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

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Abstract

Seeds from five populations of *Rhododendron vaseyi* A. Gray (pinkshell azalea), representing the entire distribution of the species, 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), 8, 12, or 24-hr (continuous light). Germination was recorded every 3 days for 30 days. Responses to light and temperature of all populations were similar. Light was required for germination regardless of temperature. As photoperiod increased, germination increased for all populations with the alternating temperature partially compensating for the light requirement. The highest cumulative germination for all populations ranged from 51 to 67% and was achieved at 30/20C with a 24-hr photoperiod. These germination percentages, although at a moderate level, were due in part to rigorous cleaning and grading of seeds collected across a broad range of plants and growing conditions prior to initiation of the study, suggesting seed viability of *R. vaseyi* is inherently low.

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

Significance to the Nursery Industry

Data are presented for the effects of light and temperature on germination of seeds of *Rhododendron vaseyi* from five populations in western North Carolina. Seeds from plants across the entire native distribution responded similarly to light and temperature treatments. Rigorous cleaning and grading techniques combined with a liberal application of seeds to a germination medium can compensate for low viability and help produce a uniform stand of seedlings. Small seed size plus the light requirement for germination dictates that seeds simply be dusted on the surface of a germination medium, and be exposed to an 8/16-hr thermoperiod of 30/20C (86/68F) with a 24-hr photoperiod (continuous light). These conditions will maximize germination, which should begin 9 to 12 days after sowing and be nearly complete by 24 days.

Introduction

Rhododendron vaseyi A. Gray (pinkshell azalea) is a rare, deciduous, ericaceous species endemic to Watauga, Avery, and Mitchell Counties in northwest North Carolina, and to Transylvania, Jackson, and Macon Counties in the southwest portion of the state at elevations above 914 m (3000 ft) (15). Found primarily in moist woodlands near mountain springs and streams, native populations of *R. vaseyi* are endemic to a relatively small region but appear abundant within this range.

From May to June, *R. vaseyi* produces attractive, pink to sometimes white, woodsy-smelling flowers in corymbs. Flowers appear before leaves in 5 to 10 cm (2 to 4 in) wide clusters of 3 to 15 flowers (8). Five to seven stamens are produced per flower, which are more than other deciduous *Rhododendron* L. spp. (azaleas) native to the southeast United States, but fewer than the 10 stamens produced by flowers of most evergreen *Rhododendron* L. spp. (rhododendrons) native to the same area (9). The corolla tube is noticeably shorter than that of most other native deciduous species within the genus (11). The attractive clusters of fragrant spring flowers, excellent deep burgundy fall leaf color, and exfoliating bark of *R. vaseyi* make it an appealing landscape plant, especially popular among native-plant enthusiasts. *Rhododendron vaseyi* is often sold by local (western North Carolina) or mail-order nurseries that propagate the plants by either seeds or to a lesser by extent stem cuttings.

The official protocol for testing seed germination of *Rhododendron* spp. requires germination for 21 days using an 8/16-hr thermoperiod of 30/20C (86/68F) with 8 hr of light daily during the high-temperature portion of the cycle or a constant 25C (77F) with 8 hr of light daily (2). Germination protocols were reported previously for two populations of *R. vaseyi* (10, 19). LeBude et al. (10) and Walker et al. (19) studied the effect of constant 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. Both studies tested seeds from the same population collected from the northernmost range of the species, and LeBude et al. (10) also included another population from the southernmost range. In each study, light was required for germination, and germination at each temperature increased with increasing photoperiod. With continuous light, seeds from the population common to both studies, as well as the additional population included by LeBude et al. (10), had total germination of 50% at 30/20C (86/68F) and 31% at 25C (77F). The light requirement was not surprising because seeds of many ericaceous species, including *Rhododendron*, have a light requirement for germination (3, 4, 12). Before conducting their studies, Walker et al. (19) and LeBude et al. (10) subjected seeds to rigorous cleaning and grading to achieve the highest germination percentages possible. Despite these

[1]Received for publication December 23, 2009; in revised form April 1, 2010. This research was funded in part by the North Carolina Agricultural Research Service, Raleigh, NC 27695-7643. Assistance of the staff of the Southeastern Plant Environment Laboratory (NC State Univ. Phytotron), Joe Conner, Ed Collins, Ron Lance, John Turner of the Southern Appalachian Highland Reserve, Chris Ulrey of the National Park Service, and Mollie E. LeBude is gratefully acknowledged. From a thesis submitted by C.J.H. in partial fulfillment of the requirements for the MS degree.

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efforts, maximum germination of 50% led Walker et al. (19) and LeBude et al. (10) to hypothesize that low overall germination for *R. vaseyi* might be due to inherently low viability. Thus, additional research with seeds from the entire native distribution is needed to test this hypothesis as this would provide evidence that low germination percentages indicate inherently poor seed viability across the entire native range. Therefore, the objective of this research was to determine the influence of light and temperature on seed germination of *R. vaseyi* representing the entire distribution of the species.

Materials and Methods

Mature seed capsules (fruit) were collected from approximately 50–75 open-pollinated plants in Fall 2007 from each of five native populations of *R. vaseyi* in western North Carolina. Populations 1 and 2 in the present study were the same populations utilized by LeBude et al. (10) and Walker et al. (19). The five populations were selected because they are isolated by mountain ranges, valleys, or long distances, yet allow access by automobiles or hikers. It was assumed that populations chosen for this study do not generally interbreed.

On November 3, capsules were collected from Pilot Mountain in Transylvania County (lat. 35°16'23.60"N, long. 82°52'02.17"W) [mean elevation = 1539 m (5050 ft)] and along the ridgeline between Jackson and Transylvania counties at the intersection of Highway 215 and Charley's Creek Road (lat. 35°16'12.37"N, long. 82°55'16.47"W) [mean elevation = 1295 m (4250 ft)]. These were pooled and designated Population 1. Capsules for Population 2 were collected the same day from plants adjacent to the parking lots of the Lynn Cove Viaduct Visitors Center (lat. 36°05'25.58"N, long. 81°48'52.37"W) and Rough Ridge (lat. 36°05'49.68"N, long. 81°47'57.00"W) at mileposts 303 and 300 [mean elevation = 1330 m (4364 ft)], respectively, of the Blue Ridge Parkway. Capsules were also collected from plants growing alongside hiking trails and adjacent forests and all capsules were pooled. On October 22, capsules were collected along Highway 107 (lat. 35°04'37.13"N, long. 83°04'00.67"W) [mean elevation = 1054 m (3460 ft)] south of Cashiers, NC, pooled, and designated Population 3. For Population 4, capsules were collected and pooled October 22 from the Southern Appalachian Highlands Reserve (lat. 35°07'51.41"N, long. 82°57'42.57"W) [mean elevation = 1067 m (3500 ft)]. Capsules for Population 5 were collected and pooled November 2 from the overlooks of John Rock, Fetterbush, and Devil's Courthouse (lat. 35°18'15.49"N, long. 82°53'16.62"W) along the Blue Ridge Parkway between mileposts 419 and 422 [mean elevation = 1655 m (5429 ft)].

Capsules from all populations were dried at 21C (70F) for 10 days after which seeds were released gently by 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 that remained after previous cleaning. Graded seeds were stored in darkness at 4C (39F) in sealed glass vials until the germination study was initiated.

On January 7, 2008, graded seeds were removed from storage and placed in covered 9-cm (3.5 in) diameter glass petri dishes. Each dish contained two pre-soaked germination blotters moistened with tap water (pH 6.8). After placement of seeds in the petri dishes, half the dishes for each population were designated for germination at 25C (77F) and the other half for germination at an 8/16-hr thermoperiod of 30/20C (86/68F), which previously had been deemed optimal for *R. vaseyi* (10, 19). All dishes were placed in black sateen cloth bags and the seeds were allowed to imbibe overnight at 21C (70F). The following day, bags were randomized within two growth chambers [C-chambers (18)] each set at the appropriate temperature. Temperatures within chambers varied ± 0.5C (0.9F) of the set point.

Within each temperature regime, seeds were subjected daily to the following photoperiods: 0 (continuous darkness), 8, 12, or 24-hr. Because previous studies with *R. vaseyi* determined that a 24-hr photoperiod resulted in the highest germination at 25C (77F) or 30/20C (86/68F) (10, 19), the present study included only four photoperiods to reduce the number of experimental units. All photoperiod treatments, with the exception of 0 and 24 hr began at 8 A.M., daily and these coincided with the transition to the high temperature portion of the cycle for the 30/20C (86/68F) thermoperiod. Growth chambers were equipped with cool-white fluorescent lamps providing 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 exposed to light in the chambers. Dishes for the 0-hr (continuous 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. Seeds showing signs of decay were removed from the dishes when recording data.

Each photoperiod and population combination within each temperature was 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 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 the mean of four replications per treatment. Data were analyzed using the general linear models procedure (PROC GLM) and regression analysis (PROC REG), if appropriate, to determine relationships between germination percentage, photoperiod, days after sowing, and population for each temperature (17). Differences for main effects and relationship trends were considered significant at $P \leq 0.10$.

Results and Discussion

Seeds from all five populations of *R. vaseyi* required light for germination regardless of temperature (Figs. 1 and 2). This requirement is consistent with previous reports of seed germination of this species (10, 19), as well as other species of *Rhododendron* and members of the Ericaceae (1, 3, 4, 12).

Analysis of variance (ANOVA) indicated that at 25C (77F) cumulative germination at day 30 was affected by the interaction of population and photoperiod and by the quadratic response to photoperiod, but not population or photoperiod

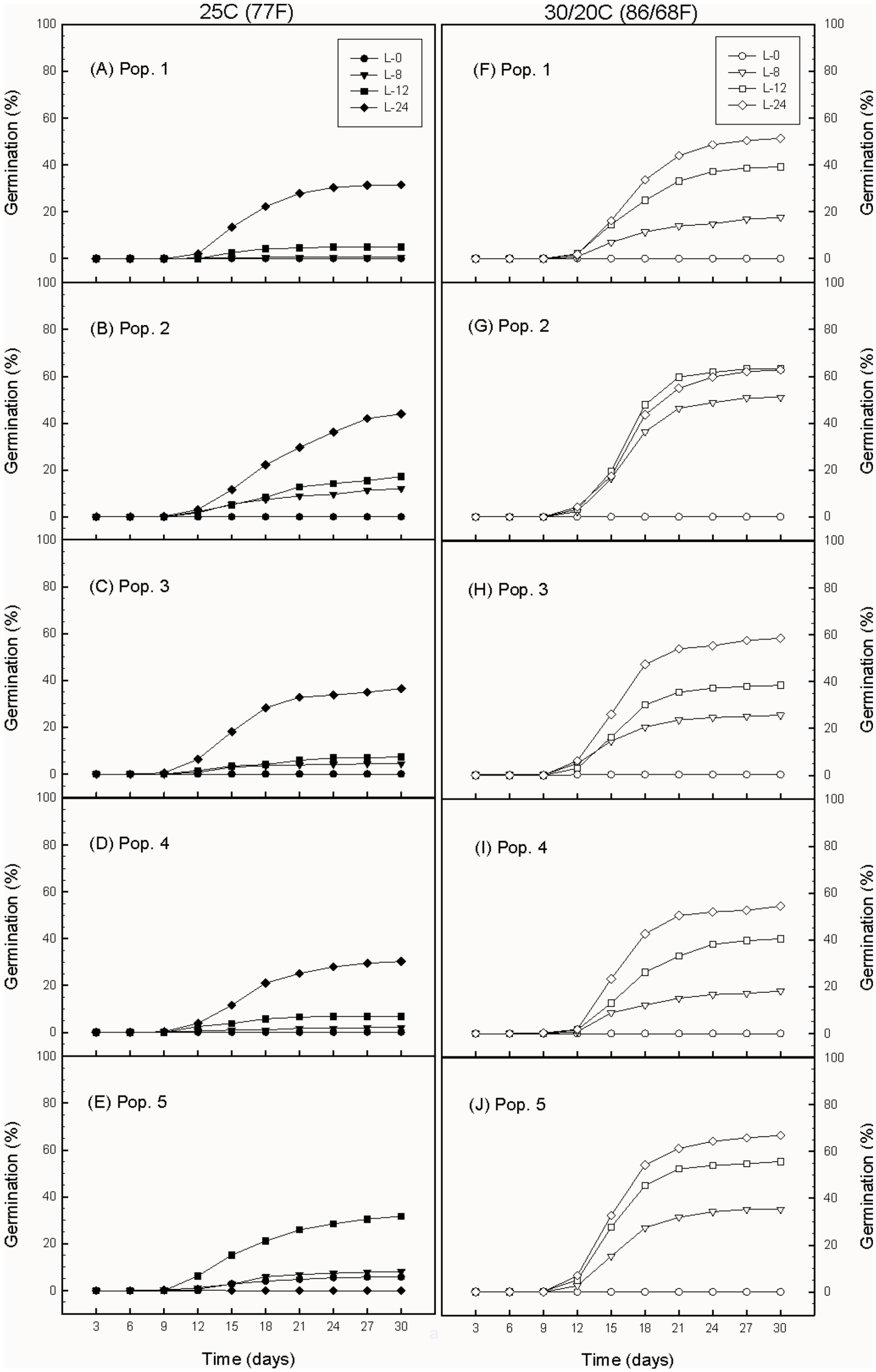

Fig. 1. Influence of light and temperature on seed germination of five populations (pop.) of *R. vaseyi*. (A) Population 1, (B) Population 2, (C) Population 3, (D) Population 4, and (E) Population 5 germinated at 25C (77F) with daily photoperiods (L) of total darkness (L-0), 8-hr (L-8), 12-hr (L-12), or 24-hr (L-24). (F) Population 1, (G) Population 2, (H) Population 3, (I) Population 4, and (J) Population 5 germinated at an 8/16-hr thermoperiod of 30/20C (86/68F) using the same photoperiods as for 25C (77F). Each symbol represents mean germination of four replicates (petri dishes), each containing 100 seeds. The legends in (A) and (F) apply to (B–E) and (G–J), respectively.

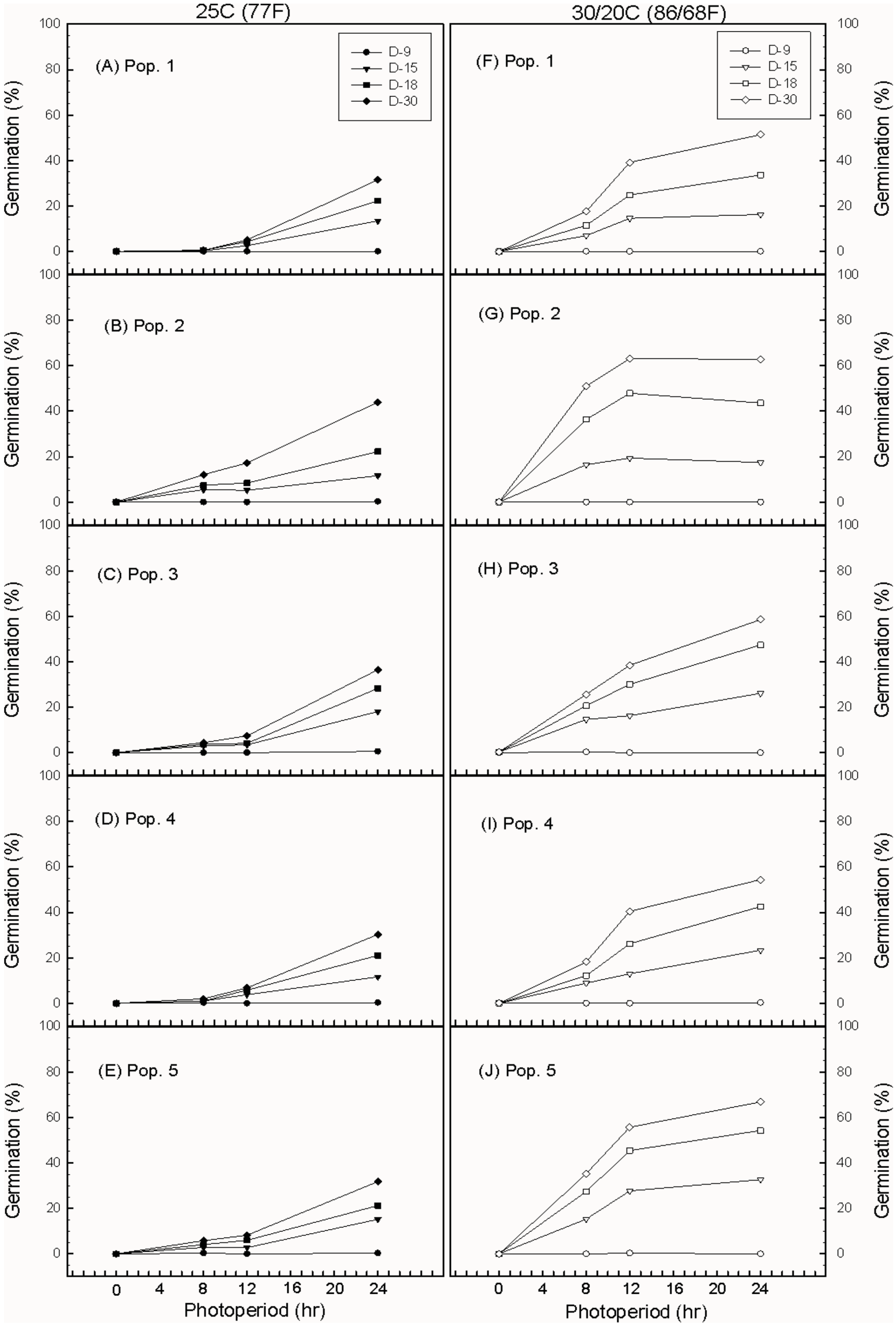

Fig. 2. Cumulative seed germination of five populations (pop.) of *R. vaseyi* as influenced by photoperiod at days (D) 9 to 30. (A) Population 1, (B) Population 2, (C) Population 3, (D) Population 4, and (E) Population 5 germinated at 25C (77F) with daily photoperiods of total darkness (0), 8, 12, or 24-hr. (F) Population 1, (G) Population 2, (H) Population 3, (I) Population 4, and (J) Population 5 germinated at an 8/16-hr thermoperiod of 30/20C (86/68F) using the same photoperiods as for 25C (77F). Each symbol is mean germination of four replicates (petri dishes) each containing 100 seeds. The legends in (A) and (F) apply to (B–E) and (G–J), respectively. Data for days 3, 6, 12, 21, 24, and 27 were omitted because germination did not occur until day 9, germination for day 12 was similar to that of day 15, and data for days 21, 24, and 27 were similar to those for day 30. Statistical significance of the responses illustrated in each graph is presented in Table 1.

separately. The data in Fig 2. for day 30 indicate that Populations 1, 3, 4, and 5 responded similarly to the 0, 8, and 12-hr photoperiods, but germination increased considerably at the 24-hr photoperiod for all populations and particularly for Population 2. At 30/20C (86/68F), cumulative germination at day 30 was affected by population, photoperiod, and the quadratic response to photoperiod (ANOVA not presented). When averaged over all photoperiods at Day 30, mean germination was similar for Populations 2 and 5 (42%) and significantly higher than the mean for Populations 1, 3 and 4 (29%), which were similar to one another. Higher cumulative germination for Populations 2 and 5 at day 30 for the 30/20C (86/68F) thermoperiod across all photoperiods indicates differences in viability among populations of these species. The response to light and temperature, however, was similar among all populations.

For each temperature, germination increased as a function of photoperiod for all populations but this depended on the population and time after sowing (days) (Fig. 2). Regardless of the population, germination after day 9 was generally highest for the 24-hr photoperiod for each temperature (Fig. 1). At day 30, cumulative germination at 25C (77F) for seeds subjected to the 24-hr photoperiod (continuous light) was highest for Population 2 (45%), followed by Population 3 (38%), Population 1 (33%), Population 5 (32%), and Population 4 (30%) (Fig. 1A–E). However, at day 30, for seeds subjected to the same photoperiod but a 30/20C (86/68F) thermoperiod, germination was highest for seeds of Population 5 (67%), followed by Population 2 (63%), Population 3 (59%), Population 4 (54%), and Population 1 (51%) (Fig. 1F–J). Despite the interaction between population and photoperiod, germination was highest for seeds exposed to all photoperiods at 30/20C (86/68F) compared to the same photoperiods at 25C (77F), with the exception of the 0-hr photoperiod of which all seeds failed to germinate.

The alternating temperature of 30/20C (86/68F) partly compensated for the light requirement for germination at photoperiods < 24 hr. For example, at 30/20C (86/68F), each population had higher cumulative germination at day 30 for the 12-hr photoperiod than at day 30 for the 24-hr photoperiod at 25C (77F) (Fig. 2A–H). The extent of this difference in germination between the two photoperiods and temperatures depended on the population. For example, Population 5 had a difference in germination of 20% (55 vs. 35%) between these conditions, whereas Population 3 had a difference of 2% (40 vs. 38%) at the same conditions (Fig. 2C, E, H, and J). An alternating temperature partially compensating for the light requirement of *R. vaseyi* has been reported previously (10, 19), as well as for other species and genera (1, 3, 4, 6, 12, 14).

Due to the significant interactions for photoperiod, time (days), and population (ANOVA not presented), cumulative germination was regressed on photoperiod within each population by temperature for each 3-day interval (Table 1). Data for total darkness were included in the regression analyses to ensure sufficient degrees of freedom when testing both the linear and quadratic terms of photoperiod.

At 30/20C (86/68F), germination began by day 9 for the 12- and 24-hr photoperiods for Populations 2 and 4. By day 9 at 25C (77F), germination began for the 8-hr photoperiod for Populations 2 and 4 and for the 24-hr photoperiod for Populations 2, 3, 4, and 5. By day 9, seeds from Populations 1 and 3 failed to germinate regardless of temperature or photoperiod, but germination began on day 12. By day 15 at 25C (77F), germination for all populations had a similar linear relationship with photoperiod, and by day 21 all populations

Table 1. Influence of photoperiod on cumulative seed germination of five populations (pop.) of *Rhododendron vaseyi* for days 3 to 30. Data for the 0 hr photoperiod treatment were included in the analysis. This table can be used to determine the statistical significance for the relationships shown in Fig. 2.

			Time (days)									
Temp.	Pop.	Photoperiod[z]	3	6	9	12	15	18	21	24	27	30
25C	1	L	—	—	—[y]	NS	*	*	*	*	*	*
		Q	—	—	—	NS	*	*	*	*	*	*
	2	L	—	—	NS	*	*	*	*	*	*	*
		Q	—	—	*	*	NS	NS	*	*	*	*
	3	L	—	—	NS	*	*	*	*	*	*	*
		Q	—	—	*	*	*	NS	*	*	*	*
	4	L	—	—	NS	*	*	*	*	*	*	*
		Q	—	—	NS	NS	*	*	*	*	*	*
	5	L	—	—	NS	*	*	*	*	*	*	*
		Q	—	—	NS	*	NS	*	*	*	*	*
30/20C	1	L	—	—	—	NS	*	*	*	*	*	*
		Q	—	—	—	NS	NS	NS	NS	NS	NS	NS
	2	L	—	—	—	*	NS	NS	NS	NS	NS	NS
		Q	—	—	—	*	*	*	*	*	*	*
	3	L	—	—	—	NS	*	*	*	*	*	*
		Q	—	—	—	NS	NS	*	*	*	*	*
	4	L	—	—	NS	*	*	*	*	*	*	*
		Q	—	—	*	NS	*	NS	NS	NS	NS	NS
	5	L	—	—	NS	*	*	*	*	*	*	*
		Q	—	—	NS	NS	NS	NS	NS	*	*	*

[z]NS,* Nonsignificant or significant ($P < 0.10$) linear (L) or quadratic (Q) response, respectively.

[y]For all populations seed germination began after day 6, and for some populations, germination began after day 9 depending on temperature and photoperiod.

shared a similar linear and quadratic relationship (Table 1, Fig. 2). At 30/20C (86/68F), the response of all populations to light was less uniform than at 25C (77F). Populations 1, 3 and 4 had similar linear responses to photoperiod after day 15 (the slope of the linear regression was 2 for each population) (equations not presented). In contrast, Population 2 had a quadratic relationship (the slope of the linear portion of the curve was 8), indicating more seeds germinated per hour increase of light. Population 5 had a similar linear response (slope = 2) to photoperiod as Populations 1, 3, and 4 but also had a quadratic response (slope of the linear portion was 6) similar to Population 2.

Mean germination of the five populations at 30/20C (86/68F) for the 24-hr photoperiod at day 30 was 59% ± 7.7% SD. Despite low viability of the species, seeds of Populations 2 and 5 had higher viability and may have possessed greater vigor as reflected by differences in their rates of germination. When subjected to similar temperature and photoperiod conditions, seeds of one provenance of *R. catawbiense* Michx. (Catawba rhododendron) germinated approximately 3 days earlier on days 6 to 9 and with cumulative germination of 98% compared to two other provenances that germinated on days 9 to 12 and with cumulative germination of 80 and 90% (16). Rowe et al. (16) concluded differences in germination between provenances were due to seed vigor rather than inherent differences between provenances in their responses to light or temperature. In the present study, seeds of Populations 2 and 5 germinated initially three (Population 5) or four (Population 2) times faster than the other three populations at 30/20C (Fig. 1) and overall about 13% higher when averaged over photoperiods. Although seeds of Population 2 had a faster initial germination rate than seeds of Population 5, cumulative germination for Population 2 was 63% compared to 67% for Population 5. The rate of germination of Populations 2 and 5 was unique when compared to the other populations but may represent greater seed vigor for these populations than any inherent fundamental physiological differences compared to the other three populations.

Generally, the germination responses to light and temperature herein of all five populations were similar, and results for Populations 1 and 2 were similar to the results reported previously by Walker et al. (19) and LeBude et al. (10). After 30 days at 30/20C (86/68F) with a 24-hr photoperiod, seeds of Population 1, as reported by Walker et al (19), had 54% germination, and seeds of Populations 1 and 2 had 45 and 50% germination, respectively, as reported by LeBude et al. (10). Mean germination for the populations in both studies was 50%. In the current study, germination was 51 and 63%, respectively, for Populations 1 and 2 (Fig. 1F and G) and the mean of the two populations was 57%. The relatively lower germination percentages observed by Walker et al. (19) and LeBude et al. (10) could have resulted from poor seed viability caused by such environmental factors as rainfall or temperature during seed development (7).

Low seed viability of *R. vaseyi* may be due to its limited range. For fragmented populations of the prairie wildflower, *Silene regia* Sims (royal catchfly), Menges (13) reported populations > 150 plants had uniformly high germination compared to smaller populations which had lower and more variable germination. In small populations there is a greater effect of genetic drift and genetic erosion on the frequency of alleles and it is possible this, combined with inbreeding depression within the population, may have an adverse effect on seed viability and vigor (5, 13). Despite its limited natural range, *R. vaseyi* appears to grow in relatively large numbers within specific habitats scattered throughout this range. Seeds in the present study were collected from at least 50 individual plants of *R. vaseyi* per population and were pooled from a number of locations within the vicinity of each population. The actual number of plants in each population is currently unknown. Nevertheless, research studying genetic diversity of the different populations of *R. vaseyi* within its natural range could determine the extent of genetic diversity within the species and yield clues about whether the species is affected by inbreeding depression or genetic drift. Alternatively, crosses within and between populations could be made to determine if germination increases when populations are outcrossed.

At the conclusion of this study on day 30, remaining nongerminated seeds in petri dishes of each population at 30/20C (86/68F) with a 24-hr photoperiod were examined under a dissecting microscope. This procedure was done to determine if these remaining seeds were possibly viable, which might indicate whether this temperature/photoperiod treatment was sufficient to elicit maximum germination. The vast majority of the remaining seeds were observed to be decayed and empty and obviously incapable of germination. Very few of the remaining seeds were nondecayed (a range of 1 to 6 seeds for each population), and of these seeds the majority appeared to lack nutritive tissue (endosperm), also indicating they were nonviable. Thus, we are confident that germinating seeds of *R. vaseyi* at an 8/16-hr thermoperiod of 30/20C (86/68F) with a 24-hr photoperiod is optimum for germination and 30-day germination illustrated in Figs. 1 and 2 with a 24-hr photoperiod was the maximum that was attainable under the environmental conditions of which these seeds were germinated. These moderate germination percentages reflected rigorous and time consuming grading of seeds before initiating this research, and without such efforts, germination would have been much lower. These findings support the hypothesis that seed viability of *R. vaseyi* is inherently low. Although seeds of *R. vaseyi* are not difficult to germinate, development of simple and less time-consuming procedures to grade seeds than those described herein would be useful to propagators.

As reported previously, seeds of *R. vaseyi*, like many species of *Rhododendron* are quite small (3) being 1 to 1.5 mm (0.04 to 0.06 in) in length (10). Small seed size plus the light requirement for germination dictates that seeds should be simply dusted on the surface of a germination medium utilizing an 8/16-hr thermoperiod of 30/20C (86/68F) with continuous light. These conditions should maximize germination with germination beginning between 9 and 12 days and nearing completion by 24 days.

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