Juvenility and Photoperiodic Flowering Requirements of *Chrysanthemum* $\times$ *superbum* 'G. Marconi' and 'Snow Lady' Grown under Short- and Long-day Conditions

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Abstract. Juvenility and flowering requirements of *Chrysanthemum* $\times$ *superbum* Bergmans ex J. Ingram 'G. Marconi' and 'Snow Lady' were examined by growing plants under short days (SDs) and transferring them to long days (LDs) upon reaching specific true leaf stages. 'G. Marconi' plants did not flower in continuous SDs and only sparse flowering occurred in plants transferred to LDs. 'Snow Lady' plants transferred from SDs to LDs at the cotyledon stage flowered fastest from seeding (75 days) and had the fewest number of main stem leaves and total leaves (9 and 15, respectively) at the time of first flower. Plants moved from SDs to LDs at the 24 true leaf stage flowered 123 days after seeding and averaged 28 leaves on the main stem and 37 total leaves at the time of first flower. To examine apical floral initiation, plants were distributed between SDs and LDs following transplanting and five plants per treatment were sampled each week thereafter. Microscopic examination revealed floral initiation in plants sampled after just 1 week in LDs and, after 5 weeks, macroscopic terminal flower buds were present. Under SDs, apical floral initiation began after 5 weeks, yet, 9 weeks were required for floral initiation in all five plants sampled.

A period of juvenility, described as a physiologically based time when a plant is insensitive to conditions that later promote floral initiation, is common in plants. For many photoperiodic herbaceous species, the passage through juvenility can be gradual, characterized by a period of increased sensitivity to daylength rather than a total inability to flower (Bernier et al., 1981). Juvenility has been quantified by determining the minimum leaf number required before the apex was capable of initiating floral organs (Purvis, 1934) and when a critical photosynthetic leaf area was formed to sustain flowering and fruiting (Schwabe, 1976; Wareing and Frydman, 1976). However, it is questionable whether foliage plays the only role in floral induction in all plants. Systematic removal of leaves, even to the extreme of leaving only the rosette stem and shoot apex intact, failed to alter the response of *Eschscholzia californica* to long-day (LD) induction, an indication that a specific node number may affect floral induction (Lyons and Booze-Daniels, 1986). In addition, Lyons and Neale (1983) identified a negative linear relationship between unfolded leaf stage and rapidity of flowering in *E. californica*. Flowering occurred most rapidly in *E. californica* (Lyons and Booze-Daniels, 1986). *Coreopsis lanceolata* 'Early Sunrise' (Damann and Lyons, 1993). *Rudbeckia hirta* 'Marmalade', and *Gaillardia pulchella* (Bourke, 1990) when 10, 16, 19, and 20 expanded true leaves, respectively, were present at the start of LDs.

Flowering status can be a simple, judgmental determination; a plant is visibly flowering or it is not. However, flowering is not a simple process and additional factors need to be considered. For instance, a plant may be induced to flower by two different treatments, flowering profusely under one, while only sparsely under the other. The rate of reproductive development can also be affected by different photoperiods. Due to the complexity of quantifying flowering, several techniques have been used, includ-

ing calculating the percentage of plants flowering, counting the number of unopened reproductive buds or terminal or axillary flowers per plant, recording the rate of development until flowering occurs, and observing apical development microscopically (Metzger, 1988).

Chrysanthemum $\times$ *superbum* (shasta daisy) cultivars are relatively insignificant to commercial floriculture, yet they have a perceived market potential as evidenced by rising exposure in garden catalogs and perennial flower guide. Reports concerning the role of photoperiod in *C. xsuperbum* flowering remain inconsistent. Flowering was hastened by increased day length (Kofranek, 1952; Laurie and Poesch, 1932), and individual cultivars were later found to be either obligate LD plants (Griffin and Carpenter, 1964) or in need of a chilling period to induce flowering (Shedron and Weiler, 1982). There is also some confusion about the flowering requirements of individual cultivars, indicating a need for research so that flowering may be better manipulated and scheduled.

The objectives of the present study were to quantify the end of juvenility in *C. xsuperbum* 'G. Marconi' and 'Snow Lady', describe vegetative and reproductive responses to photoperiod treatments, and document the apical events during floral initiation in *C. xsuperbum* 'Snow Lady' grown in continuous short day (SD) and LD conditions.

Materials and Methods

Seeds were sown in June in a medium of 2 vermiculite: 1 peatmoss (v/v) and placed under intermittent mist and a natural photoperiod at a constant 19 to 21°C day and night. Upon germination, misting ended and all seedlings were moved to SDs held predominantly at 16 to 18°C nights with the assistance of fan and pad cooling. SDs were created by covering the plants from 1700 to 0800 HR with 100% light-exclusion, black sateen cloth. LDs were created by night interruption from 2200 to 0200 HR with 60-W incandescent bulbs strung overhead to provide 3 to 4 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetic photon flux density (PPFD) at plant height. Plants were fertilized weekly during irrigation with 400 mg N/liter from 20N-6.6P-17.6K.

Juvenility and flowering requirements of C. xsuperbum 'G. Marconi' and 'Snow Lady'. Following germination, plants were

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transplanted into 500-ml (10-cm diameter) plastic pots containing a mixture of 3 peatmoss : 1 perlite : 1 vermiculite (by volume). Ten plants (replications) were transferred from SDs to LDs upon reaching the following true leaf stages: 0 (cotyledons only), 3, 6, 9, 12, 15, 18, 21, and 24, where a leaf was defined as possessing a fully expanded lamina. Ten plants of each cultivar remained in SDs for the duration of the experiment as controls. Leaf number transfer treatments were assigned randomly at the time of sowing. Data taken at first flower included the number of days elapsed from seeding and start of LDs, number of expanded leaves along the main stem, stem length (from cotyledonary node to the most distal node possessing a visible, reproductive, axillary branch), nonreproductive scape length (from the node where stem length ended to the most distal node possessing a leaf), and leafless scape length (from the node where nonreproductive scape length ended to the point of calyx attachment). First flower was defined as the date when ray florets had expanded perpendicular to the stem. Regression and ANOVA methods were used to analyze the data.

Apical examination of floral initiation in *C. ×superbum* ‘Snow Lady’. Seeds were sown in January. At about four leaves per plant, all plants were transplanted into 500-ml (10-cm diameter) plastic pots containing a mixture of 1 peatmoss : 1 perlite : 1 vermiculite (by volume). Plants were randomly selected and evenly distributed between SDs and LDs at the time of transplanting. Beginning 1 week after transplanting, five plants (replications) were sampled weekly from LDs and SDs. After all expanded leaves and roots were removed, the shoot apices were fixed in 10% formalin-% 15 acetic acid-%15 alcohol (FAA). The number of expanded leaves removed per plant was recorded. Sampling continued for 12 weeks, at which time macroscopic terminal flower buds were present.

Samples were dehydrated using a histomatic tissue processor (model 166; Fisher Scientific, Lexington, Mass.), imbedded in

paraffin blocks and sequentially and longitudinally sectioned at 8 µm on a rotary microtome (Spencer Lens Co., Buffalo, N.Y.) until reaching the approximate center of the apex. Tissue was mounted on glass slides, cleared, stained with Mayers Hemalum, and counter-stained with Erythrosin (Sass, 1958). Slides were examined via light microscopy for indications of floral initiation.

Results and Discussion

Juvenility and flowering requirements of *C. ×superbum* ‘G. Marconi’ and ‘Snow Lady’. ‘G. Marconi’ did not flower in SDs and only 14 plants did so in LDs, indicating that the scope of this experiment could not define the juvenile period for this cultivar. The limited flowering was random and could not be attributed to a specific leafnumber treatment. These plants exhibited wide variability in total height, ranging from 40 to 74 cm (data not shown).

All ‘Snow Lady’ plants flowered except for those in the 24-leaf stage and the SD-control treatments, which exhibited 70% and 90% flowering, respectively. With SD-control plants deleted from the data set, regression analysis indicated linear relationships between leaf number at transfer to LDs (x) and the number of days to flower (y) from onset of LDs ($y = 61.9 - 1.07x$, $r^2 = 0.54^{**}$) and number of days from seeding to flower (Fig. 1). Plants transferred to LDs at the cotyledon stage flowered fastest from seeding, 75 days, whereas those transferred to LDs at 24 leaves flowered 123 days after seeding. The end of juvenility has been quantified in other species by identifying the leaf number associated with the fastest flowering after the onset of inductive conditions (Bourke, 1990; Damann and Lyons, 1993; Lyons and Booze-Daniels, 1986). For *C. ×superbum*, however, this technique is inappropriate. In this experiment, plants grown in SDs to 21 leaves required 43 LDs to flower once transferred compared to only 34 LDs if moved to LDs at the 24-leaf stage. This may suggest that the most rapid flowering from the onset of LDs occurred if plants were transferred at later stages. Yet in actuality, plants in this later treatment bolted and possessed visible terminal buds in SDs before obtaining 24 expanded leaves. These observations indicated that LDs alone were unnecessary for floral initiation but likely influenced inflorescence development, with little revealed about juvenility other than it may not exist in this cultivar.

There was a quadratic relationship between leaf number at

Table 1. Number of expanded *Chrysanthemum ×superbum* ‘Snow Lady’ leaves per plant at the time of sampling.

Sampling date (week)	Long days ^a			Short days ^a		
	Leaves per plant			Leaves per plant		
	Mean	Min	Max	Mean	Min	Max
1	5.4	5	6	5.4	5	6
2	7.0	6	8	6.0	5	7
3	8.8	7	10	9.0	8	10
4	12.4	10	14	10.4	9	13
5	18.4	16	23	12.6	12	13
6				14.8	14	16
7				17.0	15	18
8				19.2	17	23
9				22.8	18	30
10				26.8	21	37
11				25.2	23	30
12				29.0	21	46

^aAll five plants sampled after 5 weeks possessed visible terminal flower buds at the time of sampling.

^bTwo of the five plants sampled after 9, 10, 11, and 12 weeks possessed visible terminal flower buds at the time of sampling.

Fig. 1. The effect of leaf number at transfer to long days on number of days from seeding to flower for *Chrysanthemum ×superbum* ‘Snow Lady’, $y = 75.4 + 1.97x$, $r^2 = 0.70^{**}$.

transfer to LDs (x) and stem leaf number (y) at first flower ($y=8.87 + 1.21x - 0.0179x^2$, $R^2 = 0.77^{**}$) and a linear relationship between leaf number at transfer to LD (x) and stem leaf number (y) at first flower ($y = 15.2 + 0.898x$, $r^2 = 0.81^{**}$). Stem leaf number at first flower ranged from nine on plants grown in LDs from the cotyledon stage, to 28 on plants moved to LDs with 24 expanded leaves. The fewest number of total leaves at first flower, 15, occurred on plants grown in LDs from the cotyledon stage. Plants held in SDs until attaining 24 expanded leaves had the most total leaves, 37. There were no significant linear or quadratic relationships between leaf number at start of LDs and scape leaf number, stem length, scape length, or total height. The responses of SD-control plants, having been excluded from regression analysis, were compared to the leaf number treatment responses via a means separation test. SD-control plants averaged 123 days from seeding to flower and had significantly more stem leaves (25) and total leaves (34) than plants transferred to LDs at 0, 3, 6, or 9 leaves (data not shown).

These results partially support the original conclusion that Shasta daisy is a LD plant (Laurie and Poesch, 1932). However, considerable differences were apparent between the responses of 'G. Marconi' and 'Snow Lady'. The former remained completely vegetative in SDs and showed only limited flowering under LDs for the 5-month duration of this experiment. This lack of response to LDs is in direct contrast with the results of Laurie and Poesch (1932), Kofranek (1952) and Griffin and Carpenter (1964) who reported an earlier or enhanced flowering under LDs. However, plant age can affect the LD flowering response of Shasta daisy. Laurie and Poesch (1932) used 2-year-old field-grown plants, and Griffin and Carpenter (1964) and Kofranek (1952) used plants obtained from rooted divisions and clonal division. The age and maturity of this plant material and the nature of overwintering conditions are not specified. The fact that most 'G. Marconi' plants in the present study remained vegetative under LDs confirms the most recent results of Shedron and Weiler (1982), who also used

Fig. 2. *Chrysanthemum x superbum* 'Snow Lady' (A) apex sampled after one week of long days (LDs), (B) floral initiation following the second and (C) third week of LD; (D) vegetative apex after 5 weeks in short days.. t = tunica; c = corpus, l = leaf primordia; pm = pith-rib meristem, i = primordial internodes; b = involucre bracts. Bar = 100 μ m.

this cultivar as seed-produced experimental material.

Floral initiation of 'Snow Lady' plants under LDs and SDs indicated a facultative rather than obligate LD requirement for floral initiation. 'Snow Lady' plants transferred to LDs at the cotyledon stage required 77 days from seeding to flower, whereas plants grown in continuous SDs averaged 123 days. While it can be argued that most obligate LD plants will eventually flower if held in SDs for an extended period of time (Evenari and Gutterman, 1966), taking less than twice the time to do so compared to the fastest plants in LDs appears to indicate a facultative response that has not been previously reported for this species.

Apical examination of floral initiation in *C. ×superbum* 'Snow Lady'. Leaf unfolding occurred more quickly on plants grown in LDs than in SDs (Table 1). The average leaf number of plants grown in SDs and sampled after 7, 8, and 9 weeks was 17.0, 19.2, and 22.8, respectively. Therefore, given the sporadic flower initiation observed via light microscopy at these sample stages, floral initiation in SDs before 24 leaves, as observed in the juvenility experiment, is not surprising. Floral initiation had begun in the five plants sampled after just 1 week in LDs, as evidenced by slight doming of the apices and primordial internode elongation (Fig. 2A). By the end of the second week in LDs, the apical doming was nearly hemispherical, internode elongation was much more pronounced, and cells of the pith-rib meristem were arranged in a columnar pattern (Fig. 2B). After 3 weeks in LDs, involucre bracts were present and a uniform mantle of cells had expanded over the entire surface of the central parenchymatous core (Fig. 2C). Although apical floral initiation was complete in some plants after 3 weeks in LDs, sampling continued through the fifth week, at

Fig. 3. *Chrysanthemum ×superbum* 'Snow Lady' (A) sampled after 5 weeks and (B) 7 weeks in short days. t = tunica; c = corpus; l = leaf primordia; pm = pith-rib meristem; i = primordial internodes. Bar = 100 µm.

which time all five plants sampled had macroscopic terminal flower buds.

Apical examination after 5 weeks in SDs revealed vegetative meristems typified by a relatively flat apex (Fig. 2D), and slight apical doming on some plants (Fig. 3A). Sampling after 6 weeks in SDs showed apical doming in four of five plants. Following 7 weeks in SDs, apices were domed and nearly hemispherical; primordial internode elongation, and pith-rib columnarization were evident, and vacuolation and elongation of the pith-rib meristem had occurred (Fig. 3B). Stages of floral initiation were similar following the eighth week in SDs, with enhanced apical doming and internode elongation. Floral initiation was evident in all plants following the ninth week of sampling. Two plants possessed macroscopic terminal buds at the time of sampling (Fig. 4A), while histological examination of the remaining three plant meristems revealed apical doming, primordial internode elongation and columnarization of the pith-rib meristem. Sampling continued through the twelfth week; each week, two plants possessed macroscopic flower buds at sampling and histological examination revealed various stages of floral initiation (Fig. 4B) in the other plants sampled.

Earlier research with *E. californica* and *R. hirta* indicated the first signs of floral initiation to occur following six LD cycles (Carter, 1986) and six to eight LD cycles (Kochankov and Chailakhyan, 1986), respectively. However, the number of inductive cycles required for floral initiation can be affected by the length of the photoperiod, the criteria used to identify the transition to flowering, the duration of the observations, and plant age. Although six to eight LDs were required for complete floral initiation in 3-month-old *R. hirta* plants, as few as three LDs induced 7-month-old plants (Kochankov and Chailakhyan, 1986). While this information may account for the lack of flowering in 'G. Marconi', it is not a valid comparison with the apical meristem examinations of 'Snow Lady' when these plants were grown for only 1 month before start of LD treatments. Although 3 weeks of LDs were needed for complete initiation in *C. ×superbum* 'Snow Lady', plants of different ages grown under these conditions could not be expected to respond to inductive conditions similarly.

The growth rate of plants in SDs, measured by an increase in expanded leaf number each week, was slower than the rate of growth in LDs (Table 1). However, the time required for apical floral initiation in all five plants within the treatment, 3 weeks in LDs compared to 9 weeks in SDs, reflects this slower development of leaves, and reveals the fact that plants grown in SDs produced more leaves per plant before floral initiation occurred. Plants sampled after 3 weeks in LDs had fewer than half as many leaves per plant compared to those grown in SDs for 9 weeks. Therefore, delayed flowering cannot solely be a growth rate issue under the less favorable SD environment, but can also be attributed to a delay in actual floral initiation. Although there was not a common leaf number per plant for floral initiation in LDs and SDs, responses in the two photoperiods can be compared to determine the number of LD or SD cycles needed to reach a particular floral initiation stage. Apical floral initiation after 2 weeks in LDs (Fig. 2B) is nearly identical to the initiation stage found after 7 weeks in SDs (Fig. 3B). Theoretically, one LD equals 3.5 SDs, or, considering the time required for floral initiation to occur in all plants within each treatment, 3 weeks of LDs are equivalent to 9 weeks of SDs. Thus, the previously proposed facultative LD flowering nature of *C. ×superbum* 'Snow Lady' is not only confirmed but also quantified. Flowering will occur in LDs and SDs; however, initiation is faster and more uniform in LDs and plants grown in SDs will possess more leaves at anthesis.

Fig. 4. *Chrysanthemum* $\times$ *superbum* *Snow Lady* (A) following 9 and (B) 10 weeks in short days, i = Primordial internodes; b = involucral bracts; m = meristematic mantle; pc = parenchymatous core; fp = floret primordia. Bar = 100 μ m.

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