

# **Crop Growth and Development in Closed and Semi-closed Greenhouses**

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# **Crop Growth and Development in Closed and Semi-closed Greenhouses**

**Tian Qian**

## **Thesis**

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# Chapter 1

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## General Introduction

## Global environmental protection and energy saving

In the last 50 years, climate change and resource scarcity raised people's concern about environmental protection and energy saving. The Club of Rome, the global policy institute, raised considerable public attention in 1972 with its report "The Limits to Growth". In 1987, the World Commission on Environment and Development, created by the United Nations, released the Brundtland report "Our Common Future" (Anonymous 1987), in which the term "sustainable development"<sup>1</sup> was defined. In 1997, the Kyoto Protocol of the United Nations Framework Convention on Climate Change, an international treaty, set binding obligations on developed countries to reduce emissions of greenhouse gases.

The European Union (EU) translated the Kyoto protocol into its own targets for 2020 (known as the EU Climate and Energy Package). In the Netherlands, the government announced the "Clean and efficient" program in 2007, aiming to improve energy efficiency in various sectors (Anonymous 2007). One of these was the agro-sector<sup>2</sup>, in which the sub-sector greenhouse horticulture contributes to about 10% of the national consumption of natural gas (CBS et al. 2014; Van der Velden and Smit 2014). Energy costs constitute about 15-30% of the total annual costs of a greenhouse (Vermeulen, 2012). In 2008, the Dutch agro-sectors signed a Public-Private Partnership with the government entitled 'Clean and Efficient Agro-sectors', agreeing to make the agro-sectors more sustainable by 2020 (Anonymous 2008). Within the context of the 'Clean and Efficient Agro-Sectors' program, the greenhouse horticultural sub-sector has signed the Greenhouse and Environment Covenant (known as GLAMI) (Anonymous 2010) with the government, which contains objectives on the performance of greenhouse horticulture with respect to energy and environment.

The objectives to be achieved are: 1) Total reduction of at least 3.3 megatons CO<sub>2</sub> emission in 2020 compared to that of 1990; 2) 2% improvement of energy efficiency yearly until 2020; 3) Contribution of sustainable energy<sup>3</sup> to the total energy input of 20% in 2020. Subsequently, in 2014 the Dutch government and greenhouse horticultural sector agreed upon a long-term agreement for energy transition in the greenhouse horticultural sector ("Meerjarenafspraak Energietransitie Glastuinbouw 2014-2020"). This agreement focuses on a target of maximum 6.2 megatons CO<sub>2</sub> emission in 2020. In 2015 the CO<sub>2</sub> emission was already reduced to 5.7 megatons. This means that the greenhouse horticulture sector has already reached the goal set for 2020 (Van der Velden and Smit 2016). In 2015, the sustainable energy was only 5.1% of the total energy consumption (Van der Velden and Smit 2016).

## Dutch greenhouse horticulture

The Netherlands has a world-leading position in greenhouse horticulture, with

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<sup>1</sup>Sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs. (Anonymous 1987)

<sup>2</sup> Agro sector is defined as the complex of businesses that are involved in agriculture (consisting of sub-sectors arable farming, livestock farming, horticulture), as well as those businesses that are involved in the chain of supply to agriculture and processing of agricultural products. (De Haas 2013)

<sup>3</sup>Sustainable energy is defined as energy that is generated through renewable processes from sun, wind, water power, earth warmth and biomass. (Anonymous 2010)



glass greenhouse area of about 10,000 hectare (Statline 2016). Mechanization and technological developments such as heating, artificial light, CO<sub>2</sub> enrichment and climate control led to high control of the production process in the greenhouses (Breukers et al. 2008). Crop productivity increased by 113% for tomato, 90% for sweet pepper, and 35% for cucumber over a period of 27 years from 1983 to 2010 (De Gelder et al. 2012). Application of technology, mainly heating and artificial light, enabled year-round production, but was also associated with high energy consumption. In 2012, 82% of the energy in the agro-sector was consumed by greenhouse horticulture (Anonymous 2012), although the energy consumption per unit of production (energy use efficiency) decreased by 70% between 1980 and 2008 (Van der Velden and Smit 2012). Over the last decades, the Dutch greenhouse horticultural sector made remarkable efforts to increase its energy use efficiency. Measures that were taken in greenhouse horticulture to reduce the energy consumption were summarized by Elings et al. (2005) and Dieleman et al. (2006). These measures included improving greenhouse insulation and improving light transmission by antireflection coatings (Hemming et al. 2012), applying temperature integration, improved energy screen opening strategies (Dieleman and Kempkes 2006) and increasing heat buffer capacity.

### **Various greenhouse concepts toward sustainability**

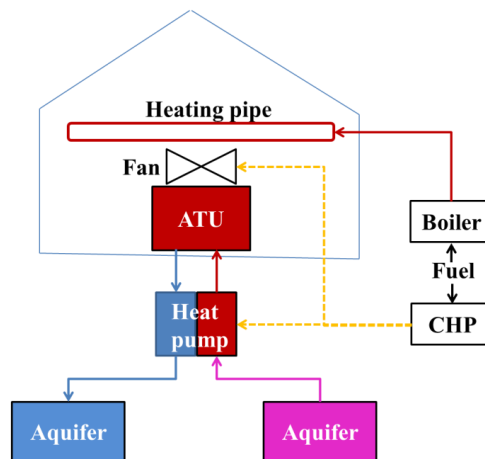
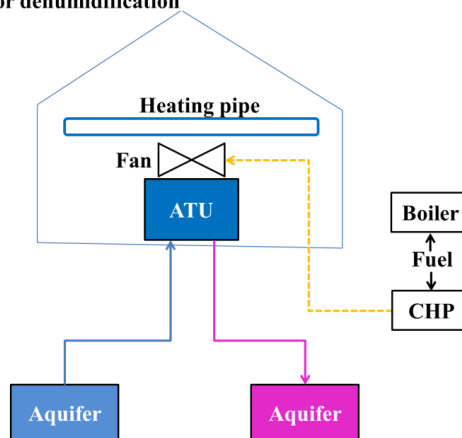
Various greenhouse concepts aiming at environmental protection and energy saving were developed in the last decades. Bot (1992) indicated that the trends in greenhouse development for the purpose of environmental protection and energy saving would reflect in the use of window ventilation and improving the technology of mechanic air treatment. Later Bot et al. (2005) described the solar greenhouse concept for high value crop production without the use of fossil fuels. Solar energy could be captured in summer, stored in an underground aquifer, and re-used in winter for heating. This concept was firstly tested in a 14,000 m<sup>2</sup> closed greenhouse for tomato production (Opdam et al. 2005; De Gelder et al. 2005). Another concept, the Watergy greenhouse (Buchholz et al. 2005), was developed for central and southern European conditions. The Watergy greenhouse concept consisted of a closed greenhouse with solar thermal energy storage, water recycling, and water desalination, using a cooling tower and a secondary heat collector. The system had constant air humidification, enabling the transfer of large amounts of energy via latent heat from the greenhouse to a thermal storage water tank. Bakker et al. (2006) developed the energy (heat) producing greenhouse concept, using advanced greenhouse covering materials (Sonneveld and Swinkels 2005) to maximize the transmission of solar radiation, and to minimize the heat loss from the greenhouse. For efficient air conditioning, a fine wire heat exchanger with a very high heat transfer coefficient was used. In 2008, the Sunergy greenhouse concept was developed and tested in a 550 m<sup>2</sup> greenhouse (De Zwart 2011). The Sunergy greenhouse was a semi-closed greenhouse that was closed only during periods with high solar radiation in order to enable harvesting of solar energy when temperatures were relatively high. During dull days and nights, outside air was taken in for dehumidification purposes. This way of dehumidification lowered the investment costs of the air treatment unit and moreover lowered the electrical demand in comparison to a completely closed greenhouse with mechanical dehumidification. Tantau et al. (2011) described the concept of Low energy greenhouse (named Zineg), aiming for energy savings of up to 90%. The Zineg concept combined many different methods that had been developed in the past. For instance, making use of solar radiation as a sustainable energy source, as in all

concepts mentioned above, thereby reducing the energy consumption. Window opening was also reduced which results in less CO<sub>2</sub> and heat loss.

### **The closed and semi-closed greenhouse concept**

In a conventional greenhouse, excess heat and moisture are released from the greenhouse by window ventilation. In this thesis, these conventional greenhouses are referred to as open greenhouses, because window opening is the only means of cooling and dehumidification. In a closed greenhouse (Fig. 1.1), cooling and dehumidification are done mechanically by an air treatment unit (ATU). The ATU is connected to an underground aquifer, which is located about 20-100 meter below the soil surface (De Gelder et al. 2012), depending on local soil conditions. The ATU contains a heat exchanger and connects to a ventilator. The ventilator sucks air from inside the greenhouse and distributes the cooled and dehumidified air back to the greenhouse. Most commonly, the ventilator distributes the air via ducts. The ducts are usually placed below the growing gutters, since placement overhead causes shading and placement within the crop interferes with the cultivation practices. The energy flow of the closed greenhouse system is presented in Fig 1.1. For cooling in summer, the cold water with a temperature of approximately 7 °C is pumped from the aquifer. The surplus heat from the greenhouse is absorbed by the cold water when passing the heat exchanger of the ATU. The collected heat in water is transported to the aquifer and stored (De Gelder et al. 2012). For heating in winter, water with a temperature of approximately 18 °C is pumped from the aquifer to a heat pump. The heat pump further increases the water temperature to 35-50 °C (Bot et al. 2005; De Zwart 2012) for greenhouse heating. In both summer and winter, if dehumidification is needed, humid air from the greenhouse passes the cold surface of the heat exchanger of the ATU, resulting in removal of air moisture by condensation. Therewith, extra (latent) heat can be harvested. Primary energy, mainly electricity, is required for the operation of the heat and other pumps, and the ventilators connected to the ATU. Electricity can be obtained from the grid, or produced by the co-generation system of a heat and power system. Co-generation of heat and power requires primary energy/fuel input, and by burning the fuel, electricity is produced for the heat pump, the duct ventilation system, and other pumps, and also heat and CO<sub>2</sub> for the greenhouse.

A fully closed greenhouse requires high investment cost for drilling the pipes toward the aquifers, for the ATUs and heat pumps. To have a high cooling capacity, the heat exchanger has to have a large exchanging surface and a high heat exchange coefficient (De Zwart and Kempkes 2008). The large exchanging surface requires plenty of materials. The high exchange coefficient is achieved by forced convection induced by ventilators, which consumes large amounts of electricity and therefore leads to high running costs. Electricity needed for cooling was mostly twice as high as that for heating (Hoes et al. 2008). To reduce these costs, the concept of semi-closed greenhouse with smaller cooling capacity was developed. In this concept, window ventilation is combined with mechanical cooling: at moments of low cooling demand all the cooling is mechanically and windows are kept closed, while at moments of high cooling demand both mechanical cooling and window ventilation are used.

**Heating****Cooling and/or dehumidification**

**Figure. 1.1** The energy flow during heating, cooling and dehumidification in the closed greenhouse. ATU is Air Treatment Unit and CHP is Combined Heat and Power.

**New climate in the closed greenhouse**

An important difference in climates between the closed greenhouse and open greenhouse is the CO<sub>2</sub> concentration in summer. When outside radiation is high, the CO<sub>2</sub> concentration in the closed greenhouse can easily be maintained at around 1000 ppm whereas in an open greenhouse the CO<sub>2</sub> concentration can fall below 400 ppm due to window ventilation (Opdam et al. 2005). Such a combination of a high CO<sub>2</sub> concentration and high radiation is typical for a closed greenhouse. Another typical climate character in a closed greenhouse is the occurrence of vertical temperature and humidity gradients, which is a consequence of the placement of the cooling ducts beneath the growing gutter. Solar radiation increases temperature at the top of the greenhouse, while the cooled and dehumidified air decreases temperature at the lower

part of the greenhouse. Air movement in a closed greenhouse is different from that in an open greenhouse. The air movement of an open greenhouse depends on the ventilation rate of the window, and ranges from 0.1 to 0.5 m s<sup>-1</sup> (Wang et al. 1999). The air movement of a closed greenhouse depends on the forced air circulation by the fans connected to ATU and ducts, ranging from 0.2 to 1 m s<sup>-1</sup> (Elings et al. 2007). In the closed greenhouse mechanical cooling and dehumidification make greenhouse air temperature and humidity independent from outside radiation. No CO<sub>2</sub> is lost via windows when outside radiation is high. In short, the use of mechanical cooling enables the decoupling of outside radiation and other climate factors, such as temperature, CO<sub>2</sub> concentration, and humidity. Therefore, a closed greenhouse has greater abilities for climate control than an open greenhouse. It might be expected that the increased CO<sub>2</sub> concentration and improved climate conditions would positively affect the production levels that could be realized in a closed or semi-closed greenhouse.

### **Expected effects of the new climate on crops**

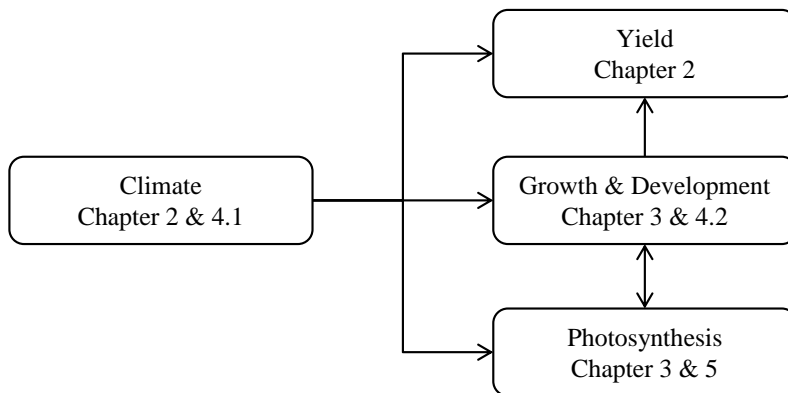
The new combinations of climate characters in the closed greenhouse may affect crop growth, development and, finally, production. Instantaneous effects are likely to have seasonal consequences. A higher CO<sub>2</sub> concentration leads to higher leaf photosynthesis (Cannell and Thornley 1998). The effect of CO<sub>2</sub> concentration on photosynthesis shows a strong interaction with the effects of other climate factors such as temperature and radiation. When radiation is the limiting factor for photosynthesis, increased CO<sub>2</sub> concentration has only a limited influence on photosynthesis while effects at high radiation are much larger (Körner et al. 2009). CO<sub>2</sub> concentration is continuously high in a closed greenhouse. Plants grown at high CO<sub>2</sub> concentrations may in the long term have a lower photosynthesis rate at a given CO<sub>2</sub> concentration than plants grown at a lower CO<sub>2</sub> concentration (Ayari et al. 2000; Hao et al. 2006; Yelle et al. 1990). This can be explained by over-accumulation of assimilates in leaves, leading to feedback inhibition on leaf photosynthesis (Poorter et al. 2009). These studies were done in open greenhouses or climate chambers, and mostly with young plants. Whether photosynthetic acclimation to high CO<sub>2</sub> concentrations occurs in the closed greenhouse when cultivating a fruit bearing crop and whether photosynthetic acclimation may nullify the effect of high CO<sub>2</sub> on photosynthetic rate has not been investigated.

The effects of vertical temperature gradients on crops in a closed greenhouse have not been investigated either. Temperature influences various crop growth and development processes, such as photosynthesis (Cannell and Thornley 1998; Yamori et al. 2010), maintenance respiration (Amthor 1989), leaf and truss initiation (Adams et al. 2001; De Koning 1994), and fruit growth (Bertin 2005; Fanwoua 2012). Thus, it was expected that a vertical temperature gradient would have effects on crop growth and development. For instance, the fruits at the lower part of the crop may be subjected to lower temperatures during ripening due to a vertical temperature gradient, which might result in longer ripening duration and higher fruit weight (Adams et al. 2001). Consequently, the combined result of all the possible effects might influence the final production in the closed greenhouse.

### Aim and outline of the thesis

When the concept of closed greenhouse was introduced, researchers initially focused on its economic and technical aspects. During the development of the closed greenhouse, scientists and growers realized that their knowledge on crop physiological processes under such new climate conditions was insufficient to fully explore the possibilities of climate control in the closed greenhouse. This PhD research, therefore, focuses on the crop physiology in closed and semi-closed greenhouses, aiming to study the effects of the new climate conditions on crop growth and development and underlying processes.

The outline of this thesis is presented in Fig.1.2. Climate conditions in the closed and semi-closed greenhouses are analyzed and compared to those of the open greenhouse in **Chapter 2** and **Chapter 4.1**. Most typical climate conditions for closed and semi-closed greenhouses are the high CO<sub>2</sub> concentration and vertical temperature gradients. Plant growth and crop production in closed, semi-closed and open greenhouses are investigated in **Chapter 2**. The effects of two most typical climate conditions, high CO<sub>2</sub> concentration and vertical temperature gradients on crop growth and development are investigated in **Chapter 3** and **Chapter 4.2**, respectively. Photosynthesis, as the main physiological process that contributes to plant growth and yield, is investigated in **Chapter 3** and **Chapter 5**. Whether photosynthetic acclimation occurs at high CO<sub>2</sub> concentration in the semi-closed greenhouse is investigated in **Chapter 3**. **Chapter 5** quantifies the photosynthesis response to light, CO<sub>2</sub> concentration, temperature, air humidity and their interactions at wide range. In **Chapter 6**, the general discussion, results of the previous chapters are summarized and discussed. Yield increase in the closed and semi-closed greenhouses is analyzed by using a component hierarchical scheme. This chapter also discusses the energy aspects and application of closed and semi-closed greenhouses in the horticultural sector.



**Figure.1.2** Schematic outline of this thesis

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# Chapter 2

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## **Comparison of climate and production in closed, semi-closed and open greenhouses**

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**Abstract**

A (semi-)closed greenhouse is a novel greenhouse with an active cooling system and temporary heat storage in an aquifer. Air is cooled, heated and dehumidified by air treatment units. The climate in (semi-)closed greenhouses differs from that of conventional open greenhouses. The aims of our research were firstly to analyze the effect of active cooling on greenhouse climate, in terms of stability, gradient and average levels; secondly to determine crop growth and production in closed and semi-closed greenhouses. An experiment with tomato crop was conducted from December 2007 until November 2008 in a closed greenhouse with  $700 \text{ W m}^{-2}$  cooling capacity, two semi-closed greenhouses with  $350 \text{ W m}^{-2}$  and  $150 \text{ W m}^{-2}$  cooling capacity, respectively, and an open greenhouse. The higher the cooling capacity, the more independent the greenhouse climate was of outside climate. As the cooling ducts were placed underneath the plants, cooling led to a remarkable vertical temperature gradient. Under sunny conditions temperature could be  $5^\circ\text{C}$  higher at the top than at the bottom of the canopy in the closed greenhouse. Cumulative production in the semi-closed greenhouses with  $350 \text{ W m}^{-2}$  and  $150 \text{ W m}^{-2}$  cooling capacity were 10% ( $61 \text{ kg m}^{-2}$ ) and 6% ( $59 \text{ kg m}^{-2}$ ) higher than that in the open greenhouse ( $55 \text{ kg m}^{-2}$ ), respectively. Cumulative production in the closed greenhouse was 14% higher than in the open greenhouse in week 29 after planting but at the end of the experiment the cumulative increase was only 4% due to botrytis. Model calculations showed that the production increase in the closed and semi-closed greenhouses was explained by higher  $\text{CO}_2$  concentration.

## Introduction

Energy consumption of Dutch greenhouse industry contributes to about 10% of the total national energy use and 79% of the total energy use of agriculture in the Netherlands (Lansink and Ondersteijn 2006). Closed and semi-closed greenhouses were innovated to reduce the energy consumption. A closed greenhouse has no window ventilation. Air is cooled and dehumidified by air treatment units (ATU), which mainly takes place in summer. Surplus heat as energy is stored in an underground aquifer and used in winter to warm the greenhouse (Opdam et al. 2005). A semi-closed greenhouse has a smaller cooling capacity than a closed greenhouse. Window ventilation is combined with active cooling when temperature is too high to be managed by the active cooling system.

The greenhouse macro- and microclimates are distinctly different in (semi-)closed greenhouses compared to that of open greenhouses. A high CO<sub>2</sub> concentration (about 1000 ppm) is one of the typical climate characteristics of the (semi-)closed greenhouse (De Gelder et al. 2005), which increases the production in the (semi-)closed greenhouse (Heuvelink et al. 2008). In particular, the combination of high CO<sub>2</sub> and high radiation that occurs during summer in a (semi-)closed greenhouse is impossible to realize in an open greenhouse. However, there is little detailed information available on climate conditions that are realized by different cooling capacities in the (semi-)closed greenhouses. In addition, a simultaneous comparison of climate and production between a (semi-)closed greenhouse and an open greenhouse is necessary to analyze processes under similar outdoor climate conditions.

The aims of our research are firstly to analyze the effect of active cooling on climate, in terms of stability, gradient and average levels, in closed and semi-closed greenhouses; and secondly to determine the production increase in closed and semi-closed greenhouses. For this reason, we evaluated climate and crop growth and production in greenhouses with different cooling capacities.

## Materials and methods

Four experimental Venlo greenhouses were located in Bleiswijk, The Netherlands. Each greenhouse was 144 m<sup>2</sup> (15 m × 9.6 m), with a gutter height of 5.5 m. From these four greenhouses, one was a conventional open greenhouse; the other three had cooling capacities of 700 W m<sup>-2</sup>, 350 W m<sup>-2</sup>, and 150 W m<sup>-2</sup>, respectively, installed. The air conditioning was controlled by a standard horticultural computer (Hoogendoorn-Economic). Greenhouse air was extracted to the ATU by five ventilators placed at the top of each greenhouse. In the ATU the air was cooled and dehumidified, and subsequently blown into the greenhouse through five plastic ducts placed beneath the growing gutters. Each duct had six holes (16 mm diameter) per meter. Cooling capacity was adjusted based on a difference between supply and return water temperature in the ATU. Cooling was achieved by controlling air speed and water temperature with a minimum temperature of 9 °C to obtain a desired greenhouse temperature. If temperature of the greenhouse air exceeded the set point for cooling (Table 1), cooling was used. When the cooling capacity could not cope with too high temperature, ventilation windows were opened to support cooling (Table 1). In the greenhouse with 700 W m<sup>-2</sup> cooling capacity the cooling capacity was high enough to keep the windows closed during the experiment. Hence, this greenhouse was defined as a closed greenhouse. The greenhouses with 350 W m<sup>-2</sup> and 150 W m<sup>-2</sup> cooling capacities represented semi-closed greenhouses, of which the latter one had more extended periods of window opening. Climate treatments started on 10<sup>th</sup> March 2008 (89 days after planting). Heating was done via the conventional heating

pipes. Average temperature set points during treatment for heating in the greenhouses with  $700 \text{ W m}^{-2}$ ,  $350 \text{ W m}^{-2}$ , and  $150 \text{ W m}^{-2}$  cooling capacities, and in the open greenhouse, were  $19.3^\circ\text{C}$ ,  $18.6^\circ\text{C}$ ,  $18.1^\circ\text{C}$ , and  $17.9^\circ\text{C}$ , respectively. Pure  $\text{CO}_2$  was supplied with a maximum capacity of  $230 \text{ kg ha}^{-1} \text{ h}^{-1}$  during daytime with a set point of 1000 ppm for all treatments. Outside solar radiation, greenhouse  $\text{CO}_2$  concentration, greenhouse air temperatures and humidity at the top of the canopy and at the growing gutter were recorded automatically at a 5 min interval. Relations between outside solar radiation and greenhouse  $\text{CO}_2$  concentration, vertical temperature gradient, and air humidity were established for the purpose of trend analysis only.

**Table 1.** Average temperature set points to start cooling, to open lee side windows and wind side windows in the greenhouses with  $700 \text{ W m}^{-2}$ ,  $350 \text{ W m}^{-2}$ , and  $150 \text{ W m}^{-2}$  cooling capacities, respectively, and in the open greenhouse.

Treatment	Cooling ( $^\circ\text{C}$ )	Open lee side windows ( $^\circ\text{C}$ )	Open wind side windows ( $^\circ\text{C}$ )
$700 \text{ W m}^{-2}$	20	28	29
$350 \text{ W m}^{-2}$	19	22	25
$150 \text{ W m}^{-2}$	18	20	23
Open		19	22

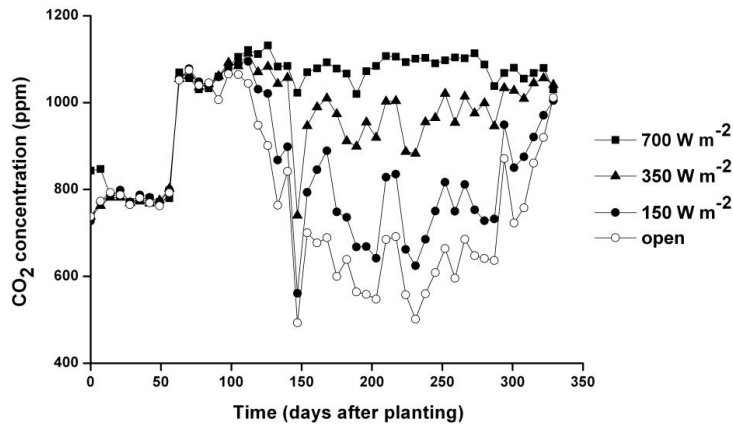
Tomato plants, cultivar Capricia (truss tomato) grafted on the rootstock Emperor, were planted in rock wool on 12 December 2007 with an initial stem density of  $2.5 \text{ stem m}^{-2}$ . In week 11 after planting, one plant out of each two developed an additional side shoot, to increase the stem density to  $3.75 \text{ stems m}^{-2}$ . Fruit harvest was started in 14 week after planting. Fresh weights of the harvested fruits were recorded weekly. Scenarios were calculated, by using the plant growth model INTKAM (Marcelis et al., 2009), to investigate the contribution of the climate factors to the final production increase. Calculation started by inputting the actual  $\text{CO}_2$  concentration, air temperature and VPD of the open greenhouse.  $\text{CO}_2$  concentration, then, was replaced by the actual  $\text{CO}_2$  concentrations of the closed greenhouse, the semi-closed greenhouse with  $350 \text{ W m}^{-2}$  cooling capacity and the semi-closed greenhouse with  $150 \text{ W m}^{-2}$  cooling capacity, respectively. The same operations were done for air temperature and VPD.

## Results and discussion

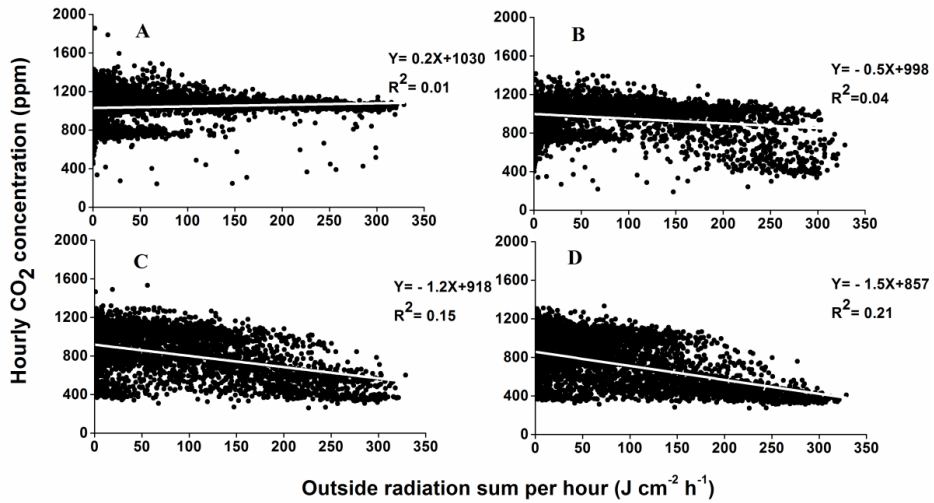
### Carbon dioxide

In summer, average day-time  $\text{CO}_2$  concentration in the closed greenhouse with  $700 \text{ W m}^{-2}$  cooling capacity was greater than 1000 ppm, while it was about 600 ppm in the open greenhouse (Fig. 1). However, the total amount of  $\text{CO}_2$  supplied to the open greenhouse was almost four times more than that of the closed greenhouse (Table 2).  $\text{CO}_2$  concentration in the closed greenhouse was independent of solar radiation, whereas in the semi-closed greenhouse with  $150 \text{ W m}^{-2}$  cooling capacity and the open greenhouse  $\text{CO}_2$  concentration decreased with increasing solar radiation (Fig. 2). The differences in  $\text{CO}_2$  concentration and  $\text{CO}_2$  supply rates between treatments were due to differences in window opening. During treatments, the average extents of lee side and wind side window opening of the closed greenhouse, the semi-closed greenhouses with  $350 \text{ W m}^{-2}$  and  $150 \text{ W m}^{-2}$  cooling capacities, and the open greenhouse, were correspondingly 0%, 6%, 18%, and 30% for lee side and 0%, 0%, 3%, and 5% for wind side (0% is fully

closed and 100% is fully open). Window ventilation during high radiation removed not only heat but also CO<sub>2</sub> and water vapour.



**Figure 1.** Weekly average day-time CO<sub>2</sub> concentrations in the greenhouses with 700 W m<sup>-2</sup> (■), 350 W m<sup>-2</sup> (▲), and 150 W m<sup>-2</sup> (●) cooling capacities, respectively, and in the open greenhouse (○).



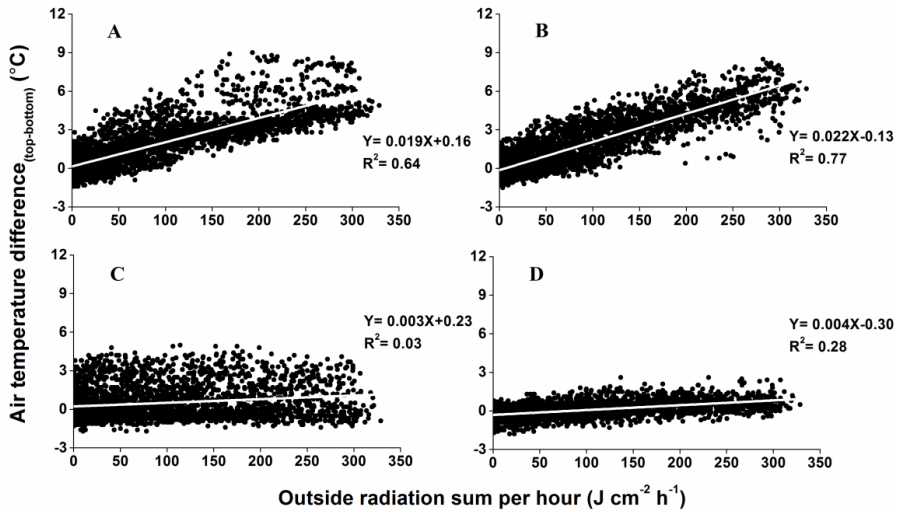
**Figure 2.** Relation between outside radiation sum and CO<sub>2</sub> concentration in the greenhouses with 700 W m<sup>-2</sup> (A), 350 W m<sup>-2</sup> (B), and 150 W m<sup>-2</sup> (C) cooling capacities, respectively, and in the open greenhouse (D). White line indicates the fitted linear curve.

**Table 2.** Early cumulative fruit production in week 29 after planting, final cumulative production in week 48 after planting, and total amount of supplied CO<sub>2</sub> in the greenhouses with 700 W m<sup>-2</sup>, 350 W m<sup>-2</sup>, and 150 W m<sup>-2</sup> cooling capacities, respectively, and in the open greenhouse. Values between brackets indicate increase compared to open greenhouse

Treatment	Early production (kg m <sup>-2</sup> )	Final production (kg m <sup>-2</sup> )	Supplied CO <sub>2</sub> (kg m <sup>-2</sup> )
700 W m <sup>-2</sup>	28 (14%)	57 (4%)	14
350 W m <sup>-2</sup>	27 (10%)	61 (10%)	30
150 W m <sup>-2</sup>	26 (6%)	59 (6%)	46
Open	24	55	55

### Temperature

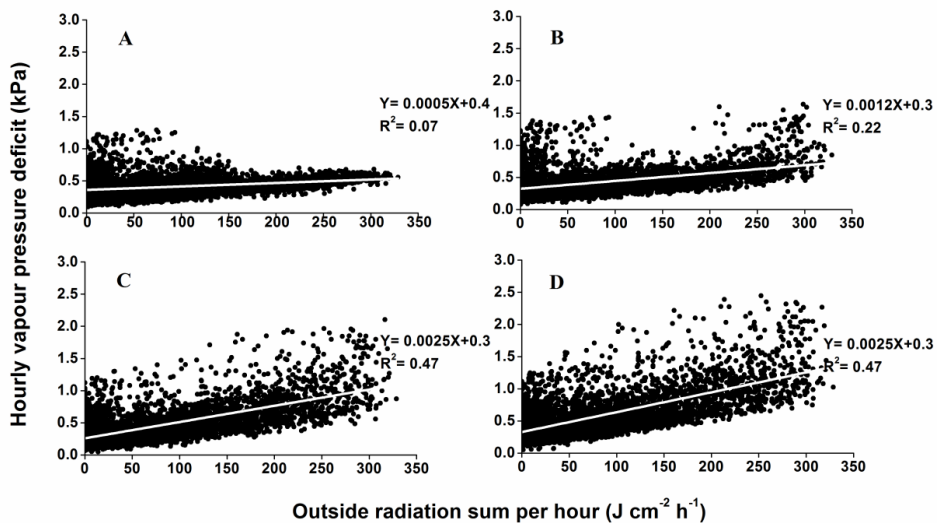
Air temperature in the greenhouse showed a positive linear relation with solar radiation in all greenhouse types (the slope being about 0.03 J cm<sup>-2</sup> h<sup>-1</sup> °C<sup>-1</sup> for the four greenhouses). Realized average day-time temperature (measured at the top canopy) was about 21.5 °C, 21.2 °C, 21.3 °C, and 21.0 °C for the closed greenhouse, the semi-closed greenhouses with 350 W m<sup>-2</sup> and 150 W m<sup>-2</sup> cooling capacities, and the open greenhouse, respectively. Since the closed and semi-closed greenhouses had higher CO<sub>2</sub> concentrations compared to that of the open greenhouse, temperature in the closed and semi-closed greenhouses was controlled to a higher level to have a higher rate of crop development. The vertical temperature gradient pattern differed remarkably between greenhouse types, especially when solar radiation was high (Fig. 3). As the cooling ducts were placed underneath the plants, cooling led to a lower temperature at the bottom of the canopy than at the top of the canopy. In addition, the vertical temperature gradient also depended on the temperature and the speed of the air blown into the greenhouse from ATU and caused the fluctuation of the vertical temperature gradient (Fig. 3). Temperature affects the partitioning of photosynthetic assimilates indirectly by affecting rate of development, such as leaf initiation, truss appearance and fruit growth duration (Pek and Helyes 2004; Adams et al. 2001; Heuvelink 1995). Since during the treatment, the average air temperature at the top of the canopy in the closed greenhouse was higher than that of the crop in the open greenhouse (21.8 °C vs. 21.4 °C), plants in the closed greenhouse formed more trusses than the plants in the open greenhouse (data not shown). However, since the average air temperature around the ripening fruits in the closed greenhouse was lower than that of the open greenhouse (19.8 °C vs. 21.2 °C), fresh weight of an individual ripen fruit in the closed greenhouse was higher than that in the open greenhouse (data not shown). The sensitivity of fruit to temperature is not equal at different fruit development stages (De Koning 2000). In closed and semi-closed greenhouses with vertical temperature gradient, fruits experienced high temperature after anthesis but low temperature during ripening. Just after anthesis, temperature does not affect fruit size significantly, due to compensation between the effects of temperature on cell number and cell size (Bertin 2005). In the last 1-2 weeks before maturity, lower temperature causes fruits to become larger due to a longer growth period (Adams et al. 2001).



**Figure 3.** Relation between outside radiation sum and vertical temperature gradient (air temperature difference) in the greenhouses with  $700 \text{ W m}^{-2}$  (A),  $350 \text{ W m}^{-2}$  (B), and  $150 \text{ W m}^{-2}$  (C) cooling capacities, respectively, and in the open greenhouse (D). Air temperature difference is the difference between the air temperatures measured at the height of the top canopy (top) and at the height of the growing gutter (bottom). White line indicates the fitted linear curve.

### Vapour Pressure Deficit

The VPD of the air inside the greenhouses with a higher cooling capacity was less dependent on outside radiation (Fig. 4). When radiation induced a temperature rise in the greenhouse, VPD strongly increased in the open greenhouse. Realized average day-time VPD (measured at the top canopy) was about 0.4 kPa for the closed greenhouse and semi-closed greenhouse with  $350 \text{ W m}^{-2}$  cooling capacity, 0.5 kPa for the semi-closed greenhouse with  $150 \text{ W m}^{-2}$  cooling capacity, and 0.6 kPa for the open greenhouse. In general, VPD within the range of 0.2–1.0 kPa has little effect on crop growth and development in tomato (Grange and Hand 1987). However, 11%, 6%, 3%, and 1% of the time the VPD was higher than 1 kPa, and 5%, 5%, 10%, and 4% of the time the VPD was lower than 0.2 kPa, in the open greenhouse, the semi-closed greenhouses with  $150 \text{ W m}^{-2}$  and  $350 \text{ W m}^{-2}$  cooling capacities, and the closed greenhouse, respectively. When VPD exceeds 1 kPa, it might promote water stress and stomatal closure, leading to a reduction of photosynthesis and transpiration (Grange and Hand 1987; Leonardi et al. 2000). On the other hand, too low VPD may also cause physiological disorder by reducing transpiration, following by less uptake of water and nutrient (Adams 1991; Del Amor and Marcelis 2006).



**Figure 4.** Relation between outside radiation sum and vapour pressure deficit in the greenhouses with  $700 \text{ W m}^{-2}$  (A),  $350 \text{ W m}^{-2}$  (B), and  $150 \text{ W m}^{-2}$  (C) cooling capacities, and in the open greenhouse (D). White line indicates the fitted linear curve.

## Production

The early cumulative production in the closed greenhouse, the semi-closed greenhouse with  $350 \text{ W m}^{-2}$  cooling capacity, the semi-closed greenhouse with  $150 \text{ W m}^{-2}$  cooling capacity were 14%, 10% and 6% , respectively, higher than that in the open greenhouse (Table 2). The final cumulative production in the semi-closed greenhouses with  $350 \text{ W m}^{-2}$  and  $150 \text{ W m}^{-2}$  cooling capacities were, respectively, 10% and 6% higher than that in the open greenhouse (Table 2). However, the final cumulative production in the closed greenhouse was only 4% higher than that in the open greenhouse, due to infection of botrytis firstly detected in week 29 after planting. Infected stems were removed to prevent spreading of botrytis, which caused a diminished increase of the production in the closed greenhouse. It was also the reason for a lower actual yield increase in the closed greenhouse in some other studies (Heuvelink et al. 2008). Stem infection by botrytis increased as a function of air humidity, especially high humidity and wound spots on the stems providing a favourable condition for the development of botrytis (Eden et al. 1996). However, high humidity is not a likely reason for the botrytis problem in the present experiment, since the semi-closed greenhouse with  $150 \text{ W m}^{-2}$  had an even higher percentage of time with high humidity, around the wound spots caused by leaf picking on the stem, than that of the closed greenhouse.

The crop model estimated the increase of production by 5%, 11% and 15% when  $\text{CO}_2$  concentration increased by 4%, 10% and 14%, respectively. These data fitted the observation well, suggesting that the difference in  $\text{CO}_2$  concentration can fully explain the difference in production. The model assumed no acclimation of photosynthesis and production to long term exposure to high  $\text{CO}_2$ . However, acclimation of photosynthesis and production to high  $\text{CO}_2$  concentration may occur (Besford et al. 1990; Peet et al. 1986). Dieleman et al. (2006) found in current Dutch greenhouse systems, photosynthesis and production did not show adaptation to high  $\text{CO}_2$  concentration.



## **Conclusions**

In conclusion, the higher the cooling capacity, the more independent the greenhouse's interior climate is of the outside climate. In addition, the active cooling from below the canopy introduced new macro and micro climate conditions in the greenhouse, which were the vertical temperature gradient and the combination of high radiation and high CO<sub>2</sub> concentration. Future work will have to be done to quantify the relations between climate factors and crop physiological processes, such as photosynthesis and transpiration.

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# Chapter 3

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## Crop morphology and physiology in response to high CO<sub>2</sub> concentration

### **Published as:**

Qian, T., Dieleman, J. A., Elings, A., Marcelis, L. F. M., 2012. Leaf photosynthetic and morphological responses to elevated CO<sub>2</sub> concentration and altered fruit number in the semi-closed greenhouse. *Scientia Horticulturae*. 145: 1–9.

### **Abstract**

Semi-closed greenhouses have been developed to reduce the energy consumption in horticulture. In these greenhouses, CO<sub>2</sub> concentration is higher than in the conventional modern greenhouses due to the reduction of window ventilation. Photosynthetic and morphological acclimation to elevated CO<sub>2</sub> has been found in many plant species with feedback inhibition being the main mechanism to explain this. The aim of this study was to investigate the occurrence of photosynthetic and morphological acclimation to elevated CO<sub>2</sub> concentration in the semi-closed greenhouse. Our hypothesis was that photosynthetic and morphological acclimation to elevated CO<sub>2</sub> concentration only occurred in plants with low sink strength. Experiments were carried out with tomato plants with varying fruit loads in a semi-closed greenhouse and a conventional modern greenhouse. Our results showed that photosynthetic acclimation to elevated CO<sub>2</sub> concentration only occurred when the number of fruits was considerably reduced. Elevated CO<sub>2</sub> as well as fruit removal reduced specific leaf area. Reduction in photosynthesis rate was associated with, but not caused by reduced stomatal conductance. The increase of dry matter production in the semi-closed greenhouse was mainly explained by a higher CO<sub>2</sub> concentration compared to the open greenhouse. We suggested that elevated CO<sub>2</sub> concentrations in the semi-closed greenhouse do not cause feedback inhibition in high producing crops, because the plants have sufficient sink organs (fruits) to utilise the extra assimilates.

## Introduction

Greenhouse horticulture has been developed to protect crops from unfavourable environmental conditions, thereby extending the growing season, at the cost of a high energy demand. Over the last decades, greenhouse concepts were developed aiming at reducing the energy consumption (De Gelder et al. 2012; Heuvelink et al. 2008). In these concepts, cooling by window ventilation was replaced by mechanical cooling. The excess solar energy was then collected and stored, to be reused to heat the greenhouse in winter (Bakker 1992; Blackwell and Garzoli 1981; Opdam et al. 2005). The greenhouse with reduced or no window opening were named as semi-closed or closed greenhouses. Further advantages of the (semi)closed greenhouse are the reduction of CO<sub>2</sub> emission and improvement of crop and pest management (Bot 1992). The reduced window ventilation in the (semi)closed greenhouses results in a continuously high air CO<sub>2</sub> concentration of about 800-1000  $\mu\text{mol mol}^{-1}$  throughout the year, while nowadays in conventional modern greenhouses the CO<sub>2</sub> concentration in summer is 400-600  $\mu\text{mol mol}^{-1}$  (Jokinen et al. 2011; Chapter 2). In the short-term, elevated CO<sub>2</sub> concentration enhances photosynthesis (Cannell and Thornley 1998). However, in the long-term, plants grown at an elevated CO<sub>2</sub> concentration may have a lower photosynthesis rate at a given CO<sub>2</sub> concentration than plants grown at a lower CO<sub>2</sub> concentration (Chen et al. 2005; Pérez et al. 2007; Zhang et al. 2009). Such a decline of photosynthesis rate at a given CO<sub>2</sub> concentration was defined as photosynthetic acclimation to elevated CO<sub>2</sub> concentration (Wolfe et al. 1998; Xu et al. 1994) and has been found in cucumber (Peet et al. 1986), tomato (Besford et al. 1990; Nederhoff 1994; Yelle et al. 1989b), strawberry (Bunce 2001), rice (Chen et al. 2005; Zhang et al. 2008), wheat (Pérez et al. 2007) and trees (Urban et al. 2003). Furthermore, photosynthetic acclimation to elevated CO<sub>2</sub> was often associated with changes in stomatal conductance and leaf morphology. For instance, decrease of stomatal conductance at elevated CO<sub>2</sub> concentration was found in cotton, tomato, and soybean (Ainsworth et al. 2002; Sasek et al. 1985; Yelle et al. 1990). Increase of leaf thickness at elevated CO<sub>2</sub> concentration, as a consequence of increased leaf weight and unaltered leaf area, was found in soybean and tomato (Besford et al. 1990; Clough and Peet 1981; Yelle et al. 1990).

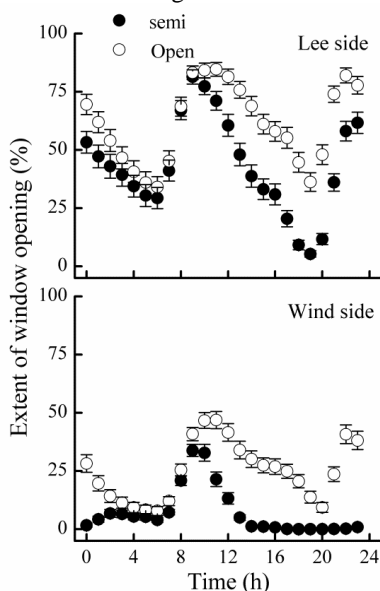
Feedback inhibition is the main mechanism that explains photosynthetic acclimation to elevated CO<sub>2</sub> concentration, in which the source-sink balance plays a role. Source organs are the net exporters of carbon assimilates (mainly leaves), and source strength is defined as the rate at which carbon assimilates are produced (photosynthesis rate) (Marcelis 1996). Sink organs are the net importer of assimilates, and sink strength is defined as the competitive ability of an organ to attract assimilates (Marcelis 1996). Photosynthesis rate increases at elevated CO<sub>2</sub> concentration in the short term, reflecting an increase of source strength. Such a high photosynthesis rate is sustained in the long term if the sink is not limiting. If the sink is limiting, over-accumulation of assimilates in leaves causes feedback inhibition on leaf photosynthesis, associated with a decrease of stomatal conductance and increase of leaf thickness (Poorter et al. 2009; Stitt et al. 1991). Species in which feedback inhibition at elevated CO<sub>2</sub> concentration was not found, had large sink organs, e.g. underground storage organs like potato (Sage et al. 1989), radish (Usuda 2006), and bulb plants (Gutjahr and Lapointe 2008); or were at high sink demand stages, e.g. reproductive stage (Davey et al. 2006) and fruit producing stage (Heuvelink 1995).

The aim of this study was to investigate whether there was photosynthetic and morphological acclimation to elevated CO<sub>2</sub> concentration in semi-closed greenhouses. Our hypothesis was that photosynthetic and morphological acclimation to elevated CO<sub>2</sub> concentration only occurred if the sink strength was low.

## Materials and methods

### Greenhouses and crop management

The experiments were performed in two adjacent experimental greenhouses of 144 m<sup>2</sup> each (15 m × 9.6 m), oriented from north to south, located at Bleiswijk, the Netherlands. A standard horticultural computer (Hoogendoorn-Economic, The Netherlands) controlled the environment inside the greenhouses. For temperature control, one greenhouse combined mechanical cooling of 350 W m<sup>-2</sup> cooling capacity with window ventilation, whereas the other greenhouse only used window ventilation for cooling. Consequently, the greenhouse with mechanical cooling had the ventilation windows more closed (defined as ‘semi-closed greenhouse’ hereafter) than the greenhouse without mechanical cooling (defined as ‘open greenhouse’ hereafter), especially in periods with a high cooling demand (Fig. 1). Pure CO<sub>2</sub> was supplied at a maximum rate of 23 g m<sup>-2</sup> h<sup>-1</sup> during day-time (between sun rise and sun set) with a set point of 1000 µmol mol<sup>-1</sup> for both greenhouses.



**Figure 1.** Diel patterns of the extents of the lee side and wind side window opening in the semi-closed and open greenhouses in 2009. Data points are average values of week 30-39 after planting. Vertical bars indicate the standard error of mean (n=69).

In the 2009 experiment, tomato plants (*Solanum lycopersicum* ‘Cappricia’), grafted on the rootstock Emperor, were planted on Rockwool® on 23 December 2008 in the two greenhouses. Stem density was initially 2.5 stems m<sup>-2</sup> (1 stem per plant). By maintaining side stems, the stem density was increased to 3.3 stems m<sup>-2</sup> at 8 weeks after planting. Truss initiation rate was 0.9 truss week<sup>-1</sup> in both greenhouses. The first truss flowered at 5 weeks after planting. Trusses were pruned to maintain 6 fruits per truss on all plants. At 32 weeks after planting, when the plants had 6 trusses, trusses of six randomly selected plants were pruned to 2 fruits per truss and of six other randomly selected plants to 0 fruit per truss in each greenhouse.

At the start of the cultivation, crops in both greenhouses were grown under identical climate conditions. The temperature control strategies (semi-closed greenhouse versus open greenhouse) started on 23 March 2009 (13 weeks after planting).

In the 2010 experiment, tomato plants ‘Cappricia’ were planted on 18 December 2009 in two other adjacent experimental greenhouses of 144 m<sup>2</sup> at the same location. Climate control and crop management in the open and semi-closed greenhouses were similar to the previous year. Temperature control strategies started on 29 March 2010 (14 weeks after planting).

### Photosynthesis measurements (Table 1)

In the 2009 experiment, photosynthesis measurements were carried out on top and middle leaves during periods when the average day-time difference in CO<sub>2</sub> concentration between the open and semi-closed greenhouses was larger than 200  $\mu\text{mol mol}^{-1}$  (Fig. 2). For the top, leaf number 5 was taken, which is the uppermost fully unfolded leaf, and which has an age of about 11 days. The middle leaf was leaf number 11, a mature leaf in the middle of the canopy, with an age of about 25 days. Photosynthesis rate (net CO<sub>2</sub> exchange rate) and stomatal conductance were measured with LCpro (ADC BioScientific Ltd. UK). Light intensity, Photosynthetic Active Radiation (PAR), in the measuring chamber was 1860  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Two CO<sub>2</sub> concentrations were set in the measuring chamber, viz., 600  $\mu\text{mol mol}^{-1}$  and 1000  $\mu\text{mol mol}^{-1}$ . Air temperature in the chamber was controlled at 27°C, and Vapour Pressure Deficit (VPD) was controlled to be less than 1 kPa. Photosynthesis rate and stomatal conductance of the plants with 6 fruits per truss were measured at 30, 33, 36 and 39 weeks after planting, to ensure that middle leaves were initiated when difference in CO<sub>2</sub> concentration was larger than 200  $\mu\text{mol mol}^{-1}$  in the two greenhouses (from week 28 after planting onwards). Photosynthesis rate and stomatal conductance of the plants with 0 or 2 fruits per truss were measured at 33, 36 and 39 weeks after planting (1, 4, and 7 weeks after fruit pruning).

**Table 1.** Overview of the measurements of photosynthesis rate described above

Year	Fruit load (fruit truss <sup>-1</sup> )	Time (week after planting)	Leaf position (leaf number)	CO <sub>2</sub> concentration ( $\mu\text{mol mol}^{-1}$ )	Replicates
2009	6	30, 33, 36, 39	5, 11	600, 1000	6
2009	2	33, 36, 39	5, 11	600, 1000	6
2009	0	33, 36, 39	5, 11	600, 1000	6
2010	6	27	4, 7, 11, 14, 17	1000	6
2010	6	27	11	50-1600	6

In the 2010 experiment, the aim was to confirm the absence of photosynthesis acclimation in plants with 6 fruit per truss. Therefore, the measurements were taken on more leaf layers, namely leaf number 4, 7, 11, 14, and 17, counting from the top to bottom. Leaf number 1 was the upmost leaf with the leaf length longer than 5 cm. Leaf number 4, 7, 11, 14, and 17 were approximately 9, 16, 25, 32 and 39 days after appearance, respectively. Differences in CO<sub>2</sub> concentration between the open and semi-closed greenhouses were smaller than 200  $\mu\text{mol mol}^{-1}$  until 24 weeks after planting (data not shown). Photosynthesis measurements were carried out on the five leaf layers at 27 weeks after planting in the two greenhouses. Average day-time CO<sub>2</sub> concentrations at 27 weeks after planting were 856  $\mu\text{mol mol}^{-1}$  in the semi-closed greenhouse and 575  $\mu\text{mol mol}^{-1}$  in the open greenhouse. Light intensity in the measuring chamber was 1860  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR. CO<sub>2</sub> concentration was set to 1000  $\mu\text{mol mol}^{-1}$ . Air temperature in the chamber was controlled at 27 °C, and VPD was controlled to be less than 1 kPa. In addition, the response of photosynthesis rate to CO<sub>2</sub> concentration was measured on leaf number 11 at CO<sub>2</sub> concentrations varying between 50 and 1600  $\mu\text{mol mol}^{-1}$  in the measuring chamber on the plants in the two greenhouses. The starting

CO<sub>2</sub> concentration was 600  $\mu\text{mol mol}^{-1}$ , followed by 400, 200, 50, 600, 800, 1200, 1600  $\mu\text{mol mol}^{-1}$ . Air temperature, VPD, and light intensity in the leaf chamber were 27 °C, <1 kPa, and 1395  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR, respectively.

### Plant measurements

In the 2009 experiment, the leaves on which photosynthesis rate was measured were removed from the plant after the measurements (week 39 after planting), and leaf area was measured immediately with a leaf area meter (LI-3100C, Li-Cor Inc. USA). Leaves were dried for 48 h at 80 °C in the oven to determine their dry weights. Specific Leaf Area (SLA) was calculated by dividing leaf area by leaf dry weight. Ripe fruits were harvested weekly, starting from 15 weeks after planting. Three bottom leaves were removed weekly, starting from 8 weeks after planting. Destructive harvests were performed at 27 and 40 weeks after planting. Each compartment was divided into 3 blocks from north to south. Two plants were randomly selected from each block. Dry weights (dried at 80 °C for over 48 h) of leaves, stems and fruits were measured. Dry weight of harvested fruits and the picked old leaves were added to the cumulative total dry weights.

### Model calculations and scenario studies

The INTKAM crop growth model, as described in more detail by Marcelis et al. (2009), was used to simulate total above-ground dry matter production. The model considers the main crop physiological processes, and consists of modules for radiation interception by the canopy, leaf and canopy photosynthesis, maintenance respiration, dry matter increase, and dry matter partitioning among plant organs (roots, stem, leaves and individual trusses of fruits).

Interception of radiation, and canopy gross photosynthesis are calculated for a multi-layered uniform canopy (Spitters 1986; Goudriaan and van Laar 1994). Extinction of radiation is calculated according to the law of Lambert-Beer:

$$I_{abs,L} = (1 - \rho)I_0(1 - e^{k \times L})$$

Where  $I_{abs}$  is radiation absorbed by the canopy ( $\text{J m}^{-2} \text{s}^{-1}$ ),  $\rho$  is canopy reflection coefficient (0.04 for diffuse light),  $I_0$  is the radiation level at the top of the canopy ( $\text{J m}^{-2} \text{s}^{-1}$ ),  $k$  is the extinction coefficient (0.77 for diffuse light), and  $L$  is leaf area index (LAI) ( $\text{m}^2 \text{m}^{-2}$ ). Instantaneous gross leaf gross photosynthesis is calculated with the biochemical model of Farquhar-von Caemmerer-Berry (Farquhar et al. 1980) for 5 leaf layers in the canopy. Instantaneous gross crop photosynthesis rate is obtained by applying a 5-point Gaussian integration over LAI (Goudriaan 1986). The five Gaussian depths are at 5%, 23%, 50%, 77% and 95% of total LAI. Instantaneous gross crop photosynthesis is computed at 5-60 minutes time intervals, depending on the availability of environmental information, and accumulated to daily gross crop photosynthesis rate.

Net assimilate production ( $P_n$ ) results from the difference between canopy gross photosynthesis ( $P_g$ ) and maintenance respiration ( $R_m$ ):

$$P_n = P_g - R_m$$

Maintenance respiration is calculated as a function of dry weights of the different plant organs, temperature and crop relative growth rate according to Heuvelink (1995). Assimilate partitioning between vegetative parts and individual trusses is simulated on the basis of the concept of sink strengths, as described by Heuvelink (1996) and Marcelis (1994). In this concept the assimilates partitioned to an organ ( $f_i$ ) is proportional to the ratio between its potential growth rate (sink strength,  $S$ ) and that of all plant parts together ( $\Sigma S$ ):



$$f_i = S/\Sigma S$$

Dry matter production of the organs is calculated as the amount of assimilates partitioned into each organ divided by the assimilate requirements for dry matter production. Rates of formation and harvest or removal of fruits and leaves is calculated as a function of temperature (De Koning 1994). Appearance rate of new sections and trusses depends on temperature solely (De Koning 1994). Early-season and late-season leaf harvest is modelled according to realized crop management practices. Otherwise, leaves and a truss from the same section are removed when the truss is harvest-ripe. All trusses are assumed to have six fruits.

Leaf area increase is potential if SLA of the whole canopy is smaller than the maximum SLA (SLA<sub>max</sub>). Potential leaf area increase is computed as the product of the potential weight of new leaf material and the minimum SLA (SLA<sub>min</sub>). If the actual SLA is greater than SLA<sub>max</sub> (if the leaf is thinner than permitted), leaf area increase is equal to the product of the weight of new leaf material and SLA<sub>max</sub>. SLA<sub>max</sub> is a constant, and SLA<sub>min</sub> is made dependent on the day of the year (*DOY*, day 1 is 1<sup>st</sup> January) (Heuvelink 1999):

$$SLA_{min} = 226 + 88 \sin(2\pi(DOY + 68)/365)$$

The model has been proven to accurately simulate tomato production in open and closed greenhouses (Heuvelink et al. 2008; Marcelis et al. 2009). Planting date, plant density, number of side stems retained and realized climate data (5-minutes averages of global radiation, CO<sub>2</sub> concentration, temperature, VPD) of the semi-closed and open greenhouses from the 2009 experiment were used as input. Scenario studies were done to investigate the contributions of CO<sub>2</sub> concentration to dry matter production increase by replacing the input values for CO<sub>2</sub> concentration of one greenhouse type by that of the other one.

### Statistical analysis

Data on photosynthesis rate, stomatal conductance, and SLA were analysed by linear mixed models and the variance components were estimated by Restricted Maximum Likelihood (REML) in Genstat (14<sup>th</sup> Edition, VSN International, UK). The linear mixed models consisted of two parts: the fixed mode and the random model. The fixed model consisted of the main factors: greenhouse (semi-closed and open greenhouses), fruit load (0, 2, and 6 fruits per truss), leaf position (top and middle leaves), CO<sub>2</sub> concentration in the measurement cuvette (600 and 1000 µmol mol<sup>-1</sup>), and time (week after planting). CO<sub>2</sub> concentration in the cuvette was not included in the analysis of SLA. The random model took into account that the plant samples from the same greenhouse were correlated. In addition, pairwise comparisons of the means were analysed by student t-test (p=0.05), comparing the means of photosynthesis rate, stomatal conductance, and SLA of the leaves from the plants with different fruit loads in the two greenhouses. Since there were no interaction effects of greenhouse × leaf position, greenhouse × CO<sub>2</sub> concentration, fruit load × leaf position, and fruit load × CO<sub>2</sub> concentration on photosynthesis rate and stomatal conductance (p>0.1), we present photosynthesis rate and stomatal conductance averaged over leaf positions and CO<sub>2</sub> concentrations, and the SLA averaged over leaf positions.

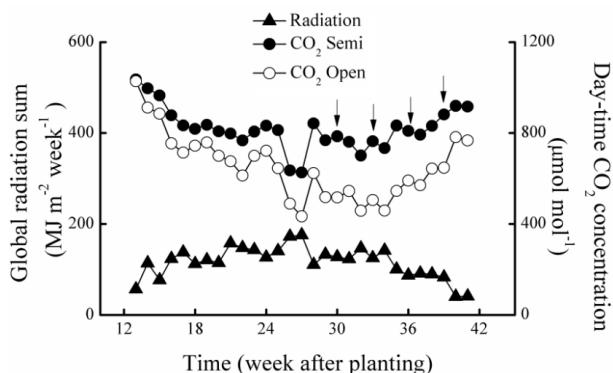
For the response of photosynthesis rate to CO<sub>2</sub> concentration, the intercellular CO<sub>2</sub> concentration (C<sub>i</sub>) was the output from the device calculated based on the function described by Von Caemmerer and Farquhar (1981). The maximum carboxylation capacity (V<sub>cmax</sub>), the electron transport rate (*J*), and the non-photorespiratory CO<sub>2</sub> release (R<sub>d</sub>) are parameters of the photosynthesis model of Farquhar-von Caemmerer-Berry (Farquhar et al. 1980) and were estimated from the data of the response of photosynthesis rate to C<sub>i</sub>, using simultaneous

estimation method and nonlinear mixed effects model in R (version 2.9.2) described in Chapter 5.

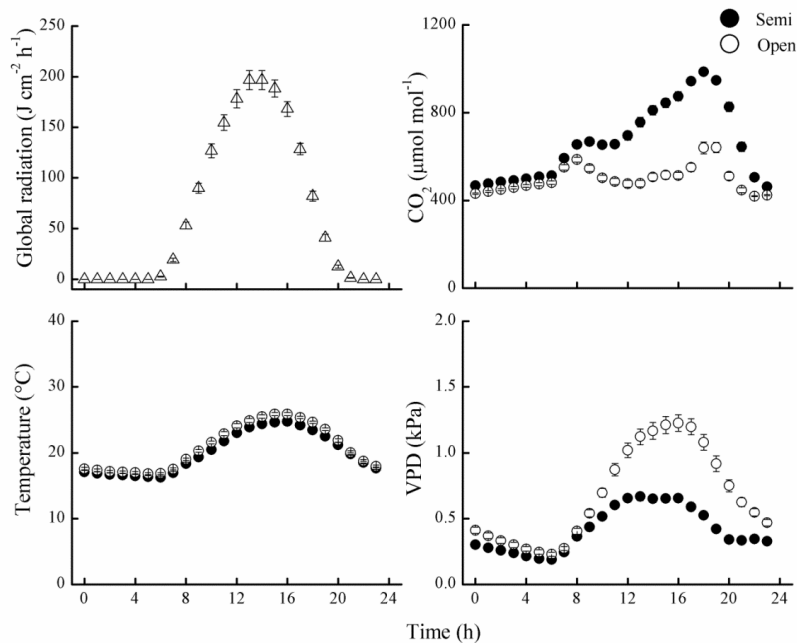
## Results

### Climate

The global radiation fluctuated during the 2009 season (Fig. 2). The realized  $\text{CO}_2$  concentration in the semi-closed greenhouse was higher than in the open greenhouse (Fig. 2), due to less  $\text{CO}_2$  loss via the window. However, since window ventilation was used in both compartments for temperature control,  $\text{CO}_2$  concentrations in both greenhouses did not reach the set point of  $1000 \mu\text{mol mol}^{-1}$  (Fig. 1). During the period of photosynthesis measurements (indicated by the arrows in Fig. 2), the average day-time difference in  $\text{CO}_2$  concentration was larger than  $200 \mu\text{mol mol}^{-1}$ . The diel pattern of the  $\text{CO}_2$  concentration (Fig. 3) was largely affected by the extent of window opening. The  $\text{CO}_2$  concentration during night was close to ambient since was no  $\text{CO}_2$  enrichment during the night. The diel pattern of global radiation, averaged over the period of photosynthesis measurements, reached a peak around mid-day (Fig. 3). The greenhouse temperature and VPD (measured at the top of the canopy) showed the same diel pattern as the global radiation. The greenhouse temperature, controlled via window ventilation or mechanical cooling, differed less than  $0.5^\circ\text{C}$  between the semi-closed and open greenhouses. During day-time, the hourly average VPD in the open greenhouse was higher than in the semi-closed greenhouse, due to the relatively drier outside air entering the greenhouse via window ventilation. In the semi-closed greenhouse, cold air was blown in via ducts placed under the growing gutters, resulting in a vertical temperature gradient (data not shown). The temperature at the lower part of the semi-closed greenhouse was about  $5^\circ\text{C}$  lower than at the bottom of the open greenhouse at high outside solar radiation round mid-day. There was virtually no vertical temperature gradient in the open greenhouse.



**Figure 2.** Weekly average day-time  $\text{CO}_2$  concentration in the semi-closed and open greenhouses in 2009 and weekly sum of the global radiation outside the greenhouses. Arrows indicate the weeks when the photosynthesis measurements were carried out.

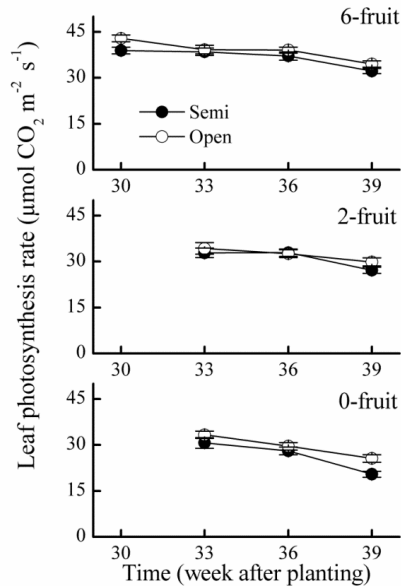


**Figure 3.** Diel patterns of the global radiation, the greenhouse CO<sub>2</sub> concentration, temperature and VPD at the top of the canopy in the semi-closed and open greenhouses. Data points are average values of week 30-39 after planting in 2009. Vertical bars indicate the standard error of mean (n=69).

In 2010, window opening between the open and semi-closed greenhouse differed as well, resulting in comparable differences in CO<sub>2</sub> concentration and VPD as in 2009 (data not shown). Temperatures at the top of both greenhouses were comparable, whereas a vertical temperature gradient of up to 5 °C was recorded in the semi-closed greenhouse.

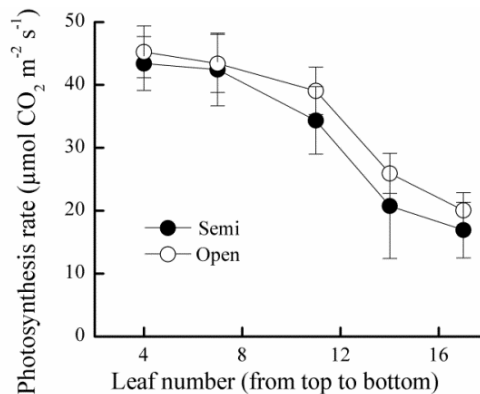
### Photosynthesis and stomatal conductance

Fruit load had a significant effect on leaf photosynthesis rate ( $p < 0.001$ ). Photosynthesis rate decreased with decreasing fruit load (Fig. 4). Such an effect of fruit load on photosynthesis rate was detected from 1 week after fruit pruning (33 weeks after planting), when the photosynthesis rates of the plants with 2 and 0 fruits per truss were significantly lower than that of the plant with 6 fruits per truss. Greenhouse type (semi-closed vs. open greenhouses) had no significant effect on leaf photosynthesis rate averaged over all measurement days, of the plants with 0 ( $p = 0.06$ ), 2 ( $p = 0.57$ ), and 6 ( $p = 0.41$ ) fruits per truss (Fig. 4). However, in the plants with 0 fruit per truss, the difference in photosynthesis rate between the semi-closed and open greenhouses became significant at 39 weeks after planting, which was 7 weeks after start of fruit pruning treatments (Fig. 4).

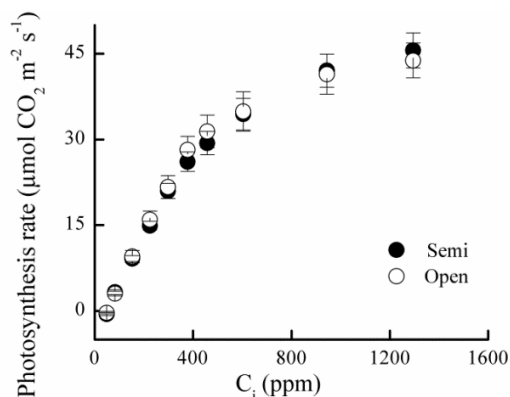


**Figure 4.** Time courses of leaf net photosynthesis rate in the semi-closed and open greenhouses. Data are average values of top and middle leaves (leaf numbers 5 and 11, respectively). Error bars indicate the standard errors of means. Fruit pruning was started at 32 weeks after planting ( $n=24$ ).

Leaf photosynthesis rate significantly decreased with increasing canopy depth ( $p<0.001$ ) (Fig. 5). Greenhouse type ( $p=0.66$ ) and greenhouse type  $\times$  canopy depth interaction ( $p=0.96$ ) had no significant effect on leaf photosynthesis. The response of photosynthesis rate to  $\text{CO}_2$  concentration of the middle leaf (leaf number 11) in the semi-closed and open greenhouses was the same (Fig. 6), since the parameters  $V_{\text{cmax}}$  ( $p=0.65$ ),  $J$  ( $p=0.75$ ), and  $R_d$  ( $p=0.61$ ) were not significantly different in the two greenhouses.  $V_{\text{cmax}}=103 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $J=196 \mu\text{mol m}^{-2} \text{s}^{-1}$ , and  $R_d=1.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ .



**Figure 5.** Leaf photosynthesis rate at different heights in the canopy in the plants with 6 fruits per truss in the semi-closed and open greenhouses at 27 weeks after planting in 2010. Vertical bars indicate the standard errors of means ( $n=6$ ).



**Figure 6.** Photosynthesis A<sub>Ci</sub> curves measured on leaf number 11 grown in the semi-closed and open greenhouses. Vertical bars indicate the standard errors of means (n=6).

Stomatal conductance decreased with decreasing fruit load ( $p < 0.001$ ). Greenhouse type had no significant effect on stomatal conductance (Table 2).

**Table 2.** Stomatal conductance of plants with three different fruit loads in the semi-closed and open greenhouses at week 39 after planting. Values were averaged over top and middle leaves, and over CO<sub>2</sub> concentrations of 600  $\mu\text{mol mol}^{-1}$  and 1000  $\mu\text{mol mol}^{-1}$  (n=24). \*

Fruit load (fruit truss <sup>-1</sup> )	Semi (mol m <sup>-2</sup> s <sup>-1</sup> )	Open (mol m <sup>-2</sup> s <sup>-1</sup> )	Average (mol m <sup>-2</sup> s <sup>-1</sup> )
0	0.37 <sup>a</sup>	0.46 <sup>a</sup>	0.42 <sup>1</sup>
2	0.53 <sup>ab</sup>	0.46 <sup>a</sup>	0.49 <sup>1</sup>
6	0.70 <sup>b</sup>	0.74 <sup>b</sup>	0.72 <sup>2</sup>
Average	0.53 <sup>A</sup>	0.55 <sup>A</sup>	

\*Superscript characters indicate if averages are significantly different ( $P < 0.05$ ). Small letters for comparing individual treatments, capital letters for comparing average values of the open versus semi-closed greenhouse and numbers for comparison among average values of fruit load treatments.

### Leaf morphology

SLA significantly decreased with decreasing fruit load (Table 3). SLA of the leaves in the open greenhouse was significantly higher than in the semi-closed greenhouse. SLA of the middle leaves was 6.4% higher than that of the top leaves (data not show).

**Table 3.** SLA of plants with three different fruit loads in the semi-closed and open greenhouses at week 39 after planting. Values were averaged over top and middle leaves (n=12). \*

Fruit load (fruit truss <sup>-1</sup> )	Semi (cm <sup>2</sup> g <sup>-1</sup> )	Open (cm <sup>2</sup> g <sup>-1</sup> )	Average (cm <sup>2</sup> g <sup>-1</sup> )
0	126 <sup>a</sup>	155 <sup>bc</sup>	140 <sup>1</sup>
2	148 <sup>b</sup>	171 <sup>c</sup>	159 <sup>2</sup>
6	173 <sup>c</sup>	195 <sup>d</sup>	184 <sup>3</sup>
Average	149 <sup>A</sup>	174 <sup>B</sup>	

\*Superscript characters indicate if averages are significantly different ( $P < 0.05$ ). Small letters for comparing individual treatments, capital letters for comparing average values of the open versus semi-closed greenhouse and numbers for comparison among average values of fruit load treatments.

### Observed and simulated dry matter production

In the 2009 experiment, the observed total above-ground dry matter production in the semi-closed greenhouse between 27 and 40 weeks after planting was 21% higher compared to

the open greenhouse (Table 4). Observed fresh weight of the ripe fruits harvested between 27 and 40 weeks after planting was 27.1 kg m<sup>-2</sup> in the semi-closed greenhouse and 21.9 kg m<sup>-2</sup> in the open greenhouse, which is an increase of 24%.

**Table 4.** Simulated and measured total above-ground dry matter between week 27 and 40 after planting, for the semi-closed and open greenhouses as input.

CO <sub>2</sub>	VPD	Temperature	Simulation (kg m <sup>-2</sup> )	Measurement (kg m <sup>-2</sup> )
open	open	open	1.42	1.33
semi	semi	semi	1.62	1.61
open	semi	semi	1.44	
semi	open	open	1.60	

Simulated total above-ground dry matter production was 14% higher in the semi-closed than in the open greenhouse (Table 4). If in model computations, the VPD and temperature of the open greenhouse were replaced by those of the semi-closed greenhouse, the simulated total dry matter production was only 1% higher than the simulated value of the open greenhouse. If the CO<sub>2</sub> concentration of the open greenhouse was replaced by the CO<sub>2</sub> concentration of the semi-closed greenhouse, the simulated total dry matter production was 13% higher than the simulated value of the open greenhouse (Table 4).

## Discussion

### Photosynthetic acclimation to elevated CO<sub>2</sub> concentration and fruit removal

Continuously high CO<sub>2</sub> concentration is one of the typical characteristics of the semi-closed greenhouse compared to the conventional greenhouse. Since photosynthetic and morphological acclimation to continuously high CO<sub>2</sub> concentration have been found in many plant species, it is necessary to investigate whether this occurs in semi-closed greenhouses.

In previous studies where photosynthetic acclimation to elevated CO<sub>2</sub> concentration was found, plants were young or were not yet producing fruits (Besford 1993; Peet et al. 1986; Sims et al. 1998). Young plants without reproductive organs are likely to be sink-limited (Arp and Drake 1991). In addition, in some studies, supplemental lighting was applied (Ayari et al. 2000b; Yelle et al. 1990). Combination of high light intensity with elevated CO<sub>2</sub> concentration enhances source strength. The limited sink cannot sufficiently use the extra assimilates, so that feedback inhibition occurs. In other studies where photosynthetic acclimation to elevated CO<sub>2</sub> concentration was not found, plants had large sinks (Arp and Drake 1991; Davey et al. 2006; Gutjahr and Lapointe 2008; Heuvelink and Buiskool 1995; Sage et al. 1989; Usuda 2006). In these cases, the sink may not have been limiting, which would explain the absence of feedback inhibition. Therefore, the occurrence of photosynthetic acclimation to elevated CO<sub>2</sub> concentration depended on the sink strength.

In our study, we also manipulated the sink strength of the plants by altering the fruit number to 6 fruits, 2 fruits and 0 fruits per truss. Six fruits per truss is the normal fruit load of this cultivar in modern greenhouse cultivation. Under normal greenhouse conditions, tomato plants that already produce fruits are source-limited (Heuvelink 2005). In our experiment, no photosynthetic acclimation to elevated CO<sub>2</sub> concentration was found on plants with 6 fruits per truss or even with 2 fruits per truss. The sink strength of tomato fruits normally comprises about 70% of the total plant sink strength (Heuvelink 1997). Reducing the number of fruits proportionally reduces the sink of all fruits together (Heuvelink 1997). Therefore, when the fruit number per truss was reduced from 6 to 2 or 0 fruits, the total fruit sink strength was reduced by 67%, or 100%, respectively. Consequently, the total plant sink strength was reduced by 47% or 70%, respectively. Hence photosynthetic acclimation to elevated CO<sub>2</sub>

concentration only occurred when plant sink strength was reduced by about 70%. We concluded that the occurrence of photosynthetic acclimation to elevated CO<sub>2</sub> concentration depended on the source sink balance.

Photosynthetic acclimation to elevated CO<sub>2</sub> concentration has been attributed to the reduction of RuBP carboxylation rate and RuBP regeneration rate in C<sub>3</sub> plants (Ainsworth and Rogers 2007; Chen et al. 2005; Urban et al. 2003; Zhang et al. 2009). Reduction of RuBP carboxylation rate, reflected by a decrease of maximum carboxylation rate ( $V_{\text{cmax}}$ ), was correlated with a decrease of Rubisco content (Makino and Mae 1999) and Rubisco activity (Portis 2003). Reduction of RuBP regeneration rate, reflected by a decreased electron transport rate ( $J$ ), was explained by a decline of cytochrome (Cyt)  $f$ , which is the key component connecting the electron transport between the photosystem II and photosystem I (Zhang et al. 2008). No photosynthetic acclimation to elevated CO<sub>2</sub> concentration was found on plants with 6 fruits per truss. This was confirmed by the identical  $V_{\text{cmax}}$  and  $J$  derived from our  $A/C_i$  curves measured on these plants in both greenhouses, reflecting no decrease of either RuBP carboxylation rate or RuBP regeneration rate.

Reduction of photosynthesis by removing sink organs has been observed in tomato (Tanaka and Fujita 1974), sweet pepper (Hall and Milthorpe 1978), cucumber (Marcelis 1991; Plaut et al. 1987), potato (Tekalign and Hammes 2005), soybean (Setter and Brenner 1980), cotton (Plaut et al. 1987), grape (Petrie et al. 2000), and fruit trees (Cheng et al. 2009; Gucci et al. 1995; Syvertsen et al. 2003; Urban et al. 2004). In our study, leaf photosynthesis rate was also decreased by fruit removal. In these studies, as well as in our study, reduction of photosynthesis was associated with lower stomatal conductance. Some studies suggested that sink limitation, induced by removing sink organs, decreased photosynthesis rate via reducing stomatal conductance (Cheng et al. 2009; DaMatta et al. 2008). In contrast, other studies suggested that decrease of photosynthesis rate by removing sink organs could not be attributed to the reduction of stomatal conductance (Petrie et al. 2000; Syvertsen et al. 2003; Urban et al. 2004). In our study, the decrease of stomatal conductance was associated with a constant intercellular CO<sub>2</sub> concentration, demonstrating that the reduction of photosynthesis rate at lower fruit load was not attributed to a stomatal-associated decrease in  $C_i$ . It is more likely that stomatal closure was a consequence of the reduced photosynthesis, as stomata tend to open and close to maintain a constant ratio between the intercellular and air CO<sub>2</sub> concentrations (Drake et al. 1997; Wong et al. 1979). We conclude that although stomatal conductance was reduced by fruit removing, this in itself did not limit photosynthesis.

The majority of the studies on photosynthesis acclimation to elevated CO<sub>2</sub> were conducted in growth chambers (Bunce 2001; Peet et al. 1986; Sims et al. 1998; Van Oosten et al. 1995) or the open field (Chen et al. 2005; Pérez et al. 2007; Zhang et al. 2008). A limited number of studies were carried out in greenhouses (Ayari et al. 2000a; Hao et al. 2006; Yelle et al. 1990). These studies used ambient CO<sub>2</sub> concentration (about 400  $\mu\text{mol mol}^{-1}$ ) as a reference (Hao et al. 2006; Yelle et al. 1990). An ambient CO<sub>2</sub> concentration of 400  $\mu\text{mol mol}^{-1}$  is not relevant for modern greenhouses, since CO<sub>2</sub> enrichment is practiced year-round (Heuvelink et al. 2008; Opdam et al. 2005), even in summer when ventilation rates are high. Therefore, we used CO<sub>2</sub> concentrations of about 600  $\mu\text{mol mol}^{-1}$  in summer time in the open greenhouses which correspond to CO<sub>2</sub> concentrations encountered in modern commercial greenhouses. Our results suggest that a continuously high CO<sub>2</sub> concentration in a semi-closed greenhouse does not cause feedback inhibition in high producing crops, because these plants have sufficient sinks (fruits) to utilise the extra assimilates.

### Morphological acclimation

Reduction of SLA at elevated CO<sub>2</sub> concentration (Besford et al. 1990; Clough and Peet 1981; Holbrook et al. 1993; Nederhoff et al. 1992) and by removing fruits (Bertin and Gary

1998; Heuvelink and Marcelis 1996; Marcelis 1991; Nii 1997) has been found in many studies as well as in our study. Lower SLA was attributed to over-accumulation of starch in the leaf at elevated CO<sub>2</sub> concentration (Yelle et al. 1989a) and by removing fruits (Poorter et al. 2009). The effect of starch accumulation on SLA might be via a regulator, trehalose-6-phosphate, linking between SLA and carbohydrate availability (Poorter et al. 2009; Schluepmann et al. 2003).

### **The semi-closed greenhouse**

Semi-closed greenhouses have been developed to reduce the energy consumption in horticulture. The three main differences in climate of the semi-closed greenhouse compared to open greenhouse in our study were a higher CO<sub>2</sub> concentration, a lower VPD under sunny conditions, and a vertical temperature gradient when outside radiation was high, which is in line with reports of De Gelder et al. (2005), Heuvelink et al. (2008), and Opdam et al. (2005). Our study main focused on CO<sub>2</sub> concentration, but the differences in VPD and temperature were also taken into account. Scenario studies were done with the INTKAM crop growth model to analyse the effects of the separate climate factors on growth and production. This showed that, although more climate factors than the CO<sub>2</sub> concentration differed between the semi-closed and open greenhouses, CO<sub>2</sub> was the most relevant climate factor involved, explaining most of the production difference.

In this study, we mainly focused on the carbon balance in the plant: dry matter production and its distribution. CO<sub>2</sub> concentration was the main factor that influenced photosynthesis, and, consequently, used to explain the increase of dry matter production in the semi-closed greenhouse (Chapter 2). Our scenario studies confirmed that the difference in dry matter production between the semi-closed and open greenhouses was mainly explained by the difference in CO<sub>2</sub> concentration (Table 4). The increase in simulated dry mass production in a semi-closed greenhouse compared to an open greenhouse was slightly higher than the measured increase. As the used simulation model did not consider feedback inhibition of photosynthesis, this gives some further support that feedback inhibition did not occur in a producing tomato crop in the semi-closed greenhouse. The vertical temperature gradient was not expected to affect photosynthesis, since the temperature difference occurred only at the bottom of the canopy, where light intensity is low. Leaf photosynthesis was less sensitive to temperature at low light intensity (Chapter 5). Lower temperature at the bottom of the canopy might influence the sink strength of the organs at the bottom. However if the sink strength of all plant organs responds proportionally to temperature, dry matter partitioning does not change with temperature (Heuvelink 1995). VPD influences photosynthesis indirectly via stomatal conductance (Acock et al. 1976; Stanghellini and Bunce 1993).

Besides the carbon balance, VPD and vertical temperature gradient influenced the water balance in the plant. A lower VPD in the semi-closed greenhouse resulted in a reduced transpiration rate compared to the open greenhouse (De Gelder et al. 2005; Joliet and Bailly 1992). In addition, low VPD could either increase leaf area by increasing leaf expansion or decreasing leaf area through calcium deficiency (Bakker et al. 1987). Stanghellini et al. (2012) showed that the transpiration rate in the lower leaf levels in the semi-closed greenhouse was lower than in the open greenhouse, due to the vertical temperature gradient.

### **Conclusions**

Our conclusions are 1) Acclimation of photosynthesis to elevated CO<sub>2</sub> concentration only occurred when the number of sinks was considerably reduced. 2) Elevated CO<sub>2</sub> as well as fruit removal reduced SLA indicating morphological acclimation of the plants. 3) Reduction in photosynthesis was associated with, but not caused by reduced stomatal



conductance. 4) Increase of dry matter production in the semi-closed greenhouse was mainly explained by elevated CO<sub>2</sub> concentration.

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# Chapter 4

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**Crop growth and development in response to vertical temperature gradients**

## **Chapter 4.1 Occurrence of vertical temperature and vapour pressure deficit gradients**

**Adapted from the publication:**

Qian, T., Dieleman, J. A., Elings, A., Van Kooten, O., Marcelis, L. F. M., 2012. Vertical temperature gradients in the semi-closed greenhouses: occurrence and effects. *Acta Horticulturae*. 927: 59-66.

### **Abstract**

Semi-closed greenhouses have been developed in which window ventilation is minimized due to active cooling, enabling enhanced CO<sub>2</sub> concentrations at high irradiance. Cooled and dehumidified air is blown into the greenhouse from below or above the canopy. Cooling below the canopy may induce vertical temperature gradients along the height of the plants. The aim was to analyse the effect of the positioning of the inlet of cooled and dehumidified air on the magnitudes of vertical temperature gradients and vapour pressure deficit (VPD) gradients in the semi-closed greenhouses. Tomato crops were grown year-round in four semi-closed greenhouses with cooled and dehumidified air blown into the greenhouses from below or above the crop. Cooling below the canopy induced vertical temperature and VPD gradients. The temperature at the top of the canopy was over 5 °C higher and VPD was 0.7 kPa lower, than at the bottom, when outside solar radiation was high (solar radiation > 250 J cm<sup>-2</sup> h<sup>-1</sup>). The vertical temperature gradients and VPD gradients were studied in seasonal pattern, and in diel pattern in detail. The vertical temperature gradients and VPD gradients positively correlated with outside radiation and outside temperature.



## Introduction

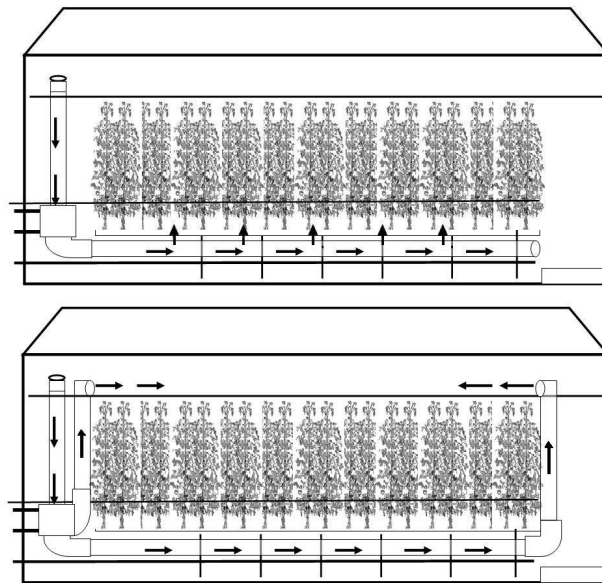
Semi-closed greenhouses were developed to save energy. Greenhouse air is cooled and dehumidified by air treatment units and returned to the greenhouse through cooling ducts (De Gelder et al. 2012). Active cooling is combined with window ventilation if temperatures are too high to be controlled by the air treatment units with limited cooling capacity. Cooling ducts are normally placed beneath the growing gutters, because placement overhead or within crops either causes loss of light or interferes with cultivation procedures (Wells and Amos 1994). However, cooling from below induces a vertical temperature gradient along the canopy (Chapter 2). The occurrence and magnitude of the vertical temperature gradient depend on the radiation (Suay et al. 2008), the cooling capacity, and temperature of the air blown into the greenhouse (Chapter 2). It may vary during a day and during the season. We carried out an experiment with tomato crops in semi-closed greenhouses where a vertical temperature gradient was present or absent. The aim was to analyse the effects of the positioning of the inlet of cool and dehumidified air on the occurrence and magnitudes of vertical temperature gradients and vapour pressure deficit (VPD) gradients in the semi-closed greenhouses. The vertical temperature gradients and VPD gradients were studied in seasonal pattern, and in diel pattern in detail.

## Materials and methods

Experiments were conducted in four adjacent semi-closed greenhouses with 350 W m<sup>-2</sup> maximum cooling capacity, located in Bleiswijk, The Netherlands. Each greenhouse measured 144 m<sup>2</sup> (15 m x 9.6 m), with a gutter height of 5.5 m. Transmission of diffuse global light was 59 %. Seven growing gutters, about 70 cm above the floor, with rock-wool slabs, were oriented from east to west, of which five were double-row and two were single-row for border plants. Air conditioning was controlled by a standard horticultural computer (Hoogendoorn-Economic). Cooling capacity was controlled by adjustment of air speed and the temperature of the cooling water. This control was based on the difference between supply and return water temperature in the air treatment units. Air temperature set points for cooling and heating were identical for all the four greenhouses. Ventilation windows were opened if the 350 W m<sup>-2</sup> cooling capacity was insufficient to keep greenhouse temperature below the critical level. Pure CO<sub>2</sub> was supplied at a maximum rate of 23 g m<sup>-2</sup> h<sup>-1</sup> during daytime with a set point of 1000 µmol mol<sup>-1</sup> for each greenhouse. Outside radiation, greenhouse CO<sub>2</sub> concentration, greenhouse air temperature and humidity were recorded automatically at a 5 min interval. In addition, temperature and humidity sensors (Hoogendoorn) were placed at four canopy heights in each greenhouse (3.5 m, 2.5 m, 1.2 m, and 0.3 m above the growing gutters, which were 0.7 m from the ground). The highest sensor (3.5 m) was above the top of the canopy. The lowest sensor (0.3 m) was between the lowest truss and the rockwool slab. The values of the two sensors in the middle (2.5 m and 1.2 m) were averaged, representing the temperature and humidity at the middle of the canopy.

Two treatments were applied, namely cooling from below and from above the canopy (Fig. 1). Each treatment was replicated in two greenhouse compartments. The difference of the realized daily average temperature between the two replicates was less than 0.5°C. In all the four greenhouses, air was extracted from the greenhouse by five ventilators placed at the top of the greenhouse, cooled and dehumidified in the air treatment units. In one treatment, treated air from the air treatment units was returned to the greenhouse through five plastic cooling ducts placed horizontally beneath the growing gutters. Each duct had six holes (16 mm diameter) per meter. In the other treatment, treated air

from the air treatment units was returned at the top of the greenhouse through the ends of five blowers at the two ends of the greenhouse.



**Figure 1.** Layout of the greenhouse with cooling below the gutter (top figure) and the greenhouse with cooling above the canopy (bottom figure).

Truss tomato plants, cultivar Cappricia grafted on the rootstock Emperor, were planted on rock wool slabs on December 23<sup>rd</sup>, 2008, at a plant density of 2.5 m<sup>-2</sup>. Initially one stem per plant was maintained. In week 8 after planting, an additional side shoot was maintained at 1/3 of the plants, increasing stem density to 3.33 m<sup>-2</sup>. Climate treatments started on 23<sup>rd</sup> March 2009.

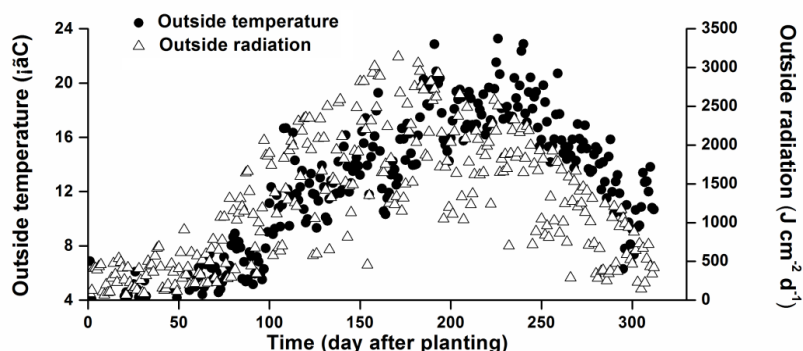
Data of the hourly outside radiation, outside temperature, hourly temperature difference between top and bottom of the canopy, and VPD difference between top and bottom of the canopy in the greenhouse with cooling from below, were analysed using SPSS version 22. Hourly outside radiation and outside temperature were set as independent variables and hourly temperature difference and VPD difference between top and bottom of the canopy were set as dependent variables. Single effect of outside radiation or outside temperature on vertical temperature difference and VPD difference was analysed by Student's t-test ( $P=0.01$ ), fitting the data with linear regression. Effects of both outside radiation and outside temperature on vertical temperature difference and VPD difference were analysed by Student's t-test ( $P=0.01$ ), fitting the data with multiple linear regression.

## Results and discussion

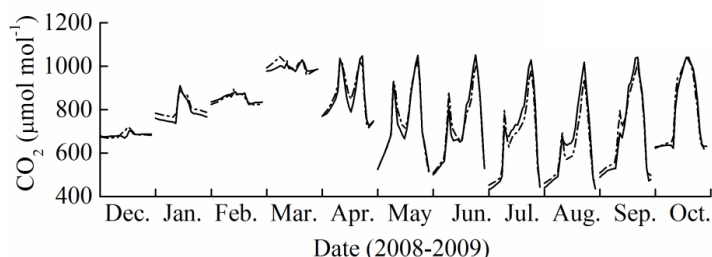
Outside global radiation and outside temperature fluctuated during the whole growing season (Fig. 2). Average climate conditions at top of the canopy were similar between the two treatments where cooling was performed from below or above the canopy. Realized seasonal average values for day/night temperature, CO<sub>2</sub> concentration, and VPD

(measured at the height of the top of the canopy) were 21.6/17.2 °C and 21.8/17.0 °C; 764  $\mu\text{mol mol}^{-1}$  and 763  $\mu\text{mol mol}^{-1}$  (Fig 3); 0.38 kPa and 0.37 kPa of the treatments with cooling from above and from below, respectively. Diel patterns of the  $\text{CO}_2$  concentrations in both treatments were similar (Fig. 3).

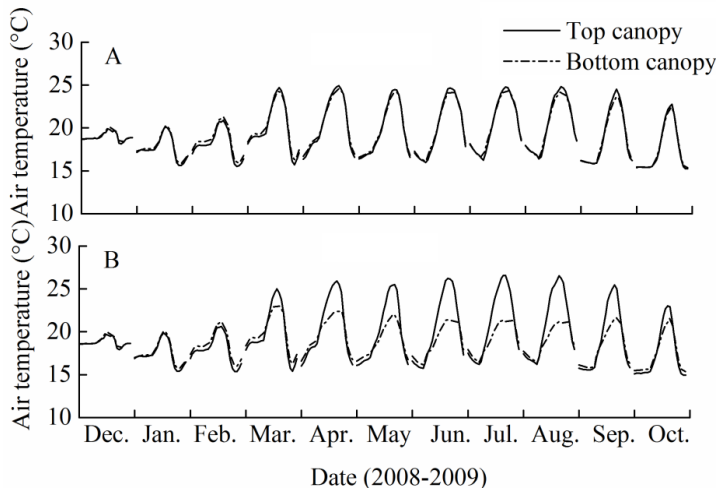
Cooling below the canopy resulted in a vertical temperature gradient which first appeared in March, when the cooling systems were turned on (Fig. 4). Temperatures at the top of the canopy were similar between treatments, but were lower in the lower part of the canopy. The gradient was most pronounced from June to September when the temperature difference between the top and the bottom of the canopy was  $> 2^\circ\text{C}$  for 55% of the time, and was  $> 5^\circ\text{C}$  for 20% of the time. In the treatment with cooling from above, air temperatures at the top and the bottom of the canopy were similar (Fig. 4). The VPD throughout the canopy hardly differed in the treatment with cooling from above, whereas in the treatment with cooling from below, the VPD in the top of the canopy was higher than in the lower part of the canopy (Fig. 5).



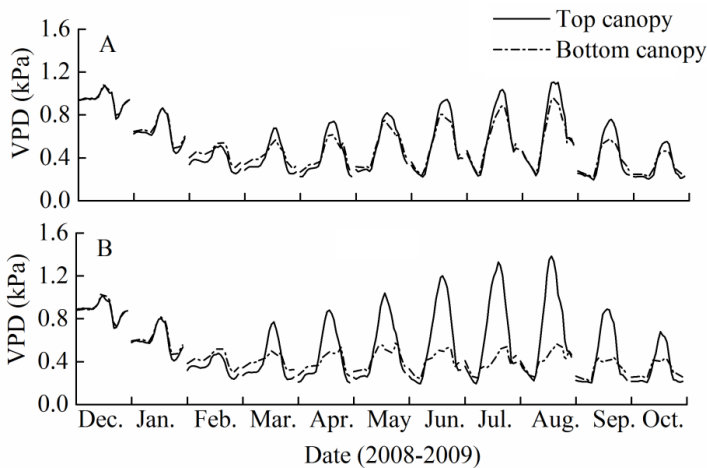
**Figure 2.** Daily average of the outside temperature (●) and outside solar radiation (Δ) throughout the growing season (December 2008 – October 2009), 23 December 2008 is considered as 0 day after planting.



**Figure 3.** Diel patterns of  $\text{CO}_2$  concentration in treatments with cooling from above (dash line) and with cooling from below (solid line) of the canopy throughout the growing season (December 2008 – October 2009). Each bell-shape curve shows the 24-h pattern of  $\text{CO}_2$  concentration averaged over the two greenhouses per treatment and over all days in the months indicated on the x-axis.



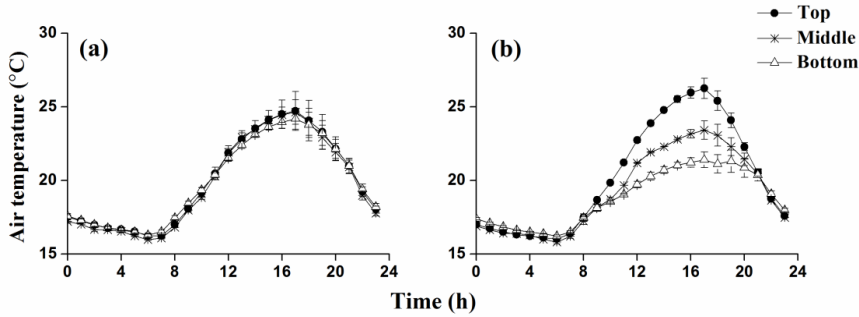
**Figure 4.** Diel patterns of air temperature at the top and bottom of the canopy in treatments with cooling from above (A) and cooling from below (B) of the canopy throughout the growing season (December 2008 – October 2009). Each bell-shape curve shows the 24-h pattern of air temperature averaged over the two greenhouses per treatment and over all days in the months indicated on the x-axis.



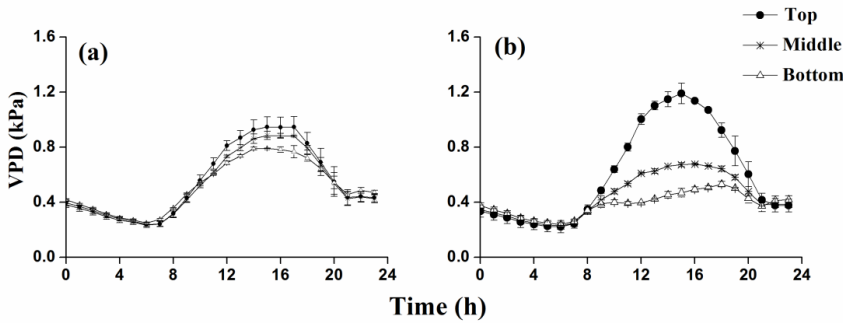
**Figure 5.** Diel patterns of vapour pressure deficit (VPD) of the air at the top and bottom of the canopy in treatments with cooling from above (A) and with cooling from below (B) of the canopy throughout the growing season (December 2008 – October 2009). Each bell-shape curve shows the 24-h pattern of VPD averaged over the two greenhouses per treatment and over all days in the months indicated on the x-axis.

The hourly temperature and VPD from July to September were averaged to investigate their diel patterns in detail. The magnitudes of vertical temperature and VPD gradients among top, middle, and bottom of the canopies reached their maxima after midday around 4 PM when temperature in greenhouses was highest (Fig. 6b and 7b). The biggest difference in VPD between top and bottom of the canopy in the treatment with cooling

from below was 0.7kPa (Fig. 7). However, no distinct vertical temperature and VPD gradients were measured in the greenhouses with cooling from above during the whole day (Figs. 6a, and 7a).



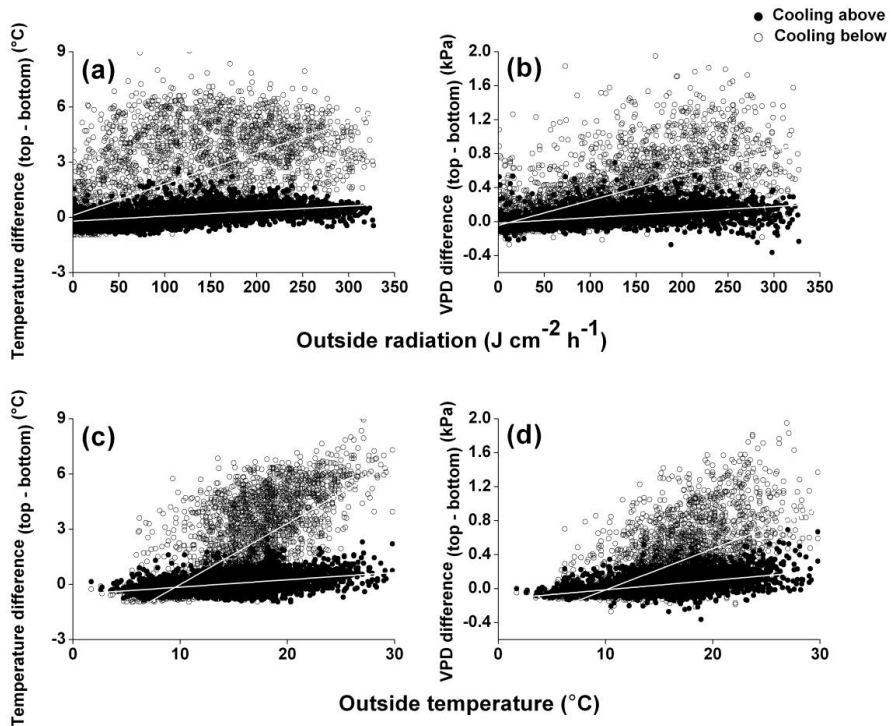
**Figure 6.** Average diel air temperature at the top (●), middle (Δ) and bottom (✱) of the canopy from July to September 2009 in the greenhouses with cooling from above (a) and from below (b). Top of canopy is 3.5 m, and bottom of canopy is 0.3 m above the gutter. The values of middle of the canopy were the average values measured at 2.5 m and 1.2 m above the gutter. Vertical bars indicate the s.e.m..



**Figure 7.** Average diel vapour pressure deficit at top (●), middle (Δ) and bottom (✱) of the canopy from July to September 2009 in the greenhouses with cooling from above (a) and from below (b). Top of canopy is 3.5 m, and bottom of canopy is 0.3 m above the gutter. The values of middle of the canopy were the average values measured at 2.5 m and 1.2 m above the gutter. Vertical bars indicate the s.e.m..

Outside temperature shows a positive linear correlation with global radiation ( $R^2=0.41$ ). In the greenhouses with cooling from below, the temperature difference between the top and the bottom of the canopy correlated positively with increasing outside global radiation (Fig. 8a,  $R^2=0.64$ ,  $P<0.01$ ). This difference was larger than 5 °C when outside solar radiation was higher than 250 J cm<sup>-2</sup> h<sup>-1</sup> (equal to 700 W m<sup>-2</sup>) (Fig. 8a). VPD differences between top and bottom of the canopy also showed a positive correlation with outside radiation in the greenhouses with cooling from below (Fig. 8b,  $R^2=0.67$ ,  $P<0.01$ ). The temperature (Fig 8c,  $R^2=0.68$ ,  $P<0.01$ ) and VPD (Fig 8d,  $R^2=0.60$ ,  $P<0.01$ ) differences between the top and the bottom of the canopy also positively correlated with outside temperature in the treatment with cooling from below.

Multiple regression of temperature gradients versus global radiation and outside temperature, which were positively correlated themselves ( $R^2 = 0.41$ ), showed that the latter two both had an effect on the vertical temperature gradient ( $R^2 = 0.62$ ,  $P < 0.01$  of both factors) and vertical VPD gradient ( $R^2 = 0.57$ ,  $P < 0.01$  of both factors). High global radiation and outside temperature led to a higher cooling demand and therefore a lower temperature of the air blown from the ducts. Low temperature of the air blown from the ducts underneath the canopy leads to the higher vertical temperature and VPD gradients.



**Figure 8.** The relationship of hourly outside radiation and outside temperature with hourly temperature difference between top and bottom of the canopy (a, c) and VPD difference between top and bottom of the canopy (b, d) in the greenhouse with cooling from above (●) and the greenhouse with cooling from below (○). Each data point is the average of the values from two replicates. White lines indicate the fitted linear curves.

## Conclusions

Cooling from below the canopy induced vertical temperature and VPD gradients. The vertical temperature and VPD gradients were most pronounced from June to September. At high radiation levels temperature at bottom of the canopy was 5°C lower and VPD was 0.7 kPa lower than at the top of the canopy, occurring after midday around 4 PM. The vertical temperature and VPD gradients were correlated with outside radiation and outside temperature. No vertical temperature and VPD gradients were detected in greenhouses with cooling from above.

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# Chapter 4

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## **Crop growth and development in response to vertical temperature gradients**

### **Chapter 4.2 Effects of vertical temperature gradients on crop growth and development**

#### **Adapted from the publication:**

Qian, T., Dieleman, J. A., Elings, A., De Gelder, A., Marcelis, L. F. M., 2015. Response of tomato crop growth and development to a vertical temperature gradient in a semi-closed greenhouse. *Journal of Horticultural Science and Biotechnology*. 90 (5): 578–584.

### **Abstract**

Semi-closed greenhouses have been developed over the last decade to conserve energy. Energy consumption is reduced by collecting the excess solar energy in summer, storing it in aquifers and reusing it in winter to heat the greenhouse. Cooling systems placed in the lower part of the greenhouse, can cause vertical temperature gradients, which can be more than 5°C at high levels of solar radiation. Given the substantial effect that air temperature has on a number of plant physiological processes, we expected to observe effects on plant growth and fruit production. Tomato plants were grown in semi-closed greenhouses with or without a vertical temperature gradient. Despite these large vertical temperature gradients, plant growth and fruit yields were mostly unaffected. Leaf and truss initiation rates did not differ between treatments, since air temperatures at the top of the canopy were comparable. The only observed response of plants to the vertical temperature gradient was the reduced rate of fruit development in the lower part of the canopy. This resulted in a longer time between anthesis and fruit harvest in the treatment with a vertical temperature gradient, and an increase in the average fruit weight in summer. However, total fruit production over the whole season was not affected. These results are important when designing greenhouses, as well as heating and cooling systems for greenhouses.

## Introduction

Air temperatures can be spatially and temporally heterogeneous in nature, in a forest (Leuzinger and Körner 2007), an orchard or in the open field (Baldocchi et al., 1983). This heterogeneity is caused by features of the canopy architecture such as plant height, plant density, and leaf orientation (Grace 1977) and by environmental conditions such as solar radiation, wind, and rainfall (Parker 1995). Spatial and temporal variations in air temperature also exist in a protected environment such as a greenhouse (Bojaca et al. 2009; Kempkes et al. 2000). This spatial variation in temperature in a greenhouse can be caused by operational actions such as window or screen opening (Soni et al. 2005), heating or cooling (Kempkes et al., 2000). Canopy architecture, in combination with leaf traits, was shown to affect leaf temperature (Leuzinger and Körner 2007). Savvides et al. (2013) showed that at a constant air temperature, the difference between the apex temperature and the air temperature in tomato plants varied from  $-3^{\circ}\text{C}$  to  $+4^{\circ}\text{C}$ , depending on air temperature, vapour pressure deficit, radiation, and wind speed, thereby affecting the rate of plant development.

Semi-closed greenhouses have been developed over the last decade to conserve energy. In such greenhouses, energy consumption is reduced by collecting the excess solar energy in summer, storing it in aquifers and reusing it in winter to heat the greenhouse (De Gelder et al. 2012; Opdam et al. 2005; Vadiée and Martin 2012). In this system, active cooling minimizes window ventilation, thereby enabling more favourable conditions for plant growth (e.g. a high light intensity with a high  $\text{CO}_2$  concentration and moderate temperatures) which have been shown to have a positive effect on production levels (De Gelder et al., 2012; Dannehl et al. 2012; 2013; Chapter 3). Cooled and dehumidified air is returned to the greenhouse through cooling ducts, which are commonly placed beneath the growing gutters (De Gelder et al. 2012). This system results in a vertical temperature gradient (VTG) that can be considerably greater than in a conventional greenhouse with window ventilation. The occurrence and magnitude of the VTG depends on the incident radiation (Suay et al. 2008), the cooling capacity of the installation, and the temperature of the air being blown into the greenhouse (Chapter 2 and Chapter 4.1).

Air temperature influences a wide range of plant growth and developmental processes. Together with the level of solar radiation and  $\text{CO}_2$ , air temperature is therefore one of the major factors affecting crop characters such as the balance between generative and vegetative growth, the rate of crop development, and, ultimately, crop yield. Thus, it can be expected that a VTG would have a noticeable effect on a number of plant physiological processes and, ultimately, on crop yield.

The influence of temperature on the growth and development of tomato plants has been studied extensively (reviewed by Van der Ploeg and Heuvelink 2005). The effect of temperature on the rate of leaf photosynthesis depends, among other things, on the intensity of the light (Chapter 5; Cannell and Thornley 1998; Yamori et al. 2010). At low light levels the effect of temperature on the rate of photosynthesis is relatively small. Light intensity is relatively low at the bottom of the canopy. So, if the temperature of the lower leaves changes due to a VTG in a closed greenhouse, the effect of this gradient on photosynthesis might be limited.

Maintenance respiration rate decreases with temperature (Amthor 1989). Consequently, a vertical temperature gradient with a lower air temperature at the bottom of the canopy may lead to greater availability of assimilates for crop growth (i.e., source strength), when the rate of photosynthesis is largely unaffected and the rate of maintenance respiration is low. However, the rate of maintenance respiration also depends on organ weight (Amthor 1989; Penning de Vries 1975). If, due to a longer duration of fruit maturation the total crop dry

weight (DW) at a specific time-point was higher, the rate of maintenance respiration would also be increased. The consequences for the final availability of assimilates are therefore difficult to estimate.

Rates of leaf and truss initiation increase linearly with increasing air temperature (Adams et al. 2001; De Koning 1994). The rate of truss initiation is approx. one truss per week at an average daytime temperature of 20°C (De Koning 1994). Cooling from above could result in lower air temperatures at the top of the canopy, and therefore in a lower rate of truss initiation.

The rate of fruit maturation is also determined by air temperature. A certain number of day-degrees (temperature sum) have to be accumulated to reach physiological maturity (De Koning 1994). In a semi-closed greenhouse with a VTG, the fruits in the lower part of the canopy experience lower temperatures, which would be expected to affect the duration of fruit growth and the average fruit fresh weight (Adams et al. 2001; De Koning 1994; Fanwoua et al. 2012). Consequently, a lower air temperature would result in delayed (as expressed in the number of days required) fruit ripening. Thus, temperature effects on truss initiation and the rate of fruit maturation must interact in their effect on fruit load, for example. Lowered temperatures may result in fewer new trusses formed per unit of time, but also in a delayed fruit ripening. The result of this interaction on the number of trusses per plant is difficult to predict.

In Chapter 4.1, it has been showed that, in semi-closed greenhouses with cooling ducts below the canopy, air temperatures in the lower part of the canopy could be > 5°C lower than in the top of the canopy. They also provided some overall values for plant and fruit growth.

This work aimed to study the effects of a vertical temperature gradient on the plant physiological processes that determine growth and fruit production (e.g. the rates of truss initiation and fruit development, dry matter (DM) production and partitioning).

## Materials and methods

### Experimental design

The experiment was conducted in four adjacent, semi-closed greenhouses (144 m<sup>2</sup> each) with a maximum cooling capacity of 350 W m<sup>-2</sup> at Bleiswijk, The Netherlands (see Chapter 4.1). Two treatments were applied, with or without a vertical temperature gradient, each replicated twice using two greenhouse compartments. In the treatment with a vertical temperature gradient (+VTG), mechanically cooled air was blown into these greenhouses through cooling ducts placed horizontally beneath the growing gutters. Each duct had six holes (16 mm in diameter) m<sup>-1</sup>. In the treatment without a vertical temperature gradient (-VTG), cooling was applied above the canopy in two greenhouses. Mechanically cooled air was blown into the top of these greenhouses through five blowers (three blowers at the eastern end and two blowers at the western end of each greenhouse). Since cold air has a higher density, this mixed with the lower layers of warmer air, resulting in no VTG (Fig. 1 of Chapter 4.1).

The air temperature set points at the top of the canopy were similar between both treatments. The greenhouse climate was controlled based on measurements of air temperature, relative humidity (RH) and CO<sub>2</sub> concentration, which were recorded automatically at 5 min time intervals, using one climate sensor (Hoogendoorn, Vlaardingen, The Netherlands) per greenhouse, placed at the top of the canopy. Pure CO<sub>2</sub> was supplied at a maximum rate of 23 g m<sup>-2</sup> h<sup>-1</sup> between sunrise and sunset with a set point of 1,000 µmol mol<sup>-1</sup> in each greenhouse.

Tomato (*Solanum lycopersicum* 'Cappricia') plants, scions grafted on Emperor rootstock, were planted on Rockwool slabs on 23 December 2008, at a plant of 2.5 plants m<sup>-2</sup>

( $n=300$  per greenhouse). Initially only one stem was maintained per plant. An additional side shoot was maintained on one-third of the plants ( $n=100$ ) 8 weeks after planting, increasing the stem density to  $3.33 \text{ stems m}^{-2}$ . Starting 8 weeks after planting, the lowest two-to-three leaves per stem were removed each week and the shoots were lowered to keep the tops of the plants at a constant height (3.5 m above the gutter). Fruit numbers per truss were manually restricted to six fruits per truss.

The  $\pm$ VTG treatments started on 23 March 2009 (13 weeks after planting) when the two cooling systems were turned on. Pests and diseases were controlled biologically, as much as possible, and their incidences were very low. Plants were topped 40 weeks after planting. The last fruit harvest occurred 7 weeks after topping (20 November 2009).

### Crop measurements

Eight plants in each greenhouse compartment were marked to record their truss number each week throughout the growing season. Plant biomass was measured by harvesting plants destructively at planting and 9, 19, 28, 40, and 46 weeks after planting. The fresh weights (FW) and dry weights (DW; oven dried at  $80^\circ\text{C}$  for  $> 48 \text{ h}$ ) of leaves, stems, and fruits were measured, as well as the leaf area per plant (LI-3100C Area Meter, LI-COR, Lincoln, NE, USA). FWs and DWs of the picked leaves and harvested fruits were recorded and added to the cumulative FW and DW values. The leaf area index (LAI;  $\text{m}^2 \text{ m}^{-2}$ ) was calculated from the leaf area per plant and the plant density. The specific leaf area (SLA;  $\text{cm}^2 \text{ g}^{-1}$ ) was calculated as the leaf area per unit leaf DW. To select plants for destructive measurements, each compartment was considered to be three blocks, evenly divided from North-to-South. Two plants were selected at random from each block. In total, six plants (four with a single main stem and two with a side shoot) were selected from each greenhouse compartment at each harvest. Plants with a side shoot were always selected from two different blocks. Weekly fruit harvests started 15 weeks after planting. Total fruit FW and the total number of harvested fruits, from one pre-selected double row were measured in each greenhouse to determine yields ( $\text{kg m}^{-2}$ ). Average fruit FW were calculated by dividing the total FW of the harvested fruits by the total number of harvested fruits. Fruit dry matter contents (DMC) were measured 19, 24, 28, 34, 37, and 43 weeks after planting. Five trusses were at random selected from the harvested trusses in each greenhouse, and the FW and DW (oven-dried at  $80^\circ\text{C}$  for  $> 48 \text{ h}$ ) of the fruits were measured to determine DMC (%).

### Measurements of fruit growth and development

Twenty-one weeks after planting, six Smartdust sensors (Wisensys, Emmen, The Netherlands) were attached to six randomly-selected trusses on which the second flower was just reaching anthesis, in one greenhouse of each treatment. Air temperature and relative humidity around the selected trusses were recorded automatically from 21 - 30 weeks after planting, when the trusses were harvested. The dates on which the second fruit on the selected truss reached colour stage '4' were recorded as 'breaker stage', and the duration of growth between anthesis and the breaker stage was determined. Colour stage was defined using a standard tomato colour chart (The Greenery, Breda, The Netherlands). Temperature sum (expressed in day-degrees;  $^\circ\text{C}$ ) was calculated using a base temperature of  $4^\circ\text{C}$  (as was previously used by e.g. De Koning 1994). This procedure was repeated twice, from 30 - 38 weeks after planting, and from 33 - 42 weeks after planting.

### Statistical analysis

Data on fruit yield, DMC, truss number, production and partitioning of DM, LAI, and SLA were analysed using a linear mixed model (Potvin et al. 1990). The variance components were estimated using Restricted Maximum Likelihood (REML) in Genstat (14<sup>th</sup> Edition; VSN International, Hemel Hempstead, UK). Each linear mixed model consisted of two parts: a fixed model and a random model. The fixed model consisted of the main factors: cooling position (above and below) and time (weeks after planting). The random model accounted for the fact that plant samples from the same greenhouse were linked. Individual data (not averaged) on truss numbers and fruit yields were fitted as a linear function of time.

The slopes and intercepts of the fitted curves of the two treatments were analysed using a Student's t-test ( $P \leq 0.05$ ). Individual data (not averaged) on DM production, DM partitioning, LAI, and SLA were fitted to non-linear curves as a function of time. The parameters of the curves of the two treatments were analysed using Student's t-test ( $P \leq 0.05$ ).

Pairwise comparisons of means were analysed using Student's t-test ( $P \leq 0.05$ ), comparing the duration of fruit growth and the cumulative degree-days (temperature sum) of fruits reaching the breaker stage in both greenhouses.

## Results

### Greenhouse climate

Diurnal and seasonal patterns of the climate in the treatments with cooling from above and below are described in Chapter 4.1.

### Truss initiation, leaf initiation and leaf growth

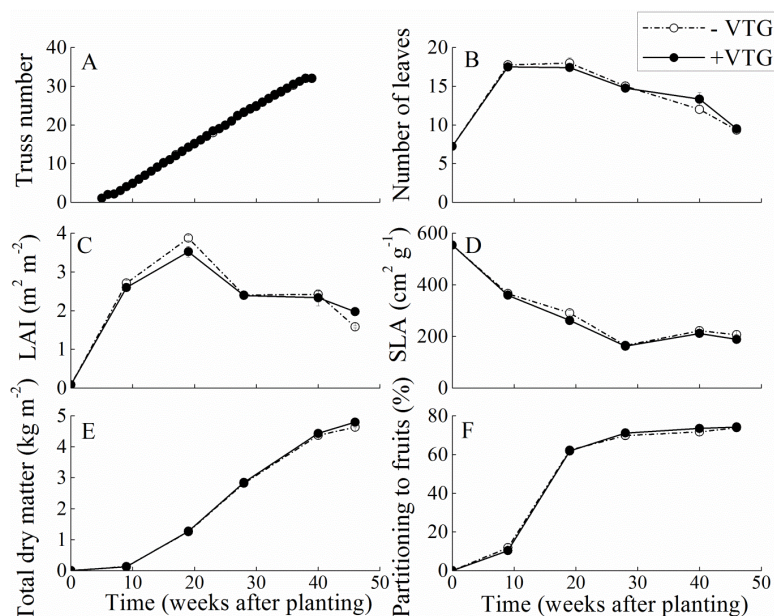
Rates of truss initiation did not differ significantly between the two treatments, as is shown by the non-significant difference in the slopes of truss number as a function of time (Fig. 1A;  $P = 0.99$ ). Given a fixed ratio of 1:3 between numbers of trusses and leaves once the first truss has appeared, the number of leaves per plant did not differ significantly either between the two treatments (Fig. 1B;  $P = 0.78$ ). LAI increased in the beginning of the growing season up to almost  $4 \text{ m}^2 \text{ m}^{-2}$  19 weeks after planting (end of April 2009; Fig. 1C). Thereafter, the LAI decreased due to leaf picking and did not differ significantly between treatments ( $P = 0.68$ ; Fig. 1C). The SLA showed a seasonal pattern with a higher value in early spring than in summer (Fig. 1D), but did not differ significantly between treatments ( $P = 0.22$ ).

### Dry matter production and partitioning

Dry matter production did not differ significantly between the two treatments ( $P = 0.16$ ; Fig. 1E). The fraction of dry matter partitioned to the fruits reached a maximum value of 72%, but did not differ significantly between the two treatments (Fig. 1F).

### Fruit growth and development

The temperature sum required from anthesis to breaker stage did not differ significantly between the two treatments (Table 1). The duration of fruit growth from anthesis to breaker stage lasted on average 1.3 days longer in the treatment +VTG compared to the treatment -VTG (Table 1).



**Figure 1.** Truss numbers (Panel A), leaf numbers (Panel B), leaf area index (LAI; Panel C), specific leaf area (SLA; Panel D), cumulative total dry matter (DM) produced (Panel E) and the percentage of DM partitioning to fruits (Panel F) throughout the growing season (December 2008 – November 2009) in treatments with (closed circles) or without (open circles) a vertical temperature gradient. Vertical bars indicate  $\pm$  standard errors of the mean.

**Table 1.** Temperature sum (expressed in degree-days;  $^{\circ}\text{C}$ ) and duration of growth of fruits from fruit set to breaker stage in treatments with or without a vertical temperature gradient

Period	Temperature sum ( $^{\circ}\text{C}$ )		Growth duration (d)	
	+ VTG	- VTG	+ VTG	- VTG
Weeks 21-30	923.7* a	931.8 a	55.9 b	54.5 ab
Weeks 30-38	894.7 a	897.1 a	54.5 ab	53.1 a
Weeks 33-42	932.0 a	931.3 a	58.7 d	57.3 c
Average	916.8 a	920.1 a	56.3 b	55.0 a

+VTG, with a vertical temperature gradient; -VTG, no vertical temperature gradient

\*Mean values (n=6) followed by different lower-case letters denote significant differences according to the Student's t-test at  $P \leq 0.05$ .

### Fruit production

The cumulative fruit production was not significantly different between treatments ( $P = 0.10$ ; Fig. 2A), being  $64.5$  and  $62.5 \text{ kg m}^{-2}$  in the treatments with or without a VTG, respectively. The average FW of harvested fruits from July to September was significantly higher ( $P = 0.02$ ) in the treatments with ( $124 \text{ g fruit}^{-1}$ ) or without a VTG ( $115 \text{ g fruit}^{-1}$ ; Fig.

2B). Throughout the growing season (47 weeks between planting and last harvest), the average FW of the harvested fruits was 118 and 112 g fruit<sup>-1</sup> for the treatments with or without a VTG, respectively ( $P = 0.06$ ). Fruit DMC did not differ between treatments at any of the six periodic harvests (Fig. 2C) and was 5.29% and 5.35% averaged over the harvests for the treatments with or without a VTG ( $P = 0.56$ ), respectively.

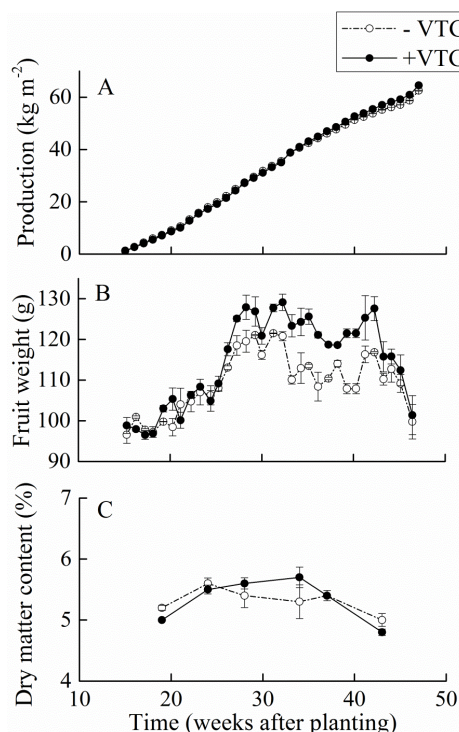


Figure 2. Pattern of cumulative fresh fruit weight (FW; Panel A), average fruit FW of harvested fruits (Panel B) and dry matter content of the harvested fruits (DMC, Panel C) throughout the season (December 2008 – November 2009) in treatments with (closed circles) or without (open circles) a vertical temperature gradient. Vertical bars indicate  $\pm$  standard errors of the mean.

## Discussion

Semi-closed greenhouses have been developed to reduce the energy consumption in horticulture, by collecting excess heat in summer, storing it in aquifers and reusing it in winter. The use of a cooling system with ducts below the gutters resulted in a VTG (Chapter 4.1; De Gelder et al. 2012). Since temperature is a main factor influencing a large number of plant physiological processes, we expected to observe significant effects of a VTG on tomato plant growth and development and underlying processes such as truss initiation rate, production and partitioning of DM, fruit growth and development. Interestingly, we found hardly any effect of the VTG on plant growth and production, even though the gradient was often  $> 5^{\circ}\text{C}$ . Effects of the VTG on underlying plant processes are discussed below.



### **Truss initiation**

In general, rates of leaf and truss initiation increase with air temperature (Adams et al., 2001). In our experiments, the greenhouse climate was controlled based on online air temperature measurements at the top of the canopy which were kept similar between treatments (Fig. 1). Therefore, processes occurring in the top of the canopy, i.e. leaf and truss initiation were unaffected.

### **Dry matter production**

Total plant DM production depends on the amount of light intercepted by the canopy and its light use efficiency. Light interception is determined by LAI and the light extinction coefficient  $k$ , which was assumed constant. In our experiment, LAI was not significantly different between treatments. Therefore, light interception by the canopy could be assumed to be similar in both treatments. In addition, the light intensity, CO<sub>2</sub> concentration and temperature at the top of the canopy did not differ between treatments in our experiment, most likely resulting in similar rates of leaf photosynthesis at the top of the canopy. At the middle and lower parts of the canopy, the leaves in treatment +VTG experienced lower temperatures. However, leaf photosynthesis is hardly sensitive to temperature at low light intensities (Chapter 5; Yamori et al. 2010). The rate of photosynthesis in the lower canopy was therefore hardly influenced by temperature due to the low light conditions. In conclusion, the effect of a VTG on net production of assimilates was negligible, and did not significantly affect the total DM production.

### **Dry matter partitioning**

DM partitioning to the fruit depends on fruit sink strength and the total number of fruits. Sink strength of the fruit is defined as the competitive ability of the fruit to attract assimilates (Marcelis 1996), and the potential fruit growth rate is a measure of sink strength of the fruit. Temperature does not influence the potential fruit growth rate of tomato (De Koning 1994; Heuvelink and Marcelis 1989). In our experiment, the lower canopy experienced lower temperatures in the treatment with a VTG. However, all plant organs (leaf, stem and fruit) at the lower part of the plant experienced the same lower temperature, and therefore, dry matter partitioning among organs was not influenced. The total number of fruits resulted from the truss initiation rate, truss number and number of fruits per truss. No differences in truss initiation rates were found between treatments, resulting in identical numbers of trusses per plant. Fruit number per truss was manually controlled at 6 fruits in both treatments. In conclusion, the fact that there were no differences in total sink strength of the fruits and in total fruit number explained the absence of treatment effect on DM partitioning to the fruit.

### **Fruit growth and development**

Temperature affects rates of fruit growth (Adams et al. 2001) and development (De Koning 2000). The temperature sensitivity of these rates depends on the stage of fruit development. During the first week after anthesis, higher temperatures increase the rate of fruit development, and therefore shorten the time to maturity (De Koning 1994; Fanwoua et al. 2012). This phase is followed by a period during which temperature hardly affects fruit growth and development (Adams et al. 2001; De Koning 1994). In the last 1 to 2 weeks before harvest, a lower temperature reduces the rate of fruit development and therefore increases the fruit growth duration and fruit FW (Adams et al. 2001; De Koning 1994). In our experiment, the number of degree-days for fruit development was identical, but the duration (number of days) of fruit growth from anthesis to breaker stage was longer in the treatment

with a VTG than without a VTG due to lower temperatures at the final stage of fruit development. This resulted in fruits with a significant higher FW during the summer months in which the VTG was largest (Figure 5B).

### **Application**

In our experiment, in which tomatoes were grown in semi-closed greenhouses with cooling ducts below the gutter, the plants experienced a VTG of  $> 5^{\circ}\text{C}$  during the day in summer, which is in agreement with results in other (semi-)closed greenhouses (Hoes et al. 2008) and in other high-wire grown crops such as cucumber and sweet pepper (De Gelder et al. 2012). Gieling et al. (2011) pointed out that a VTG requires special attention, since the response of a crop to such a microclimate was unknown. Our results show that the rate of truss initiation, production and partitioning of DM, and yield were not different in the treatments with or without a VTG, when the temperatures at the top of the canopy were the same. Higher yields were found when crops were grown in (semi-)closed greenhouses compared to conventionally ventilated greenhouses (De Gelder et al. 2012; Hoes et al. 2008; Dannehl et al. 2013), mainly due to increased concentrations of  $\text{CO}_2$  leading to higher rates of photosynthesis (Chapter 2). Furthermore, fruit quality and health-promoting compounds were increased by higher  $\text{CO}_2$  concentrations in semi-closed greenhouses (Dannehl et al. 2012; Farneti et al. 2013), implying that semi-closed greenhouses can be beneficial to plant growth, yield and product quality. Our results show that a VTG resulting from a cooling system in the lower part of the greenhouse did not affect plant growth, development and production. These results are important when designing greenhouses and their heating and cooling systems when balancing light loss due to cooling systems in the top of the greenhouse with effects of vertical temperature gradients.

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# Chapter 5

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## Quantification of photosynthesis under a wide range of climate conditions

### **Adapted from the publication:**

Qian, T., Elings A., Dieleman, J. A., Gort, G., Marcelis, L. F. M., 2012. Estimation of photosynthesis parameters for a modified Farquhar–von Caemmerer–Berry model using simultaneous estimation method and nonlinear mixed effects model. *Environmental and Experimental Botany*. 82: 66–73.

### Abstract

The aims of this paper were to modify the photosynthesis model of Farquhar, von Caemmerer and Berry (FvCB) to be able to predict light dependency of the carboxylation capacity ( $V_c$ ) and to improve the prediction of temperature dependency of the maximum carboxylation capacity ( $V_{cmax}$ ) and the maximum electron transport rate ( $J_{max}$ ). The FvCB model was modified by adding a sub-model for Ribulose-1,5-bisphosphate carboxylase (Rubisco) activation and validating the parameters for temperature dependency of  $V_{cmax}$  and  $J_{max}$ . Values of parameters for temperature dependency of  $V_{cmax}$  and  $J_{max}$  were validated and adjusted based on data of the photosynthesis response to temperature. Parameter estimation was based on measurements under a wide range of environmental conditions, providing parameters with broad validity. The simultaneous estimation method and the nonlinear mixed effects model were applied to ensure the accuracy of the parameter estimation. The FvCB parameters,  $V_{cmax}$ ,  $J_{max}$ ,  $\alpha$  (the efficiency of light energy conversion),  $\theta$  (the curvature of light response of electron transport), and  $R_d$  (the non-photorespiratory  $CO_2$  release) were estimated and validated on a dataset from two other years. Observations and predictions matched well ( $R^2=0.94$ ). We conclude that incorporating a sub-model of Rubisco activation improved the FvCB model through predicting light dependency of carboxylation rate; and that estimating  $V_{cmax}$ ,  $J_{max}$ ,  $\alpha$ ,  $\theta$ , and  $R_d$  requires data sets of both  $CO_2$  and light response curves.

## Introduction

Many studies have established the relations between photosynthesis and light intensity (Ogren and Evans, 1993; Heschel et al., 2004), CO<sub>2</sub> concentration (Cannell and Thornley, 1998), and temperature (Cannell and Thornley, 1998; Yamori et al., 2010). Most studies deal with photosynthesis response to only a few environmental factors. Integrated studies, where effects of all these environmental factors and their interactions are quantified in a wide range, are scarce.

The model of Farquhar, von Caemmerer and Berry (1980) ('the FvCB model' hereafter) is the most commonly used over the past three decades to study the response of C<sub>3</sub> photosynthesis to environment. The model predicts net photosynthesis rate ( $A$ ) at any given environmental condition. The CO<sub>2</sub> dependency of photosynthesis rate is determined as the minimum value of three distinct states, limited by Ribulose-1,5-bisphosphate carboxylase (Rubisco) for carboxylation, ribulose-1,5-bisphosphate (RuBP) regeneration, or triose phosphate utilization (TPU). The light dependency of photosynthesis rate is determined by the light response of electron transport rate ( $J$ ). The relation between  $J$  and light intensity was first described as a rectangular hyperbola function (Farquhar and von Caemmerer, 1982) and later modified to a non-rectangular hyperbola function (Farquhar and Wong, 1984; Von Caemmerer, 2000). The temperature dependency of the FvCB parameters related to kinetic properties of Rubisco is described based on the Arrhenius function (Farquhar et al., 1980; Bernacchi et al., 2001; Medlyn et al., 2002a). The original functions to describe the temperature dependency of  $V_{cmax}$  and  $J_{max}$ , were modified in many studies (Dreyer et al., 2001; Leuning, 2002; Medlyn et al., 2002b; Warren and Dreyer, 2006). The peaked function was considered the best, since it predicts the  $V_{cmax}$  and  $J_{max}$  at the super-optimal temperature with the parameter deactivation energy ( $H_d$ ) (Medlyn et al., 2002b). Parameter values for the activation energy ( $H_a$ ), deactivation energy ( $H_d$ ), and the entropy factor ( $S$ ) were estimated for different species (Harley et al., 1992b; Bunce, 2000; Bernacchi et al., 2001; Dreyer et al., 2001;).

The FvCB model assumes that Rubisco is always fully activated (Farquhar et al., 1980; Von Caemmerer, 2000). The consequence of this assumption is that the carboxylation rate of Rubisco ( $V_c$ ) is independent of light intensity. In other words,  $V_c$  is assumed to be equal to  $V_{cmax}$ . However, several studies (Taylor and Terry, 1984; Salvucci et al., 1986; Von Caemmerer and Edmondson, 1986; Brooks et al., 1988;) have shown that the fraction of Rubisco activation increases with light intensity. Using  $V_c$  as  $V_{cmax}$  derived under low light condition to determine photosynthesis rate under high light condition might cause under-estimation of photosynthesis rate. It is therefore necessary to extend the FvCB model with a sub-model of light dependency of  $V_c$ , relating  $V_c$  to Rubisco activation.

The FvCB model is often simplified to two limitations, since the TPU limitation occurs only occasionally in case of saturated photosynthesis rate or even decreased photosynthesis rate with increased CO<sub>2</sub> concentration (Long and Bernacchi, 2003; Sharkey et al., 2007). The CO<sub>2</sub> response curves are then fitted with two nonlinear functions either limited by Rubisco or RuBP regeneration, taking the minimum value of the two. The methods used to fit the curves to the data and estimate the parameters are not yet consistent in literature. One method is the disjunct segments estimation method, separately fitting the functions of Rubisco-limited photosynthesis and of RuBP-regeneration-limited photosynthesis (Manter and Kerrigan, 2004; Onoda et al., 2005; Sharkey et al., 2007). In this method, gas exchange data are divided into two subsets. Sub-setting is usually subjective, as it is not possible to unambiguously allocate data points to both processes. Arbitrary division of the two subsets has a significant effect on the estimation of the parameters (Miao et al., 2009). The second method is the simultaneous estimation method (Dubois et al., 2007), which simultaneously estimates the parameters for both functions using the entire gas exchange data set. This method avoids the need for

preliminary division of the gas exchange data before analysis. However, the simultaneous estimation method is not commonly applied to gas exchange data for the study of effects of environmental factors on photosynthesis.

Typically, data sets of light and CO<sub>2</sub> responses curves possess two characteristics. The first characteristic is that the data set usually involves repeated measurements. Gas exchange measurements are obtained on one leaf over a series of light intensities or CO<sub>2</sub> concentrations. Proper data analysis should take into account that observations obtained from the same experimental unit (one leaf) are correlated, as otherwise the estimated error variance and standard errors of parameter estimates may be wrong (Potvin et al., 1990; Peek et al., 2002). The second characteristic is the increase in variation of the photosynthesis rate with increasing light intensity or CO<sub>2</sub> concentration (Peek et al., 2002; Lin et al., 2008). If the non-constant variance is ignored, the standard deviation will be overestimated at low light intensity or CO<sub>2</sub> concentration, and underestimated at high light intensity or CO<sub>2</sub> concentration. To accommodate for these two characteristics, Peek et al. (2002) proposed the use of nonlinear mixed effects models in photosynthesis response curves. However, only a few studies applied the nonlinear mixed-effects model to their data analysis to investigate treatment differences (Peek et al., 2002; Heschel et al., 2004; McElrone and Forseth, 2004; Ozturk et al., 2011).

The aims of this paper were to modify the FvCB model to be able to predict light dependency of  $V_c$  and to improve the prediction of temperature dependency of  $V_{cmax}$  and  $J_{max}$ . Parameter estimation was based on measurements under a wide range of environmental conditions, providing parameters with broad validity. CO<sub>2</sub> response curves were analysed by the simultaneous estimation method rather than the traditional disjunctive segments estimation method. A nonlinear mixed effects model was used to account for the fact that photosynthesis response measurements involved repeated measurements on the same leaf. The simultaneous estimation method and the nonlinear mixed effects model ensured the accuracy of the parameter estimation.

## Materials and methods

### Plant cultivation

Tomato (*Solanum lycopersicum*, cultivar ‘Cappricia’) plants, grafted on the rootstock Emperor, were planted on Rockwool® on 23 December 2008 in an air conditioned greenhouse. The greenhouse had a size of 144 m<sup>2</sup> with a gutter height of 5.5 m, and was located at Bleiswijk, the Netherlands. Initial stem density was 2.5 stem m<sup>-2</sup>. Stem density was increased to 3.3 stems m<sup>-2</sup> eight weeks after planting. A standard horticultural computer (Hoogendoorn-Economic) controlled the environment inside the greenhouse. Photosynthesis measurements were conducted during July and August 2009. Daily average outside radiation in July and August 2009 was 18.17 MJ m<sup>-2</sup> d<sup>-1</sup>. Realized day/night temperatures, CO<sub>2</sub> concentration and relative humidity averaged over July and August 2009 in the greenhouse were 22.3/17.6 °C, 759/486 µmol mol<sup>-1</sup>, and 80/86 %, respectively. Water and nutrients were adequately supplied.

### Photosynthesis measurements

Leaf photosynthesis rate was measured with a portable photosynthesis device (LCpro+, ADC, UK) at two leaf positions in the canopy, namely the uppermost fully unfolded leaf (top leaf) and the leaf near the middle of the canopy (middle leaf). Light intensity, CO<sub>2</sub> concentration, temperature, and humidity were controlled in the leaf chamber of the device. Measurements were carried out between 9:00 and 15:00 to avoid photosynthesis afternoon depression.



CO<sub>2</sub> response of photosynthesis was measured at CO<sub>2</sub> concentration levels between 50 and 1600  $\mu\text{mol mol}^{-1}$ . The starting CO<sub>2</sub> concentration was 600  $\mu\text{mol mol}^{-1}$ , followed by 400, 200, 50, 600, 800, 1200, 1600  $\mu\text{mol mol}^{-1}$ . CO<sub>2</sub> concentration of the air in the leaf chamber ( $C_a$ ) was measured, and intercellular CO<sub>2</sub> concentration ( $C_i$ ) was the output from the device calculated based on the function described by Von Caemmerer and Farquhar (1981). Air temperature and vapour pressure deficit (VPD) in the leaf chamber were maintained at 27 °C and values below 1 kPa, respectively. CO<sub>2</sub> response curves were determined at 1395 and 465  $\mu\text{mol m}^{-2} \text{s}^{-1}$  incident photosynthetic active radiation (PAR). 1395  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR was considered as high light intensity at which Rubisco was fully activated, and 465  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR was considered as low light intensity at which Rubisco was not fully activated. For each light intensity and canopy depth, six leaves were randomly selected from the greenhouse for six CO<sub>2</sub> response curves. The order of light intensity and canopy depth observations was randomized.

Light response of photosynthesis was measured at PAR levels between 0 and 1860  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The starting level of PAR was 465  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , followed by 233, 93, 0, 465, 930, 1395, 1860  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR. Light response measurement did not start at the highest light intensity to avoid photo-inhibition (Leverenz et al., 1990). Air temperature and VPD in the leaf chamber were maintained at 27 °C and below 1 kPa, respectively. Light response curves were measured at four CO<sub>2</sub> concentrations, which were set to 400, 800, 1200 and 1600  $\mu\text{mol mol}^{-1}$  in the leaf chamber. For each CO<sub>2</sub> concentration and each canopy depth, six leaves were randomly selected from the greenhouse for six light response curves. The order of CO<sub>2</sub> concentration and canopy depth observations was randomized.

Temperature response of photosynthesis was measured at air temperatures of 24, 26, 28, 30, 32, 34, 36, and 38 °C. Temperature response curves were measured at two CO<sub>2</sub> concentrations (1200 and 400  $\mu\text{mol mol}^{-1}$ ) and two light intensities (1395 and 465  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR). For each temperature, light intensity, CO<sub>2</sub> concentration, and canopy depth, six leaves were randomly selected from the greenhouse. The order of temperature, light intensity, CO<sub>2</sub> concentration and canopy depth observations was randomized. VPD in the leaf chamber was maintained below 1 kPa. However, when air temperature in the chamber was increased above 30 °C, VPD could not be maintained below 1 kPa. Measurements on the VPD response of photosynthesis showed that the photosynthesis rate was not affected by VPD between 1 and 3 kPa (data not shown).

### The modified FvCB model

In our CO<sub>2</sub> response measurements, we did not detect saturated or decreased photosynthesis rate with increased CO<sub>2</sub> concentration. The model, therefore, was simplified to two limitations

$$A = \min\{A_c, A_j\} \quad (1)$$

Where  $A$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) is net photosynthesis rate,  $A_c$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) is Rubisco carboxylation limited photosynthesis rate, and  $A_j$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) RuBP regeneration limited photosynthesis rate.

$$A_c = \frac{V_c(C_i - \Gamma^*)}{C_i + K_c(1 + O/K_o)} - R_d \quad (2)$$

Where  $V_c$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) is the carboxylation capacity at certain light intensity,  $\Gamma^*$  ( $\mu\text{mol mol}^{-1}$ ) is the CO<sub>2</sub> compensation point,  $K_c$  ( $\mu\text{mol mol}^{-1}$ ) is the Michaelis-Menten constant of Rubisco for CO<sub>2</sub>,  $K_o$  ( $\text{mmol mol}^{-1}$ ) is the Michaelis-Menten constant of Rubisco for O<sub>2</sub>,  $O$  (210  $\text{mmol mol}^{-1}$ ) is the oxygen concentration,  $R_d$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) is non-photorespiratory CO<sub>2</sub> release, which comprised mitochondrial respiration.

$$A_j = \frac{J(C_i - \Gamma^*)}{4C_i + 8\Gamma^*} - R_d \quad (3)$$

Where  $J$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) is the electron transport rate at certain light intensity. The light dependency of  $J$  is determined by a non-rectangular hyperbola (Farquhar and Wong, 1984)

$$J = \frac{\alpha PAR + J_{\max} - \sqrt{(\alpha PAR + J_{\max})^2 - 4\theta J_{\max} \alpha PAR}}{2\theta} \quad (4)$$

Where  $J_{\max}$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) is the maximum electron transport rate,  $\alpha$  ( $\text{mol e}^- \text{mol}^{-1} \text{photon}$ ) is the efficiency of light energy conversion on an incident light basis,  $\theta$  (dimensionless) is the curvature of the light response of  $J$ .

$V_c$  is equal to  $V_{c\max}$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), the maximum carboxylation capacity, if Rubisco is fully activated. Literature data in combination with our own data (see 2.4) showed Rubisco activation increased with light intensity. This relationship was well described by an empirical logistic function (Fig. 1). Assuming  $V_c$  to be proportional to Rubisco activation,  $V_c$  was described by

$$V_c = V_{c\max} (24.5 + 75.4 \times (1 - \exp(-PAR / 659))) / 100 \quad (5)$$

$R_d$ ,  $K_c$ ,  $K_o$  and  $I^*$  ( $Parameter_{Tleaf}$ ) at leaf temperature  $T_{leaf}$  ( $^{\circ}\text{C}$ ) were determined by an Arrhenius function

$$Parameter_{Tleaf} = \exp(c - H_a / R(T_{leaf} + 273.15)) \quad (6)$$

Where  $c$  (dimensionless) is a scaling constant,  $H_a$  ( $\text{J mol}^{-1}$ ) is the activation energy, and  $R$  ( $8.314 \text{ J K}^{-1} \text{mol}^{-1}$ ) is the molar gas constant. The values of  $c$  and  $H_a$  for calculating  $R_d$ ,  $K_c$ ,  $K_o$  and  $I^*$  at  $T_{leaf}$  were from Bernacchi et al. (2001), and listed in Table 1.

$V_{c\max}$  and  $J_{\max}$  ( $Parameter_{Tleaf}$ ) at  $T_{leaf}$  were determined by a peaked function, which is a modified Arrhenius function (Medlyn et al., 2002a)

$$Parameter_{Tleaf} = Parameter_{28} \exp\left(\frac{H_a(T_{leaf} - 28)}{(28 + 237.15)R(T_{leaf} + 273.5)}\right) \times \frac{1 + \exp\left(\frac{S - H_d / (28 + 273.15)}{R}\right)}{1 + \exp\left(\frac{S - H_d / (T_{leaf} + 237.15)}{R}\right)} \quad (7)$$

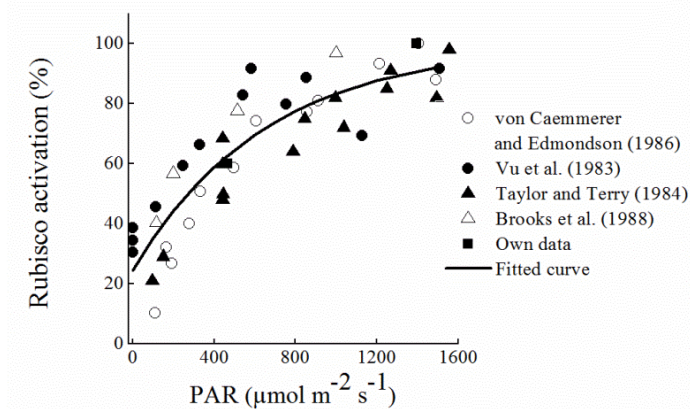
Where  $Parameter_{28}$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) is the value of the parameter  $V_{c\max}$  or  $J_{\max}$  at leaf temperature of  $28^{\circ}\text{C}$ .  $H_d$  ( $\text{J mol}^{-1}$ ) is the deactivation energy.  $S$  ( $\text{J K}^{-1} \text{mol}^{-1}$ ) is the entropy factor. The values of  $H_a$ ,  $H_d$ , and  $S$  for calculating  $V_{c\max}$  and  $J_{\max}$  at given temperatures were from Harley et al. (1992b), and listed in Table 1.

Equations 1-4 are the basic equations of the FvCB model, predicting photosynthesis response to  $\text{CO}_2$  and light. Adding our empirical eqn. 5, the model is able to predict the light dependency of  $V_c$ . Coupled with eqns. 6 and 7, the model can also predict photosynthesis response to temperature.

**Table 1.** Parameter values and literature sources used for calculating  $K_c$ ,  $K_o$ ,  $I^*$ ,  $R_d$ , of eqn. 6, and  $V_{cmax}$ , and  $J_{max}$  of eqn. 7 at given temperatures.

Parameter	$H_a$ (J mol <sup>-1</sup> )	$H_d$ (J mol <sup>-1</sup> )	$S$ (J K <sup>-1</sup> mol <sup>-1</sup> )	$c$	Reference
$K_c$	79430			38.05	Bernacchi et al. (2001)
$K_o$	36380			20.30	Bernacchi et al. (2001)
$I^*$	38830			19.02	Bernacchi et al. (2001)
$R_d$	46390			18.72	Bernacchi et al. (2001)
$V_{cmax}$	91185 <sup>1</sup>	202900	650		Harley et al. (1992b)
$J_{max}$	79500	201000	650		Harley et al. (1992b)

<sup>1</sup> The value of  $H_a$  for  $V_{cmax}$  was estimated based on temperature response curves of our own data

**Figure 1.** Dependency of Rubisco activation on light intensity. A logistic function, Rubisco activation =  $24.5 + 75.4(1 - \exp(-PAR/659))$  ( $R^2=0.79$ ), was fitted to literature and own data (the two data points ■ were estimated from our own CO<sub>2</sub> response curves at two light intensities, assuming Rubisco activation was proportional to  $V_c$ ).

### Parameter estimation and validation

The nonlinear mixed effects model was in the form

$$y_{ij} = f(x_{ij}, \beta, u_i) + e_{ij} \quad (8)$$

Where function  $f$  is the nonlinear function (eqns. 2, 3, or 4) describing the CO<sub>2</sub> or light dependency of leaf photosynthesis,  $x_{ij}$  is the covariate vector for the  $j^{th}$  observation on the  $i^{th}$  experimental unit, consisting of CO<sub>2</sub> concentration, light intensity, and canopy depth;  $\beta$  is the vector of unknown fixed effect parameters, containing  $V_{cmax}$ ,  $R_d$ ,  $\alpha$ , and  $\theta$ , with possibly different values for the two canopy depths;  $u_i$  is the vector of random effect terms for  $i^{th}$  experimental unit, consisting of random deviations  $v_i$  and  $w_i$  of the population parameter values  $V_{cmax}$  and  $J_{max}$ .  $e_{ij}$  is a vector of unknown random errors. The random deviations  $v_i$  and  $w_i$  were allowed to be correlated, with possibly different variance-covariance matrices for the two canopy depths. The resulting model is an example of a nonlinear random coefficients model.

CO<sub>2</sub> response data were used to estimate the  $V_c$  at two light intensities by using eqns. 1, 2, 3, 6 in the nlme (nonlinear mixed effects model) package of the R-software (version 2.9.2). The simultaneous estimation method described by Dubois et al. (2007) was applied. The estimated value of  $V_c$  at  $465 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR was 61% of the value at  $1395 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR. In this way, the two data points representing our own data in Fig. 1 were derived, assuming

Rubisco was fully activated at  $1395 \mu\text{mol m}^{-2} \text{s}^{-1} \text{ PAR}$ . The other light dependent activation data of Rubisco in Fig. 1 were obtained from literature. The relation between  $V_c$  and Rubisco activation was assumed to be proportional. A logistic function was chosen to describe the light dependency of Rubisco activation. The parameters of the logistic function were estimated based on the data points in Fig. 1, resulting in the empirical prediction function eqn. 5.

Light and  $\text{CO}_2$  response data were used together to estimate the FvCB parameters  $V_{cmax}$ ,  $J_{max}$ ,  $\alpha$ ,  $\theta$ , and  $R_d$  at leaf temperature of  $28^\circ\text{C}$  by using eqns. 1-6 in the nlme package of the R-software. The simultaneous estimation method described by Dubois et al. (2007) was applied.

For validation, the derived parameters by using the nonlinear mixed effect model were tested against measurements of photosynthesis rate at  $28^\circ\text{C}$  of tomato (*Solanum lycopersicum* 'Cappricia') in two other years (2008 and 2010). The photosynthesis rates of these two years were measured in a greenhouse at varying light intensities ( $0\text{-}1395 \mu\text{mol m}^{-2} \text{s}^{-1} \text{ PAR}$ ) and  $\text{CO}_2$  concentrations ( $50\text{-}1600 \mu\text{mol mol}^{-1}$  air  $\text{CO}_2$  concentration). Eqns. 1- 6 were used to calculate  $A$  from the derived parameters,  $V_{cmax}$ ,  $J_{max}$ ,  $\alpha$ ,  $\theta$ , and  $R_d$ , based on the  $C_i$ ,  $\text{PAR}$  and  $T_{leaf}$  measured with each data point.

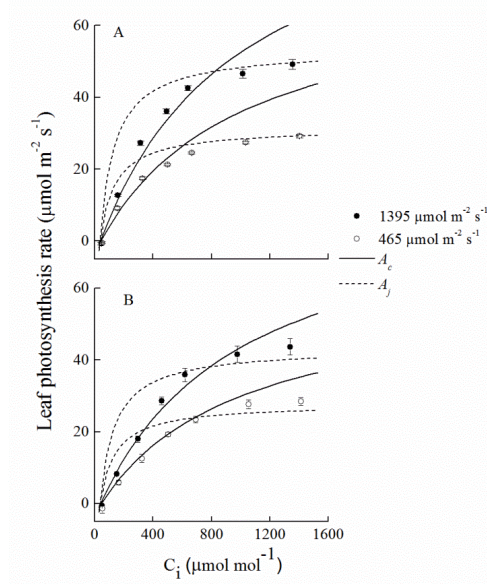
To compare the FvCB models that included and excluded the sub-model of Rubisco activation, parameter estimation was carried out by using eqns. 1, 2, 3, 4, 6 (excluding eq. 5, the sub-model of Rubisco activation). The derived parameters  $V_{cmax}$ ,  $J_{max}$ ,  $\alpha$ ,  $\theta$ , and  $R_d$ , were used to calculate the  $A$  for light response curves at four air  $\text{CO}_2$  concentrations ( $400$ ,  $800$ ,  $1200$ , and  $1600 \mu\text{mol mol}^{-1}$ ) and  $28^\circ\text{C}$  leaf temperature; for  $\text{CO}_2$  response curves at two light intensities ( $1395$  and  $465 \mu\text{mol m}^{-2} \text{s}^{-1} \text{ PAR}$ ) and  $28^\circ\text{C}$  leaf temperature; and for temperature response curves at two  $\text{CO}_2$  concentrations ( $1200$  and  $400 \mu\text{mol mol}^{-1}$ ) and two light intensities ( $1395$  and  $465 \mu\text{mol m}^{-2} \text{s}^{-1} \text{ PAR}$ ), using Eqns. 1, 2, 3, 4, 6, based on the  $C_i$ ,  $\text{PAR}$  and  $T_{leaf}$  measured with each data point.

### Incorporation of temperature dependency of $V_{cmax}$ and $J_{max}$ in the FvCB model

Estimation of three parameters,  $H_a$ ,  $H_d$ , and  $S$ , resulted in an over-parameterization problem, as often has occurred in other studies (Harley et al., 1992a; Medlyn et al., 2002b). Estimation of only  $H_a$  for  $V_{cmax}$  on the basis of temperature response data was possible, by using eqns. 1, 2, 3, 4, 5, 6, and 7.  $H_d$  and  $S$  for  $V_{cmax}$  were fixed as constant, using the value from Harley et al. (1992) (Table 1.). Calculated temperature response curves were compared with measured temperature response curves.

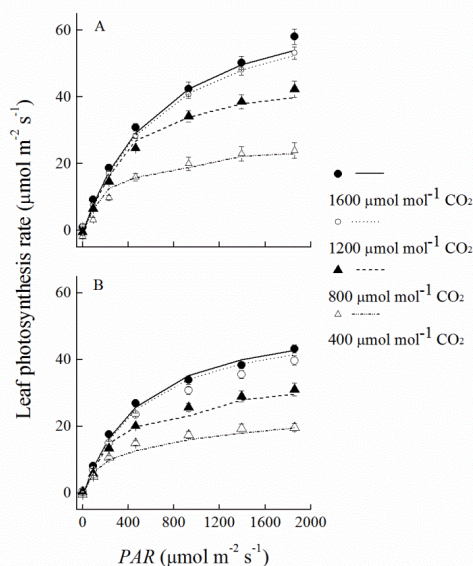
### Results

As  $\text{CO}_2$  concentration increased, the effect of light intensity on photosynthesis rate increased (Fig. 2), indicating a shift of photosynthesis from the Rubisco-limited process to the RuBP regeneration limited process. Light intensity had a significant effect on  $V_c$  (P-value  $< 0.001$ ).  $V_c$  was  $122 \mu\text{mol m}^{-2} \text{s}^{-1}$  at  $1395 \mu\text{mol m}^{-2} \text{s}^{-1} \text{ PAR}$  and  $71 \mu\text{mol m}^{-2} \text{s}^{-1}$  at  $465 \mu\text{mol m}^{-2} \text{s}^{-1} \text{ PAR}$  for the top leaf; and  $102 \mu\text{mol m}^{-2} \text{s}^{-1}$  at  $1395 \mu\text{mol m}^{-2} \text{s}^{-1} \text{ PAR}$  and  $65 \mu\text{mol m}^{-2} \text{s}^{-1}$  at  $465 \mu\text{mol m}^{-2} \text{s}^{-1} \text{ PAR}$  for the middle leaf. On average, the value of  $V_c$  at  $465 \mu\text{mol m}^{-2} \text{s}^{-1} \text{ PAR}$  was about 61% of the value of  $V_c$  at  $1395 \mu\text{mol m}^{-2} \text{s}^{-1} \text{ PAR}$ .



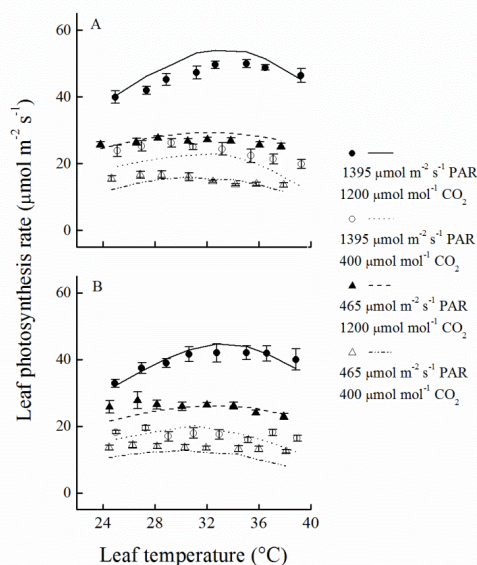
**Figure 2.** CO<sub>2</sub> response of photosynthesis of the top leaf (A) and middle leaf (B) at 1395 and 465  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR. Vertical bars indicate standard error of mean (n=6). Symbols represent measured data. Lines indicated the fitted curves of Rubisco limited photosynthesis (solid line) and RuBP regeneration limited photosynthesis (dashed line).

CO<sub>2</sub> concentration affected the light response of photosynthesis of both top leaf and middle leaf (Figs. 3A and 3B). For the top leaf, increasing the CO<sub>2</sub> concentration from 400 to 800, and from 800 to 1200  $\mu\text{mol mol}^{-1}$ , increased the maximum photosynthesis rate by 87% and 33%, respectively (Fig. 3A). For the middle leaf, increasing the CO<sub>2</sub> concentration from 400 to 800, and from 800 to 1200  $\mu\text{mol mol}^{-1}$ , increased the maximum photosynthesis rate by 65% and 35%, respectively (Fig. 3B). Further increase of CO<sub>2</sub> concentration from 1200 to 1600  $\mu\text{mol mol}^{-1}$  only increased the maximum photosynthesis rate by 6% for the top leaf (Fig. 3A) and 4% for the middle leaf (Fig. 3B). In addition, the light response curves showed no saturation at the highest light intensity 1860  $\mu\text{mol m}^{-2} \text{s}^{-1}$  when CO<sub>2</sub> concentration was equal to or higher than 800  $\mu\text{mol mol}^{-1}$ .



**Figure 3.** Observed (symbols) and predicted (lines) light response of photosynthesis of the top leaf (A) and middle leaf (B) at 1600  $\mu\text{mol mol}^{-1}$ , 1200  $\mu\text{mol mol}^{-1}$ , 800  $\mu\text{mol mol}^{-1}$ , and 400  $\mu\text{mol mol}^{-1}$   $\text{CO}_2$  concentrations. Vertical bars indicate standard error of mean (n=6).

The temperature response of leaf photosynthesis showed an optimum at about 32–36 °C at 1395  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR and 1200  $\mu\text{mol mol}^{-1}$   $\text{CO}_2$  (Fig. 4A and 4B). However, at low light or low  $\text{CO}_2$  concentration, the peak is less evident.



**Figure 4.** Observed (symbols) and predicted (lines) temperature response of photosynthesis of the top leaf (A) and middle leaf (B) at four combinations of light intensity and  $\text{CO}_2$  concentration: 1395  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR and 1200  $\mu\text{mol mol}^{-1}$   $\text{CO}_2$ , 1395  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR and 400  $\mu\text{mol mol}^{-1}$   $\text{CO}_2$ , 465  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR and 1200  $\mu\text{mol mol}^{-1}$   $\text{CO}_2$ , 465  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR and 400  $\mu\text{mol mol}^{-1}$   $\text{CO}_2$ . Vertical bars indicate standard error of mean (n=6).

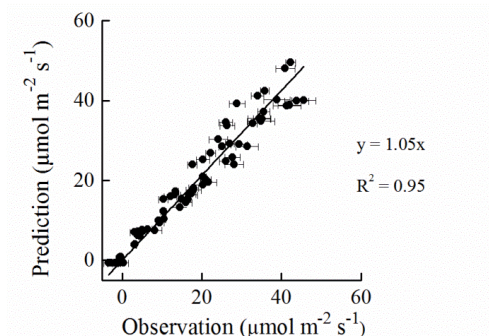
The FvCB parameters were estimated by using a nonlinear mixed effect model (Table 2). In the analysis, parameters  $V_{cmax}$ ,  $J_{max}$ ,  $\alpha$ ,  $\theta$ , and  $R_d$  were allowed to be different between leaf positions. Leaf position had a significant effect on  $V_{cmax}$  (P-value < 0.001) and  $J_{max}$  (P-value < 0.001), but not on  $\alpha$  (P-value = 0.39),  $\theta$  (P-value = 0.98), and  $R_d$  (P-value = 0.16). Therefore, the effects of leaf position on  $\alpha$ ,  $\theta$ , and  $R_d$  were removed from the model. The final model included separate values of parameters  $V_{cmax}$  and  $J_{max}$  for top and middle leaf, and random deviations of  $V_{cmax}$  and  $J_{max}$  per leaf from the population values. The FvCB parameters were also estimated by using the ordinary nonlinear model (Table 2). The analysis using an ordinary nonlinear model also showed that leaf position had a significant effect on  $V_{cmax}$  (P-value < 0.001) and  $J_{max}$  (P-value < 0.001), while no effect on  $\alpha$  (P-value = 0.82),  $\theta$  (P-value = 0.33), and  $R_d$  (P-value = 0.76) was found.

**Table 2.** Parameter values (standard error in parenthesis) of the FvCB photosynthesis model for two leaf positions in the canopy estimated on the basis of light and CO<sub>2</sub> response curves, using nonlinear mixed effect model and ordinary nonlinear model.

Parameter	Leaf position	Value estimated by Nonlinear mixed effect model		Value estimated by Ordinary model	
$V_{cmax}$	Top	117	(4.3)	125	(2.2)
	Middle	97	(3.1)	99	(2.0)
$J_{max}$	Top	315	(12.3)	331	(10.1)
	Middle	235	(10.7)	238	(14.3)
$\alpha$	Top and middle	0.43	(0.020)	0.39	(0.030)
$\theta$	Top and middle	0.19	(0.132)	0.22	(0.231)
$R_d$	Top and middle	0.70	(0.187)	0.63	(0.324)

Temperature response of photosynthesis was estimated with the FvCB parameter values obtained. The predicted and observed values were satisfactorily close at high CO<sub>2</sub> levels. A mismatch was detected at low CO<sub>2</sub> levels (data not shown) when we applied the value of  $H_a$ , 116300 J mol<sup>-1</sup>, for  $V_{cmax}$  from Harley's work (Harley et al., 1992b). This mismatch was caused by an inaccurate temperature dependency of  $V_{cmax}$  in the model. Therefore, we estimated the  $H_a$ , 91185 J mol<sup>-1</sup>, for  $V_{cmax}$  from our own temperature response data (Table 1), resulting in improved prediction (Fig. 4).

The validation of the model on data from two other years (Fig. 5.) showed that the predictions using the values derived by nonlinear mixed effect model were very close to the observed values ( $R^2=0.95$ , estimated relationship  $y = 1.05x$ ). The importance of the Rubisco activation sub-model was tested by comparing the predicted light response curves, CO<sub>2</sub> response curves and temperature response curves, using the parameters derived from the FvCB model including and excluding the sub-model of light dependency of  $V_c$ . When this sub-model was excluded,  $A$  was over-estimated near the transition point (intersection of  $A_c$  and  $A_j$ ). Consequently, the light response curves at air CO<sub>2</sub> concentration of 400  $\mu\text{mol mol}^{-1}$  showed over-estimation of  $A$  at  $PAR$  levels of about 200-500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Fig. 6A). The CO<sub>2</sub> response curve at 465  $\mu\text{mol m}^{-2} \text{s}^{-1} PAR$  showed over-estimation of  $A$  at  $C_i$  concentrations of about 300-500  $\mu\text{mol mol}^{-1}$  (Fig. 6B). As a result, the temperature response of  $A$  was over-estimated at 400  $\mu\text{mol mol}^{-1}$  air CO<sub>2</sub> concentration and 465  $\mu\text{mol m}^{-2} \text{s}^{-1} PAR$  (Fig. 6C). Apart from these data points close to the transition point, the predictions by both models including and excluding the Rubisco activation sub-model were similar and matched the observed values well for the rest of the response curves (data not shown). Similar results were observed for top and middle leaves (data not shown).



**Figure 5.** Observed and predicted photosynthesis rate using the estimated FvCB parameters (Table 2). Horizontal bars indicate standard error of mean (n=6).

## Discussion

### Validity domain

In this study, the FvCB parameters,  $V_{cmax}$ ,  $J_{max}$ ,  $\alpha$ ,  $\theta$ , and  $R_d$ , were estimated based on photosynthesis data measured at a wide range of light intensities and CO<sub>2</sub> concentrations. It broadened the validity domain of the estimated parameters for light response ranging from 0 to almost 2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and for CO<sub>2</sub> response ranging from 50 to 1600  $\mu\text{mol mol}^{-1}$ . With regards to the temperature response of photosynthesis, joint estimation of  $H_a$ ,  $H_d$ , and  $S$  suffered from over-parameterization in many studies (Harley et al., 1992a; Medlyn et al., 2002b). We used Harley's (1992b) values of  $H_a$ ,  $H_d$ , and  $S$  to determine the temperature response of  $V_{cmax}$  and  $J_{max}$ . Harley's (1992b) values were validated against our temperature response curves measured at temperature ranging from 24 to 38 °C under two light intensities and two CO<sub>2</sub> concentrations). The mismatch between some measured data points and the estimation might due to the fact that Harley's parameter values were derived based on measurements on cotton, and our data were measured on tomato. Parameter values of  $H_a$ ,  $H_d$ , and  $S$  for  $V_{cmax}$  for tomato are available in literature (Bunce 2000), but not for  $J_{max}$ . We therefore decided to use the values of  $H_a$ ,  $H_d$ , and  $S$  for both  $V_{cmax}$  and  $J_{max}$  from Harley's (1992b) work, which are the most used values in other studies.

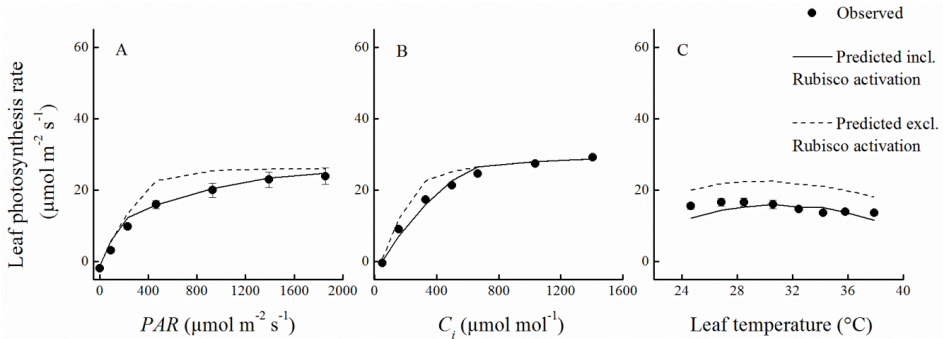
There is increasing evidence that mesophyll conductance ( $g_m$ ) might be limiting CO<sub>2</sub> diffusion from the intercellular airspace to the site of carboxylation in the chloroplast, resulting in significant lower CO<sub>2</sub> concentration at the site of carboxylation ( $C_c$ ) compared to  $C_i$  (Flexas et al., 2008). The three most commonly used approaches to estimate  $g_m$  are based on gas exchange data only (Sharkey et al., 2007), combination of gas exchange data with fluorescence data (Yin and Struik, 2009), or with data on photosynthesis response to O<sub>2</sub> (Bunce, 2009). However, estimating  $g_m$  from our gas exchange data only was risky (Pons et al., 2009), therefore we used  $C_i$  in our study as most studies do. Assuming infinite  $g_m$  in our analysis meant that an appropriate consideration was needed in choosing values of Rubisco kinetic constants ( $K_c$ ,  $K_o$ ,  $\Gamma^*$ ) (Bernacchi et al., 2002). We choose the parameter values for temperature dependency of  $K_c$ ,  $K_o$ ,  $\Gamma^*$ , from Bernacchi et al (2001) and for  $V_{cmax}$  from Harley et al (1992), as they also assumed a  $C_i$ -based FvCB model.

### Rubisco activation

In the original FvCB model,  $V_{cmax}$  was used instead of  $V_c$  in eqn. 2, assuming that Rubisco is always fully activated. Taylor and Terry (1984) found that the percentage of activated Rubisco increased from 25% to 90%, with increasing light intensity from 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  to 1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Von Caemmerer & Edmondson (1986) also found that the activated Rubisco increased with increasing light intensity, and that only 50% Rubisco was



activated at a light intensity of  $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Ogren and Evans (1993) indicated that full activation often required  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ . However, reported light intensities used in  $\text{CO}_2$  response measurements varied from  $400 \mu\text{mol m}^{-2} \text{s}^{-1}$  to over  $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ , without testing whether these light intensities were high enough to fully activate Rubisco (Wullschlegel, 1993). We modified the FvCB model by including an empirical sub-model of light dependency of  $V_c$  (eqn. 5). In this way, the estimated  $V_{cmax}$  is similar to  $V_c$  when Rubisco is fully activated by light. We conclude that the sub-model of light dependency of  $V_c$  was of importance, especially at conditions around the transition point, on the basis of a comparison of predicted photosynthesis rate when using the FvCB model including and excluding the sub-model of light dependency of  $V_c$  (Fig. 6).



**Figure 6.** Observed (symbols) and predicted (lines) light response curves at  $400 \mu\text{mol mol}^{-1}$  air  $\text{CO}_2$  concentration and  $28^\circ\text{C}$  leaf temperature (A);  $\text{CO}_2$  response curves at  $465 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR and  $28^\circ\text{C}$  leaf temperature (B); and temperature response curves at  $400 \mu\text{mol mol}^{-1}$  air  $\text{CO}_2$  concentration and  $465 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR (C) of the top leaf. The predictions used the estimated parameters of the FvCB models including (solid lines) and excluding (dash lines) the sub-model of Rubisco activation. Vertical bars indicate standard error of mean ( $n=6$ ).

Rubisco activation was found to decrease with increased  $\text{CO}_2$  concentration at low light intensity (Sage et al., 1990; Crafts-Brandner and Salvucci, 2004) and high temperature (Crafts-Brandner and Salvucci, 2004; Cen and Sage, 2005), indicating that Rubisco activation is not only a function of light intensity. However, there is no complete information on how light intensity,  $\text{CO}_2$  concentration, and temperature interact to modulate Rubisco activation. This requires more research.

### Combination of light and $\text{CO}_2$ response curves for parameter estimation

The FvCB parameters are commonly estimated only from  $\text{CO}_2$  response curves ( $A/C_i$  curves), while only incidentally combined data from  $\text{CO}_2$  and light response curves are used (Braune et al., 2009).  $J_{max}$  cannot be directly estimated if only  $\text{CO}_2$  response curves are used (Dubois et al., 2007). In some studies, the  $\text{CO}_2$  response curves were measured at very high light intensities, and was the estimated  $J$  assumed to be equal to  $J_{max}$ . Other studies estimated the FvCB parameters from the data of light response curves only (Müller et al., 2005) or even point measurements only (Kosugi et al., 2003), by assuming a constant ratio for  $J_{max}/V_{cmax}$ . However, the  $J_{max}/V_{cmax}$  ratio varies with temperature (Bernacchi et al., 2001) and species (Poorter and Evans, 1998; Leuning, 2002; Medlyn et al., 2002a). A third way for indirect estimation of  $J_{max}$  is to assume a constant value for the parameters,  $\alpha$  and  $\theta$  in the non-rectangular hyperbola function. For instance, the value of  $\alpha$  was assumed  $0.24 \text{ mol e}^- \text{mol}^{-1} \text{photon}$  (Harley et al., 1992b),  $0.18 \text{ mol e}^- \text{mol}^{-1} \text{photon}$  (Wullschlegel, 1993), and  $0.3 \text{ mol e}^- \text{mol}^{-1} \text{photon}$  (Medlyn et al., 2002b), and the value of  $\theta$  was assumed 0.9 (Medlyn et al., 2002b), 0.5 and 0.95 (Cannell and Thornley, 1998). Our estimated values of  $\alpha$  and  $\theta$  were 0.4

$\text{mol e}^- \text{mol}^{-1} \text{ photon}$  and 0.2, respectively (Table 2), which is higher, respectively lower than literature values. However, if we assign values of  $\alpha$  to  $0.3 \text{ mol e}^- \text{mol}^{-1} \text{ photon}$  and  $\theta$  to 0.7, the estimated  $J_{\max}$  is  $272 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $209 \mu\text{mol m}^{-2} \text{s}^{-1}$  for top and middle leaves, respectively. These two values differ substantially from our results (Table 2), which shows that the values of  $\alpha$  and  $\theta$  do influence the estimated value of  $J_{\max}$ . This stands in contrast with Medlyn et al. (2002b), who indicated only a slight effect. We conclude that estimation of the FvCB parameters, without the assumptions  $\alpha$ ,  $\theta$ , and  $J_{\max}/V_{\max}$ , required both data sets of  $\text{CO}_2$  and light response curves.

### Nonlinear mixed effects model

Peek et al. (2002) showed an example of misinterpretation by comparing the conclusions drawn by ordinary fixed effects and mixed effects model analysis. From the ordinary model it was concluded that light and species had significant effects on all the parameters of a light response function. However, from the mixed effects model it was concluded that light and species only had significant effect on the parameter for the maximum photosynthesis rate at saturated light intensity. This difference was due to the violated model assumption of homogeneity of variances in the ordinary model. We also compared the parameter estimates from the ordinary nonlinear model and the nonlinear mixed effects model. The analyses using the two models yielded identical conclusions. It was found in both cases that leaf position had significant effects on  $V_{\max}$  and  $J_{\max}$ , but not on  $\alpha$ ,  $\theta$ , and  $R_d$ . The parameter estimates from both models were quite comparable, but some differences in standard errors were observed (Table 2). Consequently, we advocate using nonlinear mixed effects models for estimating the FvCB parameters, because these models incorporate properties of repeated measurements experimental design of photosynthesis studies, which are neglected by fixed effects models. The analysis using a nonlinear mixed effect model for data of repeated measurements yields more realistic standard errors, since it takes into account the correlation among the data points from the same leaf and the non-constant variance.

### Conclusions

The FvCB parameters,  $V_{\max}$ ,  $J_{\max}$ ,  $\alpha$ ,  $\theta$ , and  $R_d$ , were estimated based on  $\text{CO}_2$  and light response curves, using nonlinear mixed effects model.  $H_a$  for calculation of  $V_{\max}$  was estimated from temperature response curves. The main conclusions were that (1) incorporating a sub-model of Rubisco activation improved the prediction of the FvCB model for light dependency of carboxylation rate; (2) Estimating  $V_{\max}$ ,  $J_{\max}$ ,  $\alpha$ ,  $\theta$ , and  $R_d$  requires both data sets of  $\text{CO}_2$  and light response curves.

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# Chapter 6

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## General Discussion

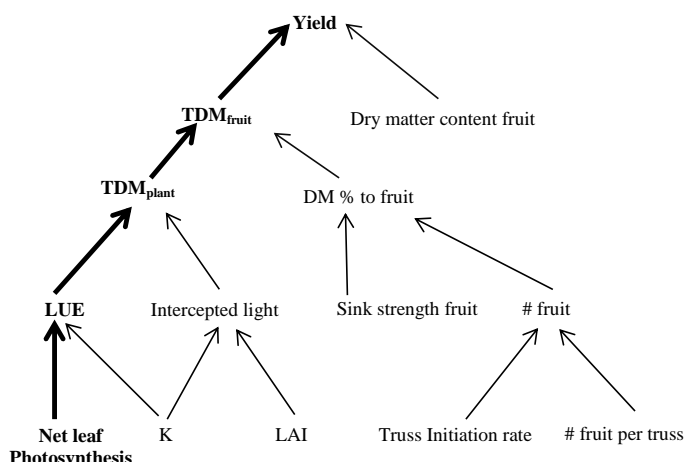
Closed and semi-closed greenhouses were developed to reduce energy consumption and increase production. In these greenhouses, ventilation windows are kept fully or largely closed, respectively. Climate conditions in these greenhouses can differ from open greenhouses. In summer, high CO<sub>2</sub> concentration is combined with high light intensity (Chapter 2) and vertical temperature gradients occur due to cooling below the gutters (Chapter 4). As described in Chapter 1, knowledge on crop growth and development and the underlying physiological processes in (semi)-closed greenhouses is the key to fully explore the advantages of these greenhouse systems. This PhD research focuses on crop physiology in closed and semi-closed greenhouses, aiming to study the effects of the new climate conditions on crop growth, development and underlying processes. For this investigation, experiments were performed in closed, semi-closed and open greenhouses. Greenhouse climate (Chapter 2 and 4.1), yield (Chapter 2), crop growth and development (Chapter 3 and 4.2), and photosynthesis (Chapter 3 and 5) were analyzed.

In this Chapter, the results of these experiments are integrated and discussed. The yield increase in a closed greenhouse, compared to that in an open greenhouse is discussed based on physiological and developmental processes. In addition, sustainability of the system is discussed in terms of energy, compared to an open greenhouse. This chapter ends with the discussion on the applicability of the closed greenhouse concept and other innovations for greenhouse energy conservation.

## 6.1 Yield analysis

The effect of the closed greenhouse system on yield is discussed by using a component hierarchical scheme (Fig. 6.1, modified from Higashide and Heuvelink (2009)). Yield increase (fresh fruit production, kg m<sup>-2</sup>) can be caused by an increase of total fruit dry matter (TDM<sub>fruit</sub>, kg m<sup>-2</sup>) and/or a decrease of fruit dry matter content (ratio between dry and fresh mass, %). Increase in TDM<sub>fruit</sub> can result from an increase of total plant dry matter (TDM<sub>plant</sub>, kg m<sup>-2</sup>) and/or an increase of the fraction (%) of dry matter partitioned into the fruit. TDM<sub>plant</sub> is determined by the light use efficiency of the plant (LUE, kg J<sup>-1</sup>), i.e. TDM<sub>plant</sub> per unit of PAR light that is intercepted by the canopy (J m<sup>-2</sup>). Dry matter partitioning to the fruit is determined by sink strengths of the fruits relative to those of the vegetative plant organs. Sink strength of a fruit is defined as the competitive ability of a fruit to attract assimilates, and the potential fruit growth rate is a measure of fruit sink strength (Marcelis 1996). Total fruit sink strength of a plant depends on sink strength of the individual fruits and the number of fruits. An increase of LUE can be the result of an increase of net leaf photosynthesis rate (μmol m<sup>-2</sup> s<sup>-1</sup>), while a lower light extinction coefficient (k) may lead to better distribution of light over the leaves and therefore also lead to higher rate of crop photosynthesis (Higashide and Heuvelink 2009). A higher light interception is the result of a higher light extinction coefficient and/or a higher leaf area index (LAI, m<sup>2</sup> m<sup>-2</sup>). An increase of number of fruits per plant can be caused by a higher number of trusses per plant, more fruits per truss and/or longer fruit growth duration.





**Figure. 6.1** Scheme of the components that determine the final yield of a tomato crop. The scheme is a modification of Fig. 2 from Higashide and Heuvelink (2009). Yield: fresh fruit production,  $TDM_{fruit}$ : Total fruit dry matter,  $TDM_{plant}$ : Total plant dry matter, DM% to fruit: fraction (%) of dry matter partitioned into the fruits, LUE: Light use efficiency (dry matter production per unit intercepted light), LAI: Leaf Area Index,  $k$ : extinction coefficient. Factors that substantially contributed to the yield increase in the closed greenhouse compared to open greenhouse are highlighted in bold.

### Yield increase

Compared to the open greenhouse, yield increases at 29 weeks after planting in the trial of Chapter 2 were 14% in the closed greenhouse, 10% in the semi-closed greenhouse with  $350 \text{ W m}^{-2}$  cooling capacity, and 6% in the semi-closed greenhouse with  $150 \text{ W m}^{-2}$  cooling capacity (Table 2 of Chapter 2). In the trial of Chapter 4, final yield increase in the semi-closed greenhouse with  $350 \text{ W m}^{-2}$  cooling capacity was 12% compared to the open greenhouse (data not shown). Production increase was also found in other studies on closed and semi-closed greenhouses. Cultivation in closed greenhouses in Belgium (Hoes et al. 2008), France (Grisey et al. 2011), and in a semi-closed greenhouse in Germany (Dannehl et al. 2014) showed increases in tomato yield of 7-11%, 34%, and 21%, respectively, compared to cultivation in open greenhouses. Differences in production increase might be due to differences in season, growing area, climate strategy, and cultivars. Summer production of cucumber in a semi-closed greenhouse was 15-23% higher than in an open greenhouse in Finland (Kaukoranta et al. 2014). Bean production in a closed greenhouse was  $1.9 \text{ kg m}^{-2}$  compared to  $1.4 \text{ kg m}^{-2}$  in an open greenhouse in Spain, which reflects 36% yield increase (Zaragoza et al. 2008). De Gelder et al. (2012a) concluded in their review paper that in closed greenhouses an average yield increase of 10-20% compared to open greenhouses is realistic.

### Botrytis

In the experiment in 2008 of Chapter 2, from 29 weeks after planting a large number of plants in the closed greenhouse were infected by *Botrytis*. At the end of the experiment, 32% of plants were removed from the closed greenhouse due to *Botrytis*, whereas only 1% of plants were removed from the open greenhouse. Due to the removal of 32% of the plants from the closed greenhouse, cumulative production was only 4% higher compared to that in the open greenhouse. *Botrytis* is a very common fungal disease in major greenhouse crops (Dik and Wubben 2007). Yield loss due to

*Botrytis* infection also occurred in other closed greenhouses reviewed by Heuvelink et al. (2008) and De Gelder et al. (2012a). Grisey et al. (2011) also reported more *Botrytis* occurrence in the closed than in the open greenhouse (plant loss of respectively 8% and 3%). De Gelder et al. (2008) reported a cucumber experiment in which 40% of the plants in the closed greenhouses and 20% in open greenhouses were infected by *Botrytis*.

High air humidity is the most likely reason for the high occurrence of *Botrytis* in experiments with closed greenhouse (Heuvelink et al. 2008; De Gelder et al. 2012a). We indeed found that in our experiments air humidity in the closed greenhouse was higher than in the semi-closed and open greenhouse (Fig. 4 of Chapter 2), with the latter two having very limited occurrence of *Botrytis*. In the 2008 experiment (Chapter 2) only 1% and 2% of the plants were affected by *Botrytis* in the semi-closed greenhouses with respectively 150 and 350 W m<sup>-2</sup> cooling capacity, which was comparable to the loss of 1% of the plants in the open greenhouse. In the experiment in 2009 (Chapter 4), no *Botrytis* infection was detected in any of the semi-closed greenhouses, which had cooling capacity of 350 W m<sup>-2</sup>.

Flower, fruit and leaf infection by *Botrytis* is related to high air humidity in particular when combined with an organ temperature that is lower than the air temperature, which causes condensation on the organs. The spores of *Botrytis* needs wind or water to spread. This can occur in the morning in a greenhouse, when air temperature is rising faster than plant temperature. In practice, the heating system is usually turned on before sunrise to gradually increase leaf temperature concomitantly with air temperature to avoid condensation, which reduces the risk of *Botrytis* infection of leaves (Dik and Wubben 2007). Stem infection by *Botrytis* is stimulated by high air humidity, but it is not linked directly to condensation. The presence of wound spots provides the fungus with sufficient moisture for rapid infection. Eden et al. (1996) showed an interaction effect of air humidity and temperature for tomato stem infection by *Botrytis*. Stem infection increased with increasing relative humidity from 56%-100%, and with decreasing air temperature from 25 °C to 15 °C. This could explain the more frequent occurrence of *Botrytis* in closed greenhouses, which is characterized by relatively humid air. High air humidity and low temperature were combined at the lower part of the canopy, where cool air was brought in in summer (Chapter 4.1). High humidity in closed and semi-closed greenhouses was also found in other experiments (Dannehl et al. 2012, Hoes et al. 2008). Such a combination of high humidity and low temperature at the lower part of the canopy can be avoided by applying the cooling above instead of below the canopy (Chapter 4). In conclusion, close attention should be paid to humidity and temperature, especially at the lower part of the canopy, in the closed greenhouse to avoid infection by *Botrytis*.

### **Yield increase due to increase of total fruit dry matter**

The yield increase in the closed and semi-closed greenhouses, compared to the open greenhouse, was the result of an increase of total dry matter production of the fruit, but not of a decrease of the dry matter content of the fruit (Fig. 6.2.). Dannehl et al. (2014) also found yield increase in tomato in the closed greenhouse compared to an open greenhouse while dry matter content of the fruit was unaffected. This is in line with the fact that increased light intensity (Kläring and Krumbein 2013) and CO<sub>2</sub> concentration (Nederhoff 1994) increased fresh and dry weight production but not the fruit dry matter content of tomato fruits. Furthermore, Heuvelink (1995) and De Koning (1994) showed that the source/sink ratio had no effect on dry matter content of tomato fruits, despite its effects on fruit growth rate. In contrast to tomato, in

cucumber a higher fruit dry matter content was found in a semi-closed greenhouse than in an open greenhouse (Luomala et al. 2008). This is not surprising as for cucumber an increase in assimilate supply (Marcelis 1993a) as well as a decrease in temperature (Marcelis and Baan Hofman-Eijer 1993) increases the fruit dry matter content and affects the developmental stage of the fruits at harvest, which further contributes to a change in dry matter content.

The air humidity in the open greenhouse is in general lower than that in the closed greenhouse. Bertin (2000) found an increase in tomato fruit dry matter content when the air humidity was lower, but only under saline conditions in summer in the south of France. Salinity induced restriction of water supply to the fruit, which promoted fruit dry matter content but had a negative effect on yield (Li et al. 2001). In several experiments under Dutch greenhouse conditions no (Bakker 1991) or only small effects (Li et al. 2001) of air humidity were found in tomato. In our experiments, plants were grown under non-saline condition with an EC in the rockwool slab of approximately  $3.7 \text{ dS m}^{-1}$  in all treatments and experiments. We conclude that the increase in tomato yield in the closed and semi-closed greenhouse, compared to the open greenhouse, was not due to an increase in fruit dry matter content, but due to an increase of total dry matter of the fruit.

### **Total dry matter production of the plant**

Increase in total fruit dry matter can be the result of an increase of total dry matter of the plant and/or an increase of dry matter partitioning to the fruit (Fig. 6.1). We showed that the increase of total fruit dry matter was the result of an increase of total plant dry matter production, rather than a larger fraction of dry matter partitioned into the fruit (Chapter 4.2). Higashide and Heuvelink (2009) compared the yield of different tomato varieties, and also found that the increase of fruit yield was not due to a change in the fraction of dry matter partitioned into fruit but that it positively correlated with total plant dry matter production. In the research of Luomala et al. (2008) with cucumber, both higher total dry matter production of the plant and higher dry matter allocation to the fruits was found in the semi-closed greenhouse compared to an open greenhouse.

### **Dry matter partitioning to the fruits**

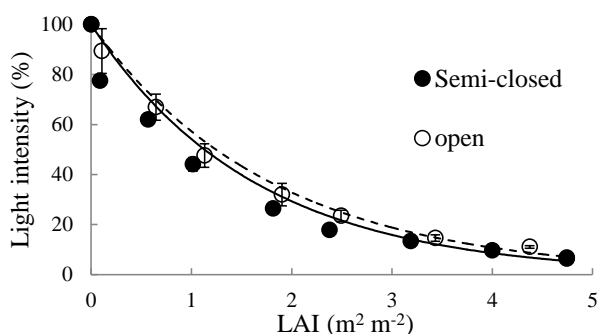
Dry matter partitioning is regulated by sink strengths of the organs. Source strength has no direct influence on dry matter partitioning to the fruit (reviewed by Marcelis 1996) which was confirmed by several studies. Radiation (Marcelis 1992),  $\text{CO}_2$  concentration (Nederhoff 1994) and plant density (indirect influence of light interception per plant, Heuvelink 1995) neither influenced the dry matter partitioning to the fruit. The importance of sink strength in determining dry matter partitioning in tomato was corroborated by experiments where fruit number per truss (fruit load) was varied (Heuvelink 1996). In our experiments, as is common practice in commercial tomato cultivation, fruit number was restricted by pruning to a fixed number per truss. Heuvelink (1996) found that temperature did not affect dry matter partitioning into the fruits in tomato. Therefore, it is not surprising that dry matter partitioning to the fruits was similar between plants grown in closed and open greenhouses in our experiments. Although also in cucumber source strength has no direct effect on dry matter partitioning to the fruits, the number of sinks increases when source strength increases, in the long run resulting in an increased partitioning to the fruits (Marcelis 1993b). Therefore, increased source strength by higher  $\text{CO}_2$  concentration in the closed greenhouse compared to open greenhouse might increase dry matter partitioning to

the fruit indirectly via an increase in fruit number on plant, where fruit number is affected by the source strength, as is the case in cucumber (Luomala et al. 2008).

### Light use efficiency

Total dry matter production of the plant depends on the light use efficiency (ratio dry matter production per unit intercepted light) and the light interception of the canopy (Fig. 6.1). The light intercepted by the canopy is determined by LAI and light extinction coefficient ( $k$ ). Light interception by the canopy is highly affected by canopy structure, which depends on plant density, leaf pruning, growth conditions and variety (Sarlikioti et al. 2011a). In our research, the LAI and  $k$  were the same for the semi-closed greenhouse and open greenhouse (Fig. 6.2). Therefore, we conclude that the increase of total dry matter production of the plant was the result of an increase in light use efficiency, rather than a higher light interception. Dannehl et al. (2014) found the LAI of a tomato crop in the closed greenhouse was increased compared to that in the open greenhouse eight weeks after planting. The authors explained the higher LAI by a higher leaf formation rate at higher temperature in the closed greenhouse compared to the open greenhouse. The higher temperature in their closed greenhouse was due to the absence of window ventilation in early spring. In our experiments the average temperature at the top of the canopy was similar to that in the open greenhouse (Fig. 3 of Chapter 3).

Nederhoff (1994) observed no effect of increased  $\text{CO}_2$  concentration on LAI of tomato in spite of a higher leaf weight at higher  $\text{CO}_2$  concentration. In our experiments the leaves in the semi-closed greenhouse were thicker (smaller specific leaf area, SLA,  $\text{m}^2 \text{kg}^{-1} \text{DM}$ ) than in the open greenhouse while LAI was equal. Hoes et al. (2008) did not measure LAI but leaf length and width. They found that the leaf length and width of the leaves in the closed greenhouse were smaller compared to the open greenhouse. The reason was not clear, but the authors assumed that this was due to stress caused by continuous air flow from the ducts. Campen et al. (2008) measuring wind speed at different places in a closed greenhouse found the wind speed was everywhere lower than  $0.9 \text{ m s}^{-1}$ . Elings et al. (2007) found that wind speed in the range of  $0.2 - 1.0 \text{ m s}^{-1}$  in the canopy had no effect on leaf area in tomato. Therefore, air movement per se in closed greenhouses is not likely to affect the leaf area.



**Figure. 6.2** Light extinction in the canopy of a tomato crop in the semi-closed greenhouse and open greenhouse measured in the experiment of Chapter 3. Light intensity in the canopy was calculated as a percentage of the light above the canopy. LAI was counted from top to bottom of the canopy. Symbols represent average value of the measurements. Lines represent fitted curves ( $Y=100e^{-kx}$ ). Estimated extinction coefficient  $k$  was 0.61 and 0.58 in the semi-closed and open greenhouses, respectively (Error bars indicate SEM,  $n=3$ ).

A lower  $k$  value means that more light reaches the lower part of the canopy. The vertical light distribution in a canopy with a lower  $k$  value is more uniform than that in the canopy with a higher  $k$  value. Sarlikioti et al. (2011b) concluded, on the basis of model calculations for five vertical light distribution scenarios in a tomato canopy that the effect of vertical light distribution on crop photosynthesis differed between winter and summer light conditions. Under summer light conditions photosynthesis of the upper leaves in the canopy is close to saturation, and deeper penetration of the light into the lower part of the canopy increases crop photosynthesis. In contrast, under winter light condition photosynthesis of the upper leaves is not saturated. With the same amount of light, upper leaves have a higher photosynthetic rate than lower leaves (in Chapter 5, our research showed top leaf had higher  $J_{\max}$  than middle leaf). Sarlikioti et al. (2011b) calculated that deeper penetration of the light into the lower part of the canopy therefore decreased the total canopy photosynthesis under winter light conditions. In our research, the  $k$  value was not different between the semi-closed and open greenhouses, therefore, it cannot be an explanatory factor for the effect on LUE.

### Leaf photosynthesis

Leaf photosynthesis is influenced by light (Ogren and Evans 1993),  $\text{CO}_2$  concentration (Cannell and Thornley 1998), and temperature (Yamori et al. 2010). In Chapter 5, photosynthetic rates of leaves at the top of the canopy and mature leaves in the middle of the canopy were quantified under a wide range of climate conditions. Light intensity and  $\text{CO}_2$  concentration are the two main environmental factors limiting photosynthetic rate by either Rubisco carboxylation (carbon reaction part) or RuBP regeneration (light reaction part) (Sharkey et al. 2007). The temperature dependency of the photosynthetic parameters is related to kinetic properties of the enzymes such as Rubisco (Farquhar et al. 1980; Bernacchi et al. 2001). In our research, leaf photosynthesis rate was insensitive to air humidity in the range of humidity that occurred in open and closed greenhouses (vapour pressure deficit ranging from 0.2 to 2.5 kPa, data not shown).

The response of leaf photosynthesis rate to light,  $\text{CO}_2$  and temperature showed strong interaction (Chapter 5). For example, the temperature response of leaf photosynthesis rate showed an optimal response curve at high light intensity and  $\text{CO}_2$  concentration, while the rate of photosynthesis was hardly affected by temperature at either low light intensity or low  $\text{CO}_2$  concentration. At high light, leaves have a higher carboxylation capacity due to an increased percentage of activated Rubisco. The most important climatic advantage for photosynthesis in the closed and semi-closed greenhouse compared to the open greenhouse is the higher  $\text{CO}_2$  concentration. Plants grown for a prolonged period at an elevated  $\text{CO}_2$  concentration may have a lower photosynthesis rate at a given  $\text{CO}_2$  concentration than plants grown at a lower  $\text{CO}_2$  concentration (Chen et al. 2005; Pérez et al. 2007; Zhang et al. 2009). Photosynthetic acclimation to elevated  $\text{CO}_2$  has been found in tomato and other crops grown at increased  $\text{CO}_2$  concentration (Peet et al. 1986; Besford et al. 1990; Nederhoff 1994; Bunce 2001). However, in our research, photosynthetic acclimation to elevated  $\text{CO}_2$  was not found in the semi-closed greenhouse. Dannehl et al. (2013) neither found photosynthetic acclimation to elevated  $\text{CO}_2$  in the closed greenhouse. This can be explained by the fact that the crops in the closed and semi-closed greenhouse had sufficient sink strength (high fruit load per  $\text{m}^2$ ) to use the extra assimilates produced at higher  $\text{CO}_2$  concentration. Li et al. (2015) showed that fully fruiting tomato plants

when grown according to common practice were source-limited. Based on the responses of leaf photosynthesis to climate conditions and the absence of a difference in light extinction between closed and open greenhouse, we conclude that the increased light use efficiency in the closed greenhouse compared to open greenhouse was caused by an increase in the rate of leaf photosynthesis due to increased CO<sub>2</sub> concentration.

### Canopy photosynthesis

In our research (Chapters 3 and 5), leaf photosynthesis rate was measured at two heights in the canopy: at the top and middle of the canopy. Parameter values of leaf photosynthetic properties of the leaves at these two canopy heights were different; for example middle leaves showed a lower maximum carboxylation capacity ( $V_{\text{cmax}}$ ) and lower maximum electron transport rate ( $J_{\text{max}}$ ). The relation between the photosynthetic parameters and the canopy photosynthesis was not studied in this research. At high light intensity and high CO<sub>2</sub> concentration in the measurement cuvette, photosynthesis of both top and middle leaves responded strongly to temperature. However, at low light intensity the temperature response of leaf photosynthesis was much weaker. These low light intensities prevail in a large part of the canopy. This implies that canopy photosynthesis, which is the integrated value of leaf photosynthesis of leaf layers at different light intensities, shows only a weak temperature response (Heuvelink et al. 2008). In addition, photosynthesis rate of different layers did not differ between the semi-closed greenhouse and open greenhouse when measured under the same set of conditions in the measurement cuvette (Fig. 5 of Chapter 3).

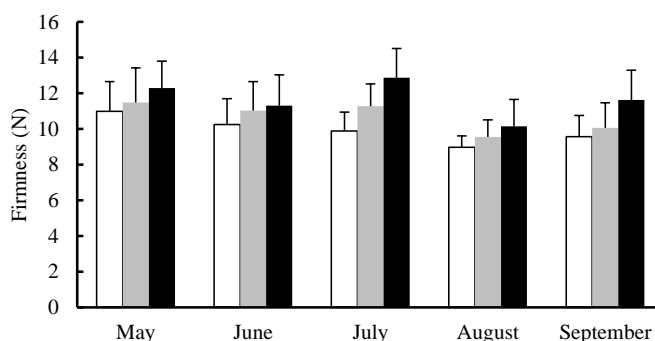
In summary, based on the yield component analysis we conclude that the yield increase of the (semi-)closed greenhouse, compared to the open greenhouse was caused by the increase of total fruit dry matter, that this increase of total fruit dry matter was due to an increase of plant total dry matter, and that this increase of plant total dry matter was due to a higher LUE, which was the result of an increase of net leaf photosynthesis (indicated as bold arrows in Fig 6.1).

### 6.2 Fruit quality

Many studies have shown that greenhouse climate during production can affect the postharvest quality of the greenhouse products (Riga et al. 2008; Fanourakis et al. 2011). In the experiment where we investigated semi-closed greenhouses with cooling from above and below (Chapter 4), the quality of the harvested tomatoes from semi-closed greenhouses and open greenhouse was tested in five different months during the growing season (Farneti et al. 2013). Quality was measured in terms of firmness (N), sugar level (mg g FW<sup>-1</sup>), and acid level (mg g FW<sup>-1</sup>). At the same color stage, the fruits harvested from the semi-closed greenhouse were firmer than those from the open greenhouse (Figure 6.3). Similarly Dannehl et al. (2014), who used consumer panels to assess fruit quality, found that the fruits from the closed greenhouse tasted firmer than those from the open greenhouse. Furthermore, Islam et al. (1996) showed that increasing CO<sub>2</sub> concentration accelerated the coloring but did not affect firmness of the fruit. This implies that tomatoes harvested from the semi-closed greenhouse with the same color stage as the open greenhouse are firmer, which is a positive quality attribute.

Sugar and acid levels of the fruits from the open greenhouse were slightly higher than those of the semi-closed greenhouses (Farneti et al. 2013). Total soluble solids followed the same trend observed for sugar but within 1% difference between

the open greenhouse and semi-closed greenhouse (Farneti et al. 2013). However, consumers will not notice the 1% difference (Harker et al. 2002). Dannehl et al. (2014) found that the soluble solids ( $\text{g kg FW}^{-1}$ ) of the fruit from the closed greenhouse were higher and were rated as sweeter by a consumer panel than that from the open greenhouse. Islam et al. (2006) also found increased soluble solids with increased  $\text{CO}_2$  concentration. In the research of Dannehl et al. (2014) the temperature in the closed greenhouse was higher than in the open greenhouse, which might explain the good taste of the fruit from the closed greenhouse. In summary, the firmer fruits in closed greenhouses in combination with the small difference in soluble solids in our research and the increased soluble solids and sweetness in the study of Dannehl et al. (2014), indicates a better quality of the fruits in closed greenhouses.

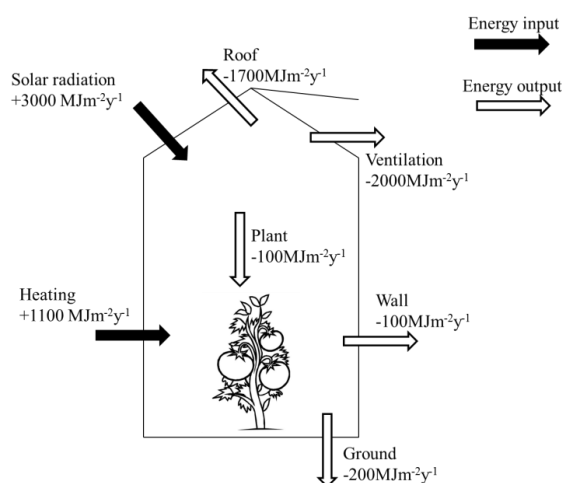


**Figure. 6.3** Firmness of the harvested fruits from open greenhouse (□), semi-closed greenhouse with cooling from above the canopy (■), and semi-closed greenhouse with cooling from below the canopy (■). Firmness was determined as the maximum force needed to compress the tomato 1 mm at  $40 \text{ mm min}^{-1}$  with a cylindrical probe with 15 mm diameter. Error bars indicate the standard deviation ( $N=45$ ). Greenhouse type and month had significant effects on firmness ( $P<0.001$ ). Figure was reprinted from Farneti et al. (2013).

### 6.3 Energy

In the Netherlands, the energy inputs of an open greenhouse consist of solar energy and burning of natural gas for heating and  $\text{CO}_2$  enrichment, as described by Elings et al. (2005). Energy outputs consist of energy loss through window ventilation, energy intercepted by crops (energy fixed as carbohydrates through photosynthesis), and energy loss through walls and roofs and to the ground. Energy fluxes of an open greenhouse are summarized in Fig. 6.4. Approximately  $2800\text{--}3000 \text{ MJ m}^{-2} \text{ y}^{-1}$  solar energy is entering a greenhouse in the northwest part of Europe (Bakker et al. 2006). An open greenhouse in the Netherlands with tomatoes consumes about  $30\text{--}40 \text{ m}^3 \text{ m}^{-2} \text{ y}^{-1}$  natural gas, equivalent to  $930\text{--}1240 \text{ MJ m}^{-2} \text{ y}^{-1}$  energy, mainly for heating (Bot et al. 2005; De Gelder et al. 2012b; De Zwart 2012). Hence, on an annual basis the solar energy entering the greenhouse is more than twice the energy consumption in the greenhouse. However, at least  $2000 \text{ MJ m}^{-2} \text{ y}^{-1}$  energy is lost through window ventilation in an open greenhouse. This consists of  $1500 \text{ MJ m}^{-2} \text{ y}^{-1}$  energy loss during sunny days for greenhouse cooling and  $500 \text{ MJ m}^{-2} \text{ y}^{-1}$  during dull days for dehumidification (de Zwart 2008). The concept of closed and semi-closed greenhouses (reducing window ventilation, using aquifer for heat and cold storage, and air treatment unit (ATU) for temperature and humidity control) enables harvesting heat that would otherwise be lost by window ventilation. If all the energy lost through the windows would be harvested, a fully closed greenhouse could

potentially harvest  $2000 \text{ MJ m}^{-2} \text{ y}^{-1}$  energy, while the energy consumption for heating of a greenhouse is only  $930\text{--}1240 \text{ MJ m}^{-2} \text{ y}^{-1}$ . It means that the harvested energy is more than enough for heating. However, energy saving by a closed greenhouse (reduction in % energy consumption compared to an open greenhouse) is not 100% because primary energy is needed for running the pumps, the ventilators of the ATU, and the heat pump (Bakker et al. 2006; De Zwart 2008; De Gelder et al. 2012). De Zwart mentioned that energy saving is less than 50% and De Gelder et al. (2012a) mentioned in their review a saving of 30–40% (heat pumps are needed to bridge the gap between the water temperature from the aquifer ( $18^\circ\text{C}$ ) and required heating water temperature (above  $40^\circ\text{C}$ ) in the ATU (Bot et al. 2005; de Zwart 2012). Electrical energy for operation of heat pump depends on the heat that the heat pump needs to supply and by the COP (coefficient of performance, ratio of heating or cooling output to work input).



**Figure. 6.4** Energy fluxes of an open greenhouse with tomatoes in the Netherlands. Values indicate energy input and output in  $\text{MJ m}^{-2} \text{ y}^{-1}$ . These are rough estimates based on Elings et al. (2005) and De Zwart (2008).

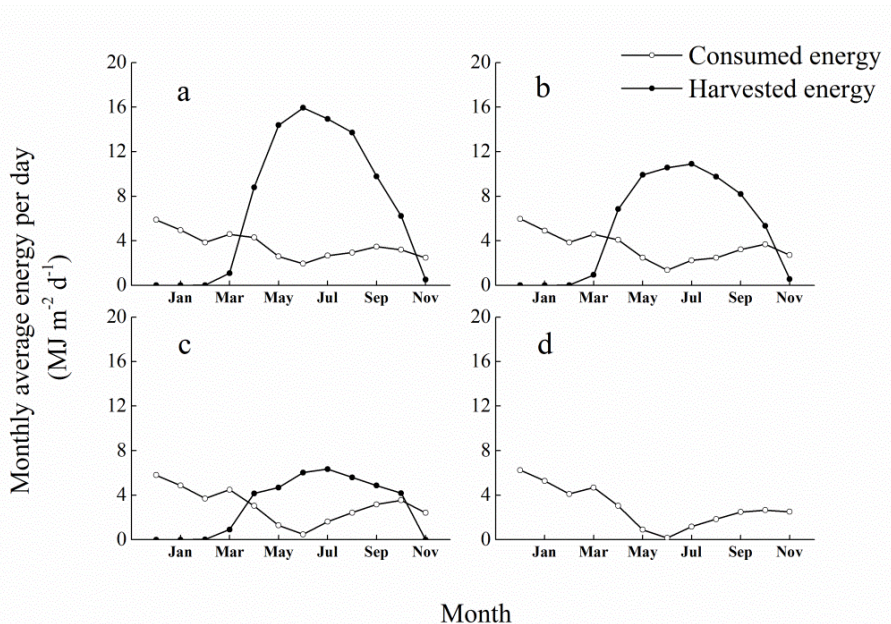
In our research, closed, semi-closed and open greenhouses were operated at the same time and same location (Chapter 2 and Chapter 4). Figure 6.5 shows the monthly average consumed and harvested energy in these greenhouses. The energy consumption calculated here was for heating, excluding the primary electricity energy for operation of the heat pump, pumps, and ventilator of ATU. The accumulated difference between harvested and consumed heat energy shows the net heat energy gained by the system. The greenhouse with a higher cooling capacity harvested more energy. On an annual basis in the fully closed greenhouse the estimated heat energy harvested was  $2605 \text{ MJ m}^{-2} \text{ y}^{-1}$ , which is equal to the heat that would be produced by burning  $84 \text{ m}^3 \text{ m}^{-2} \text{ y}^{-1}$  natural gas (Table 6.1). The energy consumption for heating in the fully closed greenhouse was equal to  $39 \text{ m}^3 \text{ m}^{-2} \text{ y}^{-1}$  natural gas. In the experiment of 2009 in Chapter 3 and 4, the semi-closed greenhouse was found to harvest less energy compared to the experiment of 2008 of Chapter 2 (Dieleman et al. 2009; Dieleman et al. 2012). A major reason for the differences in harvested energy between the two experimental years was that dehumidification was completely realized by window ventilation and that the ATU was not used for this purpose in the semi-closed



greenhouses in the second year. By dehumidification about  $500 \text{ MJ m}^{-2} \text{ y}^{-1}$  could have been harvested (De Zwart 2008).

In conclusion, the amount of harvested energy of a closed greenhouse is higher than the amount of consumed energy. The amount of the harvested energy of a (semi)-closed greenhouse depends on its cooling capacity (Table 6.1). Ideally, a closed greenhouse section should be combined with an open greenhouse section, so that the extra heat harvested by the closed greenhouse can be used in the open greenhouse section. De Zwart (2008) calculated scenarios of an open greenhouse combined with a 10-40% fraction of closed greenhouses under Dutch and Mediterranean weather conditions. He concluded that 40% fraction of closed greenhouse in Dutch conditions and 20% fraction of closed greenhouse in Mediterranean conditions were optimum in terms of financial benefit.

In different experiments with closed and semi-closed greenhouses energy savings (reduction of energy consumption as percentage of that in an open greenhouse) were 20-35 % (Opdam et al. 2005), 8-22% (Hoes et al. 2008) and 20% (Grisey et al., 2011).



**Figure 6.5** Consumption and harvest of heat energy in the closed greenhouse with  $700 \text{ W m}^{-2}$  cooling capacity (a), the semi-closed greenhouses with  $350 \text{ W m}^{-2}$  cooling capacity (b), the semi-closed greenhouse with  $150 \text{ W m}^{-2}$  cooling capacity (c), and the open greenhouse (d) during the complete growing season of a tomato crop in the year 2008 (experiment as described in Chapter2). Electricity energy consumption is not included in this figure.

**Table 6.1** Summary of the heat and electricity consumption (electricity for fans of the ducts and heat pump were not included) and harvest in the form of heat in this research. ATU stands for Air Treatment Unit

Experiment	Greenhouse type	Harvested heat (MJ m <sup>-2</sup> y <sup>-1</sup> )	Consumed natural gas (MJ m <sup>-2</sup> y <sup>-1</sup> )	Electricity consumption for ATU ventilation (MJ m <sup>-2</sup> y <sup>-1</sup> )
2008	closed	2605	1200	139
	semi-closed with 350 W m <sup>-2</sup> cooling	1920	1156	115
	semi-closed with 150 W m <sup>-2</sup> cooling	1125	1020	119
2009	open	0	958	0
	semi-closed with 350 W m <sup>-2</sup> cooling above	1078	891	73
	semi-closed with 150 W m <sup>-2</sup> cooling below	1130	880	88
	open	0	844	0

#### 6.4 Application of closed greenhouse knowledge and other innovations

The first closed greenhouse experiment has been conducted in 2002 in the Netherlands (De Gelder et al. 2005). In the beginning, the development of closed greenhouses was primarily focused on the technical possibilities of the system (Bakker et al. 2006; Buchholz et al. 2005; Bot et al. 2005). Further research on plant growth and development in the closed greenhouse followed (Hoes et al. 2008; Luomala et al. 2008; Grisey et al. 2011; De Gelder et al. 2012b; Dannehl et al. 2014), because knowledge on crop response to the climate condition in the closed greenhouse was needed for making optimal use of the new system during implementation. Improving sustainability of the greenhouse production systems needs control of growth conditions in the greenhouse to meet the demand of the crop, while the crop management should aim at a crop that suits better the growth condition in the greenhouse (Marcelis and De Pascale 2009; Dieleman and Hemming 2011).

There are several examples, in which closed greenhouse systems have been applied commercially. The first commercially applied closed greenhouse system in The Netherlands started in 2003 with 1.4 ha tomatoes. Thereafter, a number of tomato growers installed closed and semi-closed greenhouse systems (Raaphorst 2011). Semi-closed greenhouse systems were also applied to ornamentals such as cut roses, orchids and other potted plants. The largest scale in which a closed greenhouse is applied in the Netherlands is 3.4 ha, combined with 5.9 ha open greenhouse (Gieling et al. 2011). In total there are about 200 hectares of semi-closed greenhouses in the Netherlands (Kas Als Energiebron 2017). The closed greenhouse concept is also being applied in several other countries, for instance, in California of USA for tomato production (<http://www.houwelings.com/files-2/sustainability.php>). A 9 ha semi-closed greenhouse for cucumber production started in Russia in 2013, and was expanded later. A 9.4 ha semi-closed greenhouse was established in Kazakhstan in 2015 for tomato production.

Growers need several years to learn how to adjust their cultivation and climate management strategies in the new system before fully exploring the benefit of the

system. Knowledge transfer and a strong cooperation between researchers and growers are key factors in the development and application of closed greenhouses (Dieleman and Hemming 2011; De Gelder et al. 2012a).

Closed and semi-closed greenhouses with aquifer, heat pump and ATU have obvious advantages in energy saving and production increase, but the application has not been as wide as expected (De Zwart 2012). One of the reasons is the fixed investment cost for drilling to reach the aquifers, ATU and heat pump. As mentioned above, growers need several years to learn to fully exploit the new system. The profit from increased production and reduction in energy consumption might not be able to compensate for the investment costs (Ruijs et al. 2010). In addition, Combined Heat and Power (CHP) are commonly used in Dutch greenhouse. Growers purchase natural gas, by burning natural gas CHP generates electricity and heat (reject heat of the CHP engine). The heat can be used for greenhouse heating and surplus electricity, after fulfilling greenhouse electricity requirement, can be sold to the public grid. The CO<sub>2</sub> that is produced by burning natural gas is used for CO<sub>2</sub> enrichment of the greenhouse. Investment in CHP is attractive because of the relatively low natural gas price and good infrastructure for selling the electricity to the public grid (Breukers et al. 2008; De Zwart 2012). However, investment in CHP is not as interesting anymore as it was 5-10 years ago, since the price of natural gas increased from 0.16 €m<sup>-3</sup> in 2000, to 0.21 €m<sup>-3</sup> in 2015, and estimated to be about 0.28 €m<sup>-3</sup> in 2030 (Schoots et al. 2016), while electricity price was 0.22 €kwh<sup>-1</sup> in 2007 but decreased to around 0.12 €kwh<sup>-1</sup> in 2014 (Eurostat 2016).

### **The next generation greenhouse cultivation**

The knowledge obtained from closed greenhouses is currently being applied in open greenhouses (De Gelder et al. 2012a; Schuddebeurs et al. 2015; Persoon et al. 2016). This knowledge has been used in the development of the concept of the next generation greenhouse cultivation. This concept strives to reduce the energy consumption for heating without reducing yield and quality of the produce (De Gelder et al. 2012b), at much lower investment costs than the closed greenhouses. The main elements of this concept are 1) Improving insulation by application of multiple screens; 2) Controlling air humidity by forced ventilation. 3) Temperature integration by accepting higher temperature at high radiation and lowering night temperature. 4) Adjustment of the production plan by shifting planting date from December to January; 5) Reducing ventilation to retain CO<sub>2</sub> in the greenhouse (by fogging the temperature is reduced, which diminishes the need for window opening); 6) Using a combination of heat pump and aquifer for energy storage and active cooling. This concept required commercial availability and modular applicability of techniques. The next generation concept has been tested on several crops such as tomato (De Gelder and Dieleman 2012; Schuddebeurs et al. 2015), gerbera (De Gelder et al. 2014; Persoon et al. 2016), Phalaenopsis (Kromwijk et al. 2012), and Cymbidium (Raaphorst and Kromwijk 2012) and resulted in 42-50%, 40%, 14-18%, and 27% energy conservation, respectively, while production and quality were maintained compared to those of commercial growers.

### **Other innovations in the greenhouse for energy saving**

Besides the development of closed and semi-closed greenhouses and the next generation greenhouse cultivation concepts, a number of innovations are being developed for greenhouse industry to reduce energy consumption while improving production and product quality. Better understanding of crop physiology in

combination with new technologies has significantly contributed to those innovations. Dieleman and Hemming (2011) and Marcelis et al. (2014) summarized the most recent innovations: double glass with antireflection coatings was used as greenhouse cover to improve insulation without influence of light transmission (Hemming et al., 2012); diffuse glass was used as greenhouse roof cover to have uniform horizontal and vertical light distribution in the greenhouse, higher photosynthesis at the middle of the canopy and less photo inhibition and consequently higher production (Li et al. 2015; Dueck et al. 2012); LED light, which has higher efficiency in converting electricity into light than HPS lamp, was used as supplement lighting for greenhouse cultivation (Dieleman et al. 2016; Trouwborst et al. 2010). Researchers will continue to strive for lower energy consumption in greenhouses in different ways.

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## SUMMARY

Climate change and resource scarcity raised people's concern about environmental protection and energy saving. In the greenhouse horticulture sector, various concepts, aiming at sustainability and energy saving, were developed in the last decades. One of these concepts is the closed greenhouse, in which ventilation by window opening is replaced by mechanical cooling. A closed greenhouse has no window ventilation. Air is cooled and dehumidified by an air treatment unit, which mainly takes place in summer. The air treatment unit contains a heat exchanger and connects to an underground aquifer as well as to a ventilator. The ventilator sucks air from inside the greenhouse into the air treatment unit and distributes the cooled and dehumidified air back to the greenhouse. For cooling in summer, the cold water is pumped up from the aquifer. The surplus heat from the greenhouse is absorbed by the cold water and stored in the aquifer. This stored heat is used in winter to heat the greenhouse. A semi-closed greenhouse has a smaller cooling capacity than a closed greenhouse. Window ventilation is combined with active cooling when the temperature is too high to be managed by the active cooling system.

When the concept of closed greenhouse was introduced, researchers initially focused on its economic and technical aspects. During the development of the closed greenhouse concept, scientists and growers realized that their knowledge on crop physiological processes under such new climate conditions was insufficient to fully explore the possibilities of climate control in the closed greenhouse. Closed greenhouses created new climate conditions for crops, for instance, the combination of high CO<sub>2</sub> concentration and high irradiance, which is not possible in an open greenhouse, due to window ventilation. CO<sub>2</sub> concentration can be high continuously during the whole growing season in a closed greenhouse. There are vertical gradients in temperature and vapour pressure deficit (VPD) throughout the canopy in a closed greenhouse. Therefore, the effects of these new climates and their combination on crop growth and development needed to be investigated.

This thesis focuses on the crop physiology in closed and semi-closed greenhouses, aiming to study the effects of these new climate conditions on crop growth, development and underlying processes.

In **Chapter 2**, the effect of active cooling on greenhouse climate, in terms of stability, gradient and average levels was analysed. Crop growth and production in closed and semi-closed greenhouses were quantified and compared to those of an open greenhouse. An experiment with a tomato crop was conducted in a fully closed greenhouse with 700 W m<sup>-2</sup> cooling capacity, two semi-closed greenhouses with 350 W m<sup>-2</sup> and 150 W m<sup>-2</sup> cooling capacity, respectively, and an open greenhouse. The higher the cooling capacity, the more independent of outside climate the greenhouse climate was.

Cumulative production in the semi-closed greenhouses with 350 W m<sup>-2</sup> and 150 W m<sup>-2</sup> cooling capacity was 10% (61 kg m<sup>-2</sup>) and 6% (59 kg m<sup>-2</sup>) higher than that in the open greenhouse (55 kg m<sup>-2</sup>), respectively. Cumulative production in the closed greenhouse was 14% higher than in the open greenhouse in week 29 after planting but at the end of the experiment. The cumulative increase was only 4% due to *Botrytis*. Model calculations showed that the production increase in the closed and semi-closed greenhouses was explained by higher CO<sub>2</sub> concentration.

As a high CO<sub>2</sub> concentration is one of the most remarkable differences in climate between the closed and open greenhouse, **Chapter 3** focuses on CO<sub>2</sub>. Photosynthetic and morphological acclimation to high CO<sub>2</sub> has been found in many plant species with feedback inhibition being the main mechanism to explain this. **Chapter 3** investigated the occurrence of photosynthetic and morphological acclimation to high CO<sub>2</sub> concentration in the semi-closed greenhouse. The hypothesis was that photosynthetic and morphological acclimation to

elevated CO<sub>2</sub> concentration only occurred in plants with low sink strength. An experiment was carried out with tomato plants with varying fruit loads in a semi-closed greenhouse and a conventional modern greenhouse. The results showed that photosynthetic acclimation to elevated CO<sub>2</sub> concentration only occurred when the number of fruits was considerably reduced. High CO<sub>2</sub> as well as fruit removal reduced the specific leaf area. Reduction in photosynthesis rate was associated with, but not caused by reduced stomatal conductance. The increase of dry matter production in the semi-closed greenhouse was mainly explained by a higher CO<sub>2</sub> concentration compared to the open greenhouse. The results suggest that high CO<sub>2</sub> concentrations in the semi-closed greenhouse do not cause feedback inhibition in high producing crops, because the plants have sufficient sink organs (fruits) to utilise all assimilates.

Vertical temperature and VPD gradients are typical for closed and semi-closed greenhouses. In **Chapter 4.1** the effects of the positioning of the inlet of cooled and dehumidified air on the vertical temperature and VPD gradients in the semi-closed greenhouses were analyzed. Tomato crops were grown year-round in four semi-closed greenhouses with cooled and dehumidified air blown into the greenhouses from below or above the crop. Cooling below the canopy induced vertical temperature and VPD gradients. The temperature at the top of the canopy was over 5 °C higher and the VPD was 0.7 kPa lower than at the bottom, when outside solar radiation was higher than 250 J cm<sup>-2</sup> h<sup>-1</sup>. The seasonal and diel patterns of vertical temperature gradients and VPD gradients were studied. The vertical temperature gradients and VPD gradients correlated with outside radiation and outside temperature. **Chapter 4.2** investigated the effects of the vertical gradients of temperature and VPD on crop growth and development. Despite the occurrence of vertical temperature gradients, plant growth and fruit yields were mostly unaffected. Leaf and truss initiation rates did not differ between treatments, since air temperatures at the top of the canopy were comparable. The only observed response of plants to the vertical temperature gradient was the reduced rate of fruit development in the lower part of the canopy. This resulted in a longer time between anthesis and fruit harvest in the treatment with a vertical temperature gradient, and an increase in the average fruit weight in summer. However, total fruit production over the whole season was not affected. These results are important when designing greenhouses, as well as heating and cooling systems for greenhouses.

The effects of the climate factors light, CO<sub>2</sub> concentration, temperature, and humidity on leaf photosynthesis were investigated in **Chapter 5**. The photosynthesis model of Farquhar, von Caemmerer and Berry (FvCB) was modified to predict light dependency of the carboxylation capacity ( $V_c$ ) and to improve the prediction of temperature dependency of the maximum carboxylation capacity ( $V_{cmax}$ ) and the maximum electron transport rate ( $J_{max}$ ). The FvCB model was modified by adding a sub-model for Ribulose-1,5-bisphosphate carboxylase (Rubisco) activation. Values of parameters for temperature dependency of  $V_{cmax}$  and  $J_{max}$  were validated and adjusted based on data of the photosynthesis response to temperature. Parameter estimation was based on measurements under a wide range of environmental conditions, providing parameters with broad validity. The simultaneous estimation method and the nonlinear mixed effects model were applied to ensure the accuracy of the parameter estimation. The FvCB parameters,  $V_{cmax}$ ,  $J_{max}$ ,  $\alpha$  (the efficiency of light energy conversion),  $\theta$  (the curvature of light response of electron transport), and  $R_d$  (the non-photorespiratory CO<sub>2</sub> release) were estimated and validated on a dataset from two other years. Observations and predictions matched well ( $R^2=0.94$ ). We conclude that incorporating a sub-model of Rubisco activation improved the FvCB model through predicting light dependency of carboxylation rate; and that estimating  $V_{cmax}$ ,  $J_{max}$ ,  $\alpha$ ,  $\theta$ , and  $R_d$  requires data sets of both CO<sub>2</sub> and light response curves. With these parameters derived from the data obtained in closed and semi-

closed greenhouse, leaf photosynthesis rate could be estimated under a wide range of climate conditions.

At the end of the thesis, **Chapter 6**, the results were integrated and discussed. The yield increase in a closed greenhouse, compared to that in an open greenhouse was analyzed based on physiological and developmental processes. In addition, sustainability of the system was discussed in terms of energy, and compared to an open greenhouse. Applicability of the closed greenhouse concept and other innovations for greenhouse energy conservation were summarized.



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This is the end of the thesis but the colourful journey continues.



## CURRICULUM VITAE

Tian Qian (Didi) was born on February 5<sup>th</sup> 1983 in Wenzhou, Zhejiang Province of China. In 2001 she finished her secondary education in her home town. At the same year she started her study in Zhejiang University, College of Agriculture and Biotechnology, where she obtained her Bachelor degree with the thesis titled “Research on the molecular basis of heterosis by constructing and analyzing the genetic linkage map of *Brassica campestris* L.”. In August 2005 she arrived in Wageningen and started her MSc study in the Master program at the Horticultural Product Physiology Group of Wageningen University. During the internship of the Master program, she did research work in the Centre for Native Floriculture, School of Land, Crop & Food, University of Queensland in Australia. In 2007 she obtained her Master degree in Plant Sciences with the thesis titled “Biochemical and structural changes as affected by caspase inhibitor during tracheary element formation in suspension culture of *Zinnia elegans*”. In November 2007 she started her PhD project at the Horticultural Product Physiology Group of Wageningen University and Wageningen University & Research, Business Unit Greenhouse Horticulture. The PhD research was about crop growth and development in closed and semi-closed greenhouses, which resulted in this thesis. Since May 2012, she works as area manager China in Delphy B.V. in the Netherlands.



## LIST OF PUBLICATIONS

### Papers published in scientific journals:

- Qian, T.**, Dieleman, J.A., Elings, A., de Gelder, A., Marcelis, L.F.M., 2015. Response of tomato crop growth and development to a vertical temperature gradient in a semi-closed greenhouse. *Journal of Horticultural Science & Biotechnology*. 90 (5): 578–584.
- Qian, T.**, Dieleman, J.A., Elings, A., Marcelis, L.F.M., 2012. Leaf photosynthetic and morphological responses to elevated CO<sub>2</sub> concentration and altered fruit number in the semi-closed greenhouse. *Scientia Horticulturae*. 145: 1–9.
- Qian, T.**, Elings, A., Dieleman, J.A., Gort, G., Marcelis L.F.M., 2012. Estimation of photosynthesis parameters for a modified Farquhar–von Caemmerer–Berry model using simultaneous estimation method and nonlinear mixed effects model. *Environmental and Experimental Botany*. 82: 66– 73.
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- Farneti, B., Schouten, R.E., **Qian, T.**, Dieleman, J.A., Tijskens, L.M.M., Woltering, E.J., 2013. Greenhouse climate control affects postharvest tomato quality. *Postharvest Biology and Technology*. 86: 354-361.
- Damunupola, J.W., **Qian, T.**, Muusers, R., Joyce, D.C., Irving, D.E., van Meeteren, U., 2010. Effect of S-carvone on vase life parameters of selected cut flower and foliage species. *Postharvest Biology and Technology*. 55: 66-69.
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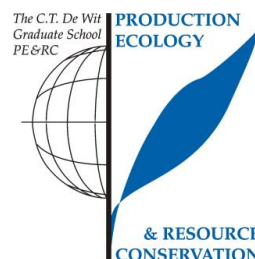
### Other publications:

- Qian, T.**, Joyce, D., He, S., 2007. China's ornamentals industry in 'full bloom'. *Australian Flower Industry*. 15: 10-11.
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- Eveleens-Clark, B.A., Dieleman, J.A., de Gelder, A., Elings, A., Janse, J., **Qian, T.**, Lagas, P., Steenhuizen, J.W., 2009. Effecten van verneveling op groei en ontwikkeling van tomaat : teelt van eind april tot eind augustus. Nota 643. Wageningen UR Greenhouse Horticulture. 34pp.

- Dieleman, J.A., **Qian, T.**, Elings, A., Marcelis, L.F.M., 2010. Crop physiology in (semi-)closed greenhouses. Final report of the Transforum scientific project “SynErgy: Monitoring and control system for conditioning of plants and greenhouse” (WP-066). Report GTB-1051. Wageningen UR Greenhouse Horticulture. 18pp.
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## PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)



### Review of literature (4.5 ECTS)

- Development of closed and semi-closed greenhouses

### Writing of project proposal (4.5 ECTS)

- Crop physiology and modelling in closed greenhouse

### Post-graduate courses (7.5 ECTS)

- Advanced statistics; PE&RC (2008)
- Applied methods in crop physiology; Aarhus University (2009)

### Deficiency, refresh, brush-up courses (3.9 ECTS)

- Greenhouse technology; Wageningen University (2008)
- Basic statistics; PE&RC (2008)

### Competence strengthening / skills courses (2.1 ECTS)

- Information literacy, including EndNote; WGS (2007)
- PhD Competence assessment; WGS (2008)
- Techniques for writing and presenting a scientific paper; WGS (2008)

### PE&RC Annual meetings, seminars and the PE&RC weekend (1.8 ECTS)

- PE&RC Weekend (2008)
- PE&RC Day (2008, 2009, 2010)

### Discussion groups / local seminars / other scientific meetings (6 ECTS)

- Frontier Literature in Plant Physiology (FLOP) (2008-2012)

### International symposia, workshops and conferences (5 ECTS)

- International symposium on high technology for greenhouse systems Greensys 2009; Quebec City, Canada (2009)
- International symposium on greenhouse 2010 and soilless cultivation IHC 2010; Lisbon, Portugal (2010)

### Supervision of MSc students

- Robert Biemans: comparison between a conventional and (semi)-closed greenhouse with respect to production and allocation of assimilates in tomato
- Ye Pan: a study on tomato performance under environmental conditions that create distinct source-sink ratios

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