

Dual induction rather than intermediate daylength response of flowering in *Echinacea purpurea*

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Echinacea purpurea cv. Bravado and Magnus have been reported to be intermediate daylength plants (IDP) which flower in response to photoperiods between 13 and 16 h. The present experiments with *E. purpurea* cv. Bravado show that *E. purpurea* is actually a dual induction short-long-day plant which flowers promptly and consistently when grown in short day (SD) followed by long day (LD) conditions, but not with the reverse sequence of photoperiods. The flowering response increased with increasing duration of both the SD and the LD treatments. A minimum of 4 weeks of SD followed by 12 LD was required for complete flowering. No flowering occurred in

continuous SD or LD, whereas a high proportion of plants flowered in continuous 14-h daylength. However, flowering was more variable in intermediate daylength than after transition from SD to LD. Furthermore, photoperiods between 13 and 16 h could satisfy both the primary SD induction and the secondary LD induction requirements. As a number of dual induction plants, both short-long-day and long-short-day plants, have such an overlapping window of effective photoperiods that can trigger both the SD and LD responses, the rationale for maintaining IDP as a separate and genuine flowering response group is seriously challenged.

Introduction

Plants have been classified into flowering response groups according to their photoperiodic requirements for flowering (Thomas and Vince Prue 1997). In their classical work on photoperiodism, Garner and Allard (1920) identified three response types: day-neutral plants (DNP) which flower irrespective of photoperiod, and short-day plants (SDP) and long-day plants (LDP), which flower or flower more rapidly when photoperiods are less than or greater than some genotype-specific critical photoperiod, respectively. Later, four additional response groups have been added. Dual induction requirements were first discovered by Dostal (1949) in *Bryophyllum* species, which are long-short-day plants (LSDP) and require a transition from LD to SD conditions for flowering, and in the short-long-day plant (SLDP) *Campanula medium* (Wellensiek 1949) which requires the reverse sequence of daylengths. Dual photoperiodic induction of both types has been demonstrated subsequently in a range of species of diverse taxonomic

affiliation. Other species have even more specialized photoperiodic responses, such as the intermediate daylength plants (IDP) which flower or flower more rapidly at intermediate photoperiods, and the ambi-daylength plants (ADP) in which flowering is promoted by both very short and very long photoperiods and with no response to intermediate ones (Thomas and Vince Prue 1997).

In comparison with the other groups, few details are known about the response mechanisms of IDP and ADP, and the rationale of this classification has been questioned. Sachs (1956) suggested that IDP are actually dual induction plants (SLDP or LSDP) in which intermediate photoperiods can satisfy both the SD and the LD requirements. Thus, in his work with the LSDP *Cestrum nocturnum* the critical photoperiod for both LD and SD induction was between 11 and 13 h. In other words, photoperiods between 11 and 13 h could satisfy both the LD and the SD induction requirements of this plant.

Abbreviations – ADP, ambi-daylength plants; DNP, day-neutral plants; IDP, intermediate-daylength plant; LDP, long-day plant; LSDP, long-short-day plant; SDP, short-day plant; SLDP, short-long-day plant.

Recently, Runkle et al. (2001) reported that the ornamental perennial *Echinacea purpurea* cv. Bravado and Magnus flowered most completely and rapidly at intermediate photoperiods of 13–16 h. Accordingly, they classified the plant as a quantitative intermediate daylength plant. Although discussing the suggestion by Sachs (1956) they rejected the possibility of a dual induction mechanism on the basis that nearly all plants remained vegetative in both 10-h and 24-h photoperiods, and therefore, ‘the flowering behaviour of *E. purpurea* cannot be considered a type of SD or LD response’. The salient characteristic of dual induction plants is, however, their need for both short and long photoperiods, either in succession or possibly, simultaneously according to the suggestion of Sachs (1956). This possibility was not tested by Runkle et al. (2001). However, their experiments with night interruption and filtered light deficient in either red or far-red, indicated that both dark-dominant SD responses and light-dominant LD responses (Thomas and Vince Prue 1997) are involved in the induction of flowering of *E. purpurea*.

On this background I decided to investigate the dual effect of SD followed by LD on the flowering of *E. purpurea*. The results reported here show that the species is a SLDP that flowers rapidly in LD preceded by SD, while remaining vegetative in the opposite sequence of daylengths as well as in continuous SD or LD.

Materials and methods

The experiments were performed in the Ås phytotron in daylight compartments combined with adjacent growth rooms for photoperiodic manipulations. Seeds of *Echinacea purpurea* Moench (syn. *Rudbeckia purpurea* L.) cv. Bravado were sown directly into 60-cell plug trays with 6-cm cell diameter. After germination, trays were thinned to one plant per cell and the plants raised in 24-h photoperiod. When the plants had produced four to five leaves (after about 9 weeks) the plants were transplanted into 12 cm plastic pots (0.9-l volume) and the experimentation started after 1 week. All plants received natural summer daylight for 10 h per day (0800–1800 h), and daylength extension was given by low-intensity light from 75 W incandescent lamps (approximately $8 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ PAR). Whenever the quantum flux in the daylight compartments became less than $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ an additional $125 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ were automatically added by high pressure metal halide lamps (Philips HPI-T; 400 W, Koninklijke Philips Electronics N.V., Amsterdam). The temperature was maintained at $21 \pm 1.0^\circ\text{C}$ with a water vapour pressure deficit of 530 Pa (78% relative humidity) throughout plant raising and experimentation. The plants were grown in a peat-based potting compost and fertilized twice weekly with a complete fertilizer solution. Otherwise the plants were watered with tap water as required.

Flowering was recorded as days to bolting (first sign of stem elongation) and days to visible bud (VB; capitulum bracts showing). The percentage flowering plants were

calculated on the basis of plants reaching VB. Plant height, number of flowering stems and total number of inflorescences were also recorded for each plant at anthesis (first open florets). In experiment I, leaf dimensions (petiole and lamina length) of the last fully developed leaf of each plant were also recorded after 4 weeks at the various photoperiods. Plants that had not bolted after 15 weeks from start of treatment were considered non-flowering.

Ten plants were used in each treatment, and one experiment (experiment 1) was duplicated in time. Results are usually presented as means \pm standard error (SE). When considered useful for evaluation of results, data were subjected to analysis of variance (ANOVA) using the Systat Inc. Version 10 software package (Systat Software Inc., Richmond, CA, USA). For experiments that were not replicated in time, the 10 parallel plants in each treatment group were treated as replicates in the analyses.

Results

Experiment 1 compared the effects of 2, 3, 4, 5 and 6 weeks of SD (10 h) treatment followed by LD (24 h) with those of continuous SD, LD and 14-h photoperiod, respectively. In SD the plants developed into compact rosettes with short leaves, whereas plants in LD developed loose rosettes with greatly elongated leaves. Those grown in the 14-h photoperiod were of an intermediate size. This photoperiodic effect was reflected in highly significant differences in leaf dimensions (Table 1). Plants grown continuously in SD or LD remained vegetative, whereas those grown in SD for a sufficient length of time bolted and flowered quickly and uniformly when transferred to LD. Flowering increased with increasing time in SD, a minimum of 4 weeks of SD being required for greater than 90% flowering (Table 2). Most plants grown continuously in 14-h photoperiod also flowered, but the flowering was less uniform than after dual induction. Such an intermediate photoperiod also resulted in shorter flower stems than did dual SD–LD induction. Dissection of plants that had been in SD for 10 weeks revealed no anatomical changes at the stem apex, demonstrating that floral initiation actually takes place after bolting has started in response to the transition from SD to LD. It was also found that no flowering took place when the continuous LD plants were transferred to SD at termination of the experiment after 10

Table 1. Dimensions of the last fully developed leaf (leaf no. 6–7) of *E. purpurea* plants grown in different photoperiods at 21°C for 4 weeks. Weighted means \pm SE of two experiments, each with 10 plants per treatment (experiment 1).

Photoperiod (h)	Length (cm)		
	Petiole	Lamina	Total
10	5.7 ± 0.5	8.7 ± 0.5	14.4 ± 0.5
14	15.0 ± 0.7	16.4 ± 0.7	31.4 ± 1.3
24	23.7 ± 1.0	17.2 ± 1.0	40.9 ± 1.3

Table 2. Flowering of *E. purpurea* plants as affected by various photoperiodic treatments. SD and LD were of 10 h and 24 h duration, respectively. All plants had been raised in 24-h LD for 10 weeks. Days to bolting are days from transfer to LD, or days from start of photoperiodic treatments. Numbers in parentheses refer to a single plant that flowered after 2 weeks of SD exposure. Means \pm SE of two experiments, each with 10 plants per treatment (experiment 1).

Photoperiodic treatments	Flowering (%)	Days to bolting		Stem height (cm)
		from transfer	from start	
2 weeks SD, then LD	5	(20)	(34)	(79)
3 weeks SD, then LD	30	28.0 \pm 7.8	49.0 \pm 7.8	93.7 \pm 2.8
4 weeks SD, then LD	90	19.0 \pm 2.1	47.0 \pm 2.1	88.9 \pm 2.4
5 weeks SD, then LD	100	19.4 \pm 3.8	54.4 \pm 3.8	92.1 \pm 2.2
6 weeks SD, then LD	100	17.0 \pm 1.5	59.0 \pm 1.5	92.6 \pm 2.0
Continuous SD	0	—	—	—
Continuous LD	0	—	—	—
Continuous 14-h photoperiod	70	—	56.6 \pm 4.3	57.6 \pm 2.9

weeks of continuous LD. In other words, while a transition from SD to LD was highly inductive, the reverse transition had no flowering effect.

A second experiment examined the number of LD cycles (days in continuous light) required for secondary induction of plants that had previously received primary induction by SD. Plants that had been in SD for 6 weeks were exposed to 3, 6, 9 and 12 LD cycles (10 + 14 h light), and then returned to SD. The results in Table 3 show that as few as 3 days of continuous light resulted in partial flowering (20%), whereas 12 LD cycles were required for 90% flowering of primary induced plants. Days to bolting and VB decreased and stem height increased with the increasing number of LD cycles (Table 3). With the shortest durations of LD the inflorescences were almost sessile.

Experiment 3 examined the critical photoperiods for the primary SD and the secondary LD inductions, respectively. Ten-week-old-plants raised in 24-h LD were exposed to photoperiods of 10, 12, 14, 16, 18 and 24 h for 6 weeks for primary induction, followed by 24-h LD. Simultaneously, plants which had been primary induced for 6 weeks in 10-h SD were exposed to the same photoperiods for 6 weeks for testing of secondary induction needs. The results in Fig. 1 demonstrate that photoperiods between about 13 and 16 h were inductive for both primary and secondary induction. After primary induction in 10-h photoperiod for 6 weeks, all plants flowered in subsequent photoperiods of 14 h or greater length. By interpolation the critical photoperiod for 50% secondary induction was estimated to be about

13 h. On the other hand, all plants were primary induced in photoperiods of 14 h or less (all plants flowered in subsequent 24-h photoperiod), while the proportion of flowering plants was reduced to 60% after primary induction in 16-h photoperiod. In photoperiods of 14 and 16 h some plants actually started to bolt during the 6-week primary induction period, thus demonstrating the capacity of these intermediate photoperiods to satisfy both primary and secondary induction needs. Optimal photoperiods for primary and secondary induction were 10–12 h and 18–24 h, respectively. As usual, days to bolting decreased, while the height of flower stems increased with increasing photoperiod during secondary induction (Table 4). On the other hand, varying photoperiods during primary induction had no significant effect on final stem height in subsequent 24-h photoperiod (data not shown), while days to heading (from transfer to 24-h photoperiod) decreased from 15.7 \pm 1.3 days with 10-h

Table 3. Effects of increasing numbers of 24-h LD cycles (days of continuous light) on flowering of primary induced *E. purpurea* plants. Days to bolting are days from transfer to LD. The plants had previously been exposed to SD for 6 weeks for primary induction (experiment 2).

Number of LD cycles	Flowering (%)	Days to bolting	Stem height (cm)
3	20	33.5 \pm 7.7	13.3 \pm 1.2
6	40	28.2 \pm 5.2	15.7 \pm 4.2
9	70	16.3 \pm 3.4	24.5 \pm 3.0
12	90	14.3 \pm 0.9	35.3 \pm 1.9

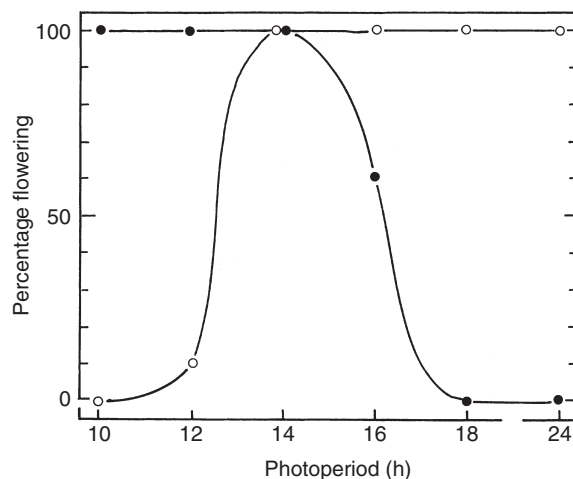


Fig. 1. Flowering percentage of *E. purpurea* plants exposed to varying photoperiods for primary (●) and secondary induction of flowering (○). Ten-week-old-plants raised in 24-h photoperiods were exposed to the various photoperiods for 6 weeks for primary induction followed 24-h LD. Plants which had been primary induced for 6 weeks in 10-h photoperiods were exposed to the same photoperiods for 6 weeks for secondary induction. Data for 10 plants in each treatment.

photoperiod to 11.7 ± 3.1 days with 16-h photoperiod during primary induction.

Discussion

The present results confirm the finding of Runkle et al. (2001) that *E. purpurea* plants flower at intermediate day-lengths of 14 h, while remaining vegetative in continuous 10-h and 24-h photoperiods (Table 2), and that no low-temperature vernalization is required for flowering of this perennial plant. However, the most consistent flowering was obtained when plants were first exposed to 10-h SD followed by exposure to 24-h LD, namely short-long-day conditions. The flowering response increased with increasing duration of both the SD and the subsequent LD exposure (Tables 2 and 3). In the natural environment these conditions are met by the seasonal changes of daylength during autumn and spring. Thus, the primary induction needs are satisfied by the short days of autumn, while secondary induction is brought about by the long days of spring and summer. The dual photoperiodic induction mode is also compatible with the results of the night interruption experiments and experiments with filtered light by Runkle et al. (2001), as these experiments indicated that both dark-dominant SD responses and light-dominant LD responses are involved in the induction of flowering of *E. purpurea*. However, dissections revealed that no inflorescence initiation takes place until bolting has started following the transition from SD to LD, a feature that is common to bolting rosette LDP (Lang 1965) as well as many dual induction grasses (Heide 1994). Because floral initiation required the transition from SD to LD, extended SD induction delayed rather than advanced the timing of bolting and flowering, even though it hastened flowering in subsequent LD (Table 2). The finding that the reverse transition from LD to SD had no flowering effect demonstrates that the highly inductive SD to LD transition (Table 2, Fig. 1) is specific for flowering in *Echinacea*.

Partial flowering resulted from as few as 2 weeks of SD, whereas 4 weeks were required for full primary induction (>90% flowering). Secondary induction of primary induced plants on the other hand, required a minimum of 3 LD cycles for partial flowering, whereas a minimum of 12 LD were required for full flowering. However, such marginal LD inductions strongly constrained flower stem elongation and delayed flower development (Table 3).

Although no flowering occurred in continuous SD or LD in the present experiments (Table 2), a small proportion (10–15%) of cv. Bravado plants flowered in 10-h and 24-h photoperiods in the experiments by Runkle et al. (2001). This difference was attributed by the authors to the fact that the plants were raised in 14–16 h daylengths, conditions which are inductive to flowering in this plant (Runkle et al. 2001). Partial induction could therefore have taken place before the experimental treatments were started.

The fact that intermediate photoperiods of 14–16 h could satisfy both the primary and secondary induction

Table 4. Effects of photoperiod during secondary floral induction on days to bolting and final height of flower stems of *E. purpurea* plants. The plants had previously been exposed to SD for 6 weeks. Numbers in parenthesis refer to a single flowering plant in 12-h photoperiod. Means \pm SE for 10 plants in each treatment (experiment 3).

Photoperiod, (h)	Days to bolting	Stem height (cm)
10	> 60	—
12	(25)	(40)
14	20.0 ± 1.1	67.1 ± 2.5
16	16.8 ± 1.1	80.7 ± 3.5
18	15.7 ± 0.9	94.1 ± 3.7
24	15.1 ± 1.0	94.0 ± 2.5

requirements of *E. purpurea* (Fig. 1), supports the suggestion by Sachs (1956) that the so-called intermediate day-length plants actually are dual induction plants in which intermediate photoperiods can satisfy the requirements for both SD and LD. The suggestion was based on the observation that the LSDP *Cestrum nocturnum* has critical photoperiods between 11 and 13 h for both LD and SD induction (Sachs 1956). Similar situations have been observed in several other dual induction plants. For example, the LSDP *Bryophyllum daigremontianum* flowers when moved from LD to a 12-h photoperiod, and when moved from SD to 12 h photoperiod and then back to SD (Resende 1956). Thus, a 12-h photoperiod acts as SD for plants coming from LD, and as LD for plants grown previously in SD. Likewise, in the LSDP *Bryophyllum crenatum* a photoperiod of 12.5 h can fulfil either the SD or the LD requirement, depending on the preceding photoperiod (Penner 1960). Furthermore, in their native habitat in Madagascar, where the annual variation in daylength is between 10.5 and 13.5 h, the photoperiodic conditions are adequate to satisfy both the LD and the SD requirement of the *Bryophyllum* species. Even on the equator, where the daylength is close to 12 h throughout the year, profuse flowering can be observed in these plants (personal observations).

Furthermore, in some SLDP that require the opposite transition from SD to LD in order to flower, there is a window of intermediate photoperiods that can satisfy both the SD and LD requirements. An example is the SLDP *Bromus inermis*, in which the critical photoperiod for both SD and LD induction is between 12 and 16 h (Heide 1984). All these results, together with the present ones with *E. purpurea*, substantiate the suggestion by Sachs (1956), that plants with an intermediate daylength flowering response are actually dual induction plants in which intermediate photoperiods can satisfy the requirements for both SD and LD. The rationale for maintaining intermediate daylength plants as a separate and genuine flowering response group is therefore seriously challenged.

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