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Flowering of *Eustoma grandiflorum* (Raf.) Shinn. Cultivars Influenced by Photoperiod and Temperature

Brent K. Harbaugh
Gulf Coast Research and Education Center, University of Florida, 5007 60th Street East, Bradenton, FL 34203

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***Abstract.* Seedling growth and flowering responses were examined for four *Eustoma* cultivars exposed to photoperiod × temperature treatments during two seedling ages. Seedlings were grown under short days (SD, 12-hour photoperiod) or long days (LD, 18-hour photoperiod) in soil at 12 or 28C from 14 to 43 days after sowing. Seedlings from each treatment were then subdivided into four lots and subjected to the same photoperiod × temperature treatments from 43 to 79 days after sowing, for a total of 16 treatments. To determine flowering response, seedlings were grown subsequently for 120 days at 22C under the same photoperiod that they received from day 43 to 79. For all cultivars and both seedling ages, seedlings were larger and had more leaves when grown at 28C rather than at 12C, but the tallest plants at flowering were from seedlings exposed to 12C. Seedlings from some treatments bolted but did not initiate visible flower buds, and some seedlings developed visible buds that later aborted, resulting in plants that did not flower by the termination of the experiment (199 days). Cultivar and interactive effects of photoperiod and temperature influenced the percentage of flowering plants. Vegetative growth and flowering responses were similar for 'Yodel White', 'Heidi Pink', and 'Blue Lisa'. They flowered as LD plants when seedlings were grown at 12C from day 14 to 43 or day 43 to 79. Seedlings of these cultivars that were grown under SD at 28C from day 43 to 79 did not flower, regardless of photoperiod or temperature treatments from day 14 to 43. However, SD photoperiod or 28C did not decrease flowering for 'GCREC-Blue'.**

Most commercial *Eustoma* cultivars have a tendency to rosette when seedlings are exposed to temperatures ≥25 to 28C (Harbaugh et al., 1992; Ohkawa et al., 1991). However, cultivars differ significantly in their sensitivity to high temperatures (Fukuda et al., 1994). The percentage of plants that bolted and formed buds after 21-day-old seedlings were exposed to 31C for 28 days was 13, 26, 56, or 97 for 'USDA Pink', 'Yodel White', 'Little Belle Blue', and 'GCREC-Blue', respectively (Harbaugh et al., 1992).

Seedling age at time of high-temperature exposure also influences the rosetting response. Ohkawa et al. (1991) reported that seedlings of 'Fukushihai' that were >6 weeks old were not sensitive to high temperatures. However, the percentage of flowering plants increased as seedling age at time of high-temperature exposure increased from 0 to 31 days for 'GCREC-Blue', but the percentage of flowering plants decreased for 'Yodel White' (Harbaugh et al., 1992).

According to recent reports on low-temperature treatment of seeds and seedlings, vernalization induces bolting in some *Eustoma* cultivars. Bolting increased when 'Fukukaen' seedlings were grown from seeds ripened on parent plants at 23C day/18C night compared to seeds ripened at 33C day/28C night (Ohkawa et al., 1993). Pergola et al. (1992) reported cold treatment (3C for 4 weeks) of imbibed seeds increased bolting of an unspecified cultivar. Similarly, cold treatment of imbibed seeds at 10C for 5 weeks resulted in 100% bolting in 'Fukushihai' and 53% bolting with 'Miyakomomo' seedlings subsequently grown at 33C day/18C night (Ohkawa et al., 1993). 'Lilac' seedlings grown for ≥3 weeks below 18C bolted rapidly when subsequently grown at >22C (Pergola, 1992). Heat-induced rosetting of 'Fukushihai' seedlings was reversed by low-temperature treatments of 15C for 4 weeks (Ohkawa et al., 1994).

Halevy and Kofranek (1984) reported that pink, blue, and white color variants of *Eustoma* flowered even under an 8-h photoperiod, and they considered *Eustoma* a day-neutral plant. Similarly, *Eustoma* F_1 hybrid blue and pink were not responsive to photoperiod and, thus, were described as natural day plants that flowered after they reached a certain age or size (Azrak, 1984). Other researchers consider *Eustoma* a quantitative long-day (LD) plant (Grueber et al., 1984; Roh and Lawson, 1984; Roh et al., 1989). Harbaugh et al. (1992) reported that more plants rosetted when seedlings were grown under short days (SD) at 28C than under LD at 28C.

My purpose was to determine interactive effects of photoperiod and temperature on seedling development and flowering of several lisianthus cultivars.

Materials and Methods

'Yodel White', 'Heidi Pink', 'Blue Lisa', and 'GCREC-Blue' seeds were sown in plastic germination trays (24 cm long × 2 cm wide × 3 cm deep) and were held for 14 days in a germination room at 22 to 24C with a photosynthetic photon flux (PPF) of 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 12 h from cool-white fluorescent lamps. Seedlings 14 days from sowing were transferred to growth chambers and grown under SDs (12-h photoperiod) or LDs (18-h photoperiod) in soil at 12 ± 1C or 28 ± 1C for 29 days (days 14 to 43). Air was maintained in the range of ±2C of the soil temperature. PPF was 150 to 190 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from cool-white fluorescent and incandescent bulbs.

After treatments from day 14 to 43, seedlings were subdivided into four lots, transplanted into 4 × 4 × 5-cm cells of a six-cell plastic container, and then exposed to the same treatment combinations for 36 days (days 43 to 79), resulting in 16 treatments (see Table 2). Seedlings then were grown for an additional 120 days at 22 ± 1C under the same photoperiod they received from day 43 to 79.

Plant width (leaf tip to leaf tip of largest opposite leaf pair) and number of expanded leaves per plant were recorded at day 43 and 79. The number of leaves when plants bolted (bolting = development of the first internode >3 mm), the number of internodes to the first flower bud, plant height to the first flower bud, the number of days to flower (first open flower),

Table 1. The influence of daylength and growth temperature from 14 to 43 days after sowing on size of *Eustoma grandiflorum* seedlings on day 43.

		Cultivar			
		Yodel White		GCREC-Blue	
Daylength (h)	Temp (°C)	Plant width (mm)	No. leaves	Plant width (mm)	No. leaves
18	28	38	7	29	6
12	28	26	6	19	6
18	12	8	4	5	3
12	12	7	4	4	2
		Analysis of variance			
Mean square treatment (3)[z]		710.9**	9.22**	453.7**	12.98**
Mean square error (6)[z]		12.8	0.36	6.6	0.57
SE		1.0	0.17	0.7	0.22

[z]Degrees of freedom are in parentheses.
**Significant at $P \leq 0.01$.

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percentage of plants that had bolted or developed visible flower buds by day 199, and percentage of normal flowering plants were recorded. Plants considered as normal flowering 1) had a terminal (central) stem that flowered first, 2) flowered by day 150 (i.e., no flowering delay), and 3) had flower buds that did not abort.

Data for each cultivar were analyzed separately because distinct cultivars were chosen initially. There were six plants per experimental unit and four replications in time for each cultivar. Plant width and number of expanded leaves per plant were not recorded for replication four at day 43, so there were only three replications for these variables. Percentage data were analyzed using arcsin-transformation, but actual data means are presented.

Results

Trends for growth and flowering responses of 'Yodel White', 'Heidi Pink', and 'Blue Lisa' were similar, but important differences existed between these cultivars and 'GCREC-Blue'. Thus, only data for 'Yodel White' and 'GCREC-Blue' are presented.

Yodel White. Seedlings were largest (expressed as plant width) at day 43 when grown under LD at 28C, smallest when grown under LD or SD at 12C, and intermediate in size when grown under SD at 28C (Table 1). On day 79, seedlings grown at 12C from day 43 to 79 had developed ≈40% fewer leaves and plant width was ≈65% less than for seedlings grown at 28C (Table 2). However, flowering plants that developed from seedlings grown at 12C from day 43 to 79 were ≈20% to 60% taller than flowering plants produced from seedlings grown at 28C.

Regardless of photoperiod, seedlings had the most leaves at bolt when grown at 28C from day 14 to 43 and at 12C from day 43 to 79. There were fewer internodes to the first flower for plants grown from day 43 to 79 under LD compared to SD. Seedlings grown from day 14 to 79 under LD at 28C required the fewest days to flower, while seedlings grown under SD at 12C took the longest to flower.

No plants bolted when seedlings were grown under SD at 28C from day 14 to 79. Regardless of treatment from day 14 to 43, ≤4% of the seedlings developed buds when grown under SD at 28C from day 43 to 79. When seedlings were grown at 12C from day

Table 2. Influence of daylength (DL) and growth temperature 14 to 43 and 43 to 79 days from sowing on *Eustoma grandiflorum* seedling size at day 79 and vegetative and flowering response after seedlings subsequently were grown for 120 days at 22C with the daylength they received from day 43 to 79.

Days 14 to 43		Days 43 to 79		Seedlings day 79		Leaves	Inter-	Days				Normal
DL (h)	Temp (°C)	DL (h)	Temp (°C)	Width (mm)[z]	Leaves (no.)	at bolt	nodes (no.)[y]	to flower[x]	Ht (mm)[w]	Bolt (%)	Bud (%)	flowering (%)[v]
						Yodel White						
18	28	18	28	122	14	10	6	107	290	58	34	21
	28	12	28	116	13	8	---	---	---	22	0	0
	28	18	12	47	9	13	7	148	424	88	75	52
	28	12	12	43	8	13	10	167	418	100	64	14
12	28	18	28	111	13	11	---	---	---	29	13	4
	28	12	28	104	12	---	---	---	---	0	0	0
	28	18	12	40	8	12	7	151	414	96	92	56
	28	12	12	38	8	12	11	171	474	100	75	13
18	12	18	28	88	11	9	4	113	353	100	80	67
	12	12	28	59	10	10	---	---	---	71	4	0
	12	18	12	30	8	8	7	140	430	100	100	88
	12	12	12	20	6	8	9	161	409	100	83	25
12	12	18	28	82	10	8	6	131	327	95	71	62
	12	12	28	52	9	11	---	---	---	42	0	0
	12	18	12	23	6	8	6	138	418	100	100	62
	12	12	12	18	6	8	8	172	435	100	92	13
						Analysis of variance						
Mean square treatment (15)[u]				5,270**	26.5**	36.7**	56.3**	14,992**	108,107**	4557**	6257**	3489**
Mean square error (45)[u]				240	1	0.9	0.6	100	1074	236	439	547
SE				1.9	0.1	0.1	0.1	1.2	4.1	1.9	2.6	2.9
						GCREC Blue						
18	28	18	28	108	16	8	4	98	275	100	100	100
	28	12	28	88	16	9	5	107	252	100	100	100
	28	18	12	45	9	9	6	136	334	100	100	100
	28	12	12	38	8	9	8	152	305	100	100	77
12	28	18	28	95	15	9	4	106	264	100	100	96
	28	12	28	87	14	10	5	126	275	100	100	100
	28	18	12	33	8	10	6	137	349	100	100	88
	28	12	12	26	8	11	7	162	334	100	90	40
18	12	18	28	61	10	9	4	125	280	100	100	100
	12	12	28	40	9	11	7	153	287	100	79	58
	12	18	12	18	6	8	6	148	313	100	100	57
	12	12	12	12	5	10	---	---	300	100	82	0
12	12	18	28	57	9	8	4	136	286	100	100	95
	12	12	28	35	8	11	7	158	300	95	95	62
	12	18	12	13	5	9	5	157	322	100	95	54
	12	12	12	9	4	10	---	---	340	100	72	0
						Analysis of variance						
Mean square treatment (15)[u]				4,074**	58.3**	4.0**	7.1**	2,330**	3,363**	6.2^{NS}	317**	4,295**
Mean square error (45)[u]				67	0.8	0.9	0.7	53	684	6.2	130	546
SE				1	0.1	0.1	0.1	0.9	3.3	---	1.4	2.9

[z]Plant width measured as distance from leaf tip to leaf tip of largest pair of leaves.
[y]Number of internodes >3 mm to the first flower.
[x]Days from sowing to first open flower if flowering occurred by day 199.
[w]Height from soil to first open flower if flowering occurred by day 199.
[v]Normal flowering; plants that had the terminal (central) stem that flowered first, flowering occurred before 150 days (i.e., no flowering delay), and flower buds did not abort.
[u]Degrees of freedom in parentheses.
$^{NS, **}$Nonsignificant or significant at $P \leq 0.001$, respectively.

43 to 79, 88% to 100% of the seedlings bolted, and 64% to 100% formed visible buds, regardless of photoperiod, although there were more normal-flowering plants under LD than SD.

GCREC-Blue. Although ‘GCREC-Blue’ seedlings were generally smaller than ‘Yodel White’ seedlings, at day 43, growth responses of ‘GCREC-Blue’ and ‘Yodel White’ seedlings were similar, with the largest seedlings resulting from growth under LD at 28C (Table 1). By day 79, plants were widest and had nearly twice as many leaves when seedlings were grown at 28C rather than 12C from day 43 to 79 (Table 2). At flowering, however, the tallest plants were produced from seedlings grown at 12C from day 43 to 79, irrespective of treatments applied from day 14 to 43.

In general, there were fewer leaves at bolting and fewer internodes at flowering for seedlings grown from day 43 to 79 under LD vs. SD when treatments were similar from day 14 to 43. Plants flowered in a minimum of 98 days when seedlings were grown from day 14 to 79 under LD at 28C and a maximum of 162 days with SD at 12C.

Nearly all (95% to 100%) of the seedlings bolted, and 72% to 100% developed visible buds for all treatments. However, only 0% to 57% flowered normally when seedlings were grown at 12C from day 14 to 43 or from day 43 to 79, irrespective of photoperiod. ‘GCREC-Blue’ seedlings grown at 28C produced more normal flowering plants at 28 than at 12C; in contrast, ‘Yodel White’ had more normal flowering plants when seedlings were grown at 12C.

Discussion

For both cultivars, seedlings were largest on day 43 when seedlings were grown from day 14 to 43 at 28C. On day 79, seedlings were largest and subsequent days to flower fewest when seedlings were grown under LD at 28C. However, flowering plants from these LDs at 28C treatments generally were shorter than plants in other treatments. Also, these plants did not appear to have the stem strength or plant vigor produced from seedlings grown at 12C from day 14 to 43 or day 43 to 79. Additional work is needed to define rapid seedling production methods that will ensure high-quality flowering plants in minimal time. My research indicates that a low-temperature treatment in the seedling stage would not only be beneficial to prevent or reverse heat-induced rosetting of some *Eustoma* cultivars but also could improve subsequent flowering crop quality, especially for cut-flower production where final height is critical for quality standards.

Photoperiod and cultivar had a more important role in flowering than previously reported for *Eustoma*. The percentage of flowering plants also was influenced by interactive effects of photoperiod × temperature. ‘Yodel White’, ‘Heidi Pink’, and ‘Blue Lisa’ flowered as LD plants when seedlings were grown at 12C from day 14 to 43 or from day 43 to 79. The combination of SD and 28C from day 43 to 79 resulted in no normal flowering plants for these cultivars, irrespective of seedling treatments from day 14 to 43. SD or 28C did not decrease normal flowering with ‘GCREC-Blue’. ‘GCREC-Blue’ flowered as a day-neutral plant when seedlings were grown at 28C. When seedlings were grown at 12C, more plants flowered normally under LD than SD.

Ohkawa et al. (1994) reported that heat-induced rosetting could be reversed with low temperatures, but they did not define any daylength requirement. In my study, ‘Yodel White’ seedlings grown from day 14 to 43 at 28C and subsequently at 12C from day 43 to 79 had ≤14% normal-flowering plants under SD and 52% normal-flowering plants under LD. Thus, reversal of heat-induced rosetting with low temperatures should be under LD and not SD. Similarly, if seedlings are grown at low temperatures (12C) from day 14 to 43 to prevent heat-induced rosetting, my results indicate that seedlings should be grown under LD from day 43 to 79 if high temperatures are expected.

Ohkawa et al. (1994) reported reversal of heat-induced rosetting with low temperature as bolting percentage. In my study, a high percentage (88% to 100%) of seedlings also bolted when grown with high temperatures (28C) from day 14 to 43 and subsequently under cool conditions (12C) from day 43 to 79. However, bolting did not ensure development of visible buds or subsequent flowering. For example, all ‘Yodel White’ seedlings bolted when they were grown from day 14 to 43 under LD at 28C, but 75% formed buds and only 8% flowered normally when subsequently grown from day 43 to 79 under SD at 12C. Flower buds developed and then aborted or plants that had bolted reverted to a rosette growth habit (i.e., the main stem elongated with several internodes forming and then internode elongation ceased as additional leaves developed). These results suggest caution in presenting or interpreting data that indicate bolting always results in normal-flowering *Eustoma* plants.

Cultivar response and interactive effects of photoperiod × temperature explain some of the differences for the long-day response found in my research compared to previous research that claimed *Eustoma* to be a day-neutral or quantitative LD plant. Seedling age, development (leaf count), and history of temperature and photoperiod are likely just as important. Halevy et al. (1984) used 110-day-old seedlings that had bolted and were pinched before the start of their photoperiod studies. Azrak (1984) used >150-day-old seedlings in his work before starting photoperiod treatments, and Grueber et al. (1984) used plugs of unspecified age or history shipped from Florida. The only descriptions of cultivars for these three studies were that they were color variants of *Eustoma*. In my study, treatments were initiated after germination (2 weeks from sowing), with many plants flowering before treatments would have been initiated in previous studies. Additional research is needed to define clearer photoperiod × temperature response for specific seedling ages or developmental stages. Cultivars used for studies must be considered and reported because my research indicates that *Eustoma* cultivars respond differently to photoperiod and temperature.

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