J. Amer. Soc. Hort. Sci. 131(3):309–312. 2006.

Day and Night Temperature Differential (DIF) or the Absence of Far-red Light Alters Cell Elongation in 'Celebrity White' Petunia

Gary R. Bachman[1] and Margaret J. McMahon[2]
Department of Horticulture and Crop Science, The Ohio State University, Columbus, OH 43210

Additional index words. light quality, photomorphogenesis, spectral filters, thermomorphogenesis, *Petunia ×hybrida*

Abstract. 'Celebrity White' hybrid petunia plants (*Petunia ×hybrida* Hort. Vilm-Andr.) were grown either in chambers constructed of $CuSO_4$-filled panels acting as spectral filters removing the far-red light (–FR) or in environmental control chambers under temperature treatments of 24 °C day/18 °C night (+DIF) or 18 °C day/24 °C night (–DIF). Growth responses for plants grown under $CuSO_4$ filter (–FR) or –DIF temperatures were similar in that both treatments resulted in decreased internode length, increased stem diameter, and decreased cell length and cell diameter in epidermal, cortical, and pith tissues. Reduced cortical cell length contributed the largest percentage to internode length reductions compared to epidermal and pith tissue for the –FR treatment while reductions in cell length of all three tissues contributed to internode reduction of –DIF-treated plants. Chlorophyll a increased for plants grown under –FR, but decreased for plants grown in –DIF when compared to the appropriate controls.

Concern about the use of agricultural chemicals to produce horticultural crops has increasingly focused attention on nonchemical methods of controlling plant growth. Alteration of light quality (Bachman and McMahon, 1995; Benson, 1992; McMahon et al., 1991; Mortensen and Strømme, 1987; Rajapakse et al., 1999) and maintaining night temperature warmer than day temperature (–DIF) (Erwin et al., 1989) have shown promise as nonchemical methods of controlling extension growth.

The manipulation of light transmitted through spectral filters that absorb far-red light from 700–800 nm decreased stem elongation and reduced plant size and increased leaf chlorophyll for various bedding plant species (Benson, 1992), *Lilium longiflorum* Thunb. 'Nellie White' (Bachman and McMahon, 1995), and *Dendranthema ×grandiflorum* (Ramat.) Kitamura 'Bright Golden Anne' (McMahon et al., 1991). This reduced growth is similar to reductions from applied chemical plant growth retardants in terms of increased chlorophyll concentration and reduced leaf size in *Rosa hybrida* L. 'Meirutral' (McMahon and Kelly, 1990), *D. ×grandiflorum* 'Bright Golden Anne' (Rajapaske and Kelly, 1992), and *Helianthus annuus* L. cultivars (Starman et al., 1989).

Manipulation of the temperature environment, more precisely the difference between day and night temperatures, termed DIF, is also a nonchemical method of influencing plant height of *L. longiflorum* (Erwin et al., 1989) and many other herbaceous crops. When day temperature is greater than night temperature (+DIF), stem elongation is promoted while the reverse situation (–DIF) inhibits stem elongation, similar to the effects of FR-absorbing filters. Other reported morphological changes from increased DIF include increased chlorophyll content, leaf area, and parenchyma cell length of *Lycopersicon esculentum* Mill. (Erwin and Pierson, 1992) and *L. longiflorum* (Erwin et al., 1994).

Previous work has shown that plant responses to light quality resulting in reduced internode elongation are similar to reduced internode growth from thermomorphogenic responses caused by –DIF (Moe, 1993; Moe and Heins, 1990). In addition, response of stem elongation to day/night temperature fluctuations can be promoted or inhibited depending on the quality of the light environment in which the plants are grown (Erwin et al., 1992). Both –DIF and FR removal reduce growth similarly to the application of chemical growth regulators (Starman et al., 1989).

Although control of internode growth using photo- and thermomorphogenic methods has been reported for other crops (Grindal and Moe, 2000), the comparison of the effects of altered light quality or DIF on stem characteristics has not been previously reported together on the same floriculture crop. Also, effects on leaf morphology and chlorophyll levels by FR-absorbing filters or exposure to –DIF indicate that photo- and thermomorphogenesis may not affect plant growth identically. The objective of this investigation was to determine differences in *P. ×hybrida* 'Celebrity White' grown under far-red spectral filters or DIF temperature treatments.

Materials and Methods

Plugs of 30-d-old *P. ×hybrida* 'Celebrity White' were pinched back to three nodes, transplanted into 10-cm-diameter square pots in MetroMix 360 media (Scotts Co., Marysville, Ohio), and placed into spectral chambers positioned on greenhouse benches or in environmental growth chambers. Petunias were chosen for this study based on responses in preliminary studies to altered light and temperature environments.

The spectral chambers were constructed using double-wall polycarbonate panels (all surfaces except bottom) to optimize light quality exposure and were either filled with 6% (w/v) $CuSO_4$ solution (McMahon et al., 1991) or left empty for chamber control treatments. Plants were grown on open greenhouse benches as a no filter treatment. Spectral irradiance for the light quality treatments is illustrated in Fig. 1. Spectral chambers measured

Received for publication 6 Jan. 2005. Accepted for publication 22 Feb. 2006. Manuscript number HCS 04-04. Salaries and research support provided by state and federal funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University. Additional funding came from The Ohio Floriculture Foundation.

[1]Former Graduate Research Associate. Present address: Illinois State University, Department of Agriculture, Normal, IL 61790-5020.
[2]Associate Professor.

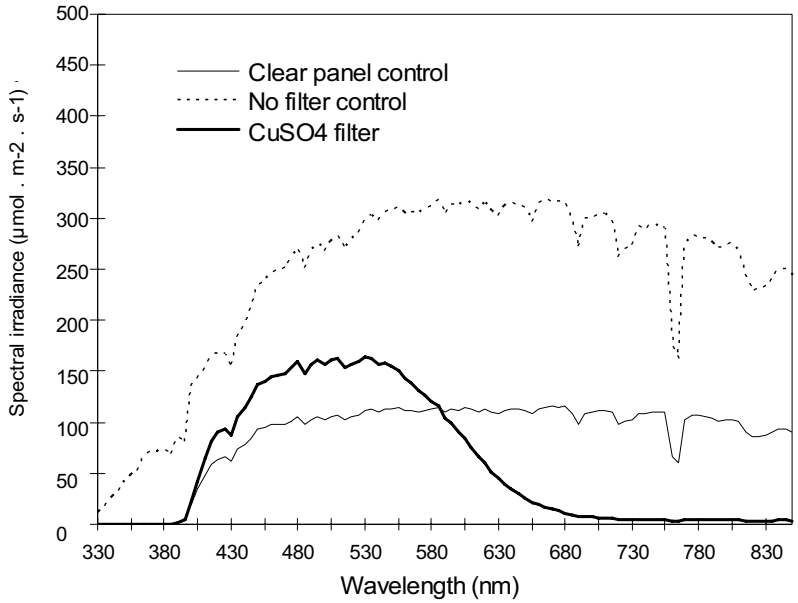

Fig. 1. Spectral scans representative of irradiance and light quality among spectral chambers and no-filter greenhouse bench treatments.

1.2 × 1.2 m with the top panel set at a 30° angle facing south. Air exchange was achieved through the expanded metal bench tops (chamber bottoms) and 10-cm gaps at the top of the front and back panels underneath a 30-cm overhang of the top panel. Set points for day and night greenhouse temperatures were 24 and 18 °C, respectively. The day temperature in the greenhouse was the same as outside ambient temperature on the days when outside temperature was above the set point. On days when outside temperature was at or below set point, then inside temperature was within 1 to 2 °C range of set point. For the night periods, the set point was nearly the same as the actual temperature except during those nights when the outside temperature remained above set point.

Environmental control chambers (Conviron, Pembina, N.Dak.) were used for the DIF treatments having temperature regimes of either 24 °C day/18 °C night (+DIF) or 18 °C day/24 °C night (–DIF). The day/night duration was 18 h day/6 h night, respectively. Lighting was achieved using both incandescent and fluorescent bulbs and provided an intensity of 590 μmol·m^{-2}·s^{-1}. The incandescent lighting was used only during the first and last 30 min of each cycle. The bulbs were maintained 30 cm above the plant canopy for the duration of the experiment. Each treatment was replicated three times with 10 plants per replicate. All treatments began 12 Aug. and ended 18 Oct., repeated beginning 10 Sept. and ended on 17 Nov.

Data collected included the internode length and stem diameter of the fourth internode from the distal end of the flowering stem. Chlorophyll content was determined following the dual wavelength procedure of Moran (1982). Epidermal, cortex, and pith cell measurements (length and width), and cell file length measurements were also made. Length and width of the pith and cortex cells were measured from the fourth internode section. Internode sections were prepared for microscopic measurements following the procedures described by Sass (1958). Longitudinal and transverse sections 20 μm thick were cut using a rotary microtome. There were 10 single-stem replications of each light quality or temperature treatment from which sections were collected.

Photographs were taken using a compound microscope (Carl Zeiss MicroImaging Inc., Thornwood, N.Y.) with a Contax 35-mm camera (ToCAD America Inc., Parsippany, N.J.) using Kodak Plus-X Pan 125 black and white print film (Eastman Kodak Co., Rochester, N.Y.). Stem diameters were measured from transverse sections magnified at 40×. Diameter was measured three times at points 120° apart, totaled, and the sum divided by 3 to accommodate any deformation of the stem tissue from the embedding and sectioning process. Cell width and length were measured using a 1-mm scale divided into 0.1 and 0.01 mm and measured from photographs taken at 100× magnification. Length and width measurements were taken of 10 cells of each replication. Cell length was measured for 10 contiguous cells within the cortex and pith tissues. In addition, the percentage of each individual cell that contributed to internode length was determined by dividing the mean cell length of epidermal, cortical, and pith cells by internode length [(cell length/internode length) × 100]. All data were analyzed using analysis of variance (ANOVA), and if significant at $P = 0.05$, data were analyzed by mean separation using least significant difference (LSD).

Results

Because the –FR treatments were applied in a greenhouse and the DIF treatments were applied in environmental growth chambers, there were inherent differences in the environmental conditions of the two regimens. Direct statistical comparisons between the light and temperature treatments were, therefore, not made, so that the results will be reported separately.

Light quality. Internode length was reduced for plants grown under –FR compared to the no filter or clear panel controls, 27% and 40%, respectively (Table 1). Stem diameter was 9% and 16% greater for plants grown under –FR compared to the no filter or plants grown under the clear panel, respectively.

Cell length of epidermal, cortex, and pith cell was reduced for plants grown under –FR compared to no filter or plants grown under the clear panel (Table 1). Cell length was greatest for plants grown under the clear panel. Epidermal and cortical cell

Table 1. Internode length, internode diameter, cell length, and cell diameter measurements of 'Celebrity White' petunia grown under far-red (FR) spectral filters or day and night temperature differential (DIF) conditions.

	Internode		Epidermis		Cortex		Pith	
	length	diam	length	diam	length	diam	length	diam
	------ mm ------		---------- μm ----------					
Spectral filters[z]								
Clear	27.0 a[y]	3.1 c	118.3 a	22.0 b	108.3 a	35.0 b	116.3 a	52.3 b
No filter	22.8 b	3.3 b	97.0 b	23.0 b	92.0 a	39.0 b	97.3 b	58.7 a
–FR	14.8 c	3.6 a	73.3 c	25.7 a	74.6 b	41.3 a	71.7 c	61.0 a
DIF[x]								
–DIF	24.3 b[w]	2.8 a	79.7 b	15.3 a	71.0 b	25.6 a	85.3 b	60.3 a
+DIF	30.7 a	2.2 b	91.3 a	11.4 b	89.0 a	21.3 b	109.0 a	49.3 b

[z]Spectral filter treatments clear (empty double-wall polycarbonate panels), no filter (open greenhouse bench), and –FR (double-wall polycarbonate panels filled with 6% $CuSO_4$ solution)
[y]Mean separation spectral filter treatments within columns by least significant difference (LSD) at$P = 0.05$. Different letters indicate significant difference, n = 10.
[x]Temperature treatments +DIF (24 °C day/18 °C night) and –DIF (18 °C day/24 °C night).
[w]Mean separation of DIF treatments within columns by LSD at $P = 0.05$. Different letters indicate significant difference.

Table 2. Individual cell length as a percentage of internode length and cell number per internode of epidermal, cortical, and pith cells of 'Celebrity White' petunia grown under far red (FR) spectral filters or day and night temperature differential (DIF) conditions.

	Proportion of internode length contributed by individual cells (%)			Cells per internode (no.)		
	Epidermis	Cortex	Pith	Epidermis	Cortex	Pith
Spectral filters[z]						
Clear	0.47 a[y]	0.36 c	0.36 b	211 a	231 a	215 a
No filter	0.46 a	0.43 b	0.40 ab	214 a	226 a	213 a
–FR	0.46 a	0.51 a	0.46 a	206 a	203 b	212 a
DIF[x]						
–DIF	0.35 a[w]	0.38 a	0.30 a	267 a	300 a	250 a
+DIF	0.24 b	0.32 b	0.25 b	307 a	315 a	257 a

[z]Spectral filter treatments clear (empty double-wall polycarbonate panels), no filter (open greenhouse bench), and –FR (double-wall polycarbonate panels filled with 6% $CuSO_4$ solution)
[y]Mean separation spectral filter treatments within columns by least significant difference (lsd) at $P = 0.05$. Different letters indicate significant difference, n = 10.
[x]Temperature treatments +DIF (24 °C day/18 °C night) and –DIF (18 °C day/24 °C night).
[w]Mean separation of DIF treatments within columns by LSD at $P = 0.05$. Different letters indicate significant difference.

width was greatest for –FR grown plants. Pith cell diameter was greatest for plants from the no filter and under –FR panels. The proportion of internode length contributed by individual cortex and pith cells was greater in plants grown under –FR than under clear panel or no filter controls, while epidermis cells were similar among the treatments (Table 2).

The number of cells per internode was similar for both epidermis and pith, but there were fewer cells per internode in the cortex for plants grown under the –FR treatment (Table 2). –FR-grown plants had greater chlorophyll a while there was no change in chlorophyll b levels among treatments (Table 3).

DIF. Stem internode length was reduced by 21% and stem diameter was increased by 23% for plants grown in –DIF conditions compared to +DIF (Table 1). Epidermal, cortex, and pith cell diameter was greater and cell lengths reduced for –DIF grown plants compared to +DIF grown plants (Table 1). The proportion of internode length contributed by individual epidermis, cortex, and pith cells grown in the –DIF treatment was greater compared to the +DIF treatment (Table 2). Cell number per internode was similar for epidermal, cortex, and pith tissues (Table 2). Chlorophyll a and total chlorophyll was reduced for the –DIF grown plants compared to the +DIF plants (Table 3).

Table 3. Chlorophyll content of 'Celebrity White' petunia grown under under far-red (FR) spectral filters or day and night temperature differential (DIF) conditions.

	Chlorophyll a	Chlorophyll b
	---------- $\mu g \cdot cm^{-2}$ ----------	
Spectral filters[z]		
Clear	54.25 c[y]	17.3 a
No filter	63.6 b	19.3 a
–FR	79.2 a	19.1 a
DIF[x]		
–DIF	49.9 b[w]	15.4 a
+DIF	62.8 a	17.9 a

[z]Spectral filter treatments clear (empty double-wall polycarbonate panels), no filter (open greenhouse bench), and –FR (double-wall polycarbonate panels filled with 6% $CuSO_4$ solution).
[y]Mean separation spectral filter treatments within columns by least significant difference (LSD) at $P = 0.05$. Different letters indicate significant difference, n = 10.
[x]Temperature treatments +DIF (24 °C day/18 °C night) and –DIF (18 °C day/24 °C night).
[w]Mean separation of DIF treatments within columns by LSD at $P = 0.05$. Different letters indicate significant difference.

Discussion

The data describing the contribution of individual cell length to the internode length (cell length/internode length) indicate that differences in both cell elongation and cell division are influenced by light quality and DIF-mediated growth. Cell length decreased in response to –FR light or –DIF; however, the percent contribution of each cell to internode length increased. Because cell length decreases in response to –FR or –DIF, then fewer cells most likely contributed to the increase in the percentage each cell contributes to the internode length.

Beall et al. (1996) reported that bean seedlings exposed to far-red light had enhanced endogenous GA_1 and GA_{20} content and increased cell elongation and division determined by comparing individual cell length as a percentage of internode length. After 7 d, pith cell length accounted for 29% or 44% of the internode length for bean seedlings grown in control or far-red light, respectively. Our data support these observations. As cell length increased in response to either exposure to FR or +DIF, the contribution of the length of individual cells decreased, with the resulting increase in internode length from a combination of cell elongation and cell division. This is different for plants grown in the absence of FR or –DIF; the subsequent internode elongation was primarily from cell elongation as observed from the percent internode of individual cell data, which increased with the absence of FR or –DIF. –FR or –DIF may act as gibberellin biosynthesis inhibitors and thus could disrupt the synergism of FR and endogenous gibberellin acting on internode elongation. The results of this study suggest the changes in internode elongation are mediated by –FR or –DIF primarily through stem cell elongation and not cell division. This observation was similar to that made by Erwin et al. (1991).

There were similarities and differences in chlorophyll content of plants grown in –FR or –DIF. Plants grown in –FR had greater amounts of chlorophyll a than control plants; however, –DIF grown plants had less chlorophyll a than plants grown in a +DIF environment. Neither –FR or DIF treatments produced any difference in chlorophyll b content. Similar results have been reported previously for chrysanthemum and bedding plants grown –FR (Benson, 1992; McMahon and Kelly, 1995) and *L. esculentum* grown under –DIF conditions (Erwin and Pierson, 1992).

Growing plants in –FR or –DIF has been shown to be effective in controlling the stem elongation of ornamental plants. Both techniques reduce internode elongation by causing a decrease in cell elongation in epidermal, cortical, or pith tissues, while these cells each contribute a greater percentage of the internode length. These two techniques, although different in the area of the growing environment altered, could be used by the commercial grower to complement growth control techniques. The use of spectral filters would have the greatest effectiveness during high light summer situations while –DIF could provide effective growth control during periods of cool, low light conditions.

Literature Cited

Bachman, G.R., and M.J. McMahon. 1995. Effects of spectral filters on growth of Easter lily. Proc. Plant Growth Regulat. Soc. Amer. 22:344–347.

Beall, F.D., E.C. Yeung, and R.P. Pharis. 1996. Far-red stimulates internode elongation, cell division, cell elongation, and gibberellin levels in bean. Can. J. Bot. 74:743–752.

Benson, J.L. 1992. Effects of spectral filters on bedding plant growth. M.S. Thesis, Clemson Univ., Clemson, S.C.

Erwin, J.E., R.D. Berghage, and R.D. Heins. 1991. Diurnal variations in temperature affect cellular elongation but not division. HortScience 26(6):105. (Abstr.).

Erwin, J.E., R.D. Heins, W. Carlson, and S. Newport. 1992. Environmental and mechanical manipulation of stem elongation. Plant Growth Regulat. Soc. Amer. Quarterly 20:1–17.

Erwin, J.E., R.D. Heins, and M.G. Karlsson. 1989. Thermomorphogenesis in *Lilium longiflorum*. Amer. J. Bot. 75(1):45–55.

Erwin, J.E. and G. Pierson. 1992. Interaction between diurnal temperature fluctuations and gibberellins on *Lycopersicum* stem elongation and chlorophyll content. HortScience 27:657. (Abstr.).

Erwin, J.E., P. Velguth, and R. Heins. 1994. Day/night temperature environment affects cell elongation but not division in *Lilium longiflorum* Thunb. J. Expt. Bot. 45(276):1019–1025.

Grindal, G. and R. Moe. 2000. The role of gibberellin and phytochrome in DIF-mediated stem elongation. Acta Hort. 514:205–211.

McMahon, M.J. and J.W. Kelly. 1990. Influence of spectral filters on height, leaf chlorophyll, and flowering of *Rosa* ×*hybrida* 'Meirutral'. J. Environ. Hort. 8:209–211.

McMahon, M.J. and J.W. Kelly. 1995. Anatomy and pigments of chrysanthemum leaves developed under spectrally selective filters. Scientia Hort. 64:203–209.

McMahon, M.J., J.W. Kelly, D.R. Decoteau, R.E. Young, and R.K. Pollack. 1991. Growth of *Dendranthema* ×*grandiflorum* (Ramat.) Kitamura under various spectral filters. J. Amer. Soc. Hort. Sci. 116(6):950–954.

Moe, R. 1993. Control of plant morphogenesis and flowering by temperature alterations. Flowering Nwsl. 15:30–34.

Moe, R. and R.D. Heins. 1990. Control of plant morphogenesis and flowering by light quality and temperature. Acta Hort. 272:81–90.

Moran, R. 1982. Formulae for determination of chlorophylous pigments extracted with *N,N*-dimethylformamide. Plant Physiol. 69:1376–1381.

Mortensen, L.M. and E. Strømme. 1987. Effects of light quality on some greenhouse crops. Scientia Hort. 33:27–36.

Rajapaske, N.C. and J.W. Kelly. 1992. Regulation of chrysanthemum growth by spectral filters. J. Amer. Soc. Hort. Sci. 117(3):481–485.

Rajapakse, N.C., R.E. Young, M.J. McMahon, and R. Oi. 1999. Plant height control by photoselective filters: Current status and future prospects. HortTechnology 9(4):618–624.

Sass, J.E. 1958. Botanical microtechnique. Iowa State Univ. Press, Ames.

Starman, T.W., J.W. Kelly, and H.B. Pemberton. 1989. Characterization of ancymidol effects on growth and pigments of *Helianthus annuus* cultivars. J. Amer. Soc. Hort. Sci. 114:427–430.