, 94 p. (Project report)

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the 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)
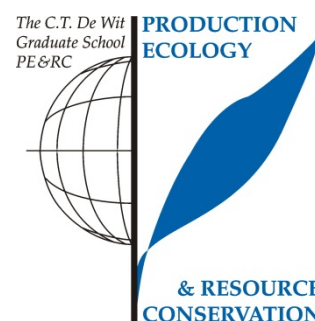
Review of literature (6 ECTS)
- Radiation use efficiency in greenhouse crops (2011)

Writing of project proposal
- Quantitative understanding and improvement of light use efficiency of greenhouse grown crops (2011)

Post-graduate courses (7.5 ECTS)
- Increasing photosynthesis in plants; PE&RC (2011)
- Applied methods in crop physiology; Odense, Denmark; GSST, Aarhus University (2012)
- Introduction to R for statistical analysis; PE&RC (2013)

Deficiency, refresh, brush-up courses (3 ECTS)
- Product quality and post-harvest physiology (2010)
- Greenhouse technology (2011)
- Research methods in crop and weed ecology (2011)
- Crop ecology (2011)
- Basic statistics (2013)

Competence strengthening / skills courses (1.8 ECTS)
- Interpersonal communication for PhD students; WGS (2011)
- Techniques for writing and presenting a scientific paper; WGS (2012)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.5 ECTS)
- PE&RC Weekend (2011)
- PE&RC Day (2011, 2013)

Discussion groups / local seminars / other scientific meetings (5.9 ECTS)
- Frontier Literature in Plant Physiology (FLOP) (2011-2014)
- International symposium on efficient utilization of light in plant production; Beijing, China (2014)

International symposia, workshops and conferences (9 ECTS)
- Workshop: Photosynthesis: from science to industry; oral presentation; Noordwijkerhout, the Netherlands (2012)
- 7th International symposium on light in horticultural systems; poster presentation; Wageningen, the Netherlands (2012)
- 4th International symposium on models for plant growth, environmental control and farm management in protected cultivation; poster presentation; Nanjing, China (2012)
- Greensys 2013: the international symposium on mew technologies for environment control, energy saving and crop production in greenhouse and plant factory; oral presentation; Jeju, South Korea (2013)

Lecturing / Supervision of practical's / tutorials (2.4 ECTS)
- Crop ecology (2013, 2014)
- Research methods in crop and weed ecology (2013, 2014)

Supervision of a MSc students (6 ECTS)
- Investigating carbohydrate content and leaf photosynthesis during plant development of three tomato cultivars
- Quantitative analysis of sink-source balance in three tomato cultivars

Sponsors

Colophon