

Initial Growth of Seedlings of Flame Azalea in Response to Day/Night Temperature

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Abstract. Seedlings of flame azalea [*Rhododendron calendulaceum* (Michx.) Torr] were grown for 12 weeks under long-day conditions with days at 18, 22, 26, or 30C for 9 hours in factorial combination with nights at 14, 18, 22, or 26C for 15 hours. Total plant dry weight, top dry weight, leaf area, and dry weights of leaves, stems, and roots were influenced by day and night temperatures and their interactions. Dry matter production was lowest with nights at 14C. Root, leaf, top, and total dry weights were maximized with days at 26C in combination with nights at 18 to 26C. Stem dry weight was maximized with days at 26 to 30C and nights at 22C. Leaf area was largest with days at 18 and 26C in combination with nights at 18 or 26C. Within the optimal, day/night temperature range of 26 C/18-26C for total plant dry weight, there was no evidence that alternating temperatures enhanced growth. Shoot : root ratios (top dry weight : root dry weight) were highest with days at 18 and 30C. Leaf area ratio (total leaf area : total plant dry weight) was highest and specific leaf area (total leaf area : leaf dry weight) was largest when days and nights were at 18C and were lower at higher temperatures. Regardless of day/night temperature, leaf weight ratio (leaf dry weight : total plant dry weight) was higher than either the stem weight ratio (stem dry weight : total plant dry weight) or root weight ratio (root dry weight : total plant dry weight). Net leaf photosynthetic rate increased with day temperatures up to 30C.

Flame azalea is a deciduous species indigenous to the Appalachian region of the United States, extending from southwestern Pennsylvania to northern Georgia (Li, 1957; Liberty Hyde Bailey Hortorium, 1976). It blooms in late spring and is considered one of the most outstanding native flowering shrubs, with flower color ranging from orange-yellow to scarlet (Liberty Hyde Bailey Hortorium, 1976). The brilliant floral display of flame azalea, in combination with heightened interest in native plants, has increased demand for the species. However, production by nurserymen has been hindered, in part by lack of knowledge of how various environmental factors, particularly light and temperature, influence growth.

In a preliminary study on the influence of temperature and photoperiod on growth of flame azalea, seedlings were placed under short-day (9-h photoperiod daily) or long-day (9-h photoperiod plus a 3-h night interruption daily from 11:00 PM to 2:00 AM) conditions with 9-h day/15-h night cycles at 22/18, 26/22, and 30/26C (Malek et al., 1989). Plants grown under long days produced more top growth than those grown under short days, with the largest top dry weight realized at 26/22C. Previous work on evergreen azaleas reported increased shoot dry weight with increased daylength (Barrick and Sanderson, 1973; Pettersen, 1972; Skinner, 1939). Thus, it appears advantageous to use long-day conditions to hasten growth of flame azalea.

The effects of temperature on growth of evergreen azaleas

has been the subject of several investigations (Barrick and Sanderson, 1973; Pettersen, 1972; Skinner, 1939). Pettersen (1972) and Skinner (1939) found maximum shoot elongation under long days at constant temperatures of 28C and 24 to 27C, respectively. Barrick and Sanderson (1973) reported higher shoot dry weight with days/nights at 29/24C compared to 24/18C under long days. These studies focused on the effect of different temperature regimes on shoot growth. No research has been reported on the influence of temperature on other growth characteristics, such as leaf and root growth.

Although temperature is undoubtedly an important factor in growth of azalea species, determination of an optimum temperature for growth is complex because, for many species, there is no single temperature or combination of temperatures that will optimize all growth. Optimum temperature is a relative term, depending on the growth measurements used (Hellmers, 1966; Hinesley, 1981). For example, Hinesley (1981) reported different temperature optima for root, shoot, and total plant dry weights in seedlings of Fraser fir [*Abies fraseri* (Pursh) Poir.].

To improve current cultural practices for production of flame azalea, research is needed regarding the influence of temperature on growth. Therefore, the objective of this research was to examine the influence of selected day/night temperatures on vegetative growth of flame azalea under extended photoperiod.

Materials and Methods

On 13 Nov. 1986 mature seed capsules were collected from a native stand of open-pollinated plants growing in Watauga County, N. C., at an elevation of 1400 m. Capsules were stored in a paper bag at 20C for 21 days. Seeds were then removed from the capsules and stored at a moisture content of 6% in a sealed bottle at 4 ± 1 C. Moisture content of seeds was determined by calculating the mean moisture content of six, 100-seed samples following drying at 105C for 24 h.

Seeds were removed from storage on 4 Aug. 1988 and surface-sown in flats containing a medium of 4 pine bark :1 peat (v/v). Flats were placed under intermittent mist (Mist-A-Matic, E.C. Geiger, Harleysville, Pa.) in a growth room at day/night temperatures of $\approx 24/18$ C with a daily 18-h photoperiod (6:00 AM to 12:00 PM) provided by cool-white fluorescent lamps. The

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lamps provided a photosynthetic photon flux (PPF) of 260 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ plus photomorphogenic radiation (PR) of 5.7 $\text{W}\cdot\text{m}^{-2}$. These and other light measurements were recorded with a cosine-corrected LI-COR LI-185 quantum/radiometer/photometer (LI-COR, Lincoln, Neb.).

Following germination, seedlings were fertilized weekly with 180 ppm N from a 15N-20P-4K water-soluble fertilizer (Rhododendron Special; Grace/Sierra, Fogelsville, Pa.) that also contained 200 ppm CaCl₂ and 75 ppm MgSO₄. When seedlings were ≈ 2 cm high, they were transplanted individually into 1-liter containers using a medium of arcillite, a calcined clay. Arcillite was selected as a medium because it allows recovery of intact root systems at harvest (Hiller and Keller, 1979). Plants were grown under natural irradiance from 8:00 AM to 5:00 PM in a greenhouse maintained at day/night temperatures of $\approx 24/16^\circ\text{C}$. From 11:00 PM to 2:00 AM daily, plants received a night interruption from incandescent lamps that provided a PPF of 3.6 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ plus PR of 0.7 $\text{W}\cdot\text{m}^{-2}$, as measured at the top of the containers. Plants were fertilized weekly as previously described. Ten days after potting, plants were pruned back to three leaves to encourage branching.

On 4 Jan. 1989, plants were transferred to the Southeastern Plant Environment Laboratory and the different temperature regimes were initiated the following day using controlled-environment A-chambers (Downs and Thomas, 1983). The study was a 4×4 factorial in a completely random design using nine single-plant replications per temperature treatment. The two main factors were four day temperatures (18; 22, 26, and 30°C) in factorial combination with four night temperatures (14, 18, 22, and 26°C). Day temperatures were maintained for 9 h each day and coincided with the daily high-irradiance light period. Temperatures were maintained within $\pm 0.25^\circ\text{C}$ of the set point. Plants were moved between chambers at 8:00 AM and 5:00 PM daily to maintain appropriate day/night temperatures. Relative humidity was $> 70\%$. Carbon dioxide concentrations were 300 to 400 ppm.

During the 9-h high-irradiance period, chambers used a combination of cool-white fluorescent and incandescent lamps that provided a PPF of 642 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ plus PR of 8 $\text{W}\cdot\text{m}^{-2}$. Incandescent lamps providing a PPF of 44 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ plus PR of 6.7 $\text{W}\cdot\text{m}^{-2}$ were used to interrupt the dark periods between 11:00 PM and 2:00 AM daily.

Initially, plants were fertilized twice weekly (Tuesday and Friday) with the standard Phytotron-nutrient solution (Downs and Thomas, 1983). Beginning on week 6, fertilization was three times weekly (Monday, Wednesday, and Friday). Plants were watered with deionized water on the remaining days.

At week 10, leaf gas exchange was measured with a LI-COR LI-6200 closed portable infrared gas exchange system between 12:00 and 3:00 PM. Photosynthetically active radiation, air and leaf temperatures, and relative humidity inside the leaf chamber were measured concurrently with gas exchange. Net photosynthetic rate was calculated using the LI-COR 6200 measurements. An attached leaf was placed in a 1-liter (1149 cm³) chamber for 20 sec. Vapor pressure deficits within the chamber were 0.92, 1.20, 1.31, and 1.71 kPa at 18, 22, 26, and 30°C , respectively. Measurement commenced immediately after the CO₂ concentration decreased. Data were recorded on three plants per treatment, and three measurements (subsamples) were taken from the most recently matured leaves on each plant.

The study was terminated after 12 weeks. Plants were harvested and divided into leaves, stems, and roots; these were dried at 70°C for 72 h and weighed. Before drying, total leaf

area was measured with a LI-COR LI-3 100 leaf area meter. Leaf, stem, and root dry weights were used to calculate the following: total plant dry weight (sum of leaf, stem, and root dry weights); top dry weight (sum of leaf and stem dry weights); shoot : root ratio (top dry weight : root dry weight); leaf weight ratio (leaf dry weight : total plant dry weight); stem weight ratio (stem dry weight : total plant dry weight); and root weight ratio (root dry weight : total plant dry weight). Data also included leaf area ratio (total leaf area : total plant dry weight) and specific leaf area (total leaf area : leaf dry weight). All data were subjected to analysis of variance procedures and regression analysis.

Results

Dry-matter production was influenced by day and night temperatures and significant day/night temperature interactions occurred for all dry-weight categories (Table 1). Optimum day temperature for total plant dry weight was 26°C (Fig. 1A). At this temperature, maximum total plant dry weight was realized when nights were at 18 to 26°C . Night temperatures other than 22°C reduced total plant dry weight with 30°C days. Regardless of day temperature, dry-matter production was lowest when nights were at 14°C (Figs. 1A–C). Response of top and leaf dry weights (data not presented) was similar to that of total plant dry weight (Fig. 1A). Stem dry weight was maximized at day temperatures of 26 to 30°C with 22°C nights (Fig. 1B). Root dry weight was generally higher with days at 22 to 26°C than at 18 or 30°C (Fig. 1C). With 26°C days, maximum root dry weight was realized when the nights were at 18 to 26°C . Similar root growth was also observed at $22/22^\circ\text{C}$. When day temperature was at 30°C , root dry weight decreased irrespective of night temperature.

Leaf area was largest with 18 and 26°C days in combination with nights at 18 or 26°C (Fig. 1D). Days at 30°C combined with nights of 14 or 26°C resulted in greatly reduced leaf area (Fig. 1D).

Day/night temperature interactions were not significant for shoot : root ratio; leaf area ratio; leaf, stem, and root weight ratio; and specific leaf area (Table 1). Shoot : root ratio in-

Table 1. Significance of growth responses of seedlings of flame azalea to day and night temperatures.

Growth criterion	Variable ^a						
	D	DL	DQ	N	NL	NQ	D \times N
Dry weight							
Total plant	**	NS	**	**	**	**	**
Top	**	NS	*	**	**	**	**
Leaf	**	NS	**	**	**	**	**
Stem	*	NS	NS	**	**	**	*
Root	**	NS	**	*	NS	**	*
Leaf area	**	**	*	**	*	**	**
Shoot : root ratio	**	NS	**	**	**	NS	NS
Leaf area ratio	**	**	**	**	NS	*	NS
Leaf weight ratio	*	NS	**	**	*	NS	NS
Stem weight ratio	*	*	**	**	*	*	NS
Root weight ratio	**	NS	**	**	**	NS	NS
Specific leaf area	**	**	NS	**	*	**	NS
Net leaf photosynthetic rate	**	**	NS	NS	NS	NS	NS

^aD = day temperature, DL = day temperature (linear), DQ = day temperature (quadratic), N = night temperature, NL = night temperature (linear), NQ = night temperature (quadratic), D \times N = interaction of D and N.

NS, *, ** Nonsignificant or significant at $P = 0.05$ or 0.01 , respectively.

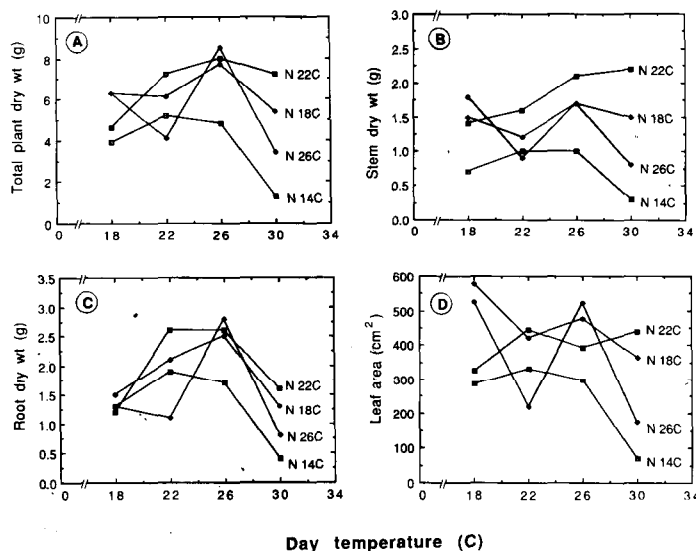


Fig. 1. Effects of day and night (N) temperatures on initial growth of seedlings of flame azalea. (A) total plant dry weight, (B) stem dry weight, (C) root dry weight, and (D) leaf area. Each datum is the mean for nine observations.

creased with increasing night temperature, and the effect of day temperature was greatest at 18 and 30C (Fig. 2A). Leaf area ratio increased curvilinearly in response to night temperature, to a maximum at 18C, but decreased with increasing day temperature (Fig. 2B).

Leaf weight ratio was consistently higher than either stem or root weight ratios throughout the whole range of day and night temperatures (Fig. 2C and D). Leaf weight ratio and stem weight ratio were lower with days at 22 or 26C, but the root weight ratio showed an opposite response (Fig. 2C). With days at 18 or 30C, 50% of total dry matter was in the leaves, with the remainder equally distributed between stems and roots (Fig. 2C). With 22 and 26C days, a higher percentage of dry matter (34%) was in the roots than in the stems (21%) (Fig. 2C). Root weight ratio decreased with increasing night temperature, whereas stem and leaf weight ratios increased (Fig. 2D). With a low night temperature (14C), the root weight ratio (0.35) was higher than the stem weight ratio (0.20) (Fig. 2D). The difference in dry matter distribution between roots and stems, however, became smaller with further increases in night temperature.

Day/night temperatures of 18C yielded the highest specific leaf area. Increasing day/night temperatures decreased specific leaf area (Fig. 2E).

Net leaf photosynthetic rate increased with increasing day temperature (Fig. 2F). Night temperatures had no effect on net photosynthetic rate, nor were there any day/night temperature interactions (Table 1).

Discussion

Generally, for nights at 18 to 26C and days at 22 to 26C, all dry-weight categories had higher values when night temperatures were lower than or equal to day temperatures (Fig. 1A and B). However, with days at 18C, most dry weights (except root dry weight, which was unaffected by night temperature) were enhanced by a higher (26C) night temperature. Higher night temperatures would be expected to be unfavorable for growth due to excessive respiration during the night (Hussey, 1965). However, Hellmers (1966) found that when nights were warmer, dry-weight production in seedlings of red fir (*Abies*

magnifica A. Murr.) was favored. Hussey (1965) reported that in seedlings of tomato (*Lycopersicon esculentum* Mill.), high night temperatures partially compensated for low day temperatures. He found that when the day temperature was suboptimal, increasing night temperature to as much as 10C above day temperature caused an increase in total dry weight. He attributed this response to increased photosynthetic area resulting from accelerated leaf growth under high night temperature. Our results showed that leaf area was near maximum under a low day/high night cycle of 18/26C (Fig. 1D). The relationships noted may account for the increased dry-weight accumulation observed under this temperature regime.

Within the optimal day/night range of 26C/18 to 26C for total plant dry weight, there was no evidence that alternating temperature enhanced growth. High total plant dry weight developed under a constant 26/26C and under alternating regimes of 26/18 and 26/22C (Fig. 1A). Friend and Helson (1976) could not find evidence for optimal thermoperiodicity in dry-weight accumulation in pea (*Pisum sativum* L.), bean (*Phaseolus vulgaris* L.), tomato, cucumber (*Cucurbita sativa* L.), corn (*Zea mays* L.), oat (*Avena sativa* L.), and wheat (*Triticum aestivum* L.).

Root dry weight was more sensitive to day temperatures than other growth characteristics. Day temperatures that were either too low (18C) or too high (30C) inhibited root growth regardless of the night temperature (Fig. 1C). Hence, even though it was possible to achieve a substantial top dry weight at 30/22 and 30/18C (data not presented), prolonged exposure to high day temperatures could lower seedling quality due to poor root growth. Further, at 30/22C, seedlings tended to have elongated internodes and atypically narrow upright leaves, whereas seedlings grown with days ≤ 26 C appeared normal. Therefore, growth under more moderate temperature combinations (e.g., 26/18, 26/22, and 26/26C) would be beneficial.

Partitioning of assimilates between leaves and other plant organs appeared to be unaffected by changes in temperature. Throughout the whole temperature range, leaf weight ratio remained higher than either stem or root weight ratios, indicating that, during initial seedling growth, dry-matter distribution favored leaves (Fig. 2C and D).

Specific leaf area is an anatomical index of leaf expansion that can also be used as an indirect measure of leaf thickness, a high ratio corresponding to a thin leaf (Friend et al., 1965). Values of specific leaf area (Fig. 2E) indicated that leaves were thinner and larger at 18C days, but thickened and became smaller as day temperatures increased to 30C (visual observation of the plants support these data).

Net leaf photosynthetic rate (Fig. 2F) was highest with 30C days, but total plant dry weight (Fig. 1A) decreased. However, temperature effects on photosynthesis are not necessarily parallel to those of dry weight gain. Potter and Jones (1977) observed that, generally, it is difficult to correlate unit area rates of photosynthesis to growth, although Friend and Helson (1976) reported that higher growth rates under a temperature regime of high day temperature was the result of higher rates of net photosynthesis. Other workers (Duncan and Hesketh, 1968; Hanson, 1971; Muramoto et al., 1965; Potter and Jones, 1977) found rates of leaf area expansion were much better correlated with growth than photosynthetic rates. This finding agrees, in part, with results reported herein. During initial seedling growth, greatest total plant dry weight occurred at 26/26C (Fig. 1A), which corresponded with a high leaf area (Fig. 1D). However, similar values for leaf area were realized at 18/26 and 18/18C

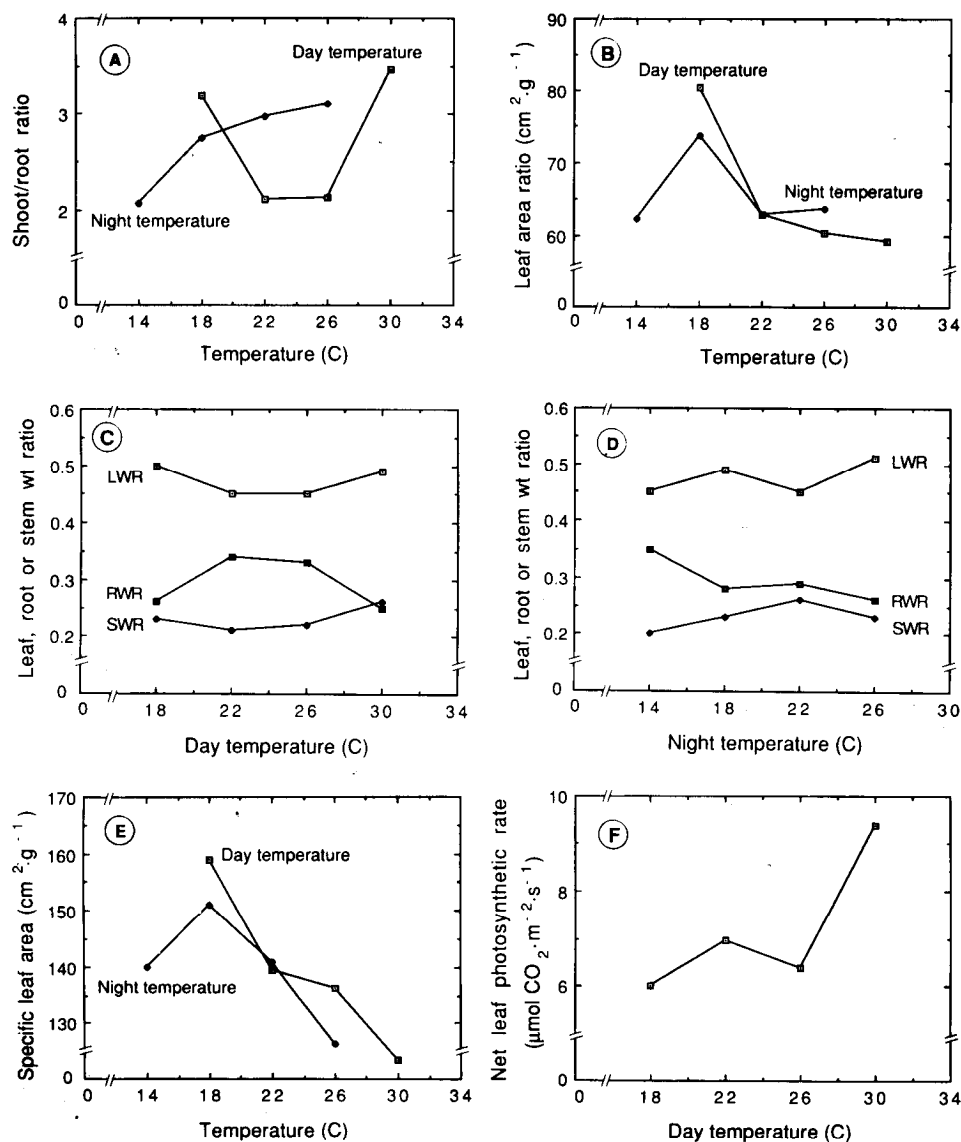


Fig. 2. Effects of day temperature (averaged over all night temperatures) and night temperature (averaged over all day temperatures) on initial growth of seedlings of flame azalea. (A) shoot : root ratio; (B) leaf area ratio; (C) leaf weight ratio (LWR), root weight ratio (RWR), and stem weight ratio (SWR) as a function of day temperature and (D) night temperature; (E) specific leaf area; and (F) net leaf photosynthetic rate as influenced by day temperature. In A–E each datum is the mean for 36 observations; in F each datum is the mean for 12 observations.

with lower total dry weight. This result suggests that even if leaf area is high, a favorable day/night temperature is necessary to maximize growth.

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