

Development of Chrysanthemum Meristems Grown under Far-red Absorbing Filters and Long or Short Photoperiods

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ABSTRACT. Two chrysanthemum [*Dendranthema ×grandiflorum* (Ramat) Kitamura] cultivars, 'Spears' and 'Bright Golden Anne', were grown under artificial short or natural long photoperiod in benchtop chambers covered with clear double-walled acrylic panels (control) or under similar panels filled with CuSO₄ (CuSO₄·5H₂O in solution at 6% w:v) that removed far-red (FR) (700 to 800 nm) light. Three times per week, a tip from one lateral branch from each of three plants per chamber was harvested and the stage of meristem development recorded. The experiment was conducted April through May and repeated May through June. For 'Spears' all short photoperiod treatments developed floral primordia at the same time and the rate of development did not differ. All plants in natural photoperiod treatments initiated flower primordia simultaneously with plants in short photoperiod treatments, but development was delayed ≈3 d in the first experiment compared to plants receiving short photoperiods. During the longer photoperiods of the second experiment, plants under FR-absorbing filters and receiving natural photoperiods initiated and developed flowers ≈2 d after plants in short photoperiod treatments initiation and development. Plants under control filters and natural photoperiods had initiation delayed by ≈4 d and development was delayed by ≈11 d. Bud development was normal for all treatments. For 'Bright Golden Anne' only short photoperiod treatments developed normal floral primordia. Plants under FR-absorbing filters and exposed to natural photoperiods eventually initiated floral primordia but development was abnormal. No floral primordia developed under natural photoperiod and control filter conditions. The results indicate that if FR-absorbing filters are used to regulate height of chrysanthemum and possibly other photoperiodic plants, the time of flowering may be affected. However, if artificial short photoperiods are imposed with the use of blackout cloth, FR-absorbing filters do not affect flowering response.

Photosensitive filters that absorb far-red (FR) (700 to 800 nm) light show promise as a nonchemical means of controlling stem elongation and improving the appearance and salability of many floral crops (McMahon et al., 1991, vanHaering et al., 1998). However, the filters have been shown to affect the photoperiodic response of chrysanthemum [*Dendranthema ×grandiflorum* (Ramat) Kitamura] and possibly other photoperiodic plants. For chrysanthemum, the effect appears to vary with season and cultivar. Flowering of the chrysanthemum cultivar 'Spears' a commercially important cultivar, is accelerated under FR-absorbing filters in natural photoperiod conditions (June), and apparently unaffected by the filter in artificial short photoperiod conditions (McMahon et al., 1991). Similar results have been observed for the 'V-14 Glory' poinsettia (*Euphorbia pulcherrima* Willd.) (McMahon and Kelly, 1998). For chrysanthemum 'Bright Golden Anne', another commercially important cultivar, flowering is delayed under FR-absorbing in natural short photoperiods (Rajapakse and Kelly, 1995). 'Spears' flowering is not affected by FR-absorbing filters when artificial short photoperiods or long nights are superimposed on the filter treatments (McMahon and Kelly, 1999). To be useful as a growth regulating technique, the

effect that FR-absorbing filters have on chrysanthemum flowering must be understood.

Chrysanthemum is a facultative short day plant. Flowering is uniform in photoperiods at or shorter than the critical length of ≈14.5 h (Furuta, 1954). Chrysanthemums will eventually become reproductive under longer photoperiods, but flowering is not uniform and often the buds and flowers do not develop normally. Furuta (1954) showed that flower development requires a shorter photoperiod, ≈13.5 h, than initiation. Development is most rapid when photoperiods are 12 h. Photoperiods less than 12 h induce flowering but if the photoperiod is not sufficient to meet photosynthetic requirements of the plant, growth and development could be negatively affected.

Cultivars vary in critical photoperiod requirement but the reports are not in agreement on why this may occur. Furuta's work and that of others (Langhans, 1964; Post, 1948) indicate that cultivars that take longer to flower after the start of short days (long response) require a shorter daily photoperiod than cultivars that take less time to develop flowers (short response). Cockshull and Kofranek (1985) did not see a correlation between cultivar response and photoperiod requirement. However, the experimental conditions between the studies were different. Furuta used day extensions to create long photoperiods (light source not stated), Cockshull and Kofranek used night breaks from incandescent lamps. Incandescent lamps have FR > red (R) (600 to 700 nm) whereas other light sources such as the sun (except for brief periods at the beginning and end of the day), and fluorescent and high intensity discharge lamps have FR < R. The timing of the light exposure and possibly the source could produce the dissimilar results because the effects of R and FR on flowering of short day plants vary with the timing of exposure. The inhibition of

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chrysanthemum flowering by a night break of R is reversed when immediately followed by FR (Cathey and Borthwick, 1957). Conversely, the flowering of chrysanthemums grown in an 8 h photoperiod of natural light are inhibited more by an end-of-day (EOD)-FR treatment than EOD-R (Kadman-Zahavi and Ephrat, 1973). It is possible that depending on timing of light exposure and the relative amounts of R and FR in the light source, cultivar sensitivity to photoperiod length may or may not be observed.

Far-red absorbing filters create a unique growing environment for plant growth and development. Few, if any, natural or artificial light conditions mimic conditions under FR-absorbing filters, i.e., nearly full spectrum photosynthetic photon flux (PPF) at near full-sun intensity, but little or no FR. It is difficult to predict from the literature how the light environment created by FR-absorbing filters will relate to studies, including cultivar response group sensitivity, where artificial light sources were used to alter the light environment.

The factor of response group sensitivity is built into the photoperiodic timing schedules developed by Yoders (1997) and others for chrysanthemum production. To be useful as a growth regulating technique, the effect that FR-absorbing filters has on the flowering of different chrysanthemum cultivars from different response groups must be understood.

It was not known if the reported differences in photoperiodic response under FR-absorbing filters of 'Spears' (8 week response) and 'Bright Golden Anne' (10 week response) resulted

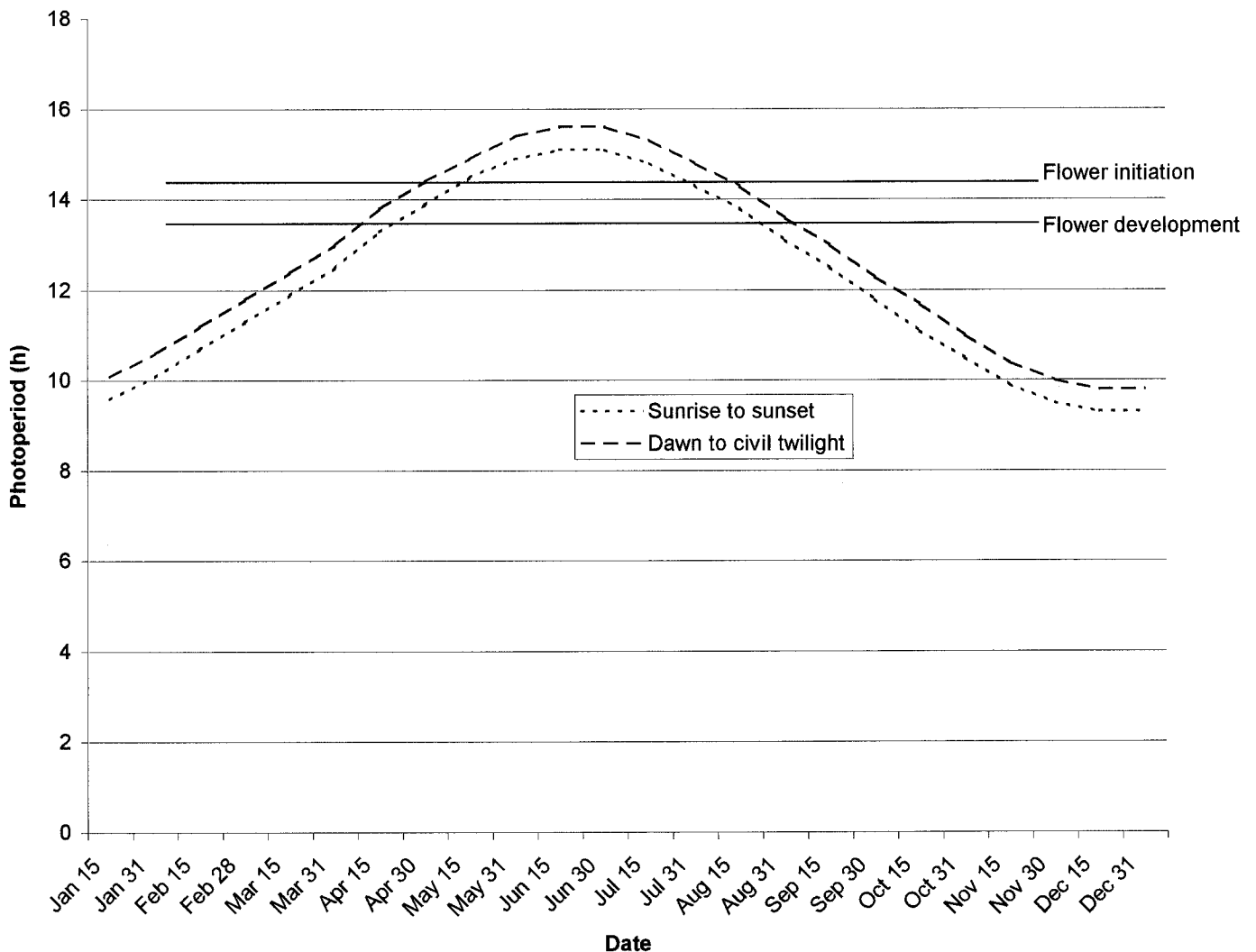
from the different seasonal or experimental conditions under which experiments were conducted or from cultivar response differences. Therefore the following experiments were conducted to study the effects of FR-absorbing filters on meristem change and development of both 'Spears' and 'Bright Golden Anne' under identical photoperiod and seasonal conditions.

Materials and Methods

Rooted cuttings of 'Spears' and 'Bright Golden Anne' (Yoder Bros., Inc., Barberton, Ohio) were planted in 11-cm pots in potting mix (Metro Mix 360, Scotts Co., Marysville, Ohio) on 22 Mar. in greenhouses located at the Ohio Agriculture Research and Development Center in Wooster, Ohio. The plants were grown under natural photoperiods and provided with a 3-h night break 2200 to 0100 HR. Plants were pinched to six nodes on 29 Mar. and continued to receive night breaks until 17 Apr. At that time, the lateral shoots were ≈ 3 cm and plants were placed in eight chambers placed on greenhouse benches.

The tops of four chambers were constructed from clear double-walled 8-mm-thick acrylic panels which were sealed at the

Fig. 1. Hours of daylight throughout the year from sunrise to under cloudless conditions for Wooster, Ohio, W081 56, N40 49, hours of daylight including 30 min of civil twilight, and critical daylengths for chrysanthemum flower initiation and for flower development.



bottom held vertically and filled with CuSO_4 ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in solution at 6% w:v) which absorbs $\approx 25\%$ radiation in the 500 to 600 nm range and $\approx 95\%$ radiation between 700 and 800 nm (Rajapkse et al., 1992). The panels were placed on the tops of 75×75 cm frames. The height of the frames was 60 cm at one end and 55 cm at the opposite end giving rise of $\approx 4^\circ$ to the panel to avoid spillage of the solution. The top panel overhung each side by several centimeters. The sides of the frames were covered with a double layer of polyethylene film, white on the inside for maximum reflection and black on the outside to prevent light contamination from nearby chambers or unfiltered light. Four additional chambers were constructed of the same materials but the acrylic panel was not filled with fluid. Earlier experiments had shown that there were no differences in chrysanthemum growth and development under clear filters that were empty or filled with water (McMahon et al., 1991).

The benches had expanded metal tops and the chambers were constructed to allow airflow through the bench tops and out the top of the chambers through a 5-cm gap between the panel and the top of the side covering. This construction kept temperatures in the chambers to near greenhouse temperatures. Light quality in the chambers was not detectably altered by having the bottoms of the chambers open and a gap between the top panel and the sides. The chambers were ≈ 25 cm apart on the benches. The distance between benches was ≈ 50 cm.

Photosynthetic photon flux was adjusted to similar levels within all chambers by placing cheesecloth over the clear chambers. Ambient greenhouse PPF was reduced $\approx 10\%$ from outside. Reduction in PPF in the chambers was $\approx 15\%$ from the ambient greenhouse level. Outside light intensity at noon on a clear day in Ohio is $\approx 1100 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in March and $\approx 1700 \text{ } \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in June. Two of each type of chamber received natural photoperiods, the other two of each type were covered with opaque blackout cloth from 1730 to 0730 HR to provide short photoperiods making a total of two replications of four treatments.

Plants were fertilized 3 times per week with a solution containing $1.25 \text{ g} \cdot \text{L}^{-1}$ of a water soluble 20N–8.6P–16.6K fertilizer (The Scotts Co., Marysville, Ohio). Plants were watered as necessary between fertilizations. The chambers were located in a glass-covered greenhouses cooled by top and side ventilators and heated with hot water circulating through overhead and peripheral lines. Temperature control set points were 23°C day and 18°C night. Night temperatures in the greenhouse were maintained near set point. Day temperatures in the greenhouse were elevated when outside temperatures rose above 23°C , reaching $\approx 30^\circ\text{C}$ during midafternoon on some clear days near the end of the final experiment. Temperatures in the chambers were 1 to 2°C above greenhouse temperatures on sunny days and equal to greenhouse temperatures during the night and under cloudy conditions. Temperatures in chambers under the black cloth were 3 to 4°C higher than greenhouse temperatures during sunny evenings.

This experiment was terminated when buds were visible or bud development appeared to be nearly static within treatments for each cultivar i.e., 5 May for 'Spears' and 20 May for 'Bright Golden Anne'. The experiment was repeated on 17 Apr., with the pinch made 24 Apr., and plants placed in the chambers on 6 May. The experiment was terminated on 3 June for 'Spears' and 30 June for 'Bright Golden Anne'.

Sunrise and sunset for Wooster, Ohio, W081 56, N40 49, was 0545 and 1910 HR, respectively, on 17 Apr. and 0500 and 2003 HR, respectively, on 30 June (Fig. 1). All times given are Eastern Standard Time. Civil twilight adds day length before sunrise

(dawn) and after sunset. Civil twilight is defined as that time from sunset until the sun is 6° below the horizon and terrestrial objects are clearly visible. (U.S. Naval Observatory, Washington, D.C.). Civil twilight adds ≈ 24 min of daylength to the beginning and ending of the day. It is not stated by the Naval Observatory what the actual light intensity is during twilight, however it is likely that the amount of light at least for part of the period is above 1 to $2 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ which is the threshold for most photoperiodic responses (Bjorn, 1986). Actual daylength varies with cloud cover (Post, 1948).

Three lateral branch tips were harvested from each chamber (each tip from a different plant) on Mondays, Wednesdays, and Fridays starting the day after placement in the chambers for the first experiment, and 2 d after placement for the repeated experiment. The meristems were placed in a preservative solution of formalin, acetic acid, and 95% ethanol (1 : 1 : 18, by vol.) then examined under a dissecting microscope at $40\times$. Stage of flower development (0–10) was recorded for each tip. Description of stages of development are from Cathey and Borthwick (1957) and are as follows:

- 0) Stem terminal flat, typical of vegetative condition.
- 1) Stem terminal slightly enlarged.
- 2) Stem terminal forming capitulum; first bracts of receptacle present.
- 3) Capitulum spherical with twelve or more bracts around its rim.
- 4) Capitulum becoming flattened; many bracts but no floret primordia present.
- 5) Two or more rows of floret primordia on rim of receptacle.
- 6) About 6 rows of floret primordia on capitulum.
- 7) Capitulum covered with floret primordia except at tip.
- 8) Entire capitulum covered with floret primordia.
- 9) A few floret primordia not yet showing beginnings of perianth.
- 10) Perianth primordia present on all florets.

The average stage of development from each treatment replication was pooled for each day's harvest and standard error calculated for each treatment.

Results

When the experiment was conducted in early spring, 'Spears' in all treatments had reached stage 1 after 8 d in chambers regardless of filter or photoperiod (Fig. 2). Plants in all treatments were at stage 5 after 13 d in the chambers. Short photoperiod plants under both filters were at stage 10 after 17 d in the chamber while natural photoperiod plants in clear chambers were at stage 8 and natural photoperiod plants in CuSO_4 chambers were at stage 9. All natural photoperiod plants were at stage 10 at 20 d.

In the repeated experiment (late spring), 'Spears' in short photoperiod treatments were at stage 2 within 10 d of the start of short photoperiods, regardless of filter (Fig. 3). Twelve d after treatments began, plants in control chambers and under natural photoperiods reached stage 1, plants in CuSO_4 chambers under natural photoperiods were at stage 3 or 4, and all plants in short photoperiod treatments were at stage 6. An approximate 2-d lag in development continued for plants in CuSO_4 and natural photoperiod conditions compared to plants in short photoperiods. For natural photoperiod plants in control chambers, development occurred but was at ≈ 11 d behind short photoperiod plants and nine days behind plants under natural photoperiods in CuSO_4 chambers. Eventually all treatments of 'Spears' developed visible, normal-appearing buds.

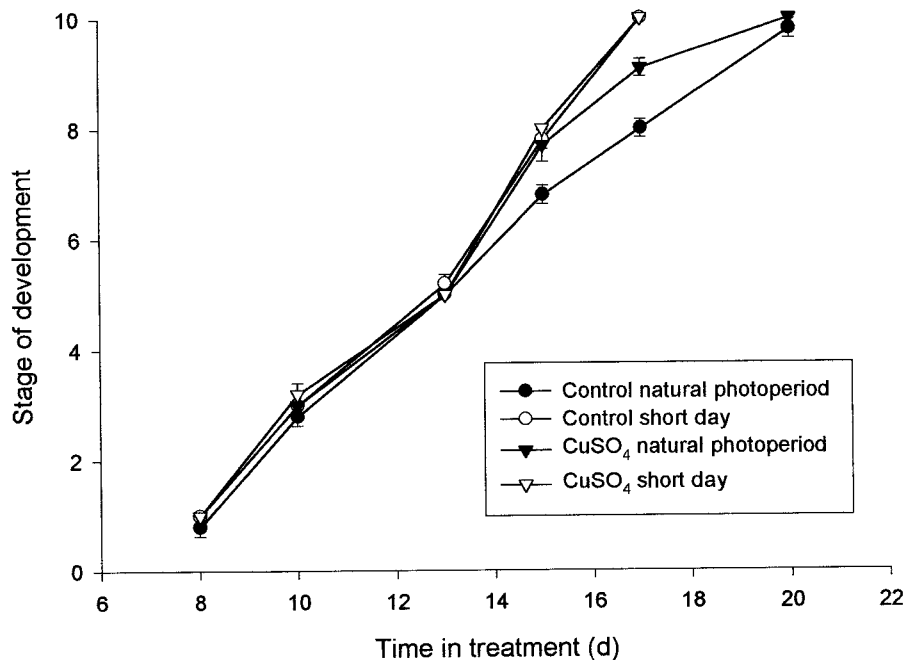


Fig. 2. Stages (1–10) (Borthwick and Cathey, 1957) of floral development of chrysanthemum 'Spears' under nonphotosensitive (control) and far-red absorbing (CuSO₄) filters in natural and artificial short (blackout cloth from 1730 to 0730 HR) photoperiods. Flower buds are visible after stage 10. Plants were placed in treatments 17 Apr. Vertical bars represent ± SE.

During the first experiment (17 Apr. to 20 May), all short photoperiod 'Bright Golden Anne' plants reached stage 1 in ≈15 d (Fig. 4). All short photoperiod plants reached stage 5 or 6 by day 27 and were at stage 10 by day 36. Plants grown in natural photoperiods under CuSO₄ filters reached stage 2 after 36 d in the treatment. Plants in control chambers and natural photoperiod conditions, did not reach stage 1. Results were similar when the experiment was repeated 17 May to 30 June, except development was ≈7 d slower for plants in short photoperiods, regardless of filter, and plants under CuSO₄ filters in natural photoperiod conditions did not develop beyond stage 1.

Discussion

'Spears', an 8 week (short) response chrysanthemum cultivar, initiated flowers simultaneously under both artificial short photoperiods and under natural daylengths considered to be photoinductive but marginally short enough for development (mid-April, ≈13.8 h) (Fig. 1) whether FR was present or absent. However, compared to plants receiving short photoperiods subsequent floral development was delayed 3 d in plants exposed to natural photoperiods regardless of the presence or absence of FR. The delay suggests that natural photoperiods during the experiment allowed uniform initiation and were sufficiently short to allow normal, if not most rapid, floral development.

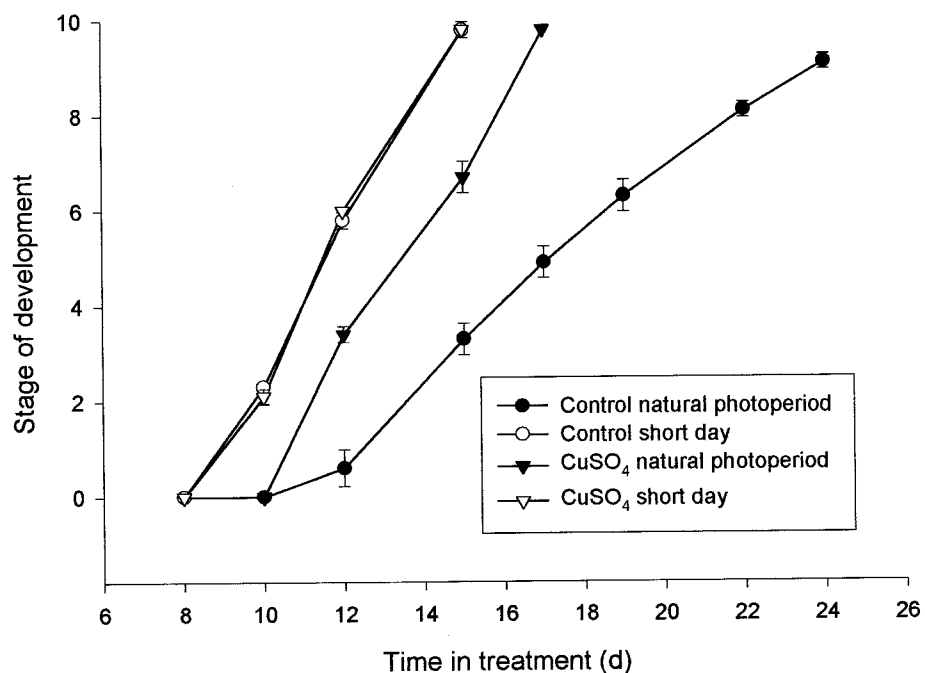
When the experiment was repeated be-

ginning 6 May, with daylength exceeding 14.5 h, flowering still occurred in all treatments for 'Spears'. However, compared to plants receiving artificial short photoperiods, plants exposed to natural photoperiods had both delayed flower bud initiation and development, with the greatest delay in plants receiving the natural light. In photoperiods that exceeded the accepted length for chrysanthemums, FR-absorbing filters promoted floral initiation and development of 'Spears' but not as effectively as artificial short photoperiods (Fig. 3). These experiments are in agreement with previous observations that 'Spears' flower sooner under FR-absorbing filters and under nonabsorbing filters in June (McMahon et al., 1991).

'Bright Golden Anne', a longer response group, did not exhibit the same response. Although floral initiation eventually occurred under natural photoperiods in a FR deficient environment in April, development was slow and probably would not have continued normally. Under longer photoperiods of late spring, initiation occurred but only stage 1 was reached and there was no indication of further development. Plants grown under natural photoperiods of early and late spring in the presence of FR light did not initiate flowers at any time. This suggests that 'Bright Golden Anne' has a greater sensitivity to critical photoperiod for initiation and development than 'Spears' which eventually flowered under the same conditions. This, too, is in agreement with Post (1948) and Furuta (1954).

The results of these experiments and others suggest that

Fig. 3. Stages (1–10) (Borthwick and Cathey, 1957) of floral development of chrysanthemum 'Spears' under nonphotosensitive (control) and far-red absorbing (CuSO₄) filters in natural and artificial short (blackout cloth from 1730 to 0730 HR) photoperiods. Flower buds are visible after stage 10. Plants were placed in treatments 6 May. Vertical bars represent ± SE.



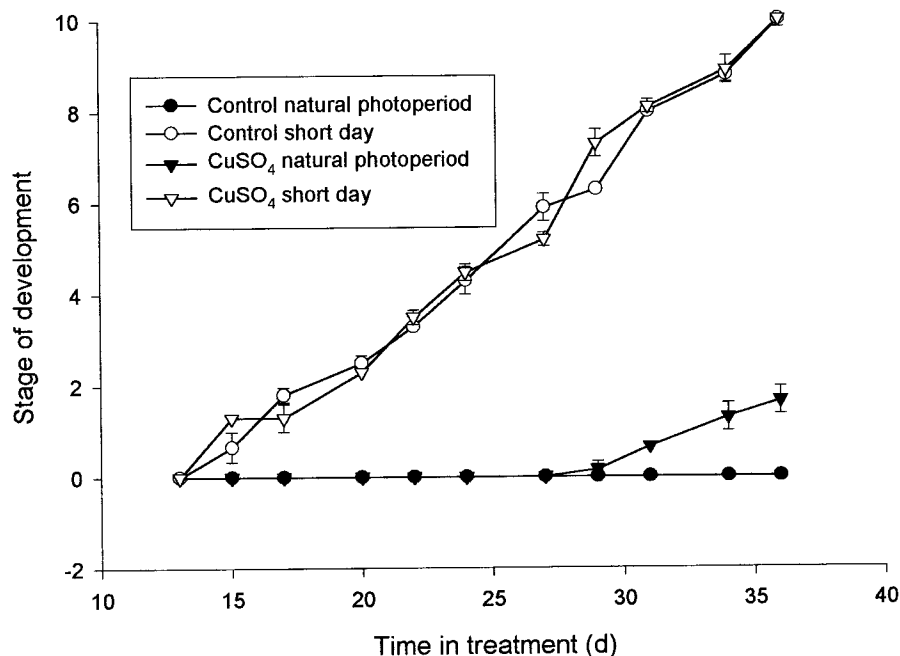


Fig. 4. Stages (1–10) (Borthwick and Cathey, 1957) of floral development of chrysanthemum 'Bright Golden Anne' under nonphotosensitive (control) and far-red absorbing (CuSO_4) filters in natural and artificial short (blackout cloth from 1730 to 0730 HR) photoperiods. Flower buds are visible after stage 10. Plants were placed in treatments 17 Apr. Vertical bars represent \pm SE.

'Spears' and perhaps other short response chrysanthemum cultivars have less sensitivity to critical daylength for flower initiation and development. They flower normally under photoperiods that inhibit flowering in long response cultivars. This could be an important factor in the production of chrysanthemums. It is possible that short response cultivars may not need blackout cloth to be applied for as much time each day as longer response cultivars. During warm and sunny conditions, heat can build up under the cloth. Night temperatures $> 27^\circ\text{C}$ can delay chrysanthemum flower development (Cathey, 1955). Shortening the daily duration of blackout cloth would reduce the heat buildup that occurs under the cloth which may prevent delayed flowering. The length of daily application may be further reduced for short response cultivars when used in conjunction with a FR-absorbing filter.

These experiments show that, regardless of cultivar, under artificial short-photoperiods time to flowering was not affected by filter. An earlier study (McMahon and Kelly, 1999) shows FR-absorbing filters do not influence the effect of night break lighting on 'Spears'. Therefore it appears that flowering schedules and

normal production techniques that have been developed for chrysanthemum may be used in conjunction with FR-absorbing filters.

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