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Effects of high intensity LED light, diffuse glass and improved climate on *Alstroemeria* photosynthesis and production

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Abstract

An innovative cultivation system for *Alstroemeria* growing, designed to be fossil fuel free for heating and lighting and to increase productivity is investigated under the working title "Cultivation of the Future". The first goal is achieved by a better isolation of the greenhouse, use of heat produced by the supplementary lights and the soil cooling machine and use of latent heat; the second goal is reached by the increase of the intensity of supplementary lights, the replacement of the HPS lights by LED and the provision of a less stressful environment. This cultivation system was compared to a "traditional" *Alstroemeria* cultivation system in the Netherlands. For the experiment, two greenhouse compartments and two *Alstroemeria* cultivars were used. During the first 11 months of the experiment, the "Cultivation of the future" had 31 and 34% higher generative stem production, for the cultivars 'Noize' and 'Virginia', respectively, than the reference cultivation system. It was concluded that production gains were caused mainly by the higher light sum in the winter months, and by increased stomatal conductance and higher photosynthesis capacity in the summer period, caused by the milder climate provided by a lower VPD and the highly diffused incident light.

Keywords: fossil fuel free; greenhouse, HPS lighting, latent heat, light interception, noise, photosynthesis, reduced VD, soil cooling, Virginia

INTRODUCTION

Greenhouse production in the Netherlands is highly dependent on fossil energy sources to supply the heat and light demands during winter. To reduce the environmental impact and become less dependent from fossil fuel, the Dutch government and the Dutch Horticultural Sector have agreed on the reduction of greenhouse horticulture CO_2 emissions by 2-3% per year, with, as ultimate goal, the achievement by 2050 of a totally energy neutral and economically viable horticulture in The Netherlands (Ministerie van Landbouw Natuur en Voedselkwaliteit, 2014). The program "Kas als Energiebron" ("Greenhouse as Source of Energy") stimulates energy saving innovations and growing concepts and the use of sustainable energy.

De Zwart et al. (2019), described in a desk study within this program, possible solutions to meet the energy requirements in fossil fuel free greenhouses with *Alstroemeria* as one of the case studies. From this study followed an economically feasible, all-electric, emission and fossil fuel free *Alstroemeria* cultivation concept. All previously successfully tested energy saving innovations for *Alstroemeria* came together in this growing concept: a diffuse glass greenhouse cover (Markvart et al., 2010; García Victoria et al., 2012; Dueck et al., 2012; Li et al., 2014, Marcelis et al., 2014), temperature integration, a dehumidification system with double insulating screens (Labrie and de Zwart, 2010), isolation of the soil, and full LED light (García Victoria et al., 2018). This growing concept was further designed in partnership with Dutch growers and was implemented as "Cultivation of the Future" in an experimental greenhouse (WUR Greenhouse Horticulture, Bleiswijk, The Netherlands), where it was compared with the crop's standard growing system in the Netherlands. The test lasted 2.5

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years.

This paper focuses on the effects on crop production and photosynthesis of the main "light and yield gaining" components of this system: high intensity LED and Diffuse Glass, during the first 11 months of cultivation.

MATERIALS AND METHODS

Greenhouses and cultivation systems (treatments)

The experiment took place in two Venlo type greenhouse compartments from the research facilities of Wageningen UR Greenhouse Horticulture in Bleiswijk (The Netherlands, 52°N, 4.5°E) with dimensions of 15×9.6 m (144 m^2) and gutter height of 5.5 m. Each greenhouse compartment represented one different treatment or cultivation system, namely, the "Cultivation of the Future" (CF) and the standard *Alstroemeria* cultivation system in the Netherlands (Reference). CF consisted on a greenhouse cover of diffuse glass with AR coating, high intensity (200 $\mu mol\ m^{-2}\ s^{-1}$) LED (8% B, 13% W, 67% R, 12% FR) and a more intense use of the misting system. The reference system had a cover of transparent glass and low intensity (80 $\mu mol\ m^{-2}\ s^{-1}$) HPS lamps. The differences between the systems are summarized in Table 1. The greenhouse climate was controlled by a Hoogendoorn iSii process computer (Hoogendoorn, Vlaardingen, The Netherlands).

Table 1. Comparison between cultivation systems.

System components	Reference	Cultivation of the Future
Covering material	Clear glass	Diffuse glass[a]
Assimilation lights	HPS[b], 80 $\mu mol\ m^{-2}\ s^{-1}$	LED[c] (RWB FR), 200 $\mu mol\ m^{-2}\ s^{-1}$
Heat supply	Natural gas	Latent and surplus heat
Isolation screen	1 screen	2 screens
Misting system set point	VD[d] 10 g kg^{-1}	VD[d] 7 g kg^{-1}
Dehumidification system	Window opening	Window opening and dehumidifier

[a] Haze of 70% and double anti-reflective coating.

[b] High pressure sodium.

[c] Light emitting diodes, (8% B,13% W, 67% R, 12% FR).

[d] Vapor deficit.

Plant material and substrate

Two cultivars of alstroemeria (*Alstroemeria* × *hybrida*) – 'Noize' (Hilverda Kooij, de Kwakel, The Netherlands) and 'Virginia' (Royal van Zanten, Rijsenhout, The Netherlands) – were planted on November 27th, in coco peat substrate beds (13×1.0×0.6 m), at a density of 3.5 plants m^{-2}. The beds were equipped with soil cooling pipes allowing root cooling. Soil temperature was set at 15.5°C after planting and was lowered to 15.0°C after the first flush.

Production and quality measurements

In order to quantify production, 12 plots (1.2×1.0 m, 3 plots $cultivar^{-1}$ $treatment^{-1}$) were designated. The first stems were harvested on the 5th of February. From then on, stems were harvested twice per week, classified as generative stems (commercial valuable stem), non-commercial stems (with less than three flowers, or with defects) or vegetative stems (without flowers). Generative stems were counted and measured (weight, length and number of peduncles). Non-commercial and vegetative stems were counted and weighted. Crop quality was evaluated monthly by destructive measurements of 15 generative stems $cultivar^{-1}$ $treatment^{-1}$) to determine leaf surface using a Li-COR 3100 (Li-Cor Biosciences, Inc., Lincoln, NE, USA) and dry weight. The production data was analyzed considering four different time periods related to the need for lamp use (the average number of hours a day that the lamps are switched on) as indicated in Table 2.

Table 2. The four periods considered for data analysis, based on the lamp use requirements.

Period	Interval	Week	Lamp use
1	5th February - 28th March	6-13	Heavy use of lamps, start of production
2	29th March - 5th June	13-23	Moderate use of lamps
3	6th June - 12th August	23-33	Lamps permanently switched off
4	13th August - 7th October	33-40	Moderate use of lamps

Photosynthetic gas exchange measurements

During summer (weeks 23 and 24), two Li-6800 portable photosynthesis systems (Li-Cor Biosciences, Inc., Lincoln, NE, USA), measured in both greenhouse compartments simultaneously net photosynthesis rate (A) and stomatal conductance (G_s) in different canopy layers (upper, middle and bottom layers). In total, 72 samples (6 leaves layer^{-1} cultivar^{-1} treatment^{-1}) were taken. For the measurement, leaves were enclosed in a 2 cm^2 chamber, with light source spectrum composed of 90% red, 10% blue light and initial intensity of 1500 µmol m^{-1}. The CO_2 concentration was set at 600 ppm and air flow to 400 µmol s^{-1}. Temperature and relative humidity (RH) were set, according to greenhouse climate conditions, to 26°C and 60%. Light response curves were obtained by measuring at 10 light levels: 1500, 1000, 750, 500, 350, 200, 150, 100, 50 and 0 µmol m^{-2} and analyzed with the help of a Li-Cor support program (Photosynthesis, Li-Cor Biosciences, Inc., Lincoln, NE, USA) to calculate light saturation (L_{sat}), maximum net photosynthesis rate (A_{max}), respiration (R), light compensation (L_c), apparent quantum yield (AQE) and curvature (ρ). Canopy net photosynthesis rate was calculated following equations given in Li-Cor Application Note (Norman et al., 1991; Li-Cor Biosciences, Inc., Lincoln, NE, USA). Leaf area index (LAI) was estimated for weeks 23 to 25, using data from production and destructive measurements.

Stomata density and size

On week 36, leaf prints were taken with the silicon rubber impressions technique (Weyers and Johansen, 1985) and analyzed with a microscope (Axio Lab.A1, Carl Zeiss, Jena, Germany). Images were taken with a microscope camera (Axiocam 105 color, Carls Zeiss, Jena, Germany) and analyzed by image processing software (ImageJ, US. National Institutes of Health, Bethesda, Maryland, USA) to determine stomata density and size on the abaxial side of leaves from the upper layer of the canopy. This measurement served as complementary data for the comprehension of stomatal conductance differences between treatments.

Statistical analysis

Photosynthesis parameters data were analyzed with Genstat (VSNI, England, UK) using two-way analysis of variance (ANOVA), with unbalanced design analyzed by Genstat regression. Significant differences were considered at *P*=0.05.

RESULTS AND DISCUSSION

Light sum and production

As light is a limiting factor for greenhouse production during periods with low light availability, the use of supplementary lighting is an important tool for greenhouse production in the Netherlands. During the experiment, lamps were used (except in period 3) for a maximum of 16 h a day whenever outside radiation was below 350 W m^{-2}. In the standard cultivation, HPS lights supplied 80 µmol PAR m^{-2} s^{-1}, while in the CF, LED lights supplied 200 µmol PAR m^{-2} s^{-1}. The higher intensity increased the light sum in the CF treatment by 68, 20.7 and 25.1% for respectively periods 1, 2 and 4 (Table 3). The total light sum during the 4 time periods considered was 20% higher in the CF treatment than in the reference treatment. The extra light was effectively converted by the crop into harvestable biomass (Table 4), which contributed to an increase in generative stems production (Table 5) of 31% ('Noize') to 34% ('Virginia'). No adverse effects were observed in the morphology of the flowers or stems as result of the used spectrum.

Table 3. Daily average PAR sum (mol day^{-1}), total PAR sum (mol m^{-2}) and PAR sum (mol m^{-2}) from the supplementary lights (SL) during the four measurement periods and the four periods together.

Period	Daily average		Total PAR sum		SL PAR sum	
	CF	Reference	CF	Reference	CF	Reference
1	17.0	10.1	879	525	544	235
2	24.2	20.0	1681	1396	352	223
3	21.3	21.4	1465	1479	-	-
4	17.2	13.7	998	784	316	125
All periods	19.9	14.0	5023	4184	1212	583

Table 4. Generative (GEN), vegetative (Veg.) and non-commercial (NCO) stems, total biomass production and difference (Dif.) between treatments "Cultivation of the Future" (CF) and reference.

	Noize			Virginia		
	Reference	CF	Dif. (%)	Reference	CF	Dif. (%)
GEN (stem m^{-2})	275	360	31.0	214	287	34.2
Veg. + NCO (stem m^{-2})	99	128	28.7	103	121	18.0
Biomass total (kg m^{-2})	30.3	39.0	28.5	25.5	34.5	35.4

Table 5. Generative stem production (stem m^{-2}) in CF and reference treatments and differences between treatments (Dif.) for the four separated analysis periods.

Period	Noize			Virginia		
	Reference	CF	Dif. (%)	Reference	CF	Dif. (%)
P1	38	44	15.6	29	40	37.3
P2	91	128	41.1	75	117	56.3
P3	103	138	34.1	74	98	31.6
P4	63	82	31.4	51	55	6.5

Production and natural light

A production peak occurred in periods 2 (spring) and 3 (summer), when respectively 80 and 100% of the supplied light was natural sunlight. The highest PAR sum (Table 3) was achieved in period 2 in CF treatment and led to stem production gains of 41 and 56% for 'Noize' and 'Virginia', respectively. During period 3, though daily light sum between treatments were equal, the higher light availability of previous periods on the CF treatment still had an influence, as plants during winter conditions could develop better and accumulate more reserves.

During period 3, as supplementary lights were not used, differences in light sum were negligible (Table 3).

In conditions of increased proportion of natural light, from spring to early autumn, the effects of diffuse light became more evident (Hemming et al., 2008). Several crops have shown production and quality gains when grown under diffuse light (Markvart et al., 2010; García Victoria et al., 2012; Dueck et al., 2012; Li et al., 2014, Marcelis et al., 2014), due to its better horizontal distribution in the greenhouse and higher penetration in the canopy layers (Hemming et al., 2008; Li et al., 2014); bringing less photoinhibition to the plants by less light peaks and lower leaf temperature (Dueck et al., 2012; Kempkes et al., 2012; Li et al., 2014), and therefore bringing higher photosynthesis capacity at leaf and canopy level (Hemming et al., 2008; Li et al., 2014).

In the CF treatment, diffuse light did not always improve light availability in deeper layers of the crop. For 'Noize', the bottom layer received slightly less light (-4%), data not shown, which was caused by higher light absorption of middle and upper layers due to increased leaf area index (LAI) of plants grown in this treatment (Table 6). For 'Virginia', light absorption was increased for the middle layers while the bottom layer was similar between treatments. García Victoria et al. (2012), reported similar results for Rose cultivation under diffuse light, as during a production flow, with increased LAI values, no differences in vertical distribution of light were observed.

Table 6. Leaf area index (LAI) calculated for weeks 23-25 in treatments CF and reference.

Week	Noize			Virginia		
	Reference	CF	Dif. (%)	Reference	CF	Dif (%)
23	2.93	4.24	44.7	1.87	2.85	52.4
24	2.77	4.04	45.8	1.71	2.68	56.7
25	2.88	4.37	51.7	1.74	2.46	41.4

Plants grown in this treatment also presented a higher A_{max} on the Top and Bottom layer for 'Noize', and on Top and Middle Layer for 'Virginia' (Table 7), leading to a A_c increase of 32 and 27.9% for 'Noize' and 'Virginia', respectively (Table 8). Li et al. (2014) also noticed an improvement on leaf net photosynthesis capacity with increasing levels of haze, however, to a lower extent, with a maximum 18.7% increase in A_{max}. Hemming et al. (2008) reported higher net photosynthesis rates in all layers of the crop, which contributed to an increase in fruit production of 11%. Diffuse light contributed to the improvement of *Alstroemeria* production in the CF treatment, however the effect was not isolated and other aspects of the cultivation may also have contributed to achieve such production gains.

Table 7. A_{max}, R, L_c, AQE, L_{sat} and ρ at 3 different crop layers for treatment CF and reference.

Cultivar	Layer	Treatment	A_{max}	R	L_c	L_{sat}	AQE	p
			(μmolCO$_2$ m^{-2} s^{-1})		(μmol m^{-2} s^{-1})			
Noize	Top	Reference	17.66^b	-0.45^{bc}	8.5	1277^a	0.049^b	2.01
		CF	23.09^a	-0.80^a	11.5	1285.5^a	0.069^a	2.10
	Middle	Reference	20.63^a	-0.55^b	8.4	1127.4^{ab}	0.067^a	2.20
		CF	18.17^b	-0.64^{ab}	13	1272^a	0.049^b	2.12
	Bottom	Reference	11.44^c	-0.25^c	6	838.5^c	0.047^{bc}	2.09
		CF	12.78^c	-0.24^c	6	1064^b	0.041^c	1.95
Virginia	Top	Reference	18.67^b	-0.57^b	13	1668.5^a	0.042^c	1.74
		CF	26.64^a	-0.94^a	13.5	1707^a	0.073^a	1.71
	Middle	Reference	19.80^b	-0.52^b	7	1005.5^{bc}	0.073^a	2.21
		CF	20.14^b	-0.47^b	7	1119^b	0.066^b	2.15
	Bottom	Reference	14.45^c	-0.32^b	4	771^d	0.071^{ab}	2.12
		CF	6.70^d	-0.34^b	16.5	856^{cd}	0.020^d	1.95

Different letters show significant differences at P=0.05.

Table 8. Canopy net photosynthesis rate (Ac) in treatments CF and reference, and difference between treatments (Dif.).

	Noize			Virginia		
	Reference	CF	Dif. (%)	Reference	CF	Dif (%)
A_c (μmol CO$_2$ m^{-2} s^{-1})	34.86	46.01	32.00	32.50	41.57	27.92

Production and reduced vapor pressure deficit

During the months with increased evaporative demand in periods 2 and 3, the CF treatment made a more intense use of the high-pressure humidification system, so that vapor pressure deficit (VPD), in this treatment, was always kept at lower levels. Also, stomatal conductance was higher in all crop layers for 'Noize' and in the top and middle layer for 'Virginia' (Table 9), while no differences were observed in neither stomata density nor size (data not shown).

Table 9. Average G_s (mol m^{-2} s^{-1}) under light intensity levels of 1500 µmol m^{-2} s^{-1} in CF and reference treatments.

	Noize		Virginia	
	Reference	CF	Reference	CF
Top layer	0.11±0.01	0.17±0.02	0.12±0.03	0.18±0.02
Middle layer	0.15±0.02	0.19±0.02	0.10±0.02	0.16±0.02
Bottom layer	0.10±0.01	0.18±0.03	0.09±0.02	0.07±0.01

Stomata play an important role in controlling CO_2 uptake and transpiration in leaves and are regulated, among other factors, by the evaporative demand of the environment or vapor pressure deficit (VPD) (Hetherington and Woodward, 2003). Experiments have shown that when VPD was held to a lower level, stomata closure was efficiently mitigated and stomata density and size increased, consequently leading to higher stomatal conductance (Zhang et al., 2015, 2017, 2018; Jiao et al., 2019). Fanourakis et al. (2015) also reported that Rose leaves grown at high relative humidity showed an increase in stomata size and density, this effect was also reported earlier for several crops (Bakker, 1991). In the CF treatment, however, lower VPD levels did not promote significant changes in stomatal size and density (data not shown), so that the increase of stomata conductance can only be explained by a higher opening.

Besides, Zhang et al. (2018) reported that growing tomato in a reduced VPD environment resulted in reduced stomatal closure, increased CO_2 acquisition and assimilation, enhanced plant photosynthesis capacity and, lastly, improved growth, biomass and fruit production. Trouwborst et al. (2017) measured net photosynthesis rates of *Alstroemeria* during summer months with or without the use of diffuse coating and humidification system and reported that the treatment combining diffuse light and lower VPD caused a higher photosynthesis rate than in the reference. A similar effect was also observed in the CF treatment as during period 2 and 3 stomata opening, photosynthesis capacity and, consequently, production gains were influenced also by the cultivation with lower VPD.

CONCLUSIONS

The *Alstroemeria* cultivars 'Noize' and 'Virginia' grown in the "Cultivation of the Future" showed 31 and 34% higher generative stem production than in the standard cultivation system during the first 11 months. The "Cultivation of the Future" consisted on a greenhouse cover of diffuse glass with AR coating, high intensity (200 µmol m^{-2} s^{-1}) LED (8% B, 13% W, 67% R, 12% FR) and a more intense use of the misting system. The reference system had a cover of transparent glass and low intensity (80 µmol m^{-2} s^{-1}) HPS lamps. The aspects that influenced a higher production in the "Cultivation of the future" varied through the seasons: in winter, spring and autumn, when natural light availability was low, the higher light intensity supplied by LED lights increased crop photosynthesis. As a result, the crop had a higher LAI. In summer when high natural light levels were available and the artificial light was not used, production gains were achieved, besides of the influence of the previous period on LAI, due to the milder climate provided by the lower VPD and the highly diffused incident light, which caused an increase in the stomatal conductance and photosynthesis capacity of the plants.

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