Temperature and Photoperiod Influence Flowering and Morphology of Four Petunia spp.

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Abstract. Flowering and morphology of four Petunia Juss. spp. [P. axillaris (Lam.) Britton et al., P. exserta Stehmann, P. integrifolia (Hook.) Schinz & Thell., and P. xhybrida Vilm.] were evaluated in response to photoperiod and temperature. Photoperiod responses were evaluated under 9-h short days (SD), 9-h photoperiod plus 4-h night-interruption lighting (NI), or a 16-h photoperiod supplemented with high-pressure sodium lamps (16-h HPS). All species flowered earlier under NI than SD and were classified as facultative (quantitative) long-day plants. Increasing the daily light integral within long-day treatments increased flower bud number for P. axillaris only. In a second experiment, crop timing and quality were evaluated in the temperature range of 14 to 26 °C under 16-h HPS. The rate of progress toward flowering for each species increased as temperature increased from 14 to 26 °C, suggesting the optimal temperature for development is at least 26 °C. The calculated base temperature for progress to flowering varied from 0.1 °C for P. exserta to 5.3 °C for P. integrifolia. Flowering of P. axillaris and P. integrifolia was delayed developmentally (i.e., increased node number below the first flower) at 14 °C and 17 °C or less, respectively, compared with higher temperatures. Petunia axillaris and P. integrifolia flower bud numbers decreased as temperature increased, whereas P. xhybrida flower bud number was similar at all temperatures. The differences in crop timing and quality traits observed for these species suggest that they may be useful sources of variability for petunia breeding programs.

Petunia (Petunia xhybrida) has long been a popular bedding plant with a wholesale value of $110 million in 2008 (U.S. Department of Agriculture–National Agricultural Statistics Service, 2009). Petunia is a facultative long-day plant for flowering (Adams et al., 1998; Piringer and Cathey, 1960), although Petunia ‘Wave Purple’ has been described as an obligate long-day plant (Erwin, 2006). Petunias are often produced in northern climates during the late winter and early spring months, when light levels are low and ambient photoperiods are short, necessitating the use of supplemental lighting to promote flowering. Breeding efforts have been successful in reducing the strength of the photoperiodic response (i.e., reducing the delay in flowering for plants grown under short days compared with night-interruption long days) of some cultivars (Pemberton and Roberson, 2006), although no day-neutral cultivars are known. In addition to photoperiod, daily light integral (DLI) can influence earliness of flowering by reducing node number below the first flower (Armitage and Tsujita, 1979; Erwin and Warner, 2002; Warner and Erwin, 2003), referred to as a facultative irradiance response (Mattson and Erwin, 2005). A survey of 40 herbaceous ornamental species identified 10 species with a facultative irradiance response, 28 species that were irradiance indifferent, and two species in which node number below the first flower increased with increasing DLI (Mattson and Erwin, 2005). Adams et al. (1999) determined that increasing DLI reduced the length of the juvenile phase of petunia ‘Express Blush Pink’.

The time required for developmental processes to occur in plants is primarily a function of accumulated thermal time, often quantified as degree-days (Bonhomme, 2000). The rate of progress toward a developmental event (such as appearance of a new node, or flowering) increases linearly between a species-specific base temperature (T_base), in which development rate, resulting in minimum development rate is nil, and an optimum temperature (Adams et al., 1997). At temperatures above the optimum, development rate declines. Growing plants at the optimum temperature for development rate, resulting in minimum production time, may be undesirable because it often results in reductions in crop quality. For example, increasing temperature from 14 to 26 °C decreased Campanula carpatica Jacc. time to flower, but also decreased flower number and flower size (Niu et al., 2001).

The genus Petunia consists of 14 currently recognized species (Stehmann et al., 2009) native to temperate and subtropical South America. Wild relative species may hold potential for improvement of the cultivated petunia (P. xhybrida). Petunia xhybrida is derived from a cross between P. axillaris and P. integrifolia (Stehmann et al., 2009), and Petunia spp. are generally cross-compatible (Ando et al., 2001; Watanabe et al., 1996, 2001), although fertility varies widely between parental species combinations. Little information is available concerning the influence of light and temperature on floral timing and crop quality characteristics of wild Petunia spp. Therefore, the objectives of work presented here were to: 1) determine the photoperiodic response group for P. xhybrida ‘Mitchell’ and three wild relative species; and 2) evaluate the influence of photoperiod and temperature on crop timing and quality parameters.

Materials and Methods
Expt. 1: Photoperiod treatments. Petunia axillaris (PI 28546; obtained from the USDA Ornamental Plant Germplasm Center, Columbus, OH), P. exserta (provided by Dr. Robert Griesbach, USDA-ARS), P. xhybrida ‘Mitchell’ (provided by Dr. David Clark, Univ. of Florida), and P. integrifolia (Diane’s Flower Seeds, Ogden, UT) seeds were sown in 128-cell (10 mL cell volume; one seed per cell) trays on 3 Feb. 2008 and placed in a greenhouse at 23 ± 1.0 °C (24-h mean ± SD) under intermittent mist. When two true leaves had unfolded, seedlings were transplanted into 10-cm diameter round pots (450 mL) containing (v/v) 70% peatmoss, 21% vermiculite, and 9% perlite (Sure-Mix; Michigan Grower Products, Galesburg, MI) and placed into one of three treatments. Treatments were: short days (SD; a truncated 9-h photoperiod obtained by covering plants with opaque cloth from 1700 to 0800 HR daily), long days provided as night interruption lighting (NI; 9-h photoperiod obtained by covering plants with opaque cloth from 1700 to 0800 HR daily plus 3 μmol·m–2·s–1 night-interruption lighting from incandescent lamps from 2200 to 0700 HR), or a 16-h photoperiod (16-h HPS; ambient light plus 90 μmol·m–2·s–1 from high-pressure sodium lamps from 0600 to 2200 HR) at 20 ± 1 °C (24-h mean ± SD) until flowering. Photosynthetic photon flux at the top of the plant canopy was measured in each treatment every 10 s with a 10-photodiode line quantum sensor (Apogee Instruments, Logan, UT) connected to a data logger (CR10; Campbell Scientific, Logan, UT). Hourly averages were stored and used to calculate DLI. The mean DLI for the SD, NI, and HPS treatments was 11.4, 11.5, and 16.5 mol·m–2·d–1, respectively.

Expt. 2: Temperature effects on crop timing and quality. Seeds of the same species were sown and transplanted as described previously and then were placed into one of five greenhouse compartments set to a constant temperature of 14, 17, 20, 23, or 26 °C under a 16-h photoperiod supplemented with HPS lamps as described for Expt. 1. Air temperature in each treatment was measured

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by a Type E thermocouple (TT-E-40; Omega Engineering, Stamford, CT) placed in an aspirated tube. Thermocouples were connected to a data logger (CR10) and data were recorded every 10 s. Hourly averages were stored. Actual mean temperatures ±sd during the experimental period were 14.0 ± 0.75, 17.0 ± 0.50, 20.0 ± 1.1, 22.6 ± 0.64, and 25.7 ± 0.60 °C. Vapor pressure deficit was maintained between 0.7 and 1.0 kPa at each temperature by steam injection.

Plants were irrigated as needed with reverse osmosis-treated well water supplemented with (mg L⁻¹): 125 nitrogen, 13 phosphorus, 125 potassium, 15 calcium, 1 iron, 0.1 boron and molybdenum, and 0.5 manganese, zinc, and copper (MSU Special: GreenCare Fertilizers, Kankakee, IL).

Experimental design, data collection, and analysis. Both experiments were fully factorial split plot designs with plants arranged in two replicated blocks of 10 plants each per species per main plot. In Expt. 1, photoperiod treatment constituted the main plot with three levels, whereas in Expt. 2, temperature was the main plot with five levels. Species was the subplot in each experiment. Block effects were not significant in any case. Therefore, data were pooled for subsequent analysis. In both experiments, at anthesis of the first flower, the date was recorded and the number of nodes on the primary shoot below the open flower, number of visible flower buds (greater than 3 mm in length), and the number of lateral branches (greater than 5 cm in length) were determined. Flowering rate in response to temperature was calculated as 1/days to flower. Analyses of variance and means separations [Tukey’s HSD(0.05)] were conducted on flowering rate as a function of temperature. The slope (b1) and y-intercept (1/b1) for each species.

Table 1. Influence of short days (SD; 9-h photoperiod), long days provided as a 4-h night interruption (NI), or long days provided by 16-h lighting with high-pressure sodium lamps (16-h HPS) on time to flower (in days), number of nodes below the first flower (nodes), number of visible flower buds at first flowering (buds), and the number of lateral branches at first flowering (branches; greater than 5 cm in length) for four Petunia spp. *

| Species          | Photoperiod | Time to flower (d) | Nodes (no.) | Buds (no.) | Branches (no.) |
|------------------|-------------|--------------------|-------------|------------|----------------|
| *P. axillaris*   | SD          | 70 ± 0.52          | 37.6 ± 2.3  | 36.7 ± 1.1 | 10.0 ± 0.4     |
|                  | NI          | 49 ± 0.53          | 17.7 ± 1.4  | 14.2 ± 1.1 | 8.4 ± 0.5      |
| *P. exserta*     | 16-h HPS    | 45 ± 0.59          | 20.0 ± 1.6  | 33.7 ± 1.7 | 7.7 ± 0.6      |
| *P. integrifolia*| SD          | 64 ± 0.54          | 25.8 ± 1.8  | 24.9 ± 1.2 | 8.9 ± 0.7      |
|                  | NI          | 52 ± 0.49          | 12.3 ± 0.8  | 13.4 ± 1.2 | 7.7 ± 0.4      |
| *P. hybrida*     | 16-h HPS    | 53 ± 0.57          | 15.0 ± 1.7  | 14.9 ± 1.4 | 7.2 ± 0.5      |

Results

Species and photoperiod treatment interacted to impact flower bud production. All species except *P. integrifolia* produced more flower buds at first flowering under SD than NI (Table 1). Growing plants under 16-h HPS increased flower bud number compared with NI for *P. axillaris* only. Branch number at first flowering varied by species and by photoperiod treatment. *Petunia axillaris* and *P. integrifolia* produced more branches under SD than under NI or 16-h HPS (Table 1). Photoperiod treatment did not significantly influence *P. hybrida* branch production, whereas *P. exserta* produced fewer branches under 16-h HPS than SD or NI.

Increasing temperature from 14 to 26 °C decreased time to flower for all species (Fig. 1). The time to flower response to temperature was best fit by a quadratic polynomial regression equation for each species. The rate of progress toward flowering increased as a linear function of temperature between 14 and 26 °C for all four species (Table 2). Species varied in the optimum temperature for rate of increase throughout the range of temperatures examined, the optimum temperature for rate of increase was best fit by a quadratic polynomial regression equation for each species. The rate of progress toward flowering increased as a linear function of temperature between 14 and 26 °C for all four species (Table 2). Species varied in the optimum temperature for rate of increase throughout the range of temperatures examined, the optimum temperature for rate of increase was best fit by a quadratic polynomial regression equation for each species.

Discussion

All four species responded as facilitative (quantitative) long-day plants, forming fewer nodes below the first flower under NI compared with SD (Table 1), although flowering occurred under all photoperiods. The similar photoperiodic responses of the evaluated *Petunia* spp. are perhaps not surprising considering that the genus is small with only 14 species and occurs naturally in a relatively narrow geographic area. In contrast, species of the genus *Hibiscus* L., with 250 to 300 species (Bates, 1965) distributed widely throughout the world, exhibit a wide range of photoperiodic responses, including obligate and facultative short-day, day-neutral, and facultative and obligate long-day species (Warner and Ervin, 2001). However, despite the similar and relatively strong photoperiodic response of the *Petunia* spp. evaluated here, it may be possible to use these species to breed for petunia cultivars with reduced photoperiod sensitivity, because interspecific hybrid populations derived from these species exhibited wide variation for floral timing traits such as node number below the first flower and days to flower (Warner and Walworth, 2010). Also, evaluation of 51 seed-propagated trailing petunia cultivars revealed that although flowering time of all cultivars was accelerated by night-interruption lighting compared with ambient

*Values followed by different letters indicate significant differences across photoperiod treatment within a species as determined by Tukey’s HSD(0.05). Means are based on two blocks of 10 plants each. **NS, * and *** indicate nonsignificance or significance at P < 0.05 or 0.001, respectively.
hours from 32 to 40 for ‘Tidal Wave Hot Pink’ to only 4 d for ‘Ramblin’ Burgundy Chrome’ (Pemberton and Roberson, 2006).

Within the long-day treatments, increasing the DLI from the NI (11.5 mol·m⁻²·d⁻¹) to 16-h HPS (16.5 mol·m⁻²·d⁻¹) treatment increased flower bud number for P. axillaris only, from 14.2 to 33.7 buds. Increasing flower bud numbers with increased DLI have been observed in other species, although the DLI resulting in the maximum flower production varies widely. Increasing DLI from 6.7 to 8.9 mol·m⁻²·d⁻¹ increased Hibiscus radiatus flower bud number from seven to 10 buds (Warner and Erwin, 2003), although further increasing DLI (up to 16.7 mol·m⁻²·d⁻¹) did not further increase flower bud number. Faust et al. (2005) evaluated several bedding plant species under mean DLIs of 5, 12, 19, and 43 mol·m⁻²·d⁻¹. Begonia ×semperflorens-cultorum ‘Vodka Cocktail’ flower number was highest at a DLI of 19 mol·m⁻²·d⁻¹ or higher, whereas Catharanthus roseus ‘Pacific Lilac’, Petunia ×hybrida ‘Apple Blossom’, Tagetes erecta L. ‘American Antigua Orange’, and Zinnia elegans L. ‘Dreamland Rose’ flower numbers were highest at 43 mol·m⁻²·d⁻¹. The optimal temperature for minimizing time to flower appears to be at least 26 °C for each Petunia species evaluated here, because time to flower decreased with increasing temperature from 14 to 26 °C, similar to results for P. ×hybrida ‘Snow Cloud’, which had an optimum temperature of 25 °C (Kaczperski et al., 1991). Differences in temperature between 14 and 26 °C (Moccaldi and Runkle, 2007). Petunia exserta node number below the first flower was greater at 26 than 14 °C, indicating that not all Petunia spp. experience developmental delay in flowering at cool temperatures. Increasing temperature reduced flower bud number of P. axillaris and P. integrifolia (Table 3). Similarly, flower bud number of nine grandiflora-type P. ×hybrida cultivars declined as temperature increased from 14 to 26 °C (Warner, unpublished data). In contrast, P. ×hybrida ‘Mitchell’ exhibited much greater thermal stability for floral production across the evaluated temperature range, because flower bud number did not decline with increasing temperature (Table 3).
Table 3. Effect of temperature on the number of nodes below the first flower (nodes), number of visible flower buds at first flowering (buds), and number of lateral shoots (branches) for four Petunia spp. grown under a 16-h photoperiod.

| Species        | Temperature (°C) | Nodes (no.) | Buds (no.) | Branches (no.) |
|----------------|------------------|-------------|------------|----------------|
| P. axillaris   | 14               | 29.1        | 42.5       | 11.1           |
|                | 17               | 21.0        | 39.4       | 8.8            |
|                | 20               | 20.1        | 31.7       | 7.7            |
|                | 23               | 19.4        | 24.9       | 9.1            |
|                | 26               | 19.3        | 20.1       | 8.2            |
| Significance   | *                | ***         | ***        | ***            |
| $P_{\text{linear}}$ | NS               | ***         | NS         | NS             |
| $P_{\text{quadratic}}$ | *               | NS         | NS         | NS             |
| P. exserta     | 14               | 12.6        | 22.7       | 11.1           |
|                | 17               | 13.1        | 16.4       | 8.4            |
|                | 20               | 14.6        | 22.2       | 6.5            |
|                | 23               | 14.1        | 30.3       | 6.8            |
|                | 26               | 15.0        | 24.0       | 9.0            |
| Significance   | *                | ***         | NS         | NS             |
| $P_{\text{linear}}$ | NS               | NS         | NS         | NS             |
| $P_{\text{quadratic}}$ | *               | NS         | NS         | NS             |
| P. xhybrida    | 14               | 24.3        | 32.2       | 12.4           |
|                | 17               | 23.7        | 33.5       | 12.7           |
|                | 20               | 25.7        | 39.7       | 11.1           |
|                | 23               | 22.9        | 37.6       | 9.9            |
|                | 26               | 21.9        | 42.6       | 8.9            |
| Significance   | *                | ***         | ***        | ***            |
| $P_{\text{linear}}$ | NS               | **         | NS         | NS             |
| $P_{\text{quadratic}}$ | NS             | NS         | NS         | NS             |
| P. integrifolia| 14               | 25.3        | 39.0       | 10.6           |
|                | 17               | 24.7        | 31.9       | 10.1           |
|                | 20               | 17.4        | 32.6       | 6.0            |
|                | 23               | 15.5        | 24.3       | 6.5            |
|                | 26               | 14.6        | 20.9       | 6.6            |
| Significance   | ***              | ***         | ***        | ***            |
| $P_{\text{linear}}$ | *               | NS         | NS         | NS             |
| $P_{\text{quadratic}}$ | NS            | NS         | NS         | NS             |

*Means are based on two blocks of 10 plants each.

$\text{NS}$, *, ** and *** indicate nonsignificance or significance at $P < 0.05$, 0.01, or 0.001, respectively.

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