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Virtuele roos: experimenteel en modelmatig onderzoek naar gewasopbouw roos

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Abstract NL

De gewasopbouw en het uitlopen van okselknoppen zijn belangrijke bepalende factoren voor productie en kwaliteit bij snijroos. Om hier meer grip op te krijgen zijn een aantal proeven gedaan. In de proeven zijn onder andere op verschillende manieren de verhouding tussen source en sink (assimilatenvraag en -aanbod), de correlatieve inhibitie (hormonale remming door plantendelen), lichtintensiteit en -spectrum bij de okselknoppen gevarieerd. De uitloop van okselknoppen nam in een aantal proeven toe als de source/sink verhouding verhoogd werd, maar in sommige ook niet. Van de onderzochte factoren bleek lichtintensiteit bij de okselknop de belangrijkste factor te zijn voor het wel of niet uitlopen van de okselknoppen nadat de bloemtak erboven geoogst was (correlatieve inhibitie verwijderd).

Een aantal rassen is geteeld op 10 locaties verspreid over Nederland, Kenia, Ethiopië, Ecuador en India. Qua productie en morfologische eigenschappen waren er duidelijke interacties tussen ras en de omgevingsfactoren. Rasverschillen in totale kilogram productie werden meer bepaald door het aantal takken dan de takgrootte van het ras.

Tevens is een gewasgroeimodel ontwikkeld dat de gewasopbouw (in 3 dimensies) kan berekenen. Het model kan de lichtabsorptie en vervolgens de fotosynthese van de verschillende bladeren van het gewas berekenen onder invloed van bijvoorbeeld verschillende belichtingssystemen.

Abstract UK

The crop architecture and breaking of axillary buds are key factor for the production of cut roses. A number of experiments have been conducted on crop architecture and bud break. In these experiments the source/sink balance, correlative inhibition, light intensity and light spectrum reaching the bud were varied by different means. In some experiments bud break increased when source/sink balance increased. The most important determinant for bud break appeared to be the light intensity at the axillary bud, after the flowering shoot above the bud was harvested (correlative inhibition removed).

Several cultivars were grown on 10 locations spread over The Netherlands, Kenya, Ethiopia, Ecuador and India. For productivity and shoot morphology distinct interactions were found between cultivar and environment. Differences in total kilogram production between cultivars was more dependent on differences in number of stems than stem weight.

A functional-structural plant model of cut rose was developed that can calculate the crop architecture in three dimensions. This model can calculate the light absorption as well as photosynthesis of all the different leaves of the crop, as influenced by for instance the lighting system.

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Samenvatting

De gewasopbouw of gewasstructuur is een belangrijke bepalende factor voor productie en kwaliteit bij roos. Met name het uitlopen van een okselknop en en de daarop volgende uitgroei tot bloemscheut hangen nauw samen met de gewasstructuur. De ideale gewasopbouw is niet gelijk voor alle rassen, terwijl nieuwe rassen elkaar in snel tempo opvolgen. Ook de ontwikkelingen op het gebied van robotisering en mobiele teeltsystemen gaan nieuwe eisen stellen aan de gewasopbouw. De gewasopbouw zal zodanig moeten zijn dat het enerzijds voldoet aan de eisen van de techniek en dat anderzijds een optimale productie en kwaliteit geleverd worden. Dit vraagt om keuzen te maken in teeltstrategieën.
Gewasopbouw is een complex proces dat niet los gezien kan worden van plantverband, raseigenschappen, snoeistrategie en klimaat.
Om hier meer grip op te kunnen krijgen is een aantal proeven uitgevoerd naar verschillen in plantopbouw.Tevens is een gewasgroeimodel ontwikkeld dat de gewasontwikkeling en gewasopbouw (in 3 dimensies) kan berekenen. Dit onderzoek is uitgevoerd in het kader van een STW project met cofinanciering van het Productschap Tuinbouw en werd begeleid door de telers S. v.d. Hulst en J. v.d. Nouweland en rozenadviseur D. v.d. Sar (Phytocare). Tevens is dit project gekoppeld aan een project naar veredeling en fenotypering van roos dat in het kader van TTI Groene Genetica werd uitgevoerd.
In een eerste proef werden twee rassen (Ilios en Akito) bij twee plantdichtheden (4 en 8 planten per m^2) geteeld om na te gaan wat invloed is van assimilatenbeschikbaarheid in de plant op vorming van bloemtakken. Akito vormde meer en grotere takken bij lagere plantdichtheid (grotere assimilatenbeschikbaarheid). Daarentegen werden de extra assimilaten bij Ilios nauwelijks gebruikt om meer takken maar vooral om veel grotere takken te vormen. Als een bloemtak geoogst wordt, loopt een variabel aantal okselknoppen uit tot nieuwe takken. De oogst van een tak verandert (1) de lichtintensiteit en (2) lichtspectrum bij de okselknoppen, (3) de correlatieve inhibitie (remmende werking van een plantendeel op groei en ontwikkeling van een ander plantendeel, veelal als gevolg van hormonale werking) en (4) de source/sink verhouding in de plant (verhouding assimilatenaanbod en -vraag). Er zijn vier proeven met het ras Akito gedaan om na te gaan welke van deze vier factoren het meest belangrijk zijn. Variaties in deze vier factoren zijn in de proeven aangebracht door bladeren of volgroeide takken weg te halen, door het aantal jonge uitgroeiende takken te variëren, door licht boven het gewas weg te schermen en door licht direct op de okselknoppen te schijnen. Een afname van de source:sink ratio leidde soms tot minder knopuitloop, maar niet altijd. Als de source: sink ratio verlaagd werd door weghalen van bladeren of volgroeide scheuten nam de knopuitloop zelfs toe. Bij de behandelingen waar meer licht bij de okselknoppen kwam (door weghalen van takken, niet wegschermen van licht of het direct belichten van knoppen), nam de knopuitloop toe. Verhoging van de rood:verrood verhouding leek hetzelfde resultaat te hebben als meer licht bij de knoppen, maar deze was veelal verstrengeld waar meer licht op de knoppen kwam. Geconcludeerd kan worden dat na verwijdering van een bloemtak, licht bij de okselknop de belangrijkst factor is die de uitloop van okselknoppen bepalen, terwijl de source:sink ratio hierbij een kleinere rol speelt.
Vervolgens zijn drie proeven gedaan om nader te onderzoeken of de lichtintensiteit of het lichtspectrum bij de okselknoppen bepalend is voor de knopuitloop. Het weghalen van de opgaande takken resulteerde zowel in een hogere intensiteit en hogere rood:verroord verhouding bij de okselknoppen als in uitlopen van okselknoppen. Als gelijktijdig met het weghalen van de opgaande takken de okselknoppen door groen crêpe papier werden beschaduwd, zodat de intensiteit en de rood:verrood verhouding gelijk bleef aan die met planten met opgaande takken, lag het aantal uitgelopen knoppen tussen dat van behandelingen met en zonder opgaande takken (en zonder papier). Dit duidt erop dat opgaande takken de knopuitloop beïnvloeden via zowel correlatieve inhibitie als licht bij de knop. Verschillende behandelingen met lichtintensiteit en rood:verrood verhouding bij de knoppen (door toepassen van LEDs en wegschermen van licht met verschillende kleuren papier) lieten een positief effect van lichtintensiteit maar niet van de rood:verrood verhouding op knopuitloop zien. Dus de lichtintensiteit die de okselknop bereikt blijkt een belangrijke bepalende factor voor knopuitloop.
Er is een functie-structuurmodel voor roos ontwikkeld. Dit simulatiemodel kan de structuur van een rozengewas in drie dimensies beschrijven. Het model kan de lichtabsorptie en vervolgens de fotosynthese van de verschillende bladeren van het gewas berekenen. Consequenties van verschillende vormen van gewasopbouw en belichting met verschillende belichtingssystemen (waaronder LED tussen of boven het gewas) voor fotosynthese en groei van het gewas zijn er onder meer door te rekenen. Het model behoeft nog wel verdere calibraties en tests om in de praktijk als beslissingsondersteuning gebruikt te kunnen worden.
Vervolgens, dwz in het TTI-GG project, heeft het onderzoek zich gericht op rasverschillen en hoe die onder verschillende

teeltomstandigheden naar voren komen. In samenwerking met de rozenveredelingsbedrijven De Ruiter, Moerheim Roses, Terra Nigra, Van Kleef Roses en Olij Roses en tuinbouw adviesbedrijf Phytocare is een aantal genotypen (rassen) van snijrozen geteeld op 10 locaties verspreid over Nederland, Kenia, Ethiopië, Ecuador en India. Op elk van de buitenlandse locaties zijn 150 genotypes geteeld terwijl op de vier Nederlandse locaties 20 cultivars zijn geteeld. Het doel van het experiment was het uitvoeren van een analyse van de productie en plantkenmerken (fenotype) van de snijroos onder vele verschillende omstandigheden. Met betrekking to productiviteit en morfologische eigenschappen van de bloemtakken waren er duidelijke interacties tussen genotypen en de omgevingsfactoren. Verschillen tussen genotypen in totale kilogram productie werden meer bepaald door het aantal takken dan de takgrootte van het genotype. Bij het vergelijken van de cultivars bleek er een significant negatieve correlatie te bestaan tussen de raseigenschappen stengel gewicht en aantal stengels per plant. In tegenstelling hiermee was er wanneer de locaties werden vergeleken, juist een positieve correlatie tussen stengel gewicht en aantal stengels per plant.
In Nederland werden bij sommige genotypes de meer gunstige omstandigheden in het voorjaar vooral omgezet in meer takken en bij andere genotypes vooral in zwaardere takken. Deze verschillende reacties op extra assimilaten was ook zichtbaar in de proeven met Ilios en Akito in Nederland. Genotypen die in Nederland grote seizoensverschillen lieten zien in productie toonden, presteerden op jaarbasis relatief minder goed.

1 Effect van plantdichtheid bij twee rassen

Dit hoofdstuk is gepubliceerd als:
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1.1 Abstract

When grown in lower density, rose plants produce more assimilates. The additional assimilates can be used to produce more and/or heavier flowering shoots.
The effect of planting density was investigated during the first five flowering flushes of a young crop.
In a heated greenhouse, two cut-rose cultivars were grown at two planting densities: 8 and 4 plants per m^2. Cultivar 'Akito' was grown on its own root while 'Ilios' had been grafted on a rootstock of 'Natal Briar'. From cuttings and stentlings, a new crop, with a bent canopy, was grown. Starting at the end of June 2007, flowering shoots were being harvested during a time span of eight months.
Based on 'flowering flushes' (times of high harvest rate) the harvesting time span could be divided into five consecutive periods. The two cultivars showed contrasting responses to planting density. In the first three flowering flushes, the response in 'Ilios' was extraordinary: At low density, plants did not produce more shoots. But the response in shoot weight was larger than in 'Akito'.
The results imply that there was a genetic difference in the effect of assimilate availability and/or local light environment. During the first three flowering flushes, these factors can not have influenced shoot number in 'Ilios', while they did in 'Akito'. It is suggested that decrease of assimilate availability in winter, caused the response in shoot number to show up for 'Ilios', after the third flowering flush.

1.2 Introduction

Planting density expresses the number of plants per unit of floor area. It is determined by the plant configuration chosen at the time of (trans)planting. In literature 'planting density' is frequently encountered under a different name. 'Plant population density' and 'plant density' have been used as synonyms. In this paper 'planting density' is sometimes abbreviated to 'density'.
This study deals with planting density in greenhouse cut-rose production, from the perspective of the individual plant. When grown in lower density, individual plants can intercept more photosyntheticcally active radiation and produce more assimilates. Extra assimilates can be used to produce a larger number of shoots and/or to produce heavier shoots. Many studies reported that rose plants respond to lower density by producing more flowering shoots (Dambre *et al.* 1998; de Hoog *et al.* 2001; Kool, 1997; Mortensen and Gislerod, 1994). Frequently the response includes an increase of (average) shoot weight as well.
Weight is an important indicator of the quality of a flowering shoot, since it is related to the size of a shoot (Marcelis - van Acker, 1994a). Heavy shoots tend to have big flowers and high (aesthetic) value (Matthijs Beelen, personal communication). By growing at lower planting density, growers can enhance shoot quality, at the cost of shoot quantity: Although individual plants produce more shoots at lower density, the number per square meter is lower.
Shoot production per square meter differs between cultivars. In case of stented plants, the background of the rootstock matters as well (de Vries and Dubois, 1990; Dieleman *et al.* 1998; Kool and van de Pol, 1992; Nazari *et al.* 2009). What can also differ is the relative size of the effect of planting density on shoot number (de Hoog *et al.* 2001; Mortensen and Gislerod, 1994). 'Akito', grown on own root, is a cultivar known to produce a large number of shoots per square meter. 'Ilios', grafted on a rootstock of 'Natal Briar', produces fewer shoots (Dick van der Sar, personal communication).
An effect of planting density is not necessarily a response to assimilate availability. The effect can also be a direct response to the local light environment: In lower density there is less mutual shading among plants, resulting in larger quantity and altered spectrum of local light. Both assimilate availability and local light can have a significant and substantial

effect on the number of flowering shoots. Strong evidence for the effect of assimilate availability came from the positive effect of CO_2 enrichment on flower number (Hand and Cockshull, 1975; Zieslin *et al.* 1972). The effect of local light was shown with supplementary light of different spectra: Low red:far-red ratio, as encountered in canopy shade, decreased flower number (Mor and Halevy, 1984).

Due to progress in canopy closure, and seasonal difference, the effect of planting density can change over time. To see the change of the effect over time, a division of the total time span should be applied in data analysis. A division can be facilitated by 'flowering flushes'. Flowering flushes are a common pattern in a (young) rose crop: Harvest rate of flowering shoots typically fluctuates with a period of 5 to 10 weeks (de Hoog *et al.* 2000).

The objective of this study was to investigate the effect of planting density on flowering shoot production by rose plants of two contrasting cultivars. How do plants use additional assimilates obtained in a lower density? Do they produce more shoots and/or heavier shoots? Is the response different between two cultivars, which are contrasting in productivity? Does the response change over time, in consecutive flowering flushes, after transplanting (in late spring)?

1.3 Materials and methods

Plant material and growth conditions

The experiment was carried out in Wageningen (the Netherlands, *latitude* 52° N) between May 2007 and February 2008. Rose plants were grown in double rows, on rockwool, in a heated experimental greenhouse. From cuttings and stentlings, a crop with a bent canopy was created.

Two cut-rose cultivars (*Rosa hybrida*) were used: 'Akito' was grown on its own root. 'Ilios' had been grafted on a rootstock of 'Natal Briar'. These two cultivars were selected because they could be grown in the same climate, but were expected to behave differently (see 'Introduction').

The distance between paired rows of plants was 25 cm (centre to centre). The distance between plants in the same row was 16.7 cm or 33.3 cm. These two spacings corresponded to planting densities of 8 and 4 plants m^{-2}.

The combination of two cultivars and two planting densities gave a total of four treatments. For each treatment four plots were set up as a part of a double row, including nine plants. The outer four plants were considered as the border of the plot, leaving five neighbouring plants per plot for data collection.

Water and nutrients were supplied to the rockwool slabs via an automatic drip fertigation system. The temperature set points for day and night were 20.0 °C and 16.5 °C respectively. Ventilation or heating started when the real temperature deviated by more than 1 °C from the set point. CO_2 was supplied if the concentration dropped below 400 ppm. Supplemental lighting by high pressure sodium lamps (Hortilux Greenpower, fitted with Philips, SON-T, 600 W light bulbs) provided a minimum photosynthetic photon flux density (PPFD) of 97 µmol m^{-2} s^{-1} at a height of 90 cm above the rockwool slabs (above the upright canopy). At a height of 28 cm above the rockwool slabs (above the bent canopy) the PPFD was 76 µmol m^{-2} s^{-1}, in the absence of an upright canopy. The natural day length was extended to 18 hours (2:00 till 20:00), with lamps being switched on automatically when outside global radiation fell below 150 W/m^2. Climate and fertigation were controlled according to commercial practice.

Daily averages of temperature, relative humidity and PPFD (at crop level) are summarized in Table 1, for consecutive periods of the experiment. Periods are explained in 'Results' and in Figure 2. Daily average CO_2 concentration was 400 ppm (standard deviation 14 ppm). The difference between periods (Table 1) was smaller than 4%.

Crop management

Cuttings and stentlings rooted in rockwool cubes were transplanted when they had a young primary shoot, on 8 May 2007. The primary shoots were bent when second order lateral shoots had appeared, on 6 June. The first flowering shoots were harvested on 30 June. From then on flowering shoots were harvested every day. Shoots were harvested when petals had started unfurling. Blind shoots were harvested as well and dealt with as other shoots. However, blind shoots were very rare (4 out of 1378 harvested shoots), so their role is negligible.

Lateral shoots, appearing before flowering of the main stem, were removed (as in commercial practice) three or four times per week.

Not all shoots were left growing until flowering. Some were bent down, far before flowering, to supplement and/or refresh the bent canopy. The decision to bend or to let grow was based on the location of the stem base. First and higher order lateral shoots of the bent primary shoot were bent (Figure 1.). Shoots appearing on the first 10 cm of the primary shoot,

outside the rockwool cube, were removed at least once per week. Flower buds of the bent canopy were removed twice per week.

Measurements

When a shoot was harvested, harvest day was recorded and fresh weight was measured. Dry weight was measured after drying for two nights in a stove at 105 °C. Plot averages were calculated for number of (harvested) flowering shoots (per plant), mean shoot fresh weight (g), and cumulative harvested dry weight (g per plant). These were calculated for each of the five periods and for all periods together.

At the end of the experiment (end of February 2008) the entire bent (primary) shoot, with all its lateral branches, was cut off from all plants. Fresh weight of green leaves was measured for all plants, and leaf area for part of the plants (53 out of 80). Plot averages were calculated first for fresh weight (g/plant). Leaf area (m^2/plant and m^2/m^2) was calculated using a linear relation between leaf fresh weight and leaf area (R^2=0.993).

Experimental design and analysis

Plots were arranged as a randomized block design. Each treatment was present once or twice in each of three double rows, considered as separate blocks.

Data processing and statistical tests were carried out with SPSS 15.0. The effect of planting density, per cultivar, was tested according to Fisher's protected LSD (least significant difference). This was a posthoc test with a linear model including cultivar and planting density combined as one factor (with four levels). In addition to a two-sided test, a one-sided test was evaluated as well. To answer the question if a quantity was larger at low density, a one-sided test is justified.

1.4 Results

During the time span of harvesting, there was a clear pattern of flowering flushes:

There was an alteration of high and low harvest rate (Figure 2.). The contrast in harvest rate was much more pronounced in 'Akito' than in 'Ilios' And flowering flushes came earlier at low planting density. Nevertheless, all treatments showed more or less synchronous fluctuation. Based on fluctuation in harvest rate (especially of 'Akito'), the total time span could be divided into five consecutive periods. Each period included one flowering flush. Since the time between subsequent flowering flushes increased in autumn and winter, the duration of consecutive periods became longer (Table 1)

Fluctuation in harvest rate was common to all plots. But the phase of the fluctuation was shifted: The timing of flowering flushes was slightly different. These differences were not merely due to planting density or cultivar. Plots of the same treatment showed differences as well. Because combining plot data would make the fluctuation less pronounced, it was preferable to represent treatments with only one plot (of the four) in Figure 2.

Cumulatively harvested dry weight (per plant) was much larger at low planting density (Table 2). This effect of density was relatively small in the first period and increased in subsequent periods. For 'Akito' the relative size of the effect increased faster than for 'Ilios'.

In period one to three, the effect on number of flowering shoots (per plant) was very different between the cultivars. For 'Akito' shoot number was much larger, at low planting density. For 'Ilios' (grafted on 'Natal Briar') density had no effect (Table 2). After the third period, both cultivars had a larger shoot number at low density, and the relative size of the effect was similar.

Mean shoot weight was larger at low planting density. However, in period one to three, the (relative) size of the effect was larger for 'Ilios' than for 'Akito' (Table 2). For 'Akito' the effect was not even significant in period 1 and 2.

At the end of the experiment, the leaf area of the bent canopy (per m^2 floor) was not significantly different between cultivars and densities ($p > 0.5$, data not shown). On average there was 1.34 m^2 leaf area per m^2 floor. Per plant, leaf area was about two times larger at low density.

1.5 Discussion

Shoot development rate depends on temperature (Marcelis - van Acker, 1994a; Mattson and Lieth, 2007). Therefore the temperature decrease in autumn and winter (Table 1) could explain the increase of the time between flowering flushes (Figure 2.). Earlier flushes at low planting density, could result from higher assimilate availability: Reduction of assimilate availability (by leaf removal), during bud development, increased the time between bud break and flowering (Marcelis - van Acker, 1994b).

Assimilate production enhances while the (bent) canopy closes. Canopy closure could explain why the relative size of the effect on cumulative harvested DW increases over the earlier periods (Table 2): Canopy closure was still progressing at low density, while it did not improve anymore at high density.

At low planting density, additional assimilates can be used by (individual) plants to produce more shoots and/or to produce heavier shoots (Dambre *et al.* 1998; de Hoog *et al.* 2001; Kool, 1997; Mortensen and Gislerod, 1994). The response of 'Ilios' (grafted on 'Natal Briar') was unexpected, especially the response in period one to three. During these periods, the response to low density did not include an increase of the number of flowering shoots, only of the weight.

Lack of response to planting density implies that assimilate availability and local light environment did not have an effect. Apparently these factors did not affect the number of flowering shoots, in 'Ilios' (grafted on 'Natal Briar'). This number must have been limited by other factors, such as correlative inhibition. After the third period, assimilate availability and/or local light environment became limiting factors, since a response to density showed up (Table 2). This could be explained by decreased assimilate availability: PPFD decreased substantially after periods two and three (Table 1).

The idea that assimilate availability became limiting to shoot number in 'Ilios', after period three, is supported by changes in cumulative harvested DW and shoot number: After period three, cumulative DW (per plant) decreased for 'Ilios', and by far the most (25%) at high density (Table 2). Shoot number decreased for 'Ilios' at high density, but not at low density, and not for 'Akito'.

If extra assimilates are not used to produce more shoots, they should be used to produce heavier shoots. Therefore it is not surprising that the effect on shoot weight was much larger for 'Ilios' than for 'Akito' (Table 2): During period one to three 'Ilios' plants could use extra assimilates only for increased shoot weight.

1.6 Conclusion

Rose plants of the two cultivars in this study showed a different response to planting density. Additional assimilates obtained at lower density, were used in a different way. 'Akito', grown on its own root, produced both a larger number of flowering shoots (per plant), and heavier shoots. 'Ilios', grafted on (rootstock) 'Natal Briar', did not produce a larger number of shoots (per plant), only heavier shoots. But the response of shoot weight was larger for 'Ilios' than for 'Akito'. The cultivar differences were pronounced in the first three flowering flushes, during summer and early autumn. After the third flush however, the relative size of the responses to density became similar. Then, a response of shoot number had shown up for 'Ilios'.

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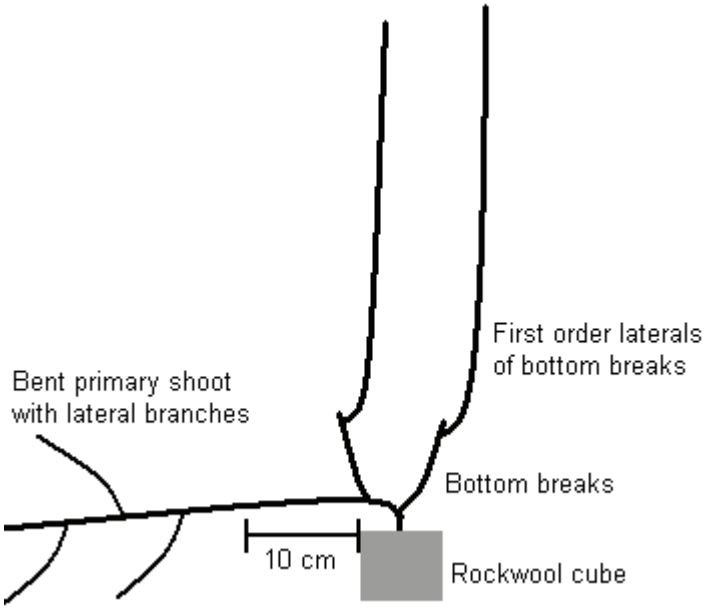

A ('Akito') B ('Ilios' grafted on 'Natal Briar')

Figure 1. Plant architecture in relation to crop management. The bar with '10 cm' indicates the part of the primary shoot, that was kept free of lateral shoots. See 'Methods' for explanation on the crop management.

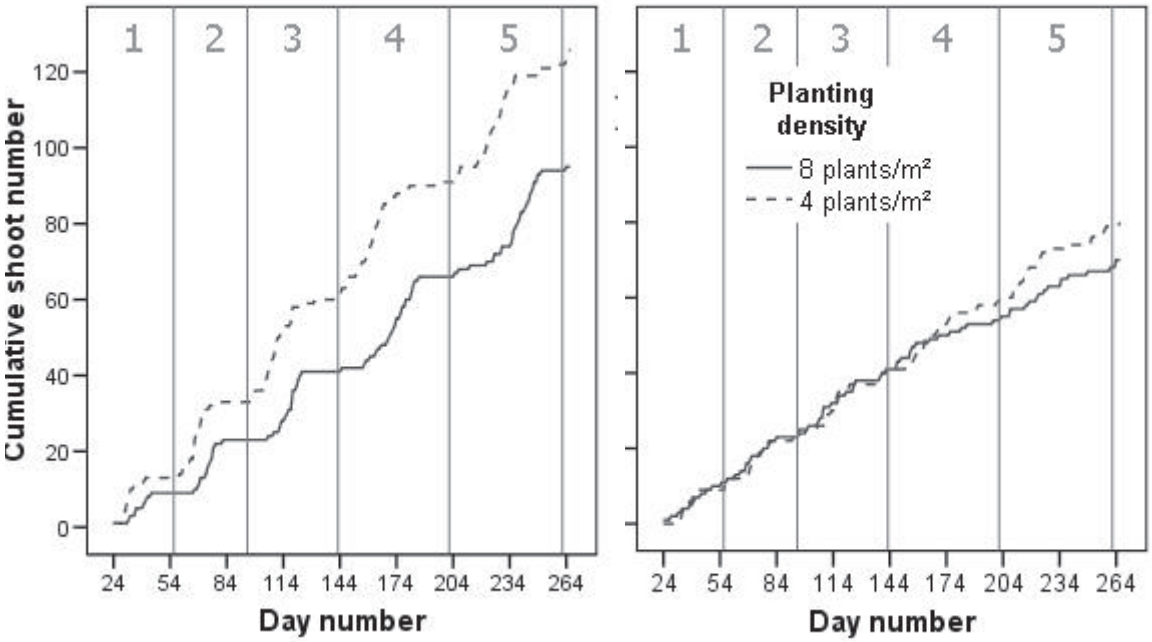

Figure 2. Cumulative number of harvested shoots plotted against day number. Day 0 is the day of bending the primary shoots, 6 June 2007. Each treatment is represented by a single plot only (one of the four). The vertical lines display the division of the harvesting time span into five periods (1-5). The division was derived from the fluctuating harvest pattern of 'Akito': Each period contains one flowering flush.

Table 1. Mean and standard deviation of the daily average of photosynthetic photon flux density (PPFD) at crop level, greenhouse air temperature (Temp.) and relative humidity (RH), in five consecutive periods of the experiment.

Period	1st day	Time	PPFD (µmol/(m² s))		Temp. (°C)		RH (%)	
		(day)	mean	st. dev.	mean	st. dev.	mean	st. dev.
1	30-jun	33	172	20	23.4	1.8	69	4
2	2-aug	39	160	21	22.6	1.6	71	4
3	10-sep	48	122	19	20.1	0.7	73	4
4	28-okt	59	86	8	18.3	0.6	77	3
5	26-dec	60	88	11	18.5	0.6	76	3

Table 2. Number of harvested flowering shoots, mean shoot fresh weight, and cumulative harvested dry weight (of the flowering shoots), in five consecutive periods of the experiment (see Table 1 and Figure 2.). For each cultivar, the relative size and the significance of the effect of planting density (P. density) are given. 'Rel. dif.' is the relative difference of low planting density (4 m^{-2}) compared to high density (8 m^{-2}).

*ns, *, ** and *** : In a two-sided test the effect was not significant, significant at 0.05, 0.01 or 0.001, respectively.*

ns[1] : The effect was significant at 0.05 in a one-sided test (Was it larger at low density?), but not in a two-sided test.

Cultivar:	Akito				Ilios			
P. density (per m²):	8	4			8	4		
Period			Rel. dif.	Sig.			Rel. dif.	Sig.
Number of harvested flowering shoots					*(per plant)*			
1	1.95	2.55	31%	ns[1]	2.30	2.05	-11%	ns
2	2.65	3.90	47%	***	2.80	2.65	-5%	ns
3	3.50	5.15	47%	***	3.40	3.15	-7%	ns
4	4.15	5.75	39%	**	2.60	3.60	38%	*
5	3.90	5.45	40%	*	2.60	3.65	40%	ns[1]
1-5	16.2	22.8	41%	***	13.7	15.1	10%	ns
Mean shoot fresh weight					*(g)*			
1	42.6	47.4	11%	ns	44.5	57.2	28%	**
2	41.0	45.7	11%	ns	45.6	65.9	45%	***
3	40.2	49.0	22%	**	48.2	72.8	51%	***
4	35.6	46.9	32%	**	45.9	60.4	32%	***
5	39.1	49.0	25%	ns[1]	45.5	56.4	24%	*
1-5	39.0	47.4	21%	***	46.2	62.2	35%	***
Cumulative harvested dry weight					*(g per plant)*			
1	18.2	26.7	47%	**	25.3	30.7	21%	*
2	23.7	38.7	63%	**	31.7	45.7	44%	**
3	30.6	57.2	87%	***	40.7	58.7	44%	**
4	32.6	59.3	82%	**	30.3	56.3	86%	**
5	34.7	59.8	72%	**	31.0	53.8	73%	**
1-5	140	242	73%	***	159	245	54%	***

2 Vier hypothesen voor uitloop van okselknoppen

Dit hoofdstuk is aan een wetenschappelijk tijdschrift aangeboden als:

Four hypotheses explaining outgrowth of axillary buds after removal of a flowering shoot in a cut-rose crop. By A. Maaike Wubs, Ep Heuvelink, Leo F.M. Marcelis, Robert C.O. Okello, Alisa Shlyuykova, Gerhard H. Buck-Sorlin, Jan Vos.

2.1 Abstract

When flower-bearing shoots in cut-rose are harvested (removed), a varying number of repressed axillary buds on the shoot remainder start to grow into new shoots (bud outgrowth). Harvesting of a shoot changes (1) light intensity and (2) light spectrum reaching buds, (3) correlative inhibition and (4) source:sink ratio of the plant. It was the goal of the paper to determine which of these factors is most important for bud outgrowth in a cut-rose crop. Four experiments were conducted, where these factors were varied by leaf removal, removal of mature shoots, varying the number of young shoots, shading of the crop and application of direct light on the buds. Increase in source:sink ratio was not consistently associated with higher bud outgrowth; if source:sink ratio was decreased by removal of leaves or a mature shoot, bud outgrowth showed even a tendency to increase. Treatments where more light reached the bud (due to less shoots, no shading of the crop, application of local light) increased bud outgrowth. Increased red:far-red ratio had the same result as more light reaching the bud, but was often interrelated with light intensity. It was concluded that after removal of the flower-bearing shoot, light intensity and spectrum were the most important factors explaining bud outgrowth on the shoot remainder, while source:sink ratio was less associated with bud outgrowth.

2.2 Introduction

Plant architecture comprises the type and relative arrangement of organs (Barthélémy and Caraglio, 2007). In a cut-rose crop, architecture of the crop is often modified by crop management. The primary shoot and, later during the cultivation, shoots without flowers are bent to create an additional source of assimilate supply for growth (De Hoog, 2001). Mature shoots (close to flowering) are harvested periodically, leaving a shoot remainder of one or two nodes. This results in outgrowth of the axillary buds on the shoot remainder, generating new shoots which constitute the next harvest. Harvesting a mature shoot alters four aspects in the crop: (1) light intensity lower in the crop canopy increases, and (2) light spectrum changes (higher intensity of red light compared to far-red light, resulting in a higher red:far-red ratio). With the harvest of the shoot, also (3) the correlative inhibition experienced by buds on the shoot remainder is removed, and (4) the source:sink ratio (i.e. the ratio between supply and demand of assimilates) changes. All these four factors can potentially explain the effect of shoot harvest on bud outgrowth.

High light levels are reported to increase the number of shoots in *Vaccinium bracteatum* (Kawamura and Takeda, 2002), and *Macadamia integrifolia* (Olesen *et al.* 2011). Rose explants exhibit a higher percentage bud outgrowth at higher light levels, while light was not required for bud outgrowth in tomato (Girault *et al.* 2008). Also light spectrum effected bud break in the rose explants: far-red LEDS inhibited bud break, whereas red, blue and white light promoted bud break. In cereals and grasses, both higher red:far-red ratio and higher light intensity increased tiller production (Evers *et al.* 2006; Belesky *et al.* 2011). The same effect was also found for shoot production in *Salvia exserta* (Mata and Botto, 2011) and bud outgrowth in wild-type *Arabidopsis* (Finlayson *et al.* 2010; Su *et al.* 2011).

Correlative inhibition is the suppression of outgrowth of axillary buds by the shoot through hormonal signalling in which auxins, cytokinins and strigolactones play a role (Domagalska and Leyser, 2011). Export of auxin from developing organs increases auxin concentration in the stem. This inhibits the export of auxin from newly formed organs and hence development of new organs is arrested (Domagalska and Leyser, 2011). For the developing bud, auxin export establishes a transport path which ensures bud outgrowth (Müller and Leyser, 2011). Alternatively, high auxin concentrations might decrease and increase upward movement of cytokinins and strigolactones, respectively (Domagalska and Leyser, 2011).

Cytokinins are produced in the shoots (Bredmose *et al.* 2005, 2008; Tanaka *et al.* 2006) and the roots when local auxin concentrations are low; they are transported acropetally where they promote bud outgrowth. The mode of action of strigolactones is still uncertain: it might reduce the auxin transport through the stem and increase competition between buds (Domagalska and Leyser, 2011) but it might also act locally by directly suppressing bud outgrowth (Dun *et al.* 2012). Harvest of rose shoots alters the source:sink ratio of the plant. Whether this affects bud outgrowth is less well known. More lateral buds developed in chrysanthemum under high light intensities. This was attributed to higher assimilation rates (Schoelhorn *et al.* 1996), and thus higher availability of assimilates (source strength). Henry *et al.* (2011) demonstrated that buds are sink organs, which need to import sugars for proper development. The uptake of sugars by buds coincides with the onset of bud outgrowth (Maurel *et al.* 2004). Young rose shoots (before appearance of the flower bud) act as sinks (Mor and Halevy, 1979). The presence of young rose shoots, therefore, decreases the availability of assimilates for buds and the source:sink ratio of the plant, and might inhibit bud outgrowth in this way. Shoots older than three weeks have enough capacity to sustain themselves without assimilate import from other plant parts, and will increase the source:sink ratio. In simulation models, source:sink ratio often determines the formation of new organs (Mathieu *et al.* 2008; Wubs *et al.* 2009).

The effects of light intensity and light spectrum on bud outgrowth have been intensively studied in model plants like *Arabidopsis* and pea (*Pisum sativum*). In roses, research is often done in simple systems. For example, Girault *et al.* (2008, 2010) studied the effect of light intensity and light spectrum on bud outgrowth with rooted stem segments from which leaves were removed. Bredmose (1997) showed the effect of light intensity and plant density on time to bud outgrowth on single node cuttings. Besides, research regarding bud outgrowth has often focussed on one aspect, keeping other aspects constant. However, the interaction and relative importance of the factors outlined above in an established crop are less well known.

This paper aims at determining the relative importance of four hypotheses for regulation of bud outgrowth in response to shoot removal in a cut-rose crop: 1) light intensity received by the bud, 2) light spectrum received by the bud, 3) correlative inhibition, and 4) source:sink ratio. Four experiments were conducted in a rose crop, designed to distinguish between the four hypotheses for regulation of bud outgrowth. The amount as well as the timing of bud outgrowth were observed.

2.3 Materials and methods

General information. Experiments were carried out in a rose crop, *Rosa x hybrida* cv. 'Akito'. The crop was grown in a 12 x 12 m^2 compartment of a multi-span Venlo type greenhouse in Wageningen, the Netherlands (52°N). The crop was established early in 2008 from one node cuttings bearing a shoot. Cuttings were inserted in rockwool cubes (7 x 7 x 7 cm, Grodan delta, Grodan B.V., the Netherlands), which were placed onto rockwool slabs (Grodan B.V., the Netherlands). Plants were placed in double rows (0.25 m apart) with a distance of 0.20 m within the rows, resulting in a planting density of 6.5 plants m^{-2}. The shoot of the cutting was bent horizontally and kept in place with wire. This so-called bent shoot is common practice in commercial cut-rose cultivation and increases assimilate production in the plants (De Hoog, 2001). From the base of the bent shoot, two buds were allowed to grow (bottom breaks), others were removed at an early stage. At maturity (shoots bearing open flower), all shoots resulting from these buds were harvested two or three nodes above their base. This was the first so-called 'flush' of harvested shoots. On the shoot remainders of these harvested shoots, buds started to grow and were pruned to four shoots per plant, forming the second flush of shoots. These were the shoots used in the first experiment. After the first experiment, an underhook cut was applied (Zieslin, 1981), in which the shoots bearing a flower were cut below their base. After this, two flushes were grown, called reset flushes. A reset flush was the period in which the crop could recover from the treatments, the bent shoot could be restored (if necessary) and plants which underwent different treatments were allowed to recover to the same shape and size. Following the reset flushes, the second experiment was conducted. Between the second and third experiment, another two reset flushes were grown. The last experiment was conducted after several other experiments and reset flushes. Experiments comprised half of the rows in the greenhouse, the other half of the rows was used for other experiments or undergoing reset flushes.

Supplemental light was provided by high pressure sodium lamps (600 W, Philips, the Netherlands) which provided 150 µmol m^{-2} s^{-1} at crop level. Supplemental light was provided between 0000 HR and 2000 HR (till 15 Dec. 2009) or between 0000 HR and 1800 HR (since 15 Dec. 2009) when global radiation was below 150 W m^{-2} outside the greenhouse (approximately 318 µmol PAR m^{-2} s^{-1} depending on light spectrum) and switched off when this radiation exceeded 250

W m^{-2} (approximately 530 µmol PAR m^{-2} s^{-1} depending on light spectrum). Heating set points were 17.5 °C during the night and 21.0 °C during the day. Realised climate conditions per experiment are given in Table 1. Water and nutrients (substrafeed E1, Yara Benelux B.V., Vlaardingen, the Netherlands) were supplied via a drip-system (nominal discharge 2 l h^{-1} per dripper; one dripper per plant).
In each experiment, bud outgrowth was observed on a shoot remainder of two nodes resulting from the harvest of a mature shoot. The shoot remainder had three positions for axillary bud outgrowth: the upper node (x1), the lower node (x2) and the basal ring (r), where the shoot was attached to the lower order branch. At the position in the ring, more than one bud could grow. Buds with a length of 1.5 cm were considered as broken, and the date when a bud reached 1.5 cm was considered as the day of bud outgrowth.

Experiment I: different number of young shoots. In this experiment, the effect of the number of young growing shoots (0, 1, 2, 3 or 4) on bud outgrowth was observed. These young shoots acted as sinks for assimilates. The experiment started with plants where four shoots were present (see description above). Approximately two weeks before they would normally be harvested, three shoots were cut two nodes above their base. There was one shoot remaining (X, Figuur 1.). Date of bud outgrowth on the shoot remainders from the harvested shoots was recorded for all buds. The shoot resulting from the uppermost bud was termed the 'GR1 shoot'. Two weeks later, the remaining shoot (X, Figuur 1.) was harvested two nodes above its base. At the same time, young shoots resulting from bud outgrowth on the shoot remainders of the three shoots harvested previously were thinned to 0, 1, 2, 3 or 4 shoots per plant (Figuur 1.). The GR1 shoot was left growing in each treatment except for the treatment where zero shoots were present. Bud outgrowth on the shoot remainder from the last harvested shoot X was observed for 21 days at least three times a week. Buds growing in other positions were removed twice per week.
Source:sink ratio of the plant was quantified by comparing the growth of the GR1 shoot in each treatment with the potential growth of a GR1 shoot. Potential growth of the GR1 shoot was measured in an additional treatment P, where only the GR1 shoot was allowed to grow. All other bud outgrowth in this treatment were removed. Growth of the GR1 shoot was measured non-destructively every week in half of the plants in each plot of each treatment (except for 0S where the GR1 shoot was not present) by measuring the length of the stem and the diameter at the stem base. From the other plants in each treatment, each week two to three GR1 shoots per plot were destructively measured; their stem length and diameter, fresh and dry weight of stem and leaves and, if present, fresh and dry weight and diameter of the flower bud were measured. From these destructive measurements, relationships were derived to estimate total dry mass of the GR1 shoot.
The experiment was set up as a completely randomised design with two plots per treatment. A plot consisted of a double row with 9-10 plants per row, with two border plants at either side of the plot. The experiment was conducted in June and July 2008.

Experiment II: role of the bent shoot. In this experiment, the effect of assimilate supply from the bent shoot on bud outgrowth was studied. Four treatments were applied:
1. bent shoot cut off
2. early shading of the bent shoot, one week before treatments 1 and 3 were initiated
3. late shading of the bent shoot, at the time bent shoot was cut off
4. control, no treatment on the bent shoot
5. Shoot remainders for observation of bud outgrowth were created when treatments 1 and 3 were initiated.

In the reset flush before the experiment, four shoots were kept per plant. Three weeks after harvest of the previous reset flush, two of the four shoots were cut back to 15 cm from the surface of the rockwool cube. Shoots sprouting from those shoot remainders were non-uniform (Fig 2A). An underhook cut was applied to remove these shoots (Figuur 2B) and at the same time one of the two remaining shoots was harvested, leaving a shoot remainder of two nodes. The other shoot was kept intact. Bud outgrowth was observed for 23 days on the shoot remainder resulting from harvest of the shoot (upper parent shoot) and on the two shoot remainders from the under-hook cut (lower parent shoots) (Figuur 2B). Early shading of the bent shoot was applied one week before the underhook cut and the harvest of one of the shoots. The other treatments started on the day the under-hook cut was applied and one of the remaining shoots was harvested. Shading was applied with shading cloth (OLS50, Ludvig Svensson BV, Hellevoetsluis, the Netherlands), which had a transmissivity of 48% for photosynthetically active radiation (PAR) and was draped over a frame.

The experiment had a randomised complete block design with two blocks and four plots per block. A plot consisted of a double row with 20 plants. Between the plots were two border plants per row. The experiment was conducted in October 2008.

Experiment III: source:sink ratio. In this experiment, five treatments were applied to alter the source:sink ratio of the plant:

1. one young shoot per plant (1S)
2. two young shoots per plant (2S)
3. two young shoots per plant and shading of the crop (Shade)
4. two young shoots per plant and all leaves removed from the bent canopy (NoL)
5. minimal assimilate supply, implying three young shoots per plant, shading of the crop and removal of leaves from the bent canopy (Min)

Treatment P was added to obtain potential growth of the GR1 shoot, as described in experiment I; in this treatment one shoot per plant was growing and the bent canopy consisted of two shoots instead of one. Newly developing shoots in this treatment P were regularly removed. Shading of the crop was done by rectangular tents consisting of shading cloth (OLS50, Ludvig Svensson BV, Hellevoetsluis, the Netherland) on a bamboo frame, 1.5 m above the bent canopy. Transmissivity of the shading cloth was 48%. Growth of the GR shoot was followed as described in Experiment I, but shoot dimensions were measured only twice. These two measurement days were two weeks apart, the first one a day before the treatments were started. Bud outgrowth was recorded 17 days after initiation of the treatments.
The experiment had a completely randomised design, with two plots per treatment. Each plot contained 17 to 20 experimental plants in a double row per treatment, with two border plants per row. The experiment was conducted in April and May 2009.

Experiment IV: local and global light. This experiment was designed to test the effect of local and global light. Local light is light reaching a bud, while global light refers to light on the crop canopy, affecting the source strength of the crop. This experiment consisted of two sub experiments (IVa and IVb). In experiment IVa, only the amount of local light was varied, while in experiment IVb both local and global light were varied.

Experiment IVa:

1. no local light on buds
2. local light on buds

Experiment IVb:

1. normal global light on the crop, no local light on buds
2. low global light on the crop, no local light on buds
3. normal global light on the crop, local light on buds
4. low global light on the crop, local light on buds

Local light on the buds was supplied by a lighting tube (Figuur 3.), which consisted of a non-transparent plastic tube (10 cm long, 4.4 cm internal diameter) in which four Light Emitting Diodes (LED) of 0.06 W each were fixed (LED light set, Gnosjö Konstsmide AB, Gnosjö, Sweden). The LEDs emitted white light, with peaks in wavelengths around 440 nm and 570 nm. The red:far-red ratio was 1.16 (wavelengths 655-665 nm for red light and 725-735 nm for far-red light) and light intensity inside the tubes was 14.7 µmol m^{-2} s^{-1} (400-700 nm) for four lights, measured with a portable spectroradiometer (Li-cor 1800, LI-COR Biosciences, Lincoln, Nebraska, USA). The top of the tube was covered with aluminium foil. The lighting tubes encapsulated the shoot remainders. A stick was attached to the tube, and the other end of the stick was placed in the rockwool cubes. Low global light on the crop was achieved by shading the crop. The shading cloth used was the same as in experiment III. In all treatments, four shoots were present, which were at least three weeks old, and which acted as source of assimilates.
Bud outgrowth was observed after 12 days. Each experiment had a randomised complete block design. There were three blocks, with four plots per block, and 18 plants per plot. Four plants in a double row were located between the plots. Experiment IVa was conducted in March 2010 and experiment IVb in May 2010.

Quantifying source:sink ratios in experiments I and III. Stem volume for destructively and non-destructively measured shoots was estimated from the length and diameter measurements of the stem assuming a cylindrical shape of the stem (equation 1).

$$V = \frac{1}{4} * D^2 * \pi * L \qquad (1)$$

V is stem volume (cm^3), *D* is stem diameter (cm) and *L* stem length (cm). Data of the destructively harvested shoots were used to derive relationships to transform estimated stem volume into shoot dry weight. A linear relationship was assumed between shoot fresh weight and estimated stem volume. R^2 was 0.99 in experiment I and 0.98 in experiment III, and the residual standard errors were 2.44 g and 1.98 g in experiment I and III, respectively. To convert shoot fresh weight to shoot dry weight, a linear relationship between dry matter content and shoot age was fitted. R^2 was 0.94 in experiment I and 0.79 in experiment III, and the residual standard error was 0.012 and 0.015, respectively.
For the shoots from which stem dimensions were measured non-destructively, the above established relationships were used to estimate shoot dry weight at the initial and final measuring date. Growth of the GR1 shoot was the dry weight increase between the initial and final measurement. Shoot growth of the GR1 shoot in treatment P was assumed to be potential (non-limiting assimilate supply). Source:sink ratio in a particular treatment was quantified as the increase in dry weight of the GR1 shoot in this treatment divided by the increase of dry weight of the GR1 shoot in the treatment P.

Statistical analyses. Total number of growing buds, number of growing buds per position (upper bud, lower bud and buds in the basal ring) and source:sink ratio were calculated per plant and were averaged per plot. In the analysis of the experiment on the role of the bent shoot, results were averaged over the three shoot remainders per plant. The total number of growing buds and the number of growing buds per position were analysed using analysis of variance (ANOVA). The number of growing buds on the upper and lower positions were arcsine-square root transformed to normalise the data. In the experiment on local and global light, the results of experiment IVa and IVb were combined, with the subexperiments as a random effect. If there was a significant treatment effect, mean separation was done applying Tukey's honest significant difference test.
For experiments I and III, source:sink ratio per plot was related to the average number of outgrowing buds per plot by Spearman's rank correlation coefficient.
Timing of bud outgrowth in experiment I and II was analysed using survival analysis (Kleinbaum and Klein, 2005). Survival curves based on growing buds only were compared using Cox's proportional hazards model. Effects of number of young shoots and bud position (experiment I) and effects of treatment on the bent shoot, position of the parent shoot and position of the bud (experiment II) on time to bud outgrowth were tested.
Statistical analyses were done in R 2.12.2 (R core development team, 2011; regressions for source:sink ratio, correlations, survival analysis) and Genstat 14th edition (ANOVA).

2.4 Results

Number of growing buds. The number of growing buds increased significantly when fewer young shoots were present (experiment I, Figuur 4A, $P < 0.001$). It did not differ between treatments with three or four young shoots, but the number of growing buds in treatments with zero, one or two young shoots differed significantly from each other. Differences in total bud outgrowth between plants with different number of young shoots were due to differences in bud outgrowth in upper buds ($P < 0.001$), lower buds ($P < 0.001$) and buds in the basal ring ($P = 0.002$) (Figuur 4A). In plants with no young shoots, often more than one bud in the ring started growing, while more than one growing bud in the ring was rare when young shoots were present. The number of growing buds was positively correlated to the estimated source:sink ratio (Figuur 4B, Spearman rho 0.83, $P = 0.02$).
Treatments on the bent shoot (experiment II) significantly affected bud outgrowth per shoot remainder ($P = 0.046$), but the effect was small (Figuur 5A); the difference between the highest and lowest number was less than one growing bud. Bud outgrowth per position was not affected by the treatment of the bent shoot for any of the positions ($P = 0.64$, $P = 0.10$, and $P = 0.32$ for upper buds, lower buds and buds in the ring, respectively). In contrast, position of the parent shoot had a strong effect on bud outgrowth: bud outgrowth was significantly higher in the upper parent shoot than in the lower

parent shoots (Figuur 5B; $P < 0.001$). Higher total bud outgrowth in the upper parent shoot was the result of higher bud outgrowth in all bud positions ($P < 0.001$ for all positions; Figuur 5B).
Treatments aimed at changing the source:sink ratio (experiment III) resulted in a slight but significant difference in the total number of growing buds ($P = 0.018$); bud outgrowth was significantly increased when leaves were removed from the canopy compared to a shaded crop, but other treatments did not significantly differ from each other (Figuur 6.). The variation in source:sink ratio between the treatments was considerable (Figuur 6.), but there was no correlation between source:sink ratio and the number of growing buds when all treatments were considered (Spearman rho 0.04, $P = 0.92$). However, there was a positive correlation between source:sink ratio and bud outgrowth (Spearman rho 0.89, $P = 0.03$) when only data were considered from the treatments where the leaves were not removed from bent canopy. In the treatments where leaves were removed from the bent shoot, Spearman rho was one, but not significant ($P = 0.08$).
Local light (experiment IV) affected the total number of growing buds ($P = 0.006$, Figuur 7, interaction $P = 0.18$), whereas the effect of global light was just not significant ($P = 0.06$). Bud outgrowth for the different bud positions was only observed in subexperiment IVb, but there was no significant effect of local nor global light on bud outgrowth in the different positions ($P > 0.05$).

Timing of bud outgrowth. The effects of number of young shoots and position of the bud (experiment I) on time to bud outgrowth were significant ($P = 0.003$ and 0.004, respectively), but small (Figuur 8A,B). There was no significant interaction between the number of shoots and bud position on time to bud outgrowth ($P = 0.11$). Bud outgrowth was delayed in plants with 2, 3 or 4 young shoots compared to bud outgrowth in plants with 0 or 1 young shoot. The upper bud (x1) started to grow first, followed first by buds in the ring and then by the lower bud. On average, the time between two successive bud outgrowths was 2.0 days.
In the experiment on the effect of the bent shoot on bud outgrowth (experiment II), there was a delay in bud outgrowth when the bent shoots were shaded ($P = 0.03$, Figuur 9A). Bud outgrowth was earlier in the lower parent shoot than in the upper parent shoot ($P < 0.001$, Figuur 9B). Bud outgrowth occurred first in the upper bud, followed by the lower bud and the buds in the ring started to grow last ($P < 0.001$, Figuur 9C). Time between bud outgrowth of two successive buds on the upper parent shoot was slightly longer (2.5 days) than on the lower parent shoot (2.3 days).

2.5 Discussion

The aim of this study was to assess the relative importance of four possible hypotheses explaining bud outgrowth in a rose crop after harvest of a mature shoot (close to flowering): change in light intensity received by the bud, change in light spectrum received by the bud, changed correlative inhibition and changed source:sink ratio. Within each experiment, it is discussed whether a hypothesis is confirmed or not. A summary of the result is given in Table 2.

Light intensity received by the bud. More shoots per plant or shading of the crop reduced the light intensity reaching the buds (although the decrease in light intensity was not quantified). In all cases where more shoots (experiments on different number of young shoots (exp. I) and on source:sink ratio (exp. III)) or shading (experiment on source:sink ratio (exp. III) and on global and local light (exp. IVb)) were present, bud outgrowth decreased, although not always significantly. The presence of the lighting tube (experiment IV on local and global light), directly supplying the buds with light, increased bud outgrowth. Shoot remainders higher in the canopy (experiment II on the role of the bent shoot) had more bud outgrowth than shoot remainders lower in the canopy. These shoot remainders higher in the canopy had presumably less foliage above them and as a consequence experienced higher light intensity than shoot remainders lower in the canopy. Thus, the assumption that higher light intensity on the bud increases bud outgrowth was supported by all experiments. These results confirm previous results of higher fraction bud outgrowth under higher light intensities (Evers *et al.* 2006; Girault *et al.* 2008; Su *et al.* 2011).

Light spectrum received by the bud. In some cases, the effects of light intensity and light spectrum received by the bud were confounded. This was the case in the experiment with different numbers of young shoots (exp. I) or when the buds were positioned lower in the canopy (exp. II). These two experiments could therefore not discriminate between the effect of light intensity and light spectrum. The lighting tube in the experiment on local light (light at bud level, exp. IV) increased

the red:far-red ratio at the bud (1.16 compared to 0.37 under five shoots), and resulted in a higher level of bud outgrowth. This supports the hypothesis that light spectrum received by the bud affects bud outgrowth. Shading the crop did not alter light spectrum but decreased light intensity. In the experiment on source:sink ratio (exp. III) and the experiment on local and global light (exp. IV), shading had the tendency to decrease bud outgrowth. This might indicate that light spectrum has no effect. Overall, no solid conclusion can be drawn about the effect of light spectrum. Experiments in which light intensity and light spectrum received by the buds are independently varied are needed to elucidate whether light intensity or light spectrum received by the buds is more important in affecting bud outgrowth in a cut rose crop.

Correlative inhibition. Growing shoots are assumed to inhibit axillary bud outgrowth due to correlative inhibition. As such, more shoots should result in less bud outgrowth. This assumption was confirmed in the experiment with different number of young shoots (exp. I). In the experiment on the effect of the bent shoot and the experiment with different source:sink ratios, source strength of the bent shoot was manipulated by removing the bent shoot or by removing its leaves, respectively. Bending of the shoot is done to increase assimilate supply (De Hoog, 2001) and is assumed to alter the hormonal balance (Cline, 1991). In contrast to the expectation, removing the bent shoot increased bud outgrowth (Figuur 5A), although the effect was small. Apparently, the bent shoot slightly inhibits bud outgrowth. Also removing all leaves from the bent shoot had a tendency to increase bud outgrowth (Figuur 6.). Leaves produce and export auxin (Cambridge and Morris, 1996; Jager *et al.* 2007), thereby contributing to the correlative inhibition. Bending a shoot did not completely remove the correlative inhibition exerted by its leaves. Alternatively, high bud outgrowth when leaves were removed from the bent shoot or the bent shoot itself was removed might be the result of a stress reaction.

Source:sink ratio. A lower source:sink ratio due to more young shoots decreased bud outgrowth (Figuur 5B). However, the positive correlation between bud outgrowth and source:sink ratio was not observed in the experiment dedicated to source:sink ratio (exp. III, Figuur 6.), unless only the treatments with the bent shoot intact were considered. On the other hand, shading of the bent shoot or cutting the bent shoot (exp. II), which were assumed to decrease assimilate availability, had no negative effect on bud outgrowth. Light intensity on the canopy (global light, which affects the source strength of the plant) affected bud outgrowth, but the effect was small (0.27 buds less due to shading) and was not significant. From this finding it can be concluded that assimilate supply or source:sink ratio was not the most important factor influencing bud outgrowth. The size (shoot weight and stem length) of mature shoots was lower when the bent shoot was shaded or when the bent shoot was cut off altogether, indicating that the treatments effectively altered the assimilate supply (data not shown). So although the process of bud outgrowth is accompanied by import of sucrose (Maurel *et al.* 2004; Henry *et al.* 2011), assimilates needed for this process are apparently sufficiently present in the plant. According to Girault *et al.* (2010), assimilates used during bud outgrowth were mobilised from the nearby stem, which is consistent with the fact that decreasing assimilate supply from the bent shoot did not affect bud outgrowth. Also in *Pisum sativum*, reduced assimilate supply did not prevent bud outgrowth, but did affect branch length of the resulting shoot (Ferguson and Beveridge, 2009). Increased branching of *Arabidopsis* under higher light intensities was also not explained by higher plant photosynthesis rates (Su *et al.* 2011). An assumption in the source:sink theory was that assimilates produced in a certain part of the plant can be made available for the whole plant (common-assimilate pool). In tomato, this assumption was confirmed (Heuvelink, 1995), but it was invalid in a woody species like grapevine (Pallas *et al.* 2009). Additional experiments in the crop showed that when half of the leaves on a shoot were removed, growth of that shoot was not affected, implying that it had access to assimilates produced elsewhere in the crop (Wubs *et al.* unpublished results). The method of quantification of the source:sink ratio assumed that the growth of one branch in comparison to unlimited growth represents the source:sink ratio of the whole plant.

Additional consideration on factors affecting bud outgrowth. There were considerable differences between the experiments in the fraction of bud outgrowth per shoot remainder. The overall fraction of bud outgrowth was high in the experiment with different source:sink ratios (exp. III) compared to the other experiments (Figuur 6.). The average radiation was highest in this experiment (Table 1.), which might explain the high overall level of bud outgrowth. The differences in bud outgrowth between the treatments were much larger in the experiment with different number of young growing shoots (exp. I) than in the other experiments. Additionally, bud outgrowth of the lower bud was low in experiment I, and it was relatively high in the buds in the ring. These effects might have to do with the young age of the crop in the first experiment. In the experiment about the role of the bent shoot (exp. II), the ages of the buds differed: buds on the upper shoot

remainder were younger than the ones on the lower shoot remainders and the younger buds had a longer time till bud outgrowth (Figuur 9B). This confirms the results of Marcelis-Van Acker (1994) that younger buds had a longer time till bud outgrowth, whereas Le Bris *et al.* (1998) found that bud age was not a major factor in the rate of bud growth.
Buds on the same shoot remainder did not start to grow at the same time, but the uppermost bud started to grow first, followed by lower positioned ones (Figs. 8,9), and a larger fraction of the upper bud than of the lower positioned buds showed growth (Figs. 4,5,7). More buds growing from the upper bud position than from the lower positioned buds was also reported by Zieslin and Halevy (1976), Zieslin *et al.* (1976) and Girault *et al.* (2008). Hence, it might be that bud outgrowth of the upper bud suppresses outgrowth of the buds positioned lower on the shoot remainder, probably through hormonal processes, of which the knowledge is still incomplete but rapidly expanding (Domagalska and Leyser, 2011). Since outgrowth of the lower buds is higher under favourable circumstances, especially high light levels on the bud, this might alleviate the hormonal suppression of outgrowth of lower positioned buds. This is supported by findings indicating that light affects hormone synthesis, transport and responsiveness to hormones, thereby influencing organ formation (*e.g.* Choubane *et al.* 2012; Yoshida *et al.* 2010; Halliday *et al.* 2009; Finlayson *et al.* 2010).

Conclusion. If a flower shoot in a cut-rose crop is harvested the buds on the shoot remainder below the cut are released from apical dominance. Light intensity and light spectrum reaching the buds then are the most important factors explaining the fraction of the buds that starts growing on the shoot remainder. Apical dominance can play a role in determining the fraction bud outgrowth among bud positions on a shoot remainder, and favourable light conditions are likely to decrease the level of apical dominance. Source:sink ratio or assimilate supply is less important in determining bud outgrowth.

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Table 1. Climate during the four experiments. Mean values ± standard deviation for radiation, temperature, relative humidity and CO_2 inside the greenhouse.

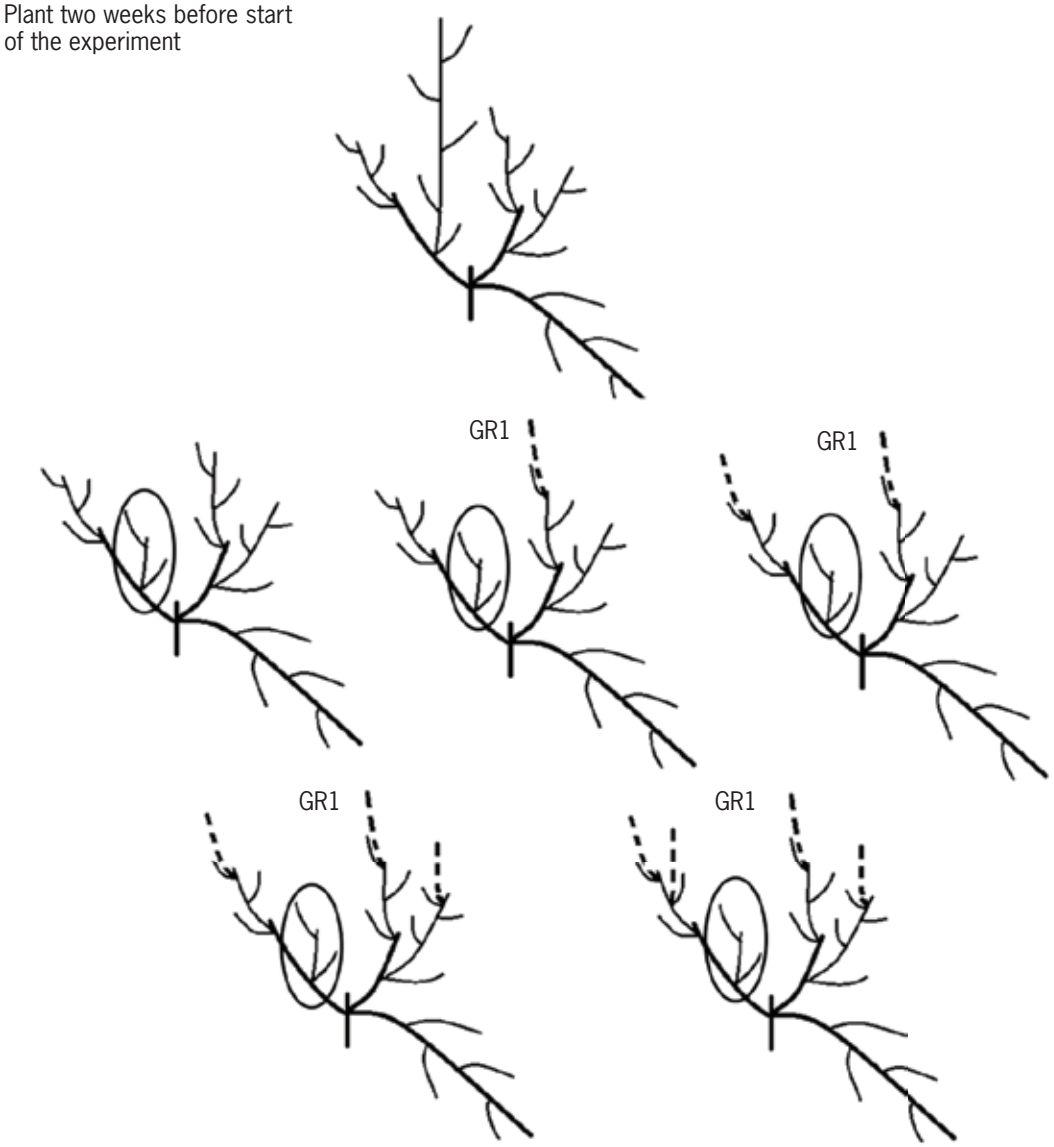
Experiment	Radiation ($\mu mol\ m^{-2}\ s^{-1}$)	Temperature (°C)	Relative humidity (%)	CO_2 ($\mu mol\ mol^{-1}$)
I	-[A]	23.1 ± 1.69	67.8 ± 4.90	441 ± 16.1
II	132 ± 12.3	19.8 ± 0.54	72.7 ± 2.69	490 ± 13.0
III	235 ± 41.6	21.5 ± 0.83	63.0 ± 4.94	461 ± 15.1
IVa	160 ± 10.9	19.2 ± 0.56	77.8 ± 3.32	461 ± 10.7
IVb	177 ± 30.3	21.3 ± 0.70	62.7 ± 4.60	460 ± 16.0

[A]data not available

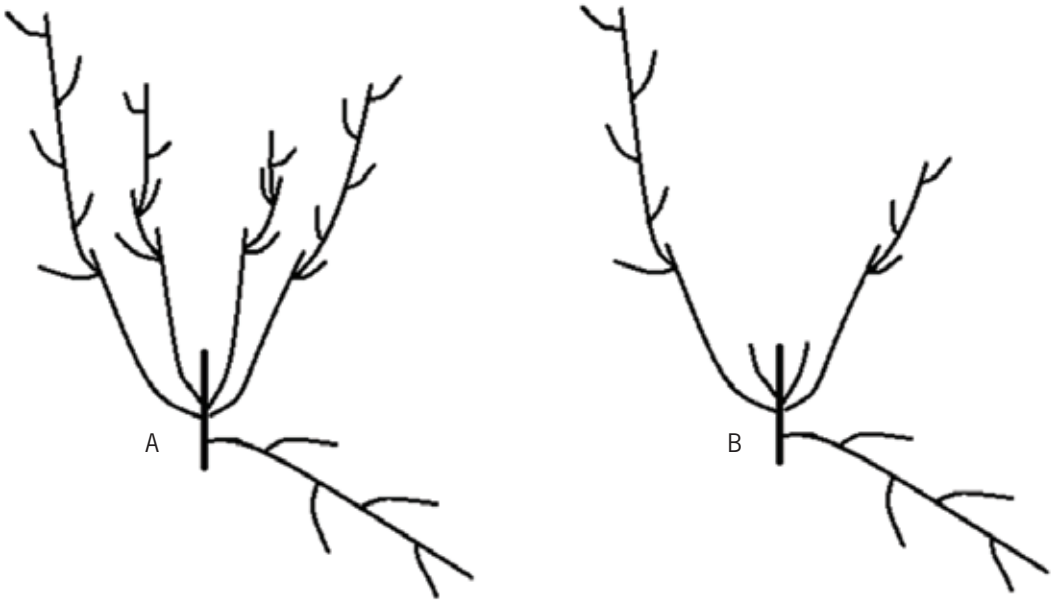
Table 2. Summary of the results of the four experiments in relation to the four hypotheses about causes of bud outgrowth.

Name experiment	No. experiment	Light intensity	Light spectrum	Correlative inhibition	Source:sink ratio
Number of young shoots	I	+[A]	+	+	+
Effect of bent shoot	II	n.t.	n.t.	+	-
Position parent shoot	II	+	+	n.t.	n.t.
Source:sink ratio	III	+	+/-	+	+/-
Local vs global light	IV	+	+/-	n.t.	+/-

[A]: + hypothesis not rejected; - hypothesis rejected; n.t. hypothesis not tested

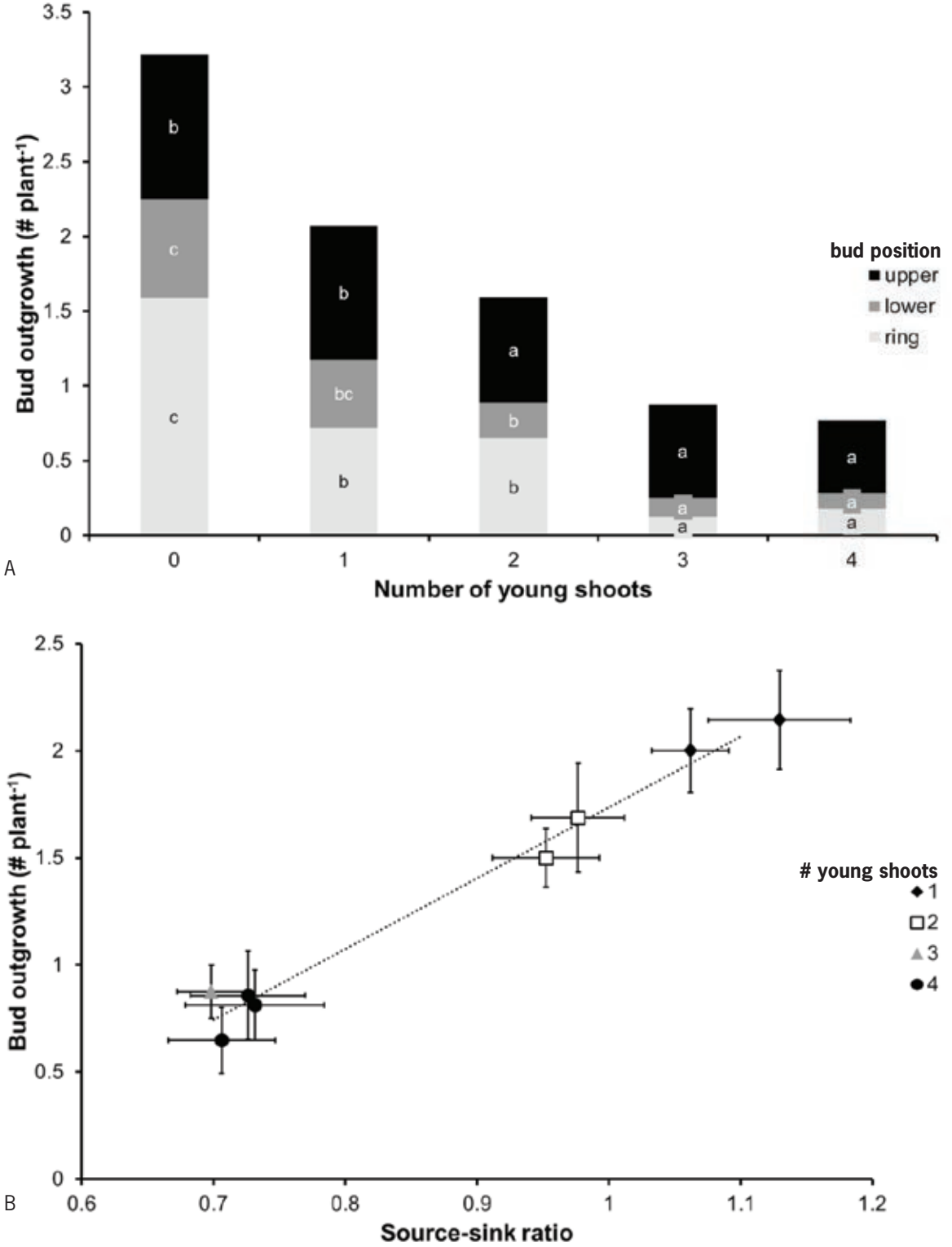


Figuur 1. Plant architecture in the experiment with different number of young shoots per plant (experiment I). Plant with the remaining shoot (X) intact two weeks before the experiment started. Other drawings show the shoot remainder of the harvested shoot on which bud outgrowth was observed (encircled) on plants with zero, one, two, three or four young growing shoots (young shoots depicted with broken line). GR1 indicates the shoot, present in plants with one or more young shoots, which was measured non-destructively over time. Source:sink ratio was quantified by comparing the growth of the GR1 shoot of each treatment with the potential growth of a GR1 shoot.

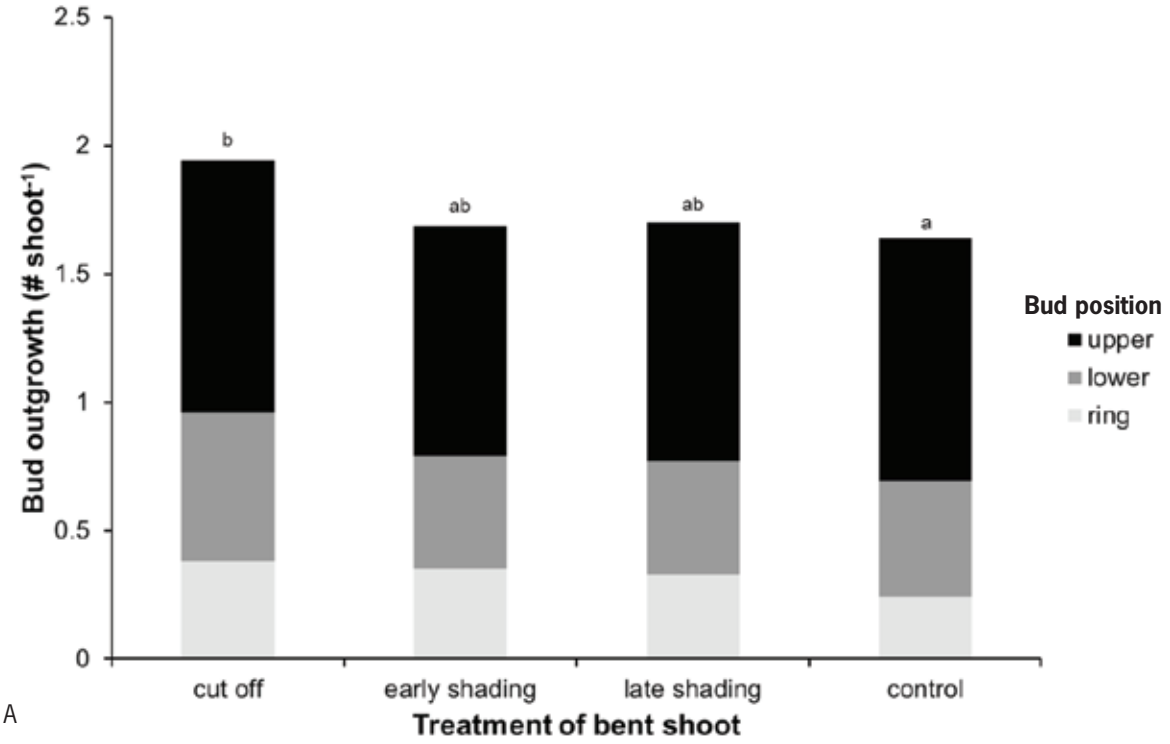


*Figuur 2. Plant architecture in experiment on the role of the bent shoot (experiment II). Plants before the start of the experiment (A) and plants from which the two inner shoots were removed with an under-hook cut and one of the other shoots harvested (B). Bud outgrowth was observed on the shoot remainders of the two lower parent shoots resulting from the under-hook cut (indicated by *) and on the shoot remainder of the upper parent shoot resulting from harvest of a shoot (indicated by **).*

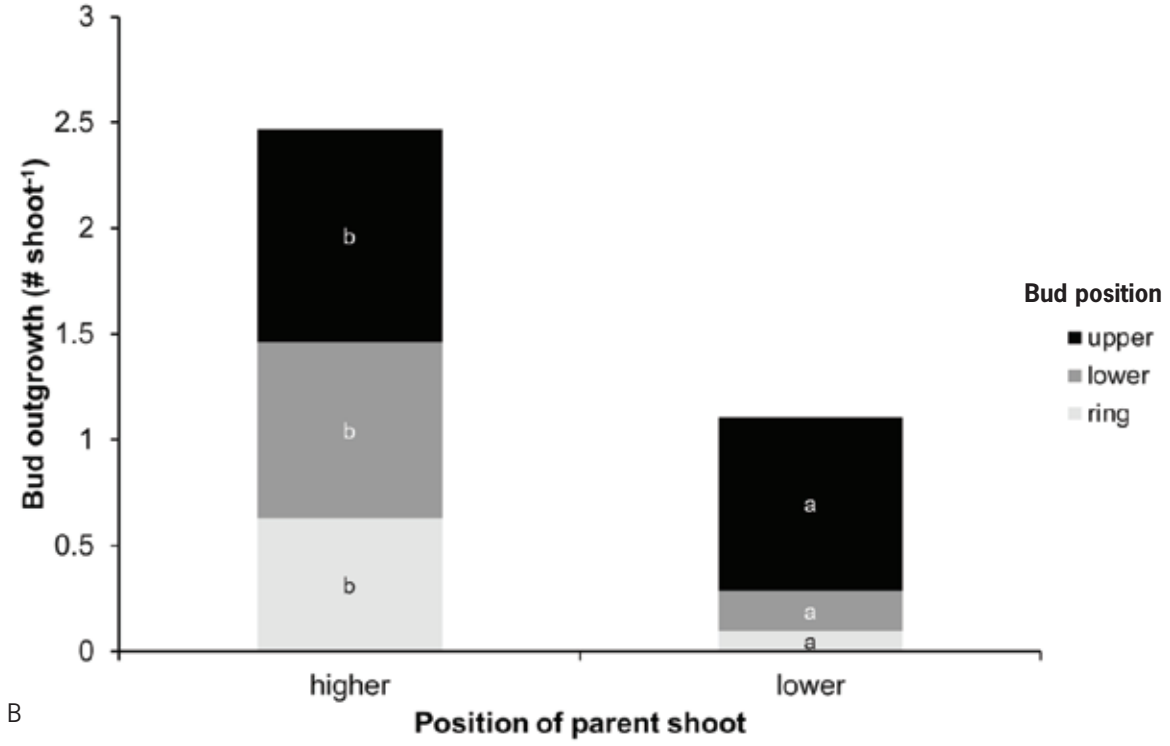
Figuur 3. Lighting tubes. Non-transparent light tubes encapsulating a shoot remainder in rose plants in the experiment on local vs global light (exp. IV). Inside the tubes were LEDs emitting white light.



Figuur 4. Bud outgrowth per shoot remainder as affected by different number of young shoots (exp. I). A) Number of growing buds per position (upper buds, lower buds and buds in the basal ring), different letters refer to significant treatment differences (P < 0.05) for total bud outgrowth (above bar) and per bud position (inside bar); B) Bud outgrowth in relation to source:sink ratio, Spearman rho=0.83, P = 0.02, different symbols refer to plants with different number of young shoots. Source:sink ratio was taken as the ratio between the growth of the GR1 shoot of a treatment divided by the growth of the GR1 shoot in a treatment where only the GR1 shoot was present. Source:sink ratio could therefore not be calculated for treatment without young shoots.

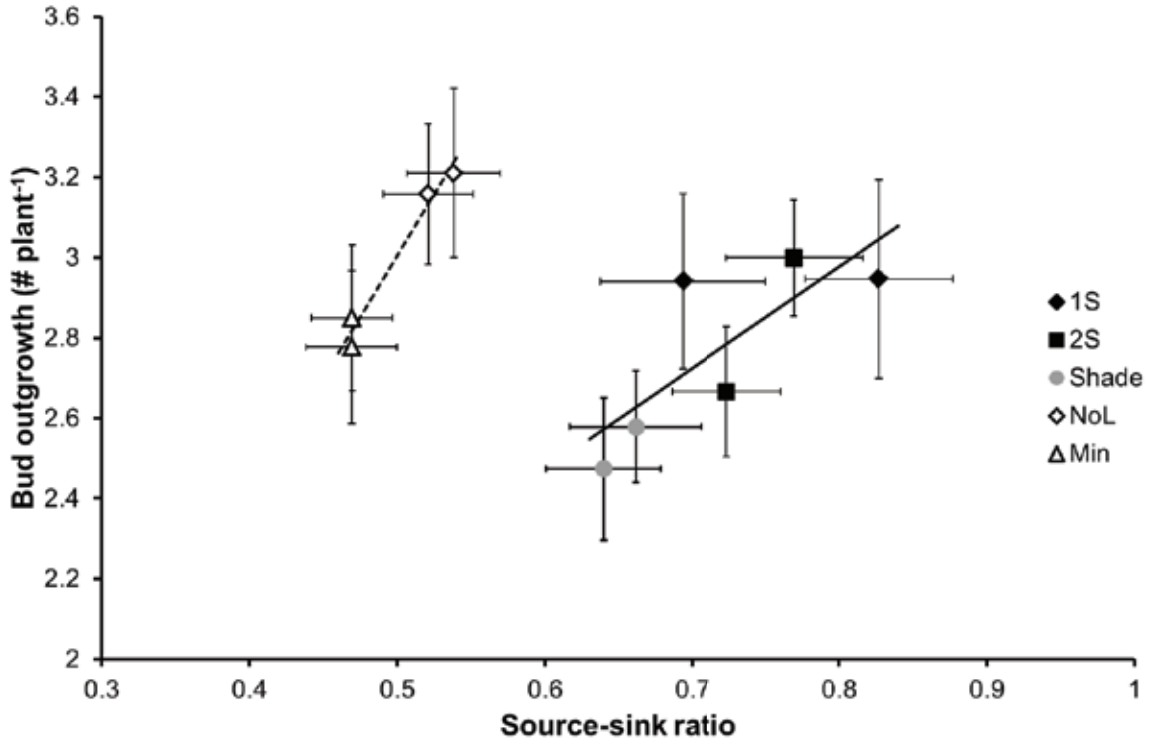


A

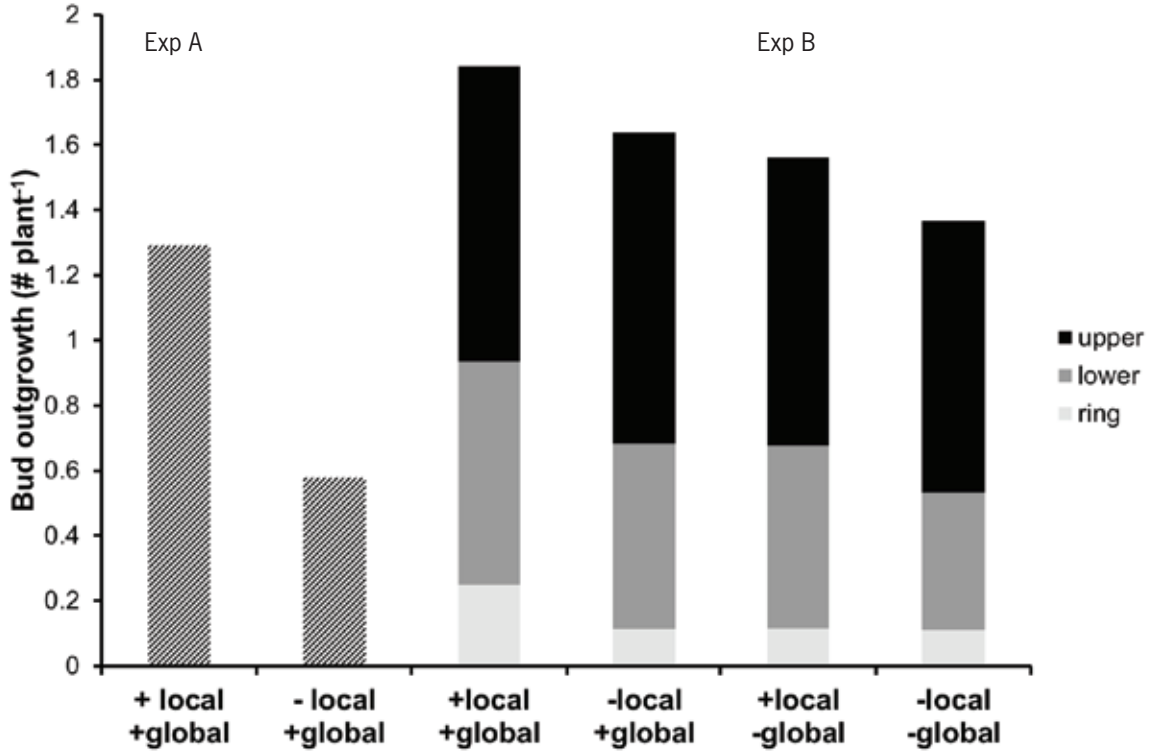


B

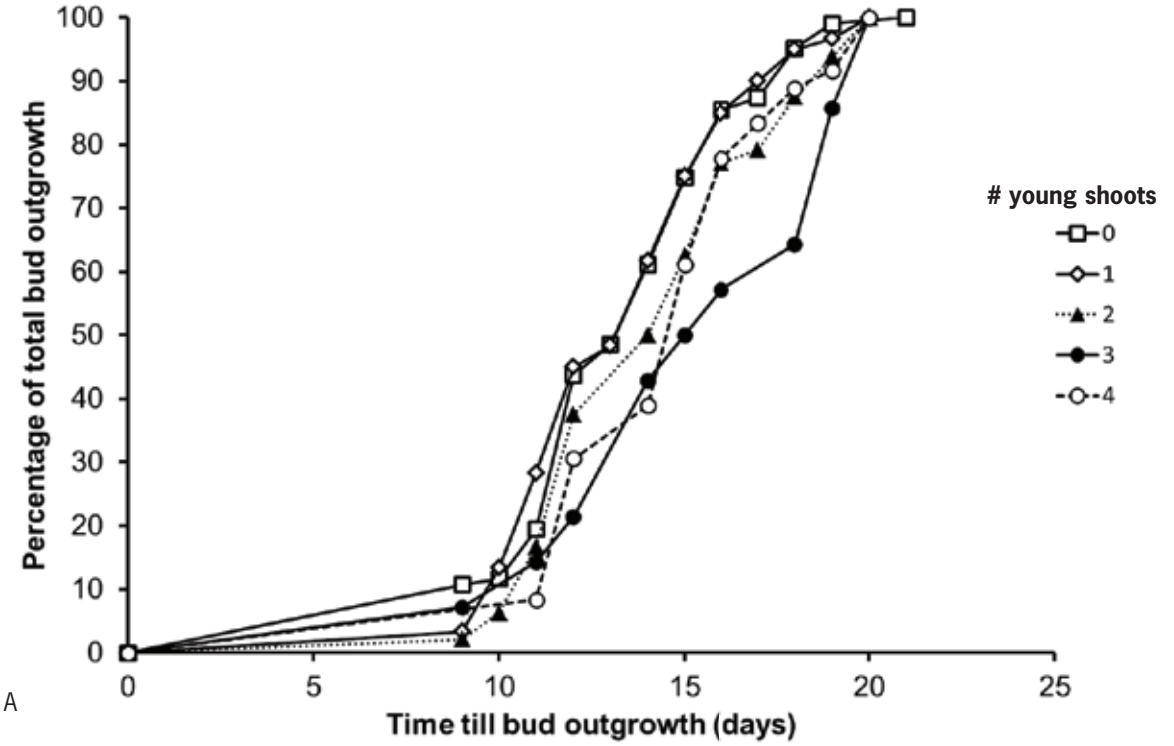
Figuur 5. Bud outgrowth per shoot remainder in experiment on role of the bent shoot (exp. II). A) Bud outgrowth as affected by shading and removing the bent shoot, letters refer to differences in total bud outgrowth per shoot remainder (P = 0.046), differences between bud positions were not significant (P > 0.05), bud outgrowth was averaged over one upper and two lower shoot remainders; B) Bud outgrowth as affected by position of the parent shoot, different letters refer to significant differences between parents shoots (P = 0.05) for total bud outgrowth (above bar) and bud outgrowth per position (inside bar). Bud outgrowth was averaged over the treatments of the bent shoot (bent shoot cut off, shading of bent shoot one week before the other treatments started, shading of bent shoot at start of other treatment, control).



Figuur 6. Bud outgrowth per shoot remainder influenced by different source:sink ratios (exp. III). Treatments: 1S) 1 shoot per plant, 2S) two shoots per plant, NoL) no leaves on the bent shoot, Shade) shading of the crop, Min) minimal assimilate supply: 3 vertical shoots per plant, no leaves on bent shoot and shading of the crop (48% light transmission).



Figuur 7. Effect of local and global light on bud outgrowth per shoot remainder (exp. IV). The experiment consisted of subexperiments A and B. Local light was altered by applying a lighting tube on the shoot remainder on which bud outgrowth was observed. Global light was altered using a shading cloth (48% light transmission) over the crop. Local light significantly affected bud outgrowth (P = 0.006), while the effect of global light was almost significant (P = 0.06) .



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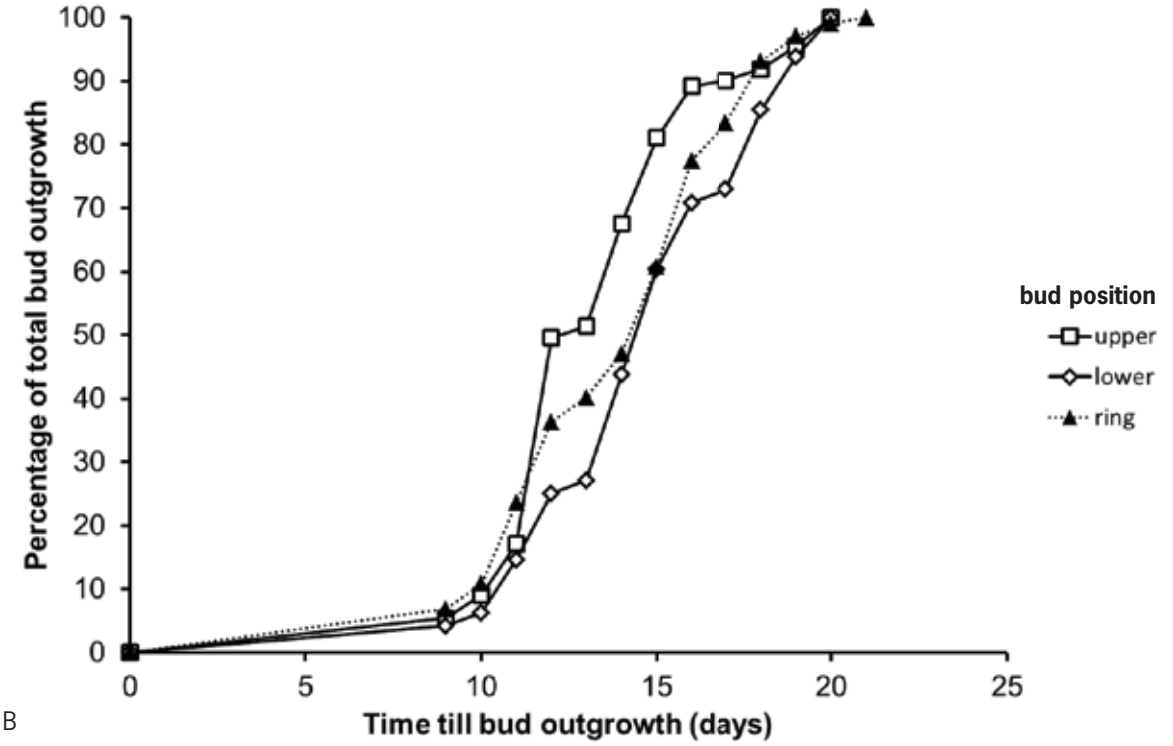
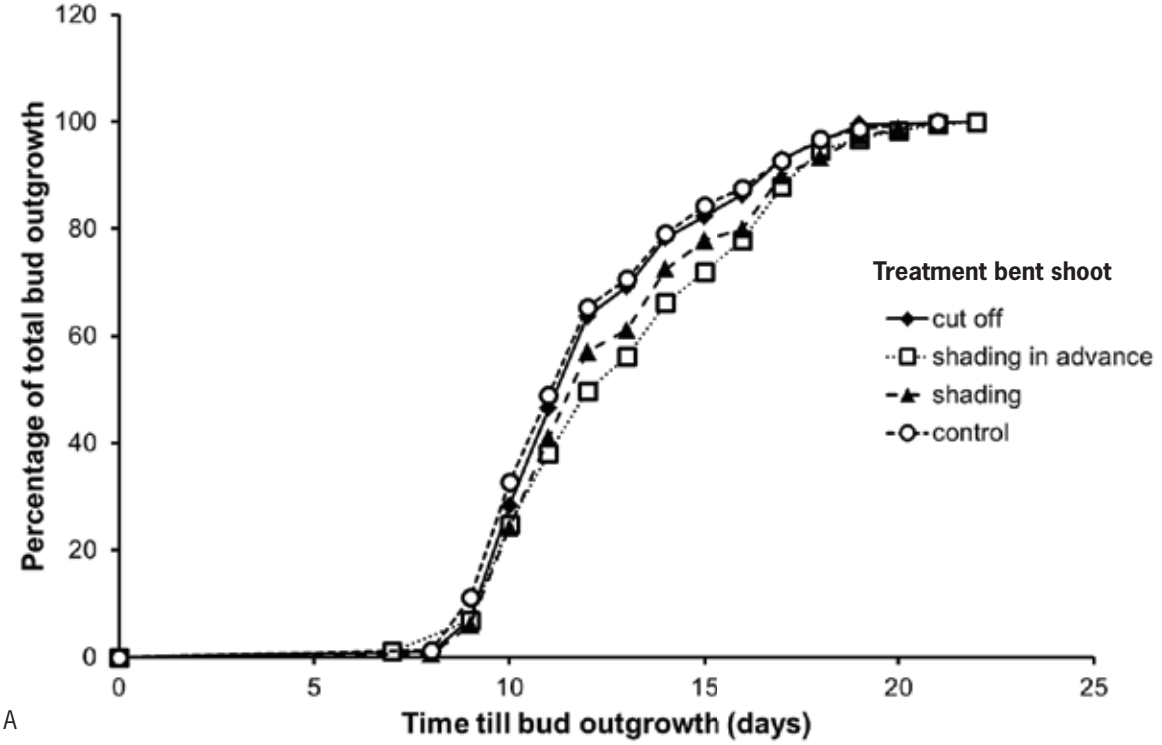
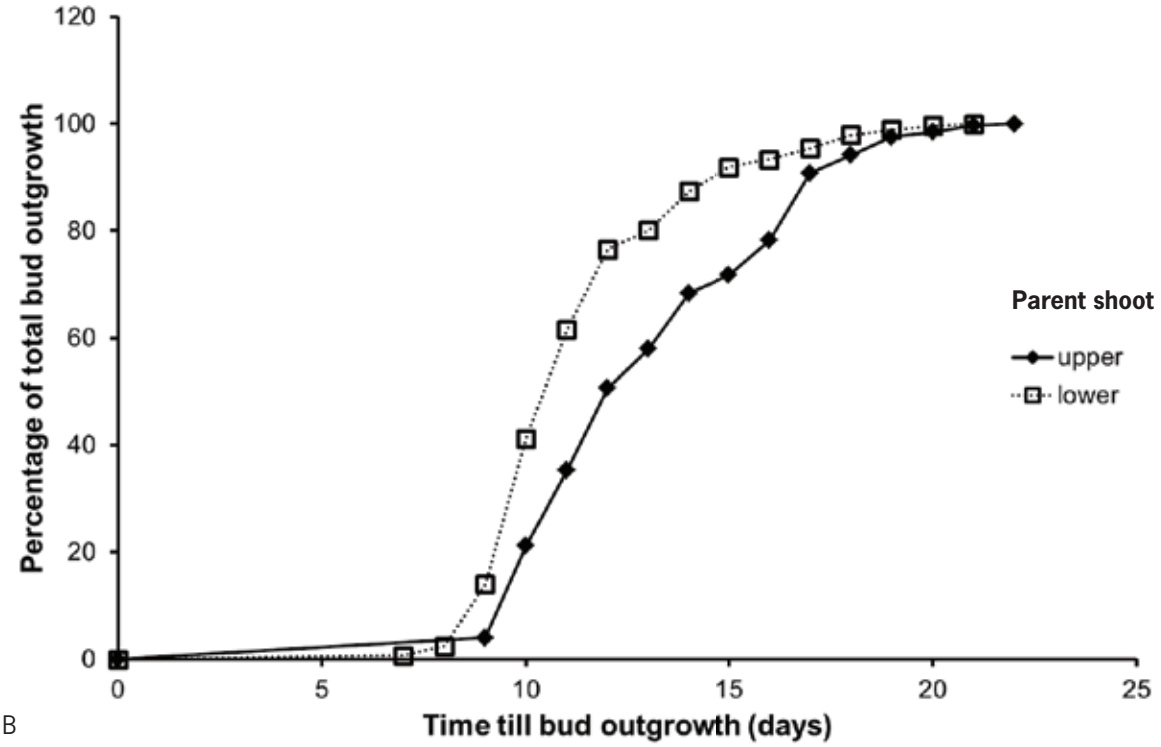


B

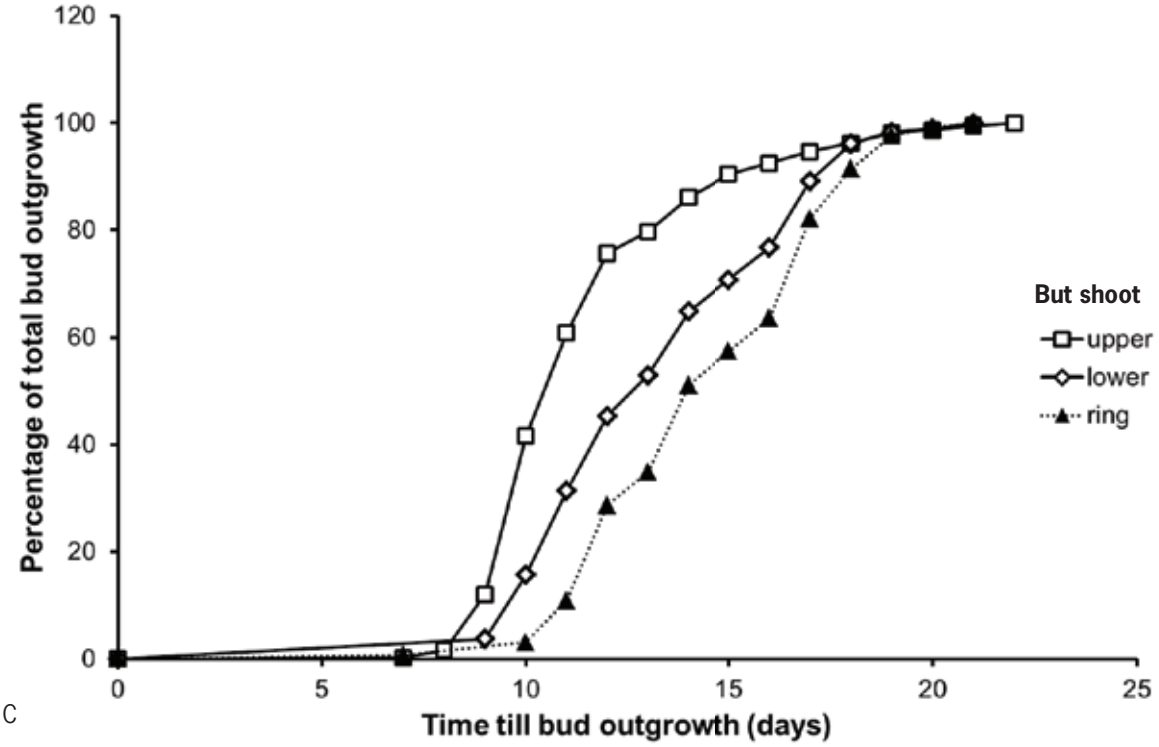
Figuur 8. Timing of bud outgrowth in experiment with different number of young shoots (exp I). Timing of bud outgrowth as affected by A) number of young shoots (P = 0.003) and B) bud position (P = 0.004). Interaction between effect of number of young shoots and bud position was not significant (P = 0.11).


Percentage of total bud outgrowth
120
100
80
60
40
20
0
0
5
10
15
20
25
Time till bud outgrowth (days)
Treatment bent shoot
cut off
shading in advance
shading
control

A


Percentage of total bud outgrowth
120
100
80
60
40
20
0
0
5
10
15
20
25
Time till bud outgrowth (days)
Parent shoot
upper
lower

B



C

Figuur 9. Timing of bud outgrowth in experiment on role of bent shoot (exp. II). Timing of bud outgrowth as affected by A) treatment on bent shoot (P = 0.03), B) position of the parent shoot (P < 0.001) and C) position of the bud (P < 0.001). Three-way and all two-way interactions between treatment of bent shoot, position of the parent shoot and position of the bud were not significant (P > 0.60 for all interactions).

3 Effect van lichtintensiteit en spectrum op uitloop van okselknoppen

Dit hoofdstuk is aan een wetenschappelijk tijdschrijft aangeboden als:
Axillary Bud Break in a Cut-rose Crop as Influenced by Light Intensity and Light Spectrum. By A.Maaike Wubs, Ep Heuvelink, Leo F.M. Marcelis, Gerhard Buck-Sorlin and Jan Vos

3.1 Abstract

When flower-bearing shoots in cut-rose are harvested, a varying number of repressed axillary buds on the shoot remainder start to grow into new shoots (bud break). Earlier experiments indicated that light reaching the bud affected the amount of bud break. The aim of this paper is to disentangle the effects of light intensity and light spectrum on bud break in a rose crop. Three experiments were conducted in a *Rosa x hybrida* crop cv. 'Akito'. In all experiments, light intensity and light spectrum at the position of the buds were quantified and related to bud break on the shoot remainders. Removal of vertical shoots increased light intensity and red:far-red ratio as well as bud break (1.9 broken buds per shoot remainder compared to 0.4 buds when five vertical shoots were present). No vertical shoots and green crepe paper over the plant base mimicked the effect of vertical shoots with respect to light intensity and light spectrum, but bud break (1.0 buds) was intermediate compared to treatments with and without shoots. This suggested that the presence of shoots exerts and inhibiting effect on bud break via both effects on light at the bud and correlative inhibition. When plants had no vertical shoots and light intensity and light spectrum at bud level were changed by different layers and colours of crepe paper, there was a positive effect of light intensity on bud break (0.3 buds more per shoot remainder) and no effect of light spectrum. Combinations of high and low light intensity with high and low red:far-red ratio on the axillary bud showed that there was a positive effect of light intensity on bud break (0.5 buds more per shoot remainder) and no effect of light spectrum.

3.2 Introduction

In cut-rose crops, harvest of a mature shoot (close to flowering) triggers axillary buds on the remainder of the shoot to grow. This bud outgrowth is an important event in a cut-rose crop as it determines the potential number of harvestable shoots. However, the amount of bud outgrowth is variable: a plant has several axillary buds which can potentially become a shoot, but not all of them do. Four hypotheses can potentially explain the degree of bud break after harvest (removal) of a shoot: higher light intensity reaching the bud (1), a change in the spectrum of light reaching the bud (2), removal of correlative inhibition (3), and a change in source-sink ratio (4). Earlier experiments in a rose crop (Wubs *et al.* unpublished results) showed that light reaching the bud is the most likely factor explaining bud break when correlative inhibition is removed due to harvest of the shoot above the buds. However, those experiments could not discriminate between the effect of light intensity and light spectrum. Both light intensity and light spectrum affected bud break in simple rose systems consisting of a nodal cutting (Girault *et al.* 2008). Comparing between rose crops illuminated with fluorescent lamps (F) and a mixture of fluorescent and incandescent light (F+I; both treatments having equal amount of PAR), Mor and Halevy (1984) found lower bud break of the lowest bud under F+I compared to F. This was attributed to differences in red:far-red ratio. In many plant species, bud break is influenced by light intensity and light spectrum, *e.g.* Arabidopsis (Finlayson *et al.* 2010; Su *et al.* 2011), Poaceae species (Belesky *et al.* 2011; Evers *et al.* 2006) and ornamental crops (Mata and Botto, 2011), but the effects are not present in all plant species (*e.g.* Crotser *et al.* 2003; Kawamura and Takeda, 2002).
The goal of this paper is to disentangle the effect of light intensity and light spectrum at bud level on axillary bud break in a cut-rose crop after the harvest of a shoot. Following the suggestion of Mor and Halevy (1984), we tested the hypothesis that axillary bud break is inhibited by unfavourable red:far-red ratio reaching the buds rather than by low light intensity. Three experiments were conducted in which light spectrum and light intensity at the level of the buds were independently varied using different methods. In each experiment, light intensity and light spectrum at bud level were quantified and

related to bud break. In contrast to Mor and Halevy (1984), light intensity and light spectrum on the rest of the crop were not changed.

3.3 Materials and methods

All experiments were executed in the same rose crop, Rosa x hybrida cv. 'Akito'. The crop was grown in a compartment (12 x 12 m^2) of a multi-span Venlo type glasshouse in Wageningen, the Netherlands (52°N). Plants were grown on Rockwool® cubes (7 x 7 x 7 cm, Grodan B.V., Roermond, the Netherlands) positioned on Rockwool® slabs, in double rows (0.25 m between centres and path width of 1.20 m) with 0.20 m distance within the rows, resulting in a plant density of 6.5 plants m^{-2}. The crop was established in spring 2008 from single node cuttings bearing a shoot. The experiments were conducted in the second half of 2009 and the first half of 2010. Between two successive experiments, the crop underwent several reset flushes (a flush is defined as the simultaneous harvest of shoots from all plants), during which it could recover from the previous experiment and the same architecture could be established in all plants.
Supplemental light was provided by high pressure sodium lamps (600 W, Philips, Eindhoven, the Netherlands) which provided 150 µmol m^{-2} s^{-1} at crop level. Lamps were switched on between 00.00 and 20.00 hr (until 15-12-2009) or between 00.00 and 18.00 hr (since 15-12-2009, change due to local legislation) when global radiation above the greenhouse was below 150 W m^{-2} and switched off when this radiation exceeded 250 W m^{-2}. Realised light intensity (natural light and supplemental light) above the canopy was estimated from outside radiation and recordings of supplemental lighting (see below). Heating set points were 17.5 °C during the night and 21.0 °C during the day. Realised climate conditions per experiment are given in Table 1.
In all experiments light intensity, red:far-red ratio and light spectrum at bud level (i.e. low in the canopy, at the level of the shoot remainder on which bud break was observed) were measured once or twice during the experiment. Light intensity was measured with a point sensor (Li-cor quantum sensor, LI-250A, LI-COR Biosciences, Lincoln, Nebraska, USA), or a line sensor (Licor LI-191). Red:far-red ratio was measured with a Skye sensor (660/730 Skye Instruments Ltd, Llandrindod Wells, UK). Light spectrum was measured with a portable spectroradiometer (Li-cor 1800). All measurements were done around midday (11.00 -14.00 hr). From the light spectrum measurements, phytochrome stationary state (PSS) was calculated according to Sager *et al.* (1988). The phytochrome stationary state (PSS) reflects the influence of all wavelengths on the balance between active and inactive phytochrome, and gives the fraction of biological active Pfr in the total amount of phytochrome (Pfr + Pr).
In all experiments, bud break was observed once, at the end of the experiment. A bud was defined as broken when leaves protruding from the scales measured more than 15mm.

3.3.1 Experiment I: manipulating light intensity and light spectrum by varying the number of shoots, application of crepe paper and removal of leaves

Six vertical shoots and three bent shoots per plant were present before the experiment started. The experiment started when the flower bud from the vertical shoots was visible (shoots were approximately three weeks old). One vertical shoot per plant was cut to 1.5cm from its base. This was the shoot remainder on which bud break was observed. Leaves were removed from this shoot remainder. Four treatments were imposed:

- No vertical shoots on the plant
- Five vertical shoots per plant
- No vertical shoots and a double layer of green crepe paper covering the base of the plants
- Five vertical shoots per plant, from which the unfolded leaves were removed

For the treatments without shoots, shoots were removed by cutting them at their base. The double layer of green paper decreased the light intensity and reduced the red:far-red ratio. The green paper only covered the plant base (part above the Rockwool® slabs) and not the bent shoots, so the source strength of the plant was not affected. The green paper was renewed after four days. Removal of the unfolded leaves increased the light intensity and increased the red:far-red ratio

on the buds. The removed leaves were mature, implying that the sink strength of the shoot stayed the same. New mature leaves were removed several times during the experiment.
The experiment was set up as a complete randomized block design, with three blocks and four plots per block. Each plot consisted of 15 plants. The experiment was conducted in September and October 2009. Bud break was observed 14 days after initiation of the treatments.
Light spectrum at bud level was measured two days after the start of the experiment with the spectroradiometer to calculate the PSS. Two and eight days after the start of the experiment, light intensity and red-far red ratio were measured with the Li-cor line quantum sensor and the Skye sensor, respectively. The light spectrum and red-far red ratio at bud level were measured at three positions per plot. At both days, it was cloudy, and the lamps were turned on during the measurements on day 8.

3.3.2 Experiment II: manipulating light intensity and light spectrum by application of crepe paper

Five vertical shoots and two bent shoots per plant were present before the experiment started. At the start of the experiment, two vertical shoots per plant were cut off 1.5cm above their base. These were the shoot remainders used for the observation of bud break. Leaves and stipules were removed from these shoot remainders. Other shoots were cut off at their base. Light intensity and spectrum were varied in four treatments by the application of different colours and layers of crepe paper over the base of the plant.

- High light intensity and high red:far-red ratio (control treatment)
- Intermediate light intensity and high red:far-red ratio, created by one layer of white crepe paper
- Low light intensity and high red:far-red ratio, created by two layers of white crepe paper and one layer of grey crepe paper
- Low light intensity and low red:far-red ratio, created by two layers of green crepe paper

The experiment was set up as a complete randomised block design, with three blocks and four plots per block. Each plot consisted of 13-14 plants. Bud break was scored 13 days after the start of the treatments.
The experiment was conducted in the first half of November 2009. Light intensity and red:far-red ratio at bud level were measured two and seven days after the start of the experiment with the Li-cor point quantum sensor and the Skye sensor, respectively, at three positions per plot. On day 7 of the experiment, measurements on light spectrum at bud level were done with the spectroradiometer to calculate the PSS (one measurement in the middle of each plot). Weather was cloudy with little natural radiation and assimilation light was switched on.

3.3.3 Experiment III: manipulating light intensity and light spectrum by application of crepe paper and far-red LEDs

Five vertical shoots and three bent shoots per plant were present before the experiment started. At the start of the experiment, two vertical shoots per plant were cut off 0.5 cm above the lowest node, at least 1.5cm from the shoot base. These shoot remainders were used for observation of bud break. Other shoots were cut 1 cm above the shoot base. All leaves from the plant base were removed. Light intensity and light spectrum were independently varied in four treatments.

- High light intensity and high red:far-red ratio (control treatment)
- Low light intensity and high red:far-red ratio, created by two layers of grey paper over the plant bases
- High light intensity and low red:far-red ratio, created by far-red LEDs above the plants
- Low light intensity and low red:far-red ratio, created by grey paper over the plant bases and far-red LEDs above the plants

Crepe paper was replaced after one week. At that time also the newly sprouting shoots on other shoot remainders were removed.
Far-red LEDs (10 W, GreenPower LED module HF far red, Philips, the Netherlands) were positioned about 50cm above

the Rockwool® slabs. Distance between the LEDS was 10cm. They provided a light intensity of 15 µmol far-red light m^{-2} s^{-1} (730-740 nm) at the top of the crop (measured with spectroradiometer). The LEDs were attached to a frame, hanging from the ceiling of the greenhouse; a row of LEDs was positioned above the centre of a plant row. In treatments without LEDs, a similar frame without LEDs was positioned above the plots.
The experiment was set up as a complete randomised block design, with three blocks and four plots per block. Each plot consisted of 15 plants. Bud break was observed 14 days after the initiation of the experiments.
The experiment was conducted in January 2010. On day 6 of the experiment, light intensity and red:far-red ratio at bud level were measured at two positions per plot with the Li-cor point sensor and the Skye sensor, respectively. Light spectrum at bud level was measured on day 7 of the experiment with the spectroradiometer at two positions per plot. On both days, weather was cloudy with little natural radiation and assimilation light was switched on.

3.3.4 Statistical analysis

Data on bud break were analysed per shoot remainder, which was the average of one (Experiment I) or two shoot remainders per plant (Experiment II and III). Light intensity and light spectrum measurements were averaged per plot and then over the two measurement days (if applicable) before analysis. PAR measurements were ln-transformed to obtain equal variances for the treatments. Data of PSS were arcsine-square root transformed to normalise the data. Differences between the treatments in bud break, light intensity, red:far-red ratio and PSS were tested using one-way analysis of variance in experiment I and II, and two-way analysis of variance in experiment III (factors being light intensity and light spectrum). Differences between treatments were tested using Tukey's Honest Significant Difference test. Analyses were done in GenStat vs 14.
Apart from the instantaneous light intensities, also average light sum per day in the greenhouse was calculated. It was calculated using the outside radiation (expressed in W global radiation m^{-2}), transmissivity of the greenhouse (60%), and data on the number of hours that the lamps were switched on. For conversion of outside radiation (W m^{-2}) to µmol m^{-2} s^{-1} a factor of 4.6 (Langhans and Tibbitts, 1997) was used, and PAR made up 48% of the global radiation. For each experiment, total light sum was the integral of the daily light sums over the total experimental period. Total light sum was than divided by the duration of the experiment (in days) to obtain average light sum per day. Daily light sum at bud level was a fraction of the light sum above the crop. This fraction was calculated from the instantaneous light intensity measurements. Average light intensity per plot was calculated from the instantaneous light intensity measurements and expressed relative to the maximum of the experiment. When light intensity was measured twice, fractions were averaged. Using this fraction, the average light sum per day at the level of the buds was calculated for each plot.

3.4 Results

3.4.1 Light intensity, red:far-red ratio and PSS.

Light intensity, red:far-red ratio and PSS at bud level differed significantly between the treatments in experiment I (P<0.001 for all variables, Table 2.). Light intensity at the bud level under five shoots was 9% of the light intensity without shoots present (Table 2.). Red:far-red ratio under five shoots was reduced to 20% of the red:far-red ratio without shoots, while PSS was reduced by 22% (Table 2.). Green paper over the plant bases decreased light intensity, red:far-red ratio and PSS at the level of the bud to values similar to or even lower than in the treatment with five shoots per plant (Table 2.). Removal of leaves from the vertical shoots increased light intensity at bud level compared to treatment where shoots were present, but it was not as high as when no shoots were present (Table 2.). Red:far-red ratio and PSS at bud level when leaves were removed from vertical shoots were similar to the treatment where complete shoots were removed (Table 2.).
Crepe paper decreased the light intensity at the bud in experiment II (P<0.001, Table 2.). One layer white paper decreased light intensity to 61% of the control. Light intensity was comparable between plants covered with two layers of white and one layer of grey paper and plants covered with two layers of green paper, and was 11-13% of control (Table 2.). However, red:far-red ratio and PSS differed between these latter two treatments, as only the green crepe paper affected light spectrum and hence red:far-red ratio and PSS (P<0.001, Table 2.).
Light intensity at the bud level was decreased with approximately 90% by the grey crepe paper compared to the control

(P<0.001) in experiment III, but red:far-red ratio and PSS were not decreased (P=0.25 and P=0.19, respectively, Table 2.). Red:far-red ratio and PSS were only affected by the far-red LEDs (P<0.001 for both variables). There was no interaction between paper and LEDs on light intensity, red:far-red ratio and PSS (P=0.28, P=0.15 and P=0.53, respectively). Red:far-red ratio and PSS were less reduced by the far-red LEDs than by the green paper in the other two experiments. Although PSS incorporates more wavelengths in defining the light spectrum than red:far-red ratio, differences between the treatments for red:far-red ratio and PSS were often similar. Only in experiment I, these differences were not similar (Table 2.): red:far-red ratio of the treatment without shoots and the plant base covered with green paper was similar to that of plants with five shoots, but the PSS was lower under green paper than under five shoots.

Table 1. Photosynthetically active radiation, temperature, relative humidity and CO_2 concentration inside the greenhouse in the three experiments (2009-2010). Means are provided and standard deviation is given between brackets.

Experiment	Time	Photosynthetic active radiation (mol m^{-2} d^{-1})	Temperature (°C)	Relative humidity (%)	CO_2 concentration (ppm)
I	21-Sept till 5-Oct	14.4 (1.12)	21.2 (0.55)	66.9 (2.51)	455 (19)
II	4-Nov till 17-Nov	12.70 (0.33)	19.0 (0.27)	73.5 (2.27)	470 (15)
III	13-Jan till 27-Jan	11.85 (0.97)	18.1 (0.65)	81.8 (2.83)	526 (26)

Table 2. Light intensity, red:far-red ratio and phytochrome photostationary state (PSS) measured at bud level in the three experiments.

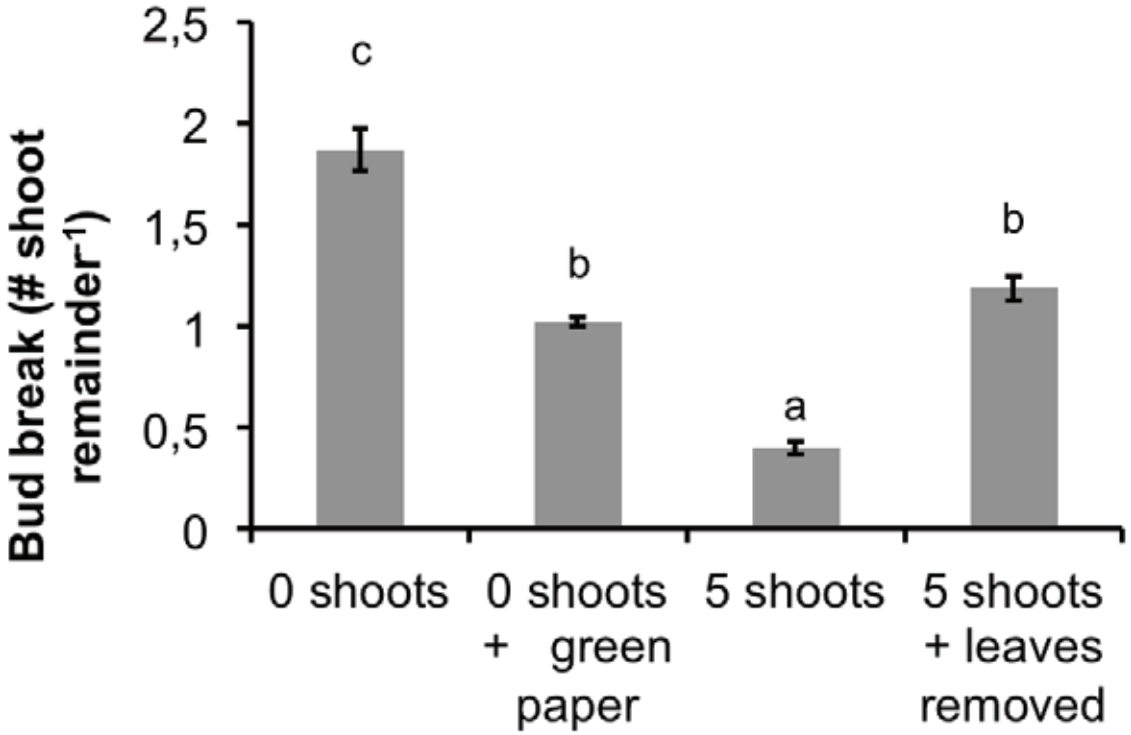
Experiment	Treatment	Light intensity (µmol m^{-2} s^{-1})*	Red:far-red ratio	PSS
I [a]	0 shoots	245 c[¶]	1.66 b	0.76 c
	0 shoots + paper	27 a	0.22 a	0.48 a
	5 shoots	21 a	0.34 a	0.60 b
	5 shoot + leaves removed	149 b	1.39 b	0.74 c
II [b]	Control	202 c	1.99 b	0.78 b
	White paper	122 b	1.97 b	0.77 b
	White + grey paper	27 a	1.96 b	0.78 b
	Green paper	22 a	0.26 a	0.52 a
III [c]	Control	165 b	2.5 b	0.79 b
	Far-red LEDs	157 b	0.4 a	0.67 a
	Paper	17 a	2.5 b	0.78 b
	Paper + far-red LEDs	18 a	0.5 a	0.67 a

*light intensity was ln-transformed for analysis
[¶] Different letters within an experiment indicate significant difference between the treatments with Tukey's honest significant difference test. Light intensity and red:far-red ratio for experiment I and II are the averages over two measurement days.
[a] Light intensity and red:far-red ratio measured on day 2 and 8, PSS on day 2
[b] Light intensity and red:far-red ratio measured on day 2 and 7, PSS on day 7
[c] Light intensity and red:far-red ratio measured on day 6, PSS on day 7

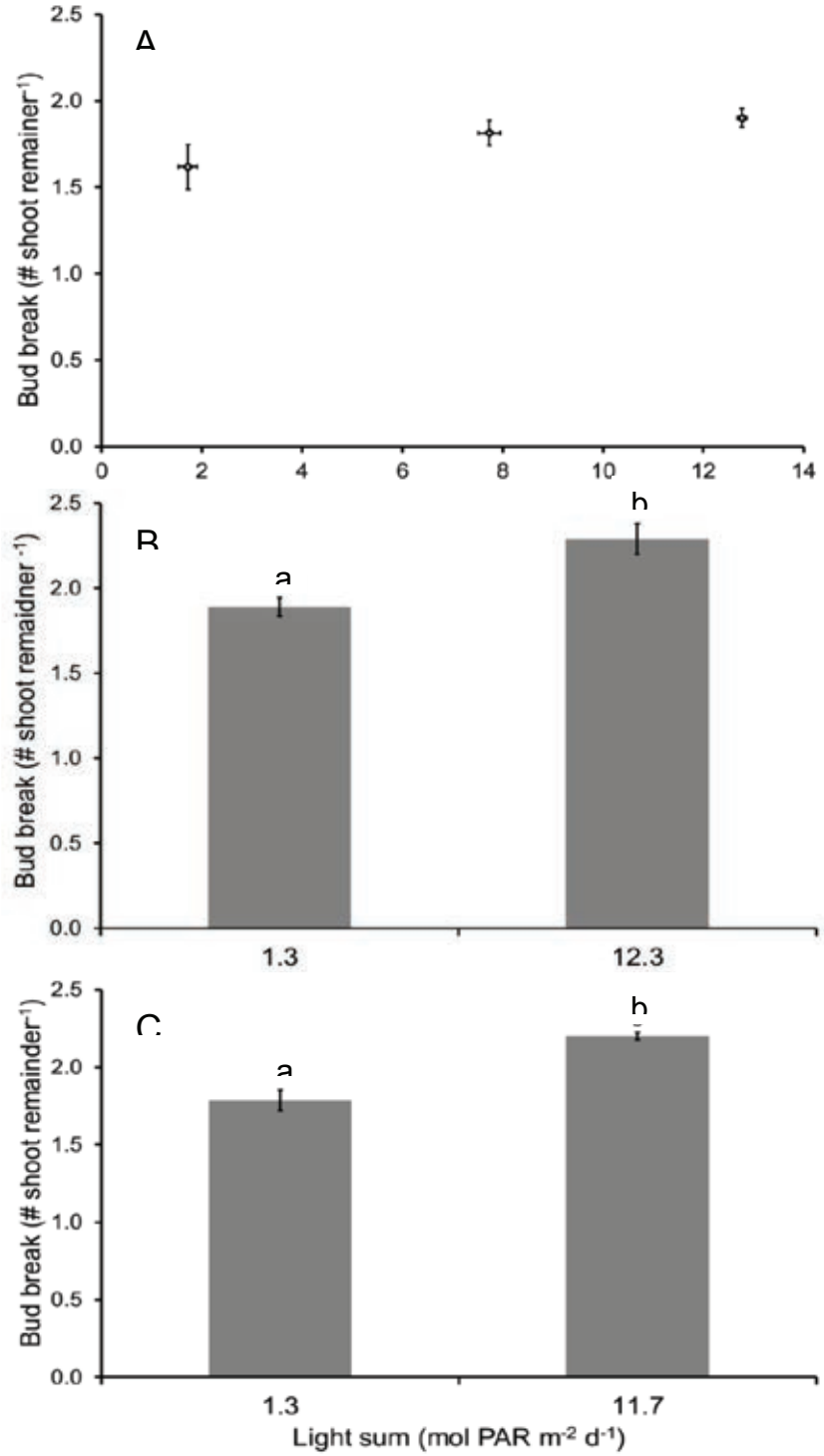
3.4.2 Bud break

In experiment I, there were significant differences in bud break between the treatments (P<0.001, Figuur 1.). Most buds broke when no shoots nor paper were present. Removal of leaves from the five vertical shoots significantly increased bud break compared to plants with five intact shoots. However, this increase was less than when all five shoots were removed. Bud break under the crepe paper was not significantly different from bud break in the treatment where leaves were removed from the vertical shoots.

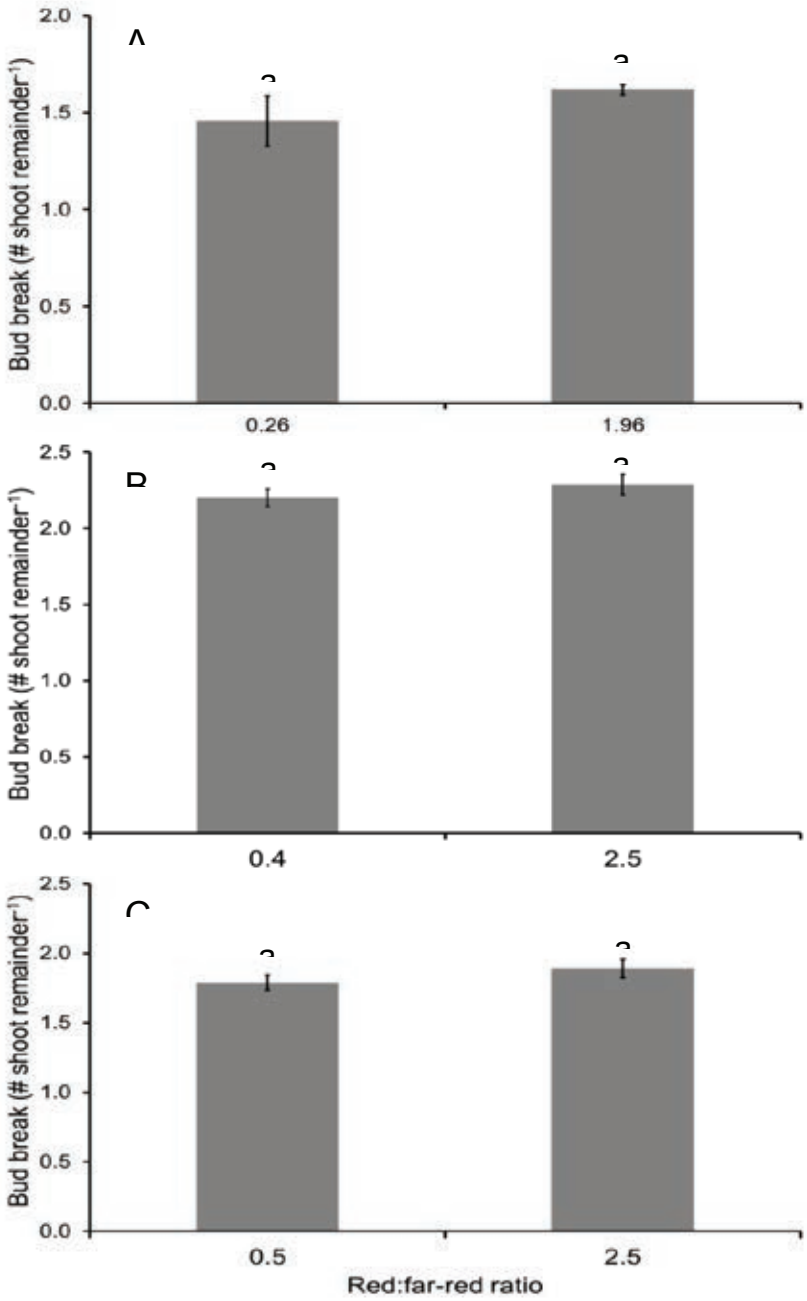
In experiment II, a significant treatment effect on bud break was found (P=0.018), but the only significant difference was between the control and the treatment with low light intensity and low red:far-red ratio (green paper). Regression analysis on the number of broken buds against the light sum for treatments with the same red:far-red ratio showed a significant positive correlation (P=0.04, Figuur 2A), although pairwise comparisons were not significant. In the same experiment, bud break for treatments with the same light intensity but different red:far-red ratios did not differ (P=0.30, Figuur 3A).
In experiment III, a decrease in light intensity at the same red:far-red ratio significantly (P=0.003) decreased bud break (Figuur 2B,C). A decreased red:far-red ratio at the same light sum did not affect bud break (P=0.35; Figuur 3B,C). There was no interaction (P=0.93) between the effect of light intensity (paper) and light spectrum (far-red LEDs) on bud break.



Figuur 1. Bud break in experiment I, where light intensity and light spectrum were varied by the number of vertical shoots (0 and 5 shoots), removal of leaves from vertical shoots (5 shoots + leaves removed) and removal of shoots combined with green crepe paper over the plant base (0 shoots + green paper). Error bars represent standard errors of the means, different letters indicate significant differences between treatments (P=0.05).



Figuur 2. Bud break in relation to average daily light sum at bud level for treatments with the same red:far-red ratio. A) Experiment II, red:far-red ratio 2.0 for all treatments, differences in the three average daily light sums were created by (from left to right) two layers of white plus one layer of grey crepe paper, one layer white crepe paper and no crepe paper (control), B) Experiment III, red:far-red ratio 2.50 for both treatments, average daily light sum of 12.3 mol PAR m^{-2} d^{1} was obtained in the control treatment, average daily light sum of 1.3 mol PAR m^{-2} d^{1} was obtained with two layers of grey crepe paper, C) Experiment III, red:far-red ratio 0.44 for both treatments, average daily light sum of 11.7 mol PAR m^{-2} d^{1} was obtained in treatment with far-red LEDs, average daily light sum of 1.3 mol PAR m^{-2} d^{1} was obtained in treatment with far-red LEDs and two layers of grey crepe paper. Error bars represent standard errors of the means. Different letters above bars indicate significant differences between treatments (P=0.05).



Figuur 3. Bud break in relation to red:far-red ratio for treatments with the same average daily light sum. A) Experiment II, light sum 1.6 mol PAR m^{-2} d^{1} for both treatments, high red:far-red ratio was obtained in treatment with two layers of white and one layer of grey crepe paper, low red:far-red ratio was obtained in treatment with two layers of green crepe paper, B) Experiment III, light sum 12.0 mol PAR m^{-2} d^{-1}, high red:far-red ratio belongs to control treatment, low red:far-red ratio was obtained in treatment with far-red LEDs, C) Experiment III, light sum 4.8 mol PAR m^{-2} d^{1}, high red:far-red ratio was obtained in treatment with two layers of grey crepe paper over the plant base, low red:far-red ratio was obtained in treatment with two layers of grey crepe paper over the plant base and far-red LEDs. Error bars indicate standard errors of the means. Different letters above bars indicate significant differences between treatments (P=0.05).

3.5 Discussion

In rose crops, shoots are harvested when the sepals of the flower bud start to open, revealing the colour of the petals. On the basal part of the shoot that is left (the 'shoot remainder'), axillary buds break and develop into the next harvestable

flower shoots. A previous study (Wubs *et al.* unpublished results) indicated that the degree of bud break on shoot remainders positively responded to more light in combination with higher red:far-red ratio received by the buds, but effects of light intensity and light spectrum could not be separated. The experiments in this study were set up to disentangle the effects of light intensity and spectrum of light reaching the axillary buds in a rose crop, trying to determine whether one or the other is more important for triggering axillary bud break crop, or that both are equally important.

The results of experiments II and III showed a small positive effect of light intensity at bud level on bud break, and no effect of light spectrum at bud level on bud break. Mor and Halevy (1984) attributed decreased bud break under illumination with a mixture of fluorescent and incandescent light (F+I), as compared to only fluorescent light (F), to the lower red:far-red ratio in the F+I light environment (0.64). In their case, the whole crop was subjected to low red:far-red ratio, while in our study, only the shoot remainders experienced low red:far-red ratios. Also experiments with other plant species where effects of light spectrum on bud break/branching were observed often changed light spectrum for the whole plant (*e.g.* Clifford *et al.* 2004; Crotser *et al.* 2003; Mata and Botto, 2011). Our research focussed on the effect of light received by the bud, as light on the bud is necessary for the bud in order to break (Girault *et al.* 2008). Changing red:far-red ratio affects the phytochrome status (Franklin and Whitelam, 2005). The contrast between the current findings (no effect of red:far-red at bud level on bud break) to literature reporting decreased bud break when the whole plant is subjected to low red:far-red ratios might indicate that not only the phytochrome status of the bud and the adjacent stem is important for bud break, but the phytochrome status of the whole plant. Also studies on the effects of light intensity on bud break also changed light intensity on the whole plant (*e.g.* Mata and Botto, 2011; Schoellhorn *et al.* 1996; Su *et al.* 2011). Our study reveals that when light intensity and light spectrum received by the plant are similar but differ at bud level, bud break was affected by light intensity and not by light spectrum.

The results of experiment I confirm the role of correlative inhibition (suppression of bud break due to the presence of plant organs including but not limited to the shoot tip) as a determinant of bud break in addition to exposure to light: light intensity and red:far-red ratio were similar in the treatment with five shoots and the treatment without shoots with green paper over the plant base (Table 2.), but bud break was lower in the treatment with five shoots. This indicates that the presence of shoots affected bud break through both their effect on light conditions at bud level and through correlative inhibition.

There was a considerable difference in bud break between the experiments: bud break was higher in experiment II and III than in experiment I, while differences between the treatments were most pronounced in experiment I. An explanation for the high overall level of bud break in experiment II and III might be the absence of correlative inhibition from other vertical shoots. Correlative inhibition relates to suppression of bud break through hormonal interactions of mainly auxin, cytokinines and strigolactones (Domogalska and Leyser, 2011). Correlative inhibition was absent in experiment II and III and this might have resulted in higher bud break. Another reason for the high overall level of bud break in experiment II and III might be the use of assimilation lamps. As a result, 82% of the light sum came from the assimilation lights, compared to 48% in experiment I. Assimilation lights increased the red:far-red ratio on the whole crop (see control treatments in Table 2.).

Apart from light, other climatic factors can also affect bud break, and might explain the difference in bud break between experiment II and III. The most likely factor is relative humidity, which was higher in experiment III, resulting in a lower VPD. A lower VPD increases bud break (Garcia Victoria *et al.* 2007). The lower temperature in experiment III would results in less bud break in the same time span (Mattson and Lieth, 2007). CO_2 levels differed as well, possibly leading to differences in assimilate production, but assimilate production had little effect on bud break (Wubs *et al.* unpublished results).

Given these effects, one should keep in mind that a plant architecture without vertical shoots, as used in these experiments, occurs during the initial phases of cultivation. In older crops, shoots of various developmental stages are present (De Hoog, 2001). Whether the effect of local light intensity and light spectrum on the bud is the same for crop with and without verticals shoots requires further experimentation.

In conclusion, when buds on decapitated shoots (shoot remainders) were exposed to light of equal red:far-red ratios, there was a positive effect of light intensity at bud level on bud break. Conversely, under similar light intensities at bud level red:far-red ratio at bud level had no effect on bud break. Difference in bud break when light conditions were similar and plant architecture was different implied that correlative inhibition modifies the effects of the light regime.

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4 Een functie-structuur model voor roos

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4.1 Abstract

- *Background and Aims* The production system of cut-rose (*Rosa x hybrida*) involves a complex combination of plant material, management practice and environment. Plant structure is determined by bud break and shoot development while having an effect on local light climate. The aim of the present study is to cover selected aspects of the cut-rose system using Functional-Structural Plant Modelling (FSPM), in order to better understand processes contributing to produce quality and quantity.

- *Methods* The model describes the production system in 3D including a virtual greenhouse environment with the crop, light sources (diffuse and direct sun light and lamps) and PAR sensors. The crop model is designed as a multiscaled FSPM with plant organs (axillary buds, leaves, internodes, flowers) as basic units, and local light interception and photosynthesis within each leaf. A Monte-Carlo light model was used to compute the local light climate for leaf photosynthesis, the latter described using a biochemical rate model.

- *Key Results* The model was able to reproduce PAR measurements conducted at different canopy positions, different times of the day, and different light regimes. Simulated incident and absorbed PAR as well as net assimilation rate in upright and bent shoots showed characteristic spatial and diurnal dynamics for different common cultivation scenarios.

- *Conclusions* The model of cut-rose as presented here allowed the creation of a range of initial structures thanks to interactive rules for pruning, cutting and bending. These static structures can be regarded as departure points for the dynamic simulation of production of flower canes. Furthermore, the model was suitable to predict local (per leaf) light absorption and photosynthesis. It can be used to investigate the physiology of ornamental plants, and in the future also for decision-support of growers and consultants.

4.2 Introduction

Cut-rose (*Rosa x hybrida*) is an important horticultural commodity worldwide. Cut-roses are grown in greenhouses, in which most environmental factors are controlled (temperature, CO_2, supply of water and nutrients, relative humidity, light). Roses are vegetatively propagated from cuttings, which consist of a piece of stem with a leaf and an axillary bud. The cutting is rooted and the bud grows out to form the primary shoot. The primary shoot is usually prevented from flowering and bent down after six to eight weeks. Secondary buds located in the axils of scale leaves at the base of the bent primary shoot grow out to form a number of shoots, the so-called 'bottom breaks'. After several cuts of bottom breaks and of descendant shoots (each time leaving a 'stump' of the harvested shoot) a 'permanent basal structure' of the plant is built with axillary buds in various positions which may break to produce new 'upright shoots' (altogether constituting the 'upright canopy'). The latter are harvested once a flower bud has advanced to the appropriate developmental stage. Small shoots and shoots without a flower are bent down. The bent shoots branch, forming the 'bent canopy' that serves to produce assimilates for 'upright shoots'. Contrary to the upright canopy, shoots in the bent canopy are prevented from forming flowers by regular removal ('pinching') of flower buds. One crop produces shoots continuously over a period of four to six years.
Growth and development also depend on management. The latter comprises harvesting time, cutting height, pruning,

bending and greenhouse climate control. In the face of continuous change in technology and cultivar characteristics, there is a strong desire for an improved understanding of the relations between the plant's architecture, the distribution of buds (as determined by the architecture) and breaking of axillary buds as well as the quality of shoots arising from these buds, with a prerequisite for the presence of a perfect flower on the top of the stem.

Functional-structural plant models (also known as virtual plants) can be defined as models explicitly describing the development over time of the 3D architecture or structure of plants as governed by physiological processes which, in turn, are driven by environmental factors (Kurth 1994; Sievänen *et al.* 1997; Vos *et al.* 2010). Commonly such models describe a plant as a set of interconnected phytomers. A phytomer is a growth unit resulting from the activity of an apical meristem and usually consisting of an internode, a leaf and an axillary bud. The organs of each phytomer have attributes like weight, shape, orientation in space and optical properties that affect the amount of light intercepted, *e.g.* for photosynthesis. This modelling approach is particularly suited to integrate and apply knowledge on plant architecture and bud break. In particular, feedback between structure and function can be implemented and verified at various levels, *e.g.* locally at the organ scale and globally at the plant or canopy scale.

When designing an FSPM of a glasshouse cut-rose crop a number of elements need to be considered, including: I. light distribution and light interception; II. photosynthesis; III. bud break; IV. Dynamics of growth and development of individual organs; V. manipulation of the plant structure by cutting and bending; VI. plant architecture; VII. Source-sink relations and allocation of assimilates to growing organs.

In the following, we will look at the first six elements:

I. Light distribution and light interception

Light received by individual leaves in the canopy comes from several sources: direct sun light and diffuse sky light penetrating the cover of the glasshouse, light from additional lamps (*e.g.* high pressure sodium (SON-T) lamps) mounted in a particular configuration at some height above the canopy. Modelling entails defining the directions and flux densities from each source as these change over the course of the day. Depending on the purpose of the study a distinction can be made between receipt of total energy or only photosynthetically active radiation (PAR), while for an understanding of photomorphogenetic effects separate simulation of red and far-red radiation is required (Evers *et al,.* 2006; Kahlen and Stützel, 2011). Optical properties of the plant and glasshouse material determine the scattering of light in the canopy and the receipt of energy at each position in the 3D structure. Buck-Sorlin *et al.* (2009) have made first steps towards the adequate modelling of these complex light regimes.

II. Simulation of the (daily) carbon assimilate production rate

The calculation of the production rate of carbon assimilates, or gross photosynthesis, depends on the simulation of the position and orientation in space of leaves, their area, photosynthetic properties and light absorption. There are various models available that calculate the leaf photosynthesis rate in rose (*e.g.* Lieth and Pasian, 1990; Kim and Lieth, 2003). These models can be applied at every time step to every single leaf unit that is distinguished in the 3D model. In the case of glasshouse production it is important that photosynthesis models are chosen that adequately quantify the effects of variable temperature and carbon dioxide concentration as these environmental variables are subject to management.

III. Bud break

In principle a model needs to keep track of all buds in the plant structure. At each time step it needs to evaluate the probability of breaking of a bud, given its position and environmental parameters. A correct quantification of bud break is essential if the model is supposed to be of value for the industry. Bud break could simply be computed as a function of the topological distance of the bud to a cutting surface, as done by Pien (2007). However, such a model can only be applied to axillary buds positioned on the stumps of harvested upright shoots ('stump buds'), as only for these sufficient data are available. There is, to our knowledge, no quantitative information about the breaking of axillary buds within the bent canopy.

IV. Dynamics of growth and development of individual organs

Meristems produce new phytomers. The organs of a phytomer (internode, petiole, leaf blade, axillary bud) exhibit a characteristic dynamics with respect to their time of initiation, their increase in weight and volume, shape and orientation in space (Fournier and Andrieu, 2000).

V. Plant manipulation

The fate of a rose plant is characterized by continuous human interference: bending of shoots, harvesting flower canes, pruning, 'pinching' (removal of flower-bearing branches). Each one of these interferences has consequences for the functioning of the plant. For instance, taking away a flower branch means removal of source contribution of its mature leaves, but also alteration in the hormonal balances governing bud break. Hence, it is essential to develop provisions to allow the interruption of the model run, to execute the type of interferences mentioned. Such interferences can be phrased as rules, which are either applied automatically and then linked to conditions, *e.g.* 'remove all mature shoots at a specified cutting height' or manually, executing a particular interference with an organ chosen interactively by the user. Properties of removed material such as leaf area, weight, length and diameter of harvested shoots need to be retrievable.

VI. Plant architecture

The recurrent application of the processes described above, mainly bud break and growth and development of organs, in combination with plant manipulation, results in the architecture of the plant. This 3D structure is modifying the local light climate, thus having an influence on local light interception and photosynthesis, and ultimately on growth and development of further structures.

The objective of the present paper is to describe a static FSPM of cut-rose focussing on the simulation of the local light climate and photosynthesis rate in connection with plant manipulation. We will show at the example of different scenarios the influence of the initial plant setup (plant density) and the history of structure management (size of the bent canopy) on light interception and canopy photosynthesis.

4.3 Materials and Methods

Overview and scope of the model

The scope of the model is the reconstruction of the structure of a mature cut-rose production system within a virtual greenhouse.

The model was written in the modelling language XL (Kniemeyer 2008) using the open-source GroIMP platform (www.sourceforge.net/projects/groimp).

Each simulated rose plant consists of a bent shoot canopy, a framework of stumps (the 'permanent basal structure') and a number of upright flower shoots, formed from axillary 'stump buds'. The root system is neglected in our model.

Light regime in the virtual greenhouse

The details of the light model used can be found in Buck-Sorlin *et al.* (2009). Essentially, an instance of the GroIMP radiation model (Hemmerling *et al.* 2008) is invoked and carried out at each model step, computing the local PAR perception of virtual sensor objects and PAR absorbing leaf objects in a 3D scene. The 3D scene consists of the virtual greenhouse with the crop and assimilation lamps (see above) inside, surrounded by a sky and sun, providing diffuse and direct light, respectively. The output of both the sky and the sun are dynamic functions of the day of the year and time of the day [h]. The sky is modelled as an array of 72 directional lights arranged in a hemisphere, (6 concentric rings each consisting of 12 lights; cf. Evers *et al.* 2010 for a similar arrangement). The sun object is another directional light, which dynamically changes its output like the sky object, but also its position. The position of the sun in normalized coordinates was computed as a function of day of year and time of day, following Goudriaan and Van Laar (1994). Inside the greenhouse 10,000 randomly arranged virtual spherical PAR sensors (radius 5 cm) were placed inside an invisible bounding volume (a rectangular cuboid of length 4 m, height 2m, and width 1.2 m or 0.65 m in the high-density scenario) around the interior double-row of the simulated rose canopy to measure incident downward PAR in the bent and upright canopy up to a height of 2 m above the greenhouse floor. The virtual sensors are a feature of the GroIMP radiation model. They are invisible, i.e. do not interfere with the path of the rays but only measure the irradiance at their surface. Furthermore, only the upper hemisphere of a sensor is used and incoming radiation is cosine-corrected, thus making the sensor a fairly correct model of the widely used PPFD quantum sensor. Such virtual sensors within the simulated crop allow the establishment of an accurate 3D map of the spatial light distribution. The bounding volume of virtual sensors was divided into two-hundred horizontal layers of one cm height, and the mean value of about fifty virtual sensors per layer was sampled. In addition,

the amount of PAR [PPFD, µmol photons m^{-2} s^{-1}] absorbed was computed by the light model at every step in each leaf. The radiation model itself (Hemmerling *et al.* 2008) is an inversed Monte-Carlo raytracer (Veach, 1998). Put simply, it produces light transport paths ('rays'), thereby connecting light sources with scene objects. The number of rays emitted by all light sources as well as the number of times a ray is followed on reflection or transmission after encounter with a scene object can be determined by the user. A combination of twenty million rays and ten reflections per ray turned out to be sufficient for our purposes. Note that during one run of the radiation model, the entire scene is bombarded with rays, including the greenhouse (see Table 1. for dimensions). On average, the different elements of the greenhouse (floor, glass walls, and roof) absorbed around 140 µmol m^{-2} s^{-1} of day light (with lamps switched off), which represented about 45% of the total light emitted by all light sources at a given moment.

The path of a ray in the scene and the likelihood with which it will be absorbed, reflected or transmitted, depends on the geometry and distribution of objects in the scene as well as on their optical properties. The latter were modelled using shaders that are mapped onto the geometrical objects representing organs. In the case of leaves these were composed of a terminal leaflet and a variable number of lateral leaflet pairs, connected to each other by a midrib. For each leaflet, a parallelogram object was used, with length and width, times a form factor (Table 1.), representing measured leaflet length and width, respectively. As a texture a so-called AlgorithmSwitchShader was used (Kniemeyer, 2008), a shader with two options: for the realistic visualization of leaflets textures were used (Figs. 2, 3), whilst for computation of light absorption a simple RGB shader (Kniemeyer, 2008) was employed, in which the measured diffuse reflection and transmission for the red, green and blue wavebands (600-700 nm, 500-600 nm, 400-500 nm, respectively) were specified (diffuse reflection for R,G,B: 6.6%, 15.2%, and 1.5%, respectively, diffuse transmission for R, G, B: 5.4%, 8.6%, and 4.7%, respectively, cf. Paradiso *et al.* 2011). The amount of PAR absorbed by a leaf, I_a, as computed by the radiation model is then:

$$I_a = I_i - I_t - I_r \qquad (1)$$

where *Ii* is the PAR incident reaching the leaf, *It* and *Ir* are the amounts of transmitted and reflected PAR, respectively.

In the case of greenhouse crops like cut-rose the path of the direct and diffuse light coming from the sky is further modified by the geometry and optical properties of the greenhouse (details in Buck-Sorlin *et al.* 2009): The light climate inside the greenhouse was modelled by reconstructing a 3D geometrical model of a greenhouse compartment consisting of side walls, roof, and shading screens; for all of these the measured optical properties were set and the transmissivity of the textures calibrated to achieve an overall transmissivity of about 63% using a simulated empty greenhouse and a comparison of output of virtual light sensors placed inside and outside the greenhouse. The interior of the greenhouse compartment was reconstructed to represent the experimental setup (see below). The virtual assimilation lamps are described in detail in Buck-Sorlin *et al.* (2009). For the lamp model, the point light object of GroIMP was extended using measured light distributions, thereby imitating the characteristic intensity of light emitted by the lamp in a particular direction.

Table 1. Model parameters

Description	Value (range[1])	unit
Greenhouse (setup and climate):		
Dimensions of greenhouse compartment (L, W, H)	12, 12, 5	m
Distance between gutter rows	1.2	m
Total width of double row	0.2	m
Length of a slab	1	m
Number of plants per slab	5	-
Number of double-rows	5	-
Number of slabs per double-row	8	-
Total number of simulated plants	400	-
Height of assimilation lamps (from ground)	3.6	m
Conversion factor daylight (PAR Watt to PAR PPFD)	4.55	µmol J^{-1}
Conversion factor SON-T lamp (PAR Watt to PAR PPFD)	4.79	µmol J^{-1}
Spacing between lamps within the same row	2	m
Spacing between lamps in different rows	3.5	m
Daylight threshold below which assimilation lamps are switched on	200	µmol PAR m^2 s^{-1}
CO_2 concentration in air	460	µmol mol^{-1}
Relative humidity of air	84	%
Daily mean temperature	20.5	°C
Plant architecture parameters:		
LAI of bent canopy	3.0	m^2 m^{-2}
Default cutting height (above base of cutting)	0.1	m
Maximum phytomer rank	(19 - 22)	-
Leaf divergence angle	54.1±16.2	º
Length of terminal leaflets	(0.0185 - 0.0541)	m
Width of terminal leaflets	(0.013 - 0.038)	m
Length of lateral leaflets	(0.02 - 0.044)	m
Width of lateral leaflets	(0.014 - 0.03)	m
Divergence angle of lateral leaflets	(55 - 65)	º
Form factor for leaflet area (= area/(length*width))	0.7038	-
Number of leaflets per leaf[2]	{1,3,5,7/9,5,3,1}	-
Plastochron	1.5	d
Internode length[2]	(0.000012 - 0.0596)	m
Internode diameter	0.004 - 0.00015*rank	m
Phyllotactic angle	124.±39.3	º

[1]A range of values is understood to follow a uniform distribution.[2] Number of leaflets and internode length are a function of the relative acropetal rank rr = rank/maximum rank.

Photosynthesis

Photosynthesis of leaves was modelled according to the model of Kim and Lieth (2003), which is based on the model of Farquhar *et al.* (1980).

Bud break model

In the model, by default, all axillary bud objects are ranked acropetally along the shoot (i.e. numbered from rank 1 at the base to rank *rmax* at the flower peduncle), and initially have a breaking probability of zero. Based on our observation that normally after a cutting event (harvest or pruning) on an upright shoot only the three uppermost axillary buds below the cut are breaking, with the most proximal one having the highest breaking probability and the two following buds a much lower probability, we implemented a model in which after interactive removal of shoots (see next section) the buds below the cut are marked as '*cut'* and given a 'proximity number' (*pn*) which thus reflects the topological proximity to the cut surface (e.g., the bud below the cut has *pn* 1, the one below it *pn* 2, and so on). Breaking probability *PBB* is then computed as an exponential function of *pn*, conditional on *Q*:

$$P_{BB} = a \cdot e^{pn \cdot b} \mid Q \qquad (2)$$

where *a* and *b* are factors and *Q* is a cutting event that needs to have taken place above the bud (Figuur 2.). This probabilistic bud break model is currently only applied to axillary buds on stumps, as there are no data on bud break in the bent canopy. Typical values for *a* and *b* are 4.433 and -1.564 (derived from observations in the experiment, data not shown). This resulted in bud break probabilities of 92.8%, 19.4%, and 4.1%, for the first three buds on the stump below the cut, and much less than 1% for all further (i.e. lower) buds.

Interaction with the plant structure

Growers' interaction with the plant structure is manifold but can be broken down to two main activities, namely cutting and bending. Mostly the targeted structure is a whole shoot (pruning and harvesting), sometimes a single organ ('pinching'). Bending is the singular or repeated application of force to the base of a shoot with the result that the shoot will grow horizontally for a while until the tip of the shoot bends upwards again (following the natural orthotropic tendency of all rose shoots). We modelled bending in two principal ways, automatic or interactive: In the case of automatic bending a basal internode within a (primary) shoot is identified via a rule in XL, i.e.:

```
Bud (<--)+ im:Internode, rv1:RV (<--)+ im1:Internode < rl:RL < rh:RH < (<--)
{4} rv2:RV, (im[rank]==3) ::>
      {
            rl[angle] = 50;
            rv1[argument] = BS;
            rv2[argument] = BS/2;
      }
```

The rule identifies a subgraph (part of the data representation of the simulated structure), consisting of an Internode named *im* which is accompanied by other organs (Bud, a preceding internode *im1*), with the condition that the rank of *im* should be 3 (i.e. the basal internode which is usually in the zone chosen for bending). In the case of interactive bending the condition (im[rank]==3) on the left-hand-side of the rule is replaced by another condition (isSelected(im)) which checks whether the internode *im* belonging to the subgraph specified on the left-hand-side has been selected by the user.

Harvest and pruning of shoots as well as removal of undesired flowers in the bent canopy constitute cutting, i.e. an event in which part of the structure is removed. Consider the following simplified rule:

```
x:Internode, (* x (<--)+ : (rt:Root) *),
      (x.getIndiID()==rt.getIndiID() && x.getBent()==false &&
distance(x,rt)>=CH) ==>> cut;
```

On the left-hand-side of this rule an internode *x* is searched for, with a context (within symbols (* *)) of a root *rt* at its base, fulfilling the following criteria: internode and root should belong to the same plant, the internode should not belong to a bent shoot, and the distance between *x* and *rt* should be bigger than a parameter *CH*. The effect of this query is that all

upright shoots above a specified cutting height *CH* will be cut. This rule also marks buds below the cut (not shown) so that they can break with a specified probability (see above).

Simulation of the initial production system

For many applications it is important to explore future developments given an initial architecture. Therefore, we developed provisions allowing the creation of an 'instant canopy'. The number of previous flushes, the frequency, sequence, and height of harvest and pruning events will all contribute to this architecture and will equally have an influence on the following generation of harvestable shoots. By employing a 'rapid mockup' technique, a specific initial production situation can be recreated: In the frame of this method an instant canopy consisting of upright shoots is produced, in which each upright shoot is created from a basal bud within one step, and consisting of the proper organs (leaves, internodes, flowers). Organ dimensions are set using stochastic variables derived from detailed measurements (Table 1.) carried out in another experiment in May to July 2009, which will be described in detail in a subsequent paper. For bud break the same probabilities are applied as described above. In addition to the formation of the instant upright canopy (essentially a flush of harvest-ripe shoots) the bent canopy can be enhanced by instantly forming more lateral shoots until a user-specified leaf area index is obtained. More specifically, the uppermost ten axillary buds of the bent primary shoot break to form second-order side shoots with up to ten phytomers, which are also instantly bent down. Alternatively, the bent canopy can be reduced in size by applying a specific cutting rule. Note that these rules are simply there to design an initial structure as a departure point for subsequent simulation of shoot production and are themselves not the outcome of the feedback of photosynthesis with simulated sink functions.

The formation of an instant canopy can be achieved by combining structure formation rules in one method "*initialCanopy()*", with automatic application of a specified sequence of the interaction methods described above. Thus, if it were desired to simulate the third flush of a production system, the *initialCanopy()* method could consist of the following sequence of commands:

```
buildCanopy(); pickBentFlower(); flushHarvest(); buildCanopy();
increaseCH(0.03); flushHarvest(); buildCanopy();
```

Figuur 3. illustrates the principle of the rapid mockup technique.

Model implementation

The model consists of several modules: the main module 1) loads and initialises global parameters from external files (e.g., species- and management-specific parameters, as well as greenhouse and general climate data, see Table 1.); 2) initialises environment, plant individuals and canopy; 3) controls information flow (simulates processes at different temporal resolutions for light model, photosynthesis and morphology); 4) provides interactive harvest and pruning functions (continuous or flush, Buck-Sorlin *et al.* 2011) that allow the user to interact with the structure at any time during the simulation; 5) creates output files and charts. All plant organs are implemented in a Modules file. Objects are defined for different hierarchical scales, from aggregated organs such as Canopy, Individual or Shoot down to basic organs like FlowerMeristem, Root, Leaf, Bud, Internode (including flower peduncle) and Flower, and on an even lower level Leaflet and Petiole. All basic organs like leaf, internode, root and flower are implementing the same organ interface thereby ensuring that all organ types are equipped with the same functionality and can be handled in the same straightforward way.

Experimental setup and measurements

Experiments to collect certain model parameters (including light distribution and photosynthesis rate) and data for model testing were conducted in the glasshouse compartments of Wageningen University and Research Center, Wageningen, The Netherlands (51° 58' N / 5° 40' E). The cultivar used in the research was a white rose, *Rosa x hybrida* 'Akito' (Tantau). The crop was planted in one glasshouse compartment (12 m x 12 m, wall height 5 m) on 25 Feb 2008 in double rows at a plant density of 6.5 plants m^{-2}. Each row consisted of ten one-metre long rockwool slabs (width 15 cm, height 7.5 cm) with a total of 50 plants. The distance between the centres of two adjacent double-rows was 0.2 m, the path width was 1.2 m (from edge to edge of two bordering slabs). Supplementary lighting was provided in the form of 24 Hortilux HS2000 Green Power (Hortilux, Pijnacker, The Netherlands) high-pressure sodium lamps (600 W, leading to PPFD level of about 171 µmol m^{-2} s^{-1} at a distance of 1.2 m below the lamps, with a spacing of 3.5 x 2 m. These lamps were switched on during the day

and part of the night (except between 2000 h and 0300 h) when the solar PAR level was below a threshold value (200 W m^{-2}). The set temperature was 19 °C at night and 23 °C during the day, relative air humidity was around 75%, and pCO_2 about 450 µmol mol^{-1} at night and 500 µmol mol^{-1} during the day.

Leaf photosynthesis measurements were made with an LCpro+ Advanced Photosynthesis System (ADC BioScientific Ltd., Great Amwell, Hertfordshire, UK) between 6 and 15 Oct 2008. Light response curves were obtained for different leaf positions in the upright and bent canopy (see Table 2.) at three CO_2 concentrations (400, 600 and 800 µmol mol^{-1}) and a leaf temperature of 25 °C.

Light-response (LR) curves at three external CO_2 concentrations, for different leaf positions in the canopy were determined on plants with almost harvest-ready upright shoots from experiment 1. Details about the measurements and the key parameters can be found in Table 2. The photosynthesis model was calibrated with the measured values, by tuning the following photosynthetic parameters using a genetic algorithm (Fogel 1998; Goldberg 1989): maximal rate of electron transport (J_{max} [µmol e^- m^{-2} s^{-1}]), curvature of the light response curve (α, [-]), quantum efficiency (θ [µmol e^- $µmol^{-1}$]), and maximum carboxylation rate (V_{cmax} [µmol CO_2 m^2 s^{-1}]).

Measurements of radiation distribution in the glasshouse were made on 26 Sept and 2 Oct 2008, between 1200 h and 1630 h under different direct and diffuse light conditions, with or without assimilation lamps switched on, in the experimental compartment in the presence of a fully grown flower crop, using a LI-190 quantum sensor (LI-COR, Lincoln, Nebraska USA), which measures PAR (400 to 700 nm waveband).

Table 2. Model parameters defining net photosynthesis rate (Farquhar - von Caemmerer - Berry model) of leaves at different position in the canopy. Parameters are based on measurements done in a developed 'Akito' rose canopy in October 2008.

Position:	J_{max} [µmol e^- m^{-2} s^{-1}]	Vc_{max} [µmol CO_2 m^{-2} s^{-1}]	Vc_{max}/J [-]	α [µmol e^- $µmol^{-1}$]	θ [-]	$J_{calc}{}^X$ [µmol e^- m^{-2} s^{-1}]	$J_{calc}{}^X$, relative rank ^{Y} [µmol e^- m^{-2} s^{-1}]
Top	178.7	78.8	0.44	0.53	0.49	181.91	174.58
Middle	126.7	54.0	0.43	0.46	0.82	120.89	130.77
Base	106.5	44.9	0.42	0.43	0.83	106.70	110.43
Light bent	102.4	45.4	0.44	0.50	0.77	103.40	97.95
Shade bent	67.0	30.5	0.46	0.38	0.56		

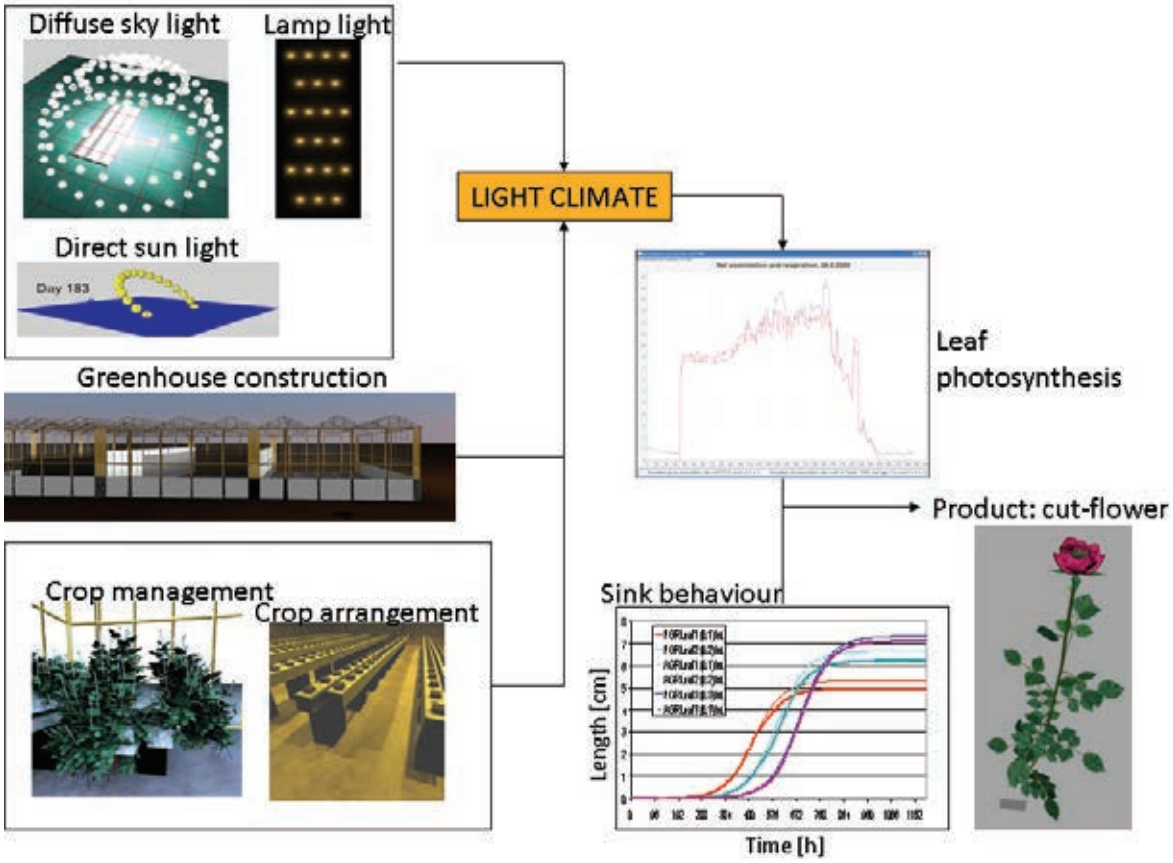
[X]calculated *Jmax* values, after tuning using a genetic algorithm (Fogel 1998; Goldberg 1989)
[Y]Relative rank is the absolute acropetal rank of a leaf, divided by the maximum rank, yielding a normalized positional index [0..1].

Scenarios

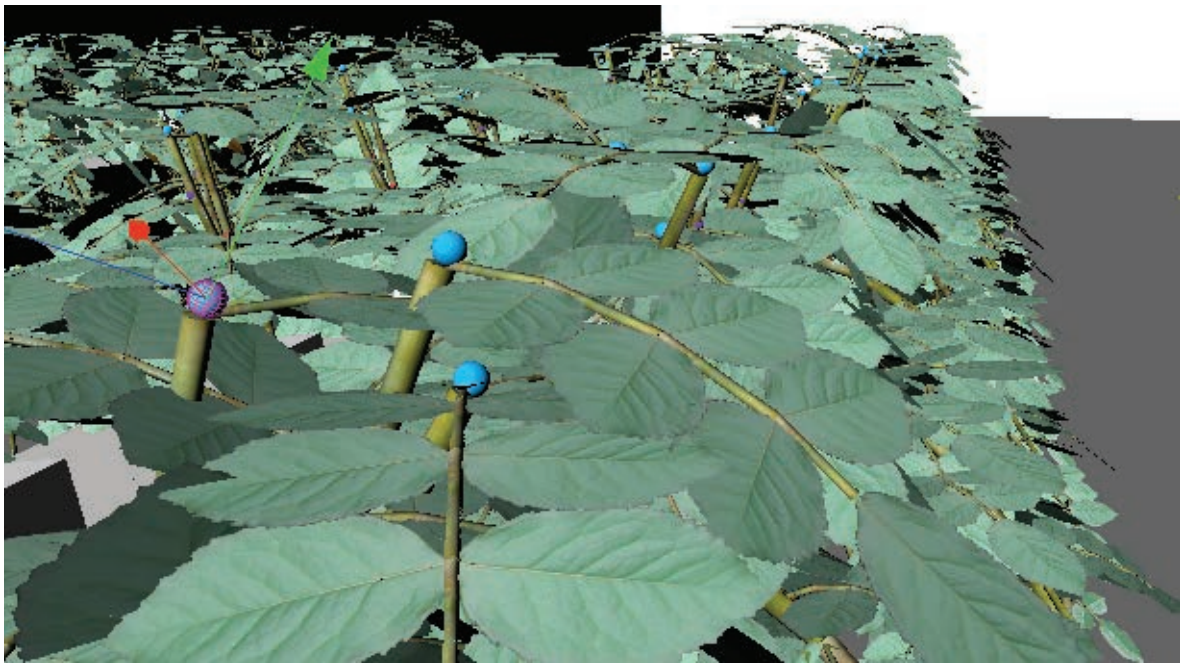
We designed three different simulation scenarios for plant arrangement to test the influence of a static structure on the spatial distribution of PAR and photosynthesis. We wanted to test the hypothesis put forward by rose growers, that reducing leaf biomass increases light use efficiency by improving light penetration. In the practice of rose production part of the greenhouse area is wasted by either keeping the path open, or by maintaining too much biomass in order to sustain enough assimilate production, the latter leading to an increase in respiratory costs. Some growers are using a production system in which the roses are grown on rolling gutters, thereby eliminating walking paths altogether, while in other companies the bent canopy is allowed to grow out to cover the entire path.

Simulation scenario 1 (control) was the reconstruction of the original measured canopy structure (Table 1.), i.e. with a path width of 1.2 m, and a standard bent canopy (LAI about 3.0). In scenario 2 the s control structure was used but the path between the rows was reduced from 1.2 m to 0.6 m, thereby creating a completely closed simulated bent canopy with bent shoots intertwining. Plant density per square metre was thus increased from 7.14 plants m^{-2} to 12.5 plants m^{-2} as the number of plants per slab was kept the same. In the third scenario again the plant architecture of the control was used but with the leaf area of the bent canopy reduced in the simulation by 50% compared to the control. This was achieved by invoking a model rule that removes part of the bent shoot above rank seven with a probability of 85%. The LAI of the entire simulated canopies was 4.7, 4.7, and 3.2 m^2 m^{-2}, respectively, for scenarios 1 to 3. Simulated plant age was 70 days at the moment that PAR absorption and photosynthesis were determined. All scenarios were carried out employing the

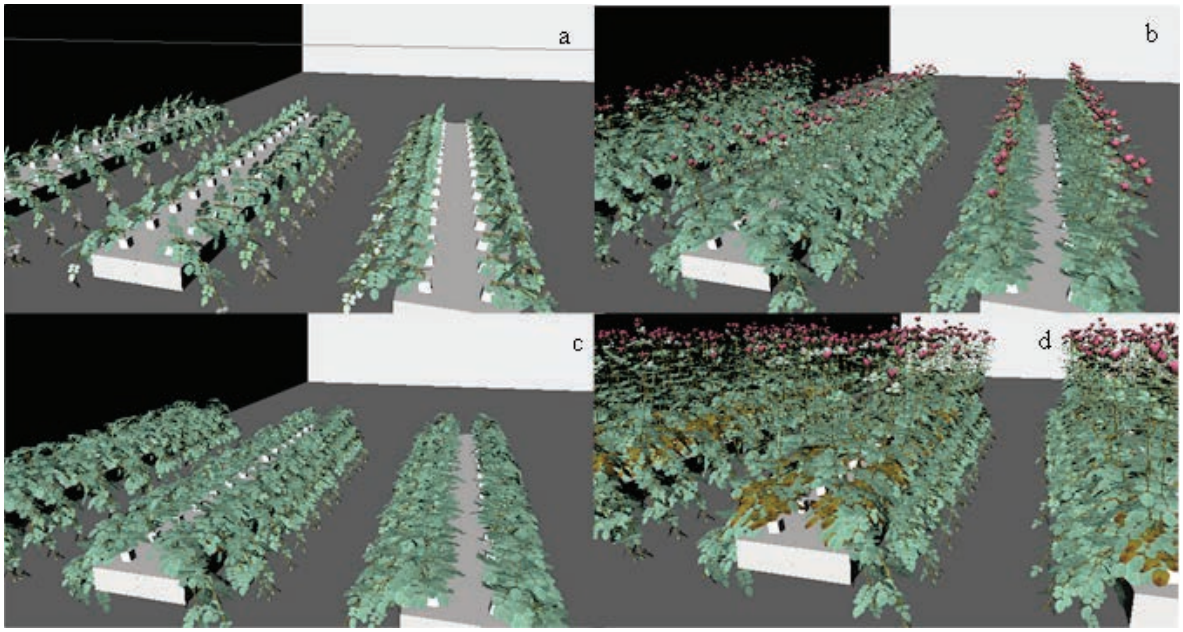
initialCanopy() method, i.e. an initial structure was simply reconstructed *in silico* (based on the measurements). Hourly mean values of measured climate data (PAR, temperature; from 10-minute averages) for 2 Oct 2008 were used, an overcast day (daily total global radiation: 19.5 MJ m^{-2}, daily total radiation measured on the ground: 7.5 MJ m^{-2}, average atmospheric transmissivity 0.39, total complementary radiation by lamps: 4.8 MJ m^{-2}, average PPFD: 216.4 µmol m^{-2} s^{-1}), to compute instantaneous light interception and photosynthesis rate. The amount of PAR intercepted by the entire canopy was estimated as the level of PAR incident above the canopy (1.80 m above greenhouse floor) minus the level of PAR transmitted below the bent canopy (0.30 m above the greenhouse floor). The time scheme for the simulated assimilation lamps was the same as in the experimental compartment (see above), i.e. in the model the SON-T lamps were switched on when the reading of a virtual sensor array on top of the roof of the greenhouse fell below a threshold value of 200 W m^{-2}.



Figuur 1. Principal elements of the cut-rose FSPM, with an emphasis on light climate.



Figuur 2. Close-up of a simulated stump after flush harvest: Buds below a cut surface are marked and the first three of them visualized as coloured spheres. Buds are assigned a proximity number (pn) and their probability for breaking determined as a function of that number. A bud with pn = 1 has been selected.



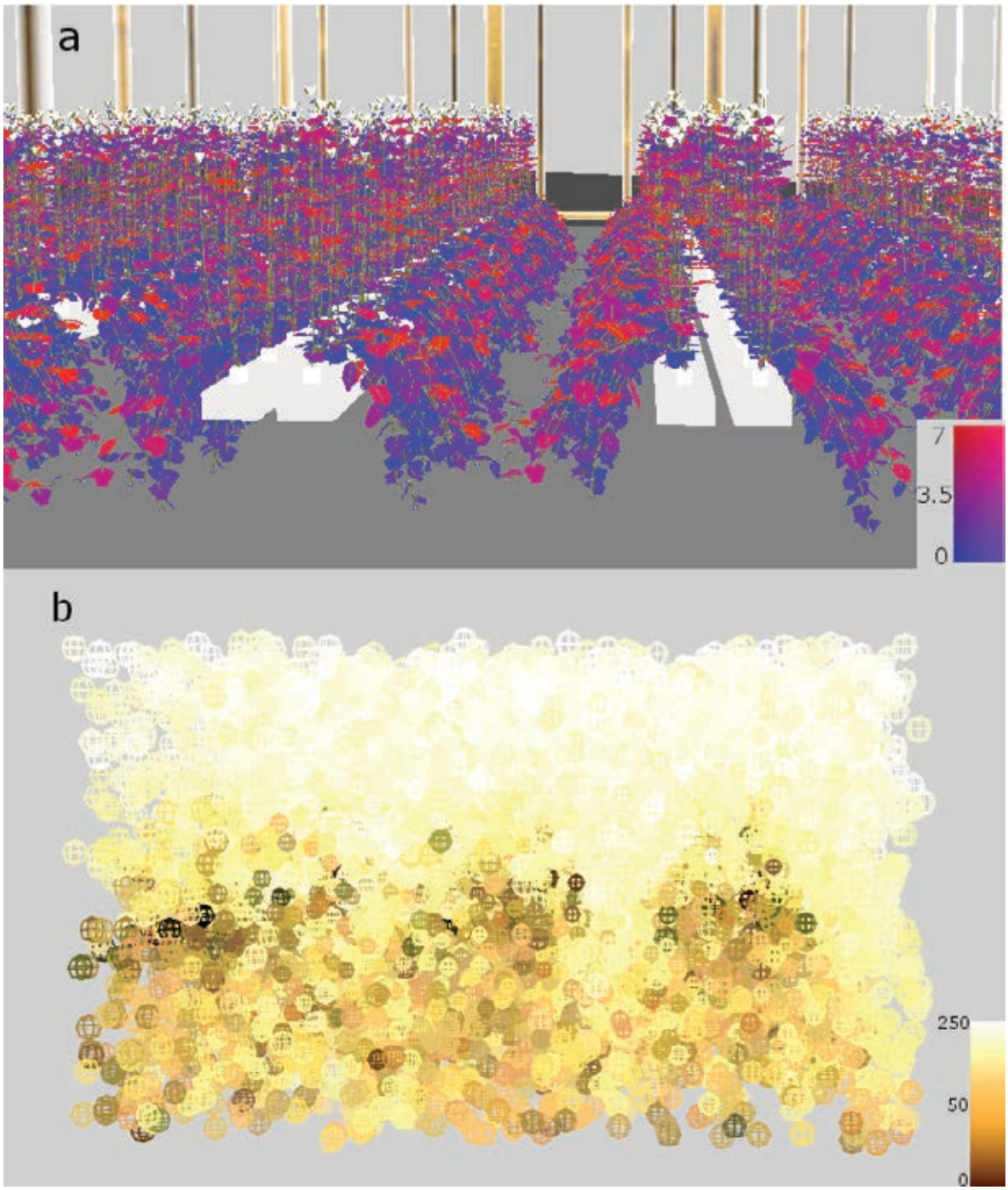
Figuur 3. Illustration of the rapid mockup technique, showing different states of the canopy: a) after bending of primary shoot, b) after creation of instant first flush, plus growth of bent canopy (branching of bent shoot, plus rebending of upright shoots), c) after harvest of first flush, d) second flush (note senescence of stump leaves).

4.4 Results

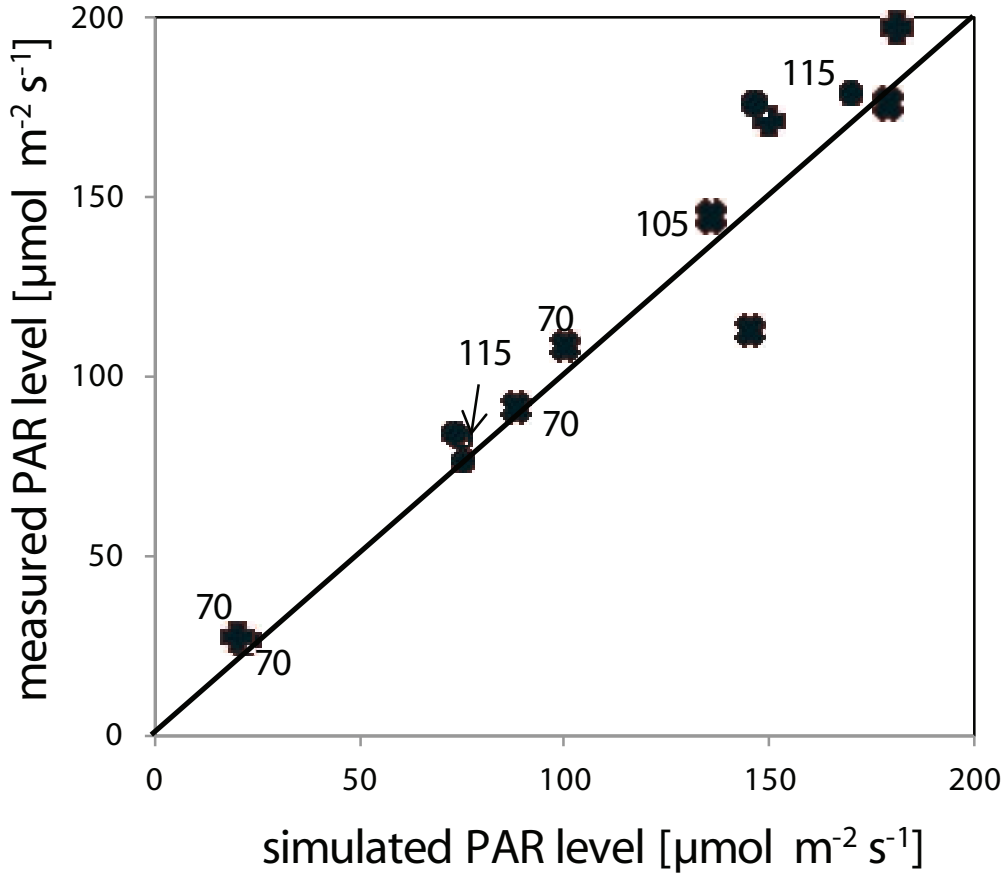
Spatial distribution of PAR in the canopy

A simulated rose canopy with false colours indicating the amount of PAR absorbed per m^2 leaf area is shown in Figuur 4a. The amount and distribution of PAR transmitted and incident in the same scene is represented with 10,000 randomly distributed virtual sensors (Figuur 4b). The simulated PAR levels at different heights above the ground exhibited some variation, which was due both to the stochasticity of the radiation model and the heterogeneity of the simulated canopy. Figuur 5. shows a comparison of measured and simulated incident PAR values, for different heights and light regimes:

The simulated canopy (age: 70 days) represented the mature (preharvest) first flush, produced using the instantCanopy method (see Material and Methods), the latter using stochastic parameters based on the measured architecture. Simulated incident downward PAR values were sampled as an average of about 50 sensor readings (as described before) from a horizontal layer of the measured height. Generally, the simulated incident PAR values within the upright canopy matched the measurements rather well (Figuur 5.).



Figuur 4. Simulated rose canopy, with (a) colour gradient (blue to red) indicating increasing amount of PAR [µmol photons m^{-2} leaf area s^{-1}] absorbed per leaf, and (b) randomly distributed virtual sensors indicating the amount of incident PAR sensed within the sensor radius (5 cm), as well as colour of incident PAR [µmol photons m^{-2} sensor area]. The snapshot shows a canopy at 1200 h, with a mixture of ambient daylight and lamp light from SON-T lamps emitting predominantly orange light.



Figuur 5. Measured versus simulated incident PAR in a rose canopy. Measurements were carried out using a LI-190 quantum sensor, on 26 Sept (direct light) and 2 Oct 2008 (diffuse light) at different heights in the canopy, and at various times between 1200 h and 1630 h, with varying light regimes: P direct light; **+** *diffuse light;* **x** *diffuse light plus SON-T light. Numbers near the data points indicate height above the greenhouse floor at which the measurement was taken. Unlabelled data points indicate a measuring height of 130 cm (just above the canopy). Simulations are mean values from 100 randomly distributed virtual sensors, s.d. of the simulations varied between 28.7 (70 cm) and 33.04 (115 cm) µmol m^{-2} s^{-1}.*

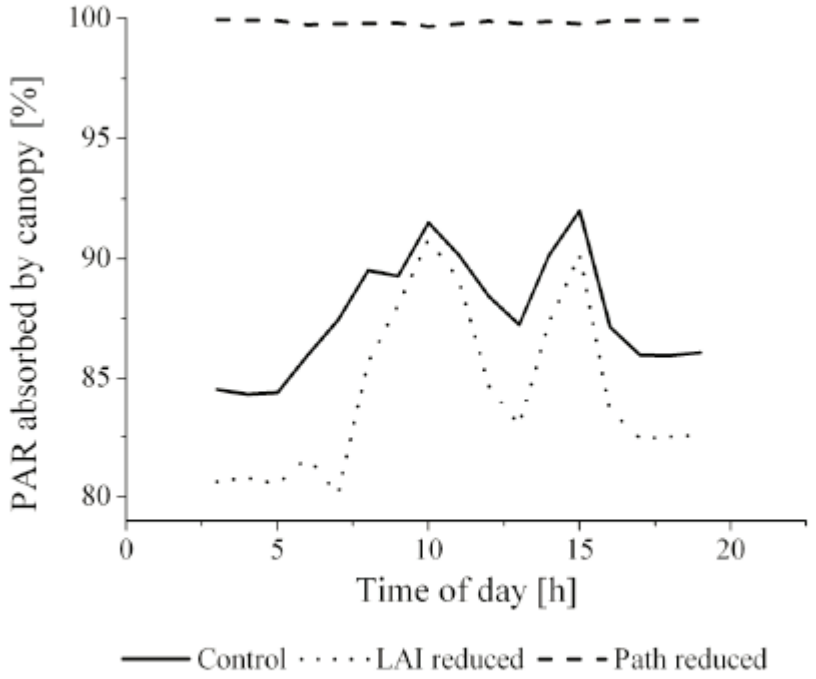
Note that in the remaining results we are reporting the outcome of the simulation scenarios, thus all parameters referred to are model output:

The simulated percentage absorbed PAR exhibited some dynamics during the day in the three scenarios (Figuur 6.): In the control, PAR absorption ranged between 84% and 92%, with two clear peaks at 1000 h and 1500 h, and lowest absorption was observed in the early morning and evening when light was provided by SON-T lamps only. A similar dynamics can be seen in scenario 3 where the bent canopy was reduced by 50%, only that the percentage range was wider (80 - 91%) and that there was a clear difference in absorption between the control and scenario 3 during times when the SON-T lamps were the only light source. In contrast to this, in scenario 2 in which the bent canopy was completely closed due to the narrow paths, almost no PAR was reaching the ground, and there was no diurnal dynamics, i.e. more than 99% of PAR was intercepted by the canopy at all times.

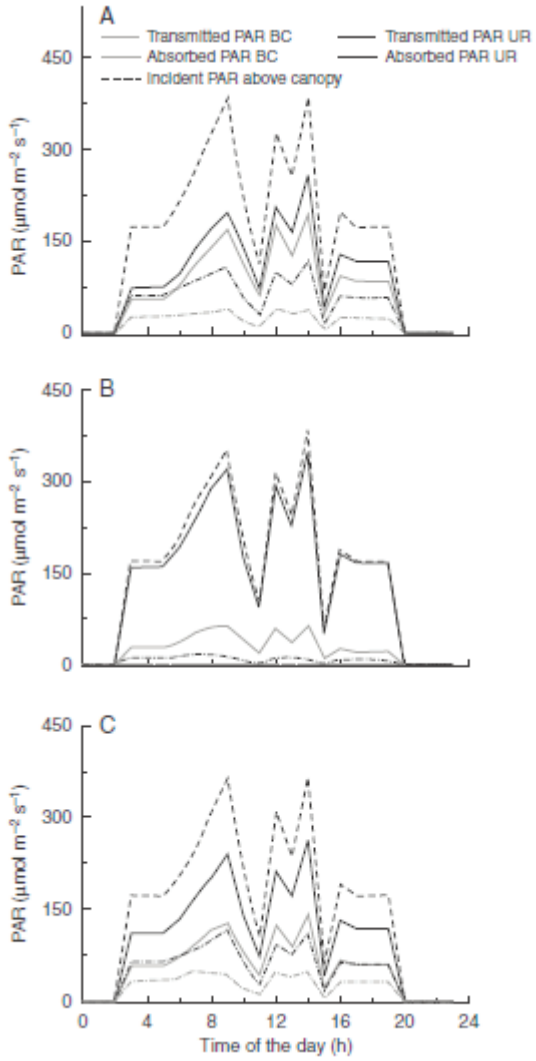
According to the model, PAR levels (absorbed and transmitted) were higher in the upright than in the bent canopy (Figuur 7.). In all scenarios, incident PAR above the canopy was computed to be around 170 µmol m^{-2} s^{-1} in the morning and evening when the only light sources were the assimilation lamps, fluctuating strongly during the day, with two peaks at 0900 h and 1400 h, and two dips at 1100 h and 1500 h. In all simulation scenarios the levels increased in the following order: transmitted PAR below the bent canopy < PAR absorbed by bent canopy < transmitted PAR at the bottom of the

upright canopy < PAR absorbed by upright canopy < incident PAR above upright canopy. Also, the sum of absorbed PAR (BC and UR leaves) matched the difference between incident PAR level above the canopy and transmitted PAR level below the bent canopy, showing that there was no significant loss of PAR to other structures (*e.g.* benches and irrigation pipes, which have black surfaces). In scenario 2 the upright canopy was absorbing nearly the entire PAR available leaving almost nothing for the bent canopy (Figuur 7b). In the control scenario the levels of PAR absorbed in the bent canopy were at all times about 20 µmol m^{-2} s^{-1} below that of the upright canopy, whereas this difference was bigger (50 - 100 µmol m^{-2} s^{-1}) and more variable in scenario 3 (Figuur 7c), obviously due to the reduced leaf area in this scenario. In scenario 3 the amount of PAR absorbed by the bent canopy quite closely followed the level of transmitted PAR in the upright canopy. Contrary to this in the control the level of transmitted PAR in the upright canopy deviated strongly (by up to 50 µmol m^{-2} s^{-1}) from the amount of PAR absorbed by the bent canopy (Figuur 7a).

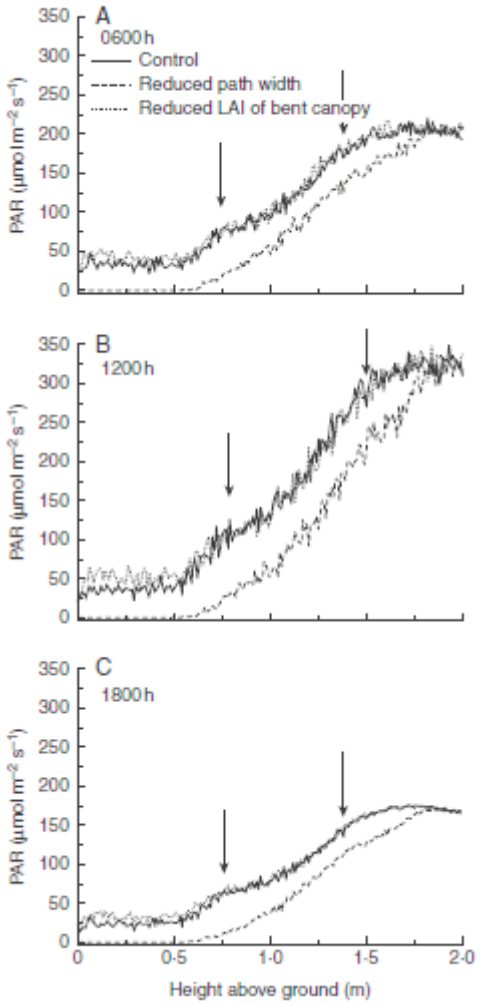
Profiles of transmitted and incident sensed PAR at three different times of the day (0600 h, 1200 h, and 1800 h) are shown for the three simulation scenarios in Figuur 8: PAR levels were highest and the gradient steepest at noon, peaking at about 320 µmol m^{-2} s^{-1}, with diffuse day light prevalent, whereas it was highest and shallowest at 1800 h when the lamps were the only light sources. As expected, the incident PAR levels below the bent canopy were lowest in simulation scenario 2, in which the path was completely covered with leaves of the bent canopy. Also, along the entire profile (0 to 2 m above the ground) the level of transmitted PAR was about 50 µmol m^{-2} s^{-1} lower than in the other two simulation scenarios which was clearly due to the higher density of plants and, therefore, of flower canes, whereas the slopes of all gradients were equal for a given time of the day. The simulated differences between the levels of transmitted PAR in the bent canopy were rather small in the control and scenario 3 with reduced bent canopy (only about 20 µmol m^{-2} s^{-1} at noon and 10 µmol m^{-2} s^{-1} at the other times), with differences tending to decrease in the upper bent canopy (0.5 - 0.7 m above the floor).



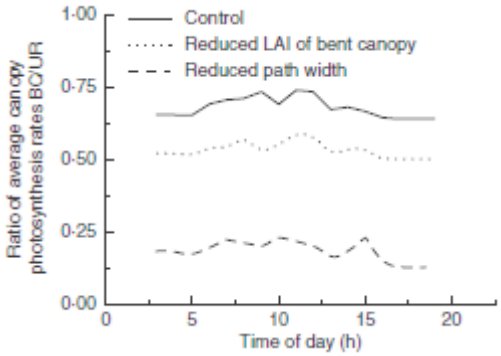
Figuur 6. Diurnal course of simulated PAR [%] absorbed by the entire canopy, computed as (PAR $_{above\ canopy}$ - PAR $_{transmitted\ below\ BC}$)/ PAR $_{above\ canopy}$.
Management scenarios were: control; reduced path width; reduced LAI of bent canopy (for more details see text).



Figuur 7. Simulation of incident, absorbed and transmitted PAR levels at different heights above, inside and below a bent and upright rose canopy during the course of the day (2 Oct. 2008). The PAR levels incident were sampled from readings of randomly distributed virtual sensors at specified heights above the floor (level incident above canopy: 1.8 - 1.82 m; bent canopy: 0.38 - 0.4 m; upright shoots: 0.7 - 0.72 m). . Management scenarios were: a) control; b) reduced path width; c) reduced LAI of bent canopy (for more details see text). BC = Bent Canopy; UR = Upright canopy.



Figuur 8. Simulated PAR level at different heights and three different times of the day (0600 h, 1200 h, and 1800 h) perceived by randomly distributed virtual sensors in a simulated rose canopy, as a function of different management scenarios: a) control; b) reduced path width; c) reduced LAI of bent canopy. The arrows indicate the height of the slab and the tip of the upright canopy.



Figuur 9. Ratio between simulated net assimilation rate of leaves from upright and bent shoots during the course of one day (2 Oct 2008). Scenarios as in Figs. 7 and 8.

Photosynthesis

Overall, simulated net assimilation rates of leaves of the bent and upright canopies were rather low. For leaves of upright shoots they were ranging between 6 and 14 µmol CO_2 m^{-2} s^{-1} in the control, 10 - 16 µmol CO_2 m^{-2} s^{-1} in scenario 2, and 8 - 14 µmol CO_2 m^{-2} s^{-1} in scenario 3, whereas in the bent canopy rates were 4 - 10, 2 - 4, and 4 - 8 µmol CO_2 m^{-2} s^{-1}, respectively. This was due to the relatively low levels of absorbed radiation. The simulated diurnal dynamics of

photosynthesis closely followed that of simulated absorbed PAR, because of the linearity of the light response at these PAR levels (results not shown). In all simulation scenarios photosynthesis rates of bent shoot leaves were lower than those of leaves from upright shoots, reaching about 70% in the control, 50% - 60% in scenario 3, and only about 20% in scenario 2, with ratios tending to increase and decrease again during the day (Figuur 9.), thereby mirroring the dynamics of the PAR transmission rate in the upright canopy (Figuur 7.). The very low ratio observed in scenario 2 was clearly due to the low PAR level transmitted to the bent canopy from above through a much denser upright canopy, whereas the low ratio in scenario 3 can be explained with the reduced LAI of the bent canopy. Also, leaves in the bent canopy and the lower upright canopy exhibited a lower light response of photosynthetic rate (Table 2.) than leaves from the higher upright canopy, which is another contributing factor to be taken into account when comparing photosynthetic rates of the bent and upright canopy.

4.5 Discussion

Light interception and photosynthesis

We chose to verify and test the plausibility of the present FSPM at the example of the local light climate, interception of PAR by leaves and local photosynthesis. Simulated local light climate, apart from being determined by greenhouse construction (*e.g.* transmissivity of glass) was influenced by diffuse and direct day light as well as light coming from assimilation lamps, all of which were more or less strongly fluctuating during the course of a day. Our model was able to reproduce these dynamics in a virtual greenhouse setup. The model was equally suitable to visualize parameters such as local PAR interception per leaf on a square-metre basis (Figuur 4a) or to generate a 3D view of downward PAR distribution in and around a simulated canopy with the help of randomly distributed virtual sensors (Figuur 4b). Local and global light interception (per individual plant or per square metre) and, subsequently, local assimilate production are important drivers for shoot production (apart from the availability of buds ready to break and thus to act as sinks), and there is always potential for an increase of source strength by optimizing management. We showed here that the initial setup of the producing canopy (plant density as well as extent of the bent canopy) has an effect on local light interception (with potential repercussions on photosynthesis and shoot production): The strongest effect was observed in scenario 2, in which plant density was increased from 7.14 plants m^{-2} to 12.5 plants m^{-2}. Light absorption of the canopy was nearly 100% which might mean that the density chosen for this scenario was too high. At such a rather high density one could expect feedback by the plant in the form of an altered bud break and shoot development pattern in both the bent and upright canopy. However, such a feedback is currently not implemented in our model.

A normal path width in combination with a reduced bent canopy (scenario 3) only slightly increased the level of transmitted PAR by the bent canopy (Figuur 8.). This means that the amount of PAR lost to the floor (transmitted PAR below the bent canopy) was not much bigger when the bent canopy was reduced, probably because the structure of the upright canopy was the same as in the control and thus the levels of transmitted PAR in the upright canopies of both control and scenario 3 were very similar. This was the impression that could be gained by analysing the sensor output only (Figuur 8.). However, when comparing this with the amount of simulated PAR absorbed by the leaves of the different canopy types (bent and upright), it appears that there was a bigger difference between the control and scenario 3 with the reduced bent canopy (Figuur 7.). In scenario 3 less PAR was absorbed in total, as expected, also having repercussions on the average net assimilation rate of the bent leaves (results not shown) and the ratio of assimilation rates between upright and bent shoots (Figuur 9.).

The simulated diurnal curves of the incident and absorbed PAR (Figuur 7.) reflected the dynamics of the light environment in the greenhouse quite accurately: during the early morning hours light came exclusively from assimilation lamps. From about 0700 h onwards the amount of (diffuse and direct) daylight was increasing, and lamps were eventually switched off once a specified threshold of external daylight was reached (as measured by virtual sensors on the roof of the greenhouse). The rather drastic dips in simulated light levels at 1100 h and 1500 h were probably due to the hourly time resolution, which was not taking into account fast changes in PPFD around the threshold level to which the lamps would react within minutes by switching on or off. The day chosen for the simulations was very overcast and, therefore, SON-T assimilation lamps were switched on during about 6 hours, resulting in a rather typical PAR curve with a slight dip whenever lamps were switched off. Typical base values (measured and simulated in our study) were around 140 to 170 $\mu mol\ m^{-1}\ s^{-1}$.

Our measurements (Table 2.) clearly showed that the maximum photosynthesis rate increased with increasing rank in upright shoots and from bent to upright shoots, with a clear difference between lit and shaded bent shoot leaves. Such differences in light response curves for different canopy positions were also found by Gonzales-Real and Baille (2000) and were ascribed by these authors to the decrease in leaf nitrogen content, i.e. the bottom leaves of the plant had 35% less nitrogen than the top leaves. The simulated canopy photosynthesis rates obtained with our model (about 10 - 12 µmol CO_2 m^{-2} s^{-1} for leaves of upright shoots, and 3 - 5 µmol CO_2 m^{-2} s^{-1} for leaves of bent shoots) appeared to be within the range observed by other authors (Kim and Lieth, 2001; 2002). Kim and Lieth (2001) found maximum rates of 13 µmol CO_2 m^{-1} s^{-1} for whole plant net photosynthesis in the cut-rose cultivar 'Kardinal', whereas Kim and Lieth (2002) who determined the diurnal response of canopy gross photosynthesis in the same cultivar found maximum rates of 29 µmol CO_2 m^{-2} s^{-1} for leaves of upright shoots, and 24 µmol CO_2 m^{-2} s^{-1} for leaves of bent shoots on a spring day in California, with an LAI of the entire canopy of 7.6 m^2 m^{-2}. However, a direct comparison is also difficult, given the differences in climate, cultivar, and management. Incidentally, the model by Kim and Lieth (2002) is to our knowledge also the only one which considered - in a simplified way and still in the frame of a process-based model - the structure of the upright and bent canopy and their influence on the distribution of incident PAR, thereby also distinguishing between north-south and east-west row directions. Sarlikioti *et al.* (2011) modelled plant architecture traits and row structure, light interception and canopy photosynthesis in an FSPM of tomato and found that especially the explicit description of leaf divergence angles significantly improved the prediction of canopy photosynthesis compared to an unstructured process-based model considering the Beer-Lambert law of light absorption as a function of cumulative LAI alone.

Computational cost of running the light model

To compute the local light environment around the rose canopy in the virtual greenhouse we used the built-in radiation model of GroIMP. This model is based on a unidirectional Monte-Carlo raytracer, i.e rays are traced from a light source in one direction to an object in a scene. Such models are stochastic and computationally expensive, because a large number of rays (several millions) and their fate (paths of reflection and transmission until final absorption by a medium) have to be traced in order to gain a reliable estimate of locally absorbed and available light. As an example, let us consider the computational cost to run the scenarios presented in this paper: For most of the computations, we used a Dell Precision WorkStation T7500, with an Ubuntu Linux operating system running on it, which supported symmetric multi-processing (SMP). This workstation had a total of 8 CPUs (4 cores per CPU, 2 threads per core), a clocking rate of 2.4 GHz, and 12 GB RAM. The GroIMP platform was running on a 64-bit server Java Virtual Machine. In all three scenarios the scene comprised about 145000 plant objects (of which 48900 leaves and internodes each and 45600 buds) and 26 light sources (24 lamps, one diffuse, and one direct). One model step involved the built-up of the entire canopy consisting of 400 plants and one run of the light model. Per scenario 18 hourly steps were computed. All scenarios were computed using the so-called headless mode of GroIMP, which allowed the platform to be run without a graphical user interface from a batch file. Computation times varied among the scenarios, being one hour for the control and scenario 2, and 50 minutes for scenario 3 (reduced bent canopy), or on average 2.8, respectively, 3.5 minutes per step.

Further applications of the model

We also tested the influence of cutting height in another set of simulation runs (results not shown), taking the control setup (scenario 1) as a starting point and varying the height at which the first flush of upright shoots was cut. This cutting height was defined as the distance of a point on the stump to the base of the stump (see Materials and Methods). Four cutting heights were tested, 0.15, 0.2, 0.25, and 0.3 m, and an entire canopy simulated with a mature second flush of upright shoots present (simulated plant age about 110 days). Both PAR absorbed and net assimilation rates tended to be highest at a cutting height of 0.2 m (results not shown), but the effect was not significant. It remains to be further investigated by modelling and experimentation if canopy management in terms of varying the cutting height of harvested shoots does noticeably influence light interception.

Bud break

Bud break in cut-rose is due to a complex variety of external and internal factors such as: apical dominance and modifying factors, *e.g.* light (directly or through its effect on source strength: Marcelis-van Acker (1994a)); the position of the buds along the shoot (Marcelis-van Acker 1994b) in one of the three zones, basal, median, or subapical (Khayat and Zieslin, 1982; Zamski *et al.* 1985); manipulation (pruning, 'pinching') and initial crop management (planting density: Kool and

Lenssen, 1997; Burema *et al.* 2010). The two basic mechanisms underlying bud break are essentially developmental "readiness" of the bud and lack of correlative inhibition (Zieslin and Halevy, 1978; Khayat and Zieslin, 1982; Marcelis-van Acker, 1994b). In our model we used the general observation (Pien, 2007) that 1) bud break of axillary buds on stumps of harvested upright shoots was induced by cutting (harvest or pruning) of (flowering) upright shoots, probably by lifting the correlative inhibition exerted by the terminal bud or growing shoot that was removed and 2) that after lifting of correlative inhibition the first bud below the cut will break with a high probability, the following with much lower probability, suggesting that the shoot emerging from the uppermost bud rapidly inhibits its subjacent neighbours.
Cultivars clearly differ in frequency of bud break, i.e. the probabilities of the top, second or third bud below a cut surface to break. One of the future promises of this model is to explore consequences of bud break behaviour in terms of numbers of flower shoots produced and their quality (length, diameter of stem and flower bud).
Once a bud is broken and a shoot beginning to develop, this will not only change the local light climate but also shift the source/sink balance, by increasing the demand. Results from our own experiments (not shown) showed that the local level of incident PAR perceived by an unbroken bud, in combination with the number of competing sinks, influences the total number of buds breaking per shoot stump, per plant, or per square metre. As the present model is completely object-oriented and modular it will be very straightforward to incorporate new processes reflecting mechanisms of bud break.

4.6 Conclusions And Outlook

The model of cut-rose presented here allowed the creation of a wide range of initial structures thanks to simple interactive rules for pruning, cutting and bending. We used the generated structures to show we can model the distribution in the canopy of light from a complex radiation environment, consisting of the solar track and the virtual glasshouse with a particular configuration of lamps. Local carbon assimilation followed from local light distribution using the Farquhar model I. In order to predict shoot production dynamically concepts are being implemented on the source- sink relations and bud break. Detailed information on phytomer growth has been collected and is partly used in the current model but will be reported in future papers.

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5 Fenotypering van rozenrassen

Phenotyping cut rose cultivars at different locations, written by B.A. Eveleens, G.W.A.M. van der Heijden,J.T.N.M. Thissen, E. Meinen, G. Buck-Sorlin, J. Vos, & L.F.M. Marcelis

In cooperation with the breeding companies De Ruiter (DR), Moerheim Roses (MR), Terra Nigra (TN), Van Kleef Roses (VK) and Olij Roses (OL) and the advisory company Phytocare a number of cut rose genotypes were grown at 10 locations spread over The Netherlands (NL), Kenya (KE), Ethiopia (ET), Ecuador (EC) and India (IN). Each of the five breeding companies provided plant material from 30 cultivars for the cuttings. In Kenya, Ethiopia, Ecuador and India 150 cultivars (genotypes) were grown, while on four Dutch locations 20 cultivars were grown. The locations in The Netherlands were in the region of Aalsmeer in the greenhouses of De Ruiter, Moerheim Roses & Trading, Terra Nigra and Van Kleef. In Keny there were three locations around Navaisha (1900 m above sea level): De Ruiter East Africa Ltd., Color Vision Roses Ltda (Terra Nigra), and Bilashaka Flowers Ltd. (Van Kleef Kenya Ltd.). There was one location in Ethiopia (Olij Ethiopia) at Debre Zeyit (1900m). The location in Ecuador (Olij Ecuador Cia. Ltd.) was at Cayambe (2800m) and the location in India (Essar Agrotech Ltd. (Moerheim) was at Bangalore (800 m).
The aim of the experiment was to phenotype cut roses under a large range of conditions and to study the interaction between genotype and environment.
The cuttings in the Netherlands (NL) were planted at the four locations in May 2009;. The cuttings at the six overseas locations were planted in June 2009. This report is on the analysis of the data collected from 1st January 2010 until 31st December 2010. Two types of measurement were done, weekly production data (weight, length and number of stems). Other plant characteristics such as stem dimensions, leaf number, leaf width and number of leaflets and flower characteristics were measured during a limited period of the year. From the leaf dimensions width and number of leaflets, leaf area of a stem was calculated.

Cultivars differed in yield determining properties. Whereas one cultivar had a large total harvested weight per plant due to the large dimensions of the stem another cultivar had a large total harvested weight due to the large number of stems per plant (Figuur 5.1. and 5.2).

The cultivars with the highest number of stems per plant also showed the lowest weight per stem.
The total weight per plant was more dependent on number of stems than on individual stem weight.

Nearly all the plant characteristics seemed to be larger in NL than in KE except number of stems and flower width. There seemed to be large differences in averages of the plant characteristics between these two countries, but most of these differences were not statistically significant because of the enormous variance (spread) between the nurseries within a country. A test on the effect of cultivars was very significant and a test on the interaction between cultivar and country was also very significant. This trend was observed for all characteristics, except that leaf area and average stem length were just about significantly larger in NL whereas flower width was smaller. In general the interaction of genotype (G) x environment (E) on total stem weight is due to G x E interaction on number of stems and to a lesser extent on weight per stem. The stem weight and stem length (significantly correlated at all locations) are fairly conservative plant characteristics and although the actual levels can vary the ranking between the locations is similar. This was also the case for leaf area of the harvested stems. The number of stems had a stronger determining effect on the total weight per plant than stem weight. The number of stems could not be correlated to the number of bottom breaks.

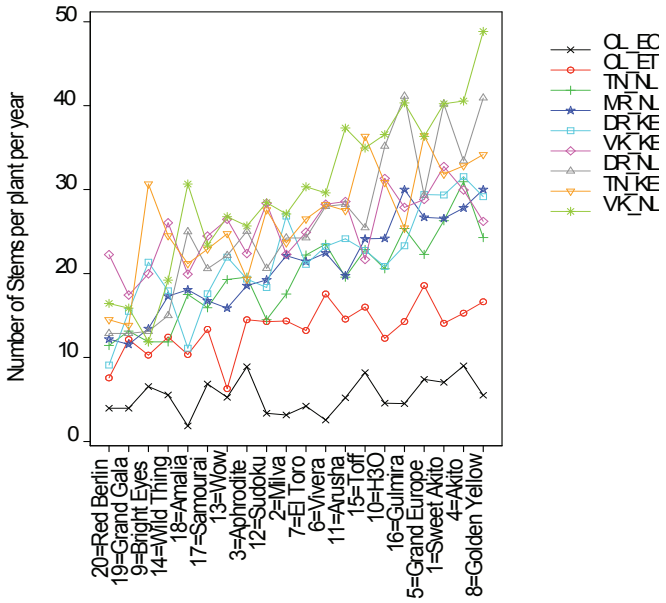
There was a large difference in the average total harvested weight per plant between the locations. Cultivars can react differently at the various locations. This is known as genotype x environment interaction (G x E). When there is no or little interaction it means that the highest producing cultivars are the same at all locations and that the lowest producing cultivars are also the same. In general the interaction in NL was smaller than in KE. At all Dutch locations there was a clear negative relationship (no interaction between cultivar and location) for most cultivars between number of harvested stems and average weight of a stem. There was little effect of the Dutch location on the stem length between cultivars, therefore the specific stem weight (g/cm) decreased per cultivar as more stems were produced. In Kenya there was an

interaction of the effect of the cultivar and location on specific stem weight. At one location (TN_KE) there was a clear negative relationship for most cultivars between number of harvested stems and average weight of a stem. The specific stem weight decreased as more stems were produced. The plants at this location showed a similar reaction to the Dutch locations. These relationships were less visible for the other two locations in Kenya where all stems were harvested much shorter.

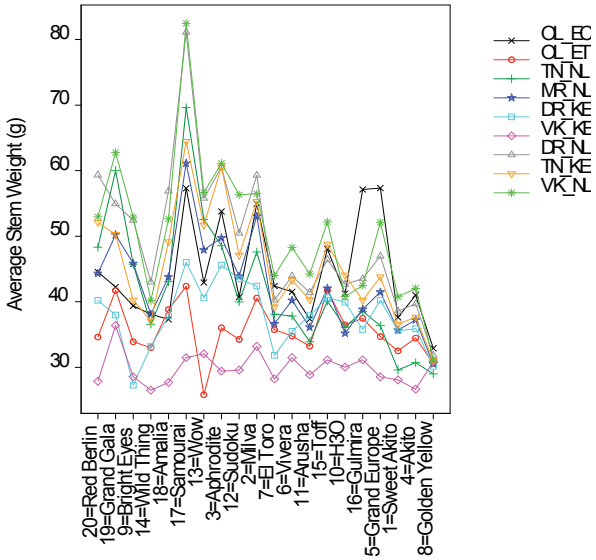
In NL the seasonal variation was visible for some cultivars whereas others showed little variation. Large seasonal variation can be due to large seasonal variations in number of stems or large seasonal variation in stem weight. The seasonal variation in weight was greater for the cultivars with heavier stems. The total weight in NL was the largest is in the second quarter but the number of stems per plant was highest is the third quarter.
The seasonal variation in Kenya for total weight per plant was difficult to summarise because the variation within and between the Kenyan locations were very large.
When cultivars were compared there was a significant negative correlation between the stem weight (g) and number of stems per plant. In contrast when the locations were compared there was a positive correlation between stem weight and number of stems. Within a location there was a trade-off between stem weight and stem number. However, among different locations the growth conditions may have varied leading to variations in total growth of the plant. This variation in total growth obviously resulted in similar variation in stem weight and number.
All locations/countries showed a significantly positive correlation between specific stem weight (g/cm stem) and stem weight.

To analyse the influence of the climate on crop production, a crop simulation model can be used. In this model analysis all other factors that may influence crop production were assumed to be equal at the different locations. The aim was to investigate if climate can explain the differences in production at the 9 nurseries using the Intkam crop model. For the Dutch growers, scenario calculations were carried out to determine the contribution of the different climate factors to the total production. For the Dutch nurseries, the scenario analyses by the crop model showed that the measured production differences could be explained mainly by differences in climate. These scenario studies showed that the production of Location B can be increased by screening less, using more assimilation lighting and use of CO_2. The lower production for Location C can be totally explained by less light in the greenhouse compared to Location A. Production of Location 4 can be increased by allowing more light in the greenhouse and by using more CO_2.

For the foreign countries, the simulated production was much higher than measured. Other factors than climate play a role and are limiting for production. Simulated production in the foreign countries was higher compared to NL. Based on the radiation, more production was simulated in quarters 1 and 4 compared to the Dutch nurseries. The simulation results suggest that at the overseas locations there is ample room for production improvement.



Figuur 5.1: Number of stems per plant per year for 20 cultivars at 9 locations. The x-axis indicates the 20 genotpes, which are ordered according to an increasing average number of stems over all of the locations.



Figuur 5.2: Average stem weight per cultivar (average over whole year) for 20 cultivars at nine locations. The x-axis indicates the 20 genotpes, which are ordered according to an increasing average number of stems over all of the locations.

Table 5.1. measured and simulated production of the four Dutch locations, expressed as percentage of the production of location A. Seven model scenarios were analysed where some of the climatic conditions of Location A were used for the the other locations: the effects are expressed as percentage production increase.

row				**Location A**	**Location B**		**Location C**		**Location D**	
1	Experiment	Measured		100	60		54		84	
2		Simulated		**100**	**61**		**61**		**76**	
3	Scenario 1	Total light VK			**+27**		**+39**		**+17**	
4	Scenario 2		Transmissivity			-1		+10		+3
5	Scenario 3		Ass. Light			+11		+19		+6
6	Scenario 4		Screen			+17		+2		+1
7	Scenario 5		Whitewash					+7		+8
8	Scenario 6	CO_2 VK			**+8**		**+1**		**+4**	
9	Scenario 7	Temp VK			**-1**		**-1**		**+1**	

Productschap Tuinbouw

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