Seedling Growth of Catawba Rhododendron. I. Temperature Optima, Leaf Area, and Dry Weight Distribution

D. Bradley Rowe¹, Stuart L. Warren², and Frank A. Blazich³

Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609

Additional index words. *Rhododendron catawbiense*, native plants, Ericaceae, optimum temperature, thermoperiod, provenance

Abstract. *Catawba rhododendron* (*Rhododendron catawbiense* Michx.) seedlings of two provenances, Johnston County, N.C. (35°45'N, 78°12'W, elevation = 67 m), and Yancey County, N.C. (35°45'N, 82°16'W, elevation = 1954 m), were grown in controlled-environment chambers for 18 weeks with days at 18, 22, 26, or 30C in factorial combination with nights at 14, 18, 22, or 26C. Shoot and root dry weights and total leaf areas of seedlings of the Yancey County provenance (high elevation) exceeded ($P \leq 0.05$) those of the Johnston County (low elevation) provenance at all temperature combinations. Leaf area was maximal at 22/22C, 18/26C, and 22/26C and minimal at 30/14C (day/night). Shoot dry weight responded similarly. Root dry weight decreased linearly with increasing day temperature, but showed a quadratic response to night temperature. Leaf weight ratio (leaf dry weight : total plant dry weight) increased, while root weight ratio (root dry weight : total plant dry weight) decreased with increasing day temperature. Leaf weight ratio was consistently higher than either stem or root weight ratios. Day/night cycles of 22 to 26/22C appear optimal for seedling growth.

Catawba rhododendron is a broad-leaf, ericaceous, evergreen species indigenous to the mountains of West Virginia and Virginia, and it extends south to North Carolina, Georgia, and Alabama (Liberty Hyde Bailey Hortorium, 1976). It is a prized landscape plant, blooming in late spring with showy flowers ranging from lilac-purple to paler lilac-rose and occasionally white. Where moderate temperatures and a moist climate prevail, catawba rhododendron generally grows as a shrub, reaching a height of 3 m. In addition to outstanding landscape characteristics, the species has been used as a parent in many breeding programs to provide cold-hardy cultivars for the northeastern United States (Liberty Hyde Bailey Hortorium, 1976).

Catawba rhododendron exhibits greater adaptability to warmer climates than most species of rhododendron (Leach, 1961; Thornton, 1990), although its southern range is still limited (Dirr, 1990) due to factors such

as high night temperatures (Harden, 1990). An isolated provenance of the species occurs at a low elevation (67 m) in eastern North Carolina and was suspected to possess a higher temperature optima for growth than higher-elevation provenances (>1500 m) located in western North Carolina (Arisumi et al., 1986). If the low-elevation provenance exhibits a higher temperature optimum, the possibility then exists to expand the landscape range of catawba rhododendron to warmer regions of the southeastern United States or to use this germplasm to develop heat-tolerant cultivars.

Doorenbos (1955) reported that plants of catawba rhododendron grown under daily photoperiods of 18 to 24 h grew twice as fast as those grown under natural daylength. However, to our knowledge, no work has been reported on the influence of temperature on seedling growth. Therefore, the following study was conducted to 1) determine if differences in temperature optima exist between two provenances of catawba rhododendron, each occurring at different elevations, and 2) determine the effects of selected day/night temperatures on seedling growth.

Materials and Methods

Mature seed capsules were collected from native stands of open-pollinated plants of catawba rhododendron growing in Johnston County, N.C. (lat. 35°45'N, long. 78°12'W, elevation = 67 m), and Yancey County, N.C. (lat. 35°45'N, long. 82°16'W, elevation = 1954 m), on 31 Oct. and 20 Nov. 1989, respectively. The limited area occupied by the Johnston County provenance allowed collection of capsules from the majority of plants at that location. However, due to the vast area

comprising the Yancey County provenance, capsules were collected from representative plants, selected on visual appearance, within the provenance. Capsules were stored in paper bags at 20C for 21 days. Relative humidity was not controlled. Seeds were then removed from the capsules and stored at a moisture content of 6% in sealed glass bottles at 4 ± 1 C. Mean moisture content of the seeds was determined by calculation based on six, 100-seed subsamples of each provenance following drying at 105C for 24 h.

Seeds were removed from storage 10 Jan. 1990 and surface-sown in flats containing a medium of milled (<13 mm) pine bark. Flats were placed in a greenhouse under intermittent mist (Mist-A-Matic; E.C. Geiger, Harleysville, Pa.) with natural irradiance of $395 \text{ W} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$. Day/night greenhouse temperatures were 24 ± 4 C/ 16 ± 4 C. Seeds germinated in 6 to 9 days. After germination, seedlings received a night interruption from 2300 to 0200 HR daily from incandescent bulbs that provided a photosynthetic photon flux (PPF) of $3.6 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, as measured at the top of the flats. These and other light measurements were recorded with a cosine-corrected LI-185 quantum/radiometer/photometer (LI-COR, Lincoln, Neb.).

Seedlings were fertilized weekly with 180 mg N/liter supplied by a 15N–20P–4K water-soluble fertilizer (Grace/Sierra, Fogelsville, Pa.) that also contained 100 mg CaCl_2 /liter and 50 mg MgSO_4 /liter. At 584 days after germination (DAG), uniform seedlings were selected and transplanted individually into 1-liter containers using a medium of arcillite (Turface; AIMCOR, Deerfield, Ill.), a calcined clay, which allowed recovery of intact root systems at harvest (Hiller and Koller, 1979). Plants were grown under the same temperature and light conditions as the seedlings in the flats and were fertilized as described above.

At 646 DAG, uniform seedlings were transferred to the Southeastern Plant Environment Laboratory (Phytotron), and temperature treatments were initiated the following day [0 days after initiation (DAI) of experiment in the Phytotron] using controlled-environment A-chambers (Downs and Thomas, 1983). Seedlings were arranged as a 4×4 factorial in a completely random design using nine single-plant replications per temperature treatment per provenance. The two main factors were day (18, 22, 26, and 30C) and night (14, 18, 22, and 26C) temperatures provided to seedlings as 9/15-h thermoperiods. Temperatures were maintained within ± 0.25 C of the set point. Plants were moved between chambers at 0800 and 1700 HR daily to maintain appropriate day/night temperatures. Plants exposed to the same day and night temperature were also moved daily to different areas of the chamber to simulate transient mechanical perturbations and to avoid possible gradient effects within chambers. Relative humidity was >70%, and CO_2 concentration was 300 to 400 $\mu\text{mol} \cdot \text{mol}^{-1}$.

During the 9-h portion of the thermoperiod, chamber irradiance was provided by a combination of cool-white fluorescent lamps and

Received for publication 9 Dec. 1993. Accepted for publication 10 June 1994. This research was funded by the North Carolina Agricultural Research Service (NCARS), Raleigh. Use of trade names in this publication does not imply endorsement by the NCARS of products named, nor criticism of similar ones not mentioned. From a thesis submitted by D.B.R. in partial fulfillment of the MS degree requirements. Assistance of William M. Reece, Juan R. Acedo, and the staff of the Southeastern Plant Environment Laboratory is gratefully acknowledged. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

¹Graduate Research Assistant.

²Associate Professor.

³Professor.

incandescent bulbs resulting in a PPF of 642 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Incandescent bulbs providing a PPF of 44 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ were used as a dark interruption between 2300 and 0200 HR daily. Long-day conditions have been shown to maximize vegetative growth in previous studies with various species of rhododendron (Barba and Pokorny, 1975; Barrick and Sanderson, 1973; Malek et al., 1989).

Initially, plants were fertilized twice weekly with standard Phytotron nutrient solution (Downs and Thomas, 1983). To prevent iron deficiency, plants were sprayed to runoff with 0.3 g FeSO_4 /liter once a week beginning 15 DAI. At 36 DAI, fertilization was increased to three times weekly. Plants were watered with deionized water on days they were not fertilized. At 68 DAI, plants were sprayed to runoff with a coordination product of zinc ion, manganese, and ethylene biodithiocarbamate (Manzate 200 foliar; DuPont, Wilmington, Del.) at 1.9 g/liter⁻¹, and the growing medium was drenched with *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetal) alanine methyl ester (metalaxyl) (Ridomil 2E; Ciba-Geigy Corp. Agr. Div., Greensboro, N.C.) at 0.1 ml/liter⁻¹. At 103 DAI, plants were sprayed to runoff with methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl) (Benlate 50 DF, DuPont) at 0.5 g/liter⁻¹. Both fungicide applications were to deter possible fungal growth on leaves.

The study was terminated 126 DAI. Plants were divided into leaves, stems, and roots, and total leaf area was measured with a LI-COR LI-3100 leaf area meter. Then, all plant organs were dried at 70°C for 72 h and weighed. The data were used to calculate the following dry weights: total plant (sum of leaf, stem, and root), shoot (sum of leaf and stem); also, leaf weight ratio (leaf dry weight : total plant dry weight), stem weight ratio (stem dry weight : total plant dry weight), root weight ratio (root dry weight : total plant dry weight), and specific leaf area (total leaf area : leaf dry weight). For growth measurements affected by a sig-

nificant day $\times$ night (D $\times$ N) temperature interaction (Table 1), treatment means were based on nine, single-plant replications (Figs. 1–3). When the D $\times$ N interaction was nonsignificant, treatment means were averaged over levels of the independent variable, and the analyses were based on 36 single-plant replications (four treatments $\times$ nine replications per treatment). Data were subjected initially to analysis of variance (ANOVA) procedures. Linear and quadratic regression equations were then fit to the data, and correlations among specified variables were examined (SAS Institute, 1990).

Results and Discussion

Dry weights of entire seedlings, shoots, roots, and total leaf areas of the high-elevation provenance consistently exceeded ($P \leq 0.05$) those for seedlings of the low-elevation provenance at all temperature combinations (data not presented). This result suggests that the low-elevation provenance does not possess a higher temperature optimum for growth than the high-elevation provenance. In addition, seedlings from the high elevation produced more vigorous growth at all thermoperiods. In a previous study, Rowe et al. (1994) reported that seeds of the Yancey County provenance exhibited greater vigor, i.e., they germinated at a faster rate with greater cumulative germination than those from the low elevation. Reduced growth of the low-elevation provenance may be due, in part, to lack of genetic diversity resulting from a relatively small gene pool. This isolated provenance consists of 100 plants, whereas the high-elevation provenance consists of thousands.

There were no significant interactions with provenances. Therefore, data were averaged over provenances and reanalyzed to provide information regarding optimum day/night temperatures for seedling growth. Leaf area was significantly influenced by day and night temperatures and by their interactions (Table 1).

In contrast, Starrett et al. (1993) reported no significant day temperature effect on seedling growth of rosebay rhododendron (*Rhododendron maximum* L.). Leaf area showed a quadratic response with increasing day temperature at all night temperatures except 26°C (Fig. 1A). Maximum leaf area was produced at 22/22°C, 18/26°C, and 22/26°C, whereas thermoperiods of 18/14°C, 18/18°C, and 30/14°C resulted in reduced leaf area. Malek et al. (1992a) reported a similar response for seedling growth of flame azalea (*Rhododendron calendulaceum* Michx. Torr.), with leaf area maximized at a thermoperiod of 18/26°C. Our data agree with their report. For rosebay rhododendron, Starrett et al. (1993) reported the largest and smallest leaf areas with nights of 18 and 14°C, respectively.

Shoot dry weights were strongly correlated with leaf area ($r \geq 0.72$, $P \leq 0.001$) (Fig. 1B). This result agrees with the findings of Dale (1964), Malek (1992a), and Potter and Jones (1977), who reported that, in the vegetative stage, dry matter production in diverse species was strongly associated with leaf area development because growth of young plants depends on the amount of light intercepted by the leaves (De Koning, 1988). During seedling growth of catawba rhododendron, shoot dry weight was highest at 22/22°C and 26/18°C, temperatures that nearly maximized leaf area.

With nights at 26°C, shoot dry weight decreased at all day temperatures $>22^\circ\text{C}$ (Fig. 1B). Presumably, higher night temperatures resulted in greater loss of respiratory carbohydrates, which reduced dry weight accumulation (Deal et al., 1990; Kramer and Kozlowski, 1979) since temperature is the most important environmental factor influencing dark respiration (Levitt, 1980). However, with days at 18°C, nights at 22 and 26°C resulted in higher shoot dry weight. This relationship agrees with findings of Hellmers (1966) and Hinesley (1981), who reported that dry weight production in seedlings of red fir (*Abies magnifica* A. Murr.) and Fraser fir [*Abies fraseri* (Pursh)

Table 1. Growth responses of catawba rhododendron seedlings to selected day/night temperatures.

Growth measurement	Analysis of variance			Regression equations							
				x = day temp				x = night temp			
	D ^z	N	D $\times$ N	Night temp (°C)	Intercept	DL ^z	DQ ^z	Day temp (°C)	Intercept	NL ^z	NQ ^z
Leaf area	***	***	***	14	-3192.55	344.76***	-7.24***	18	194.14	31.07***	---
				18	-2924.00	308.24***	-6.04**	22	591.55	18.47**	---
				22	---	---	---	26	---	---	---
				26	1521.67	-24.92***	---	30	-1947.00	276.18***	-6.67***
				14	-65.25	7.22***	-0.15***	18	---	---	---
Shoot dry weight	***	NS	**	18	-59.53	6.47***	-0.13***	22	---	---	---
				22	---	---	---	26	26.96	-0.40**	---
				26	22.94	0.24*	---	30	-37.36	5.41***	-0.13***
				14	18.56	-0.39***	---	18	-5.28	1.54**	-0.04***
Root dry weight	***	*	NS	14	53.47	0.47*	---	18	33.84	1.94***	---
Specific leaf area	NS	***	***	18	50.78	0.73**	---	22	48.61	1.13***	---
				22	111.41	-1.50**	---	26	49.54	1.06***	---
				26	94.82	-0.79**	---	30	---	---	---
				14	-0.12	0.04***	0.01***	18	0.73	-0.03**	0.01**
Leaf weight ratio	***	*	NS	14	1.21	-0.01***	0.01***	18	---	---	---
Root weight ratio	***	NS	NS	14	---	---	---	18	---	---	---

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

¹Response was nonsignificant (NS).

NS, *, **, *** Nonsignificant or significant at $P \leq 0.10$, 0.05, or 0.01, respectively.

Fig. 1. Effects of day and night (N) temperatures on seedling growth of catawba rhododendron. (A) Leaf area and (B) shoot dry weight.

Fig. 2. Effects of day and night temperatures on root dry weights of catawba rhododendron. [Data were averaged over respective day (D) or night (N) temperatures because of lack of a significant D $\times$ N temperature interaction.]

Poir.] was favored when warm nights were combined with cool days. Hussey (1965) reported high night temperatures partially compensated for low day temperatures in seedling growth of tomato (*Lycopersicon esculentum* Mill.). While this relationship may account for our results at specific thermoperiods, a day/night cycle of 30/14C resulted in the smallest leaf area and lowest shoot dry weight. Low night temperatures also reduced CO_2 assimilation rates in rose (*Rosa hybrida*) (Khayat and Zieslin, 1986), ryegrass (*Lolium perenne* L.) (Wilson, 1970), and sorghum (*Sorghum vulgare* Moench.) (Pasternak and Wilson, 1972), possibly reducing growth.

Root growth was more sensitive to high day temperatures than shoot growth. Root dry weight decreased linearly with increasing day temperature but exhibited a quadratic growth response to night temperature, with a maximum at 18C (Fig. 2). Continued exposure to high day temperatures might eventually lower seedling quality due to reduced root growth. High day temperatures also reduced root dry weight of flame azalea (Malek et al., 1992a) and Fraser fir (Hinesley, 1981).

Specific leaf area is a morphological index of leaf expansion that can also be used as an indirect measure of leaf thickness, where a high ratio corresponds to a thin leaf (Friend and Helson, 1965). Values of specific leaf area indicated leaves were thinner and larger with days at 18 or 22C in combination with nights of 22 or 26C, and at 26/26C (Fig. 3). With nights at 22 and 26C, leaves became thicker and smaller as day temperatures increased to 30C. In contrast, with nights at 14 and 18C, leaves became thinner with increasing day temperature. The same response was observed in flame azalea (Malek et al., 1992a). However, Starrett et al. (1993) reported that day temperature did not affect specific leaf area of rosebay rhododendron, but nights of 22 and 26C similarly reduced specific leaf area.

Leaf weight ratio increased with increasing day temperature from 0.41 to 0.50, an increase of 22% (Fig. 4A), and was maximized at nights of 14 and 26C (Fig. 4B). Root weight ratio decreased with increasing day temperature (Fig. 4A), but was unaffected by night temperature (Fig. 4B). As day temperature increased, the percentage of leaf dry matter increased at the expense of root dry weight. Leaf weight ratio was consistently higher than either stem (data not presented) or root weight ratios for all day/night temperatures, indicating that during seedling growth, dry matter distribution favored leaves. Leaves represented nearly 50% of total plant dry weight across all night temperatures. Stem weight ratio was fairly constant over thermoperiods (data not presented). Similar responses have been noted for flame azalea (Malek et al., 1992a), mountain laurel (*Kalmia latifolia* L.) (Malek et al., 1992b), and rosebay rhododendron (Starrett et al., 1993).

Determination of an optimal temperature for growth is complex, as it may change with the stage of plant development or with the part of the plant measured. For example, Fraser fir possesses different temperature optima for

Fig. 3. Effects of day and night (N) temperatures on specific leaf area of catawba rhododendron seedlings.

Fig. 4. Effects of day temperature averaged over all night temperatures (A) and night temperatures averaged over all day temperatures (B) on leaf weight ratio (LWR) and root weight ratio (RWR) of catawba rhododendron seedlings.

root, shoot, and total plant dry weights (Hinesley, 1981). Malek et al. (1992a) found a common optimum day/night temperature for root, leaf, shoot, and total plant dry weight, but different temperature optima for leaf area and stem dry weight in seedlings of flame azalea. Although temperature optima varied for particular growth measurements of catawba rhododendron, thermoperiods of 22 to 26/22C appeared to optimize growth of seedlings.

Literature Cited

- Arisumi, K., E. Matsuo, Y. Sakata, and T. Tottoribe. 1986. Breeding for the heat resistant rhododendrons. Part II: Differences of heat resistance among species and hybrids. J. Amer. Rhododendron Soc. 40:215-219.
- Barba, R.C. and F.A. Pokorny. 1975. Influence of photoperiod on the propagation of two rhododendron cultivars. J. Hort. Sci. 50:55-59.
- Barrick, W.E. and K.C. Sanderson. 1973. Influence of photoperiod, temperature, and node position on vegetative shoot growth of greenhouse azaleas, *Rhododendron* cv. J. Amer. Soc. Hort. Sci. 98:331-334.
- Dale, J.E. 1964. Some effects of alternating temperature on the growth of French bean plants. Ann. Bot. (NS) 28:127-135.
- Deal, D.L., J.C. Raulston, and L.E. Hinesley. 1990. Leaf color retention, dark respiration, and growth of red-leafed Japanese maples under high night temperatures. J. Amer. Soc. Hort. Sci. 115:135-140.
- De Koning, A.N.M. 1988. The effect of different day/night temperature regimes on growth, development and yield of glasshouse tomatoes. J. Hort. Sci. 63:465-471.
- Dirr, M.A. 1990. Manual of woody landscape plants: Their identification, ornamental characteristics, culture, propagation and uses. 4th ed. Stipes Publishing Co., Champaign, Ill.
- Doorenbos, J. 1955. Shortening the breeding cycle of rhododendron. Euphytica 4:141-146.
- Downs, R.J. and J.F. Thomas. 1983. Phytotron procedural manual for controlled environment research at the Southeastern Plant Environment Laboratory. North Carolina Agr. Res. Serv. Tech. Bul. 224 (revised).
- Friend, D.J.C. and V.A. Helson. 1965. Changes in the leaf area ratio during growth of Marquis wheat, as affected by temperature and light intensity. Can. J. Bot. 43:15-28.
- Harden, W. 1990. Growing rhododendrons in warm climates. J. Amer. Rhododendron Soc. 45:140-142.
- Hellmers, H. 1966. Temperature action and interaction of temperature regimes in the growth of red fir seedlings. For. Sci. 12:90-96.
- Hiller, L.K. and K.C. Koller. 1979. Potato growth responses in arcillite and sand. HortScience 14:534-536.
- Hinesley, L.E. 1981. Initial growth of Fraser fir seedlings at different day/night temperatures. For. Sci. 27:545-550.
- Hussey, G. 1965. Growth and development in the young tomato. III. The effect of night and day temperatures on vegetative growth. J. Expt. Bot. 16:373-385.
- Khayat, E. and N. Zieslin. 1986. Effect of different night temperature regimes on the assimilation, transport and metabolism of carbon in rose plants. Physiol. Plant. 67:608-613.
- Kramer, P.J. and T.T. Kozlowski. 1979. Physiology of woody plants. Academic, New York.
- Leach, D.G. 1961. Rhododendrons of the world and how to grow them. Charles Scribner's Sons, New York.
- Levitt, J. 1980. Responses of plants to environmen-

- tal stresses. 2nd ed. Academic, New York.
- Liberty Hyde Bailey Hortorium. 1976. Hortus third: A concise dictionary of plants cultivated in the United States and Canada. 3rd ed. Macmillan, New York.
- Malek, A.A., F.A. Blazich, S.L. Warren, and J.E. Shelton. 1992a. Initial growth of seedlings of flame azalea in response to day/night temperature. J. Amer. Soc. Hort. Sci. 117:216–219.
- Malek, A.A., F.A. Blazich, S.L. Warren, and J.E. Shelton. 1992b. Initial growth of seedlings of mountain laurel as influenced by day/night temperature. J. Amer. Soc. Hort. Sci. 117:736–739.
- Malek, A.A., F.A. Blazich, S.L. Warren, and J.E. Shelton. 1989. Growth response of flame azalea as influenced by temperature and photoperiod. Proc. Southern Nurserymen's Assn. Res. Conf., 34th Annu. Rpt. p. 47–48.
- Pasternak, D. and G.L. Wilson. 1972. After effects of night temperatures on stomatal behaviour and photosynthesis of sorghum. New Phytol. 71:683–689.
- Potter, J.R. and J.W. Jones. 1977. Leaf area partitioning as an important factor in growth. Plant Physiol. 59:10–14.
- Rowe, D.B., F.A. Blazich, S.L. Warren, and T.G. Ranney. 1994. Seed germination of three provenances of *Rhododendron catawbiense*: Influence of light and temperature. J. Environ. Hort. 12:155–158.
- SAS Institute. 1990. SAS/STAT user's guide. vol. 2. SAS Inst., Cary, N.C.
- Starrett, M.C., F.A. Blazich, and S.L. Warren. 1993. Initial growth of rosebay rhododendron seedlings as influenced by day and night temperatures. HortScience 28:705–707.
- Thornton, J.T. 1990. Breeding rhododendrons for the Gulf South. J. Amer. Rhododendron Soc. 44(2):91–93.
- Wilson, D. 1970. Starch and apparent rate of photosynthesis of leaves of *Lolium perenne* grown at different temperatures. Planta 91:274–278.