Seed Germination of *Rhododendron chapmanii*: Influence of Light and Temperature[1]

Luis O. Arocha[2], Frank A. Blazich[3], Stuart L. Warren[3], Mack Thetford[4], and James B. Berry[5]
Department of Horticultural Science
North Carolina State University, Raleigh, NC 27695-7609

Abstract

Seeds of *Rhododendron chapmanii* A. Gray (Chapman's rhododendron) were germinated at 25C (77F) or 8/16 hr thermoperiods of 25/15C (77/59F) or 30/20C (86/68F) with daily photoperiods of 0 (total darkness), ¼, ½, 1, 2, 4, 8, 12 or 24 hr. For all temperatures, no germination occurred during a 30-day period for seeds not subjected to light. At 25C (77F) germination was a function of photoperiod. The longer the photoperiod the greater the germination with 30-day germination ≥ 90% for photoperiods ≥ 8 hr. Alternating temperatures, particularly 25/15C (77/59F), enhanced germination when light was limiting. The highest germination (80%) by day 30 for seeds at 30/20C (86/68F) was realized with a 24-hr photoperiod, whereas 30-day germination ≥ 90% occurred at 25/15C (77/59F) and 25C (77F) for photoperiods of 8 and 12 hr and 8, 12, and 24 hr, respectively.

Index words: sexual propagation, Chapman's rhododendron, Ericaceae, native plants, rare and endangered species.

Significance to the Nursery Industry

Quantitative data are presented concerning the influence of light and temperatures of 25C (77F), 25/15C (77/59F), and 30/20C (86/68F) on seed germination of the rare and endangered *R. chapmanii*, a plant of considerable landscape merit. Seeds were relatively easy to germinate although light was required. Photoperiods that maximized germination varied depending on the temperature. Germination > 90% occurred at 25/15C (77/59F) and 25C (77F) for particular photoperiods whereas 80% germination occurred at 30/20C (86/68F) for seeds subjected to constant light. When propagating this plant by seeds, seeds should simply be dusted on the surface of the germinating medium because of their small size [approximately 815,000 seeds per 28 g (1 oz)] and light requirement.

Introduction

Chapman's rhododendron (*Rhododendron chapmanii* A. Gray) is an evergreen, lepidote (scaly) rhododendron (Ericaceae) native to the sandy coastal plains of northern Florida (9, 11). It is a shrub with an open growth habit and reaches a height of approximately 2 m (6.6 ft) (9, 11).

Flowering of *R. chapmanii* occurs in April prior to new shoot growth. The attractive flowers are borne in terminal inflorescences (trusses). The rose-pink corollas of individual flowers are about 3 cm (1.2 in) long (9, 11). Leaves are primarily obovate or ovate with tapering bases and rounded or obtuse apices. Individual leaf blades vary in size but are usually 3–4 cm (1.2–1.6 in) long and 2–3 cm (0.8–1.2 in) wide with entire margins. Leaves have a distinctive wrinkled appearance (9).

The present taxonomic treatment of *R. chapmanii* is problematic (1, 8, 11), and it is the only evergreen species of *Rhododendron* L. (rhododendron) native to Florida (9). Since 1979 it has been classified as 'rare and endangered' and is currently under federal protection (1). Its endangered status has resulted in large measure from commercial development, overcollection of wild plants, and tree farming. Tree farming has altered large areas of native woodlands in northern Florida for culture of pines (*Pinus* L. sp.) in plantations which are utilized for pulp wood production (1, 9, 11).

At present, there are three main populations of *R. chapmanii*. They occur in Florida's Clay and Gulf Counties and on the Gadsden-Liberty County line (1, 9, 11). The smallest and most geographically isolated of these populations is the one in Clay County, where plants occur within a National Guard installation (1, 9, 11).

Despite its rare and endangered status, *R. chapmanii* can be propagated sexually (by seed) and asexually (vegetatively) by stem cuttings (10) and micropropagation (tissue culture) (2). Vegetative methods would permit cloning of superior genotypes. These techniques are somewhat destructive since they require removal of stem material from stock plants. On the other hand, sexual propagation may be the safest and most cost-effective method to increase the number of plants of *R. chapmanii* for recovery efforts and to enhance its survival and maintain genetic diversity. Seed propagation would also be important for breeding efforts to facilitate exploitation of desirable characteristics such as heat tolerance. However, no definitive work to date has been published on the influence of various environmental factors (e.g., light and temperature) on seed germination of the species.

Blazich and co-workers conducted research in 1991 on seed germination of *R. chapmanii* (unpublished data). Results indicated a light requirement for germination and other effects of light and temperature. These data were not published because of lack of sufficient treatment replication and the fact that the seeds were collected from a single cultivated plant of *R. chapmanii* growing in Mobile, AL. A more meaningful study would have utilized seeds collected from one or more of the three major populations of *R. chapmanii* in northern Florida. In 1996, the authors were able to obtain such

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[2]Graduate Student.

[3]Professor.

[4]Assistant Professor, University of Florida, Milton Campus, P.O. Box 3634, Milton, FL 32572-3634.

[5]Director, Plant Development Services, Inc., 17325 County Road 68, Loxley, AL 36551.

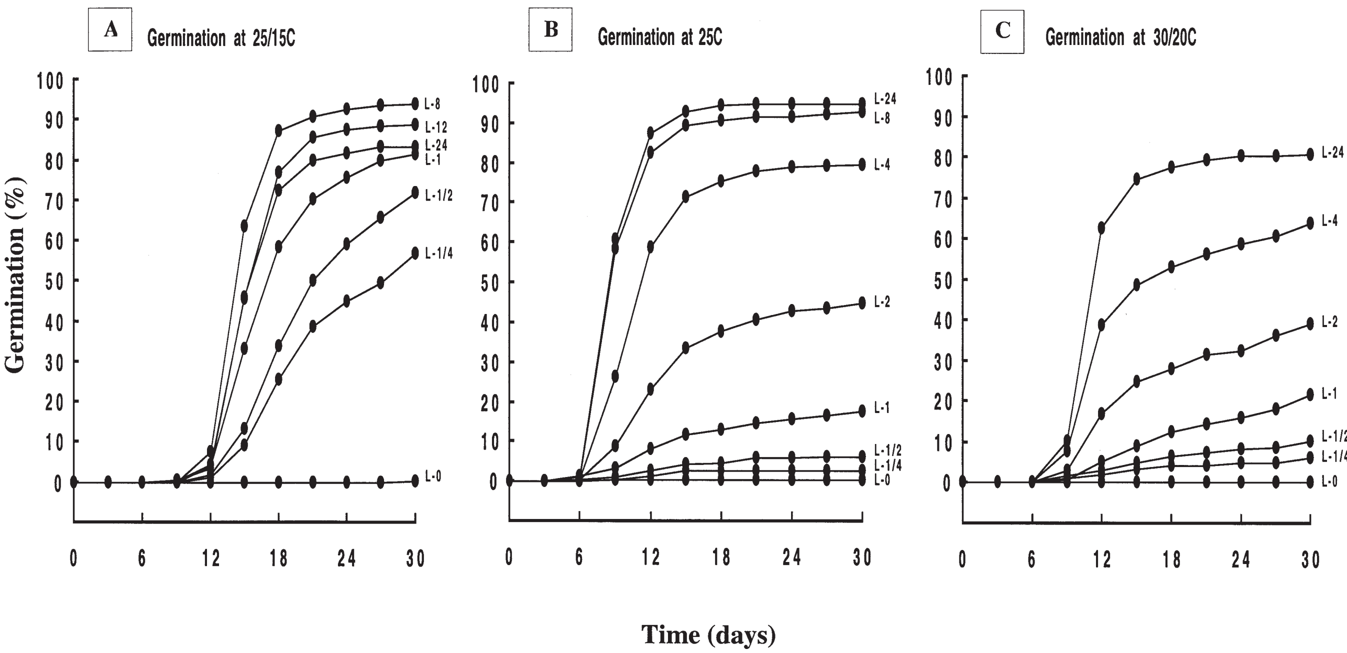

Fig. 1. Influence of light and temperature on seed germination of *R. chapmanii* with daily photoperiods (L) ranging from total darkness (L–0) to 24 hr (L–24) at all temperatures. (A) germinated at 25/15C (77/59F). Data for the 2- and 4-hr photoperiods were omitted since germination at the 2-hr photoperiod was similar to the 1-hr photoperiod, and the 4-hr photoperiod was similar to the 8-hr photoperiod. (B) germinated at 25C (77F). Data for the 12-hr photoperiod were omitted since germination was similar to the 24-hr photoperiod. (C) germinated at 30/20C (86/68F). Data for the 8- and 12-hr photoperiods were omitted since germination was similar to the 24-hr photoperiod.

seeds permitting the following research, which investigated the influence of light and temperature on seed germination of this unique and interesting plant.

Materials and Methods

On November 7, 1996, mature seed capsules (fruits) were collected from a native population of open-pollinated plants of *R. chapmanii* growing at the edge of a slash pine (*Pinus elliottii* Englem.) plantation in northern Florida on the Gadsden-Liberty County line. Capsules were dried for 5 hr at 32C (90F) followed by additional drying for 8 weeks at 21C (70F). The capsules were then placed in plastic bags and shipped to Raleigh, NC. Upon arrival, capsules were removed from the bags and dried for 2 weeks at 21C (70F) followed by seed extraction and storage at a moisture content of 5.5% in a sealed glass bottle at 4C (39F). Moisture content of the seeds was determined by calculating the mean moisture content of six 200-seed samples following drying at 105C (221F) for 24 hr.

In September 1997, seeds were removed from storage and graded under a dissecting scope, which allowed removal of abnormal, damaged, or undersized seeds, and any debris. Graded seeds [approximately 815,000 pure seeds per 28 g (1 oz)] were placed in covered 9-cm (3.5 in) glass petri dishes, each containing two prewashed germination blotters moistened with tap water. Following placement of seeds in the dishes, a third of the dishes was designated for germination at 25C (77F), a third for germination at an 8/16 hr thermoperiod of 25/15C (77/59F), and the remaining third for germination at an 8/16 hr thermoperiod of 30C/20C (86F/68F). All dishes were placed in black sateen cloth bags and the seeds allowed to imbibe overnight at 21C (70F). The following day, bags were randomized within three chambers [C-chambers (7)] set at the appropriate temperatures. Chamber temperatures varied within ± 0.5C (0.9F) of the set point.

Within each temperature regime, seeds were subjected daily to the following nine photoperiods: 0 (total darkness), ¼, 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 total darkness and 24 hr irradiation, began with the transition to the high temperature portion of the cycle.

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). 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. For the 24-hr photoperiod treatment, the petri dishes remained continuously unbagged in open chamber conditions. Regardless of the photoperiod, temperatures within the petri dishes, as measured by a thermocouple, never exceeded ambient by more than 1C (2F). Petri dishes representing the total darkness treatment remained in black cloth bags throughout the experiment, and all watering and germination counts were performed under a green safelight. Germination blotters were kept moist with tap water throughout the experiment. Seeds showing signs of decay were removed immediately from the dishes.

Each photoperiod was replicated four times within a temperature regime with a replication consisting of a petri dish containing 100 seeds. Germination counts were recorded every 3 days for 30 days. A seed was considered germinated when the emerging radicle was ≥ 1 mm (0.04 in).

Percent germination was calculated as a mean of four replications per treatment. Within each temperature, data were subjected to analysis of variance procedures and regression analysis (SAS Institute, Inc., Cary, NC).

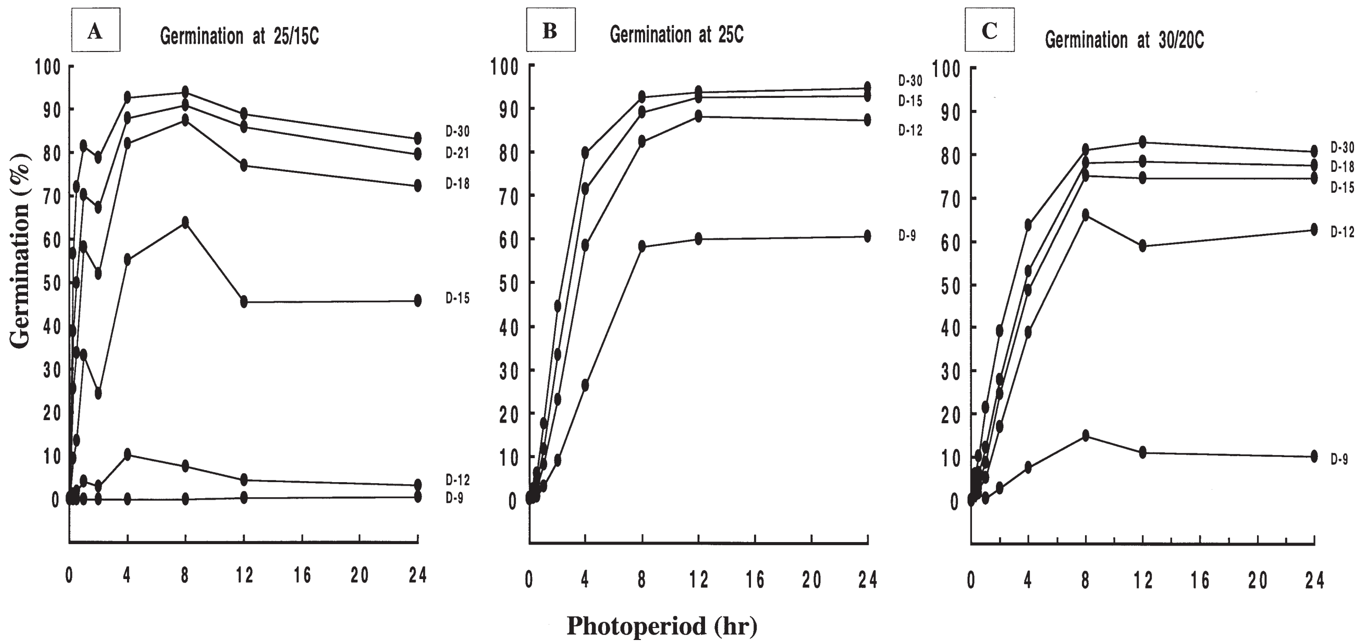

Fig. 2. Cumulative seed germination of *R. chapmanii* as influenced by photoperiod at days (D) 9 to 30. (A) germinated at 25/15C (77/59F). Data for days 24 and 27 were omitted since they were similar to day 30. (B) germinated at 25C (77F). Data for days 18, 21, 24, and 27 were omitted since they were similar to day 30. (C) germinated at 30/20C (86/68F). Data for days 21, 24, and 27 were omitted since germination at day 21 was similar to day 18, and days 24 and 27 were similar to day 30.

Results and Discussion

Seeds of *R. chapmanii* required light for germination regardless of the germination temperature (Figs. 1 and 2). This light requirement is not surprising since for many species of *Rhododendron* L. and members of the Ericaceae, light stimulates or is an absolute requirement for germination (3, 4, 5, 6, 12, 13, 14, 15).

Germination at 25C (77F) was a function of photoperiod with increasing photoperiods resulting in increased germination (Fig. 1B). At 25C (77F) germination ≥ 90% was realized for photoperiods ≥ 8 hr. For the alternating temperatures of 25/15C (77/59F) and 30/20C (86/68F), the germination response was different in comparison to that at 25C (77F) as both alternating temperatures partly compensated for the light requirement when photoperiod was limiting (Figs. 1A and 1B). This is best illustrated by comparing 30-day germination at 25C (77F) for photoperiods of ¼, ½, and 1 hr with that for the same photoperiods at 25/15C (77/59F) and 30/20C (86/68F). At 25C (77F), 30-day germination for photoperiods of ¼, ½, and 1 hr was 3%, 6%, and 17% compared to 55%, 72%, and 82% and 6%, 9%, and 21% for identical photoperiods at 25/15C (77/59F) and 30/20C (86/68F), respectively. Interestingly, 25/15C (77/59F) was much more stimulatory in terms of partly compensating for the light requirement than 30/20C (86/68F). Partial substitution of a light requirement by a germination temperature of 25/15C (77/59F) has also been demonstrated for other ericaceous species such as *Kalmia latifolia* L. [mountain laurel (13)], *R. calendulaceum* Michx. Torr. [flame azalea (12)], *R. carolinianum* Rehd. [Carolina rhododendron (5)], *R. maximum* L. [rosebay rhododendron (3)], and *Leucothoe fontanesiana* (Steud.) Sleum [drooping leucothoe (4)].

Analysis of variance showed that for each temperature, photoperiod, time (days), and their interactions were highly significant. Thus, regression analysis was conducted on cumulative germination within each temperature for each 3-day interval. The analysis did not include data for total darkness.

At 25C (77F) and 30/20C (86/68F) highly significant linear and quadratic responses were noted beginning on day 9 and continuing to day 30 (Table 1). Similar responses occurred at 25/15C (77/59F) except the highly significant quadratic response began on day 12 instead of day 9.

Although light was required for germination at all three temperatures, photoperiods of 12 and 24 hr. appeared to inhibit germination at 25/15C (77/59F) and 30/20C (86/68F) (Figs. 2A and 2C). This was not observed at 25C (77F). Beginning at day 12 and continuing to day 30, germination at 25/15C (77/59F) with photoperiods of 12 and 24 hr was always less than germination with an 8-hr photoperiod. A similar response occurred at 30/20C (86/68F) for days 9 and 12. However, by day 15 there was no inhibition. Similar responses have been noted for seeds of *R. carolinianum* (5), *R. maximum* (3), and *L. fontanesiana* (4) when germinated at 25C (77F) or 25/15C (77/59F).

For all temperatures, no germination was observed by day 3. Between days 3 to 6 germination commenced at 25C (77F) for seeds exposed to light. At 30/20C (86/68F) germination was delayed by 3 days and by day 9 germination was observed for seeds subjected to light. At 25/15C (77/59F) no appreciable germination was noted until day 12. Despite the delay in commencement of germination at 25/15C (77/59F), 30-day germination for photoperiods of 8 and 12 hr was comparable to 30-day germination at 25C (77F) for photoperiods of 8, 12, and 24 hr (Figs. 1A and 1B).

For years, taxonomists have debated the taxonomic classification of the lepidote rhododendrons of the southeastern United States. Many separate the Southern plants into three species, *R. minus* Michx. (Piedmont rhododendron), *R. carolinianum*, and *R. chapmanii*. The most recent treatment

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

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

[z]NS, ** indicates nonsignificant, and highly significant ($P \geq 0.01$) linear (L) or quadratic (Q) response, respectively.

[y]No germination occurred by day 3.

[x]No germination occurred by day 6 at 25/15C(77/59F) and 30/20C(86/68F).

of the *R. minus* complex was published in 1962 by Duncan and Pullen (8). They concluded there is only one species of lepidote rhododendron in the southeastern United States, *R. minus* with two varieties, *R. minus* Michx. var. *minus* (which includes *R. carolinianum*) and *R. minus* Michx. var. *chapmanii* (A. Gray) Duncan and Pullen. This latest treatment, however, is much debated and further study is warranted to resolve this issue.

In 1993, Blazich et al. (5) reported results of a seed germination study with *R. carolinianum.* Seeds were germinated at 25C (77F) or an 8/16 hr thermoperiod of 25/15C (77/59F) with daily photoperiods ranging from 0 (total darkness) to 24 hr. If one compares the data of Blazich et al. (5) with those in Figs. 1 and 2, some similarities and differences are noted although it appears viability of the seeds of *R. carolinianum* used in the earlier study of Blazich et al. (5) was less than that of *R. chapmanii* in the present study. Nevertheless, when seeds of *R. carolinianum* were germinated at 25C (77F), Blazich et al. found that germination was a function of photoperiod similar to that illustrated in Fig. 1B for *R. chapmanii*. The longer the photoperiod the greater the germination. When seeds of *R. carolinianum* were germinated at 25/15C (77/59F), the alternating temperature partly compensated for the light requirement when photoperiod was limiting. However, Blazich et al. (5) reported that 30-day germination for particular photoperiods at 25/15C (77/59F) was much greater than identical photoperiods at 25C (77F). For example, at 25C (77F) germination of 20%, 27%, and 38% was observed for photoperiods of 8, 12, and 24 hr, compared to 75%, 69%, and 71% germination at 25/15C (77/59F) for the same photoperiods, respectively. Thus, greater germination of *R. carolinianum* occurred at the alternating than the constant temperature. However, in the present study, comparable germination of *R. chapmanii* was achieved at 25C (77F) or 25/15C (77/59F) depending on the photoperiod.

Blazich et al. (5) also observed a response with *R. carolinianum*, which is similar to that in Figs. 2A and 2C when photoperiods ≥ 8 hr caused some inhibition of germination. Results of their study with *R. carolinianum*, and the data herein, suggest possible differences in the manner seeds of these plants respond to light and temperature during germination. Such differences are worthy of further investigation.

As mentioned previously, seeds of *R. chapmanii* are very small. Based on a storage moisture content of 5.5%, we estimated approximately 815,000 seeds per 28 g (1 oz). Small seed size plus the light requirement for germination warrants that when seeds are sown, they should be dusted on the surface of the germinating medium. Despite the light requirement, seeds were not difficult to germinate which should simplify sexual propagation of this most unusual rhododendron.

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