End-of-day Lighting with Different Red/Far-red Ratios Using Light-emitting Diodes Affects Plant Growth of *Chrysanthemum* × *morifolium* Ramat. ‘Coral Charm’

Janni Bjerregaard Lund¹

Department of Agricultural Sciences, Copenhagen University, Højebykkegaardalle 21, DK- 2630 Taastrup, Denmark

Theo J. Blom

Department of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Jesper Mazanti Aaslyng

AgroTech A/S, Taastrup, Denmark

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Abstract. Controlling plant height without the use of plant growth retardants is one of the goals in future production of potted plants. Light quality with a low red to far-red ratio (R:FR) increases plant height. In this trial, the effects of light quality [R:FR ratio of 0.4, 0.7, and 2.4 (R = 600–700 nm, FR = 700–800 nm)] at the end of day were investigated on potted chrysanthemums using growth chambers. After a 9-h photoperiod, the 30-min end-of-day lighting was provided by light-emitting diodes at low irradiance by maintaining either red = 1 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (R_{con}) or far-red = 1 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (FR_{con}). After 3 weeks of end-of-day lighting, plants given the lowest end-of-day ratios (R:FR of 0.4 or 0.7) were taller than control plants (R:FR = 2.4). For low ratios of R:FR (0.4), the actual intensities of R and FR did not affect plant height, whereas for higher ratios of R:FR (0.7 and 2.4), plant height was greater for FR_{con} than for R_{con}. Leaf area of the lateral side shoots was lower for plants treated with an R:FR of 0.4 compared with those of controls. Dry weight, stem diameter, number of internodes, and number of lateral branches were unaffected by the end-of-day ratio.

One of the environmental concerns in the production of potted plants is the use of plant growth retardants (PGRs) for the control of plant height. In the search for alternatives to PGRs, changes in light quality [e.g., increased red to far-red ratio (R:FR ratio)] have been shown to limit elongation growth (Khattak and Pearson, 2006; Mortensen and Strømme, 1987; Rajapakse and Kelly, 1992). However, the effect of changes in light quality is not always positive. A decrease in the R:FR ratio at the end of the day occurs naturally during twilight. This has been shown to increase stem elongation in different plant species (Blom et al., 1995; Blom and Kerec, 2003) comparable to end-of-day far-red (EOD-FR) treatments.

The photoreceptor, phytochrome, is responsible for the physiological responses incited by changes in red (600–700 nm) and far-red (700–800 nm) light. The phytochrome molecule exists in two photorever-

sible states, P_{fr} and P_r. Irradiation with high levels of far-red light increases the proportion of the molecule in the P_r state, whereas a high amount of red light increases the proportion of the P_{fr} form. Light quality determines the R:FR ratio and phytochrome photoequilibrium (ϕ), which in turn determines plant morphology (Holmes and Smith, 1977). In *Arabidopsis*, phytochrome is represented by five members: phytochrome A (phyA) to phytochrome E (phyE) (Quail, 2002; Smith, 2000). Phytochrome B is assumed to play a role at all stages of the life cycle (Smith, 2000), including the increase in stem elongation in response to EOD-FR treatments (Smith, 2000; Smith and Whitelam, 1990).

Under outside conditions, the R:FR ratio changes from ≈ 1.15 during daylight to 0.7 during twilight in the evening (Holmes and Smith, 1977). Outside measurements at lat. 42°N (Feb. 1) showed that the R:FR ratio was 1.37 at official sunset with photosynthetic photon flux (PPF) of 3.8 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, whereas R:FR decreased to 0.69 with PPF 0.06 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (near darkness) over a period of ≈ 30 min (Blom et al., 1995). The twilight period depends on the time of the year and latitude. Near summer and winter

solstices at lat. 56°N, it lasts for up to 54 and 44 min, respectively, whereas year-round twilight lasts for ≈ 20 min near the equator (List, 1971). Eliminating twilight using black-out curtains in experiments during early spring in Canada (lat. 42°N) resulted in a decrease of 10% to 25% in height of easter lilies compared with ambient (Blom et al., 1995; Blom and Kerec, 2003). A similar experiment in Norway (lat. 60°N) with chrysanthemum and tomato had no or only a small effect on plant height (Mortensen and Moe, 1992). The latter experiment was done during summer with photoperiods varying between 12 and 18.5 h. Knowledge from experiments with EOD-FR is useful in understanding the effect of twilight, and EOD-FR response has been suggested to be the most effective in increasing the stem elongation rate during short photoperiods (Downs et al., 1957; Vince-Prue, 1977).

The effects of twilight on elongation are not clear and might depend on factors like the season and irradiance. Photoreceptors serve not only as quality detectors, but also as photon counters (Smith and Whitelam, 1990). Therefore, we wanted to test whether small differences in irradiation for the same ratios of R:FR would affect plant growth and/or development.

The objective of this study was to investigate whether EOD light quality as found during twilight affects growth and developmental characteristics of potted chrysanthemums and whether elongation depends on R:FR alone or on the level of irradiance of R and FR as well. For this purpose, it was decided to create artificial twilight using light-emitting diodes (LEDs) in growth chamber experiments. Light-emitting diodes give the possibilities of small and precise changes in intensity and spectral distribution of the EOD light treatment.

Materials and Methods

Plant material. Rooted cuttings of *Chrysanthemum morifolium* Ramat. ‘Coral Charm’ were planted singly in 10-cm pots filled with standard peat mix (Pindstrup 2; Pindstrup, Denmark). In the growth chambers, six batches of plants were placed during 2004 (5 July, 27 July, 24 Aug., 21 Sept., 16 Nov., 14 Dec.). Each batch was kept for 21 d in the growth chambers. From each batch, 72 nonpinched plants were selected for uniformity and distributed among three plots arranged in two growth chambers (1.3 m × 2.5 m, PGV36; Conviron, Winnipeg, Manitoba, Canada) after being cultivated for 4 weeks in a greenhouse (16-h daylength, 22 °C day/18 °C night) at Copenhagen University (Copenhagen, Denmark). The two growth chambers were divided into four sections of 0.8 m² cultivation area (black plastic covered with white plastic on either side from the top to the bottom of the chamber). Only three of the four sections were used. In each section, 24 plants were randomly distributed, 12 border plants and 12 plants for measurements (30 plants/m²).

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¹To whom reprint requests should be addressed; e-mail jbl@agrotech.dk

Growing conditions. One week before the start of the experiment, the plants were placed in the growth chambers applying a 16-h photoperiod at 21 °C day/18 °C night. Irrigation and fertilization were done simultaneously (fertigation) using drippers. In the first week, plants were fertigated once a day (Pioneer Macro 14N-3P-23K + Mg combined with Pioneer Micro; pH 5.5; electrical conductivity 1.3; Broste, Denmark). Later with increasing plant size, fertigation was done twice daily. Metal halide lamps (400 W HQI-T; Osram, München, Germany) and incandescent bulbs (40 W Osram, Germany) were used as the main light source and switched on and off according to the needs of the different experimental treatments. The mean *PPF* at the top of the plant canopy was $120 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (± 10) (measured under the LED racks) and the daily light integral delivered in the chambers ($3.9 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$) represents a winter day in northern Europe. The R:FR ratio of the artificial lighting was 2.4 ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}/\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) (Murakami et al., 1997) (Fig. 1).

For measurements of the artificial light in the photoperiod and the EOD light treatment, a fiberoptic cosine-corrected spectrometer (Avaspec-2048; Avantes, Eerbeek, The Netherlands) was placed at the top of the canopy. After 9 h of daylight, the plants received EOD light for 30 min with an R:FR ratio of 0.4, 0.7, or 2.4 (control, R:FR as in the photoperiod). In the first experiment, FR was kept at $1 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (FR_{con}) for all treatments and the R intensity was 0.4, 0.7, and $2.4 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively. In the second experiment, the same ratios were used with R irradiation at $1 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and FR at 2.5, 1.4, and $0.4 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (R_{con}). The different R:FR ratios were created using LEDs with wavelength peaks at 660 nm (L-53SRC-F; Kingbright, Cincinnati, Ohio) and 735 nm (L735-03AU; Epitex, Marubeni, Japan). The LEDs were placed in holes in transparent acrylic plastic rails ($130 \times 50 \times 1.5$ cm) 10 cm apart and interchanging between R and FR LEDs. The R and FR LEDs were connected individually. Ten rails were placed in a metal frame in each setup. Using two programmable four-channel power supplies (HM 7044; Hameg Instruments, Hameg GmbH, Germany), the irradi-

Fig. 1. Spectral photon distribution of artificial light (metal halide lamps and incandescent bulbs) used in the growth chambers during the photoperiod.

ation of R and FR was adjusted. The LEDs' spectral distribution did not change in the range of the electrical current (0–2.3 A).

Plant measurements. Nondestructive measurements were carried out on the plants at days 1, 7, 14, and 21 from start of the light treatments. Number of lateral branches and internodes (greater than 1 cm) were counted and height (from soil level to apical bud) was measured. Both nondestructive and destructive measurements were carried out for the final harvest (day 21) using all 12 plants. Leaf area (LI-3100 Area meter; LI-COR, Lincoln, NE) and dry weights (86 °C for 48 h) of leaves from the main stem, leaves from lateral branches, stem of lateral branches, and the main stem were determined. At the final harvest, stem diameter was measured at 8 cm from the apical point using a caliper.

Statistical analysis. Treatments were rerandomized between replicates and compartments. Experimental data on plant growth were analyzed statistically using Duncan's. Height measurements, which were repeated measurements of height over time (elongation rates), were analyzed using a mixed factorial model (Proc Mixed; SAS Institute, Cary, NC) with the three factors R:FR ratio, series (R constant, FR constant), and week together with their interactions. The three replicates were included as random block effects.

Results

All treatments resulted in plants growing linearly (R^2 0.96 to 0.97) in height during the 3 weeks (Fig. 2). Plant height was significantly greater ($P < 0.0001$) when treated with a low (0.4) compared with a higher EOD R:FR ratio (2.4) (Fig. 2). The effect of the ratio was very clear, but there was an interaction between the ratio and how the ratio was achieved (FR_{con} at $1 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ or R_{con} at $1 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). For R:FR = 0.4, there was no difference on plant height in how the ratio was achieved, but for R:FR = 0.7 and 2.4, plants were taller with FR_{con} compared with R_{con} ($P < 0.05$) (Fig. 2). The FR_{con} treatment significantly increased internode number and number of lateral breaks compared with the R_{con} for all EOD treatments, whereas there was no difference between the different EOD R:FR ratios on the same parameters (Table 1). The average internode length at R:FR = 0.4 on plants treated with FR_{con} was 1.6 cm, whereas it was 1.8 cm when treated with R_{con} ($P < 0.05$). For R:FR = 0.7 and 2.4, there was no difference in average internode length when comparing FR_{con} with R_{con}.

Stem diameter and leaf area on the main stem were unaffected by the EOD R:FR ratio. However, the leaf area on the lateral breaks decreased 15% and 25% when the EOD R:FR decreased from 2.4 to 0.4. There were no significant differences in total dry weight of the aerial plant parts between plants from the different treatments nor the allocation of dry weight measured at leaves from the main stem, leaves from lateral branches, stem of lateral branches, and the main stem (data not presented).

Fig. 2. Effect of end-of-day treatments with red/far-red (R:FR) ratios of 0.4, 0.7, or 2.4 on increase in plant height of *Chrysanthemum morifolium* 'Coral Charm' during 3 weeks of daily treatments of 30 min each. SE is based on the means of three plantings ($n = 36$). Regressions lines that were not significantly different were pooled (R_{con} and FR_{con} = 0.4), so data represent means of 72 plants. FR_{con} 2.4: $Y = -0.01 + 4.81 X$, $R^2 = 0.96$, FR_{con} 0.7: $Y = 0.21 + 6.13 X$, $R^2 = 0.97$, R_{con} 2.4: $Y = -0.15 + 4.02 X$, $R^2 = 0.97$, R_{con} 0.7: $Y = -0.05 + 5.41 X$, $R^2 = 0.97$, R_{con}+FR_{con} 0.4: $Y = 0.11 + 6.69 X$, $R^2 = 0.96$. SEM of the slope is 0.09. Plant height at day 1 (start of experimental treatment) was in average 12.4 cm.

Table 1. Increase in number of internodes and number of lateral breaks of *Chrysanthemum morifolium* Coral Charm after 3 weeks of daily 30-min light exposures with different ratios of red/far-red (R:FR, R = 600–700 nm, FR = 700–800 nm) at the end of the day.²

R:FR	Internodes		Lateral breaks	
	FR _{con}	R _{con}	FR _{con}	R _{con}
0.4	12.6a	11.1a	14.5a	12.9a
0.7	12.5a	11.0a	16.2a	12.1a
2.4 (control)	11.8a	9.9a	15.9a	12.3a

Means within columns followed by different letters are significantly different at $P \leq 0.05$.

²For R_{con}, the red light irradiance was $1 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, whereas for FR_{con}, the far-red light was $1 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Discussion

The EOD treatment with an R:FR ratio of 0.7 was a simulated twilight. The duration of the treatment (30 min) was comparable to the duration of an average twilight, whereas the daily light integral and the duration of the photoperiod was comparable to a winter day in the Northern hemisphere. The EOD twilight causing increased elongation contradicts earlier findings with chrysanthemums by Mortensen and Moe (1992). Mortensen and Moe (1992) did not show a significant effect from EOD elimination of twilight using black-out curtains on plant height. Experiments with chrysanthemum were performed during summer in Norway. Downs et al. (1957) showed that increasing photoperiod [or daylight light integral (DLI) because this increased with increasing photoperiod] decreased the effect of an EOD-FR treatment on elongation in beans with a rapid decrease from a 6- to 12-h photoperiod. The Norwegian experiment was conducted during summer months with photoperiods between 18.5 h and 12 h. The effect of the duration of

the previous photoperiod or DLI on EOD responses might explain the difference between their results and ours. The effect of the EOD treatment at 9 h compared with experiments at longer days might also be related to plants increasing sensitivity to altered light quality attributable to flower initiation. However, the effect of this is expected to be limited because plants received long days until the beginning of the experiment and already after 1 week, a significant effect of the EOD treatments was observed.

The R:FR ratio in daylight is ≈ 1.15 , but in our growth chambers, it was 2.4 during the photoperiod. This would have resulted in an estimated phytochrome photoequilibrium, ϕ , of 0.57 and 0.65, respectively (calculations according to Hayward, 1984). This difference could also explain part of the difference in results between the effect of the EOD treatment in the growth chambers and natural conditions.

Under a low R:FR ratio (0.4) at EOD, there was no difference in final plant height between the situations in which either R or FR remained constant. However, when the ratio of R:FR at EOD was high (2.4), the stem extension under FR_{con} was 20% greater than under R_{con} . The FR_{con} caused an increase in internode number for all EOD treatments compared with R_{con} , whereas the average internode length did not differ between FR_{con} and R_{con} at 0.7 and 2.4. However, at R:FR = 0.4, there was a decrease in average internode length with FR_{con} having shorter internodes than R_{con} . This decrease in the average internode length at FR_{con} (R:FR = 0.4) eliminated the effect of the increase in number of internodes and caused a similar plant height as in R_{con} . In other words, the number of internodes was determined by the FR_{con} and R_{con} at all R:FR ratios, but when the ratio of R:FR was low (0.4), then the intensity of the R and FR light also influenced the average internode length. This implies that the estimated photoequilibrium of the phytochrome, ϕ , cannot be described by the R:FR ratio alone (Hayward, 1984) or ϕ does not explain elongation growth fully, e.g., the signal transduction pathway might be affected by irradiance level of the EOD R and FR.

Earlier experiments with light quality have most often been conducted with one R:FR ratio and one irradiance level. In these experiments, the EOD light had been applied using different artificial light sources and filters (Cathey, 1974; Downs et al., 1957; Khattak and Pearson, 2006; McMahon et al., 1991; Murakami et al., 1997; Rajapakse and Kelly, 1992) or FR and R fluorescent tubes (Xiong et al., 2002). When using LED, like in the presented experiments, it was possible to design the light quality of the EOD light without technical limitations. The LED technology has not been used with this purpose in earlier experiments and it has therefore been difficult to compare the results with these experiments. In some nurseries, incandescent lamps are used for day extension at the EOD. The R:FR ratio of this lamp is ≈ 0.63

(Murakami et al., 1997), or 0.70 (pers. data) which is comparable to the ratio during twilight. As seen in this experiment, it is advisable to use light sources with a high R:FR ratio for photoperiod extension [e.g., high-pressure sodium lamps with R:FR = 4.06 (Murakami et al., 1997)]. However, some long-day plants need some FR light for rapid flower initiation (Moe and Heins, 1990). To eliminate the effect of twilight, red light can be used during sunrise and sunset (Heo et al., 2001) or, like in the lily production in Canada, twilight can be eliminated using black-out curtains (Blom et al., 1995). One has to bear in mind that recent experiments with chrysanthemum have shown that they do not follow a normal shade avoidance response (Carvalho and Heuvelink, 2003; Khattak et al., 2004), e.g., plant height did not increase with plant density. Because EOD-FR treatments are comparable to shade avoidance responses (Fankhauser and Casal, 2004; Smith and Whitelam, 1990) and most light quality responses are very species-dependent (Casal, 1994), the response might not be directly transferable to other plant species.

In rose plants, EOD-FR partitioned more dry matter into the stems than into the leaves and a low R:FR reduced the number of lateral branches (Maas and Bakx, 1995). As was expected, there was a decrease in leaf surface area on the lateral branches in treatments with EOD R:FR = 0.4, but no effect on the number of lateral breaks. In chrysanthemum, height increase was strongly related to the EOD R:FR ratio and fluence rate of the light in the simulated twilight, whereas total dry weight, dry weight distribution, number of lateral breaks and internodes, leaf area on the main stem, and stem diameter were unaffected after the 3 weeks of EOD lighting. Leaf area on the lateral breaks decreased with decreasing EOD R:FR. More attention should be given to the effect of twilight on elongation rate in the production of potted plants when aiming at decreasing the use of PGRs. In a future experiment, the effect of a simulated twilight will be investigated in growth chambers using different durations for the photoperiod and light integrals.

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