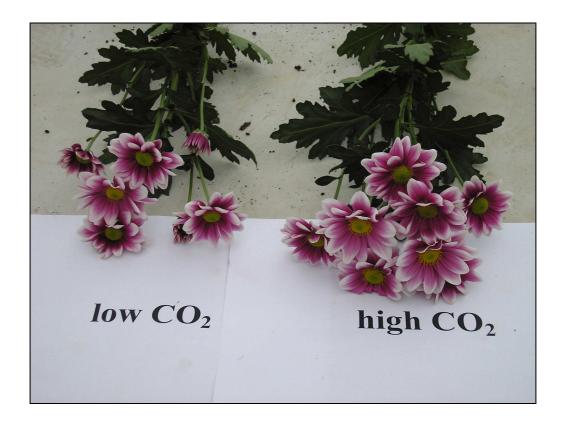
ANALYSIS OF GENETIC VARIATION IN CUT CHRYSANTHEMUM RESPONSE TO HIGH CARBON DIOXIDE CONCENTRATION (CLOSED GREENHOUSE): UNDERLYING PHYSIOLOGICAL AND MORPHOLOGICAL PARAMETERS

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PREFACE

Nowadays, the closed greenhouse concept offers new possibilities for controlling the climate, in ranges that in current commercial production are technically not possible or economically not feasible. One of these possibilities is the improved CO₂ fertilization in terms of dose, but also duration of application. In this point of view the effects of elevated carbon dioxide, except from the physiological interest, become important for applicable purposes. This concept in combination with the genotypic variation is an excellent discipline to experiment with.

The present report was written in the framework of my internship for the MSc study program I followed in the Horticultural Production Chains Group, Department of Plant Sciences, Wageningen UR, during the academic years 2005-2007. The experiment took place in Bleiswijk, part of the infrastructure of Wageningen UR, during the period February to April 2007, and was financed by Productschap Tuinbouw and OCAP. Experiment's planning, working in the greenhouse, discussions with my colleagues, as well as the writing of the report offered me valuable knowledge and experience on scientific research. I would like to thank Ruud Maaswinkel, my daily supervisor, for our discussions, his ideas and the Dutch I learnt, the time we spend together. I would also like to acknowledge my professor Dr Ep Heuvelink for his advice during the experiment, his contribution in overcoming several obstacles and for his critical comments on the report. It would be an omission not to mention the PhD student Govert Trouwborst, and my colleague, Zhichao Zhang, who made possible the photosynthesis measurements; Fred van Leeuwen, for arranging the cultivation measures; and Peter Lagas, for his kind assistance during the measurements. Finally, I wish to acknowledge the foundation Alexander S. Onassis for the financial support during my studies.

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ABSTRACT

Seventeen cut chrysanthemum cultivars were used to evaluate the effects of elevated CO_2 concentration (1500ppm), compared to the concentration (500ppm) used in commercial chrysanthemum production in The Netherlands, on growth, development and quality aspects in an experiment conducted between February and April 2007.

CO₂ enrichment increased the aboveground fresh weight (2-28%) of plants in all cultivars, significantly only for two, an increase that was partially due to higher stem length (0-12%), but also increased fresh weight of plants at 80 cm fixed length (1-22%). Moreover, the elevated CO₂ regime increased the aboveground dry weight per plant (5-40%), significantly only for 5 cultivars. This increase in the dry weight came from an increase of the Relative Growth Rate (RGR) during the LD period (first 2 weeks; due to increased NAR, despite the decreased LAR) and the period 2 to 6 weeks (due to increased NAR, despite the decreased LAR), and an increase in the Crop Growth Rate (CGR) in the period 6 weeks to final harvest (8 to 10 weeks depending on the cultivar). The increased CGR indicates an increased Light Use Efficiency in the high CO₂ regime. The increase in the dry weight was higher than the increase in the fresh weight, and this is because the elevated CO₂ decreased the Water Content (WC) (0.5-2%), and thus increased the percentage of dry mass present in the fresh mass. Furthermore, it was found that the growth in the high CO₂ regime promoted the number of flowers and flower buds per plant (3-48%).

The increased CO₂ concentration did not affect the number of leaves present on the main stem in any of the cultivars examined. This, in combination with the increase in stem length implies that the CO₂ enrichment increased the internode length. Moreover, the reaction time, and as a consequence the flower development rate, were hardly affected.

No consistent response was observed on the effect of CO_2 enrichment on the variances in the characters: stem length, number of flowers and flower buds and fresh weight.

The effect of elevated CO_2 on the stomatal density (measured in the cultivars Reagan Elite White and Feeling Green) was cultivar dependent (negative and positive trend respectively). Increased stomatal density in high CO_2 does not directly result in higher photosynthesis rates, since parameters like the leaf area and the stomata dimensions (stomatal aperture) should also be taken into account.

The CO_2 enrichment during cultivation increased the maximum leaf photosynthetic rate by 50% on average (measured in the cultivars Reagan Elite White and Feeling Green), and the increased photosynthesis rates were (at least) partially responsible for the higher production achieved. A negative slope in the P_{max} over time of cultivation was recorded, that was the result of the combined effects of aging, increased shading, and adaptation to the high CO_2 concentration. The adaptation to the high CO_2 concentration took place in week 4 or even earlier (no measurements conducted before week 4).

In conclusion, a strong genotype \times (CO₂ concentration) interaction was observed for the aboveground fresh and dry weight, and number of flowers and flower buds per plant, where a positive trend was present in all cases. A weaker genotype \times (CO₂ concentration) interaction was found for stem length and water content, the reaction time was hardly affected, while the number of leaves on the main stem was not changed by the CO₂ enrichment.

GENERAL INTRODUCTION

The development of the closed greenhouse is one of the major innovations in Dutch horticulture in the last years (Gelder et al., 2005). The system is described in Opdam et al. (2005). The commercialization of the technique requires a good growth result, sufficient for the return of the investment. Growth in a closed greenhouse (high light level and high CO_2 concentration) is new. There is no experience with growing crops in such a system, besides tomato (Gelder et al., 2005), sweet pepper and strawberry. The only experience in such a closed system for flower crops is in growth chambers. Therefore an experiment was designed to evaluate the effect of such a system, in respect of CO_2 concentration, on growth and quality aspects of an ornamental crop, while at the control compartment the carbon dioxide concentration, used in current commercial production and technically possible without the closed greenhouse concept, was released. In this template the interaction of genotype \times environment (CO_2 concentration) was investigated.

Chrysanthemum (Dendranthema grandiflorum syn. Chrysanthemum morifolium) is world-wide an important greenhouse crop not only as a cut flower but also as a pot plant (Carvalho and Heuvelink, 2001). Around one fifth of the entire Dutch area of cut flower production is occupied by chrysanthemum (Lee, 2002). Chrysanthemum is available in a wide range of cultivars, so many that a system of classification is used to categorize and identify them. The classification is based on the type of florets and their growth pattern. Chrysanthemum is an obligate Short Day Plant (SDP), however nowadays the protected cultivation is performed in a year-round basis. The cultivation takes place in intensive, industrialized, fully scheduled production systems (Lee, 2002). Such systems target on controlling most of the environmental components which affect the production namely light, temperature, CO₂ concentration and relative humidity. In this way, the growers regulate crop's growth and development in order to achieve the preferable quality and quantity of the yield.

Many studies have shown that CO_2 concentrations well above ambient can benefit plant growth. Typically, a three- to four-fold increase in CO_2 concentration yields a 10% to 25% increase in plant growth. Supplemental carbon dioxide (CO_2) refers to the addition of concentrated CO_2 to the greenhouse atmosphere to provide more raw material for photosynthesis. Supplemental CO_2 can be viewed as an additional crop input, no different from light or nitrogen. In fact, some authors refer to supplemental CO_2 as " CO_2 fertilization" or " CO_2 enrichment."

Growth and development of chrysanthemum in enriched CO₂ regimes

Application of CO₂ increases the relative growth rate (RGR) (Mortensen and Moe, 1983; Mortensen, 1986), resulting in enhancement of growth, as well as affects the visible quality aspects (external quality; Carvalho and Heuvelink, 2001) (Figure 1). External quality of chrysanthemum is usually evaluated in terms of stem and leaf morphology and flowering aspects (Carvalho and Heuvelink, 2001), and usually determines the prices. Therefore it is important the influence of CO₂ application on the specific quality aspects to be thoroughly examined.

Stem morphology: CO_2 enrichment promotes both stem and shoot elongation (Lindstrom, 1968; Mortensen and Moe, 1983; Eng et al., 1985; Mortensen, 1986) and stem diameter (Eng et al., 1985; Mortensen, 1986), resulting in increased total shoot dry weight (Mortensen, 1986). Moreover, high CO_2 concentration favors stem weight over elongation, resulting in higher stem strength (Heij and De Lint, 1987; Mortensen, 1986).

Leaf morphology: High CO₂ concentration tends to induce larger leaf area (LA) per plant (Gislerød and Nelson, 1989; Mortensen, 1986), resulting from a slight increase in LA on both main and lateral stems (Gislerød and Nelson, 1989). This is mainly the result of an increased area of the individual leaves (Gislerød and Nelson, 1997) and marginally due to higher number of leaves (Mortensen and Moe, 1983; Mortensen, 1986; Heij and De Lint, 1987). Moreover CO₂ enrichment results in decreased specific leaf area (thicker leaves) (Mortensen, 1986).

Figure 1. Effects of high CO₂ on the different external quality aspects of chrysanthemum.

	the different external	1
	EXTERNAL QUA	ALITY ASPECTS
CO ₂ enrichment		 length ↑
	\rightarrow STEM	 diameter ↑
		 strength ↑
	\rightarrow LEAF	 number ↑
		 size ↑
	\rightarrow FLOWERS	number ↑
		 size ↑

Flowering aspects: Increasing CO₂ concentration results in more lateral shoots (Hicklenton, 1985; Mortensen, 1986; Gislerød and Nelson, 1989), more flower buds and flowers per plant (Mortensen, 1986; Gislerød and Nelson, 1989) and increased flower size (Potter, 1980). However, the CO₂ level does not affect time to flowering in chrysanthemum (Potter, 1980; Mortensen, 1986).

Cultivar dependence: Most of the studies with chrysanthemum are focused on one cultivar, and only one in-depth systematic comparison between cultivars (cv Refour, Dark Flamengo and Cassa) is available (Mortensen, 1986). This work highlights that there is a cultivar \times (CO₂ concentration) to the aforementioned parameters (Table 1; Mortensen, 1986), and in this way the CO₂ enrichment benefits are not the same between the cultivars. For instance the CO₂ application did not change the number of flower buds and flowers in the cultivar Dark Flamengo (-0,8%), while the same treatment induced a 40% increase in the cultivar Cassa.

Table 1. Classification of the growth parameters according to their response to high CO₂ expressed in % relative difference compared to the low CO₂ growth (Mortensen, 1986).

strong response	small to no response	medium response		
Total shoot dry weight: 14.7- 32.1% Leaves/stem ratio: -(0.16- 26.7)% Lateral brakes: 8.5- 34.7% Shoot length: 3.5- 10.9% N. of flowers and flower buds: -0.8- 40.1% Main stem dry weight: 26.6- 50.4%	Areal leaf weight: 6.9-7.7% Days to flower: -(0- 2.6)% No. of leaves: 0.01- 4% Total leaf area: 0.1- 4.4%	RGR: 4.2- 8.8% Leaves' dry weight: 7.8- 11.7% Main stem: 22.1- 35.9% (thickness)		

CO₂ concentration

In long days (LDs), there was a significant increase in photosynthetic rate when the CO_2 concentration increased from 330 to 900 ppm CO_2 , while further increase in CO_2 did not give any additional effect. However, in short days (SDs), increasing the CO_2 concentration from 900 to 1500 ppm CO_2 gave additional increase in the photosynthetic rate (Mortensen and Moe, 1983). The authors concluded that the increase in the CO_2 saturation level maybe caused by an increase in photorespiration with age, since during short days, development of new leaves stops and aging of the total leaf mass takes place (Mortensen and Moe, 1983).

Adaptation to the high [CO₂]

Short-term experiments with CO₂ enrichment show very large effects on the net photosynthesis rate (Mortensen and Moe, 1982, 1883a, b), which are not reflected to the same extent in the relative growth rate as determined in long-term experiments (Mortensen 1983, 1986; Mortensen and Moe, 1982, 1883a, b). In short-term experiments, photosynthetic rate might be increased 50-100% by CO₂ enrichment, while in long-term experiments, the relative growth rate

is not increased proportionally. An adaptation to the high CO₂ conditions takes place resulting in a lower photosynthetic efficiency.

Mortensen (1982) reported that during the six weeks of experimental period a gradual decrease of net CO_2 uptake per unit leaf area (P_N) was observed in both high and low CO_2 conditions (increased self-shading, longer distance from the lower part of the canopy, aging). After 1 week treatment, the steady state P_N (the photosynthesis measurement was done in $[CO_2]$ of growth) of the high CO_2 plants was significantly higher than the normal CO_2 plants. Measured at similar CO_2 the high CO_2 plants were slightly more effective than the normal CO_2 plants. After 2 weeks the steady state P_N of high CO_2 plants was slightly higher, while in similar conditions the low CO_2 plants were more effective. From the 3^{rd} week and there after the steady state P_N was the same, between the treatments, while in similar conditions the P_N of low CO_2 plants was much higher (Mortensen, 1982).

The steady state P_N was similar from the 3^{rd} week and on. This indicates that the process of acclimation took place the first 2 weeks and that any difference on growth comes from this period. The high CO_2 plants after 2 weeks in normal air are much less efficient. In what extent this adaptation is the same between the cultivars in terms of the timing and the degree of the response remains unknown, as well as if this adaptation is reversible.

The high CO_2 acclimation (inhibition of photosynthesis) can be attributed neither to photorespiration (Mortensen, 1982) nor to a higher amount of carbohydrates (sugars + starch; Mortensen, 1982). Mortensen (1982) concluded that the inhibition of photosynthesis came from an increase in stomatal and mesophyll resistance, however there were no experimental data to support this claim.

AIM OF THE RESEARCH

The research questions we are called to address are: are there genetic differences in response to CO_2 enrichment on growth, development, and quality aspects of chrysanthemum, and if so how big are these differences? Furthermore, we are called to identify the underlying (morphological and/or physiological) factors being responsible for the possible differential responses between genotypes in growth and development. For this purpose 17 cultivars from 4 breeding companies were selected and evaluated.

MATERIALS AND METHODS

Experimental set-up

The experiment was conducted in two compartments ($15 \text{ m} \times 9.6 \text{ m}$) from a multispan Venlo-type glasshouse (Bleiswijk, The Netherlands, lat. 52°N), between February and April 2007. Before the experiment the nutrient condition was adjusted based on a soil analysis (BLGG, Naaldwijk, The Netherlands) and soil was steam-sterilized. From this point and further the timing of the application or treatment will be numbered (dated) by the number of days from planting, where 1^{st} of February is day 1. The same system will be followed with the numbering of weeks.

Block-rooted cuttings of Chrysanthemum `Reagan Elite White', `Calabria', `Tobago', `Paintball', `Vyking' (Royal van Zanten, Valkenburg, The Netherlands), `Anastasia', `Zembla', `Biarritz', `Noa' (Deliflor, Maasdijk, The Netherlands), `Euro', `Timman', `MonaLisa', `Snowflake' (Dekker, Hensbroek, The Netherlands), `Arctic Queen', `Bacardi', `Feeling Green', and `Ibis' (Fides, De Lier, The Netherlands) were planted in soil beds on 1st of February (day 1; 14 days after rooting). Each compartment contained six parallel soil beds (1 m × 1 m per bed), from which the outer two (border) were not taken account (Appendix 1). All the cuttings were rooted under the same conditions (temperature: 20-22°C; relative air humidity: 90-99% -covered with plastic- for 24h during 18th to 25th January; 90-99% for 23h and 80 – 90% -in the greenhouse- for 1h in 26th of January; 90-99% for 22.5h and 80 – 90% for 1.5h in 27th of January; 90-99% for 22h and 80 – 90% for 2h in 28th of January; 80 – 90% for 24h in 29th of January until the end of the rooting period), while rooting was visible on 18th of January.

Single-stem plants were grown supported by a wire mesh (0.125 m \times 0.125 m), connected to a movable frame that included the heating pipes. Plants were initially submitted to LD conditions (20 hours/day; from 0000 to 2000 hours), followed by a short-day (SD) (11 hours/day; from 0600 to 1700 hours) period up to the final harvest. A density of 64 plants m^2 and a LD period of 12 d were used. High-pressure sodium lamps (HPS Philips SON-T Agro, 8000 lux) were applied to the crop to extend the natural photoperiod, and to supplement natural sunlight. PAR was measured at a constant height of 0.5 m from the ground, using a 1.0 m line quantum sensor (LI-COR, model LI-191SA; Lincoln, USA). The lamps were switched on when outside global radiation was lower than 150 W m^{-2} , and switched off when outside global radiation was higher than 250 W m^{-2} . The SD conditions were achieved closing the blackout screen for 13 h a day (from 1700 to 0600 hours).

Table 2. The growth regulator, the day after planting of application and the dosage (gr/L) used.

cultivar			growth				
	20	22	27	29	30	34	regulator
Reagan Elite White, Calabria			3.5				Alar 64
Zembla		1.0				3.0	Alar 64
Biarritz			1.5				Alar 64
Euro	2.5		3.5			2.0	Alar 64
Timman			3.5			3.0	Alar 64
Mona Lisa	2.5		3.5			3.5	Alar 64
Snowflake					2.0	2.0	Alar 64
Tobago			3.5			1.5	Alar 64
Vyking						1.5	Alar 64
Arctic Queen				2.0			Dazide 85
Bacardi				2.5			Dazide 85
Feeling Green				3.5			Dazide 85
Ibis		1.5		3.0			Dazide 85

Irrigation was provided as required by two-pipe systems with micro sprinklers (overhead irrigation). Plant protection was applied by using chemical agents. In the cultivars `Anastasia', `

Paintball', and `Noa' no growth regulators were applied. For the remaining cultivars the treatments are shown in Table 2.

The terminal flower bud was pinched as soon as it was separated from the other crown buds (<5 mm). Plants were harvested when the first row of disc florets had reached anthesis in at least three inflorescences (flowers) per plant (stage 3 according to the auction scale).

Greenhouse climate

Day temperature was set at 18°C and night temperature at 19°C, which is common practice in commercial chrysanthemum production in The Netherlands. Ventilation temperature set point was 19°C, which was adjusted to 24°C when outside global radiation was higher than 300 W m⁻², until day 33 after planting (5th of March), while after this day (day 34 to the final harvest day), when outside global radiation was higher than 300 W m⁻², was set at 26.5°C. This change in the ventilation temperature set point, when outside global radiation was higher than 300 W m⁻², aimed to preserve the high CO₂ concentration. OCAP CO₂ was supplied, at daytime (20 hours/day until day 12, and 11 hours/day from day 13 to the end of cultivation period) when CO₂ concentration in the greenhouse was lower than 400 ppm (μmol mol⁻¹) and dosing was stopped at 600 ppm in compartment 1, while in compartment 2 (high CO₂ treatment) CO₂ was supplied when CO₂ concentration in the greenhouse was lower than 1400 ppm and dosing was stopped at 1600 ppm.

Greenhouse temperature was measured using PT500 elements. CO_2 concentration was measured with a CO_2 analyzer (URAS G, Hartmann & Braun; Frankfurt, Germany). Greenhouse temperature, relative humidity and CO_2 concentration were automatically recorded each 4 min by a commercial computer system (Hoogendoorn, Vlaardingen, The Netherlands). Daily outside global radiation was obtained from a meteorological station located outside from the greenhouse. The actual figures (mean values/ day) of temperature, Relative Humidity, CO_2 concentration, and outside global radiation are presented in the Appendices section (Appendices 16, 17, 18, 19, 20, and 21). Additionally, the actual values (each 4 min) of CO_2 concentration, and outside global radiation along one randomly selected day of cultivation (10^{th} of March) are presented in the Appendices section (Appendix 22).

Treatments

Two levels of CO_2 concentration (500 and 1500 ppm) were applied to 25 different cultivars, resulting in a total of 50 treatments.

Destructive measurements

Plants were destructively harvested on days 1, 14, 42 after planting and at final harvest (spreading from 59 to 71 d after planting, depending on the cultivar; when in at least two out of four plots, in the two CO₂ compartments, 75% of the plants had reached the stage 3 according to the auction scale).

Six (3rd destructive), eight (2nd destructive), twelve (final harvest), and twenty (1st destructive) plants were selected from an experimental unit, resulting in 12-40 plants per treatment. Plants were selected, excluding at least one row between different cultivars (2nd destructive measurement: the entire 9th row, 8 plants; 3rd destructive measurement: the 2nd row excluding the two border plants on each side of the bed, 6 plants; 4th destructive measurement: the 4th, 5th and 6th plant from the 4th, 5th, 6th and 7th row, 12 plants; Appendix 2). Within a given cultivar, data were collected on all plants at the same time. Between cultivars, harvest dates differed because of the different rates of flower development.

In the first destructive measurement the fresh weight of each experimental unit (20 plants together, excluding the roots), the stem length and the leaf area (LI-COR, Model 3100 Area Meter; Lincoln, NE, USA) of each experimental unit were recorded. In the second and third

destructive measurement the fresh mass of individual plant, excluding the roots, the number of leaves on the main stem (≥ 10 mm), the stem length and the leaf area were recorded. In the final harvest the fresh mass of individual plants, excluding the roots, the plant height, the number of flower buds (> 5 mm) and open flowers, and the reaction time (time from start of SD period to harvest) were recorded. Total dry mass of each experimental unit (20 plants for the 1^{st} , 8 plants for the 2^{nd} , 6 plants for the 3^{rd} , 12 plants for the 4^{rth} destructive measurement, excluding the roots), was measured (ventilated oven, 105° C for at least 15 h).

Growth analysis

Using the average values of the leaf area and dry weight from the different treatments the following growth parameters were calculated:

- 1. Relative Growth Rate $(RGR) = (lnW_{t2} lnW_{t1})/(t_2 t_1)$, calculated for the periods 0 to 2 (initial stages, when plants do not mutually compete for resources) and 2 to 6 weeks.
- 2. Crop Growth Rate (CGR) was calculated as the slope of the linear relationship between the crop mass (g m⁻²) and duration of cultivation, calculated for the period week 6 to final harvest (8 to 10 weeks, depending on the cultivar) (calculated during the period, when LAI>2-3).
- 3. Leaf Area Index (LAI) = $(LAP \times 64)/10.000$, calculated for the periods 0 to 2 and 2 to 6 weeks.
- 4. Leaf Area Ratio (LAR) =LAI/W, calculated for the periods 0 to 2 and 2 to 6 weeks.
- 5. Net Assimilation Rate (NAR) = $(W_2-W_1)/\{[LA_{P1}+LA_{P2}]/2\}*(t_2-t_1)$, calculated for the periods 0 to 2 and 2 to 6 weeks.

Stomatal density measurements

In the final harvest 3 fully expanded healthy leaves (leaf order 9; leaf order was assessed from top to bottom) per treatment were selected for the cultivar Reagan Elite White (A) and Feeling Green (M). Per leaf two impressions were taken from the abaxial side (lower surface) of each leaf for stomata density measurements and fixed on a preparate. Xantopren L and Activator universal (Heraeus Kulzer GmbH&Co, Hanau, Germany) were mixed and put on the leaf before hardening, as leaf imprint. After hardening the imprint was taken from the leaves and a layer of nail polish was applied to be used for the preparate. With a Leitz Aristoplan microscope (Leitz, Westlar, Germany), Nikon DXM1200 digital camera (Nikon Corp., Tokyo, Japan), Nikon ACT-1 software (version 2.00, Nikon Corp., Tokyo, Japan) and Imaging Tool (version 2.02, UTHSCSA, San Antonio, USA) the number of stomata on 4 randomly chosen sites per leaf (magnification 100x), so two sites per impression, were counted.

Photosynthesis measurements

Once a week in three leaves (1 leaf/ plant) of the cultivar Reagan Elite White (A) (week 4, 5, 6, 7, 8 after planting) and Feeling Green (M) (week 5, 6, 7, 8 after planting), from each of the two CO_2 treatments, the light-saturated rate of photosynthesis was measured first at the CO_2 level at which the plants were grown, and afterwards at the other CO_2 concentration. The light-saturated rate of photosynthesis was measured by making a light response curve in 6 light steps (100, 300, 600, 900, 1200, 1500 μ mols m⁻² s⁻¹), each of 5 minutes, while afterwards 2 light steps (1500, 1500 μ mols m⁻² s⁻¹) were used for the other CO_2 concentration (temperature: 22- 25 °C, RH of about 70%). In every week the same leaf/ plant was used (Reagan Elite White: week 4: leaf order 6, week 5: leaf order 9, week 6, 7, 8: leaf order 13; Feeling Green: week 5: leaf order 9, week 6, 7, 8: leaf order 10; leaf order was assessed from top to bottom). The CO_2 exchange was measured by a portable photosynthesis measurement system LI-6400 (LI-COR Biosciences, Nebraska, USA).

Statistical design and analysis

The statistical design was laid out in two replicates, where each replicate consisted of 1 soil bed, randomly placed in each CO_2 compartment. Each cultivar is examined in 2 replicates per compartment, except from the cultivar Reagan Elite White (A) (4 replicates at low CO_2 ; 3 replicates at high CO_2), and cultivar Bacardi (L) (2 replicates at low CO_2 ; 1 replicate at high CO_2) (Appendix 1). An analysis of variance was conducted and treatment effects were tested at 5% probability level (per cultivar). Mean separation was done using Student's t-test (P = 0.05). The statistical software package Genstat 5 (VSN International Ltd, Herts, UK) was used.

RESULTS

Plant development and quality aspects

Number of leaves on the main stem

The number of leaves on the main stem was assessed in week 6 after planting (30 days after the SD treatment; second destructive measurement), since the plants had already developed the terminal flower bud, so no new leaves were formed after that stage. Final number of leaves of all 17 cultivars did not change significantly in response to CO_2 enrichment. The differences in the number of leaves between the treatments are in the range of -5.6% to + 3.5% (Appendix 8). The number of leaves clearly is not affected by the CO_2 concentration in the range of concentrations examined (Figure 2; Appendix 7).

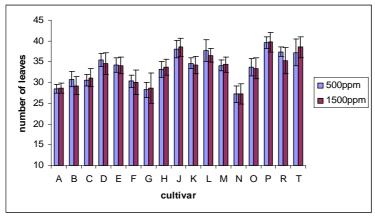


Figure 2. The number of leaves present on the main stem at final harvest in the two CO_2 compartments. Vertical bars indicate standard error of mean.

Leaf area index, Leaf Area Ratio and Net Assimilation Rate

The leaf area at the time of planting (day 1) was on average 67 cm², resulting in a Leaf Area Index (LAI) of 0.44 (Appendix 3). In week 2 the LAI was found to be decreased (1-9%) in the high CO₂ compartment in 7 out of 17 cultivars, not to be affected in 1 cultivar, while was increased (3-15%) in 9 cultivars (Appendix 5). The mean value of LAI in week 2 was 1.6, and was on average 2.5% higher in the high CO₂ regime. In week 6 after planting, the LAI was found to be decreased (0-3%) in the high CO₂ compartment in 5 out of 17 cultivars, while was increased (1-23%) in 12 cultivars. The mean value of LAI in week 6 was 5.1, and was on average 6.4% higher in the high CO₂ regime (Appendix 8). When LAI exceeds 3-4, maximum light interception has been achieved and extra leaves do not contribute to faster production rate (Heuvelink et al., 2004), fact that took place in the period week 2 to 6.

In week 2, the Leaf Area Ratio (LAR) decreased (0-10%) in 14 out of 17 cultivars, while the Net Assimilation Rate (NAR) increased (2-14%) in 13 out of 17 cultivars (Appendix 5). In week 6 after planting, the Leaf Area Ratio (LAR) decreased (1-22%) in all 17 cultivars, while the Net Assimilation Rate (NAR) increased (3-44%) in all 17 cultivars (Appendix 8).

Stem length

The stem length was increased (0-12%) at high CO_2 concentration in all 17 cultivars, except for cultivar O (-2%) (Figure 3; Appendix 11). The increase in the stem length was significant only for the cultivars C, J, M, and V. CO_2 enrichment hence resulted in taller plants.

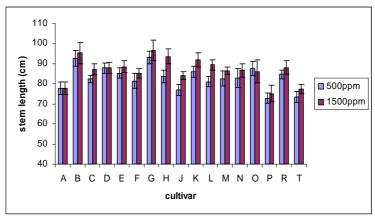


Figure 3. The stem length at final harvest in the two CO₂ compartments. Vertical bars indicate standard error of mean.

The variance (gives a measure about the variability of the examined characteristic in the treatment) for the stem length at final harvest increased (2-210%) with the CO_2 enrichment in 8 out of 17 cultivars, while it decreased in the 9 other cultivars (14-87%) (Figure 4; Appendix 12). So, no consistent response was present.

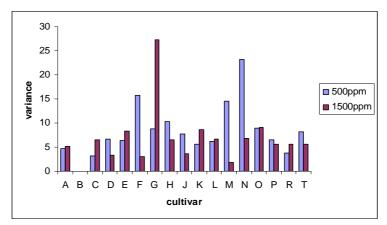


Figure 4. The variance for the stem length at final harvest in the two CO₂ compartments.

Number of flowers

At commercial anthesis, the number of flowers and flower buds was increased (3- 48%) at 1500ppm CO_2 compartment in 15 out of 17 cultivars, except from the cultivars E and B, which were not affected (Figure 5; Appendix 11). The increase in the number of flowers and flower buds was significant only for the cultivars A, F, P, and R (Appendix 13). CO_2 enrichment resulted in plants, which acquired higher number of flowers.

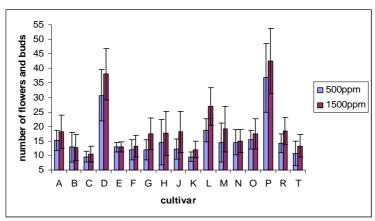


Figure 5. The number of flowers and flower buds at final harvest in the two CO₂ compartments. Vertical bars indicate standard error of mean.

The variance for the number of flowers and flower buds at final harvest increased with the CO_2 enrichment in 10 out of 17 cultivars (17-241%), while decreased in 7 cultivars (1-39%) (Figure 6; Appendix 12). So, no consistent response was present.

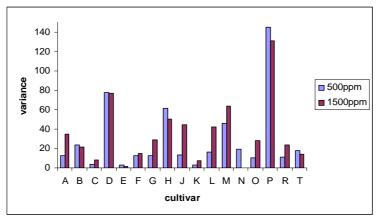


Figure 6. The variance for the number of flowers and flower buds at final harvest in the two CO_2 compartments.

Reaction time and flower development rate

The effect of CO₂ enrichment on the average number of days to reach commercial harvest stage from the onset of SD period (reaction time), was very small in all cultivars (maximum of 2 days difference) (Table 3; Appendix 13a).

Table 3. The effect of elevated CO₂ on the reaction time in the various cultivars.

difference in days	cultivar	number of cultivars
-2	Anastasia, Paintball	2
-1.5	Calabria	1
-1	Timman, ArcticQueen	2
-0.5	Bacardi	1
0	Biarritz, Noa, Ibis, Tobago, Vyking	5
+0.5	ReaganEliteWhite, Euro, MonaLisa, Snowflake	4
+1	Zembla, FeelingGreen	2

Mass production

Fresh weight

Elevated CO₂ increased (2-28%) the fresh weight in all 17 cultivars (Figure 7; Appendix 11). The increase in fresh weight was significant only for the cultivars G, and R.

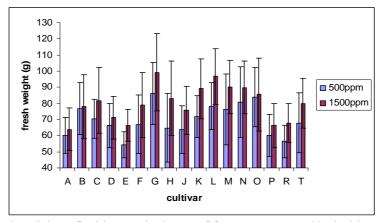


Figure 7. The fresh weight at final harvest in the two CO_2 compartments. Vertical bars indicate standard error of mean.

The variance for the fresh weight at final harvest increased (3-176%) with the CO_2 enrichment in 11 out of 17 cultivars, while decreased in 6 cultivars (2-51%) (Figure 8; Appendix 12). Again, no clear tendency was present.

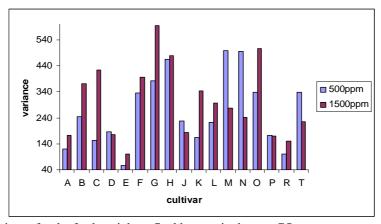


Figure 8. The variance for the fresh weight at final harvest in the two CO₂ compartments.

Fresh weight 80 cm length

Although, it can be argued that the pattern of the fresh weight from plants with 80 cm length (adjusted to this length due to market needs) is partly covered by the previous cession, it is important to be examined separately, since this determines the price, and furthermore it will make clear if the increased weight in the elevated CO_2 compartment is due to the increased stem length solely or not.

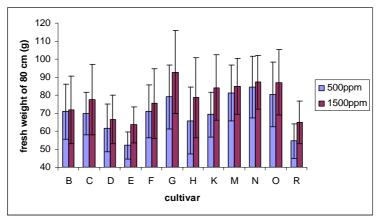


Figure 9. The fresh weight of plants with fixed length of 80 cm at final harvest in the two CO₂ compartments. Vertical bars indicate standard error of mean.

Not all cultivars exceeded (or reached) the 80 cm level. Further discussion in this cession refers to the cultivars that acquire at least 8 plants (out of 12) in both CO₂ compartments (12 out of 17 cultivars; Appendix 10).

The elevated CO_2 increased (1-22%) the fresh weight from plants with 80 cm length in all 12 cultivars (Figure 9; Appendix 11). This increase was significant only for the cultivars G, and R. Hence, the increase in fresh weight found in the enriched CO_2 compartment is not solely due to taller plants.

Dry weight

Elevated CO₂ increased (5-39%) the aboveground dry weight in all 17 cultivars (Figure 10; Appendix 11). This increase was significant only for the cultivars A, E, G, K, and R.

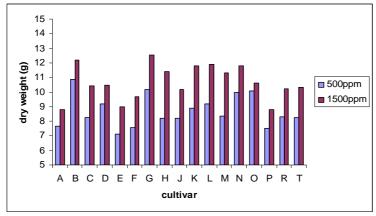


Figure 10. The dry weight at final harvest in the two CO₂ compartments.

Water content

Cultivation in elevated CO_2 decreased the Water Content (WC) in all 17 examined cultivars, and thus increased the dry matter present in a certain fresh mass. This decrease was significant only for the cultivars A, C, E, F, and J (Figure 11; Appendix 11).

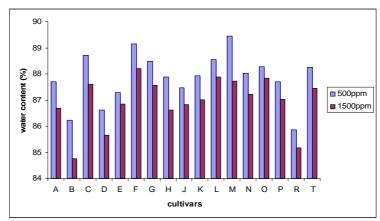


Figure 11. The water content at final harvest in the two CO₂ compartments.

Growth analysis

Relative growth rate

The Relative Growth Rate (RGR; g g⁻¹ d⁻¹) was calculated over the period 0 to 2 weeks after planting (very close to the beginning of the SD treatment, starting on day 13), when plants did not mutually compete for resources. The RGR during the aforementioned period (RGR₀₋₂), was higher (1-13%) in the elevated CO_2 compartment in 15 out of 17 cultivars, while decreased slightly in 2 cultivars (-0.5, and -2%) (Figure 12; Appendix 14a).

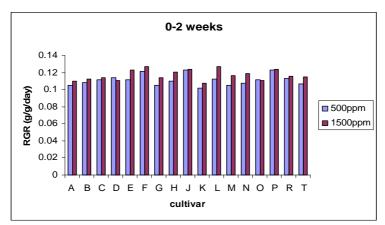


Figure 12. The RGR in the period 0 to 2 weeks in the two CO₂ compartments.

Crop growth rate

The Crop Growth Rate was calculated in the period from week 6 to final harvest (week 8 to week 10; depending on the cultivar), since the LAI at this period was higher than 3, and light fraction intercepted remained more or less constant. CO_2 enrichment increased (7-52%) the Crop Growth Rate (CGR_{6-f}; g m⁻² d⁻¹) in 14 out of 17 cultivars, while it was decreased in 3 cultivars (-5, -6, and -16%) (Figure 13; Appendix 14b).

Crop Growth Rate= (Light Use Efficiency)/ (light intercepted). Since the light intercepted was the same in both treatments, the increased CGR, implies higher LUE in the high CO_2 compartment.

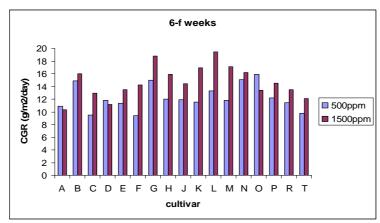


Figure 13. The CGR in the period 6 weeks to final harvest (8 to 10 weeks, depending on the cultivar) in the two CO_2 compartments.

Morphological and Physiological parameters

Stomatal density measurements

Measurements on stomata were only performed on the abaxial (lower) surface of the leaves, because a number of preliminary observations indicated that the ratio of stomatal density of the adaxial and abaxial side of the leaves used is about 1:7 (determined for Reagan Elite White; Appendices 15a, 15b).

Table 4. The number of stomata per mm² and the per cent change induced by elevated CO₂.

Cultivar	Number of s	change	
	500ppm	1500ppm	(%)
Reagan Elite White	44	33	-22%
Feeling Green	36	41	+19%

The growth in the high CO₂ compartment has a cultivar specific effect on the stomatal density (Table 4; Appendices 15b, 15c), since in the cultivar Reagan Elite White it had a negative effect, while in the cultivar Feeling Green a positive one.

Photosynthesis measurements

A negative slope with time in the light saturated rate of photosynthesis (P_{max}) is present in both cultivars, independent of the CO_2 concentration of measurement (Figures 14, 15). The P_{max} of the plants grown in high CO_2 concentration, is the same with the plants grown in the low CO_2 concentration, when the CO_2 concentration during measurement is high, expect for week 8, where the P_{max} of the plants grown in the low CO_2 concentration is higher in both cultivars, and week 7 for the cultivar Reagan Elite White only. This pattern is different when the CO_2 concentration during measurement is low, where during the whole examined period, the plants grown in low CO_2 concentration have higher P_{max} in both cultivars.

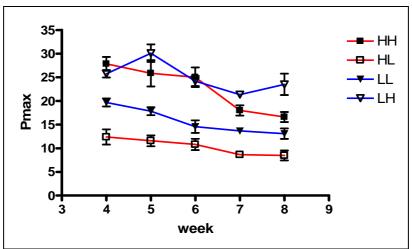


Figure 14. The light saturated rate of photosynthesis (P_{max} , μ mol CO_2 cm⁻² s⁻¹) as a function of weeks after planting for the cultivar Reagan Elite White. The filled squares are plants grown in high CO_2 and measured in high CO_2 (HH), the open squares plants grown in high CO_2 and measured in low CO_2 (HL), the filled triangles plants grown in low CO_2 and measured in low CO_2 (LL), and the open triangles plants grown in low CO_2 and measured in high CO_2 (LH). Each point represents the mean value of 3 plants (1 leaf/ plant) per treatment. In week 7, the points HH and HL are the mean value of 2 plants (1 leaf/ plant). Vertical bars indicate standard error of mean when larger than patterns.

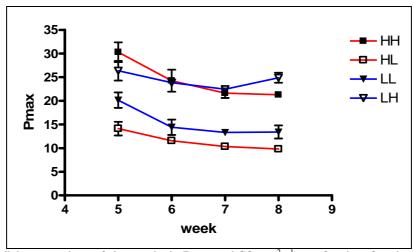


Figure 15. The light saturated rate of photosynthesis (P_{max} , μ mol CO_2 cm⁻² s⁻¹) as a function of weeks after planting for the cultivar Feeling Green. The filled squares are plants grown in high CO_2 and measured in high CO_2 (HH), the open squares plants grown in high CO_2 and measured in low CO_2 (HL), the filled triangles plants grown in low CO_2 and measured in low CO_2 (LH). Each point represents the mean value of 3 plants (1 leaf/ plant) per treatment. In week 8, the points HH and HL are the mean value of 2 plants (1 leaf/ plant). Vertical bars indicate standard error of mean when larger than patterns.

Figure 16 includes the P_{max} of both cultivars, where the CO_2 concentration of measurement was equal with that of cultivation, and in this way it reflects the conditions where growth took place. The P_{max} over time of cultivation of plants grown in low CO_2 concentration (and measured in low CO_2) in both cultivars is equal. Regarding the P_{max} over time of cultivation of plants grown in high CO_2 concentration (and measured in high CO_2) in both cultivars overlaps for the weeks 5 and 6, while the last 2 weeks of measurement the cultivar Feeling Green acquires higher P_{max} .

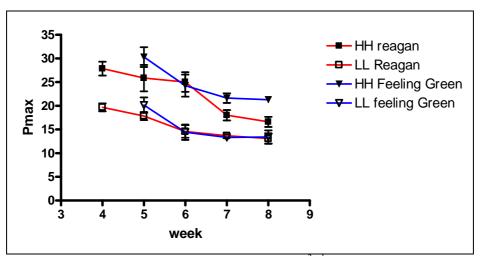


Figure 16. The light saturated rate of photosynthesis (P_{max} , μ mol CO_2 cm⁻² s⁻¹) as a function of weeks after planting for the cultivar Reagan Elite White and Feeling Green. The filled squares are plants grown in high CO_2 and measured in high CO_2 (HH) of Reagan Elite White, the open squares plants grown in low CO_2 and measured in low CO_2 (LL) of Reagan Elite White, the filled triangles plant plants grown in high CO_2 and measured in high CO_2 (HH) of Feeling Green, and the open triangles plants grown in low CO_2 and measured in low CO_2 (LL) of Feeling Green. Each point represents the mean value of 3 plants (1 leaf/ plant) per treatment. In week 7, the point HH for the cultivar Reagan Elite White, and in week 8, the point HH for the cultivar Feeling Green are the mean value of 2 plants (1 leaf/ plant). Vertical bars indicate standard error of mean when larger than patterns.

DISCUSSION

Plant development and quality aspects

Number of leaves on the main stem

Determinate plants are characterized by a shift from vegetative to reproductive growth once flowering begins, because flowering occurs from the terminal meristems (Harper 1977). Since this sift is manipulated by changes in the photoperiod (and not the CO₂ concentration) (Raven, Evert & Eichhorn 1999), the number of leaves on the main stem (formed before the terminal meristem is transformed to flower bud) is not influenced.

The number of leaves on the main stem was found not to be affected by the CO_2 regime in all cultivars examined (Figure 2). This agrees with earlier observations (Mortensen and Moe, 1983; Mortensen, 1986).

Stem length

In 3 cultivars no growth regulators were applied. In the remaining 14, the application of the growth regulators was in equal dose and timing (Table 2) in the two CO₂ compartments, so it can be assumed that this application cannot explain possible differences in stem length between the CO₂ treatments.

The stem length was found to be positively affected by the CO_2 enrichment in 16 out of 17 cultivars, while in 1 it was marginally decreased (cultivar O, -2%) (Figure 3; Appendices 10, 11). This result is in accordance with literature, where it is reported that CO_2 enrichment promotes stem elongation (Mortensen and Moe, 1983; Mortensen, 1986). Since the final number of leaves (equals to number of internodes) was not affected by the CO_2 enrichment and the stem length increased, it can be concluded that the internode length was increased by the CO_2 enrichment. Significant differences were only reported in 4 cultivars (C, J, M, and V), while in the rest the variation between the plots is responsible for not been recorded as significant. However, the positive effect is clear in all cases. A weak genotype \times (CO_2 concentration) interaction was observed.

The increased CO₂ regime decreased the variance for the stem length at final harvest in 9 out of 17 cultivars (Appendix 12).

Number of flowers and flower bubs

Increased CO₂ concentration resulted in more flowers and flower buds per plant in 15 out of 17 cultivars (Figures 5; Appendices 10, 11), except from the cultivars E (0%) and B (2%). The same is reported in previous experiments (Gislerød and Nelson, 1989; Mortensen, 1986).

A strong genotype \times (CO₂ concentration) interaction was present.

No measurements were conducted related to flower size, but it has been reported that high CO₂ concentration increased flower size (Potter, 1980).

Increased CO₂ concentration increased the variance for the number of flowers and flower buds at final harvest in 10 out of 17 cultivars (Appendix 12).

Reaction time and flower development rate

Developmental rates in *Chrysanthemum morifolium* and other determinate short-day plants are generally unaffected, or even slowed, by growth in elevated CO₂ (Reekie, Hicklenton & Reekie 1994), and can be manipulated through changes in photoperiod (Raven, Evert & Eichhorn 1999) and temperature.

The average number of days to reach commercial harvest stage from the onset of SD period was hardly affected by the CO_2 regime (Table 3), which agrees with earlier findings (Potter, 1980; Mortensen, 1986a).

Mass production

Fresh weight

 CO_2 enrichment increased the fresh weight of plants, excluding the roots, in all 17 cultivars (Figure 7), which agrees with previous work (Mortensen, 1986). The increased fresh weight came from an increase in the dry weight, but to a less extent, since the water content decreased. A strong genotype \times (CO_2 concentration) interaction was present.

The variance for the fresh weight at final harvest increased with the CO₂ enrichment in 11 out of 17 cultivars (Figure 8; Appendix 12).

Fresh weight 80 cm length

The CO₂ enrichment increased the fresh weight of plants with 80 cm fixed length in all 12 cultivars (which exceed the 80 cm limit and were adjusted to this length) (Figure 9; Appendix 11). This result implies that the increase in fresh weight found in the enriched CO₂ compartment is not only attributed to the higher stem length. Possible candidates for the additional weight can be the higher number of flowers, and/ or the higher leaf area (according to the literature), and/ or the higher number of side shoots (according to the literature).

Dry weight

The CO₂ enrichment increased the dry weight of plants, excluding the roots, in all 17 cultivars (Figures 10; Appendices 10, 11), which agrees with earlier findings (Mortensen, 1986). The increased dry weight is the result of increased RGR in the periods 0 to 6 weeks (increased NAR, despite the decrease in LAR) and increased CGR in the period 6 weeks to final harvest.

A strong genotype \times (CO₂ concentration) interaction was present.

Water content

The growth in the elevated CO_2 increased the dry matter present in a certain fresh mass (decrease WC) in all 17 examined cultivars (figure 11). A weak genotype \times (CO₂ concentration) interaction was observed, since the range of the response was medium.

Growth analysis

Relative growth rate and Crop Growth Rate

The RGR was assessed in the initial stages of growth (first 2 weeks), where it was found that the CO_2 enrichment increased the RGR_{0-2} in 15 out of 17 cultivars, by on average 5.3 %. The higher RGR is reflected in the higher dry mass production observed in the elevated CO_2 regime.

RGR=LAR*NAR, where the LAR is influenced by the morphology of the plant, while the NAR by the physiology (rate of photosynthesis) (Heuvelink et al., 2004). Since in the majority of the cultivars the LAR decreased, while the NAR increased in the period 0 to 2 weeks (Appendix 5), it can be concluded that the higher rate of photosynthesis in the 1500ppm compartment is responsible for the increased RGR₀₋₂. The same can be concluded for the period 2 to 6 weeks, where in all 17 cultivars the LAR decreased, while the NAR increased in this period.

The CGR was assessed in the latter examined period (week 6 to final harvest), where the LAI was higher than 3, which ensures that maximum light interception has been achieved, and it was found that the $\rm CO_2$ enrichment increased the $\rm CGR_{6-f}$ in 14 out of 17 cultivars, by on average 22 %.

A strong genotype \times (CO₂ concentration) interaction was present in both growth parameters (RGR and CGR).

Morphological and Physiological parameters

Stomatal density measurements

The effect of elevated CO_2 on the stomatal density was cultivar dependent (Table 4; Appendices 15b, 15c), and a strong genotype \times (CO_2 concentration) interaction was obvious. Increased stomatal density in high CO_2 does not directly results to higher photosynthesis, since parameters like the leaf area and the stomatal size and functioning are also important.

Photosynthesis measurements

We decided to measure the light saturated rate of photosynthesis (P_{max}), since it reflects the potential rate of photosynthesis, and is independent of the light conditions during the measurement. We measured the light saturated rate of photosynthesis in the CO_2 concentration of growth, to have a picture of the photosynthesis rates during cultivation, and in the other CO_2 concentration to investigate if adaptation to the high CO_2 concentration takes place.

In order to examine the adaptation, that according to literature takes place in the high CO_2 regimes resulting in lower photosynthesis rates, compared to short term CO_2 concentration increases, we decided to measure the same leaf every week (1 leaf/ plant; 3 leaves/ treatment). In this way, except from the effect of adaptation to the high CO_2 concentration, the results include the effects of aging and shading (the shading of the leaves chosen increased in time, as the leaf order increased). In this point of view it is not possible to separate the aforementioned effects, but comparisons between the treatments can be made, since the effects of aging and shading are expected to be similar.

In all cases, with the exception of the last point of the curve which consists the measurements of plants grown in low CO_2 concentration and measured in high CO_2 concentration of the cultivar Reagan Elite White, a negative slope in the P_{max} over time is obvious (Figures 24, 25) that is the combined effects of the aforementioned 3 factors.

When the CO_2 concentration of measurement is high, lower P_{max} , indication of adaptation to the high CO_2 concentration, is only evident in week 8 for cultivar Feeling Green, and in weeks 7 and 8 for cultivar Reagan Elite White. If we only examine these curves, we would conclude that the adaptation to the high CO_2 concentration took place 1 week earlier in the cultivar Reagan Elite White. However, if we examine the curves, where the CO_2 of measurement is low, the adaptation to the high CO_2 concentration took place on week 4 or even earlier (no measurements conducted before week 4). When the CO_2 of measurement is low, the plants grown in high CO_2 concentration are much less efficient.

The pattern obtained is not that expected where the adaptation is clear in low CO_2 measurement conditions, where the CO_2 is limiting for photosynthesis, while is absent in high CO_2 measurement conditions, in the week 4, 5 and 6 for the cultivar Reagan Elite White and week 5, 6 and 7 for the cultivar Feeling Green.

Between the cultivars, differences in the P_{max} , when CO_2 concentration during measurement was that of growth, Feeling Green was more efficient in the high CO_2 conditions only the last 2 weeks of cultivation (Figure 26). However, a comparison like this is risky, since the last two weeks the leaves of the cultivar Reagan Elite White (leaf order 13) were more shaded than the leaves of the cultivar Feeling Green (leaf order 9), and this discrepancy might be due to the adaptation to lower light conditions. It seems that there is no genotype \times (CO_2 concentration) interaction, at least in the 2 measured cultivars.

 CO_2 enrichment during cultivation increased the P_{max} 50% on average, and the increased photosynthesis rates are (at least) partially responsible for the higher production achieved.

CONCLUSION

In summary, cultivation in 1500ppm, compared to cultivation in 500ppm, did not affect the number of leaves on the main stem in any of the cultivars, while hardly affected the reaction time in some of the cultivars. CO_2 enrichment promoted stem length (through increased internode length, and not number) in 16 out of 17 cultivars (0-12%; weak genotype \times CO_2 concentration interaction), while slightly decreased the water content (0.5-2%; weak genotype \times CO_2 concentration interaction). Increased CO_2 concentration increased the number of flowers and flower buds per plant in 15 out of 17 cultivars (3-48%; strong genotype \times CO_2 concentration interaction). Moreover, the CO_2 enrichment increased the fresh weight (2-28%; strong genotype \times CO_2 concentration interaction) and the dry weight (5-40%; strong genotype \times CO_2 concentration interaction) of plants, excluding the roots, in all 17 cultivars. This increase in the dry weight came from an increase of the RGR in the period 0 to 6 weeks (through increased NAR, despite the decreased LAR), and an increase of the CGR in the period 6 weeks to final harvest (increased LUE).

The per cent increase in fresh weight, induced by the CO_2 enrichment at final harvest, is well correlated (R^2 =0.84) with the per cent increase of dry weight. The same did not happen with the per cent increase of number of flowers and flowers buds at final harvest, which is not correlated neither with the per cent increase of fresh weight (R^2 =0.14) nor the per cent increase of dry weight (R^2 =0.1). In other words, the cultivars that gave the higher per cent increase in fresh (or dry) weight by the CO_2 enrichment, did not gave the higher per cent increase in the number of flowers and flower buds at final harvest.

From this work it can be concluded that a number of cultivars responds more efficiently to the 1500ppm concentration in terms of growth (fresh weight), but also in certain quality aspects (number of flowers and flower buds, and in a lower degree stem length). The higher per cent increase in fresh weight, in most of the cases, is compensated by a lower per cent increase in the number of flowers and flowers buds.

The return, but also the profitability, of the closed greenhouse investment in chrysanthemum cultivation requires that certain characters, which are benefited from the CO_2 enrichment, achieve a better price in the market, and that efficient cultivars are used for cultivation. The efficient cultivars in achieving higher fresh weight are less efficient in bearing more flowers. The selection of the respective efficient cultivars has to be done experimentally, while the market trends (form the prices) will give the criteria.

Limitations and Suggestions

The present experiment conducted under limited setup (only 2 compartments, so no real repetitions), and it was the first one to set an idea of genotype \times environment interaction. Based on the obtained results, further research can be designed with a lower number of cultivars, the most contrasting cultivars, and on a larger scale setup.

Since the growth analysis (through NAR), in agreement with the photosynthesis measurements performed, indicated the photosynthesis rates as responsible for the increased RGRs, we believe that photosynthesis measurements must be included in later work. Moreover, the dry matter distribution (partitioning) will give a deeper insight in the reaction of the different genotypes to the high CO₂ concentration, while we propose an increased number of destructive measurements (weekly) during the period 2 to 6 weeks (period, where LAI exceeded 3).

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APPENDICES

Appendix 1. Experimental design. Names of cultivars and numbers are given for each plot. The border plots are not depicted. Each cultivar is examined in 2 plots per compartment, except for the cultivar Reagan Elite White (A) (4 plots at 500ppm CO_2 ; 3 plots at 1500ppm CO_2), and cultivar Bacardi (L) (2 plots at 500ppm CO_2 ; 1 plot at 1500ppm CO_2). The plots 62 and 63 were not taken into account, since they were planted with more than 1 cultivars.

500 j	ppm CO ₂	,

300 ppm CO ₂									
9	M	18	A	27	L	36	E		
	FeelingGreen		REliteWhite		Bacardi		Noa		
8	K	17	В	26	D	35	O		
	ArcQueen		Anastasia		Biarritz		Calabria		
7	R	16	Е	25	P	34	J		
	Paintball		Noa		Tobago		Snowflake		
6	D	15	J	24	A	33	С		
	Biarritz		Snowflake		REliteWhite		Zembla		
5	N	14	P	23	R	32	G		
	Ibis		Tobago		Paintball		Timman		
4	Н	13	С	22	N	31	T		
	MonaLisa		Zembla		Ibis		Vyking		
3	G	12	L	21	В	30	M		
	Timman		Bacardi		Anastasia		FeelingGreen		
2	A	11	О	20	K	29	F		
	REliteWhite		Calabria		ArcQueen		Euro		
1	T	10	F	19	Н	28	A		
	Vyking		Euro		MonaLisa		REliteWhite		

1500 ppm CO₂

			1500 pp		- 2		
45	F	54	M			72	J
	Euro		FlingGreen				Snowflake
44	K	53	Н			71	K
	ArcQueen		MonaLisa				ArcQueen
43	C	52	J	61	E	70	R
	Zembla		Snowflake		Noa		Paintball
42	L	51	O	60	P	69	C
	Bacardi		Calabria		Tobago		Zembla
41	T	50	R	59	M	68	Н
	Vyking		Paintball		FlingGreen		MonaLisa
40	N	49	G	58	G	67	N
	Ibis		Timman		Timman		Ibis
39	D	48	E	57	T	66	В
	Biarritz		Noa		Vyking		Anastasia
38	В	47	A	56	D	65	F
	Anastasia		REliteWhite		Biarritz		Euro
37	A	46	P	55	O	64	A
	REliteWhite		Tobago		Calabria		REliteWhite

Appendix 2. The plants selected in the second, third, and fourth destructive measurement from a certain plot. Each square (10X8 plants) represents one plot, and 9 plots consist 1 bed. The orientation of the figure is the same like Appendix 1. For example at the plot 38, the 1st row is next to plot 37 and the 10th row is next to plot 39.

	X	X	X	X	X	X	X	X	10
second measurement	X	X	X	X	X	X	X	X	9
	X	X	X	X	X	X	X	X	8
	X	X	X	X	X	X	X	X	7
	X	X	X	X	X	X	X	X	6
	X	X	X	X	X	X	X	X	5
	X	X	X	X	X	X	X	X	4
	X	X	X	X	X	X	X	X	3
	X	X	X	X	X	X	X	X	2
	X	X	X	X	X	X	X	X	1
	X	X	X	X	X	X	X	X	10
third measurement									9
	X	X	X	X	X	X	X	X	8
	X	X	X	X	X	X	X	X	7
	X	X	X	X	X	X	X	X	6
	X	X	X	X	X	X	X	X	5
	X	X	X	X	X	X	X	X	4
	X	X	X	X	X	X	X	X	3
	X	X	X	X	X	X	X	X	2
	X	X	X	X	X	X	X	X	1
	X	X	X	X	X	X	X	X	10
fourth measurement									9
	X	X	X	X	X	X	X	X	8
	X	X	X	X	X	X	X	X	7
	X	X	X	X	X	X	X	X	6
	X	X	X	X	X	X	X	X	5
	X	X	X	X	X	X	X	X	4
	X	X	X	X	X	X	X	X	3
	X							X	2
	X	X	X	X	X	X	X	X	1

Appendix 3. The initial fresh weight (FW), stem length, leaf area (LA), dry weight (DW), and water content (WC) for the rooted cuttings.

Cultivar/ treatment	FW	Stem length	LA	DW	WC
	(g)	(cm)	(cm ²)	(g)	(%)
A- ReaganEliteWhite	3.0	13.3	73	0.26	91.3
B- Anastasia	3.0	10.3	73	0.23	92.3
C- Zembla	2.2	10.5	58	0.21	90.2
D- Biarritz	2.9	11.3	72	0.22	92.5
E- Noa	2.4	10.4	60	0.19	92.1
F- Euro	2.4	12.7	58	0.19	92.1
G- Timman	2.8	12.5	65	0.24	91.3
H- MonaLisa	2.5	13.1	68	0.22	91.3
J- Snowflake	2.1	10.0	49	0.16	92.3
K- ArcticQueen	3.2	11.6	79	0.28	91.2
L- Bacardi	3.1	11.7	71	0.23	92.7
M- FeelingGreen	3.4	12.6	86	0.28	91.5
N- Ibis	3.3	12.7	84	0.31	90.6
O- Calabria	3.0	11.1	68	0.25	91.5
P- Tobago	2.7	12.7	73	0.20	92.6
R- Paintball	2.5	10.1	62	0.22	91.2
T- Vyking	2.8	10.9	73	0.21	92.4

Appendix 4. The fresh weight (FW), number of leaves (No. of leaves), stem length, leaf area (LA), dry weight (DW), and water content (WC) 2 weeks after planting. Values within the column and within the cultivar followed by different letters are significantly different at P=0.05 level (tested per cultivar).

Cultivar/ treatment	FW No. of leaves		antly different at P= Stem length	LA	DW	WC
	(g)		(cm)	(cm ²)	(g)	(%)
A- ReaganEliteWhite	(8/		(****)	(6111)	\8/	(70)
High	11.56	14.0	28.7	203	1.23	90.0a
Low	11.00	12.7	27.1	209	1.15	90.1b
B- Anastasia						
High	11.72	14.7	19.5	243	1.12	90.0
Low	11.82	15.8	20.0	253	1.06	90.8
C- Zembla						
High	10.78	13.9	26.2	218	1.05	89.9
Low	10.31	12.9	25.0	212	1.01	89.9
D- Biarritz						
High	10.99	16.1a	23.7	222	1.05	90.2
Low	11.82	16.6b	25.7	245	1.09	90.4
E- Noa		2000				
High	12.64	16.9	27.2	248	1.05	91.5
Low	10.42	15.9	26.0	215	0.89	91.2
F- Euro	· · · -		_5.0		/	
High	11.95	15.7a	29.0	233	1.11	90.6
Low	11.43	14.6b	28.0	233	1.02	90.5
G- Timman						
High	13.08	17.2	27.5	232	1.18	90.7
Low	11.97	15.9	26.1	220	1.04	91.1
H- MonaLisa						,
High	13.39	17.7	30.5	284	1.19	90.9
Low	11.81	16.8	28.2	269	1.03	91.0
J- Snowflake						,
High	10.90	18.1	28.2	203	0.94	91.0a
Low	10.95	17.6	26.9	195	0.93	91.5b
K- ArcticQueen	10.50	17.0	20.7	1,0	0.55	, 1.0.0
High	13.67	17.3a	28.0	249	1.26a	90.6
Low	12.55	16.1b	25.8	253	1.16b	90.5
L- Bacardi						
High	17.00	19.6	27.3	318	1.34	91.7
Low	14.40	17.3	26.4	285	1.10	92.1
M- FeelingGreen						
High	16.30	17.8	32.2	296	1.44	90.9
Low	14.70	17.7	31.3	266	1.24	91.3
N- Ibis						
High	15.74	14.0	33.7	301	1.62a	89.4
Low	14.11	13.94	32.7	275	1.39b	89.9
O- Calabria						
High	13.96	15.8	27.7	267	1.21	91.1
Low	14.63	15.7	28.2	283	1.22	91.5
P- Tobago						
High	12.65	19.4	28.4	256	1.15	90.5
Low	13.04	19.1	28.2	265	1.14	91.1
R- Paintbal						
High	11.47	18.0	21.2	246	1.11	89.9
Low	11.41	18.1	20.7	259	1.08	90.2
T- Vyking		10.1	-0.,		1.00	, v. -
High	12.19	19.2a	21.8	272	1.06	90.9
Low	11.13	18.2b	21.0	247	0.94	91.2

Appendix 5. The per cent (%) relative difference in fresh weight (FW), number of leaves (No. of leaves), stem length, leaf area (LA), dry weight (DW), leaf area ratio (LAR), and net assimilation rate (NAR) of plants grown in the 1500ppm compartment, compared to plants grown in the 500ppm CO_2 compartment 2 weeks after planting.

Cultivar/ treatment	FW (%)	No. of leaves (%)	Stem length (%)	LA (%)	DW (%)	LAR (%)	NAR (%)
A-							
ReaganEliteWhite	5.09	9.83	6.13	-2.87	6.96	-7.58	9.35
B- Anastasia	-0.85	-6.70	-2.35	-3.95	5.66	-3.13	1.99
C- Zembla	4.56	7.26	5.13	2.83	3.96	-1.65	3.48
D- Biarritz	-7.02	-3.02	-7.79	-9.39	-3.67	-2.54	-2.27
E- Noa	21.3	6.67	4.34	15.3	18.0	-4.91	13.9
F- Euro	4.55	8.17	3.46	0.00	8.82	-4.35	5.74
G- Timman	9.27	7.84	5.519	5.45	13.5	-3.49	7.53
H- MonaLisa	13.4	5.23	8.30	5.58	15.5	-6.88	12.0
J- Snowflake	-0.46	2.84	4.98	4.10	1.07	4.58	-3.72
K- ArcticQueen	8.92	7.78	8.37	-1.58	8.62	-9.64	13.3
L- Bacardi	18.0	13.3	3.52	11.6	21.8	-5.48	12.6
M- FeelingGreen	10.9	0.34	3.10	11.3	16.1	0.35	5.14

3.15

-1.67

0.99

1.97

3.57

9.45

-5.65

-3.40

-5.02

10.1

16.5

-0.82

0.88

2.78

12.8

-1.88

-1.12

-0.41

-5.51

0.54

7.25

-1.27

-1.16

4.92

4.56

N- Ibis

O- Calabria

R- Paintball

P- Tobago

T- Vyking

11.5

-4.58

-2.99

0.52

9.52

0.43

0.76

1.67

-0.33

5.48

Appendix 6. The variance of the fresh weight (FW), number of leaves, stem length, and leaf area (LA) 2 weeks after planting.

Cultivar/ treatment	FW	No. of leaves	Stem length	LA
A- ReaganEliteWhite		110. 01 leaves	Stem length	2.1
High	1.79	0.45	2.44	493.0
Low	2.10	0.31	1.52	558.0
B- Anastasia	2.10	0.51	1.32	330.0
High	3.58	0.77	1.07	1226
low	4.04	0.81	1.23	1672
C- Zembla	7.07	0.01	1.23	1072
high	2.99	0.82	3.00	1171
low	1.14	0.49	1.18	538.0
D- Biarritz	1.11	0.17	1.10	230.0
high	2.99	1.20	3.21	1046
low	2.82	0.85	2.42	980.0
E- Noa	2.02	0.05	2.12	700.0
high	1.87	0.81	2.20	748.0
low	0.78	0.62	2.34	416.0
F- Euro	2.70	5.0 2		0.0
high	6.23	7.78	15.7	517.0
low	2.41	0.99	2.30	929.0
G- Timman	=			
high	3.42	1.78	5.63	898.0
low	3.11	1.10	1.94	843.0
H- MonaLisa				
high	2.32	0.78	1.98	1021
Low	2.84	1.92	5.95	1311
J- Snowflake				
high	2.96	2.27	5.03	888.0
low	2.52	1.62	3.94	569.0
K- ArcticQueen				
High	2.95	0.95	4.66	746.0
Low	3.72	0.63	3.85	1436
L- Bacardi				
High	1.28	1.41	1.28	349.0
low	3.23	1.24	2.52	1156
M- FeelingGreen				
High	4.59	1.06	4.68	1545
Low	5.26	1.05	3.03	2094
N- Ibis				
High	6.68	0.86	11.21	1257
low	3.02	0.92	7.01	1380
O- Calabria				
high	4.25	0.88	4.60	1207
low	1.91	1.20	3.76	777.0
P- Tobago		_		
high	2.15	0.74	2.69	864.0
low	1.91	1.05	2.94	790.0
R- Paintball	=			
high	1.67	0.86	1.64	899.0
low	1.75	0.28	3.53	1650
T- Vyking		0.10	2.51	0.5= 0
high	2.39	0.48	3.24	967.0
low	1.49	1.34	2.19	688.0

Appendix 7. The fresh weight (FW), number of leaves (No. of leaves), stem length, leaf area (LA), dry weight (DW), and water content (WC) 6 weeks after planting. Values within the column and within the

cultivar followed by different letters are significantly different at P=0.05 level (tested per cultivar).

Cultivar/ treatment	FW	No. of leaves	leaves Stem length		DW	WC
	(g)		(cm)	(cm ²)	(g)	(%)
A- ReaganEliteWhite		•	` '			` ′
High	46.4a	28.6	65.8	731	5.40a	89.2a
Low	38.3b	28.5	62.1	639	4.08b	90.4b
B- Anastasia						
High	47.7	29.2	72.3	789	5.20	89.7
Low	43.3	30.8	69.3	808	4.31	90.7
C- Zembla						
High	50.9	31.1	70.2	833	5.38	90.1
Low	43.2	30.5	64.8	837	4.51	90.4
D- Biarritz						
High	50.3	34.5	75.9	728	6.12a	88.4
Low	40.5	35.4	73.0	754	4.58b	89.9
E- Noa						
High	51.5a	34.1	80.2	798a	5.41a	90.1
Low	38.2b	34.2	76.2	650b	4.12b	90.1
F- Euro						
High	50.3	30.0	68.8	757	5.00	90.7
Low	46.9	30.3	64.4	750	4.48	91.2
G- Timman						
High	56.1	28.7	76.5	807	5.50	90.7
Low	47.3	28.2	72.5	764	4.59	91.0
H- MonaLisa						
High	52.5	33.7	79.5a	921	5.95	89.3
Low	40.8	33.2	65.8b	836	4.04	90.9
J- Snowflake						
High	47.4	38.5	70.4	737	5.44	89.2
Low	37.6	38.0	64.4	668	4.30	89.6
K- ArcticQueen						
High	52.8	34.2	78.8	764	5.71	89.9
Low	45.7	34.6	72.8	776	4.77	90.4
L- Bacardi						
High	57.2	36.5	78.3	963	5.19	91.4
Low	48.2	37.7	70.9	820	4.64	91.1
M- FeelingGreen						
High	58.5	34.3	74.9a	879	5.71	90.8
Low	47.5	34.1	71.2b	809	4.46	91.3
N- Ibis						
High	58.6	27.3	74.2	997	6.48	89.6
Low	47.2	27.2	71.8	872	5.02	90.2
O- Calabria						
High	52.9	33.4	73.7	825	5.78	89.7
Low	45.0	33.7	68.6	802	4.33	91.1
P- Tobago						
High	41.56	39.7	66.2	714	4.93	89.2
Low	40.31	39.6	64.0	740	4.30	90.3
R- Paintbal						
High	44.74a	35.3	75.2	751	5.37a	88.8a
Low	36.81b	37.4	70. 7	690	4.18b	89.7b
T- Vyking						
High	53.1a	38.5	65.1a	954	4.81	91.4
Low	42.9b	37.2	58.1b	891	3.83	91.7

Appendix 8. The per cent (%) relative difference in fresh weight (FW), number of leaves (No. of leaves), stem length, leaf area (LA), dry weight (DW), leaf area ratio (LAR), and net assimilation rate (NAR) of plants grown in the 1500ppm compartment, compared to plants grown in the 500ppm CO_2 compartment 6

weeks after planting.

Cultivar/ treatment	FW	No. of	Stem	LA	DW	LAR	NAR
	(%)	leaves	length	(%)	(%)	(%)	(%)
	, ,	(%)	(%)	, ,	, ,		. ,
A-							
ReaganEliteWhite	21.1	0.35	5.96	14.4	32.3	-5.57	15.9
B- Anastasia	10.2	-5.19	4.33	-2.35	20.6	-11.3	17.5
C- Zembla	17.8	1.97	8.33	-0.48	19.3	-15.5	21.7
D- Biarritz	24.2	-2.54	3.97	-3.45	33.6	-22.2	44.1
E- Noa	34.8	-0.29	5.25	22.8	31.3	-8.94	15.7
F- Euro	7.25	-0.99	6.83	0.93	11.6	-5.89	7.35
G- Timman	18.6	1.77	5.52	5.63	19.8	-10.9	15.3
H- MonaLisa	28.7	1.51	20.8	10.2	47. 3	-14.4	23.7
J- Snowflake	26.1	1.31	9.32	10.3	26.5	-12.5	25.7
K- ArcticQueen	15.5	-1.16	8.24	-1.54	19.7	-14.8	19.9
L- Bacardi	18.7	-3.18	10.4	17.4	11.8	-1.04	2.59
M- FeelingGreen	23.1	0.59	5.20	8.65	28.0	-11.8	17.7
N- Ibis	24.1	0.37	3.34	14.3	29.1	-7.91	14.4
O- Calabria	17.5	-0.89	7.43	2.87	33.5	-12.5	27.4
P- Tobago	3.10	0.25	3.44	-3.51	14.6	-6.41	9.84
R- Paintball	21.5	-5.61	6.36	8.84	28.5	-10.4	24.7
T- Vyking	23.8	3.49	12.0	7.07	25.6	-13.5	19.5

Appendix 9. The variance of the fresh weight (FW), number of leaves, stem length, and leaf area (LA) 6 weeks after planting.

Cultivar/ treatment	FW	No. of leaves	Stem length	LA
A- ReaganEliteWhite				
High	25.9	1.00	2.00	8379.0
Low	30.5	0.89	3.39	9523.0
B- Anastasia				
High	47.7	29.2	72.3	789.00
low	72.8	3.53	26.1	21533
C- Zembla				
high	70.9	5.08	14.1	9384.0
low	19.3	1.97	6.90	6273.0
D- Biarritz				
high	18.8	4.80	1.00	5954.0
low	47.0	2.48	6.19	9234.0
E- Noa	17.0	2.10	0.17	7231.0
high	9.83	2.62	0.90	975.00
low	23.4	2.62	7.08	6706.0
F- Euro	23.4	2.02	7.00	0700.0
high	295	9.77	19.2	58114
low	62.7	1.63	10.9	18241
G- Timman	02.7	1.03	10.7	10241
high	165	13.2	20.2	21491
low	87.5	3.23	19. 6	13600
H- MonaLisa	67.5	3.23	17.0	13000
high	117	3.82	18.5	21059
Low	163	3.02	30. 0	50443
J- Snowflake	103	5.02	30.0	30443
high	40.2	4.97	4.09	5360.0
low	63.6	4.47	9.90	14926
K- ArcticQueen	05.0	7.7	7.70	14920
High	135	4.43	12.2	21074
Low	35.4	1.82	7.57	8390.0
L- Bacardi	33.4	1.62	1.51	6370.0
High	119	2.70	11.7	12378
low	85.5	6.22	17.9	15090
	65.5	0.22	17.9	13090
M- FeelingGreen	330	3.53	28.6	48469
High Low				
N- Ibis	42.2	1.62	7.98	7640.0
	127	6.12	12.1	13915
High low	127 101			
	101	3.12	17.8	26435
O- Calabria	115	£ 20	160	10011
high	115	5.28	16.8	18311
low	128	4.42	21.9	25675
P- Tobago	111	- co	20.0	101.12
high	111	5.62	28.9	18142
low	33.8	1.68	8.82	7379.0
R- Paintball		25.5		
high	44.7	35.3	75.2	751.00
low	53.4	1.08	17.1	11880
T- Vyking				
high	43.6	6.12	3.30	8973.0
low	50.9	9.97	11.3	19093

Appendix 10. The fresh weight (FW), stem length, number of flowers and flower buds, fresh weight at 80 cm fixed length, number of plants that exceeds the 80cm, dry weight (DW), and water content (WC) at final harvest (8 to 10 weeks after planting, depending the cultivar). Values within the column and within

the cultivar followed by different letters are significantly different at P=0.05 level (tested per cultivar).

Cultivar/ treatment	FW		N flowers and	ent at P=0.05 level (t FW 80cm length	N plants	DW	WC
Cultivar/ treatment	(g)	Shoot length (cm)	buds	(g)	>80cm	(g)	(%)
A- ReaganEliteWhite	(8)	(CIII)	buus	(g)	>00CIII	(8)	(70)
High	63.9	77.9	18.22a	66.8	2.7	8.78a	86.7a
Low	60.2	77.7	15.1b	69.4	2.5	7.65b	87.7b
B- Anastasia			10.120				0
High	78.0	95.3	12.7	71.9	12.0	12.19	84.8
Low	76.8	92.8	12.9	70.9	12.0	10.84	86.2
C- Zembla							
High	81.8	87.2a	10.4	77.5	12.0	10.44	87.6a
Low	70.4	82.3b	9.5	69.7	11.0	8.23	88.7b
D- Biarritz							
High	71.3	88.0	38.0	66.6	12.0	10.47	85.7a
Low	66.2	87.8	30.7	61.8	12.0	9.20	86.6b
E- Noa							
High	66.5	88.5	12.9	63.5	12.0	9.00a	86.9a
Low	54.3	85.4	12.9	52.2	12.0	7.13b	87.3b
F- Euro							
High	78.9	85.1	13.2a	75.4	11.5a	9.67	88.2
Low	67.0	81.4	12.0b	70.9	8.0b	7.55	89.2
G- Timman							
High	99.2a	96.7	17.5	92.7a	12.0	12.56a	87.6
Low	86.2b	93.0	12.0	79.0b	12.0	10.19b	88.5
H- MonaLisa							
High	83.2	93.6	17.6	78.6	12.0	11.41	86.6
Low	64.8	83.6	14.5	65.9	9.0	8.18	87.9
J- Snowflake							
High	75.7	84.0a	18.1	74.5	11.0a	10.17	86.8a
Low	63.8	76.9 b	12.2	74.9	1.0b	8.20	87.5b
K- ArcticQueen							
High	89.1	92.0	12.0	84.2	12.0	11.79a	87.0
Low	71.9	86.0	9.6	69.2	12.0	8.90b	87.9
L- Bacardi							
High	96.7	89.5	26.9	91.7a	12.0	11.88	87.9
Low	78.2	81.0	18.6	80.6b	6.5	9.21	88.6
M- FeelingGreen							
High	90.3	86.6a	19.1	84.9	12.0	11.33	87.7
Low	76.4	82.5b	14.5	81.2	9.0	8.34	89.4
N- Ibis	00.0	067	15.0	07.0	11.5	11.70	07.0
High	89.8	86.7	15.0	87.2	11.5a	11.79	87.2
Low	80.8	82.7	14.5	84.5	9b	9.97	88.0
O- Calabria	05.5	0.6.1	17.5	07.1	10.5	10.60	07.0
High	85.5	86.1	17.5	87.1	10.5	10.60	87.8
Low	83.8	87.6	15.5	80.4	11.5	10.06	88.3
P- Tobago	66.2	75.1	12 5-	81.4	0.5	0.70	97.0
High	66.2		42.5a		0.5	8.79	87.0
Low P. Pointhall	60.2	72.8	36.8b	-	0.0	7.53	87.7
R- Paintball	67 C-	00 1	10 4-	65 A-	12.0	10.22-	05.2
High	67.6a	88.1	18.4a	65.0a	12.0	10.23a	85.2
Low T. Wyking	56.5b	84.8	14.2b	54.5b	11.5	8.28b	85.9
T- Vyking	80.0	77.40	12.2	89.4	1.5	10.20	Q7 1
High		77.4a	13.3		1.5	10.30	87.4
Low	67.9	73.4b	10.7	=	0.0	8.27	88.3

Appendix 11. The per cent (%) relative difference in fresh weight (FW), stem length, number of flowers and flower buds, fresh weight at 80 cm fixed length, number of plants that exceeds the 80cm, dry weight (DW), and water content (WC) of plants grown in the 1500ppm compartment, compared to plants grown in

the 500ppm CO₂ compartment at final harvest (8 to 10 weeks after planting, depending the cultivar).

Cultivar/ treatment	FW	Stem	N flowers	FW 80cm	N plants	DW	WC
	(%)	length	and buds	length	>80cm	(%)	(%)
		(%)	(%)	(%)	(%)		
A- ReaganEliteWhite	6.15	0.25	20.7	-	8.000	14.8	-1.15
B- Anastasia	1.56	2.69	-1.55	1.41	0.000	12.4	-1.70
C- Zembla	16.2	5.95	9.47	11.2	9.090	26.8	-1.25
D- Biarritz	7.70	0.23	23.8	7.77	0.000	13.8	-1.11
E- Noa	22.5	3.63	0.00	21.6	0.000	26.2	-0.48
F- Euro	17.8	4.54	10.0	6.35	43.70	28.1	-1.07
G- Timman	15.1	3.98	45.8	17.3	0.000	23.2	-1.03
H- MonaLisa	28.4	12.0	21.4	19.3	33.30	39.5	-1.44
J- Snowflake	18.6	9.23	48.4	-	1000	24.0	-0.74
K- ArcticQueen	23.9	6.98	25.0	21.7	0.000	32.5	-1.03
L- Bacardi	23.6	10.5	44.6	-	84.60	29.0	-0.76
M- FeelingGreen	18.2	4.97	31.7	4.56	33.30	35.8	-1.92
N- Ibis	11.1	4.84	3.45	3.19	27.70	18.2	-0.93
O- Calabria	2.03	-1.71	12.9	8.33	-8.690	5.37	-0.50
P- Tobago	9.97	3.16	15.5	-	-	16.7	-0.77
R- Paintball	19.6	3.89	29.6	19.3	4.350	23.5	-0.80
T- Vyking	17.8	5.45	24.3	-	-	24.5	-0.93

Appendix 12. The variance of the fresh weight (FW), stem length, fresh weight at 80 cm fixed length, and number of flowers and flower buds at final harvest.

Cultivar/ treatment	FW	Stem length	FW 80cm length	N flowers and buds
	F VV	Stem length	r w ouch length	14 Howers and buds
A- ReaganEliteWhite	172	5.20	256	24.4
High	173	5.20	256	34.4
Low B- Anastasia	121	4.76	79.9	12.8
	270	10.7	220	21.1
High	370	10. 7	339	21.1
Low	243	13. 7	208	23.3
C- Zembla	422	C 15	204	7.01
High	423	6.45	394	7.81
Low	153	3.14	142	3.54
D- Biarritz	176	2.26	170	76.5
High	176	3.26	178	
Low	185	6.63	170	77.4
E- Noa	00.7	0.21	101	1.00
High	99.7	8.31	101	1.80
Low	56.7	6.38	49.6	2.93
F- Euro	207	2.07	274	146
High	397 225	3.07	374	14.6
Low	335	15.8	218	12.5
G- Timman	595	27.3	551	28.8
High				
Low	381	8.80	328	12.5
H- MonaLisa	470	(5 (445	50.0
High	479	6.56	445	50.0
Low	466	10.3	341	61.6
J- Snowflake	102	2.66	1.4.4	44.2
High	183	3.66	144	44.3
Low	227	7.75	101	13.0
K- ArcticQueen	343	0.57	220	7.21
High	343 164	8.57 5.59	320 159	7.31 2.75
Low L- Bacardi	104	5.59	159	2.75
	297	6.64	257	41.9
High low	223	6.17	237 171	16.4
	223	0.17	1/1	10.4
M- FeelingGreen High	277	1.87	251	63.8
Low	498	14.5	231 247	46.1
N- Ibis	470	14.3	∠ + /	40.1
High	242	6.84	212	15. 7
low	496	23.2	308	19.1
O- Calabria	770	23.2	500	17.1
high	505	9.14	360	28.4
low	303	8.93	310	10.7
P- Tobago	331	0.93	510	10.7
high	169	5.57	107	131
low	172	6.47	-	145
R- Paintball	1/2	0.47	-	143
high	151	5.54	147	23.6
low	101	3.84	96.1	10.9
T- Vyking	101	J.0 1	70.1	10.9
high	225	5.62	3.46	14.1
low	338	8.18	J.40	17.4
10W	330	0.10	-	1 / .4

Appendix 13a. The reaction time in the various cultivars in the two ${\rm CO_2}$ compartments.

cultivar	days to flower fr	om SD treatment
	500ppm	1500ppm
Tobago, Noa	47.0	47.0
Ibis	51.0	51.0
Reagan Elite White, Snowflake, Euro	51.0	51.5
FeelingGreen	51.0	52.0
MonaLisa	51.5	52.0
Bacardi	52.5	52.0
ArcticQueen	53.5	52.5
Paintball	54.0	52.0
Calabria	54.0	52.5
Timman	55.0	54.0
Biarritz	55.0	55.0
Zembla	55.0	56.0
Anastasia	59.0	57.0
Vyking	59.0	59.0

Appendix 13b. The rate of flower development in the various cultivars in the two CO_2 compartments.

cultivar	rate of flower o	levelopment (d ⁻¹)
	500ppm	1500ppm
Tobago, Noa	0.0213	0.0213
Ibis	0.0196	0.0196
Reagan Elite White, Snowflake, Euro	0.0196	0.0194
FeelingGreen	0.0196	0.0192
MonaLisa	0.0194	0.0192
Bacardi	0.0190	0.0192
ArcticQueen	0.0187	0.0190
Paintball	0.0185	0.0192
Calabria	0.0185	0.0190
Timman	0.0182	0.0185
Biarritz	0.0182	0.0182
Zembla	0.0182	0.0178
Anastasia	0.0169	0.0175
Vyking	0.0169	0.0169

Appendix 14a. The Relative Growth Rate (RGR) in the periods 0 to 2 weeks (RGR₀₋₂), 2 to 6 weeks (RGR₂₋₆), 6 weeks to final harvest (8 to 10 weeks after planting, depending the cultivar; RGR_{6-f}), and from

planting to final harvest (RGR_{0-f}) for the various cultivars in the two CO₂ compartments.

	RG	R_{0-2}	RG	R ₂₋₆	RG	R_{6-f}	RG	R_{0-f}
cultivar	(g g ⁻¹	day ⁻¹)						
	low CO ₂	high CO ₂						
ReaganEliteWhite	0.1048	0.1096	0.0452	0.0528	0.0180	0.0139	0.0534	0.0556
Anastasia	0.1085	0.1124	0.0501	0.0548	0.0220	0.0203	0.0549	0.0566
Zembla	0.1115	0.1143	0.0534	0.0583	0.0154	0.0170	0.0546	0.0581
Biarritz	0.1137	0.1110	0.0513	0.0629	0.0179	0.0138	0.0556	0.0575
Noa	0.1112	0.1230	0.0547	0.0585	0.0177	0.0164	0.0616	0.0656
Euro	0.1210	0.1270	0.0528	0.0537	0.0149	0.0188	0.0587	0.0626
Timman	0.1047	0.1138	0.0530	0.0550	0.0210	0.0217	0.0568	0.0600
MonaLisa	0.1099	0.1202	0.0488	0.0575	0.0196	0.0181	0.0564	0.0616
Snowflake	0.1233	0.1241	0.0547	0.0627	0.0184	0.0179	0.0619	0.0654
ArcticQueen	0.1019	0.1078	0.0505	0.0540	0.0169	0.0196	0.0533	0.0576
Bacardi	0.1127	0.1268	0.0514	0.0484	0.0190	0.0230	0.0579	0.0618
FeelingGreen	0.1054	0.1161	0.0457	0.0492	0.0179	0.0196	0.0537	0.0585
Ibis	0.1078	0.1187	0.0459	0.0495	0.0196	0.0171	0.0552	0.0579
Calabria	0.1117	0.1111	0.0452	0.0558	0.0228	0.0164	0.0565	0.0573
Tobago	0.1229	0.1235	0.0474	0.0520	0.0181	0.0186	0.0612	0.0638
Paintball	0.1135	0.1154	0.0483	0.0563	0.0185	0.0174	0.0558	0.0590
Vyking	0.1064	0.1150	0.0502	0.0540	0.0179	0.0177	0.0516	0.0547

Appendix 14b. The Crop Growth Rate (CGR) in the period 6 weeks to final harvest (8 to 10 weeks after planting, depending the cultivar; CGR_{6-f}) for the various cultivars in the two CO₂ compartments.

cultivar	CGR _{6-f} (g m ² day ⁻¹)			
Cuitivui	low CO ₂	high CO ₂		
ReaganEliteWhite	10.88	10.30		
Anastasia	14.92	15.98		
Zembla	9.52	12.95		
Biarritz	11.83	11.13		
Noa	11.33	13.51		
Euro	9.36	14.23		
Timman	14.93	18.83		
MonaLisa	12.04	15.88		
Snowflake	11.88	14.41		
ArcticQueen	11.49	16.92		
Bacardi	13.29	19.46		
FeelingGreen	11.82	17.13		
Ibis	15.08	16.18		
Calabria	15.94	13.41		
Tobago	12.16	14.53		
Paintball	11.40	13.52		
Vyking	9.80	12.11		

Appendix 15a. The number of stomata in 4 randomly chosen sites per leaf (2 impressions, 2 pictures/impression; area of 0.9483 mm²/ site; magnification 100x), in the adaxial surface of the leaf (leaf order 9) in the cultivar Reagan Elite White, taken from the 500ppm compartment.

Site	number of stomata Low CO ₂					
	Leaf 1	Leaf 2	Leaf 3			
11	7.0	4.0	4.0			
12	8.0	5.0	3.0			
21	6.0	12	3.0			
22	5.0	10	8.0			
mean value/ site	6.5	7.7	4.5			

Appendix 15b. The number of stomata in 4 randomly chosen sites per leaf (2 impressions, 2 pictures/impression; area of 0.9483 mm²/site; magnification 100x), in the abaxial surface of the leaf (leaf order 9) in the cultivar Reagan Elite White, from the 500 (LL1, LL2, and LL3) and 1500ppm (HL1, HL2, and HL3) treatment.

Site	number of stomata						
	Low CO ₂			High CO ₂			
	Leaf 1	Leaf 2	Leaf 3	Leaf 1	Leaf 2	Leaf 3	
11	41.0	51	37.0	38.0	39.0	26.0	
12	48.0	39	39.0	36.0	38.0	31.0	
21	39.0	42	41.0	40.0	38.0	28.0	
22	41.0	40	42.0	43.0	26.0	26.0	
mean value/ site	42.2	43	39.7	39.2	35.2	27.7	

Appendix 15c. The number of stomata in 4 randomly chosen sites per leaf (2 impressions, 2 pictures/impression; area of 0.9483 mm²/ site; magnification 100x), in the abaxial surface of the leaf (leaf order 9) in the cultivar Feeling Green, from the 500 (LL1, LL2, and LL3) and 1500ppm (HL1, HL2, and HL3) treatment.

site	number of stomata					
	Low CO ₂			High CO ₂		
	Leaf 1	Leaf 2	Leaf 3	Leaf 1	Leaf 2	Leaf 3
11	36.0	28	28	43.0	39.0	32.0
12	36.0	35	37	42.0	47.0	33.0
21	31.0	33	30	38.0	42.0	35.0
22	31.0	28	25	39.0	39.0	39.0
mean value/ site	33.5	31	30	40.5	41.7	34.7

Appendix 16. The amount of carbon dioxide (per hour/ ha, and per day/ ha) supplied, and the radiation (per day) in the two CO₂ compartments in the period week 2 to week 6 after planting.

day) in the two	CO ₂ compartment	500ppm	500ppm	1500ppm	1500ppm	
		amount CO ₂	amount CO ₂	amount CO ₂	amount CO ₂	radiation/day
photoperiod	day after planting	(kg/ day/ ha)	(kg/ hour/ ha)	(kg/day/ha)	(kg/ hour/ ha)	(J/cm ²)
LD	7	0.00	0.0	198.9	9.90	630.0
LD	week 2	0.00	0.0	1060	52.0	155.0
	8	0.00	0.0	1060	53.0	155.0
LD	9	0.00	0.0	839.8	42.0	360.0
LD	10	0.00	0.0	1193	59.7	153.0
LD	11	0.00	0.0	1105	55.3	333.0
LD	12	22.1	1.1	1282	64.1	435.0
SD	13	66.3	6.3	1812	172	423.0
SD	14	0.00	0.0	198.9	18.9	152.0
SD	week 3 15	22.1	2.1	221.0	21.0	924.0
SD	15 16	132	12	685.1	65.2	803.0
SD	16 17	44.2	4.2	486.2	46.3	651.0
SD	18	0.00	0.0	861.9	82.1	286.0
SD	19	0.00	0.0	265.2	25.3	337.0
SD	20	44.2	4.2	663.0	63.1	495.0
SD	20 21	0.00	0.0	331.5	31.6	254.0
SD	week 4	0.00	0.0	331.3	31.0	234.0
SD	22	66.3	6.3	596.7	56.8	711.0
SD	23	265	25	972.4	92.6	655.0
SD	24	66.3	6.3	1547	147	329.0
SD	25	817	78	1348	128	267.0
SD	26	596	57	1127	107	402.0
SD	27	22.1	2.1	419.9	40.0	192.0
SD	28	177	17	1967	187	773.0
	week 5					
SD	29	309	29	1613	153	970.0
SD	30	265	25	1149	109	823.0
SD	31	862	82	1503	143	509.0
SD	32	88.4	8.4	663.0	63.1	651.0
SD	33	155	15	1259	120	970.0
SD	34	44.2	4.2	994.5	94.7	250.0
SD	35	66.3	6.3	618.8	58.9	471.0
an-	week 6					
SD	36	177	17	817.7	77.9	1219
SD	37	66.3	6.3	663.0	63.1	430.0
SD	38	155	15	1238	117	1352
SD	39	133	13	1017	96.8	1118
SD	40	199	19	1238	118	1469
SD	41	265	25	1392	133	1271
SD	42	155	15	928.2	88.4	1075

Appendix 17. The amount of carbon dioxide (per hour/ ha, and per day/ ha) supplied, and the radiation (per day) in the two CO₂ compartments in the period week 7 to week 10 after planting.

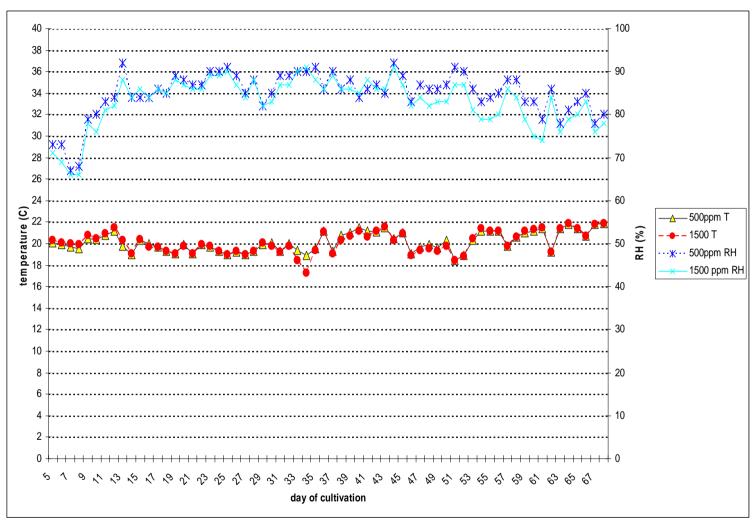
		500ppm	500ppm	1500ppm	1500ppm	
		amount CO ₂	amount CO ₂	amount CO ₂	amount CO ₂	radiation/day
photoperiod	day after planting	(kg/ day/ ha)	(kg/ hour/ ha)	(kg/day/ha)	(kg/ hour/ ha)	(J/cm ²)
	week 7					
SD	43	199	18.9	1127	107	1453
SD	44	110	10.5	663.0	63.1	1095
SD	45	309	29.5	1613	153	1304
SD	46	287	27.4	2386	227	1066
SD	47	707	67.4	1878	179	852.0
SD	48	177	16.8	1635	156	1166
SD	49	177	16.8	1459	139	1244
	week 8					
SD	50	243	23.2	1525	145	1472
SD	51	110	10.5	928.2	88.4	382.0
SD	52	133	12.6	1060	101	534.0
SD	53	376	35.8	1834	175	1341
SD	54	376	35.8	2011	191	1638
SD	55	354	33.7	2011	191	1607
SD	56	354	33.7	2121	202	1542
	week 9					
SD	57	199	18.9	1547	147	850.0
SD	58	287	27.4	1746	166	1139
SD	59	376	35.8	2122	202	1523
SD	60	376	35.8	2166	206	1725
SD	61	420	40.0	2232	213	1704
SD	62	155	14.7	1127	107	737.0
SD	63	309	29.5	1812	173	1880
	week 10					
SD	64	354	33.7	1901	181	1770
SD	65	265	25.3	2099	200	1621
SD	66	199	18.9	1193	114	1186
SD	67	597	56.8	2210	210	1931
SD	68	928	88.4	2387	227	1920

Appendix 18. The mean temperature, Relative Humidity (RH) and CO_2 concentration ([CO_2]) during light period in the two CO_2 compartments in the period week 2 to week 6 after planting.

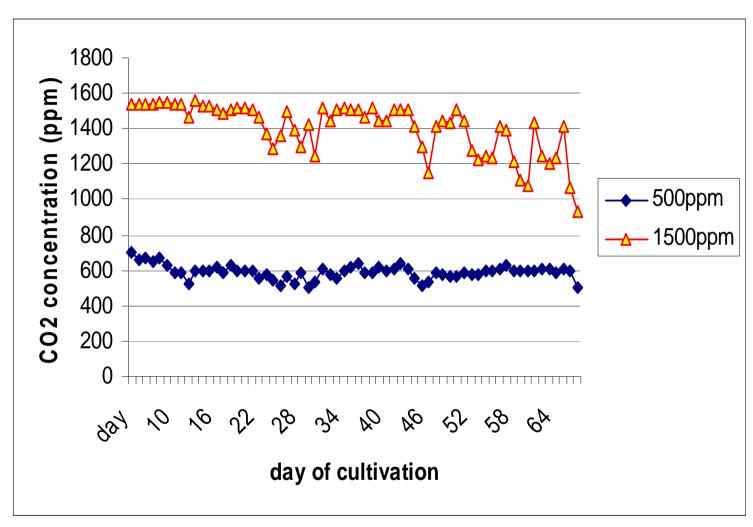
period	d in the two CO ₂ compartments in the period week 2 to week 6 after planting. 500ppm 1500ppm								
			Suupp	m	1500ppm				
		temperature	RH	mean [CO ₂]/ day	temperature	RH	mean [CO ₂]/ day		
photoperiod	day after planting	(°C)	(%)	(ppm)	(°C)	(%)	(ppm)		
LD	5	20.1	73	706	20.3	71	1538		
LD	6	19.9	73	664	20.1	69	1537		
LD	7	19.7	67	672	20.0	66	1538		
	week 2								
LD	8	19.5	68	653	19.9	66	1535		
LD	9	20.5	79	669	20.8	78	1545		
LD	10	20.4	80	633	20.5	76	1545		
LD	11	20.8	83	581	20.9	81	1541		
LD	12	21.2	84	583	21.5	82	1538		
SD	13	19.8	92	524	20.3	88	1463		
SD	14	19.0	84	600	19.1	84	1558		
CD	week 3	20.2	0.4	505	20.4	0.5	1520		
SD	15	20.3	84	595	20.4	86	1529		
SD	16	20.0	84	601	19.7	84	1527		
SD	17	19.7	86	617	19.7	86	1512		
SD	18	19.3	85	586	19.3	85	1485		
SD	19	19.1	89	632	19.1	88	1507		
SD	20	19.9	88	600	19.8	87	1520		
SD	21 week 4	19.1	87	595	19.1	86	1521		
SD	week 4 22	19.9	87	597	19.9	86	1509		
SD	23	19.7	90	560	19.8	89	1465		
SD	24	19.3	90	575	19.3	89	1370		
SD	25	19.0	91	542	19.0	90	1288		
SD	26 26	19.2	89	517	19.3	87	1361		
SD	27	19.0	85	561	19.0	84	1496		
SD	28	19.3	88	528	19.3	88	1387		
	week 5	17.3	00	320	19.3	00	1307		
SD	29	19.9	82	586	20.1	82	1293		
SD	30	20.1	85	505	19.8	83	1422		
SD	31	19.3	89	533	19.2	87	1248		
SD	32	20.0	89	612	19.8	87	1519		
SD	33	19.4	90	572	18.4	90	1442		
SD	34	18.9	90	560	17.3	91	1503		
SD	35	19.5	91	599	19.4	88	1519		
	week 6								
SD	36	21.2	86	622	21.1	86	1510		
SD	37	19.3	90	635	19.1	89	1509		
SD	38	20.8	86	582	20.3	86	1460		
SD	39	21.0	88	587	20.7	86	1513		
SD	40	21.5	84	622	21.2	85	1441		
SD	41	21.2	86	594	20.6	88	1446		
SD	42	21.1	87	609	21.2	86	1511		

Appendix 19. The mean temperature, Relative Humidity (RH) and CO₂ concentration ([CO₂]) during light period in the two CO₂ compartments week 7 to week 10 after planting.

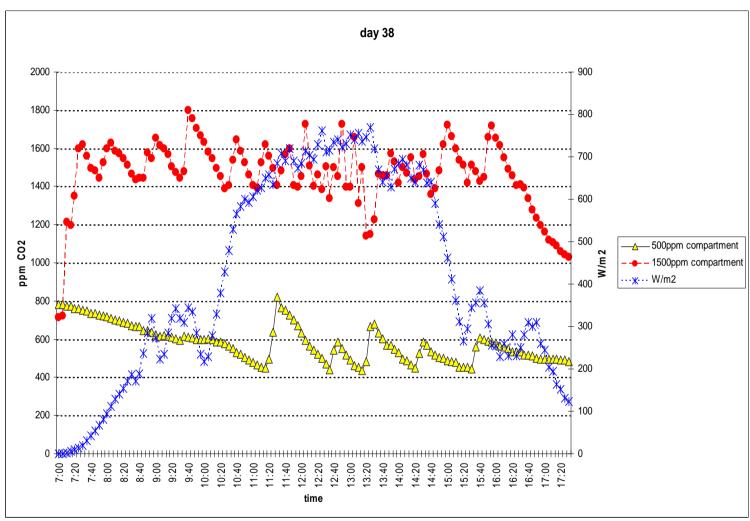
period in the two CO ₂ compartments week 7 to week 10 after planting.										
		500ppm			1500ppm					
photoperiod	day after planting	temperature (°C)	RH (%)	mean [CO ₂]/ day (ppm)	temperature (°C)	RH (%)	mean [CO ₂]/ day (ppm)			
TT	week 7	(0)	(70)	(PP)	(0)	(/0)	(PP)			
SD	43	21.5	85	638	21.6	86	1510			
SD	44	20.5	92	605	20.3	91	1502			
SD	45	21.0	89	553	20.9	87	1408			
SD	46	19.1	83	512	18.9	82	1294			
SD	47	19.7	87	531	19.4	84	1146			
SD	48	19.9	86	581	19.5	82	1410			
SD	49	19.6	86	580	19.3	83	1447			
	week 8									
SD	50	20.3	87	566	19.8	83	1437			
SD	51	18.4	91	566	18.4	87	1511			
SD	52	18.9	90	583	18.8	87	1443			
SD	53	20.3	86	571	20.5	81	1273			
SD	54	21.2	83	578	21.4	79	1220			
SD	55	21.2	84	596	21.2	79	1241			
SD	56	21.2	85	593	21.2	80	1237			
an a	week 9	10.0	0.0	510	10.0	0.5	1.100			
SD	57	19.8	88	610	19.8	86	1408			
SD	58	20.6	88	627	20.6	84	1388			
SD	59	21.0	83	598	21.2	79	1214			
SD	60	21.2	83	592	21.3	75	1110			
SD	61	21.4	79	598	21.5	74	1080			
SD	62	19.2	86	593	19.2	84	1435			
SD	63	21.4	78	607	21.4	76	1245			
SD	week 10 64	21.8	81	608	21.9	79	1204			
SD	65	21.4	83	589	21.4	80	1233			
SD	66	20.7	85	605	20.7	83	1413			
SD	67	21.8	78	594	21.8	76	1071			
SD	68	21.8	80	506	21.8	78	929.0			
שט	08	41.7	80	300	41.7	70	747.U			



Appendix 20. The mean temperature and Relative Humidity (RH) in the two CO₂ compartments over the day of cultivation.



Appendix 21. The mean CO₂ concentration in the two CO₂ compartments during light period over the day of cultivation.



Appendix 22. The CO₂ concentration in the two CO₂ compartments, and the outside radiation during the day 38 (10th of March).