Interpretation of Light Quality Measurements and Plant Response in Spectral Filter Research

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Abstract. Experiments were conducted to correlate the response of chrysanthemum [*Dendratema × grandiflorum* (Ramat.) Kitamura] plants to light environment based on various quantitative light quality parameters by growing plants under 6% or 40% CuSO₄ and water spectral filters. Using a narrow band width (R = 655-665 and FR = 725-735 nm) or a broad band width (R = 600-700 and FR = 700-800 nm) for R : FR ratio calculation, 6% CuSO₄ filter transmitted light with a higher R : FR ratio than 40% CuSO₄ or water filters. Light transmitted through 40% CuSO₄ and water filters had similar narrow band R : FR ratios (≈ 1.2), but the broad band R : FR ratio (2.0) of 40% CuSO₄ filter was higher than that of water filters. The estimated phytochrome photoequilibrium (ϕ) value varied considerably with the photochemical properties of phytochrome used for estimations. Final height and internode length of plants grown in 6% or 40% CuSO₄ chambers was $\approx 30\%$ less than of plants in corresponding control chambers. Leaf and stem dry weights were reduced by light transmitted through CuSO₄ filters. The results suggest that broad band R : FR ratio correlated more closely to above plant responses than the narrow band R : FR ratio. Blue (B) : R and B : FR ratios (not absolute amount of blue wavelengths) correlated well with plant response, suggesting that involvement of blue light should not be ignored in expressing plant response to light transmitted through CuSO₄ filters. At present, the presentation of complete spectral data would be the most useful in explaining plant response to light environment.

Despite the increasing interest in the physiological responses of plants to variations in light quality, comprehensive agreement on a numerical parameter for correlating plant response and light quality has not emerged from the literature. The most complete method would be the presentation of the entire spectral profiles, but this method lacks the conciseness and precision of quantitative measurements such as R : FR ratio, estimates of phytochrome photoequilibrium (ϕ) [i.e., the amount of phytochrome present in FR absorbing form (P_{fr}) relative to total phytochrome (P_{tot})], or phytochrome cycling rate (i.e., phytochrome interconversion relative to specific spectral quality and irradiance). At present, the numbers being used to compare plant responses to light environment vary considerably and rely on the preference of the researcher.

The R : FR ratio for a given light source varies with the wavelengths selected to represent R and FR measurements; a wide range of wavelengths has been used for R : FR

ratio calculations in the literature. Some researchers have used narrow wavelengths for R : FR ratio calculations [i.e., R = 655-665 nm, 660 nm and FR = 725-735 nm, 730 nm (Smith, 1982); R = 640-650 nm and FR = 725-735 nm (Kasperbauer et al., 1963)], while others have used broad wavelengths [i.e., R = 600-700 nm and FR = 700-800 nm (Mortensen and Stromme, 1987)].

Some researchers suggest that the R : FR ratio of a light source is less accurate in correlating plant response to light quality than estimates of in vivo ϕ because of the interacting effects of wavelengths (350 to 850 nm) in phytochrome photoconversion (Kelly

and Lagarias, 1985; Sager et al., 1988). The estimates of ϕ are derived from measured optical properties of purified phytochrome over a broad spectrum (i.e., from 350 to 850 nm). Methods for estimating ϕ from spectroradiometric data have been described by several researchers (Gardner and Graceffo, 1982; Kelly and Lagarias, 1985; Sager et al., 1988). Smith and Holmes (1977), however, suggested that ϕ can be accurately estimated from R : FR ratio, because established ϕ is mainly related to the energy in R and FR wavelengths. Pratt and Briggs (1966), on the other hand, have shown that phytochrome undergoes absorbance changes in blue wavelengths, indicating that phytochrome transformation may be sensitive to blue wavelengths of the spectrum. Because of the variations in R : FR ratio and ϕ , it is difficult to select a numerical quantifier for correlating plant response to a given light environment.

The difficulties in correlating quantitative light quality parameters and plant response emerged as a result of experiments conducted to explore the use of spectral filters for plant growth regulation as an alternative means to the use of chemical growth regulators. In our previous work, light transmitted through 6% CuSO₄ filters produced short, compact plants similar in appearance to plants treated with chemical growth regulators, such as daminozide (Rajapakse and Kelly, 1991). However, light measurements under several CuSO₄ solutions indicated that the spectral quality and quantitative light quality parameters varied with CuSO₄ concentration.

The objectives of this experiment were to determine if chrysanthemum plants grown under 6% and 40% CuSO₄ filters (which gave different spectral characteristics of transmitted light) have similar morphology and to correlate the response of chrysanthemum to several quantitative light quality parameters.

Determination of spectral quality. The spectral energy flux (350 to 850 nm in 5-nm increments) of sunlight transmitted through double-layered polycarbonate panels (with 6 × 11-mm channels) filled with 0% [water (control)], 2%, 4%, 6%, 8%, 10%, 15%, 20%, 30%, or 40% CuSO₄·5H₂O solutions was measured on a clear day between 1200

Fig. 1. Spectral distribution curves for 0% (control), 2%, 6%, 10%, 20%, and 40% CuSO₄ filters.

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Table 1. Variation in red : far red (R : FR) ratio, as influenced by band width, and blue : red (B : R) and B : FR ratios of light transmitted through CuSO₄ filters.

CuSO ₄ concn (%)	R : FR ^z				
	NB1	NB2	BB	B : R ^y	B : FR ^x
0	1.1	1.2	1.1	0.7	0.9
2	1.9	2.1	2.1	1.0	2.0
4	2.8	3.4	3.3	1.2	4.1
6	4.0	5.0	5.2	1.4	7.7
8	4.7	6.1	6.4	1.7	11.6
10	4.7	6.3	6.7	2.1	14.0
15	3.5	5.6	6.0	3.2	19.1
20	1.7	2.7	3.0	3.8	14.0
30	1.3	1.7	2.8	7.7	18.6
40	1.1	1.3	2.0	11.4	20.9

^zNB1 = narrow band R : FR ratio using R = 655-665 nm and FR = 725-735 nm (Smith, 1982). NB2 = narrow band R : FR ratio using R = 640-650 nm and FR = 725-735 nm (Kasperbauer et al., 1963). BB = broad band R : FR ratio using R = 600-700 nm and FR = 700-800 nm (Mortenson and Stromme, 1987).

^yB : R ratio using B = 400-500 nm and R = 600-700 nm.

^xB : FR ratio using B = 400-500 nm and FR = 700-800 nm.

Table 2. Variation in estimated phytochrome photoequilibrium (ϕ) of light transmitted through CuSO₄ filters using different photochemical properties of phytochrome.

CuSO ₄ concn (%)	ϕ^z		
	SSEC	K&L	G&G
0	0.72	0.71	0.55
2	0.77	0.76	0.61
4	0.80	0.79	0.65
6	0.81	0.80	0.67
8	0.81	0.80	0.69
10	0.80	0.80	0.70
15	0.79	0.78	0.70
20	0.76	0.75	0.67
30	0.73	0.72	0.65
40	0.70	0.70	0.63

^zSSEC, D&L, and G&G represent ϕ values estimated by Sager et al. (1988), Kelly and Lagarias (1985), and Gardner and Graceffo (1982) methods, respectively.

Table 3. Influence of control (cont.) and CuSO₄ filters on leaf area and internode length.

CuSO ₄ concn	Plant Internode		
	Shade (%)	ht (cm)	length (cm)
0 (cont. 1)	37	34.8	1.8
(cont. 2)	58	32.3	1.6
6%	37	24.4	1.2
40%	58	21.6	1.1
Contrasts ^a			
6% CuSO ₄ vs. cont. 1		***	***
40% CuSO ₄ vs. cont. 2		***	***
Cont. 1 vs. cont. 2		*	*
CuSO ₄ 6% vs. 40%		**	*

^aSingle-degree-of-freedom contrasts.

*, **, ***Significant at $P = 0.05$, 0.01 , or 0.001 , respectively.

and 1400 HR with a LI-1800 spectroradiometer fitted with a LI-1800-10 remote cosine sensor (LI-COR, Lincoln, Neb.). The procedure was repeated on another clear day. Photosynthetic photon flux (PPF) of transmitted light was determined as photon flux (PF) integrated between 400 and 700 nm. Percent shading (in 400-700 nm range) by CuSO₄ solutions was determined by comparing them with irradiance under the water (control) panel. The R : FR ratio of transmitted light was determined as the ratio of PF between 655 and 665 nm (R) and 725 and 735 nm (FR) (narrow band R : FR) and as the ratio of PF between 600 and 700 nm (R) and 700 and 800 nm (FR) (broad band R : FR). In addition, blue : red (B : R) and blue : far red (B : FR) ratios were determined as ratio of PF between wavelengths 400 and 500 nm (B), 600 and 700 nm, 400 and 500 nm, and 700 and 800 nm, respectively. Phytochrome photoequilibrium under spectral filters was estimated using spectroradiometric data and phytochrome properties described by Gardner and Graceffo (1982), Kelly and Lagarias (1985), and Sager et al. (1988). Gardner and Graceffo (1982) used etiolated corn (*Zea mays* L.) coleoptiles to determine an in vivo action spectrum for phytochrome transformation, and ϕ was determined as the sum of the product of photon fluence rate (PFR) of the light source and the relative quantum efficiency of phytochrome photo-transformation. Kelly and Lagarias (1985) and Sager et al. (1988) used purified phytochrome from etiolated oat and rye (*Secale cereale* L.) tissues to determine in vitro action spectra, and ϕ was determined as the sum of the product of PFR and the photochemical cross sections of phytochrome.

Evaluation of plant growth. Rooted 'Bright Golden Anne' chrysanthemum shoot cuttings with three to four leaves (Yoder Bros., Pendleton, S.C.) were planted (Sept. 1990) in 0.6-liter square plastic pots containing a commercial potting mix (Mix 3B, Fafard Inc., Anderson, S.C.). After a 1-week establishment period, plants were transferred to four growth chambers, one each with 6% or 40% CuSO₄·5H₂O and two with water (control) "fluid roofs" (Rajapakse and Kelly, 1992) placed inside a glass greenhouse. Light transmission into the chamber only occurred through the fluid roof. PPF under 6% and 40% CuSO₄ filters was reduced by $\approx 37\%$ and 58% , respectively, of natural light in the greenhouse, which averaged $\approx 1500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. A neutral shading material (cheesecloth) was used in each control to ensure the same PPF level as in the corresponding CuSO₄ chamber. Plants were irrigated once daily with 18N-3.5P-5K mM from Peter's 20-20-20 fertilizer (W.R. Grace, Foggsville, Pa.). Average daily maxima inside 6% and 40% CuSO₄ chambers were $30 \pm 4^\circ\text{C}$ and $27 \pm 3^\circ\text{C}$, respectively, and the minimum was $18 \pm 4^\circ\text{C}$ for both.

At the end of the 4-week growing period, plant height (height from soil level to apex), number of fully expanded leaves, and leaf and stem dry weights were recorded. For dry

weight measurements, tissue was oven dried at 85°C for 48 h. Average internode length was estimated as plant height/number of leaves.

The experiment was repeated once. Four light treatments were randomly assigned to chambers with five plants per treatment. Data were subjected to analysis of variance. Differences among means were tested using single-degree-of-freedom contrasts.

CuSO₄ solutions reduced both R (600-700 nm) and FR (700-800 nm) wavelengths of transmitted light, but the reduction of FR was much greater than the reduction of R wavelengths (Fig. 1). Increasing CuSO₄ concentration from 0% to 10% reduced transmission of most of the FR wavelengths. Further increase in CuSO₄ concentration (from 10% to 40%) reduced transmission of R wavelengths. Photon flux in the B wavelengths (400-500 nm) was relatively unaffected by CuSO₄ filters.

R : FR ratio varied considerably with the band width selection (Table 1). R : FR ratio under CuSO₄ filters was slightly higher when calculated using broad band widths than narrow band widths. Both narrow and broad band R : FR ratios increased as CuSO₄ concentration increased from 0% to 10% due to relatively greater reduction of FR wavelengths of transmitted light. Further increase of CuSO₄ concentration reduced R : FR ratio due to continued reduction of R wavelengths. The narrow band R : FR ratio of light under 40% CuSO₄ filter was similar to that of the control (water) due to reduction of R wavelengths above 640 nm; the broad band R : FR ratio, however, was slightly higher under 40% CuSO₄ than under the control. B : R and B : FR ratios increased as the concentration of CuSO₄ increased because of the relatively unchanged PF in B wavelengths and large reductions of R and FR wavelengths of transmitted light with increasing concentration (Table 1). The highest B : R or B : FR ratios were under 40% CuSO₄ filter.

In general, the estimated ϕ light transmitted through CuSO₄ filters increased as concentration increased from 0% to $\approx 10\%$ and decreased thereafter (Table 2). The estimated values of ϕ using the methods described by Sager et al. (1988; SSEC) and Kelly and Lagarias (1985; K&L) were not significantly different from each other but higher than the values estimated using the Gardner and Graceffo (1982; G&G) method. The estimated ϕ for light transmitted through 40% CuSO₄ filter (0.70) was slightly lower than that for the control (water) filter (≈ 0.71) when calculated using the SSEC or K&L methods; however, when calculated using the G&G method, light transmitted through 40% CuSO₄ filter had a higher estimate for ϕ (0.63) than the control filter (0.55). Generally, estimated values of ϕ were slightly higher when calculated by using the method described by SSEC or K&L than by that of G&G.

In our previous experiments, we noted that the plants grown under CuSO₄ filters (4%, 6%, 8%, or 16% CuSO₄) were significantly

Table 4. Influence of control (cont.) and CuSO₄ filters on leaf and stem dry weights. Number in parentheses indicates the percentage of total dry weight accumulated in leaves or stems.

CuSO ₄ concn	Shade (%)	Dry wt (g)		
		Total	Leaf	Stem
0 (cont. 1)	37	3.5	2.3 (66)	1.2 (34)
(cont. 2)	58	3.1	2.2 (71)	0.9 (29)
6%	37	2.5	2.0 (80)	0.5 (20)
40%	58	1.9	1.5 (79)	0.4 (21)
Contrasts ²				
6% CuSO ₄ vs. cont. 1		***	**	***
40% CuSO ₄ vs. cont. 2		***	***	***
Cont. 1 vs. cont. 2		*	NS	**
CuSO ₄ 6% vs. 40%		***	***	**

²Single-degree-of-freedom contrasts.

NS, *, **, ***Nonsignificant or significant at $P = 0.05$, 0.01 , or 0.001 , respectively.

shorter than plants grown under water filters (Rajapakse and Kelly, 1991, 1992). Light transmitted through 4%, 6%, 8%, or 16% CuSO₄ filters had higher narrow or broad band R : FR ratios and higher estimates for ϕ (using any of the above methods) than the control; therefore, plant height reduction under these filters was attributed to a higher R : FR ratio and higher ϕ . Spectral data in this study indicated that light transmitted through 40% CuSO₄ filter had a similar narrow band R : FR ratio and slightly lower ϕ than that of the control when calculated using the method of SSEC or K&L. However, broad band R : FR ratio and estimated ϕ using the G&G method were higher for light transmitted through 40% CuSO₄ than the control filter. Because of the variability in these quantitative estimates for light quality under 40% CuSO₄ and control filters, we decided to compare the morphology of chrysanthemum plants grown under 40% CuSO₄ to those grown under 6% CuSO₄, which has already been shown to alter morphology (Rajapakse and Kelly, 1991).

Final height of plants grown under 6% and 40% CuSO₄ filters was $\approx 30\%$ and 33% less than that of plants grown under corresponding control filters (Table 3). A height difference between plants grown under CuSO₄ and control filters was visible within 1 week after treatment and the difference increased as exposure time increased (data not shown). Plants receiving more PPf were significantly taller than plants receiving lower PPf in both control and CuSO₄ treatments.

There were 19 or 20 leaves per plant, regardless of treatments (difference NS at $P = 0.05$). Light under 6% and 40% CuSO₄ filters resulted in about 31% and 33% reduction in average internode length, respectively.

Total shoot dry weight was reduced under CuSO₄ filters compared to corresponding control filters. Total dry weight reduction (39%) under the 40% CuSO₄ filter was greater than that under the 6% CuSO₄ filter (28%) (Table 4). Both leaf and stem dry weights were reduced by light in CuSO₄ chambers compared with the corresponding control. The reduction of stem dry weight was greater than the reduction of leaf dry weight. Leaf dry weight reduction (32%) was greater under 40% CuSO₄ filter than under 6% CuSO₄ filter (13%). Stem dry weight reduction was

basically unchanged for the 6% and 40% CuSO₄ filters (56% and 58%, respectively). CuSO₄ filters reduced percent dry matter accumulation into stems (from $\approx 31\%$ to $\approx 20\%$) and increased it into leaves (from $\approx 70\%$ to $\approx 80\%$) (Table 4).

Light transmitted through 6% or 40% CuSO₄ filters had similar effects on plant morphology. In our previous experiments, we showed that 6% CuSO₄ spectral filters reduced plant height, internode length, growth rate, and total dry weight of chrysanthemum. These responses were attributed to the high R : FR ratio and ϕ of transmitted light (Rajapakse and Kelly, 1992). Light high in R wavelengths shifts ϕ toward production of more P_{fr} , which is assumed to be the active form in controlling plant responses. Light high in R relative to FR wavelengths has been reported to reduce stem elongation of maize (Vanderhoef et al., 1979) and reduce stem dry weight of tomato (*Lycopersicon esculentum* Mill.) (Hurd, 1974).

Although most of the research on light quality and plant responses has focused on R : FR ratios and ϕ , involvement of B light in regulation of plant response under CuSO₄ filters cannot be neglected. Blue light has been shown to reduce stem elongation, and FR light in combination with B light has been shown to reverse B-light-induced responses (Kadman-Zahavi and Ephrat, 1976). Red light, in the absence of B, may not be effective in reducing stem elongation (McMahon et al., 1991). Therefore, B light, acting on the phytochrome system or through a blue light receptor, might have elicited the observed reduction in plant height and internode length under the CuSO₄ filter. CuSO₄ filters used in this study did not significantly alter PF in B wavelengths (400-500 nm). Because of the relatively unchanged B wavelengths and continued reduction of R and FR wavelengths, ratios of B : R and B : FR of transmitted light increased as the CuSO₄ concentration increased from 0% to 40%; light under 40% CuSO₄ had the highest B : R or B : FR ratio (Table 1). The morphological differences observed under 6% and 40% CuSO₄ filters could be better explained (higher correlation) by the higher B : R or B : FR ratios of transmitted light under these filters. In this study, both control and CuSO₄ filters had about the same amount of PF in B wave-

lengths, but the amount of B wavelengths relative to R or FR wavelengths was much higher under CuSO₄ filters. The amount of B wavelengths relative to R or FR wavelengths in light possibly may be more important in regulating the plant response than the absolute amount of B wavelengths received by the plant.

R : FR red ratio and estimates of ϕ in a given light environment vary considerably with the wavelengths and the photochemical properties of phytochrome used in the calculations. Based on narrow band R : FR ratio or ϕ estimated by the Sager et al. (1988) or Kelly and Lagarias (1985) method, one would expect no difference in plant height between 40% CuSO₄ and the control filter. However, 40% CuSO₄ filters reduced plant height and internode length, although narrow band R : FR ratio and ϕ values were similar to those of the control filter. This result suggests that these two quantitative light quality parameters did not correlate well with the plant response observed under 40% CuSO₄ filter. The broad band R : FR ratio and ϕ estimated using the Gardner and Graceffo (1982) method, however, were better correlated with the morphological differences observed between control and CuSO₄ filters. The data also suggest that involvement of B light should not be ignored in expressing plant response to light quality.

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