Seed Germination of Two Populations of *Rhododendron vaseyi:* Influence of Light and Temperature[1]

Anthony V. LeBude[2], Frank A. Blazich[3], Lela C. Walker[4], and Sonya M. Robinson[5]
Department of Horticultural Science, Mountain Horticultural Crops Research and Extension Center
North Carolina State University, Mills River, NC 28759

Abstract

Seeds of two populations of *Rhododendron vaseyi* A. Gray (pinkshell azalea) were germinated at 25C (77F) or an 8/16 hr thermoperiod of 30/20C (86/68F) with daily photoperiods at each temperature of 0 (total darkness), ½, 1, 2, 4, 8, 12, or 24 hr. Germination was recorded every 3 days for 30 days. Light was required for germination regardless of population or temperature. As photoperiod increased, germination increased. The highest cumulative (30-day) germination percentages were realized at 30/20C with a 24-hr photoperiod; germination percentages for populations 1 and 2 were 45 and 50%, respectively. Temperature and photoperiod requirements for germination did not generally differ between the two populations.

Index words: seeds, sexual propagation, native plants, Ericaceae, pinkshell azalea.

Significance to the Nursery Industry

Quantitative data are presented concerning the influence of light and temperatures of 25C (77F) or an 8/16 hr thermoperiod of 30/20C (86/68F) on germination of seeds of *Rhododendron vaseyi* (Ericaceae Juss.) collected from two populations in western North Carolina. Light was required for germination and continuous light (24 hr) resulted in the highest germination for both populations at 30/20C (86/68F). Generally, the light and temperature requirements for germination did not differ between the populations and seeds from both populations germinated at approximately 50%. Because of their small size and light requirement, seeds should be simply dusted on the surface of a germination medium.

Introduction

Rhododendron vaseyi (pinkshell azalea) is a rare, deciduous ericaceous species endemic to Watauga, Avery, and Mitchell Counties in northwest North Carolina and Transylvania, Jackson, and Macon counties in the southwest portion of the state (13). The primary habitats of this plant are swamps and bogs above 914 m (3000 ft), but plants have been observed in high elevation spruce forests and on southern or southeastern slopes of Grandfather Mountain above 1524 m (5000 ft) (8, 10, 13, 16).

In May to June, *R. vaseyi* produces attractive pink to sometimes white, woodsy smelling flowers. Flowers appear during leaf development in 5 to 10 cm (2 to 4 in) wide corymbs of 3 to 15 flowers (8). Five to seven stamens are produced per flower, which are more than other deciduous azaleas (*Rhododendron* L. sp.) native to the southeast United States, but fewer than the 10 stamens produced in flowers of most evergreen rhododendrons (*Rhododendron* L. sp.) native to the same area (9). The corolla tube is noticeably shorter than most other native deciduous rhododendrons (10). Recently, botanists have placed *R. vaseyi* in sub-genus *Pentanthera* (G. Don) Pojark, section *Rhodora* (L.) G. Don, along with *R. canadense* (L.) Torr. (rhodora) (7).

Plants of *R. vaseyi* can grow 3 to 5 m (10 to 15 ft) tall with long slender stems having exfoliating bark that cracks and peels in long grayish to grayish-brown strips. Although rooted shallowly in their native habitat, nursery grown plants transplant well, are cold hardy to USDA hardiness zones 5–8, and become attractive specimens in the landscape. The long, slender, elliptical and acuminate leaves may appear reddish on emergence before reaching 7 to 15 cm (4 to 6 in) in length and turning bright crimson in autumn. Because of its attractive landscape attributes, plants of *R. vaseyi* are in demand thus providing opportunities for production by the nursery industry.

Construction of vacation homes, housing developments, and stream desecration have decreased natural habitats of *R. vaseyi* with subsequent loss of plants (8). As a consequence, propagation protocols need to be developed to ensure survival and permit production by the nursery industry. Nurserymen have reported *R. vaseyi* produces copious quantities of seeds each year (6), and sexual propagation could be one means to ensure survival of the species and allow the nursery industry to meet the demand for plants. However, to develop protocols for sexual propagation of *R. vaseyi* will require research on the influence of various environmental factors on seed germination of the species, which up to recently has been lacking. A recent preliminary study by Walker et al. (19) reported seeds of *R. vaseyi* require light for germination and suggests seed viability of the plant may be inherently low based on maximum germination of 54%. The light requirement is not surprising because seeds of many ericaceous species including rhododendrons have a light requirement (1, 2, 3, 4, 5, 11, 12, 14, 19). To suggest viability is inherently low, however, raises a number of questions and may explain why the species has such a limited distribution. Viability may also vary among populations of *R. vaseyi* as Walker et al. (19) only worked with seeds from one of the six counties where plants are endemic. To determine whether viability is poor would also be important in knowing how dense seeds should be sown during propagation and whether grading of seeds is necessary to achieve high germination. Therefore, the following research was conducted to study the influence

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[2]Assistant Professor. anthony_lebude@ncsu.edu

[3]Alumni Distinguished Graduate Professor. frank_blazich@ncsu.edu

[4]Graduate Student.

[5]Undergraduate Student.

of light and temperature on germination of seeds of *R. vaseyi* from two populations in western North Carolina.

Materials and Methods

On October 15, 2006, mature seed capsules (fruits) were collected from four native locations of open-pollinated plants of *R. vaseyi* in western North Carolina. The first two locations were collected on Pilot Mountain in Transylvania County [lat. 35°21′N, long. 83°14′W, elevation 1231 m (4040 ft)], and along the ridgeline between Jackson and Transylvania counties at the intersection of Highway 215 and Charley's Creek Road [lat. 35°26′N, long. 82°92′W, elevation 1280 m (4200 ft)]. All capsules were pooled and designated Population 1. The second two locations of seeds were collected from plants growing adjacent to the parking lots of the Lynn Cove Viaduct Visitors Center [lat. 36°09′N, long. 81°81′W, elevation 1231 m (4040 ft)] and Rough Ridge [lat. 36°10′N, long. 81°79′W, elevation 1231 m (4040 ft)] at mileposts 305 and 300, respectively, of the Blue Ridge Parkway in Avery County. Capsules were also collected from plants growing beside hiking trails and adjacent forests. Capsules were pooled from these two locations and designated Population 2. The distance between the two populations was approximately 193 km (120 miles).

Capsules were dried at 21C (70F) for 10 days after which seeds were released using a rolling pin. Chaff and other debris were removed using sieves and a dissecting microscope. Cleaned seeds were graded further under a dissecting microscope to remove abnormal and damaged seeds and any debris not removed by previous cleaning. Graded seeds were stored at 4C (39F) in sealed glass vials until the experiment was initiated

On November 1, 2006, graded seeds were removed from storage and placed in covered 9 cm (3.5 in) diameter glass petri dishes, each dish containing two pre-soaked germination blotters moistened with tap water. After placement of seeds in the petri dishes, half the dishes were designated for germination at 25C (77F) and the other half for germination at an 8/16 hr thermoperiod of 30/20C (86/68F). All dishes were placed in black sateen cloth bags and the seeds were allowed to imbibe overnight at 21C (70F). The following day, the bags were randomized within two growth chambers [C-chambers (18)] each set at the appropriate temperature at the Southeastern Plant Environment Laboratory (N.C. State University Phytotron). Temperatures within chambers varied ± 0.5C (0.9F) of the set point.

Within each temperature regime, seeds were subjected daily to the following eight photoperiods: 0 (total darkness) ½, 1, 2, 4, 8, 12, or 24 hr. Regardless of temperature, photoperiod treatments were administered the same time each day. All photoperiod treatments, with the exception of the 0 and 24-hr treatments began at 8 A.M. daily and this coincided with the transition to the high temperature portion of the cycle for the 30/20C thermoperiod. Growth chambers were equipped with cool-white fluorescent lamps that provided a photosynthetic photon flux (400–700 nm) of approximately 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (3.2 klx) as measured outside the dishes at dish level with a cosine-corrected LI-COR LI-185 quantum/radiometer/photometer (LI-COR, Lincoln, NE).

Daily photoperiod treatments were regulated by removal and placement of the petri dishes into black sateen cloth bags. Petri dishes for the 24-hr photoperiod treatment remained continuously unbagged in the chambers. Regardless of the photoperiod, temperatures within the petri dishes, as measured by a thermocouple, never exceeded ambient by more than 1C (2F). Petri dishes for the 0 hr (total darkness) treatment remained bagged throughout the experiment and all germination counts and moistening of the blotters for this treatment were performed in a dark room utilizing a fluorescent lamp equipped with a #122 Roscolux green diffusion filter (Rosco Laboratories, Inc., Stamford, CT). Germination blotters were kept moist with tap water throughout the experiment. Within a temperature regime, photoperiods were replicated four times with a replication consisting of a petri dish containing 100 seeds. Germination counts were recorded every 3 days for 30 days and germinated seeds, as well as seeds showing signs of decay, were removed from the dishes. A seed was considered germinated when radicle emergence was ≥ 1 mm (0.04 in) in length. Percent germination was calculated as a mean of four replications per treatment. Data were subjected to analysis of variance procedures (ANOVA) and regression analysis (SAS v. 9.1) (15).

Results and Discussion

Seeds of both populations of *R. vaseyi* required light for germination regardless of temperature (Figs. 1 and 2). This is consistent with previous reports of seed germination of various species of *Rhododendron* and other members of the Ericaceae (1, 2, 3, 4, 5, 11, 12, 14, 19).

Germination increased as a function of photoperiod for the two populations at both temperatures (Figs. 1 and 2). Generally, regardless of temperature, germination at any particular day, with the exception of days 3 and 6, was always greater for seeds under the 24-hr photoperiod (Fig. 2). Cumulative germination was highest on day 30 at 30/20C for seeds of Population 1 (45%) and Population 2 (50%) under continuous light (Fig. 2C and D). Seeds from Population 2 germinated at slightly higher percentages compared to seeds from Population 1 at both temperatures and all photoperiods. The magnitude of difference between populations, however, was not consistent as indicated by the significant interaction between population, temperature, and photoperiod (ANOVA not presented). By day 30 at 25C, germination differed between populations by 12% at the 8-hr photoperiod (15% vs. 3%) (Fig. 2A and B), but 21% for the same photoperiod at 30/20C (37% vs. 16%) (Fig. 2C and D). The difference was 5% between populations at day 30 for the 24-hr photoperiod at both temperatures [31% vs. 26% at 25C (Fig. 2A and B); 50% vs. 45% at 30/20C (Fig. 2C and D)]. Previous research reported germination of *R. calendulaceum* (Michx.) Torr. (flame azalea), *R. chapmanii* A. Gray (Chapman's rhododendron), and *Kalmia latifolia* L. (mountain laurel) was a function of photoperiod at a constant temperature of 25C (77F), but was not a function of photoperiod at alternating temperatures of 25/15C (77/59F) or 30/20C (86/68F) (1, 11, 12). Similar results have been reported for two of three provenances of *R. catawbiense* Michx. (Catawba rhododendron) (14). In the aforementioned reports, the highest germination occurred for an alternating temperature treatment, yet at a photoperiod less than 24 hr. Thus, it appears seeds of *R. vaseyi* require longer photoperiods to maximize germination.

The alternating temperatures of 30/20C (86/68F) partly compensated for the light requirement for germination at shorter photoperiods. The amount of compensation depended on the population and the photoperiod treatment. For example, at day 30, the difference in germination for Population 2 at 30/20C (86/68F) and 25C (77F) for the 4-hr photoperiod

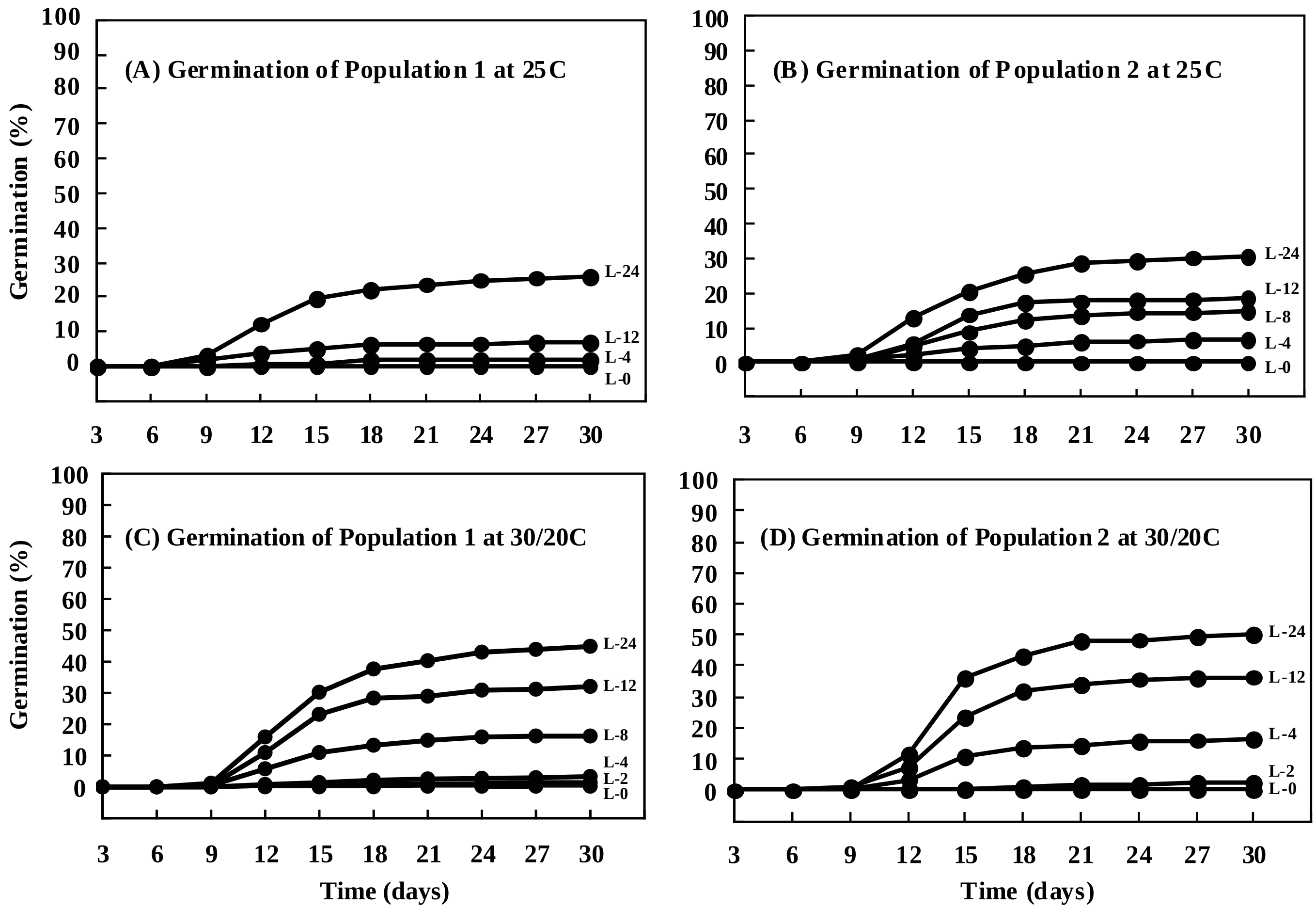

Fig. 1. Influence of light and temperature on seed germination of *Rhododendron vaseyi*. (A) Population 1 germinated at 25C (77F) with daily photoperiods (L) ranging from total darkness (0 hr) to 24 hr. Data for the ½, 1, 2, and 8-hr photoperiods were omitted because they were similar to 0, 4, or 12 hr. (B) Population 2 germinated at 25C (77F) utilizing the same photoperiods as in (A). Data for the ½, 1, and 2 hr photoperiods were omitted because they were similar to 0 or 4 hr. (C) Population 1 germinated at 30/20C (86/68F) utilizing the same photoperiods as in (A). Data for the ½ and 1 hr photoperiods were omitted because they were similar to 0 or 2 hr. (D) Population 2 germinated at 30/20C (86/68F) utilizing the same photoperiods as in (A). Data for the ½ and 1 hr photoperiods were omitted because they were similar to 0 or 2 hr.

was 10% (17% at 30/20C vs. 7% at 25C) (Fig. 1B and D), whereas the difference in germination for Population 1 under similar conditions was 1% (3% vs. 2%) (Fig. 1A and C). At the 12-hr photoperiod, however, the difference between temperatures was 25% for Population 1 (32% at 30/20C vs. 7% at 25C) (Fig. 1A and C) and 18% for Population 2 (36% at 30/20C vs. 18% at 25C) (Fig. 1B and D) at day 30. Alternating germination temperatures have partly compensated for the light requirements of other species of *Rhododendron* and members of the Ericaceae (1, 3, 5, 11, 12, 14). In those reports, cumulative germination was much higher overall and partial compensation occurred at much lower photoperiods, for example, maximum germination of 80 to 100% occurred in treatments ranging from ½ to 8 hr. In the present study, it is possible that use of an alternating thermoperiod of 30/20C (86/68F) rather than 25/15C (77/59F) may have affected germination. In studies utilizing both alternating temperatures, there was only a 10 to 20% reduction in overall germination at 30/20C compared to 25/15C (77/59F) (1). Thus, low overall germination in the present study may not have been due to the absence of a 25/15C (77/59F) treatment.

ANOVA showed that interactions for each temperature, photoperiod, time (days), and population were highly significant (ANOVA not presented). Therefore, cumulative germination was regressed on photoperiod within each population by temperature for each 3-day interval. Data for total darkness were not included.

Beginning with day 9 and continuing to day 30, germination had a highly significant linear and quadratic response to photoperiod for each population by temperature combination with the exception of the linear response of Population 2 on day 9 at 30/20C (86/68F) (Table 1). For both temperatures and populations, no germination occurred between days 3 and 6. By day 9, germination occurred at both temperatures for each population at the 4, 8, 12, and 24-hr photoperiods.

Effects of light and temperature on seed germination of *R. vaseyi* were very similar to other *Rhododendron* sp. except cumulative germination percentages were much lower (1, 5, 11, 14). Fifty percent cumulative germination for *R. vaseyi* in this experiment and similar germination of the species as reported by Walker et al. (19) may have resulted from poor seed viability despite grading of the seeds by Walker et al. (19) prior to initiation of their experiment and also grading of seeds in the present investigation. Another possibility may have been the two germination temperatures and the various photoperiod treatments at each temperature employed

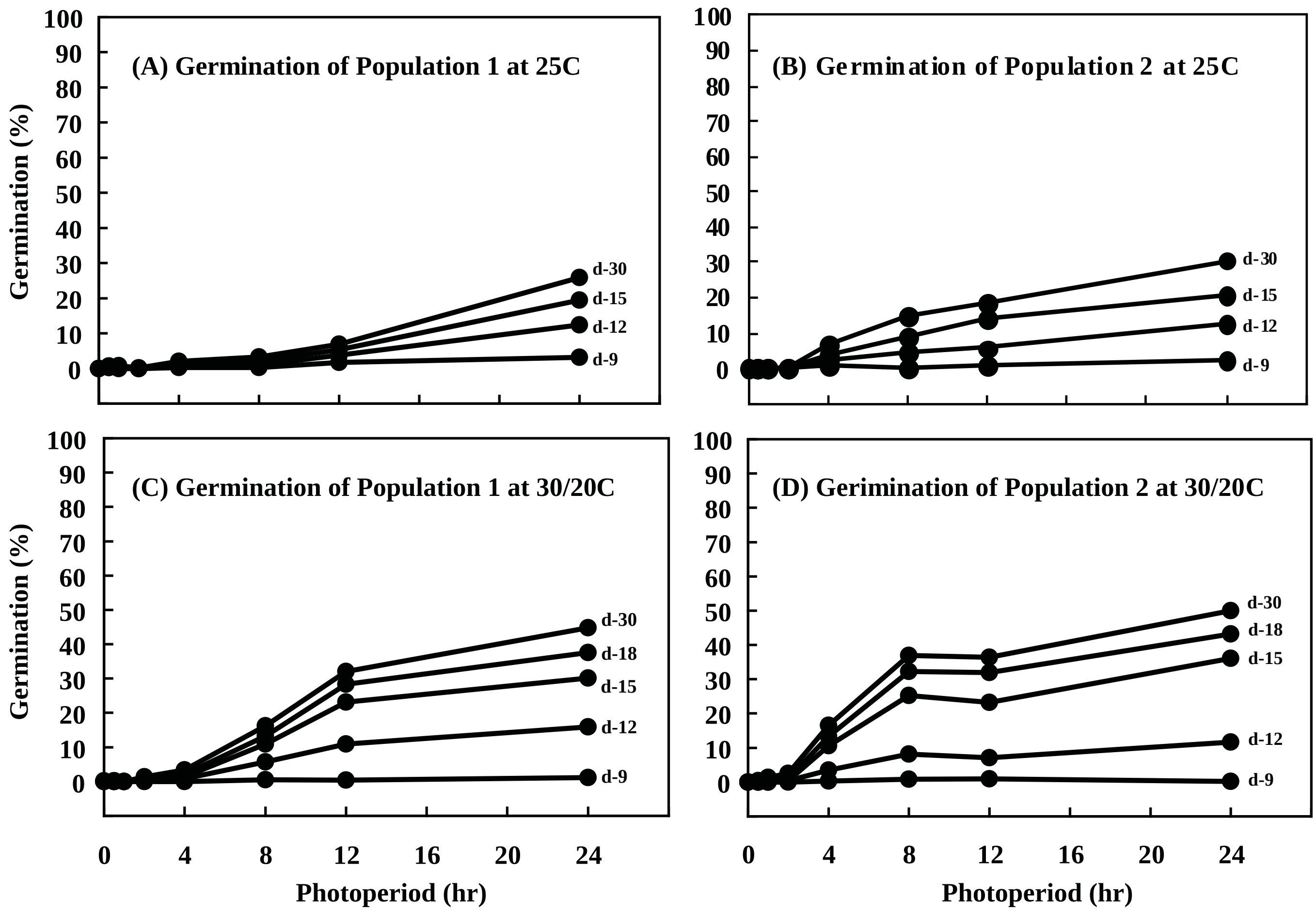

Fig. 2. Cumulative seed germination of two populations of *Rhododendron vaseyi* as influenced by photoperiod at days (d) 9 to 30. (A) Populations 1 and (B) 2 germinated at 25C (77F). Data for days 3 to 6 and 18 to 27 were omitted from (A) and (B) because germination had not occurred until day 9 and germination at days 18 to 27 was similar to day 30. (C) Populations 1 and (D) 2 germinated at 30/20C (86/68F). Data for days 3 to 6 and 21 to 27 were omitted from (C) and (D) because germination had not occurred until day 9 and germination at 21 to 27 days was similar to day 30.

by Walker et al. (19) and the research reported herein, did not permit maximum germination. The authors, however, discount this possibility for several reasons, one of which is that during the present study many decayed seeds were removed from the pertri dishes when recording data. Such seeds not only exhibited signs of fungal growth but also appeared to be empty and would not have germinated even if fungal growth had been controlled or suppressed by various seed treatments. Also, at the conclusion of the study on day 30, remaining seeds of both populations in petri dishes maintained at 30/20C (86/68F) under a 24-hr photoperiod were examined under a dissecting scope as this particular temperature-photoperiod treatment resulted in the highest cumulative germination for both populations. This was done to determine whether these nongerminated seeds were viable and perhaps if subjected to different environmental

Table 1. Influence of photoperiod on cumulative seed germination of two populations of *Rhododendron vaseyi* for days 3 to 30.

			Time (days)									
Temp. (C)	**Population**	**Photo-period[z]**	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**27**	**30**
25	1	L	—	—[y]	**	**	**	**	**	**	**	**
		Q	—	—	**	**	**	**	**	**	**	**
25	2	L	—	—	**	**	**	**	**	**	**	**
		Q	—	—	**	**	**	**	**	**	**	**
30/20	1	L	—	—	**	**	**	**	**	**	**	**
		Q	—	—	**	**	**	**	**	**	**	**
30/20	2	L	—	—	NS	**	**	**	**	**	**	**
		Q	—	—	**	**	**	**	**	**	**	**

[z]NS, **, Nonsignificant or highly significant ($P \leq 0.01$) linear (L) or quadratic (Q) response, respectively.
[y]No germination occurred by day 6.

conditions of temperature and light may have germinated. Examination of these remaining, nongerminated seeds indicated the vast majority were empty and thus nonviable. Very few remaining seeds appeared to have any nutritive tissue strongly indicating they were nonviable and incapable of germination. Thus, the authors feel the 30/20C (86/68F) thermoperiod in combination with a 24-hr photoperiod was sufficient to elicit maximum germination and the fact many seeds did not germinate under this treatment was related to lack of viability.

It is possible seed viability of *R. vaseyi* may be inherently low and methods to determine/estimate viability prior to germination, in addition to development of grading procedures that permit separation of viable and nonviable seeds, would be useful. Total germination of seeds of *Pieris floribunda* (Pursh ex Sims) Benth. and Hook. (mountain pieris) was increased by removing nonviable seeds prior to administering temperature and photoperiod treatments (17). It is intriguing to suggest the limited range of *R. vaseyi* and its rare status may be linked to its low seed viability. The biology of seed production by this species is unknown and the seeds are quite small. Although the authors did not determine the number of seeds per unit weight at a particular moisture content, each seed is approximately 1 to 1.5 mm (0.04 to 0.06 in) in length and the number of seeds per gram would range in the thousands similar to other species of rhododendron (2). Fifty percent germination would still produce many seedlings. It is also possible, but less likely, there are other barriers to germination, in addition to the light requirement, that warrant further investigation. Additionally, germination tests of other populations within North Carolina might indicate if low seed viability is widespread within the species.

Because of the small size of the seeds and the light requirement for germination, seeds of *R. vaseyi* should simply be dusted on the surface of a germination medium. An 8/16 hr thermoperiod of 30/20C (86/68F) with constant light should maximize germination and the majority of seeds will germinate in 12 to18 days.

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