Effect of the "Cultivation of the future" on Alstroemeria production, stomata conductance and photosynthesis capacity

Graduation Thesis in Agronomic Engineering

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Rapport WPR-1141

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WAGENINGEN UNIVERSITY & RESEARCH

KAS ALS ENERGIEBRON

Glastuinbouw Nederland
morgen groeit vandaag

Ministerie van Landbouw, Natuur en Voedselkwaliteit

ESALQ
Luiz de Queiroz College of Agriculture
University of São Paulo

Referaat
De "Teelt van de Toekomst" is een innovatief, fossielvrij en klimaatneutraal teeltsysteem voor Alstroemeria teelt, ontwikkeld door Wageningen University & Research (WUR) in het kader van "Kas als Energiebron" en in overleg met Nederlandse telers. Het teeltsysteem is beproefd in vergelijking met een referentie teeltsysteem in Nederland. Voor het experiment zijn twee kascompartimenten van WUR Glastuinbouw en twee Alstroemeria cultivars gebruikt. Tijdens het eerste jaar van het experiment liet de "Teelt van de toekomst" een 31% en 34% hogere generatieve stengelproductie zien, voor respectievelijk de rassen Noize en Virginia. Verschillen in klimaatomstandigheden, intensiteit en kwaliteit van het invallende licht waren de belangrijkste factoren die van invloed waren op de opbrengstwinst. Tijdens perioden met weinig natuurlijk licht, verhoogde de hogere lichtintensiteit van LED-lampen de gewas assimilatie. Op momenten met veel natuurlijk licht, was het mildere klimaat door een lagere VD en het sterk diffuse invallende licht die voor een hogere huidmondjesgeleidbaarheid zorgde en meer fotosynthese capaciteit. Het project is mogelijk gemaakt met inspiratie en financiële steun van Kas als Energiebron, het Nederlandse energietransitieprogramma voor de glastuinbouw.

Abstract
The "Cultivation of the Future", is an innovative, fossil free and climate neutral cultivation system for Alstroemeria growing developed by Wageningen University & Research (WUR) in the framework of the program "Greenhouse as Source of Energy" in consultation with Dutch growers. This growing system was tested and compared to a reference cultivation system in The Netherlands. For the experiment, two greenhouse compartments of WUR and two Alstroemeria cultivars were used. During the first year of the experiment, the "Cultivation of the future" showed 31% and 34% higher generative stem production, for respectively the varieties Noize and Virginia. Differences in climate conditions, intensity and nature of the incident light were the main factors influencing such yield gains. During periods with low natural light availability, the higher light intensity supplied by LED lights increased photosynthesis and crop assimilation. At moments with high natural light availability, was the milder climate provided by a lower VD and the highly diffused incident light, what caused an increase in the stomata conductance and in the photosynthesis capacity of the plants. The project was made possible with inspiration and financial support of 'Kas als Energiebron', the Dutch energy transition program for the greenhouse sector.

Rapportgegevens

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Disclaimer

Adresgegevens

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1 Introduction

Alstroemeria L., described by Linnaeus (1762), is a neotropical genus with approximately 65 species with natural distribution in South America (ASSIS, 2009). Believed to be a promising cut flower, due to the high ornamental value, good post-harvest quality, high production and low energy growing requirements, breeders developed, in the 1960's, the first varieties for greenhouse production.

Production was then seasonal, with flows during spring (March – June) and fall (October – November) (VONK NOORDEGRAAF, 1975). Therefore, to overcome seasonality, efforts were made towards a deeper understanding of the photoperiod and temperature influences on generative and vegetative stem production (HEINS; WILKINS, 1979; VONK NOORDEGRAAF, 1981; HEALY; WILKINS, 1982), and further towards isolated influence of soil temperature and the soil cooling technique on flower production (KEIL-GUNDERSON *et al.* 1989; BLOM; PIOTT, 1990; VAN DE WIEL, 1990; VAN LABEKE; DAMBRE, 1993; STAPEL; MOURIK, 1997; VAN NOORT, 2009; HEIJ *et al.* 2004).

Though a bigger focus was given to crop flowering induction factors and techniques, other cultivation aspects of the crop were also discussed, as the use of assimilation lighting (VAN LABEKE; DAMBRE, 1993; UITERMARK *et al.* 1996; DIJKSTRA, 2009; GARCIA VICTORIA *et al.* 2017), CO_2 enrichment (VAN NOORT, 2004), adequate nutrition (DE KREIJ *et al.* 1997; VAN DER HELM *et al.* 2013) and cultivation methods (BAKKEN, 2000). Heij (2002) also summarized ten years of applied research with Alstroemeria in The Netherlands (HEIJ, 2002). Regarding Alstroemeria's photosynthesis characteristics, Leonardos *et al.* (1994) analyzed, in experimental conditions, the influence of irradiance, CO_2 concentration, and temperature on leaf and crop net photosynthesis rates, as well as their optimum levels, and then, in 1996, the effects of leaves (source) and roots (sink) temperatures on net photosynthesis, carbon export and partitioning rates (LEONARDOS *et al.* 1994; 1996). Trouwborst *et al.* (2015) analyzed photosynthesis efficiency and its saturation levels by two Alstroemeria cultivars, in order create a CO_2 enrichment and energy-efficient lighting strategy for the crop (TROUWBORST *et al.* 2015). Later, Trouwborst *et al.* (2017) evaluated the influence of diffuse coating and misting system on net photosynthesis rates of Alstroemeria, by minimizing light stress during summer (TROUWBORST *et al.* 2017).

Moreover, as result of an increase of incentives to a more energy efficient and energy clean cultivation in horticulture, Labrie and de Zwart (2010) designed a new cultivation system for Alstroemeria, with improved energy efficiency by the use of temperature integration, negative difference between day and night temperatures, dehumidification system and double screening (LABRIE; DE ZWART, 2010). Later, experiments done at growers facilities brought energy efficiency improvement by the use of LED in hybrid assimilation light systems (GARCIA VICTORIA *et al.* 2015; GARCIA VICTORIA *et al.* 2018), by providing less humidity input in double screen greenhouses, via better isolation of the soil, discard of residual wastes and by use of ventilation systems (HELM *et al.* 2012; GARCIA VICTORIA *et al.* 2017).

Though energy use in horticulture has lowered, as outcome of the past years of research, the goal of achieving fossil free cultivation is still a challenge. Therefore, de Zwart (2019) described possible solutions for warmth necessity in fossil free greenhouses (DE ZWART, 2019), and designed, in partnership with the project "Alstroemeria of the future, close by", a fossil free cultivation system for Alstroemeria, the "Cultivation of the Future". This system was built in one experimental greenhouse compartment in the Business Unit Greenhouse Horticulture and Flower Bulbs (Bleiswijk, The Netherlands) and compared to a reference compartment, which simulated the crop's standard growing system in The Netherlands.

During this study, focus has been given to the effects of the "Cultivation of the Future" on production, photosynthesis and stomata conductance of two Alstroemeria's cultivars grown for one year in this system.

2 Objectives

By the comparison between the "Cultivation of the Future" and the reference systems, this study aims to evaluate the influence of this new system on:

- Production in terms of number of generative, vegetative and non-commercial stems and biomass.
- Crop photosynthesis response to light and CO_2 .
- Stomata conductance, density and size.
- Light stress.

3 Material and methods

The experiment was done during one year and information about the cultivation and measurements are explained as follows.

3.1 Plant Material and Cultivation

Two cultivars of Alstroemeria (Alstroemeria x 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 (13x1x0,6m), with constant soil temperature of 15.5°C, at a density of 3,5 plants per m² in two Venlo type greenhouse compartments in Bleiswijk (the Netherlands, 52 °N, 4.5 °E) with dimensions of 15 x 9.6m (144 m²) and gutter height of 5.5 m. The greenhouse climate was controlled by a Hoogendoorn iSii process computer (Hoogendoorn, Vlaardingen, the Netherlands) and climate data was analysed in four different periods, as Table 1 shows.

Table 1
Climate analysis periods.

Period	Interval	Week
1	5th February - 28th March	6 – 13
2	29th March - 5th June	13 - 23
3	6th June - 12th August	23 – 33
4	13th August - 7th October	33 - 40

3.2 Treatments

The experiment consists in a comparison of two different cultivation systems. The first is the "Cultivation of the Future", designed previously in a desk study by de Zwart *et al.* (2019), with the aim of achieving a climate neutral, fossil free cultivation with better production and quality during the winter. The second represents the standard Alstroemeria cultivation system in the Netherlands, which served as reference. Each greenhouse compartment represented one cultivation system, and their main differences are highlighted in Table 2.

Table 2
Comparison between cultivation systems.

System Components	Reference	Cultivation of the Future
Covering material	Clear glass	Diffuse glass (70% haze) with double anti-reflective coating
Assimilation lights	HPS, 80 μmol.m^{-2}.s^{-1}	LED (RWB FR), 200 μmol.m^{-2}.s^{-1}
Warming system	Gas	Electricity, latent and surplus heat
Screening	1 Screen (Harmony 7247 FR)	2 screens (Harmony 7247 FR and Luxous 1147 FR)
Humidification system	Misting system (150 g.m^{-2}.h^{-1}) with 50% RH as set point	Misting system (150 g.m^{-2}.h^{-1}) with 60% RH as set point
Dehumidification system	Window opening	Window opening and input of outside heated air

Besides the increase in photosynthetic active radiation (PAR) from 80 $\mu mol.m^{-2}.s^{-1}$ to 200 $\mu mol.m^{-2}.s^{-1}$ in the "Cultivation of the future", there was also a substantial change in light spectra between the HPS and LED lights. For the experiment, a previous test was made in the Light Innovation and Demonstration Centre (Bussiness Unit Greenhouse Horticulture and Flowerbulbs, Bleiswijk) for the selection of a suitable spectrum for Alstroemeria (WEERHEIM *et al.* 2018), measured light spectra in both treatments is shown in Figure 1 and Figure 2.

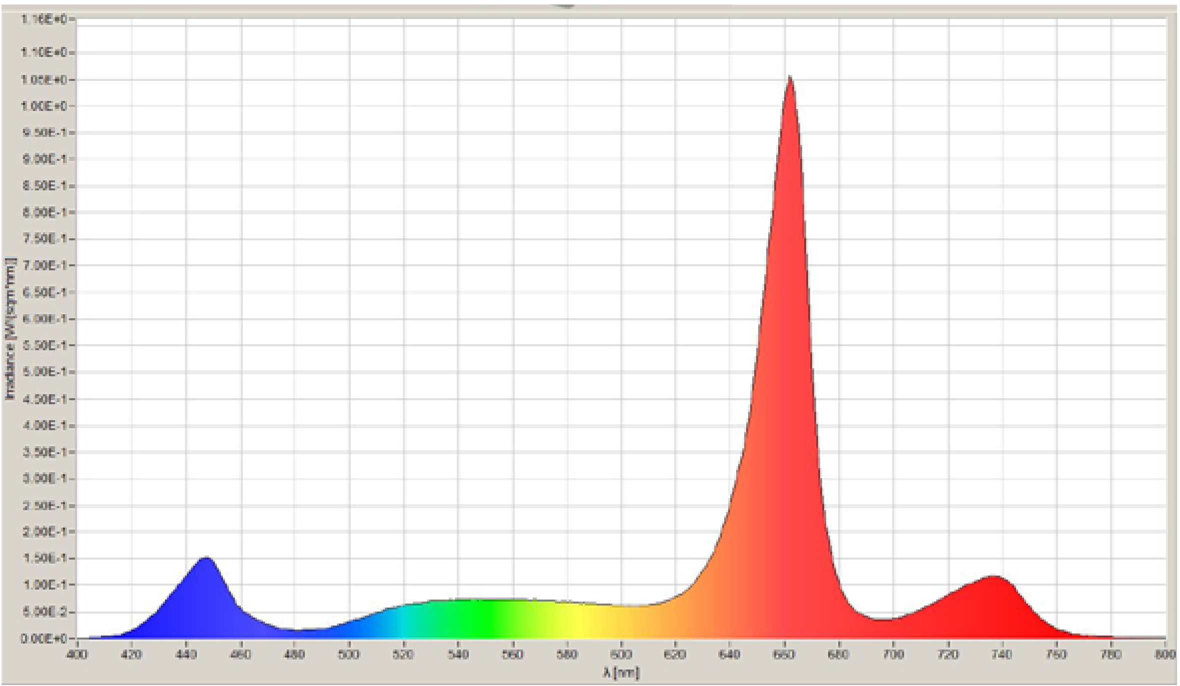


Figure 1 *Light spectra in the Cultivation of the Future.*

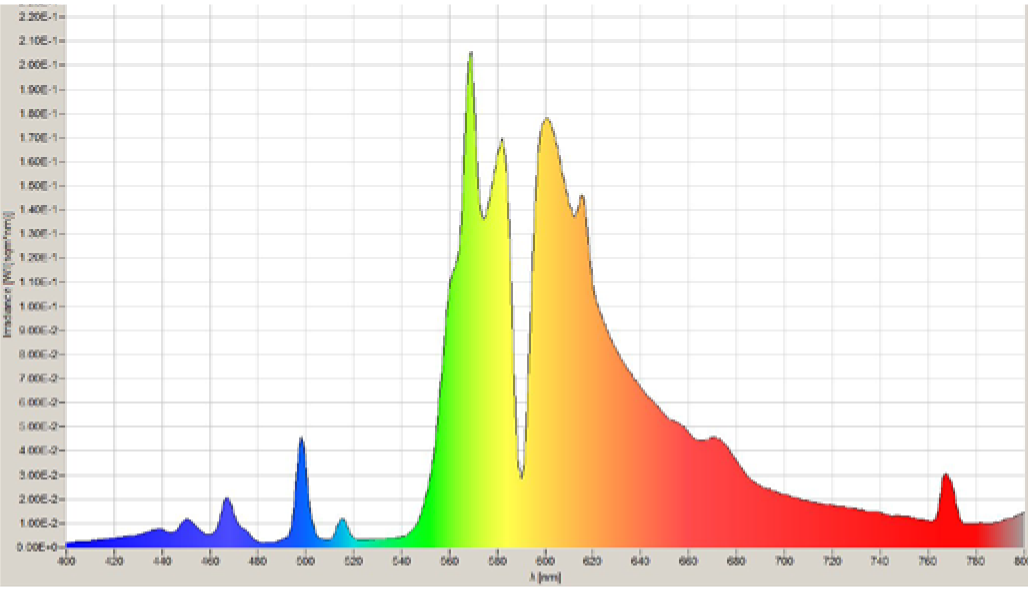


Figure 2 *Light spectra in the Cultivation of the Future (Reference).*

3.3 **Production Quantification**

As shown in Figure 3, a total of 12 plots (1,2x1,0m, 3 $plots.variety^{-1}.treatment^{-1}$) were used to quantify production during the cultivation period. Stems were harvested twice per week, classified as generative stems (commercial valuable stem), non-commercial stems (with less than 3 flowers, or with defects) or vegetative stems (with no flowers). Generative stems were then measured (weight, length and number of peduncles). Non-commercial and vegetative stems were counted and weighted.

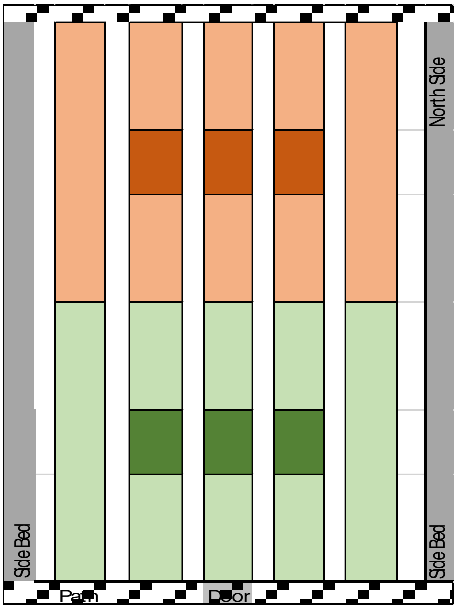


Figure 3 *Experimental layout. Variety Virginia (Orange), Noize (Green) and measurement fields (dark orange and dark green).*

3.4 Destructive measurement

Monthly, in order to evaluate the crop's quality, 15 stems.variety^{-1}.treatment^{-1} were harvested to determine weight of stem, buds and leaves, number of leaves, leaf surface using a Li-COR 3100 (Li-Cor Biosciences, Inc., Lincoln, NE, USA) and dry weight.

3.5 Generation time

Every other week, 10 shoots.variety^{-1}.treatment^{-1}, with a length of 5 cm above the soil, were labeled. After achieving ripeness, stem's length was measured and date of the harvesting was registered, making possible to calculate the generation time (from new shoot to ripe stem) and growth rate of the stems.

3.6 Leaf Absorbance

On week 38, stems were harvested in the morning and supplied with water in order to keep stems hydrated. Later in the same day, upper layer leaves (6 leaves.variety^{-1}.treatment^{-1}) were detached from the stems and transmittance and reflectance of light were measured in 5 nm steps using a Spectrophotometer (LAMBDA 950 UV/VIS, PerkinElmer, Waltham, MA, US) in a range from 400 to 800 nm. Afterwards, absorbance was calculated as 1 – reflectance – transmittance. This values served as basis for light response curves analysis to calculate photosynthesis parameters.

3.7 Crop light interception and Leaf Area Index

During weeks 23 and 24, light interception by the crop was measured using a line sensor (SS1 SunScan, Delta-T, Cambridge, UK). The canopy was divided in 3 layers for Noize (0, 35 and 70cm) and 4 layers for Virginia (0, 35, 70, 105cm) and the measurements took place in 6 points of each greenhouse compartment. From this method is possible to determine the amount of light reaching different layers of the crop, an important factor to calculate canopy net photosynthesis rates. Besides, leaf area index (LAI) was estimated for weeks 23 to 28, using data from production and destructive measurements.

3.8 Photosynthetic gas exchange measurements

During the weeks 23 and 24, two Li-6800 portable photosynthesis systems (Li-Cor Biosciences, Inc., Lincoln, NE, USA), were used simultaneously, in both greenhouse compartments, to measure net photosynthesis rate (A) and stomatal conductance (G_s) in different canopy layers (upper, middle and bottom layers, Figure 4), light intensities and CO_2 concentrations, in a total of 72 samples (6 leaves.layer $^{-1}$.variety^{-1}.treatment^{-1}).
For the measurement, leaves were enclosed in a 2 cm² chamber, with light source spectrum composed of 90% red and 10% blue light and initial intensity of 1500 µmol.m^{-1}, CO_2 initial concentration was 600ppm, temperature was set to 26°C, relative humidity (RH) to 60%, and air flow to 400 µmol.s^{-1}. Temperature, RH and CO_2 concentration were chosen according to climate during the measurement period.

Figure 4 *Example of stems of Noize (above) and Virginia (below) and crop layers (red boxes).*

After achieving stable conditions inside the measurement chamber, CO_2 concentration varied to 50, 100, 150, 200, 400, 600, 800, 1000, 1500 and 2000 ppm, while the light levels were kept constant to 1500 µmol.m^{-2}.s^{-1}, in order to generate a CO_2 response curve. Subsequently, CO_2 levels were held constant to 600 ppm, while light levels varied to 1500, 1000, 750, 500, 350, 200, 150, 100, 50 and 0 µmol.m^{-2}, in order to generate the light response curve.

Light response curves were then analyzed via a LiCor support program (Photosynthesis, Licor Biosciences, Inc., Lincoln, NE, USA) and the parameters light saturation (L_{sat}), maximum net photosynthesis rate (A_{max}), respiration (R), light compensation (L_c), apparent quantum yield (AQE) and curvature (ρ) were calculated. For stomatal conductance (G_s) analysis, values were selected from the light response curve at light step of 1500 µmol.m^{-2}.s^{-1}, at which plants were light saturated or close to saturation point, what according to Medrano *et al.* (2002) makes possible a more standardized analysis of the relations between photosynthetic parameters and G_s (MEDRANO *et al.* 2002).

3.9 Canopy net photosynthesis rate

Canopy net photosynthesis rate was calculated following equations given in Li-Cor Application Note (Norman *et al.* Li-Cor Biosciences, Inc., Lincoln, NE, USA). First net photosynthesis rate (A) was calculated as follows in Equation 1,

$$A = \frac{(Aqe.I_a)}{[1+(Aqe.I_a)^{\rho}]^{\frac{1}{\rho}}} \qquad (1)$$

Where I_a is the light absorbed quanta, calculated as incident light (Q) multiplied by a leaf absorbance coefficient (Abs) (Equation 2).

$$I_a = Abs.Q \qquad (2)$$

Net photosynthesis rate of the layer (A_{layer}) is then calculated as shown in Equation 3 and canopy net photosynthesis rate (A_c) is calculated as the sum of the tree experiment layers (Equation 4)

$$A_{layer} = A\left(\frac{LAI}{3}\right) \qquad (3)$$

$$A_c = \sum_{i=1}^{3} A_{layer_i} \qquad (4)$$

3.10 Stomata density and size

On week 36, the silicon rubber impressions technique (WEYERS; JOHANSEN, 1985) was used to make leaf prints, which were analyzed with a microscope (Axio Lab.A1, Carl Zeiss, Jena, Germany) images were done with a camera coupled in the microscope (Axiocam 105 color, Carls Zeiss, Jena, Germany) and later images were analyzed using an image processing software (ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA) to determine stomata density 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.

3.11 Chlorophyll fluorescence

Maximum PS II Yield (Fm/Fv) of both varieties was measured in 30 minutes intervals using a fluorescence meter (Plantivity, Plant Dynamics, Randwijk, The Netherlands). Measurements were done first in the reference treatment, during the months April and May and on June and July, in the “Cultivation of the Future”. Days with the highest PAR sums were then selected and analyzed, in order to see the presence of photoinhibition during extreme summer days.

3.12 Statistical analysis

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

4 Results

During the experiment period, several data was measured and are shown as follows.

4.1 Climate

4.1.1 Light

Treatment "Cultivation of the Future" showed higher values of PAR intensity (Figure 5 to Figure 8) and PAR sum (Table 3) during periods 1, 2 and 4 (see Table 1). Differences are related to the number of hours of assimilation lights use and to the higher intensity of the LED lights. During period 3, when all the light provided was natural, no differences were noticed between treatments.

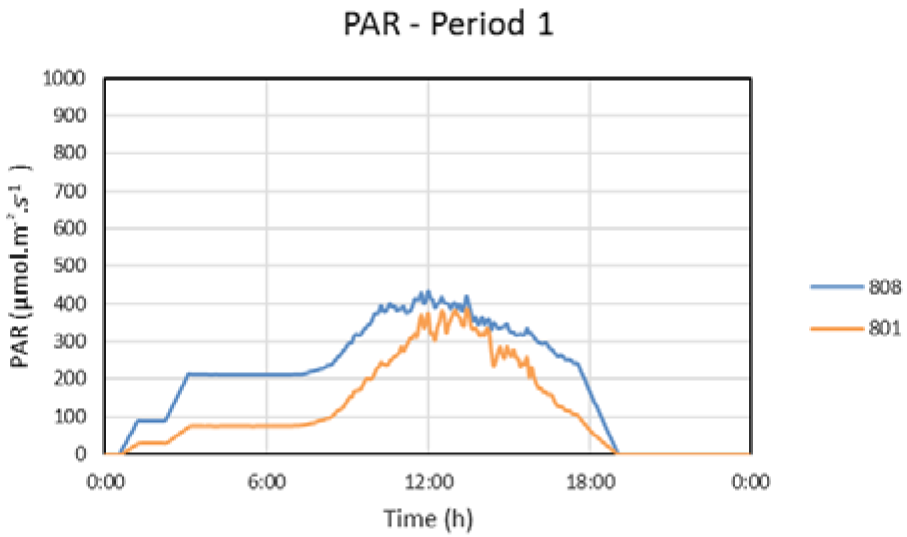


Figure 5 *Cyclic average PAR intensity for period 1 in "Cultivation of the Future" (808) and reference (801) treatments.*

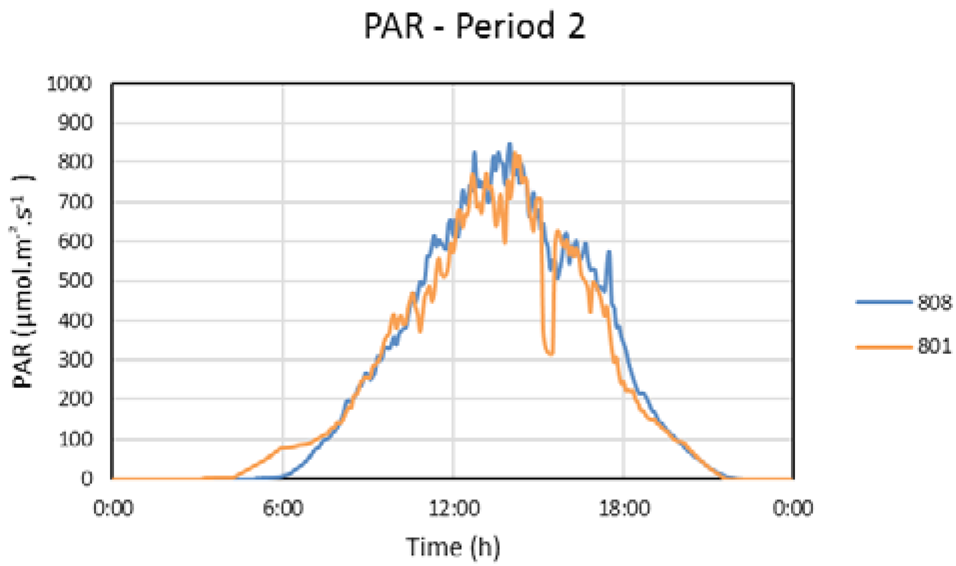


Figure 6 *Cyclic average PAR intensity for period 2 in "Cultivation of the Future" (808) and reference (801) treatments.*

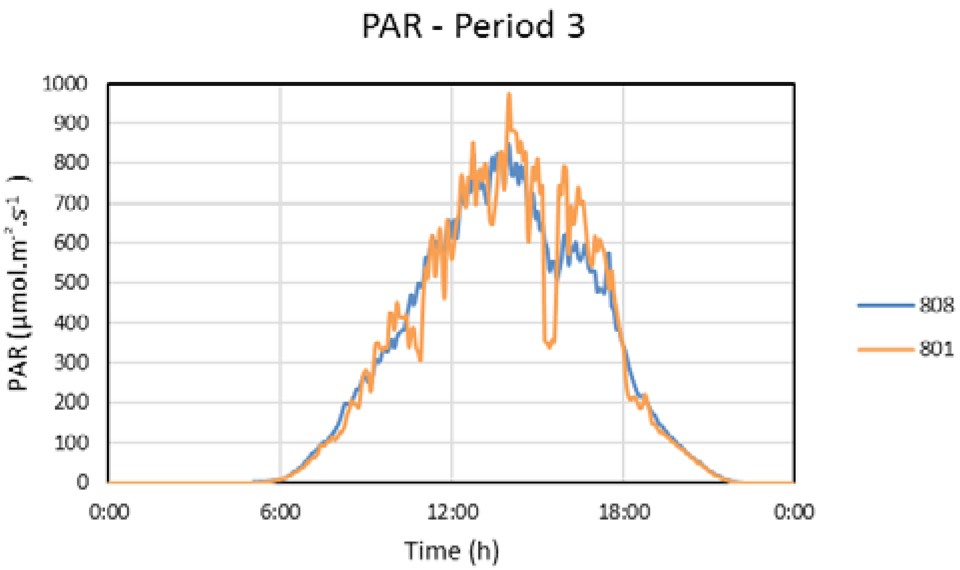


Figure 7 *Cyclic average PAR intensity for period 3 in "Cultivation of the Future" (808) and reference (801) treatments.*

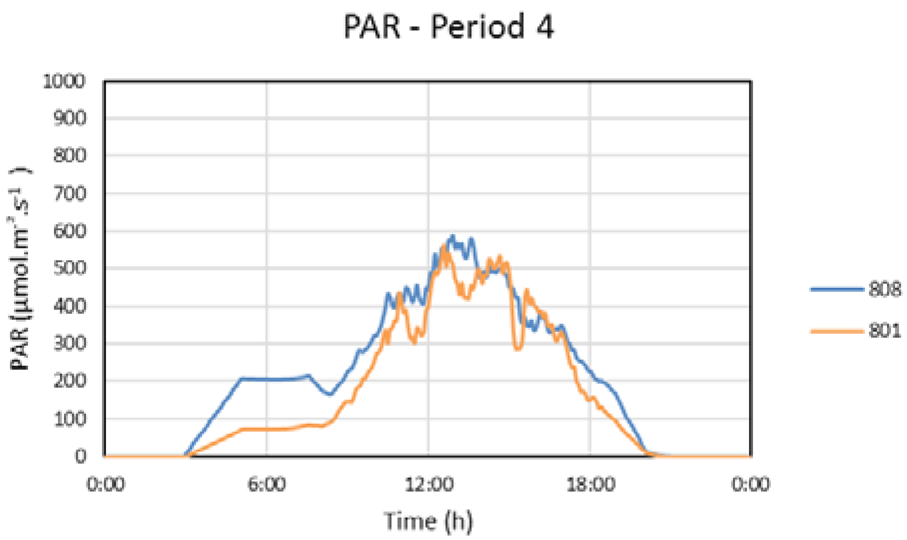


Figure 8 *Cyclic average PAR intensity for period 4 in "Cultivation of the Future" (808) and reference (801) treatments.*

Table 3
Daily average, total and supplementary lights (SL) PAR sum (mol) during measurement periods.

	Daily average		Total PAR sum		SL PAR sum	
Period	CF	Reference	CF	Reference	CF	Reference
1	17.01	10.13	878.67	525.06	543.78	235.344
2	24.20	20.05	1680.82	1395.58	351.72	223.32
3	21.30	21.42	1465.28	1479.44	-	-
4	17.17	13.72	997.95	784.46	315.90	124.68

4.1.2 Temperature

Temperature variation during a cyclic day of four different periods is shown in Figure 9 to Figure 12. The experiment followed the Floriconsult Temperature Table (DE GROOT, 2017, unpublished), which states that average temperature should increase proportionally to the light intensity. Therefore, in the "Cultivation of the Future", average temperature (Table 4) was higher in periods 1, 2 and 4, due to also higher light intensity values during these periods.

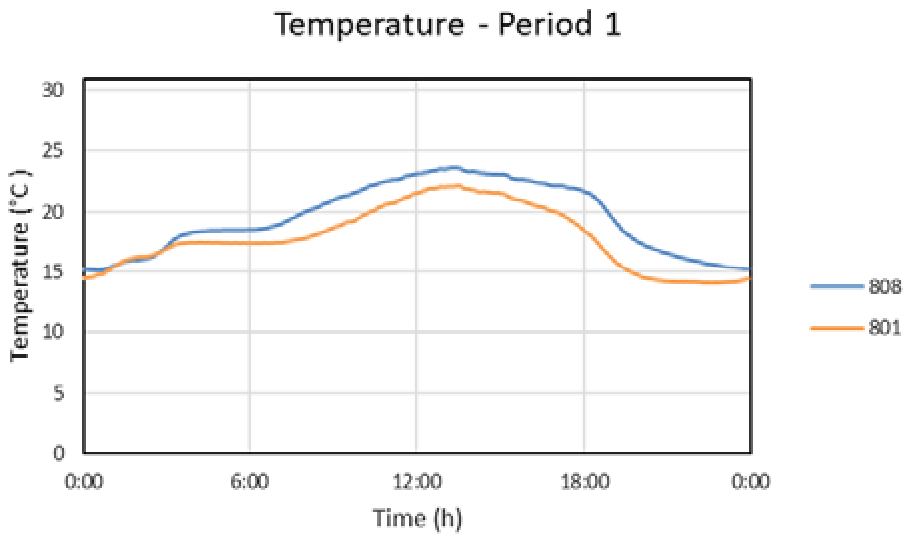


Figure 9 *Cyclic average temperature for period 1 in treatments "Cultivation of the future" (808) and reference (801).*

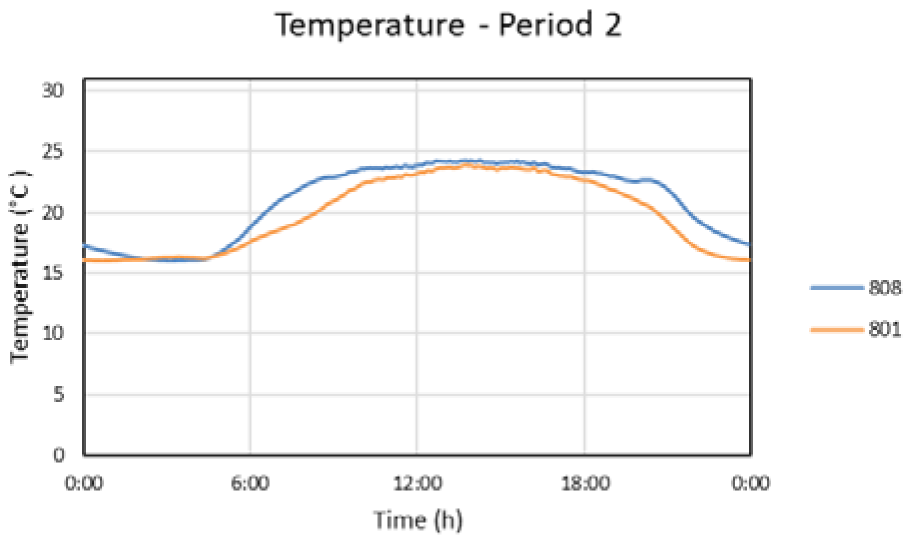


Figure 10 *Cyclic average temperature for period 2 in treatments "Cultivation of the future" (808) and reference (801).*

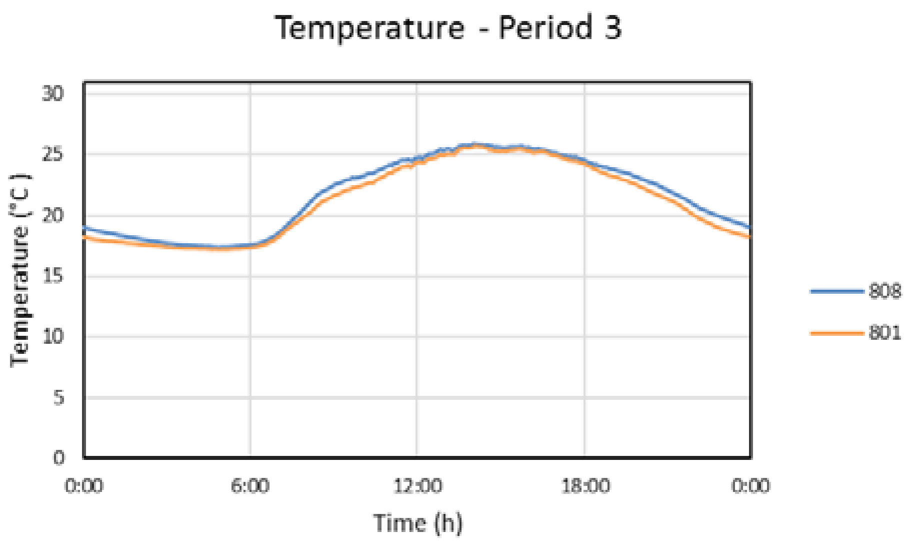


Figure 11 *Cyclic average temperature for period 3 in treatments "Cultivation of the future" (808) and reference (801).*

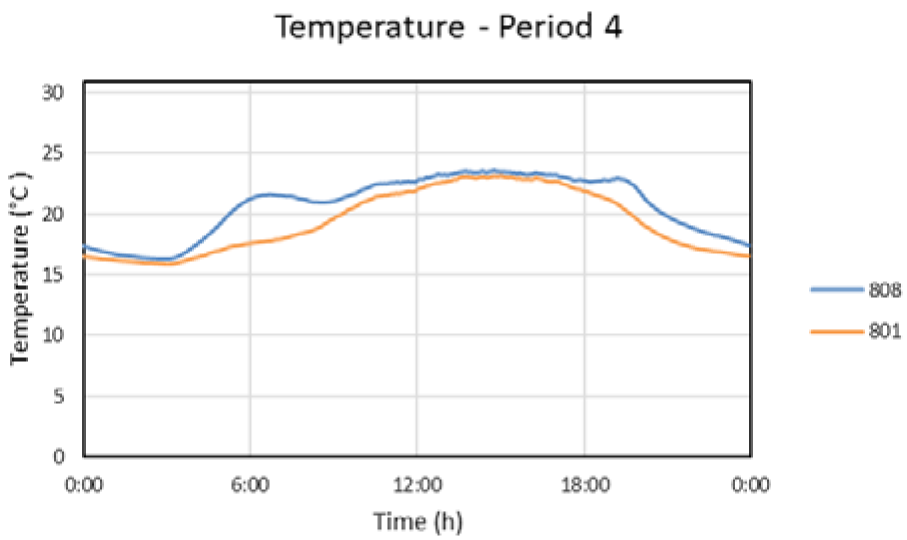


Figure 12 *Cyclic average temperature for period 4 in treatments "Cultivation of the future" (808) and reference (801).*

Table 4

Average temperature (°C) during four experiment periods in treatments "Cultivation of the Future" (CF) and reference.

	Treatment	
Period	CF	Reference
1	19.5	17.9
2	21	19.9
3	21.8	21.3
4	20.8	19.5

4.1.3 Vapour Deficit (VD)

While in period 1, VD values were similar between greenhouses, periods 2, 3 and 4, showed lower values for treatment "Cultivation of the Future" (Figure 13 to Figure 16), which means that, in this treatment, plants had a potentially higher stomatal conductance capacity due to a milder and less stressful environment. This is result of a more intense use of the misting system in this treatment, due to activation set point at higher RH values.

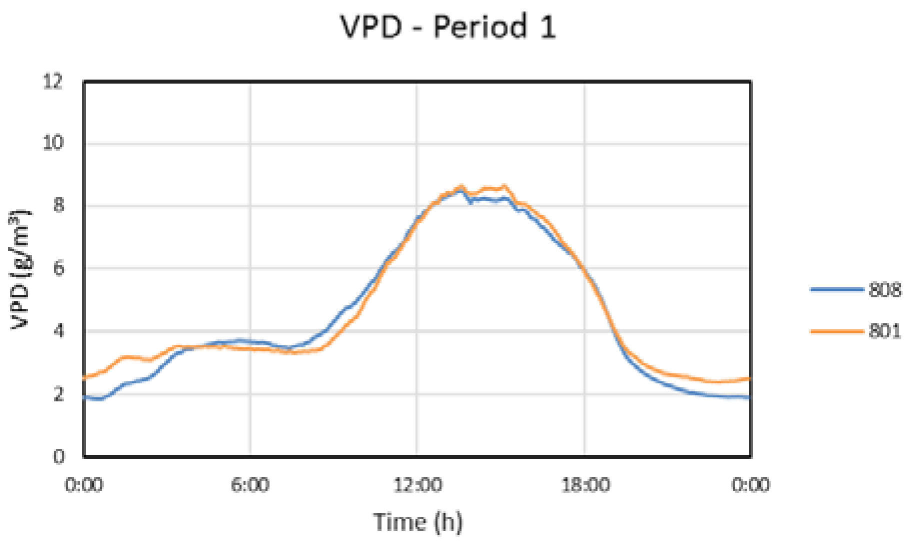


Figure 13 *Cyclic average vapour deficit (VD) for period 1 in treatments "Cultivation of the future" (808) and reference (801).*

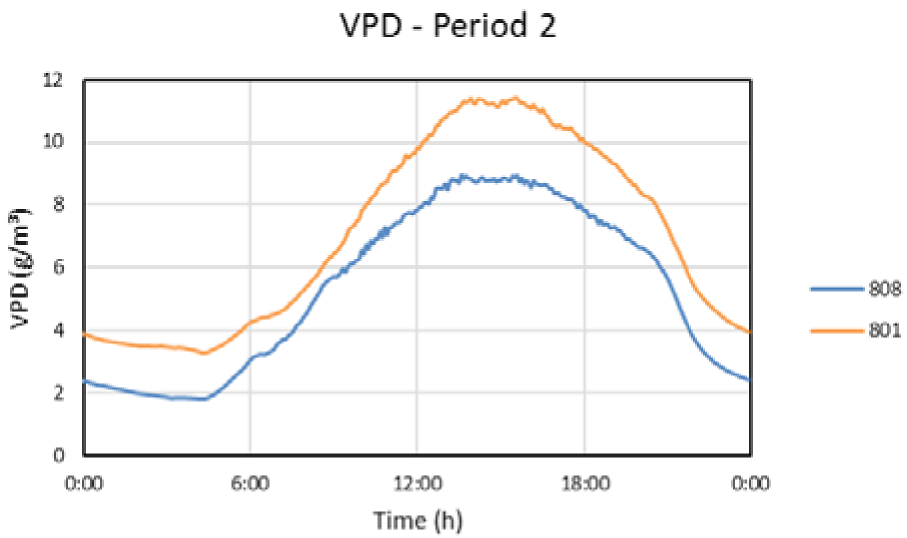


Figure 14 *Cyclic average vapour deficit (VD) for period 2 in treatments "Cultivation of the future" (808) and reference (801).*

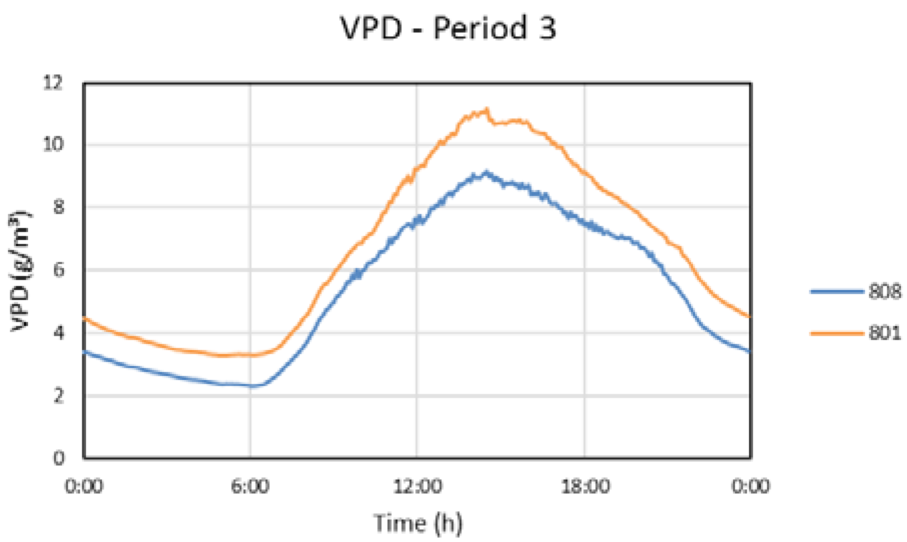


Figure 15 *Cyclic average vapour deficit (VD) for period 3 in treatments "Cultivation of the future" (808) and reference (801).*

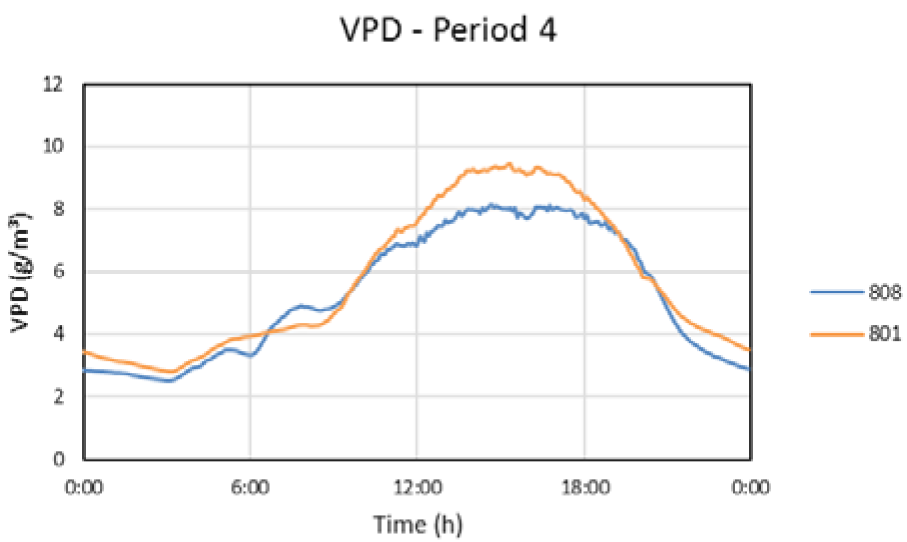


Figure 16 *Cyclic average vapour deficit (VD) for period 4 in treatments "Cultivation of the future" (808) and reference (801).*

4.1.4 CO_2 Concentration

CO_2 concentration fluctuated during the day (Figure 17 to Figure 20), the variation was caused by ventilation in the greenhouse compartments, which was higher or lower according to the cooling necessities. During night period, CO_2 concentration was higher in treatment "Cultivation of the Future" as a result of higher isolation capacity of the greenhouse, given by the double screening, and maybe as a result the higher amount of night respiration rates in this greenhouse, since the crop in this treatment had higher biomass production (Table 5) and higher respiration rates (Table 11).

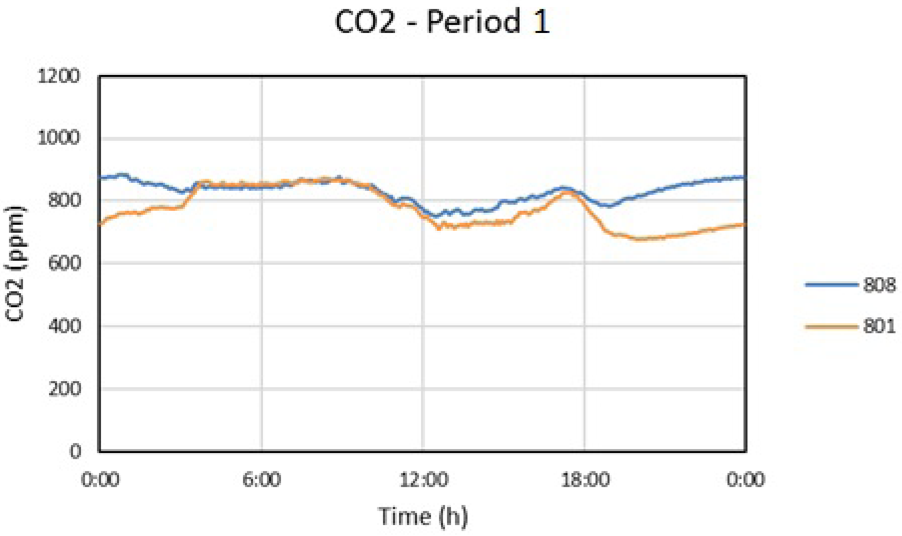


Figure 17 *Cyclic average CO_2 concentration for period 1 in treatments "Cultivation of the future" (808) and reference (801).*

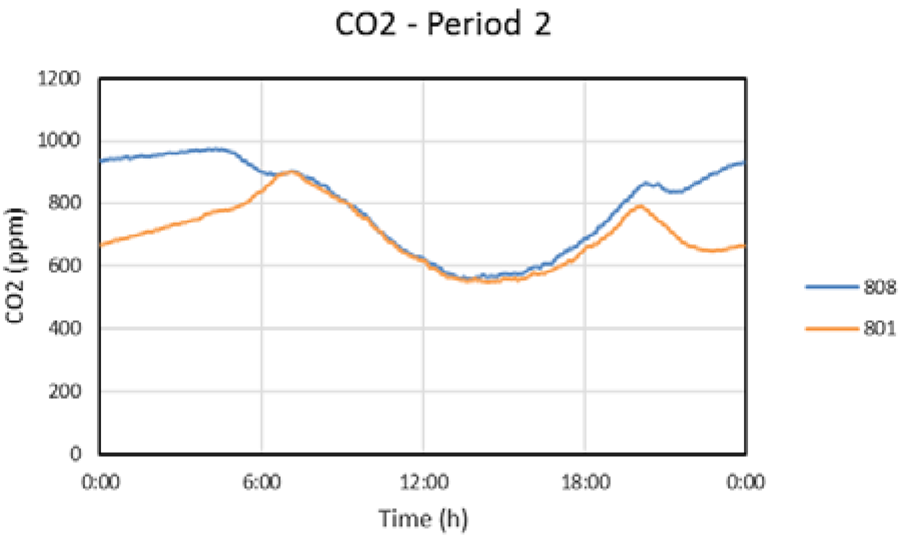


Figure 18 *Cyclic average CO_2 concentration for period 2 in treatments "Cultivation of the future" (808) and reference (801).*

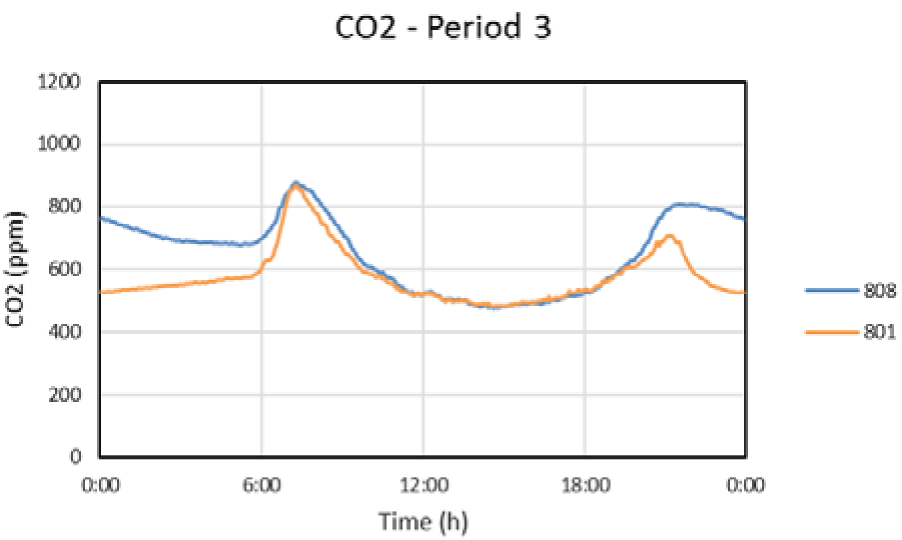


Figure 19 *Cyclic average CO_2 concentration for period 3 in treatments "Cultivation of the future" (808) and reference (801).*

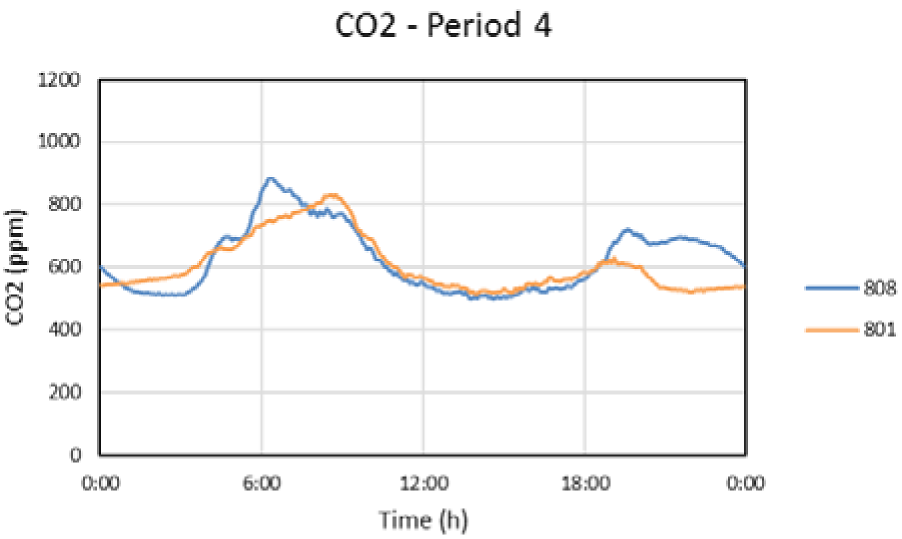


Figure 20 *Cyclic average CO_2 concentration for period 4 in treatments "Cultivation of the future" (808) and reference (801).*

4.2 Production and Quality

4.2.1 Production

Cumulative production of commercial (Figure 21), vegetative and non-commercial stems (Figure 22) were determined from first week of production until week 40. During this period, the "Cultivation of the Future" showed higher generative stem production for cultivars Noize (+30,97%) and Virginia (+34,17%), and higher vegetative and non-commercial stem production for Noize (+28,68%) and Virginia (+18,23%) (Table 5). Besides, production in different experiment periods are shown in Table 6.

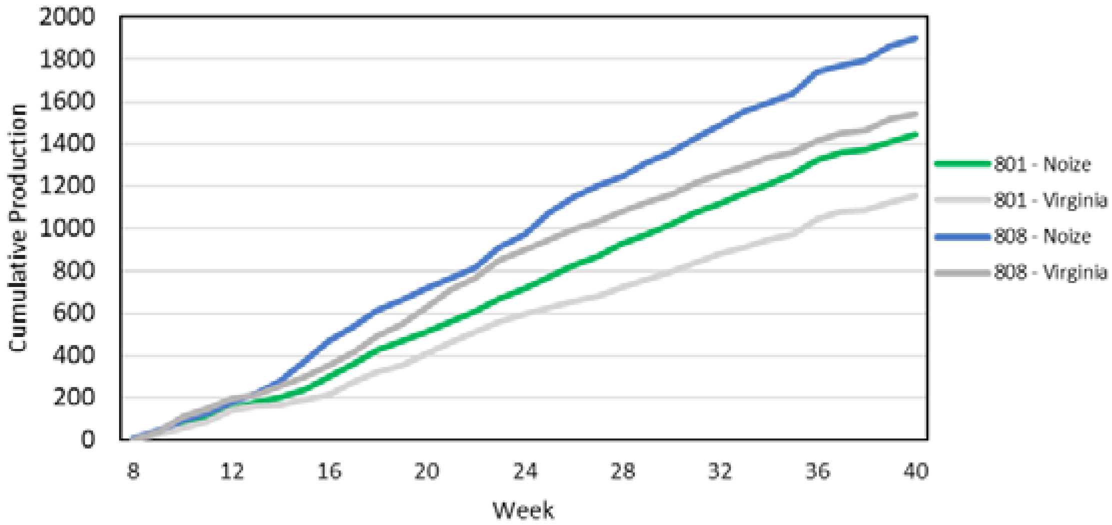


Figure 21 *Cumulative stem production of measurement fields from week 8 to week 40 of treatments "Cultivation of the future" (808) and reference (801).*

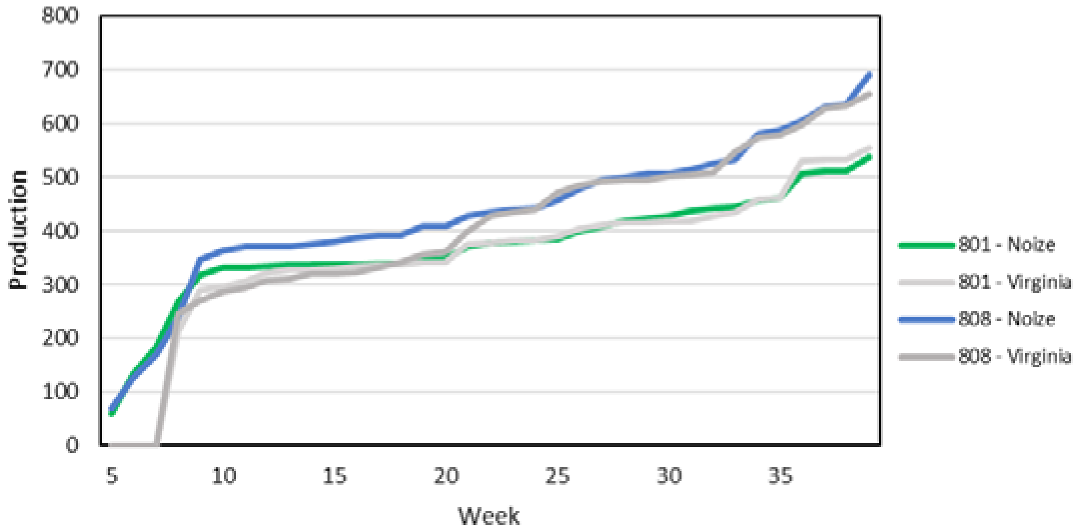


Figure 22 *– Cumulative vegetative and non-comercial stem production of measurement fields from week 5 to week 40 of treatments "Cultivation of the future" (808) and reference (801).*

As expected, higher stem production in the "Cultivation of the Future" also brought higher biomass production (Figure 23 and Table 5). In comparison to the reference, treatment "Cultivation of the Future" showed an increase of total biomass production of 28,48% for Noize and 35,45% for Virginia (Table 5).

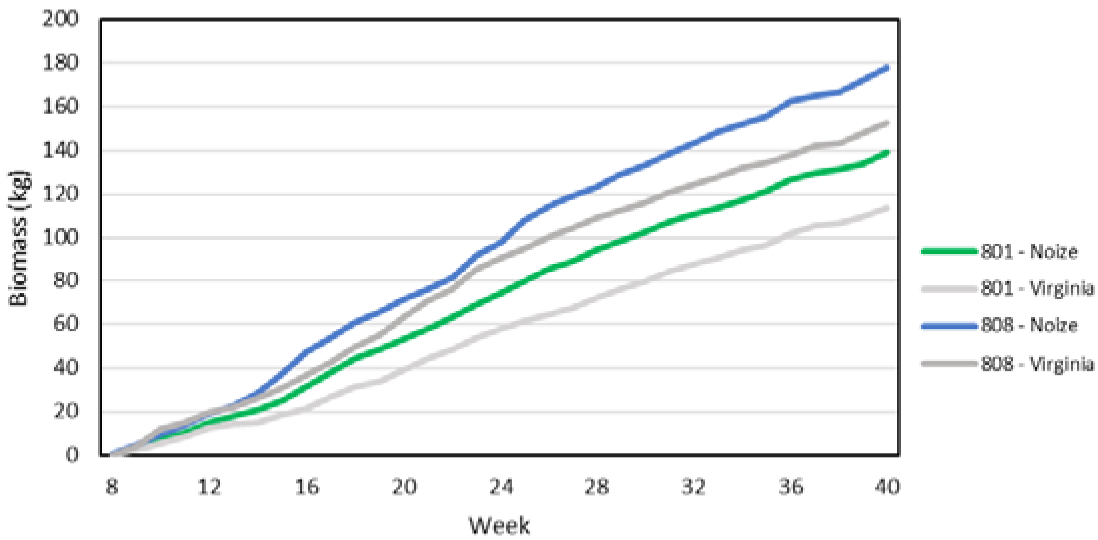


Figure 23 *Cumulative generative stem biomass production of measurement fields from week 8 to week 40 of treatments "Cultivation of the future" (808) and reference (801).*

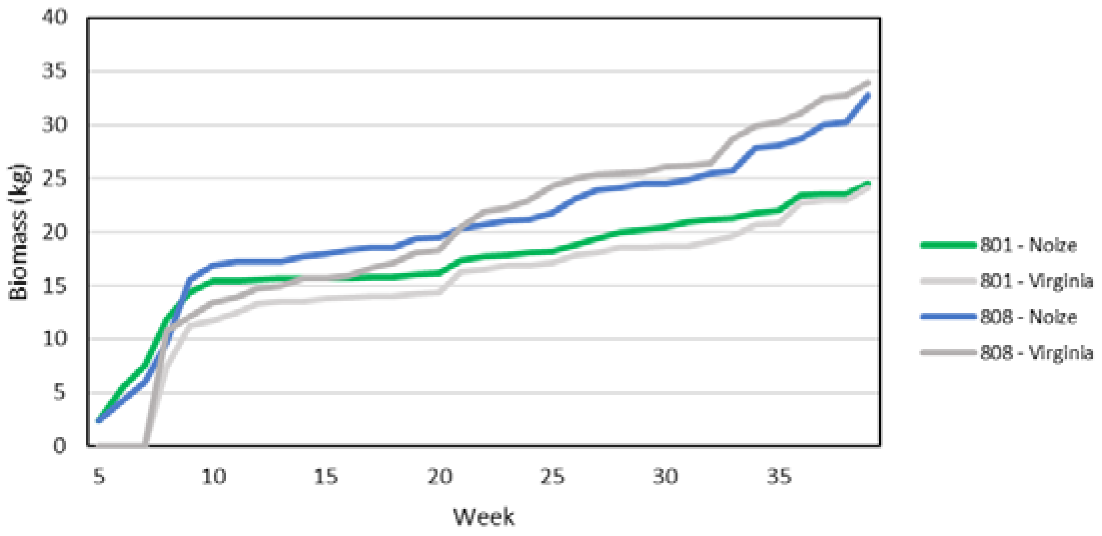


Figure 24 *Cumulative vegetative and non-comercial biomass production of measurement fields, from week 5 to week 40 of treatments "Cultivation of the future" (808) and reference (801).*

Table 5

Generative (GEN), vegetative (Veg.) and non-commercial (NCO) stem production (stem/m²), biomass production (kg/m²) and difference (Dif.) between treatments "Cultivation of the Future" (CF) and reference.

	Noize			Virginia		
	Reference	CF	Dif. (%)	Reference	CF	Dif. (%)
GEN production	275	360.18	30.97	214	287.22	34.17
Veg. + NCO production	99	128	28.68	103	121	18
Biomass (Veg. + NCO)	4.55	6.07	33.44	4.47	6.27	40.07
Biomass (GEN)	25.78	32.90	27.61	21.01	28.25	34.46
Biomass Total	30.33	38.97	28.48	25.49	34.52	35.45

Table 6

Generative stem production (stem.m^{-2}) during measurement periods in the "Cultivation of the future" (CF) and in the reference treatment and differences between treatments (Dif.).

	Noize			Virginia		
	Reference	CF	Dif. (%)	Reference	CF	Dif. (%)
P1	37.96	43.89	15.61	29.26	40.19	37.34
P2	90.56	127.78	41.10	74.63	116.67	56.33
P3	102.59	137.59	34.12	74.44	97.96	31.59
P4	62.59	82.22	31.36	51.48	54.81	6.47

4.2.2 Light Use Efficiency

Total light use efficiency and supplementary light use efficiency were calculated as a ratio between light sum and generative stem biomass during different measurement periods (Table 7 and Table 8). Differences in total light use efficiency vary between periods with low natural light and high natural light. Besides, LED supplementary lights that in the "Cultivation of the future" provided a higher light intensity, always showed a lower efficiency than HPS lights with lower intensity.

Table 7
Total light use efficiency expressed in in g.mol^{-1}.m^{-2} (generative stems) during measurement periods in the treatments "Cultivation of the Future" (CF) and reference, and difference between treatments (Dif.).

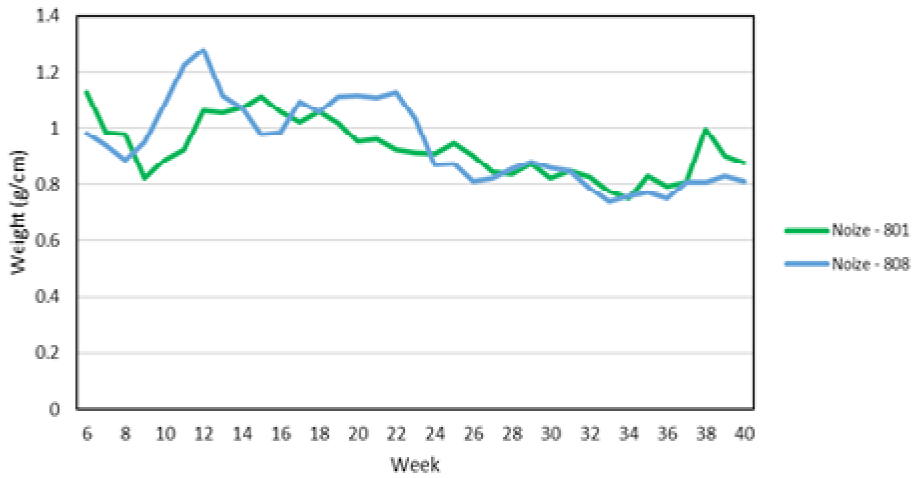
	Noize			Virginia		
Period	Reference	CF	Dif. (%)	Reference	CF	Dif. (%)
1	7.23	5.10	-29.56	5.04	4.61	-8.42
2	6.53	7.10	8.69	5.03	6.47	28.55
3	5.69	7.43	30.50	4.61	5.74	24.59
4	6.07	5.84	-3.78	5.78	4.93	-14.65

Table 8
Supplementary light use efficiency expressed in g.mol^{-1}.m^{-2} (generative stems), during measurement periods in the treatments "Cultivation of the Future" (CF) and reference, and difference between treatments (Dif.).

	Noize			Virginia		
Period	Reference	CF	Dif. (%)	Reference	CF	Dif. (%)
1	16.14	8.23	-48.99	11.24	7.46	-33.67
2	40.81	33.91	-16.89	31.45	30.91	-1.70
3	-	-	-	-	-	-
4	38.21	18.46	-51.69	36.34	15.57	-57.14

4.2.3 Crop Quality

Weight of stems, here expressed as the ratio between weight and length (Figure 25), is an important quality variable of the production, as heavier stems have a better market price. The ratio showed, for both varieties, the tendency to decrease as the temperature and light intensity increased and differences between treatments are not clear.



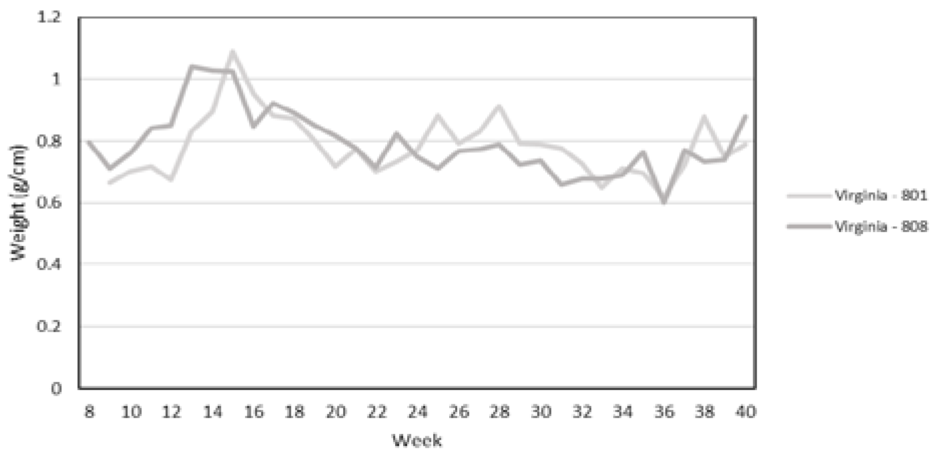


Figure 25 *Ratio between weight and length of cultivars Noize (above) and Virginia (below) in treatments "Cultivation of the future" (808) and reference (801). Data collected twice per week.*

Other crop aspects, as dry weight (Figure 26), leaf surface (Figure 27) and number of leaves (Figure 28), appear to be cultivar dependent, with negative response to an increase on temperature and light intensity (ex: dry weight for both varieties; leaf surface and number of leaves for Noize).

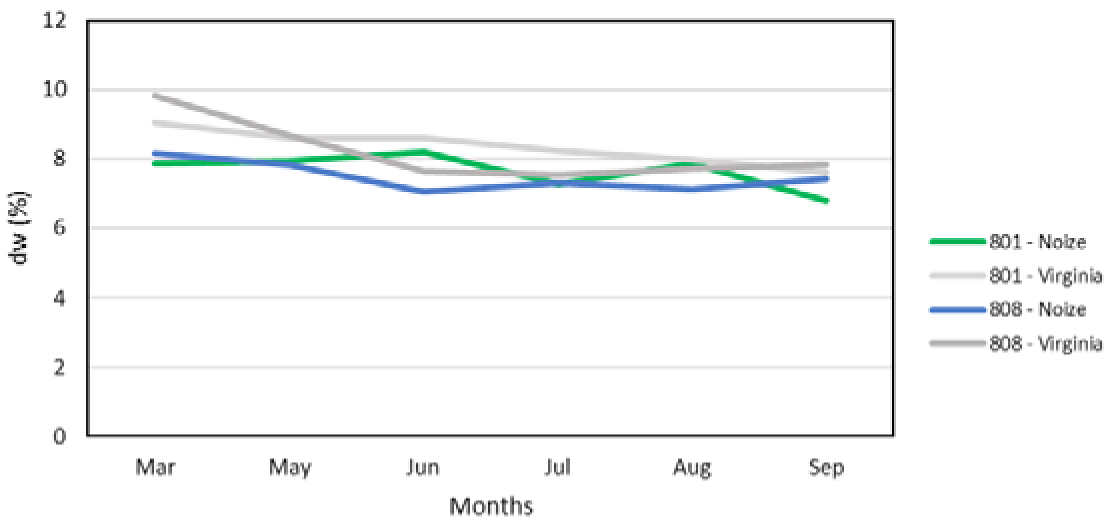


Figure 26 *Average of dry weight percentage in treatments "Cultivation of the future" (808) and reference (801). Values are means of 15 stems collected once per month.*

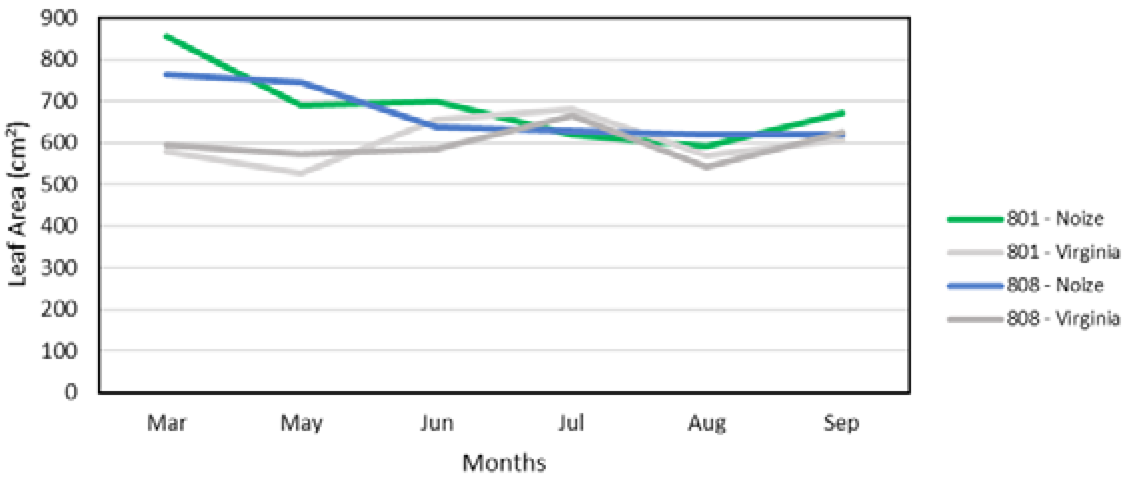


Figure 27 *Average of total leaf surface in treatments "Cultivation of the future" (808) and reference (801). Values are means of 15 stems collected once per month.*

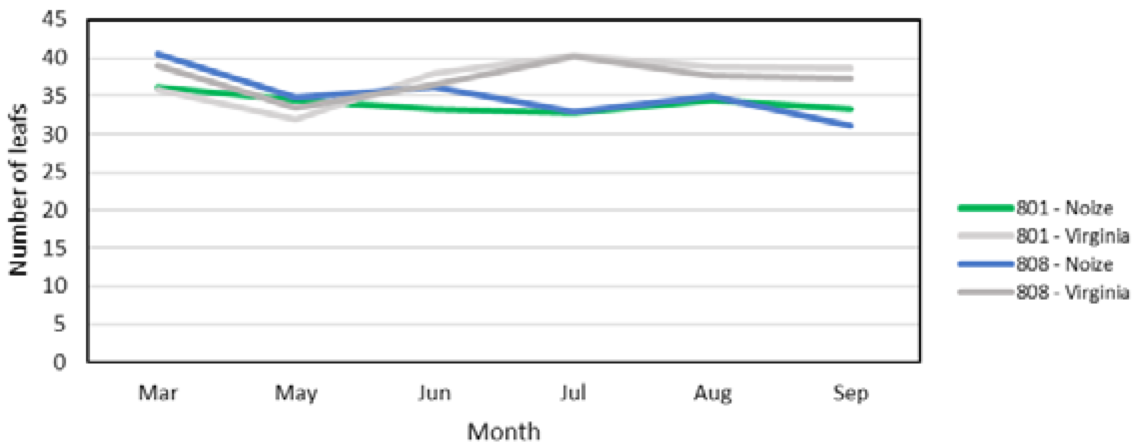


Figure 28 *Average of number of leaves in treatments "Cultivation of the future" (808) and reference (801). Values are means of 15 stems collected once per month.*

4.2.4 Generation Time

Generation time (Figure 29), the development time from new shoot until ripe flower, varied along experiment periods (Table 1) in response to climate changes. For Noize, during Period 1 and 2, the higher values of light sum, average temperature and CO_2 concentration may have influenced a faster stem development in treatment "Cultivation of the Future". In Period 3, when climate between compartments was similar, stems showed similar generation time. For Virginia, generation time increased during Period 4, but showed no differences between treatments.

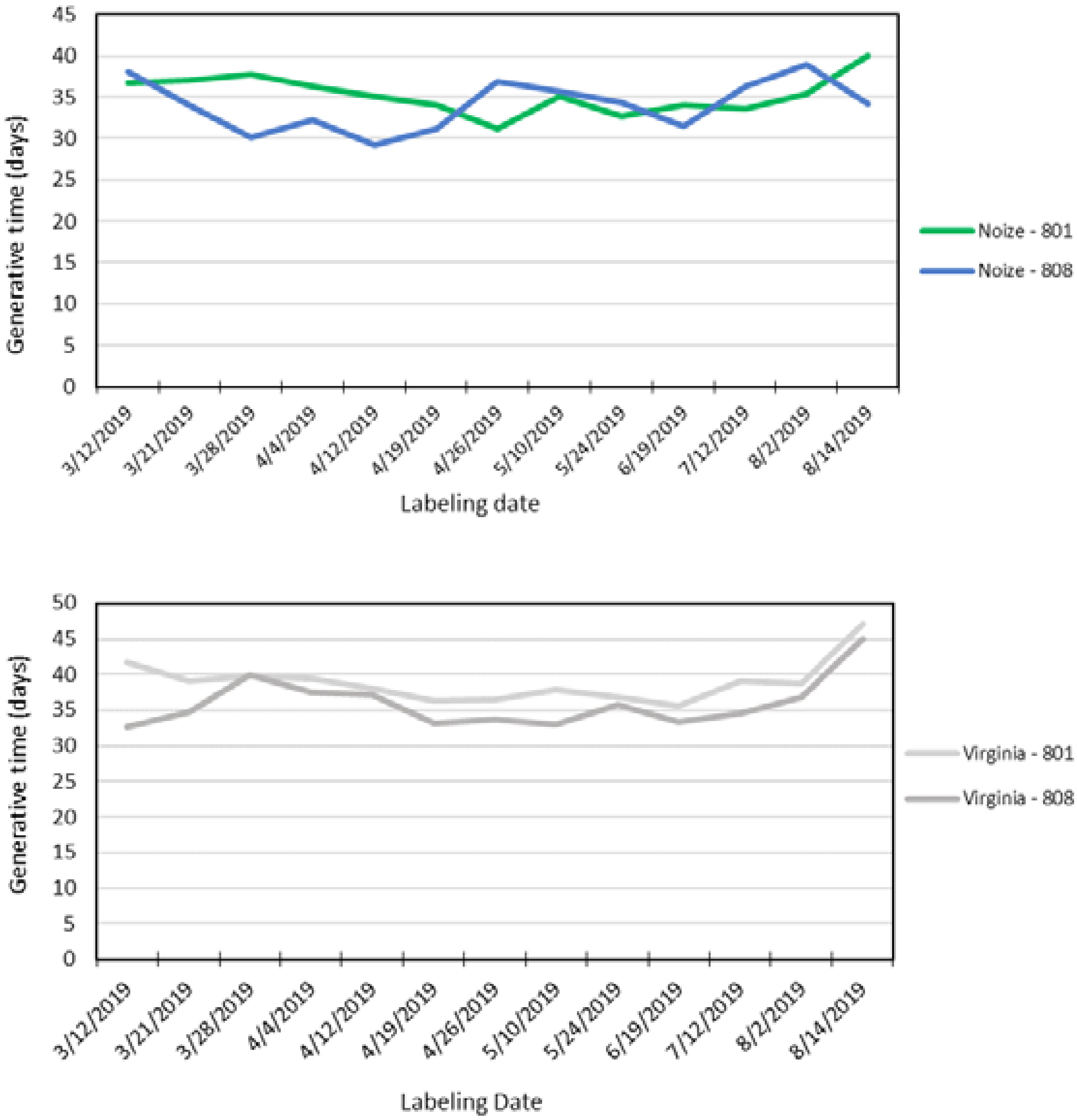
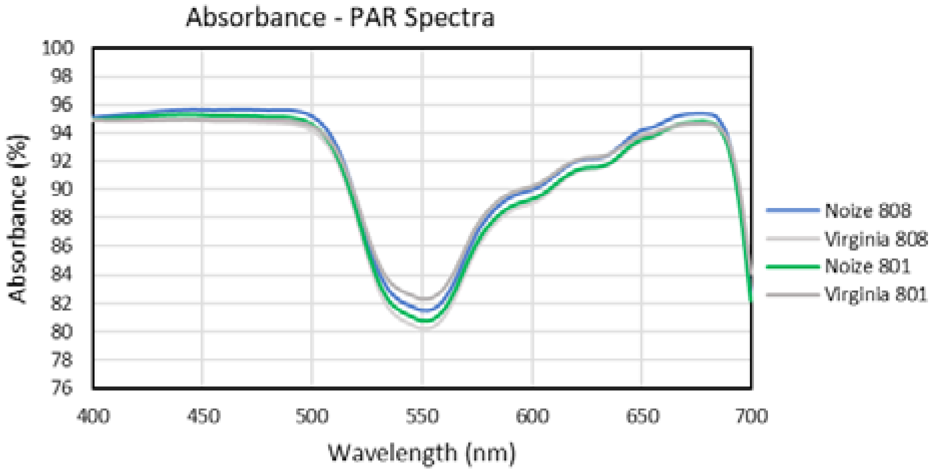


Figure 29 *Generation time in treatments "Cultivation of the future" (808) and reference (801). Stems labeled with maximum heigth of 5 centimeters and harvested when ripeness was achieved.*

4.3 Leaf Absorbance

In order to calculate photosynthesis parameters, leaf absorbance of top layer leaves was measured (Figure 30). Values do not show significant differences between treatments and varieties (Table 9).



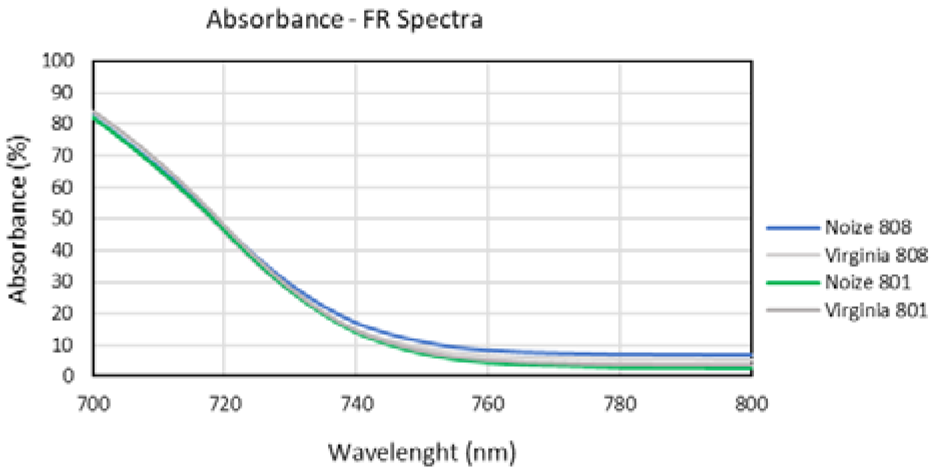


Figure 30 *Leaf Absorbance in the PAR light spectra (above) and in the FR spectra (below) for treatments "Cultivation of the Future" (808) and reference (801).*

Table 9

Average of leaf absorbance in different spectra ranges for treatments "Cultivation of the Future" (CF) and reference.

	Noize		Virginia	
	CF	Reference	CF	Reference
Absorbance Blue (400-480nm)	95.51	95.21	94.85	94.91
Absorbance Green and Yellow (485-600nm)	88.18	87.52	87.07	88.40
Absorbance Red (605-700nm)	92.67	92.08	91.94	92.57
Absorbance FR (705-800nm)	22.65	19.77	20.86	20.74
Absorbance PAR (400-700nm)	91.69	91.16	90.84	91.58

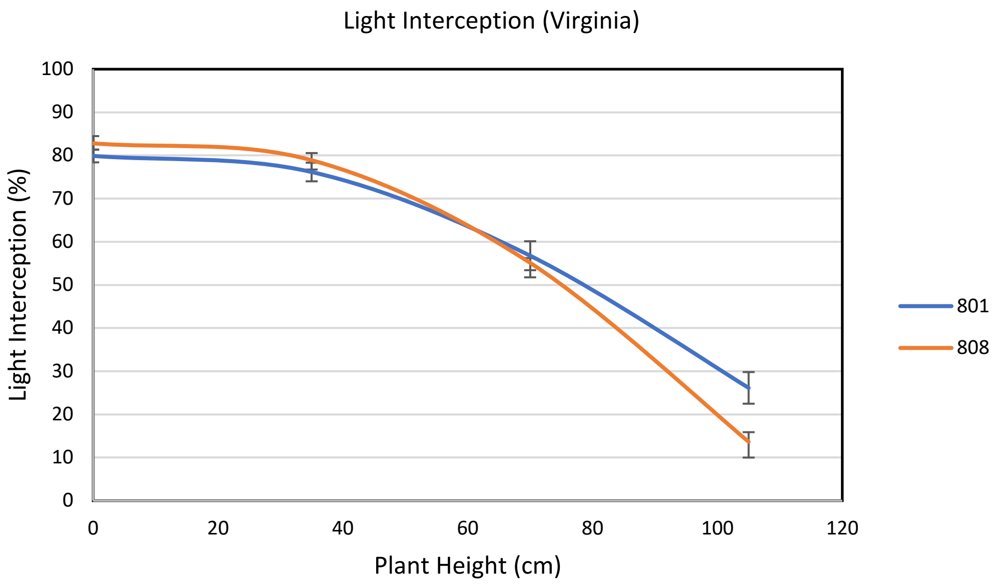
4.4 Light Interception and Leaf Area Index

Light interception, measured in a cloudy day, was higher in all layers of the crop for Noize in treatment "Cultivation of the Future", while for Virginia, light interception was similar between treatments, with slightly higher value in the bottom layer for "Cultivation of the future". Light interception is directly related to canopy density, here expressed in leaf area index (LAI) of the canopy (Table 10), which was higher for both varieties grown in the "Cultivation of the future".

Table 10

Leaf area index (m²/m²) calculated for weeks 23-28 in treatments "Cultivation of the future" (CF) and reference.

	Noize		Virginia	
Week	Reference	CF	Reference	CF
23	2.93	4.24	1.87	2.85
24	2.77	4.04	1.71	2.68
25	2.88	4.37	1.74	2.46
26	3.05	3.31	1.80	2.17
27	2.82	2.74	1.76	1.89
28	2.27	2.74	1.80	2.10
Average	2.79	3.57	1.78	2.36



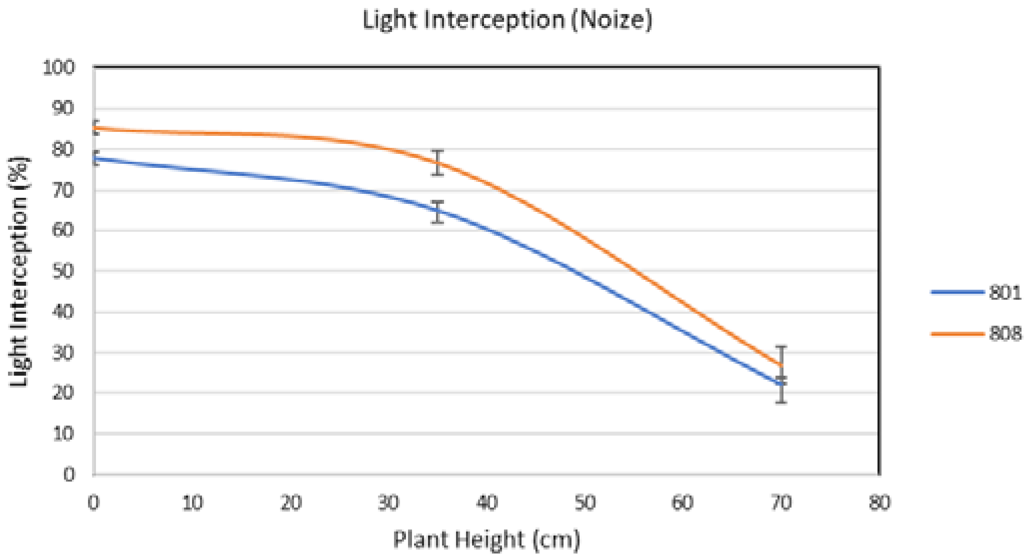


Figure 31 *Light interception of Noize and Virginia in the treatments "Cultivation of the Future" (808) and reference (801).*

4.5 Photosynthesis

4.5.1 Light response

Leaves from different varieties, crop layer and treatments exhibited different responses to varying light levels (Figure 32). The parameters A_{max}, R, L_c, AQE and L_{sat} were calculated for each light response curve.

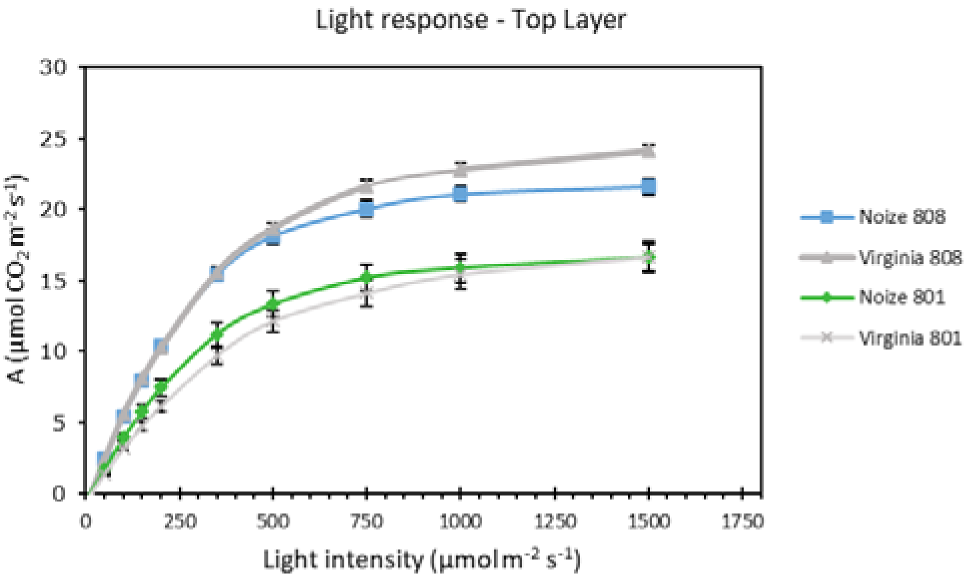
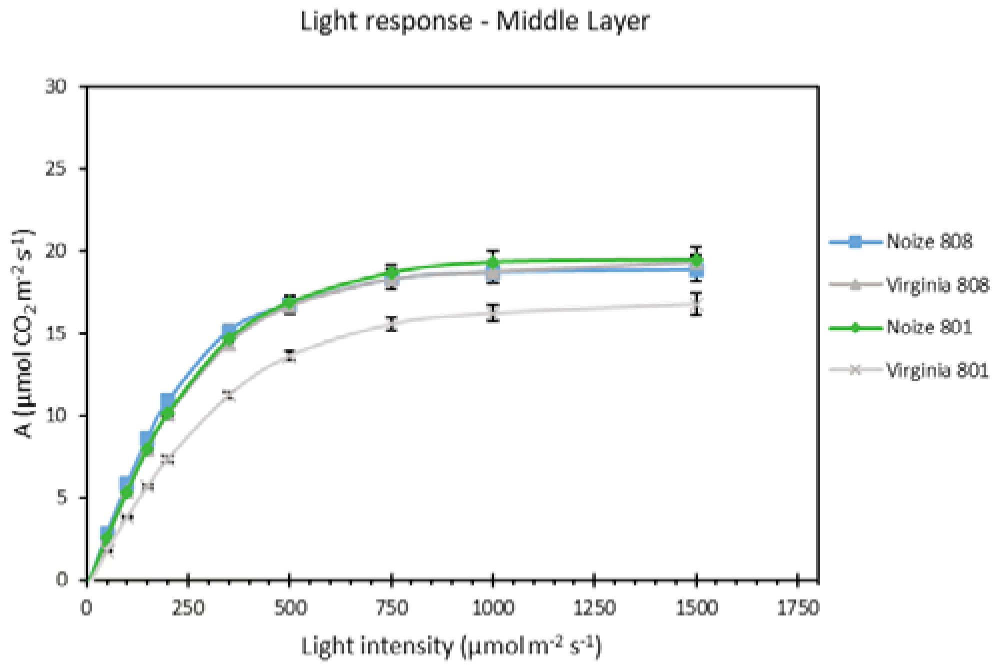


Figure 32 *Light response curve of leaves from 3 different crop layers in treatments "Cultivation of the future" (808) and reference (801).*



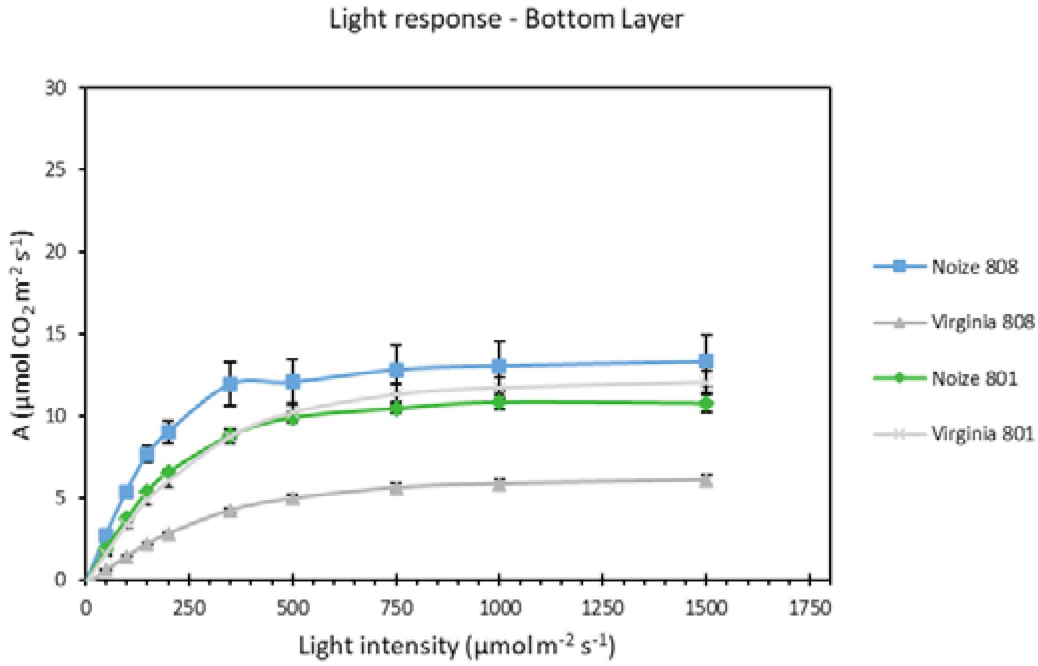


Figure 33 *Light response curve of leaves from 3 different crop layers in treatments "cultivation of the future" (808) and reference (801).*

For the top layer of both varieties, plants grown in the "Cultivation of the Future" had a significant higher photosynthesis capacity. For middle layer, in contrary, Noize grown in the reference showed significant higher Amax values, while for Virginia, there were no differences between treatments. Plants grown in the "Cultivation of the Future", showed significant differences between layers, with the tendency to decrease at each level. In the reference, however, there was an increase in photosynthesis capacity from top to middle layer, while bottom layer showed the lowest values. L_{sat} values (Table 11) in top and middle layer of Noize were similar in both treatments, while in the bottom layer values were significantly lower.

Table 11

Light saturation, maximum net photosynthesis rate (A_{max}), Respiration (R), Light compensation (L_c), Apparent quantum yield (AQE), Light saturation (L_{sat}) and convexity (ρ) at 3 different crop layers for treatment "Cultivation of the Future" (CF) and reference. Different letters show significant differences at P=0.05.

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

For Virginia, however, L_{sat} had the tendency to decrease in the middle and bottom layers of the canopy. AQE values (Table 11) showed contrasting responses in the top and middle layer, in the first, treatment "Cultivation of the Future" showed significantly higher values, while in the second, the reference. R values (Table 11) were significantly higher on top layer leaves from treatment "Cultivation of the Future", but, for L_c no significant difference was found between treatments and layers.

4.5.2 CO_2 response

Net photosynthesis rates (A) were also measured for different CO_2 levels and crop layers (Figure 33). In the top layer higher A values were observed for treatment "Cultivation of the Future". Comparison between layers show a decrease in A values, except in the middle layer of Noize from treatment Reference.

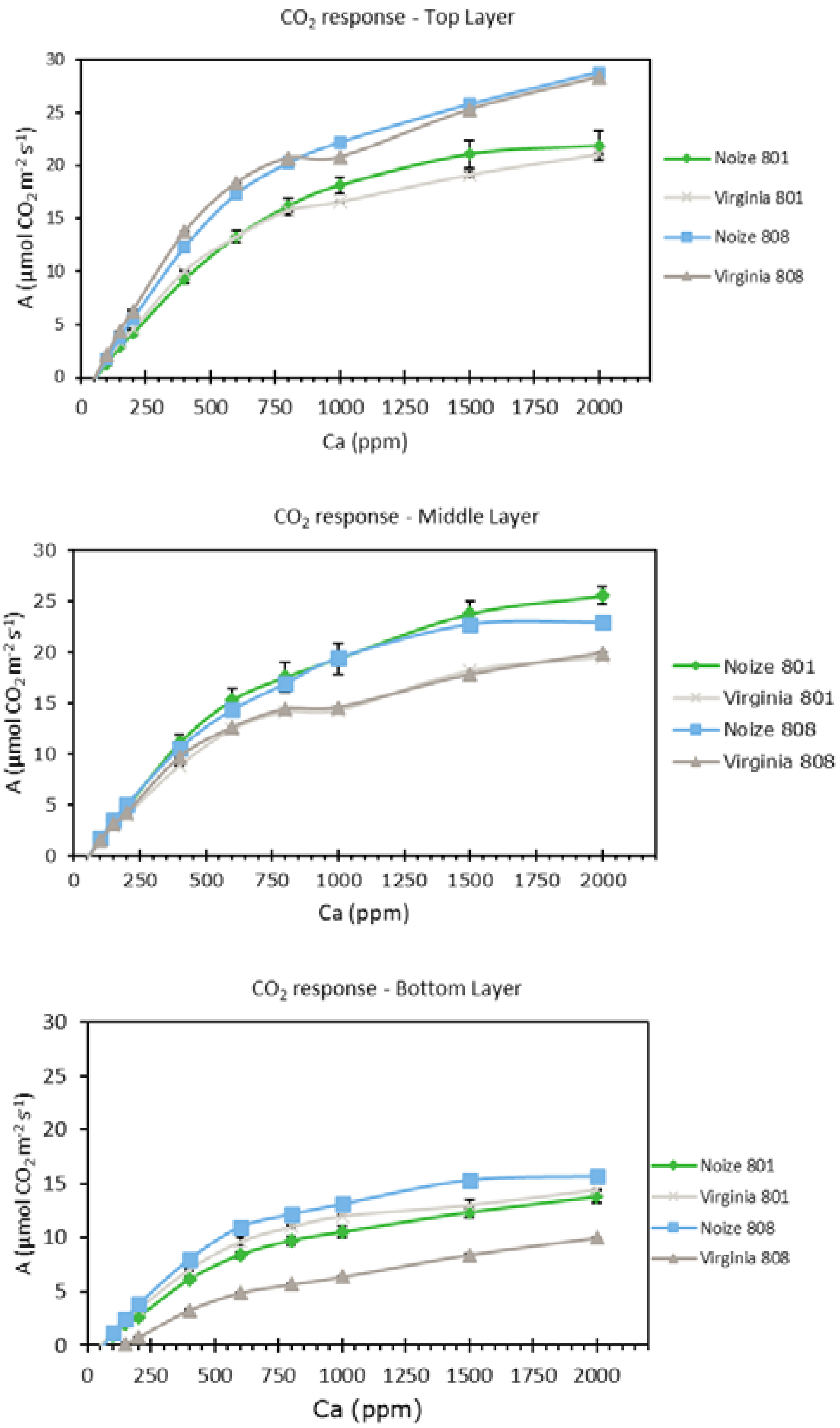


Figure 34 *CO_2 response curve of leaves from 3 different crop layers in treatments "Cultivation of the future" (808) and reference (801).*

4.5.3 Canopy net photosynthesis rate (A_c)

Canopy net photosynthesis rate, calculated for incident light (Q) of 1500 µmol.m^{-2}.s^{-1}, was 32% and 27.92% higher in the "Cultivation of the future" for cultivars Noize and Virginia, respectively (Table 12). Therefore, in this treatment, plants have a higher photosynthesis capacity, even though net photosynthesis rates at leaf level are not always higher (e.g. middle layer Noize, Table 11).

Table 12

Canopy net photosynthesis rate (A_c) in treatments "Cultivation of the future" (CF) and reference, and difference between treatments (Dif.).

Noize			Virginia		
Reference	CF	Dif. (%)	Reference	CF	Dif (%)
34.86	46.01	32.00	32.50	41.57	27.92

4.5.4 Stomatal Conductance (G_s)

G_s values (Table 13) were selected from light response curve at light step 1500 µmol.m^{-2}.s^{-1}, at which all, but Virginia's top layer, were light saturated. In all Noize layers and for Virginia top and middle layers, G_s was significantly higher in the "Cultivation of the Future". Which can be a response to the lower VPD values, in this treatment, during the measurement.

Table 13

Average G_s under light intensity levels of 1500 µmol.m^{-2}.s^{-1} in the "Cultivation of the Future" (CF) and in the reference treatments.

	Noize		Virginia	
	Reference	CF	Reference	CF
Top Layer	0.112	0.166	0.119	0.176
Middle Layer	0.151	0.186	0.102	0.156
Bottom Layer	0.101	0.183	0.090	0.066

4.6 Stomata density and size

Stomata parameters were measured from microscopy images (Figure 34), no differences between treatments were found, but stomata in Noize showed a higher average length, indicating a possible variety effect (Table 14). Leaf prints were done in late summer when the extreme climate conditions were already attenuated, therefore a new trial should be done in order to confirm results presented here.

Table 14

Stomata density and Length in treatments "Cultivation of the future" (CF) and reference

	Noize		Virginia	
	Reference	CF	Reference	CF
Density (stomata/mm²)	54.12	52.78	55.49	55.25
Length (µm)	54.31	54.97	50.81	50.64

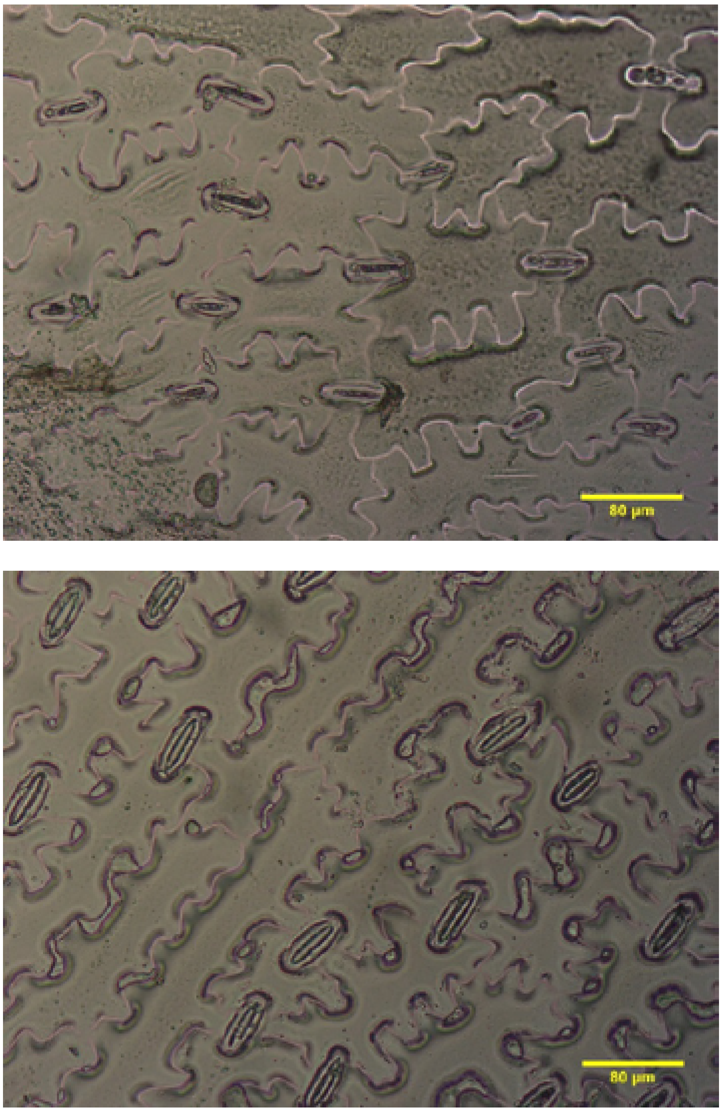


Figure 35 *Microscopy images of leaf prints magnified 40x.*

4.7 Chlorophyll fluorescence

From the measured data, the 5 days with the highest PAR sum were selected and then processed as cycle mean (Table 15 and Figure 35). Yield of the PSII did not vary between varieties and treatments, and even after day periods with high light intensities, efficiencies returned to values above 81%, during the night period, which shows that photoinhibition did not take place. Figure 35 also shows, for the reference treatment, a decrease in PAR intensity between 12am and 2pm, which was caused by shading of the measuring equipment. Although the equipment was placed in the same position in the “Cultivation of the Future”, shades in this compartment were attenuated due to the high haze property of the glass cover.

Table 15
Selected days with respective PAR sum during chlorophyll fluorescence measurement in both treatments.

"Cultivation of the Future"				Reference	
Noize		Virginia		Noize and Virginia	
Date	PAR Sum (mol)	Date	PAR Sum (mol)	Date	PAR Sum (mol)
5/29/2019	32.3	5/29/2019	32.3	4/29/2019	24.5
6/1/2019	34.2	7/5/2019	27.1	5/11/2019	27.7
6/2/2019	29.4	7/22/2019	31.7	5/13/2019	28.8
7/5/2019	27.1	7/23/2019	34.3	5/14/2019	31
7/25/2019	28.5	7/31/2019	29	5/15/2019	31.1
Average	30.3	Average	30.88	Average	28.62

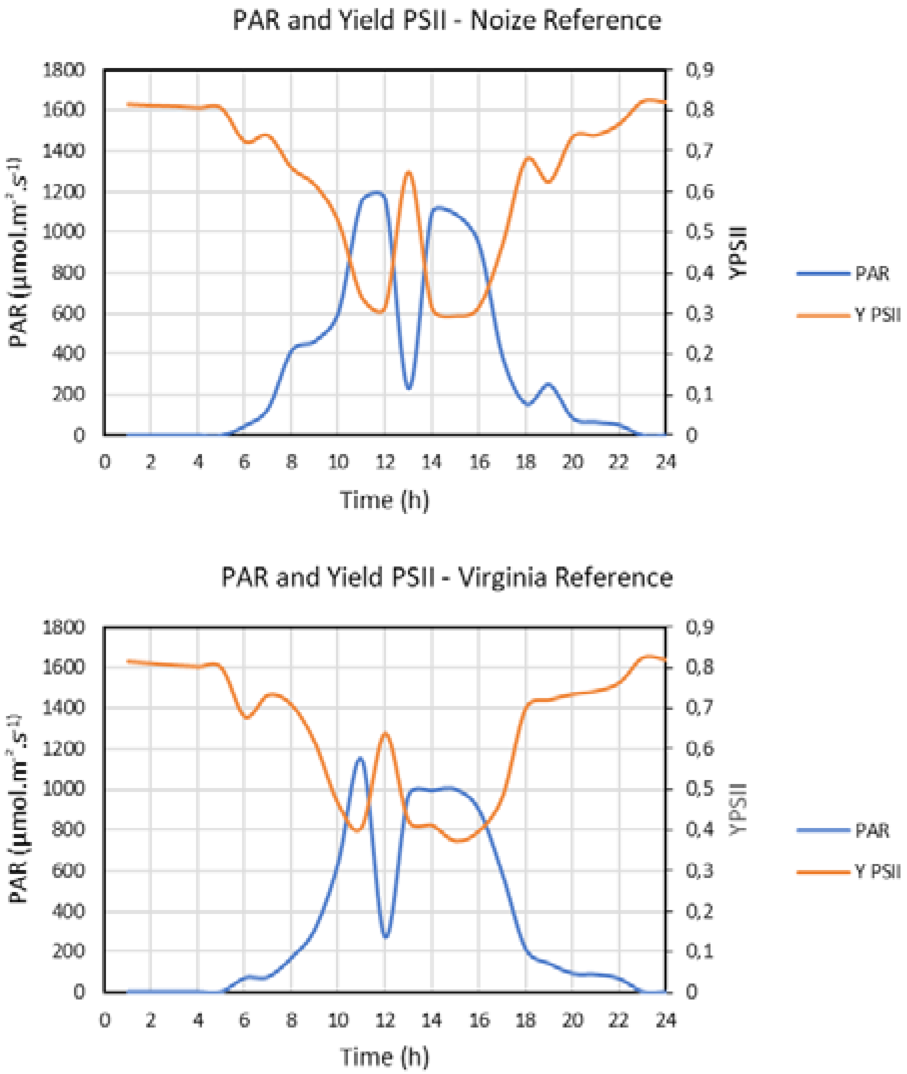


Figure 36 *Cycle mean of PAR intensity and Yield PSII during five selected days with high PAR Sum.*

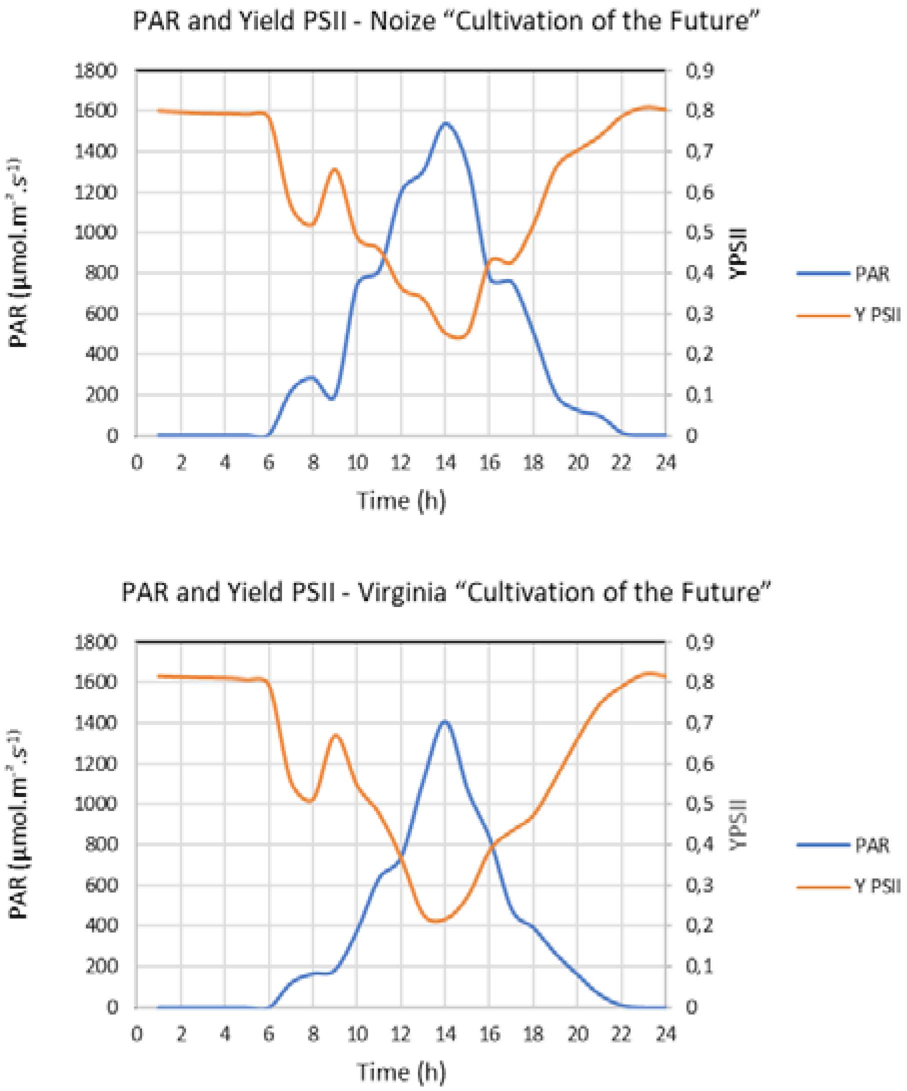


***Figure 37** Cycle mean of PAR intensity and Yield PSII during five selected days with high PAR Sum.*

5 Discussion

The “Cultivation of the Future” has proved to be an effective system for Alstroemeria growing. During the test period, generative stem production was 30,97% higher for Noize (Table 11) and 34,17% for Virginia (Table 11), with similar quality and generation time (Figure 29). Production gains were caused by higher light sum (Table 3), higher photosynthesis capacity (Table 12) and increased stomatal conductance (Table 13).

5.1 Production and Supplementary Light

Light is a limiting factor for greenhouse production during periods with low light availability, thus the use of supplementary lighting is an important tool for greenhouse production in The Netherlands, and has been studied for application in Alstroemeria production (STAPEL-CUIJPERS, 1995). However, supplementary lighting use is limited by higher production costs related to its initial investment, maintenance, and energy consumption (RAMIREZ *et al.* 2019; KUBOTA *et al.* 2016; MARCELIS *et al.* 2002; VADIEE; MARTIN, 2014).
Therefore, with the development of more energy efficient lamps, such as LED, growers expect to be able to save energy or to increase light intensity with lower increase in energy use when compared to the traditional HPS lights at same light intensities, nevertheless increases in light intensity should be followed by satisfactory plant response in terms of light use efficiency (KUBOTA *et al.* 2016).

In order to determinate the limits for an effective supplementary lighting strategy, Trouwborst *et al.* (2015) measured net photosynthesis rate on different light intensities for two Alstroemeria varieties, and reported that the limit for maximal photosynthesis efficiency was 120 $\mu mol.m^{-2}.s^{-1}$ (TROUWBORST *et al.* 2015). Besides, Garcia *et al.* (2018) reported that in a practice research where different light systems were compared, the plants in the systems that used LED presented higher light use efficiency, even though in higher light intensities, showing that, with LED, light intensities could still be increased (GARCIA *et al.* 2018).

In contrast with the standard cultivation, in which HPS lights supplied 80 $\mu mol.m^{-2}.s^{-1}$, in the “Cultivation of the future”, LED lights supplied an increased intensity of 200 $\mu mol.m^{-2}.s^{-1}$, which caused an increase in light sum of 68%, 20,7% and 25,14% for periods 1, 2 and 4 (Table 3), respectively, while during period 3, as supplementary lights were not used, differences in light sum were not present. In this treatment, although all periods had a higher production (Table 6), as expected in higher light availability conditions (MARCELIS *et al.* 2006), the total light use efficiency (Table 7) was lower in periods 1 and 4, when production was also lower and the use of supplementary lights was more intense.

The experiment also shows that the supplementary light use efficiency was always lower in the “Cultivation of the future” (Table 8), confirming what Trouwborst *et al.* (2015) stated, as an increase of supplemental light intensity at values higher than 120 $\mu mol.m^{-2}.s^{-1}$, was accompanied by a lower light use efficiency of the plants (TROUWBORST *et al.* 2015), and also with Marcelis *et al.* (2002) which also reported lower efficiencies values at higher supplementary light intensities for a tomato crop (MARCELIS *et al.* 2002). Moreover, the increase in light efficiency use during period 2 (Table 8) shows the adaption of Alstroemeria plants to the increasing natural light intensity, already observed previously (TROUWBORST *et al.* 2015).

5.2 Production and Natural Light

Production peak occurred in periods 2 and 3, spring and summer, when, respectively, 80% and 100% of the supplied light was natural. The highest PAR sum (Table 3) was achieved in period 2 in the “Cultivation of the future” and led to stem production gains of 41% and 56% for Noize and Virginia, respectively. During period 3, light sum between treatments was equal, as the artificial light was not use.

Although in both treatments an increase in production was observed during these periods, different response in light use efficiency was observed between treatments (Table 7), while in the reference treatment efficiencies were lower in period 2 and 3, in the "Cultivation of the future" these values were then the highest. During period 3, canopy photosynthesis was 32% and 27.92% higher in varieties Noize and Virginia, respectively (Table 12), generating an increase in stem production of up to 30%.

In conditions of increased proportion of natural light, from spring to early autumn, the effects of diffuse light become 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.* 2006, 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 "Cultivation of the future", diffuse light did not improve light availability in deeper layers of the crop (Figure 31), in contrary middle and bottom layers received less light, which was caused by higher leaf area index (LAI) of this treatment (Table 10), Garcia 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 (GARCIA VICTORIA *et al.* 2012).

Plants which were grown in this treatment also presented a higher net photosynthesis rate at light saturation level, with 30,7% and 42,7% higher values for Noize and Virginia, respectively. Li *et al.* (2014) also noticed an improvement on leaf net photosynthesis capacity with increasing levels of haze, however, in a lower extent, with maximum 18,70% increase in maximum net photosynthesis rates (LI *et al.* 2014). Hemming *et al.* (2008) reported higher net photosynthesis rates in all layers of the crop, which contributed to increase fruit production in 11% (HEMMING *et al.* 2008). Therefore, diffuse light played an important role in improving Alstroemeria production in the "Cultivation of the future", even though other variables also contributed to achieve such gains of productivity.

5.3 Production and Reduced Vapour Deficit

During the months with increased evaporative demand, in periods 2 and 3, the "Cultivation of the future" made a more intense use of the high pressure humidification system, so that vapour deficit (VD), in this treatment, was always kept at lower levels (Figure 13). Moreover, stomata conductance was higher in all crop layers for Noize and for top and middle layer for Virginia (Table 13), while no changes were observed neither in stomata density nor size.

Stomata plays an important role on controlling CO_2 uptake and transpiration on leaves (HETHERINGTON; WOODWARD, 2003) and is regulated, among other factors, by the evaporative demand of the environment, or vapour pressure deficit (VPD) . Experiments have shown that when VPD was held to a lower level, stomata closing 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 length and density (FANOURAKIS *et al.* 2015), this effect was also reported, earlier, for several crops (BAKKER, 1991). In the "Cultivation of the future", however, relative humidity levels were not sufficiently high to promote changes in this stomatal parameters (Table 14), so that the increase of stomata conductance can only be explained by a higher stomata opening.

Besides, Zhang *et al.* (2018) reported that growing tomato in a reduced VPD environment, reduced stomatal closure, increased CO_2 acquisition and assimilation, enhanced plant photosynthesis capacity and, lastly, improved growth, biomass and fruit production (ZHANG *et al.* 2018). This effect was also observed in the "Cultivation of the future", therefore photosynthesis capacity and production gains, during period 2 and 3, were possibly caused by an integrated effect of diffuse light and lower VPD on the plants.

5.4 Light Stress

Hogewoning *et al.* (2015) recomended for two Alstroemeria varieties during summer conditions, Virginia and Roma, that above light intensity of 900 µmol m^{-2} s^{-1} the crop should be shaded, avoiding photoinhibition and damage of the photosystem II (PSII) (HOGEWONING *et al.* 2015). During periods 2 and 3, chlorophyll fluorescence was measured, and then days with the highest light sum were selected. Although photoinhibition during the day occurred, light stress was not sufficient to damage to the PSII, as night yield values were always higher than 0,8 (Figure 35). These results agree with the light saturation values, derived from the light response curves, which were around 1300 µmol m^{-2} s^{-1} and 1700 µmol m^{-2} s^{-1} for Noize and Virginia, respectively, and, also, to the fact that also no visible damage in the leaves occurred, even though no shading technique was used during cultivation. However, Alstroemeria varieties differ greatly in their exigencies, so that a study that includes a higher genetic diversity would be necessary to draw solid conclusions.

6 Conclusions

In the present study, two Alstroemeria varieties grown in the “Cultivation of the future” had higher stem production than the reference system, while quality and generation time was equal. The main factors that influenced the crop productivity varied through different experiment periods. When low natural light was available, the higher light intensity supplied by LED lights increased the plants photosynthesis assimilation. At moments with high natural light intensity, production gains were achieved due to the milder climate provided by a lower VD and the highly diffused incident light, which caused an increase in the stomata conductance and in the photosynthesis assimilation capacity of the plants. Besides, the two studied varieties were shown to be resistant to a light stressing environment, but this response may vary in other Alstroemeria genotypes.

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