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Seed Germination of Three Provenances of *Rhododendron catawbiense*: Influence of Light and Temperature¹

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- Abstract -

Seeds of three provenances of *Rhododendron catawbiense* Michx. (Catawba rhododendron) were collected during Fall 1992 from the following localities in the southeastern United States: Cherokee Co., GA [34°20'N, 84°23'W, elev. = 320 m (1050 ft)], Johnston Co., NC [35°45'N, 78°12'W, elev. = 67 m (220 ft)], and Yancey Co., NC [35°45'N, 82°16'W, elev. = 1954 m (6410 ft). Following drying for 1 month and storage at 4°C (39°F), seeds were removed from storage in January 1993 and germinated at 25°C (77°F) or an 8/16 hr thermoperiod of 25°/15°C (77°/59°F) with daily photoperiods of 0, $\frac{1}{2}$, 1, 2, 4, 8, 12, or 24 hr. Regardless of temperature and provenance, seeds required light for germination. Negligible germination for all provenances in total darkness was overcome by daily photoperiods as short as $\frac{1}{2}$ hr. All provenances commenced germination earlier at 25°C (77°F) than at 25°/15°C (77°/59°F). Mean germination at day 24 for both temperature treatments and for all photoperiods with the exception of total darkness was 98%, 90%, and 80% for the Yancey, Johnston, and Cherokee Co. provenances respectively. Light and temperature requirements for seed germination of all provenance exhibited greater vigor; they germinated at a faster rate with greater cumulative germination.

Index words: seeds, sexual propagation, Catawba rhododendron, Ericaceae, native plants.

Significance to the Nursery Industry

Quantitative data are presented regarding the influence of light and temperatures of 25°C (77°F) and 25°/15°C (77°/ 59°F) on seed germination of three provenances of *Rhododendron catawbiense* representing diverse geographical and altitudinal distributions. Generally, light and temperature requirements for germination of different provenances appear similar. Regardless of temperature, seeds require light for germination, and daily photoperiods as short as ¹/₂ hr will maximize germination. The major difference in germination response of different provenances is related to seed vigor. Seeds of a high elevation provenance germinated at a faster rate with greater cumulative germination than seeds of lower elevation provenances.

Introduction

Rhododendron catawbiense Michx. (Catawba rhododendron) is a broad-leaf, ericaceous evergreen species indigenous to the mountains of West Virginia and Virginia, extending south to North Carolina, Georgia, and Alabama (7). 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, *R. catawbiense* generally grows as a shrub reaching a height of 3 m (9.8 ft). 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 (7).

Traditionally, the principal means of meeting demand in the Southeast for R. *catawbiense* has been harvesting of mature, native plants or 'cutbacks', produced when the tops

of mature plants are removed (cut back) to within 8 to 10 cm (3 to 4 in) of the soil surface (2). The latter are then dug and replanted in the field for further growth prior to sale. Both of these practices deplete native stands which result in an insufficient supply of native plants to meet demand. Other methods of production, such as sexual propagation, have not been widely utilized in the Southeast due in part to lack of published protocols regarding such practices and the inability of nurserymen to successfully propagate the species by seed.

In recent years, popularity and demand for R. catawbiense have intensified due to increased interest in native plants. To help satisfy demand, many nurserymen are attempting to utilize sexual propagation. Recently, Blazich et al. (3) reported on the influence of light and temperature on seed germination of a provenance of R. catawbiense located in western North Carolina. The population from which this research was based is located in Buncombe Co., NC [35°42'N, 82°22'W, elev. = 1860 m (6100 ft)]. However, in North Carolina and other areas of the southeast, isolated provenances of R. catawbiense occur at much lower elevations. These populations may possess desirable horticultural traits (e.g. greater heat tolerance) not found in germplasm originating at the higher elevations (1). Differences regarding influence of various environmental factors (e.g. light and temperature) on seed germination may also be present. Therefore, the objective of this research was to examine the influence of varying photoperiods and constant versus alternating temperature on seed germination of three provenances of R. catawbiense representing diverse geographical and altitudinal distributions.

Materials and Methods

Mature seed capsules were collected from native stands of open-pollinated plants of *R. catawbiense* growing in Johnston Co., NC [$35^{\circ}45'N$, $78^{\circ}12'W$, elev. = 67 m (220 ft)], Cherokee Co., GA [$34^{\circ}20'N$, $84^{\circ}23'W$, elev. = 320 m (1050 ft)], and Yancey Co., NC [$35^{\circ}45'N$, $82^{\circ}16'W$, elev. = 1954 m (6410 ft)] on October 6, October 12, and November

¹Received for publication December 6, 1993; in revised form April 24, 1994. Assistance of William M. Reece, Juan R. Acedo, Everett P. Whitman, and the staff of the Southeastern Plant Environment Laboratory (Phytotron) is gratefully acknowledged. This paper is based on a portion of a thesis submitted by the senior author in partial fulfillment of the requirements for the MS degree. ²Graduate Research Assistant, Professor, Associate Professor, and Assistant Professor, resp.

10, 1992, respectively. The limited area occupied by the Johnston Co. and Cherokee Co. provenances allowed collection of capsules from the majority of plants at each location. However, due to the vast area comprising the Yancey. Co. provenance, capsules were collected from a representative population within the provenance.

Following collection, capsules were stored in paper bags at 20°C (68°F) for 30 days. Seeds were then removed from the capsules and stored in sealed glass bottles at 4°C (39°F). At storage, seed moisture content of the Yancey, Johnston, and Cherokee Co. provenances was 10%, 9%, and 7%, respectively [approximately 190,000, 160,000, and 140,000 pure seeds per 28 g (1 oz), resp.]. Moisture content of each provenance was determined by calculating the mean moisture content of six, 100 seed samples following drying at 105°C (221°F) for 24 hr.

In January 1993, seeds of all provenances were removed from storage and graded by manual removal of abnormal, damaged, and undersized seeds. Graded seeds were sown in covered 9 cm (3.5 in) glass petri dishes containing two prewashed germination blotters (Filtration Sciences Corp., Mt. Holly Springs, PA) moistened with tap water. Seeds were placed in the dishes (50 seeds per dish) with half designated for germination at 25°C (77°F) and the other half for germination at an 8/16 hr thermoperiod of 25°/15°C (77°/59°F). All dishes were placed in black sateen cloth bags and seeds were allowed to imbibe overnight at 21°C (70°F). The next day, bags were randomized within two growth chambers [Cchambers (6)] set at the appropriate temperatures. Chamber temperatures varied within ± 0.5 °C (0.9°F) of the set point.

Within each temperature regime, seeds were subjected daily to the following eight photoperiods: total darkness, $\frac{1}{2}$, 1, 2, 4, 8, 12, or 24 hr. Regardless of temperature, photoperiod treatments were administered at the same time each day. All photoperiod treatments for the alternating temperature of $25^{\circ}/15^{\circ}C$ ($77^{\circ}/59^{\circ}F$), with the exception of total darkness and 24 hr, began with the transition to the high temperature portion of the cycle.

Growth chambers were equipped with cool-white fluorescent lamps which provided a photosynthetic photon flux (400-700 nm) of 28 μ mol·m⁻²·s⁻¹ (2.2 klx) as measured at dish level with a cosine-corrected LI-COR LI-185 quantum/ radiometer/photometer (LI-COR, Lincoln, NE). All photoperiod treatments, except total darkness and the 24 hr irradiation, were regulated by removal and placement of the petri dishes in black sateen cloth bags. Petri dishes remained continuously unbagged in open chamber conditions for the 24-hr photoperiod treatment. Regardless of the photoperiod, temperatures within the petri dishes, as measured by a thermocouple, never exceeded ambient by more than 1°C (2°F). Constant darkness treatment was maintained by keeping petri dishes in the black cloth bags throughout the experiment. All watering and germination counts were performed in a darkroom utilizing a fluorescent lamp equipped with a green acetate filter (Rosco Laboratories, Port Chester, NY). Germination blotters were kept moist with tap water throughout the duration of the experiment. Seeds showing signs of decay were immediately removed from the dishes.

Each photoperiod treatment was replicated four times with a replication consisting of a petri dish containing 50 seeds. Germination counts were recorded every 3 days for 30 days. A seed was considered germinated when the emerging radicle was $\geq 1 \text{ mm} (0.04 \text{ in})$. Percent germination was calculated as a mean of four replications per treatment. Within each temperature, for a provenance, data were subjected to analysis of variance procedures (11). Analysis of variance showed that for each provenance, photoperiod, time (days), and their interactions were highly significant. Thus, regression analysis was conducted on cumulative germination for a provenance within each temperature starting at day 6 for each 3day interval. Regression analyses did not include data for total darkness.



Fig. 1. Influence of light and temperature on seed germination of the Yancey Co., NC provenance of *R. catawbiense*. (A) germinated at 25°C (77°F) with daily photoperiods ranging from total darkness (0 hr) to 24 hr. Data for the 1, 2, 4, 8, and 12-hr photoperiods were omitted since germination was similar to the ¹/₂-hr treatment. (B) germinated at 25°/15°C (77°/59°F) utilizing the same photoperiods as in A. Data for the 1, 2, 4, and 8-hr photoperiods were omitted since germination was similar to the ¹/₂-hr treatment. Legend in (A) applies to both figures.



Fig. 2. Influence of light and temperature on seed germination of the Cherokee Co., GA provenance of *R. catawbiense*. (A) germinated at 25°C (77°F) with daily photoperiods ranging from total darkness (0 hr) to 24 hr. Data for the 8, 12, and 24-hr photoperiods were omitted since germination at 8 and 12 hr was similar to 1 hr and the 24-hr photoperiod was similar to the ¹/₂-hr treatment. (B) germinated at 25°(15°C (77°/59°F) utilizing the same photoperiods as in A. Data for the 2 and 8-hr photoperiods were omitted since germination at 2 hr was similar to ¹/₂ hr and the 8-hr photoperiod was similar to the 1-hr treatment. Legend in (A) applies to both figures.

Results and Discussion

With some exceptions, which will be addressed, the germination responses of all provenances were similar. Therefore, only data from the Yancey Co., NC and Cherokee Co., GA provenances will be presented.

Regardless of temperature or provenance, seeds required light for germination (Figs. 1 and 2). The light requirement for seed germination of R. catawbiense agrees with previous reports dealing with various native ericaceous species of the southeastern United States including Rhododendron maximum L. [rosebay rhododendron (3)], Leucothoe fontanesiana (Steud.) Sleum. [drooping leucothoe (4)], Rhododendron carolinianum Rehd. [Carolina rhododendron (5)], Rhododendron calendulaceum Michx. Torr. [flame azalea (8)], and Kalmia latifolia L. [mountain laurel (9)]. However, it contrasts with earlier work on R. catawbiense by Blazich et al. (3) who reported 30-day germination of 5% and 64% was achieved in total darkness at 25°C (77°F) and 25°/15°C (77°/59°F), respectively. Blazich et al. (3) speculated that seeds of R. catawbiense may lose light sensitivity with time (aging), since seeds used in their study had been in storage for 29 months. They also speculated that germination response in darkness may be influenced by environmental conditions under which the seeds develop. Seeds used in the present study were in storage for only 2 to 3 months which may account for the differing responses. Regardless of temperature, subjecting seeds in the present study to daily photoperiods as short as 1/2 hr increased total germination to 98% for the Yancey Co. provenance (Fig. 1), 85% to 92% for the Johnston Co. provenance (data not presented), and 87% to 91% for the Cherokee Co. provenance (Fig. 2).

Seeds from Yancey Co. exposed to photoperiods $\geq \frac{1}{2}$ hr began germinating between days 3 to 6 at 25°C (77°F) compared to days 6 to 9 at 25°/15°C (77°/59°F) (Fig. 1). However, seeds from Johnston Co. (data not presented) and Cherokee Co. exposed to photoperiods $\geq \frac{1}{2}$ hr began germinating between days 6 to 9 at 25°C (77°F) compared to days 9 to 12 at 25°/15°C (77°/59°F) (Fig. 2). A similar delay in



Fig. 3. Cumulative seed germination of the Yancey Co., NC provenance of *R. catawbiense* as influenced by photoperiod at days (D) 6 to 30. Data for seeds germinated in total darkness were omitted.
(A) germinated at 25°C (77°F). Data for days 15, 18, 21, 24, and 27 were omitted since they were similar to day 30. (B) germinated at 25°C (77°/59°F). Data for days 6, 18, 21, 24, and 27 were omitted since no germination occurred by day 6, and days 18, 21, 24, and 27 were similar to day 30.

germination for the species at 25°/15°C (77°/59°F) was reported by Blazich et al. (3). Regardless of temperature and photoperiod, seeds of the Yancey Co. provenance consistently germinated earlier and had greater cumulative germination.

At 25°C (77°F), photoperiods > 8 hr inhibited germination of seeds from Yancey Co. (Fig. 3A). At 12 days, inhibition was still present for the 24 hr photoperiod, but by day 15 germination for all photoperiod treatments except total darkness was 97%. Inhibition of germination at 25°/15°C (77°/59°F) was more pronounced, since an alternating temperature can partially substitute for the light requirement for some species (12) (Fig. 3B). Inhibition was first noted at day 9 for photoperiods > 2 hr, but by day 18, cumulative germination for all photoperiods except total darkness ranged from 95% to 99%.

Seeds of the Cherokee Co. provenance also exhibited inhibition of germination at particular photoperiods and this inhibition lasted for a longer period of time. Seeds germinated at 25°C were inhibited by photoperiods > 2 hr; however, by day 18 germination for all photoperiods with the exception of total darkness ranged from 74% to 88% (Fig. 4A). At 25°/15°C (77°/59°F) inhibition was first noticed at day 12 for photoperiods > 2 hr which continued throughout the study (Fig. 4B). The inhibitory affect of the 24-hr photoperiod was again more pronounced at 25°/15°C than 25°C. Cumulative germination by day 30 for all photoperiod treatments except total darkness ranged from 70% to 91% with maximum germination of 91% occurring at the 2-hr photoperiod. Inhibition of germination at 25°C (77°F) and 25°/ 15°C (77°/59°F) by particular photoperiods also has been reported by Blazich et al. for R. catawbiense (3) and other ericaceous species including R. maximum (3), L. fontanesiana (4), and R. carolinianum (5).

Generally, for equivalent photoperiods, inhibition when present, will be more pronounced at 25°/15°C (77°/59°F) than at 25°C (77°F) and will dissipate by the end of a 30day germination period (3, 4, 5). However, inhibition of seed germination of the Cherokee Co., GA provenance by photo-



Fig. 4. Cumulative seed germination of the Cherokee Co., GA provenance of *R. catawbiense* as influenced by photoperiod at days (D) 9 to 30. Data for seeds germinated in total darkness were omitted. (A) germinated at 25°C (77°F). Data for days 18, 21, 24, and 27 were omitted since they were similar to day 30. (B) germinated at 25°/15°C (77°/59°F). Data for days 9, 24, and 27 were omitted since no germination occurred by day 9, and days 24, and 27 were similar to day 30.

 Table 1.
 Effect of photoperiod and temperature on seed germination of the Yancey Co., NC and Cherokee Co., GA provenances of Rhododendron catawbiense at days 12 and 24.

Provenance	Photoperiod (hr)							
	0	1/2	1	2	4	8	12	24
	Germination (%)							
	Temp = 25°C, Day 12							
Yancey Co., NC	0	96.5	96.1	95.6	96.0	95.0	95.1	90.4
Cherokee Co., GA	0	46.5	47.2	64.8	61.1	65.6	68.3	49.8
F test ^z	NS	***	***	**	**	***	***	***
	Temp = 25°/15°C, Day 12							
Yancey Co., NC	0	76.7	84.2	85.7	83.5	82.4	67.8	50.7
Cherokee Co., GA	0	7.0	8.0	9.0	8.0	4.0	0	0
Ftest	NS	***	***	***	***	***	***	***
	Temp = 25°C, Day 24							
Yancey Co., NC	0	97.5	96.6	96.6	96.5	97.5	98.5	98.5
Cherokee Co., GA	0	83.8	79.8	86.1	75.0	77.5	78.9	85.0
Ftest	NS	**	**	*	***	**	***	**
		Temp = 25°/15°C, Day 24						
Yancey Co., NC	1.0	99.1	98.0	98.6	99.0	99.0	99.0	97.0
Cherokee Co., GA	0.5	90.7	77.2	88.5	84.6	75.6	74.3	68.8
Ftest	NS	**	***	*	***	**	***	***

²NS,*,**,*** nonsignificant or significant at P = 0.10, 0.05, or 0.01, respectively.

periods > 2 hr at 25°C (77°F) and 25°/15°C (77°/59°F) was still observed at day 30 (Figs. 2 and 4). This suggests that germination response of the Cherokee Co. provenance to light and temperature might be unique.

By day 12, regardless of temperature, germination of the Yancey Co. provenance was greater than germination for both the Johnston Co. (data not presented) and Cherokee Co. provenances at all photoperiods except total darkness (Table 1). By day 24, regardless of temperature, seeds from Yancey Co. still exhibited higher germination, but not all photoperiods were significantly greater than Johnston Co. (data not presented). These rate data (Table 1) and the aforementioned germination time courses (Figs. 1 and 2) demonstrate that seeds of the Yancey Co. provenance exhibited greater vigor than those collected from the isolated provenances in Johnston Co., NC and Cherokee Co., GA. Seeds of the Yancey Co. provenance germinated faster and with greater cumulative germination.

Greater seed vigor of the Yancey Co., NC provenance also extends to seedling growth. Rowe (10) compared seedling growth of the Yancey and Johnston Co. provenances over a range of day/night temperature regimes. Total seedling dry weights of the Yancey Co. provenance were greater (p < 0.05) at all temperature combinations compared to seedlings of the Johnston Co. provenance. In addition, 2 years after the aforementioned study was conducted, visual observations of containerized plants representing these two provenances confirmed previous results.

Generally, light and temperature requirements for seed germination of the three provenances of *R. catawbiense* were similar although seeds of the Yancey Co., NC provenance possessed greater vigor. Thus, it appears the major difference in the germination response of seeds of different provenances of *R. catawbiense* is related to vigor and not to light and temperature requirements.

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