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Irradiance during Vegetative Growth Phase Affects Production Time and Reproductive Development of *Phalaenopsis*

A. B. Hückstädt and S. Torre
(Norwegian University of Life Sciences, Department of Plant and Environmental Sciences, Ås, Norway)

Summary

In this study, *Phalaenopsis* 'Mukalla' and 'Malaga' were grown in growth chambers at low irradiance (LI): 50 μmol m^{-2} s^{-1}, moderate irradiance (MI): 125 μmol m^{-2} s^{-1} and high irradiance (HI): 200 μmol m^{-2} s^{-1} for 16 h daily (8 h darkness) at constant high temperatures (28 °C) during the vegetative growth phase. The aim was to study growth rate and carbohydrate levels of vegetative plants and verify if the irradiance during vegetative growth phase can cause after-effects on the reproductive growth of *Phalaenopsis*. Here we show that the growth rate was significantly increased and the 6th leaf appeared 6–7 weeks earlier under MI and HI compared to LI in 'Malaga' and 'Mukalla'. Due to the faster leaf appearance rate the total production time was reduced by 50 days. An increased content of soluble carbohydrates (sucrose, glucose and fructose) in vegetative source leaves was measured in 'Malaga' with increased irradiance but 'Mukalla' contained similar levels of carbohydrates regardless of the irradiance. At the 6th leaf stage the plants were transferred to cooling for flower initiation (18 °C) and finishing phase (21 °C) until flowering to study the plants ability to produce multiple inflorescences, differences in inflorescence development and morphology. 'Mukalla', which often produces multiple inflorescences, developed similar number of flower stems irrespective of the irradiance during vegetative growth and negligible after-effects were found on time from cooling to visible inflorescence. 'Malaga', which has difficulty producing multiple inflorescences, initiated fewer flower stems when the vegetative growth phase was performed under HI compared to LI or MI. 'Malaga' also developed shorter and thinner inflorescence with fewer branches and flowers when grown under LI during vegetative growth phase compared to MI and HI. A positive relationship was found between the carbohydrate content in source leaves at the start of cooling and time to visible inflorescence (VI) in 'Malaga' but no such effects were detected in 'Mukalla'. In conclusion, by increasing the irradiance from 50 μmol m^{-2} s^{-1} to 125 μmol m^{-2} s^{-1} during the vegetative growth phase the total production time was reduced extensively mainly by reducing the vegetative growth phase. The irradiance during vegetative growth phase can induce after-effects on the number of inflorescences per plant, time from cooling to VI, and on inflorescence morphology but the effect was hybrid dependent.

Key words. flowering – fructose – glucose – light – morphology – orchids – sucrose

Introduction

Phalaenopsis, or moth orchids, are slow growing crassulacean acid metabolism (CAM) plants with a monopodial habit of growth. The high popularity of orchids as ornamentals has led to large-scale production in climate-controlled greenhouses. Production time is approximately 50–70 weeks from small *in vitro* cultivated plantlets until flowering and the cultivation is divided into three phases, consisting of (1) a period of vegetative growth, (2) flower bud initiation, and (3) inflorescence development (finishing phase). In the vegetative growth phase, the leaves are differentiated alternately on opposite sides of the stem while bud primordia are formed in the axilla of each leaf. The bud primordium develops only to a certain stage, after which it becomes dormant (Rotor 1952). High day temperature (≥ 26 °C) is required to maintain the vegetative phase and develop new leaves (Sakanishi et al. 1980; Yoneda et al. 1992; Chen et al. 1994). A period of "cooling" (17 to 20 °C) is necessary to stimulate *Phalaenopsis* flower bud initiation. During this phase, the meristem activity increases and rapid elongation of the inflorescence occurs (Rotor 1952). Cooling is a key factor in scheduling the flowering date and uniform inflorescence emergence can be achieved when plants are grown at 17–20 °C for 4–5 weeks (Sakanishi et al. 1980).

Once the plants have initiated inflorescences, it normally takes 10–15 weeks at 20 to 23 °C to develop it until flowering (Sakanishi et al. 1980; Blanchard and Runkle 2006).

All upper bud primordia along either side of the main axis can develop into inflorescence, but only one spray of flowers can grow from each leaf axilla (Rotor 1952). The inflorescence usually emerges from the third and often the fourth node below the apical leaf (Sakanishi et al. 1980). An increased price is paid for a multi-stem over a single-stem plant, but it is not well understood how the inflorescence number is environmentally regulated in *Phalaenopsis*. It is well known, however, that the ability to generate multiple inflorescences is, to some degree, controlled genetically as different hybrids have tendencies to generate single or multiple inflorescences plants. On the other hand, environmental factors during the cooling phase have been found to influence the number of flower stems. Too high temperatures or fluctuations in temperatures during cooling lead to fewer flower stems (Newton and Runkle 2009). Also, high irradiance during cooling is necessary to induce an inflorescence (Kataoka et al. 2004) and plants do not develop inflorescences at all when exposed to low light or in complete darkness (Wang 1995). Several studies also indicate that the assimilation or mobilization of photosynthates plays an important role in the reproductive growth of *Phalaenopsis*. However, these studies have primarily focused on the cooling or the finishing phase (Kubota and Yoneda 1993; Chen et al. 1994; Kataoka et al. 2004; Chen et al. 2008), not the vegetative phase.

The recommended light level for *Phalaenopsis* during vegetative growth varies from 60 to 400 μmol m^{-2} s^{-1} and the wide interval is probably also a result of hybrid differences (Konow and Wang 2001; Lin and Hsu 2004; Lopez and Runkle 2005; Van Der Knaap 2005). Several studies have found that the photosynthesis of *Phalaenopsis* saturates at about 130–180 μmol m^{-2} s^{-1} (Ota et al. 1991; Lootens and Heursel 1998), and exposure to irradiance higher than 200 μmol m^{-2} s^{-1} results in a significant photoinhibition (Lin and Hsu 2004). However, information on how the irradiance during vegetative growth phase influences on production time and reproductive growth is scarce. Thus, the aim of this study was to investigate how the irradiance influences growth rate and leaf carbohydrate content of vegetative plants. Further, after-effects of the irradiance during vegetative growth phase on the plants' ability to produce multiple inflorescences, inflorescence morphology and flowering time were investigated.

Plants were grown at different light levels (50, 125 and 200 μmol m^{-2} s^{-1}) during the vegetative phase and moved to common environment for floral induction and development. The experiments were performed with two hybrids of *Phalaenopsis*, one with a tendency to make single inflorescence ('Malaga') and one which more often makes multiple inflorescences ('Mukalla').

Materials and Methods

Abbreviations

DLI = daily light integral; HI = high irradiance; LI = low irradiance; MI = medium irradiance; PAR = photosynthetic active radiation; PPF = photosynthetic photon flux; VI = visible inflorescence.

Plant material and acclimatization conditions

Young plants (10–14 cm leaf span, 3 fully developed leaves) of *Phalaenopsis* hybrids 'Mukalla' and 'Malaga' from Anthura® (The Netherlands) were transplanted into 12 cm, transparent pots in a mixture of bark pieces and sphagnum. The plants were acclimatized in growth chambers under 50 μmol m^{-2} s^{-1} for three weeks. The plants were watered daily for sufficient soaking of the growing media.

Growing conditions during the vegetative phase

After acclimatization the plants were transferred to three growth chambers with different irradiance: Low Irradiance (LI): 50 ± 5 μmol m^{-2} s^{-1}, Medium Irradiance (MI): 125 ± 10 μmol m^{-2} s^{-1} and High Irradiance (HI) 200 ± 10 μmol m^{-2} s^{-1} provided by a Powerstar® HQI®-BT 400W/D metal halide lamp (Osram, The Netherlands) for 16 hours daily (eight hours of darkness). The daily light integral (DLI) was of 2.9, 7.2 and 11.5 mol day^{-1} for LI, MI and HI respectively. The irradiance levels were measured with a Li-Cor, Model L1-185, quantum sensor (LI-COR Inc, Lincoln, Nebraska, USA). The temperature set point in the chambers was 28 °C (± 1.5 °C) and the relative air humidity (RH) 90 % (± 5 %). The leaf temperature was 2 °C higher at HI than LI and was measured with the use of a thermocouple thermometer (model HD 9016, Delta OHM SRL, Caselle Di Selvazzano, Italy). The climate in the growth chambers was controlled by a Priva computer (Priva, De Lier, Netherlands). The plants were fertilized with 50 % Pioner Orkide and Pioner Mikro Plus (Bröste group, Denmark) and 50 % Calcinit (Yara, Norway). The nutrient solution had a conductivity of 1.2 mS cm^{-1} and the pH was adjusted to 5.7 by using nitric acid (HNO_3). The plants were watered twice per week under LI and MI and three times per week under HI. All chambers were equipped with CO_2 fertilization with the enrichment beginning one hour before the dark period and ending one hour after the light was turned on. The set point was 800 ppm, but the CO_2 varied between 600 and 1000 ppm during the experimental period.

Conditions during cooling and finishing phase

The plants were transferred to cooling into new chambers for flower initiation when the sixth new leaf was visible (< 1 cm) and then into another chamber for the finishing phase. The temperature during cooling was 18 °C (± 1 °C)

and the temperature was 21 °C (± 2 °C) during the finishing phase. The irradiance was 125 ± 10 μmol m^{-2} s^{-1} and the RH 70 % (± 5 %) in both cooling and the finishing phases. No additional CO_2 was added during cooling or finishing phase.

Measurements during vegetative and generative phase

The number of days until transfer from vegetative growth phase to cooling was recorded. To measure the vegetative growth, leaf length and leaf width (measured at the middle of the leaf) on new developing leaves (leaf number 4 and 5) and leaf appearance of leaf number 5 was measured on five plants in the three different light treatments once per week. Sections from the middle areas of fully developed leaves from the three different irradiance levels were cut horizontally to the main nerve of the leaf. The samples were fixed and embedded in Low Resin White as described in Torre et al. (2003). The leaf anatomy was studied and the leaf thickness was measured on sections cut with a glass knife under a Leitz Aristoplan light microscope with use of video microscopy. The pictures were analyzed with digital image processing (ImageTool; Version 3.00; The University of Texas Health Science Center, San Antonio, Texas, USA).

During cooling the numbers of visible inflorescence (VI) were recorded every second day (inflorescence length ≥ 5 mm). At this stage, the plants were moved to the finishing phase. The plants were grown until the first open flower and the number of days from VI to first open flower were recorded. At the first open flower stage different morphological characteristics of the inflorescence were measured. The length (cm) of the flower stem was measured from the base to the first open flower of the main stem and the diameter of the stem was measured 10 cm above the base of the stem. The number of buds with visible peduncle, including the first open flower and closed buds, were counted. The diameter of the plant was measured and defined as the distance (cm) between the two largest leaves of the plant.

Carbohydrate measurements

Fructose, glucose and sucrose were measured in fully developed source leaves. The samples were taken in the middle of the photoperiod at the end of the vegetative growth phase before the transfer to cooling. Each sample consisted of 6 fully developed source leaves. The samples were frozen immediately in liquid nitrogen and maintained at −80 °C before freeze dried and ground in a mortar with a pestle. For analyzing the sugar content 200 mg portions from the samples were mixed with 10 ml of distilled water. The soluble carbohydrate was analysed spectrophotometrically by a Sucrose, D-Fructose and D-Glucose Kit (Megazyme International Ireland Ltd.), where D-Fructose and D-Glucose was determined by hydrolysis with ß-fructosidase, and sucrose content calculated by the difference in D-Glucose content before and after hydrolysis.

Statistics

Statistical analysis was conducted using analysis of variance (ANOVA) on Minitab (Version 16). Means were separated using Tukey's test at the 5 % level of significance. In the study of vegetative leaf growth and leaf thickness, five plants were examined, and in the study of production time and inflorescence morphology 10 plants were examined from each hybrid in each treatment. In the study of inflorescence emergence (Fig. 3), 37–40 plants from each light level in both hybrids were examined and the plants with one, two or three inflorescence were counted. A nonparametric Mann-Whitney test was used to test for difference in mean number of inflorescence at $P = 0.05$. This nonparametric test was used to avoid assumptions on data distribution. The same nonparametric test was used to test the light levels against each other. Then, the data were analyzed separately for each hybrid. The experiment was done once in climate controlled chambers with full control of all climate parameters.

Results

In the vegetative phase the growth of the first (4^{th}) and the second (5^{th}) leaf was clearly influenced by irradiance (Fig. 1 and 2). The leaf appearance rate of the 5^{th} leaf was significantly faster in 'Mukalla' than 'Malaga' at HI ($P < 0.05$). At HI leaf number four and five appeared almost at the same time in 'Mukalla' but in 'Malaga' leaf number five appeared one week later than leaf number four (Fig. 1 and 2). The growth rate of leaf number five increased significantly with increasing irradiance in 'Mukalla' (Fig. 2) but in 'Malaga' no accelerated leaf growth rate was found by increasing the irradiance from MI to HI (Fig. 1). Leaf number 5 reached the maximum length approximately ten weeks after the first appearance at MI and HI, but leaves grown under LI were still growing 13 weeks after appearance (Fig. 1 and 2). A slightly different leaf shape was observed and a tendency of narrower, longer and thinner leaves was found under LI compared to MI and HI in both hybrids. However, the plants at HI or MI were not more compact because the diameter (defined as the distance in cm between the two largest leaves of the plant) was not significantly different in the three light levels (data not shown) but the leaf thickness increased significantly with increasing irradiance in both hybrids (Table 1).

When the 6^{th} leaf appeared, the plants were transferred to cooling for inflorescence formation but plants were randomly picked out for carbohydrate analysis. The results clearly show that 'Malaga' contained more soluble carbohydrates with increased irradiance and a significantly higher content of sucrose, glucose and fructose

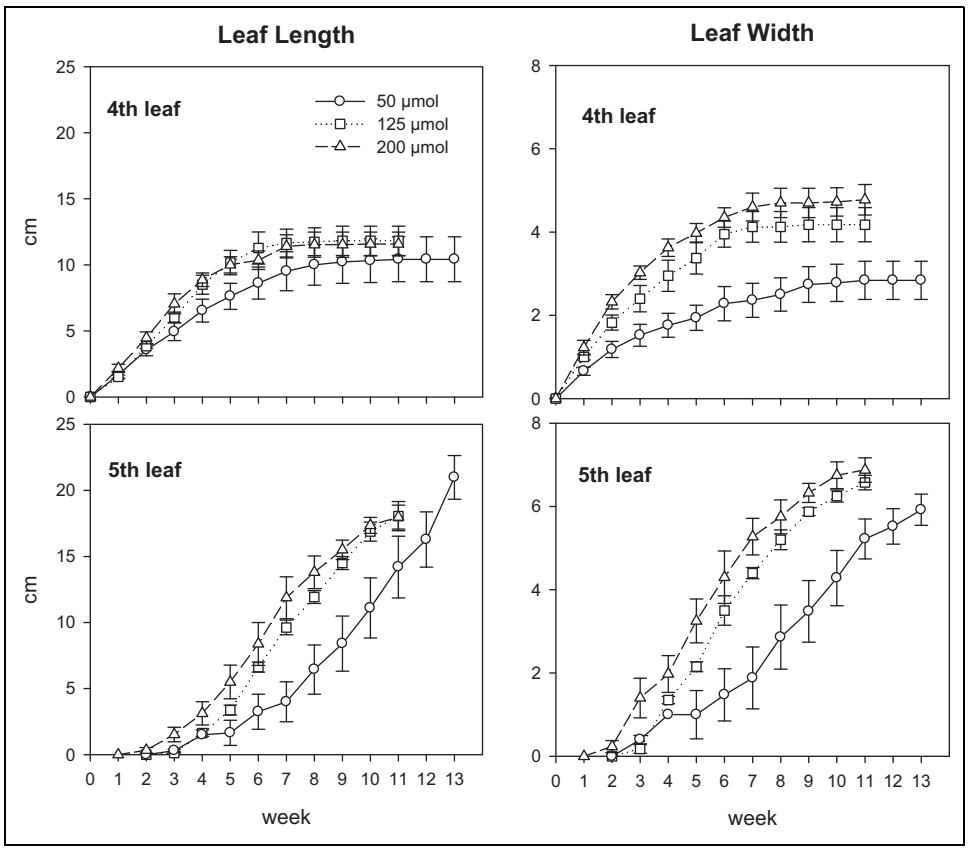


Fig. 1. The effect of irradiance on final leaf length and final leaf width on leaf numbers 4 and 5 measured on *Phalaenopsis* 'Malaga' leaves developed under LI (50 µmol m^{-2} s^{-1}), MI (125 µmol m^{-2} s^{-1}) and HI (200 µmol m^{-2} s^{-1}) in growth chambers. The measurements began when the 4th and 5th leaf appeared (week 0) and stopped after 13 weeks (Bar = SE, n = 5).

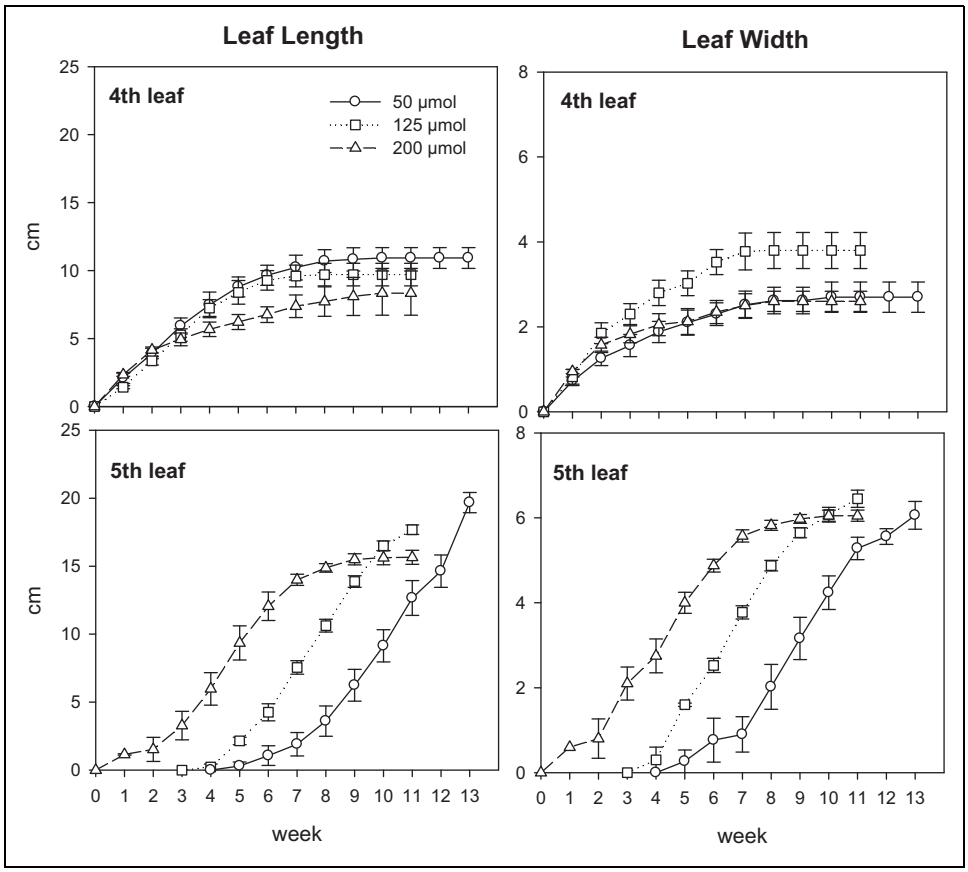


Fig. 2. The effect of irradiance on final leaf length and final leaf width on leaf numbers 4 and 5 measured on *Phalaenopsis* 'Mukalla' leaves developed under LI (50 µmol m^{-2} s^{-1}), MI (125 µmol m^{-2} s^{-1}) and HI (200 µmol m^{-2} s^{-1}) in growth chambers. The measurements began when the 4th and 5th leaf appeared (week 0) and stopped after 13 weeks (Bar = SE, n = 5).

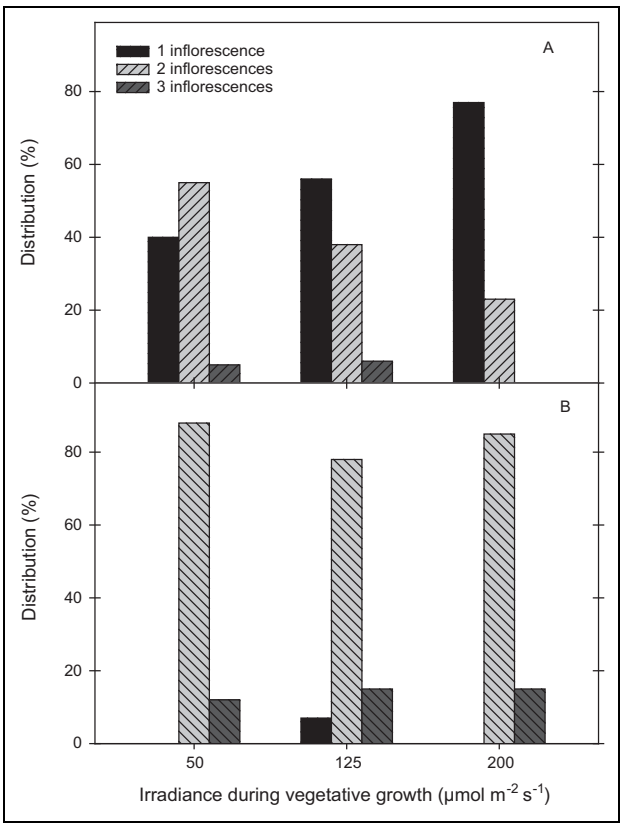


Fig. 3. The effect of irradiance during vegetative growth phase on the distribution of plants (%) with one, two or three inflorescences developed during cooling and finishing phases. Phalaenopsis 'Malaga' (A) and 'Mukalla' (B) were grown under LI (50 µmol m^{-2} s^{-1}), MI (125 µmol m^{-2} s^{-1}) and HI (200 µmol m^{-2} s^{-1}) in the vegetative growth phase before transfer to cooling and finishing phase in growth chambers (n = 36–40).

was measured in source leaves of vegetative plants grown at HI compared to LI (Table 2). The sucrose content in the source leaves of 'Malaga' was 6 and 14 times higher at MI and HI compared to LI (Table 2). In 'Mukalla' no significant effect of irradiance on the content of carbohydrate was found (Table 2) and the plants contained almost the same level of carbohydrate irrespective of the irradiance.

'Mukalla' developed more inflorescence per plant compared to 'Malaga' ($P < 0.05$) and the average number of inflorescences was 2.2 and 1.5 for 'Mukalla' and 'Malaga', respectively. In 'Mukalla', the number of inflorescence was not significantly affected by the irradiance during vegetative growth phase ($P > 0.05$) (Fig. 3B). In contrast, increased irradiance during vegetative growth reduced the numbers of inflorescence per plant in 'Malaga' and a significant difference was found between LI and HI ($P < 0.05$) (Fig. 3A). When growing 'Malaga' at LI during the vegetative phase, 55 % of the plants developed two inflorescences but only 23 % of the plants from HI developed two inflorescences (Fig. 3A).

The two hybrids also displayed different responses in time from start of cooling to visible inflorescence (VI). In 'Malaga', spiking was observed 20 days earlier when the plants were developed at HI during vegetative growth compared to LI. No such effect was seen in 'Mukalla', but the inflorescence emergence occurred faster than in 'Malaga' regardless of the irradiance during vegetative growth (Table 3). The total production time was also significantly shorter at MI and HI in 'Mukalla' compared to 'Malaga' (Table 3).

The inflorescence morphology was affected by the irradiance during vegetative growth, 'Malaga' developed significantly shorter and thinner inflorescence when grown under LI during vegetative growth compared to MI and HI (Table 4).

Neither the length nor the diameter of the inflorescence was significantly affected by irradiance during vegetative growth in 'Mukalla'. However, more branches and flower buds where found when grown at LI during the vegetative phase. Only small differences were found between MI and HI (Table 4). 'Malaga' displayed the opposite results and produced fewer branches and flowers on plants developed at LI compared to MI and HI (Table 4). As expected, the number of flower buds was closely related to the number of branches, and the more branches the more flower buds (Table 4).

Discussion

By increasing the irradiance from 50 to 125 µmol m^{-2} s^{-1}, the total production time was reduced by 50–60 days in both hybrids. An after-effect of the irradiance during vegetative phase was found on time from cooling to VI in 'Malaga' and the inflorescence appeared 3 weeks earlier when the vegetative growth phase were performed at HI compared to LI. However, no after-effects were found on time from cooling to VI in 'Mukalla'. Hence, the irradiance during vegetative growth affects the production time primarily by affecting the leaf growth rate and the length of the vegetative growth phase.

In Northern Europe supplementary lighting is commonly used in production of *Phalaenopsis* in periods when the natural light conditions are low. The recommended light level for *Phalaenopsis* during vegetative growth varies from 60 to 400 µmol m^{-2} s^{-1} (Konow and Wang 2001; Lin and Hsu 2004; Lopez and Runkle 2005; Van Der Knaap 2005). Several studies have found that the photosynthesis of *Phalaenopsis* saturates at about 130–180 µmol m^{-2} s^{-1} (Ota et al. 1991; Lootens and Heursel 1998). However, Lootens and Heursel (1998) found that the saturating PPF varied with temperature. At 20 °C, a saturating PPF was around 130 µmol m^{-2} s^{-1}, but at higher temperatures (25 to 30 °C) no saturating PPF was found at 300 µmol m^{-2} s^{-1}. In this study, the temperature was a constant 28 °C. To maintain this high temperature (> 28 °C) during vegetative growth of *Phalaenopsis* is

Table 1. The effect of irradiance on the vegetative growth expressed as final leaf length and final leaf width and ratio between leaf length and leaf width measured on leaves developed under LI (50 µmol m^{-2} s^{-1}), MI (125 µmol m^{-2} s^{-1}) and HI (200 µmol m^{-2} s^{-1}) in growth chambers. The data presented are the average of the 4th and 5th leaf in weeks 11 (MI and HI) and 13 (LI).

Irradiance (µmol m^{-2} s^{-1})	Leaf length (cm)	Leaf width (cm)	Leaf length/ leaf width	Leaf thickness (mm)
		'Mukalla'		
50	15.7 a	4.4 b	3.6 ab	1.43 e
125	13.7 a	4.4 b	2.9 abc	1.98 c
200	12.2 a	5.0 b	2.8 abc	2.15 a
		'Malaga'		
50	15.7 a	4.3 b	3.8 a	1.25 f
125	14.6 a	6.6 a	2.5 bc	1.53 d
200	14.3 a	5.7 ab	2.2 c	2.04 b

LI: low irradiance (50 µmol m^{-2} s^{-1}); MI: medium irradiance (125 µmol m^{-2} s^{-1}) and HI: high irradiance (200 µmol m^{-2} s^{-1})
+ Different letters in similar columns indicates significant differences. Means were separated using Tukey`s test at the 5 % level of significance, n = 5 (leaf length and leaf width), n = 20 (leaf thickness)

Table 2. The effect of irradiance on the content of sucrose, glucose, fructose and total soluble carbohydrates (mg g^{-1} dry matter) of source leaves from 'Mukalla' and 'Malaga' grown under LI (50 µmol m^{-2} s^{-1}), MI (125 µmol m^{-2} s^{-1}) and HI (200 µmol m^{-2} s^{-1}) in the vegetative phase. The measurements were taken on fully developed leaves prior to cooling.

Irradiance (µmol m^{-2} s^{-1})	Sucrose	Glucose	Fructose	Total soluble carbohydrates
		'Mukalla'		
50	0.82 ab	0.18 bcd	0.13 b	1.14 ab
125	0.93 ab	0.07 d	0.04 b	1.10 b
200	1.02 a	0.15 cd	0.09 b	1.28 ab
Mean	0.92	0.13	0.09	1.11
		'Malaga'		
50	0.09 b	0.27 bc	0.21 ab	0.57 ab
125	0.59 ab	0.33 b	0.16 ab	1.09 ab
200	1.32 a	0.53 a	0.25 a	2.11 a
Mean	0.66	0.37	0.20	1.25

LI: low irradiance (50 µmol m^{-2} s^{-1}); MI: medium irradiance (125 µmol m^{-2} s^{-1}) and HI: high irradiance (200 µmol m^{-2} s^{-1})
+ Different letters in similar columns indicates significant differences. Means were separated using Tukey's test at the 5 % level of significance, n = 6

costly, especially during winter and fall. Reports on how to optimize day (DT) and night temperatures (DT) during vegetative growth to save energy in the production of *Phalaenopsis* have been published recently (Blanchard and Runkle 2006; Pollet et al. 2011). However, the presented results and others (Konow and Wang 2001) show that the light conditions during vegetative growth influence the total production time and this needs to be taken into consideration. Optimal light conditions during vegetative growth phase can reduce the production time extensively and, therefore save greenhouse space and energy.

The two hybrids responded different to higher irradiance (200 µmol m^{-2} s^{-1}) and it appeared that 'Malaga' cannot utilize HI as efficiently as 'Mukalla' during the vegetative phase. Increasing the irradiance from 125 µmol m^{-2} s^{-1} to 200 µmol m^{-2} s^{-1} induced an even faster leaf growth rate in 'Mukalla' and only minor after-effects were found on the leaf morphology and reproductive growth

Table 3. The effect of irradiance during vegetative growth phase on time to visible inflorescence (VI) after transfer to cooling, time to first open flower, and total production time of *Phalaenopsis* 'Mukalla' and 'Malaga', measured on plants grown under LI (50 µmol m^{-2} s^{-1}), MI (125 µmol m^{-2} s^{-1}) and HI (200 µmol m^{-2} s^{-1}) before transfer to cooling (18 °C) and finishing phase (20 °C).

Irradiance during vegetative growth (µmol m^{-2} s^{-1})	Time from start of cooling to VI (days)	Time from VI to first open flower (days)	Total production time (days) (including vegetative phase)[1]
		'Mukalla'	
50	25.6 b	95.2 c	289 b
125	26.9 b	101.8 bc	240 c
200	25.9 b	101.7 bc	240 cb
		'Malaga'	
50	54.4 a	114.3 ab	316 a
125	43.5 ab	117.4 a	255 bc
200	34.7 b	114.2 ab	252 bc

LI: low irradiance (50 µmol m^{-2} s^{-1}); MI: medium irradiance (125 µmol m^{-2} s^{-1}) and HI: high irradiance (200 µmol m^{-2} s^{-1})
[1] The vegetative phase lasted until the plants had developed six visible leaves
[+] Different letters in similar columns indicates significant differences. Means were separated using Tukey's test at the 5 % level of significance, n = 10

Table 4. The effect of irradiance during vegetative growth phase on inflorescence morphology of *Phalaenopsis* 'Mukalla' and 'Malaga' grown under LI (50 µmol m^{-2} s^{-1}), MI (125 µmol m^{-2} s^{-1}) and HI (200 µmol m^{-2} s^{-1}) before transfer to cooling (18 °C) and finishing phase (20 °C). The length (cm) of the flower stem was measured from the base to first open flower on the main stem and the diameter was measured 10 cm above the base of the stem. The number of buds with visible peduncle was counted including the first open flower (n = 10).

Irradiance during vegetative growth (µmol m^{-2} s^{-1})	Inflorescence length at first open flower (cm)	Inflorescence diameter at first open flower (mm)	Number of branches on the inflorescence at first open flower	Number of flower buds
		'Mukalla'		
50	30.8 b	5.1 a	3.30 a	45.0 a
125	31.2 ab	5.3 a	2.7 ab	36.7 b
200	31.0 ab	5.2 a	1.90 b	30.7 bc
		'Malaga'		
50	25.0 c	4.0 b	0.81 c	12.4 e
125	31.8 ab	5.1 a	1.80 bc	23.7 cd
200	35.6 a	5.2 a	2.10 b	20.5 d

LI: low irradiance (50 µmol m^{-2} s^{-1}); MI: medium irradiance (125 µmol m^{-2} s^{-1}) and HI: high irradiance (200 µmol m^{-2} s^{-1})
[+] Different letters in similar columns indicates significant differences. Means were separated using Tukey's test at the 5 % level of significance

of this hybrid. On the other hand, 'Malaga' was found to have a vegetative growth rate which was not significantly faster at HI compared to MI. Also, a negative effect on the reproductive growth was found and a lower number of inflorescences per plant were found at HI compared to MI and LI. The number of flower stems is an important quality parameter of *Phalaenopsis* and a much better prize is paid for a multi spiked than a single spiked plant. From these results we conclude that excessive light can result in a suppression of flower bud initiation and reduce the possibility for multiple inflorescences in light sensitive hybrids.

Carbohydrates are known to have numerous roles in the reproductive growth of plants, from energy source to signal molecules (Gibson 2005). Several studies have

demonstrated the importance of carbohydrates in determining time to inflorescence emergence of *Phalaenopsis* (Kubota and Yoneda 1993; Chen et al. 1994; Kataoka et al. 2004; Chen et al. 2008). However, these studies primarily focused on the cooling or finishing phase. In this study, we tested how the carbohydrate status of vegetative plants prior to the cooling influenced the reproductive growth. No significant difference in average carbohydrate content between 'Malaga' and 'Mukalla' were found. However, 'Malaga' contained more carbohydrates with increasing irradiance and a significantly higher content of sucrose, glucose and fructose was measured in vegetative plants grown at HI compared to LI. In 'Mukalla' no effect of irradiance on the content of soluble carbohydrates were found and the levels were high irrespective of the irradiance. 'Mukalla' is known to make multiple inflorescence easily and it developed in average 2.2 flower stems per plant in contrast to 'Malaga' which developed only 1.5 Further, in 'Mukalla', the numbers of inflorescence were not significantly affected by the irradiance during vegetative growth. In contrast, increased irradiance during vegetative growth reduced the number of inflorescences per plant in 'Malaga'. It was hypothesized that high levels of carbohydrates at the start of cooling could promote the development of multiple inflorescences on plants. However, our hypothesis was rejected since the opposite situation appeared and a negative relationship between carbohydrate level and inflorescence number was observed in 'Malaga'. Thus, the possibility of inducing multiple inflorescences is not connected to a high content of soluble carbohydrates in the source leaves prior to cooling, at least not the content of sucrose, fructose or glucose. On the other hand, the fact that 'Mukalla' produces high levels of carbohydrates and makes 2 flower stems per plant irrespective of the irradiance during vegetative growth can indicate a relationship between robustness to adapt to different light levels and the ability to produce multiple inflorescences. However, the reason why 'Malaga' develops fewer inflorescences when grown at HI in the vegetative phase is not known. The cooling was performed at optimal temperatures (constant 18 °C) before the plants were moved to finishing phase (20 °C). Thus, the exposure time to cooling was not too short. Rather, the buds failed to develop under moderate temperature conditions (18–20 °C) which usually lead to inflorescence initiation (Sakanishi et al. 1980).

Plant size and maturity has been discussed in relation to reproductive growth of *Phalaenopsis*. It is recommended that the plants should have a minimum average leaf span of 25 cm to get uniform inflorescence production (Runkle et al. 2005). The plant diameter was not significantly different in this study and all the plants were > 25 cm irrespective of the irradiance during vegetative growth phase (results not shown). In general, the leaf morphological characteristics like leaf length and leaf width were only slightly affected by increasing the irradiance from 50 to 200 μmol m^{-2} s^{-1} showing that the leaf morphology and plant size is rather robust within this light interval. A 2 °C difference was measured in leaf temperature between LI and HI which could influence the physiological status. The growth data from the vegetative phase of 'Malaga' is similar in MI and HI, which indicates a saturating light level at 125 μmol m^{-2} s^{-1}. A higher light level (> 200 μmol m^{-2} s^{-1}) can cause light stress in *Phalaenopsis* (Lin and Hsu 2004; Ali et al. 2005) and induce a deeper dormancy of the buds. Plant hormones, such as abscisic acid (Wang et al. 2002) gibberellins (GAs) (Su et al. 2001) and cytokinins (Blanchard and Runkle 2008) are involved in flower induction of *Phalaenopsis*. Flower development requires optimal levels of endogenous GAs, and *Phalaenopsis* grown under high temperatures contained lower levels of GA in the shoot apical meristem area than plants from lower temperatures (Su et al. 2001). Detailed anatomical studies as well as studies of hormonal changes of leaves and buds are necessary to better understand how inflorescence number is controlled in *Phalaenopsis*.

It has been suggested that sucrose may be the primary photoassimilate that affects bud initiation (Lee and Lee 1996; Kataoka et al. 2004; Tsai et al. 2008). In the study of Kataoka et al. (2004), a relationship between sucrose content in the leaves and days to spiking was found in plants grown under different light levels during cooling. Plants grown under high light conditions (100 μmol m^{-2} s^{-1}) during cooling contained more sucrose than plants grown under low light (15 μmol m^{-2} s^{-1}) and spiked 40 days earlier (Kataoka et al. 2004). In the presented study, the sucrose content was evidently more affected by the irradiance than glucose and fructose and large differences in sucrose content was found between plants from LI and HI in 'Malaga'. Therefore, it can be assumed that the sucrose content in the source leaves before the start of cooling can induce after-effects on the time to VI if the levels are very low. Kataoka et al. (2004) concluded that the sucrose content had to be under a certain threshold level (< 0.1 mg cm^{-2}) to induce a noticeable delay in time to spiking. Very low sucrose levels were found in 'Malaga' developed at LI (0.09 mg g^{-1} dry matter) and 54.4 days passed from start of cooling to VI compared to 34.7 days until spiking and a sucrose content of 1.32 mg g^{-1} dry matter at HI.

The inflorescence morphology was affected by the irradiance during vegetative growth phase and 'Malaga' developed shorter and thinner inflorescence with fewer branches and flowers when grown under LI during vegetative growth phase compared to MI and HI. In 'Mukalla' neither the length nor the diameter of the inflorescence was significantly affected by irradiance during vegetative growth phase but more branches and flowers on the inflorescence were found when vegetative plants were grown at LI compared to HI. As discussed above, the number of leaves or the size of the plants when transferred to cooling were not different, and cannot explain the differences in inflorescence morphology. Hence, we conclude that the irradiance during vegetative growth phase induces

after-effect on the ability to produce multiple inflorescence, inflorescence morphology and development but the effect appears to be hybrid dependent.

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Addresses of authors: Arne Björn Hückstädt and Sissel Torre (corresponding author), Norwegian University of Life Sciences, Department of Plant and Environmental Sciences, P.Box 5003, 1432 Ås, Norway, e-mail (corresponding author): sissel.torre@umb.no.