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Research Reports

Secondary Seed Dormancy of *Rhododendron catawbiense* and *Rhododendron maximum*[1]

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Abstract

Seeds of *Rhododendron catawbiense* Michx. (Catawba rhododendron) and *Rhododendron maximum* L. (rosebay rhododendron) were germinated at 25C (77F) or an 8/16 hr thermoperiod of 25/15C (77/59F) with constant light after imbibed seeds were maintained in total darkness for 0, 9, 18, 27, 36, 45, 54 or 63 days at the same temperatures. Maintenance of imbibed seeds of *R. catawbiense* in darkness at 25C (77F) for up to 63 days caused no induction of secondary dormancy while induction occurred for seeds in darkness at 25/15C (77/59F). When imbibed seeds of *R. catawbiense* were subjected immediately to light following imbibition, 30-day germination at 25C (77F) was 98% compared to 95% for imbibed seeds maintained in darkness for 63 days and then exposed to light. If germinated at 25/15C (77/59F), immediate light exposure resulted in 99% germination which decreased significantly to 76% after 63 days of dark treatment. Seeds of *R. maximum* maintained in darkness developed secondary dormancy at both temperatures. Thirty day germination of seeds subjected immediately to light following imbibition at 25C (77F) was 82% which decreased to 29% after dark treatment for 9 days. Further reductions in germination continued as the length of dark treatment increased with < 10% germination after maintenance in darkness for 27 days. At 25/15C (77/59F) induction of secondary dormancy was not as dramatic as that at 25C (77F). Without dark treatment, 30-day germination at 25/15C (77/59F) was 99% which decreased significantly to 88% after dark treatment for 18 days. Reductions in germination continued up to 63 days with 67% germination. Partial removal of secondary dormancy in seeds of *R. maximum* was achieved by subjecting seeds to moist-chilling.

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

Significance to the Nursery Industry

Seeds of *Rhododendron catawbiense* (Catawba rhododendron) and *Rhododendron maximum* (rosebay rhododendron) can be germinated when mature without any pretreatment but they require light for germination. Our research demonstrates, however, that when germinating seeds of these species, the seeds must be subjected immediately to light following imbibition. If imbibed seeds are not exposed to light or if exposure is delayed, secondary seed dormancy may be induced. Such dormancy will prevent germination and will require treatment for removal.

Introduction

When mature, seeds of various indigenous, ericaceous species such as *Kalmia latifolia* L. (mountain laurel), *Leucothoe fontanesiana* (Steud.) Sleum. (drooping

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leucothoe), *Rhododendron calendulaceum* (Michx.) Torr. (flame azalea), *Rhododendron carolinianum* Rehd. (Carolina rhododendron), *Rhododendron catawbiense* Michx. (Catawba rhododendron), and *Rhododendron maximum* L. (rosebay rhododendron) do not require any pretreatment and will germinate immediately when subjected to appropriate environmental conditions (moisture, proper temperature, oxygen, and in most cases light) (1, 2, 3, 5, 6, 8, 9). However, unpublished research by the authors has shown that imbibed seeds of *R. maximum* will not germinate despite being held at a favorable temperature if they are not subjected immediately to a proper photoperiod. If imbibed seeds of *R. maximum* are maintained in darkness at a favorable germination temperature for extended periods, they appear to develop secondary dormancy and will not germinate when exposed to environmental conditions conducive to germination including light.

Mayer and Poljakoff-Mayber (7) reported that secondary dormancy is sometimes induced if seeds are provided all conditions necessary for germination except one, for example light. Observations that secondary dormancy may be induced in imbibed seeds of *R. maximum* by maintaining the seeds in darkness despite providing other conditions necessary for germination raises several questions. Therefore, the following research was undertaken to determine how long imbibed seeds of two ericaceous species, *R. catawbiense* and *R. maximum*, must be maintained in darkness to induce secondary dormancy and once it has been induced, how might it be removed.

Materials and Methods

In October 1994, mature seed capsules from native populations of open pollinated plants of *R. catawbiense* and *R. maximum* were collected in Buncombe and Avery County, North Carolina at elevations of 1860 and 914 m (6100 and 3000 ft), respectively. Capsules were stored in paper bags at 20C (68F) for 30 days. Seeds were then removed from the capsules and stored in glass bottles at 4C (39F). At storage, seed moisture content of *R. catawbiense* and *R. maximum* was 5.0% and 4.5%, respectively. Moisture content was determined by calculating the mean moisture content of six, 100-seed samples of *R. catawbiense* and six, 200-seed samples of *R. maximum* following drying at 105C (221F) for 24 hr.

Following drying and moisture determinations for both species, a viability (germination) study was conducted. Results indicated that seeds of *R. catawbiense*, and *R. maximum* were capable of germination > 90%. Prior to conducting this germination test, seeds of each species were graded under a dissecting scope which allowed removal of abnormal, damaged or undersized seeds and any debris.

Once the initial germination test was completed, seeds of each species were graded as described previously and sown in covered 9-cm (3.5 in) glass petri dishes. Each dish contained two prewashed germination blotters (Filtration Sciences Corp., Mt. Holly Springs, PA) moistened with tap water. Following placement of seeds in the dishes, half were designated for germination at 25C (77F) and the other half to be germinated at an 8/16 hr thermoperiod of 25/15C (77/59F). All dishes were placed in double layer, black sateen cloth bags and the seeds allowed to imbibe overnight at 21C (70F). The next day, bags were randomized within two growth chambers [C-chambers (4)] set at the appropriate temperatures. Chamber temperatures varied within ± 0.5C (0.9F) of the set point. Temperatures in the petri dishes never deviated from ambient temperature by more than 1C (2F) as measured by a thermocouple.

On day 1 and at 9 day intervals for 63 days, four dishes of a species in each growth chamber were removed from the black cloth bags and exposed to constant light for 30 days. If secondary dormancy occurred, as indicated by a decrease in germination, the dishes with nongerminated seeds were then placed in black sateen cloth bags and the seeds subjected to moist-chilling at 4C (39F) for 30, 45 or 60 days followed by germination at 25C (77F) under constant light for 30 days. The second 30 day germination period utilized the same germination temperature [25C (77F)] for both initial temperature regimes. Within each temperature regime, seeds were subjected to a photosynthetic photo flux of approximately 42 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (3.1 klx) provided by cool-white fluorescent lamps. Light was measured at dish level with a cosine corrected LI-COR LI-185 quantum/radiometer/photometer (LI-COR, Lincoln, NE).

Each treatment was replicated four times and a replication consisted of a petri dish containing 100 seeds. Data were recorded every 3 days throughout the investigation. Seeds maintained in darkness were examined under darkroom conditions utilizing a green safelight, a fluorescent lamp equipped with a green acetate filter (Rosco Laboratories, Port Chester, NY). A seed was considered germinated when radicle emergence was ≥ 1 mm (≥ 0.04 in). Decayed seeds were removed promptly from the dishes.

Percent germination was calculated as a mean of four replications per treatment and data for each species were subjected to analysis of variance procedures and regression analysis (10).

Results and Discussion

Secondary dormancy was induced in seeds of *R. catawbiense* and *R. maximum* by not subjecting seeds immediately to light following imbibition. However, the degree of dormancy varied depending on the length of time seeds were maintained in darkness and the temperature at which the dark treatments were imposed and the seeds germinated.

Seeds of *R. catawbiense* did not develop secondary dormancy when maintained in darkness for up to 63 days at 25C (77F) and then germinated at the same temperature with constant light (Table 1). Germination of seeds held initially in darkness from 0 to 63 days and then exposed to light ranged from 93% to 98%. In contrast, significant reductions in germination occurred for seeds maintained in darkness at 25/15C (77/59F) then germinated at the same temperature with light. However, reductions in germination were small until seeds were maintained in darkness for periods ≥ 45 days. After dark treatment for 45 days, followed by exposure to light, germination was 88% in comparison to 99% without dark treatment. Following dark treatment for 63 days, germination decreased to 76% which suggests that longer durations of darkness may have decreased germination further. This indeed may have been the situation at 25C (77F) with much longer dark periods necessary to induce secondary dormancy. Although the data for 25/15C (77/59F) indicate that germination was reduced significantly after dark treatment for 63 days, the authors did not view the 23% reduction of sufficient magnitude to warrant removal of secondary dormancy.

Table 1. Effect of darkness on induction of secondary dormancy in imbibed seeds of *Rhododendron catawbiense* germinated at 25C and 25/15C.

	Thirty day germination after dark treatment (%)[z]	
Days of darkness	Germinated at 25C	Germinated at 25/15C
0	98.0	98.7
9	96.8	93.3
18	96.7	96.8
27	95.1	93.2
36	95.3	95.1
45	93.3	87.5
54	93.6	82.0
63	94.7	75.5
Significance[y]		
Linear	NS	0.0001
Quadratic	NS	NS

[z]Each value represents mean germination of four petri dishes each containing 100 seeds.

[y]Regression analysis of days of darkness, NS = $P > 0.05$.

Response of seeds of *R. maximum* was noticeably different from that of *R. catawbiense*, as secondary dormancy was induced at both temperatures with shorter durations of darkness. When imbibed seeds of *R. maximum* were subjected immediately to continuous light, 30-day germination at 25C (77F) was 83% (Fig. 1A). However, after only 9 days in darkness followed by exposure to continuous light, germination was reduced to 29%. With longer durations of darkness, germination continued to decrease and at periods ≥ 27 days, germination was < 10%. At 25/15C (77/59F) significant reductions in germination were also observed following dark treatment but the treatments required to induce secondary dormancy were longer than those at 25C (77F).

Germination of *R. maximum* at 25/15C (77/59F) after maintenance in darkness for 0 and 9 days was 99% and 96%, respectively (Fig. 1B). After 18 days of darkness, a reduction in germination to 88% was observed which continued with increased durations of darkness. However, after 63 days of dark treatment, germination was relatively high (67%) compared to those seeds (2%) maintained in the dark for the same length of time at 25C (77F).

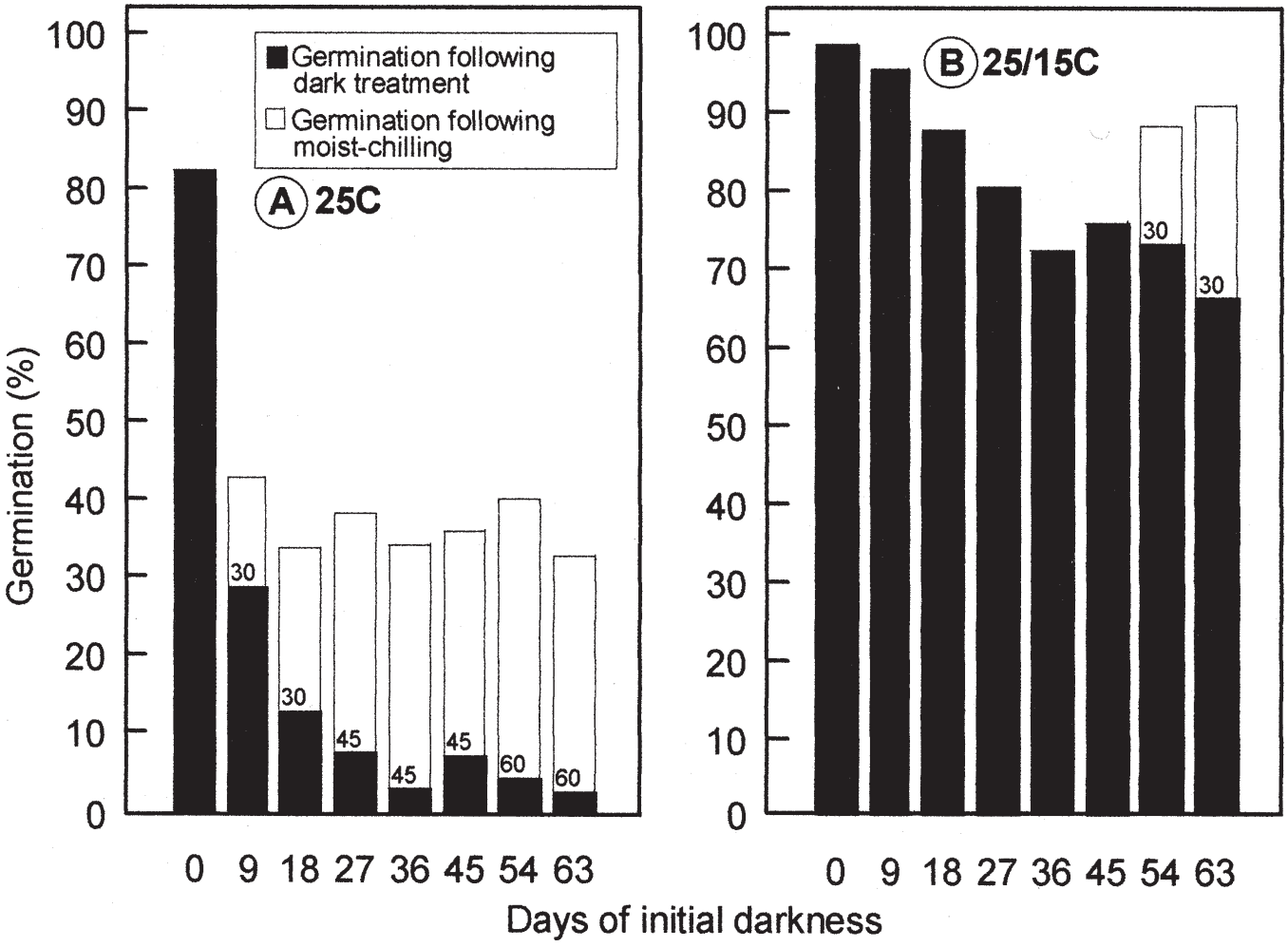

Fig. 1. Effect of darkness on induction of secondary dormancy in imbibed seeds of *Rhododendron maximum* maintained initially in darkness for 0 to 63 days at (A) 25C or (B) 25/15C followed by exposure to constant light at the same temperatures for 30 days. Regression equations for germination at 25C (77F) or 25/15C (77/59F) are $y = 69.4 - 10.08x + 0.04x^2$ ($r^2 = 0.85$) and $y = 97.3 + 0.60x - 0.02x^2$ ($r^2 = 0.83$), respectively. After 30 days, seeds which did not germinate were moist-chilled at 4C for 30, 45 or 60 days followed by germination at 25C under constant light for 30 days. The number inside the white bars represents the duration (days) of moist-chilling.

In an effort to remove/break secondary dormancy imposed by maintaining imbibed seeds of *R. maximum* in darkness at 25C (77F), petri dishes containing seeds which did not germinate after 30 days were placed in darkness (moist-chilled) at 4C (39F) for 30 days. This was followed by an additional 30-day germination period at 25C (77F) with continuous light. After moist-chilling, additional germination of 14% and 21% was realized for seeds maintained in darkness for 9 and 18 days, respectively, and germinated initially at 25C (77F) (Fig. 1A). Since dormancy was not removed completely, moist-chilling was increased to 45 days for some of the other dark treatments. This longer duration of moist-chilling resulted in increases of 31%, 31%, and 29% for dark treatments of 27, 36, and 45 days, respectively. Increased germination percentages suggested that longer periods of moist-chilling might stimulate additional increases in germination. Thus, the chilling treatment was increased to 60 days for the dark treatments of 54 and 63 days. Germination of seeds maintained in darkness for 54 and 63 days and moist-chilled for 60 days increased only 36% and 31%, respectively. It appeared moist-chilling for 60 days was no better than 45 days although both treatments were more effective than 30 days.

Moist-chilling for 30 days was also utilized in attempts to remove secondary dormancy of seeds of *R. maximum* maintained in darkness for 54 and 63 days at 25/15C (77/59F) (Fig. 1B). Following chilling, the seeds were placed at 25C (77F) with continuous light. Total germination after 30 days increased 15% and 25% for seeds maintained initially in darkness for 54 and 63 days, respectively (Fig. 1B). However, total germination for both treatments was less than seeds which were never maintained in darkness following imbibition.

Induction of secondary dormancy by maintaining imbibed seeds in darkness for varying lengths of time was more pronounced in *R. maximum* than *R. catawbiense*. This could have been due to failure to use dark treatments of sufficient length to induce secondary dormancy in *R. catawbiense*. Previous work by Blazich *et al.* (1) demonstrated that although most seed lots of *R. catawbiense* have an obligate light requirement, daily 1/2 hr photoperiods will maximize germination whether seeds are germinated at 25C (77F) or an 8/16 hr thermoperiod of 25/15C (77/59F). On the other hand, seeds of *R. maximum* also have an obligate light requirement. However, the daily photoperiod necessary to elicit maximum germination is longer than that for *R. catawbiense*. When germinated at 25C (77F), seeds of *R. maximum* require daily photoperiods $\geq$ 12 hr to maximize germination whereas at an 8/16 hr thermoperiod of 25/15C (77/59F), daily photoperiods $\geq$ 4 hr are necessary. It is interesting to note that seeds of *R. catawbiense* do not need as many hours of light daily to germinate in comparison to *R. maximum* (1, 9). This may explain why shorter periods of maintenance in darkness induced greater secondary dormancy in seeds of *R. maximum* as opposed to *R. catawbiense*.

Results herein appear to be the first report of secondary dormancy in any ericaceous species. Whether this phenomenon exists in other members of the Ericaceae is deserving of further study. Our results may also have important ecological and practical significance. From an ecological standpoint, secondary dormancy may explain why seeds of rhododendron can remain viable for several years under natural conditions (8). Since seeds of many species of rhododendron such as *R. catawbiense* and *R. maximum* are capable of germination without pretreatment in the fall when mature, induction of secondary dormancy may serve as a seed banking/conservation mechanism. It may also permit a postponement of germination unless conditions are favorable for survival of seedlings.

On a practical level, knowledge of secondary dormancy might prove useful in situations if an individual attempted to germinate seeds of *R. catawbiense* and *R. maximum* and did not provide adequate environmental conditions (e.g., light) necessary for germination. For example, if sufficient time had lapsed since the seeds were sown it could lead to a situation where secondary dormancy would be induced thus preventing germination. At this point, providing a satisfactory photoperiod would not stimulate germination unless the dormancy was removed.

One could argue that the phenomenon observed by the authors and described herein could simply have been due to seed decay. While the imbibed seeds were maintained in darkness it is possible they were subject to attack by various seed pathogens resulting in seed mortality. Thus, reductions in germination by withholding light may have been due to seed decay and not secondary dormancy. The authors, however, dismiss such speculation since little if any seed decay was ever observed. Furthermore, many seeds of *R. maximum* which did not germinate following dark treatment and subsequent exposure to light, germinated after moist-chilling. Germination of *R. maximum* following moist-chilling never approached that of imbibed seeds subjected immediately to light. This suggests that additional treatments may be necessary to completely remove secondary dormancy.

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