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Influence of Storage Temperatures on Long-term Seed Viability of Selected Native Ericaceous Species[1]

Christopher T. Glenn[2], Frank A. Blazich[3], and Stuart L. Warren[3]
Department of Horticultural Science
North Carolina State University, Raleigh, NC 27695-7609

Abstract

Following harvest of capsules, drying, and seed extraction, seeds of *Kalmia latifolia* L. (mountain laurel), *Leucothoe fontanesiana* (Steud.) Sleum (drooping leucothoe), *Rhododendron carolinianum* Rehd. (Carolina rhododendron), *Rhododendron catawbiense* Michx. (Catawba rhododendron), and *Rhododendron maximum* L. (rosebay rhododendron) were stored for 0, 1, 2, 3, 4 or 5 years at –18, 4 or 23C (0, 39 or 73F) and then germinated at 25C (77F) or an 8/16 hr thermoperiod of 25/15C (77/59F) with daily photoperiods of 0, 1 or 24 hr. Storage at –18 or 4C (0 or 39F) were most effective for maintaining seed viability of all species. After 5 years storage at –18 or 4C (0 or 39F), viability of *L. fontanesiana*, *R. catawbiense*, and *R. maximum* was relatively unchanged with total germination of 59%, 87%, and 88%, respectively. The same was noted for seeds of *K. latifolia* and *R. carolinianum* with total germination of 77% and 91%, respectively, after storage for 4 years at the same temperatures. Storage at 23C (73F) was the least effective for maintaining viability. After storage for 1 year at 23C (73F), germination decreased significantly for all species except *R. carolinianum*. By year 3, storage at 23C (73F) reduced seed viability of *L. fontanesiana* to essentially zero. The same occurred by year 4 for seeds of *R. catawbiense* and *R. maximum* stored at 23C (73F). Viability of *K. latifolia* also decreased under storage at 23C (73F) with germination of 14% noted by year 4. Viability of *R. carolinianum* did not decrease as rapidly as the other species when stored at 23C (73F) with total germination of 77% occurring by year 4. Regardless of storage duration, the photoperiod and temperature requirements for maximum germination of all species did not change.

Index words: sexual propagation, *Kalmia latifolia, Leucothoe fontanesiana, Rhododendron carolinianum, Rhododendron catawbiense, Rhododendron maximum*, native plants.

Significance to the Nursery Industry

Seed viability of *Kalmia latifolia* (mountain laurel), *Leucothoe fontanesiana* (drooping leucothoe), *Rhododendron carolinianum* (Carolina rhododendron), *Rhododendron catawbiense* (Catawba rhododendron), and *Rhododendron maximum* (rosebay rhododendron) can be maintained relatively constant for 4 to 5 years when seeds are dried to moisture contents of 4% to 7% and the seeds stored in sealed containers under freezer [–18C (0F)] or refrigerated [4C (39F)] conditions. For all species, except *R. carolinianum*, room temperature storage [23C (73F)] should be avoided as viability is lost rapidly. Lack of change in seed viability following storage for 4 to 5 years at –18 or 4C (0 or 39F) suggests these storage conditions should permit maintenance of viability for periods greatly exceeding these lengths of time. Results also demonstrated that the photoperiod and temperature requirements for maximum germination of all species remained constant; they did not change with storage duration.

[1]Received for publication February 18, 1998; in revised form July 8, 1998. This research was funded in part by the North Carolina Agricultural Research Service, Raleigh, NC 27695-7643 and by grants from the North Carolina Association of Nurserymen, Inc., P.O. Box 400 Knightdale, NC 27545. Assistance of Juan R. Acedo, William M. Reece, William H. Swallow, 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 C.T.G. in partial fulfillment of the requirements for the MS degree.

[2]Graduate Research Assistant.

[3]Professor.

Introduction

Many woody ericaceous species native to the Appalachian Mountains of the United States are desirable landscape plants, including *Kalmia latifolia* L. (mountain laurel), *Leucothoe fontanesiana* (Steud.) Sleum (drooping leucothoe), *Rhododendron carolinianum* Rehd. (Carolina rhododendron),

Table 1. Seed sources (county in NC and elevation), date of collection (month/year), moisture content at storage, and number of pure seeds per 28 g (1 oz) for the various species.

Species	Seed source and elevation	Collection date	Moisture content (%)	No. pure seeds per 28 g (1 oz)[z]
K. latifolia	Buncombe Co., 700 m (2300 ft)	11/91	5[y]	1,300,000
L. fontanesiana	Buncombe Co., 700 m (2300 ft)	10/90	7[x]	550,000
R. carolinianum	Burke Co., 1100 m (3600 ft)	11/91	4[y]	670,000
R. catawbiense	Buncombe Co., 1860 m (6100 ft)	11/90	6[x]	170,000
R. maximum	Avery Co., 950 m (3100 ft)	11/90	5[y]	320,000

[z]Based on moisture content at storage.

[y]Based on six, 200-seed samples.

[x]Based on six, 100-seed samples.

Rhododendron catawbiense Michx. (Catawba rhododendron), and *Rhododendron maximum* L. (rosebay rhododendron). Each is evergreen and generally grows as a shrub. Due to a number of ornamental characteristics, they are highly prized landscape plants.

Most of these species are produced by 'cutbacks' harvested from native stands on public and private lands (1). However, native stands are finite. Production by seeds represents an alternative system for producing high quality plants. Because large quantities of viable seeds are not always available on a yearly basis, growers attempt to collect large amounts during bountiful years and store them for future use.

Many reports have appeared in the literature regarding seed storage of particular ericaceous species. However, such recommendations are based primarily on personal observations. Few quantitative data have been reported to show the extent to which viability, as influenced by storage conditions for various ericaceous species, varies with time. Leach (12) commented that seeds of *Rhododendron* L. (rhododendron) are 'not designed by nature to endure long in good condition.' From personal experience he reported that seeds lose 50% viability a year when stored at 'room temperature' and the seeds that remain viable, germinate slowly. Using 2-year-old seeds, Leach (12) reported that, following sowing, so few seeds germinated that his flats containing the seeds were discarded. He also noted that storage in sealed vials in a refrigerator at 3.5C (38F) prolonged viability.

Other reports have also appeared regarding storage and subsequent viability of seeds of *Rhododendron*. Brydon (6) noted that seeds of *Rhododendron* retain viability for several years when stored under cool, dry conditions or in a refrigerator. Similarly, Valder (17) reported that refrigerated seeds of *Rhododendron* 'will remain viable for at least 2 years and perhaps much longer.' Bowers (5) reported that seeds will remain viable for about 2 years at 'room temperature,' but after 1 year in storage many fail to germinate. Young and Young (18) also observed that seeds remain viable for about 2 years when stored at 'room temperature.' Olson (14) observed that dried seeds will remain viable for 2 years when stored at 'room temperature'; however, the ideal method is to store the seeds at –6C (20F) to ensure prolonged viability.

Jaynes (11) reported that seeds of *K. latifolia*, if stored under cool, dry conditions, will remain viable for many years. In addition, he noted that of 28 lots of seed stored for ≥ 10 years in glassine and coin envelopes at 4C (39F), 75% had germination > 50%. In contrast, it appears no information has been reported regarding seed storage of *L. fontanesiana*.

With the increasing popularity of native plants and the general lack of knowledge pertaining to seed storage of particular ericaceous species, the following investigation was initiated in Fall 1990. The objective of the research was to study the influence of storage temperatures on long-term seed viability of *K. latifolia, L. fontanesiana, R. carolinianum, R. catawbiense*, and *R. maximum*.

Materials and Methods

In October and November 1990, mature seed capsules from native populations of open pollinated plants of *L. fontanesiana*, *R. catawbiense*, and *R. maximum* were collected in western North Carolina (Table 1). The following year, November 1991, mature seed capsules of *K. latifolia* and *R. carolinianum* were also collected from the western part of the state (Table 1). Following collection of capsules of each species, the capsules were stored in paper bags at 20C (68F) for 30 days.

Seeds were then removed from the capsules, moisture content of each species was determined, and seeds stored at 21C (71F) in sealed glass bottles. Moisture content was determined by calculating the mean moisture content of six samples, each with 100 or 200 seeds, following drying at 105C (221F) for 24 hr (Table 1).

Prior to conducting germination tests, seeds of each species were removed from storage and graded under a dissecting scope which allowed removal of abnormal, damaged or undersized seeds, and any debris. Two viability (germination) studies were conducted; a preliminary study followed immediately by a more rigorous germination test [time 0 (Table 2)] conducted at the Southeastern Plant Environment Laboratory [Phytotron (8)]. Results of the studies were in

Table 2. Initial (time 0) seed germination (%) of *K. latifolia*, *L. fontanesiana*, *R. carolinianum*, *R. catawbiense*, and *R. maximum*.[z]

	Germination temperature					
	25C (77F)			25/15C (77/59F)		
	Photoperiod			Photoperiod		
Species	0 hr	1 hr	24 hr	0 hr	1 hr	24 hr
K. latifolia	0.0	1.7	74.0	0.0	3.5	76.7
L. fontanesiana	0.0	33.5	54.0	0.0	61.2	56.7
R. carolinianum	0.0	0.5	90.9	0.0	18.4	92.1
R. catawbiense	3.5	91.4	90.0	1.5	88.6	90.1
R. maximum	0.0	0.3	81.6	0.0	41.7	95.7

[z]All values represent the mean germination percentage of four petri dishes each containing 100 seeds.

agreement and indicated that seeds of *K. latifolia*, *R. carolinianum, R. catawbiense*, and *R. maximum* were capable of germination ≥ 75% whereas, seeds of *L. fontanesiana* germinated at ≈ 55%.

Once the more rigorous germination tests were initiated at the Phytotron, seeds of each species were divided into three equal lots (seeds in sealed glass bottles) and stored at the following temperatures: –18, 4 or 23C (0, 39 or 73F). The initial Phytotron germination studies were conducted in the following manner and were repeated yearly up to 1996. Annual tests for a species included seeds stored at each of the three storage temperatures. Prior to conducting the yearly tests, seeds were graded as described previously.

Seeds of each species were sown in covered 9-cm (3.5 in) glass petri dishes containing two germination blotters (Filtration Science Corp., Mt. Holly Springs, PA) moistened with tap water. Following placement of the seeds in the dishes, half of the dishes 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 (8)] 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 photoperiods with a photosynthetic photon flux (400–700 nm) of ≈ 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (2.8 klx) provided by cool-white fluorescent lamps: total darkness, 1 or 24 hr. Light was measured at dish level with a cosine corrected LI-COR LI-185 quantum/radiometer/photometer (LI-COR, Lincoln, NE). Photoperiod treatments were regulated by removal and placement of the petri dishes in black sateen cloth bags. The 1-hr photoperiod treatment at the alternating temperature of 25/15C (77/59F) began with the transition to the high temperature portion of the cycle. Temperature in the petri dishes never deviated from ambient temperature by more than 1C (2F) as measured by one thermocouple per chamber. The constant darkness treatment was maintained by keeping the petri dishes in the black cloth bags throughout the experiment. 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).

Each photoperiod treatment was replicated four times and a replication for a species, with the exception of *R. carolinianum*, consisted of a petri dish containing 100 seeds. A replication for *R. carolinianum* utilized 50 seeds per petri dish. Germination data were recorded every 3 days for 30 days. 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 (16). The analyses did not include data for the initial germination tests (time 0). All mean separations were performed by least significant difference (LSD) procedures at P = 0.05.

Results and Discussion

Analysis of variance showed that for each species, storage temperature, duration of storage, photoperiod, and their interactions were highly significant ($P \leq 0.002$). Viability, expressed as total germination percentage, was influenced by storage temperature with room temperature storage [23C (73F)] being the least effective means of maintaining viability over time (Figs. 1 to 5).

After 1 year at room temperature [23C (73F)], a significant decrease in total germination was noted for seeds of *K. latifolia*, *L. fontanesiana, R. catawbiense*, and *R. maximum*, in comparison to seeds stored in a refrigerator [4C (39F)] or freezer [–18C (0F)] (Figs. 1, 2, 4, and 5). On the other hand, viability of *R. carolinianum* remained relatively unchanged regardless of storage temperature (Fig. 3). Storage at room temperature dramatically reduced seed viability of *L. fontanesiana*; by year 3 germination was negligible (Fig. 2). The same was noted by year 4 for seeds of *R. catawbiense* and *R. maximum* stored at room temperature. Although viability of *K. latifolia* decreased rapidly at room temperature, germination of 14% still occurred by year 4. The rapid loss in viability of seeds of *R. catawbiense* and *R. maximum* when stored at room temperature agrees with observations of Leach (12).

Seed viability of all species remained relatively constant for storage under refrigerated [4C (39F)] or freezer [–18C (0F)] conditions. For each species there were specific instances when yearly germination differed between freezer-stored seeds and refrigerated storage. However, with few exceptions, the differences were too small to suggest that one storage temperature was better than the other. Perhaps if viability had been monitored for longer periods of time (e.g., 10 years or longer), more pronounced differences would have been observed. Although seed viability of *L. fontanesiana*, *R. catawbiense*, and *R. maximum* was studied for 5 years and that of *K. latifolia* and *R. carolinianum* for 4 years, the data suggest that viability of these species can be maintained for much longer periods of time by refrigerated or freezer storage.

When estimating viability of a particular lot of seeds one must consider both total germination within a specific period of time and the rate of germination. In the present study both variables were considered although only data for total (30-day) germination are presented. Decreases in viability were normally accompanied by reductions in germination rate. Conversely, when no changes in viability occurred, germination rates remained relatively constant.

Even though seed viability of all species stored under refrigerated or freezer conditions remained relatively constant, there was one exception. For seeds of *R. maximum* germinated at 25C (77F) with constant light, it appeared that by year 3, viability decreased greatly for seeds stored under refrigerated or freezer conditions (Fig. 5). However, no similar decrease in germination occurred for seeds germinated at 25/15C (77/59F) with the same photoperiod. By year 4, germination at 25C (77F) increased to previous levels and remained basically the same through year 5. The cause of this decrease in germination at year 3 is unknown. It is possible that environmental conditions in the 25C (77F) growth chamber were not identical to years 1, 2, 4 or 5 which decreased germination.

Of the five ericaceous species utilized in this investigation, seeds of *R. carolinianum* exhibited the least decrease in viability when stored at room temperature (Fig. 3). Although viability decreased at room temperature storage between years 1 and 2, by year 4 total germination of 86% and 68% was noted for seeds germinated with constant light at 25C (77F)

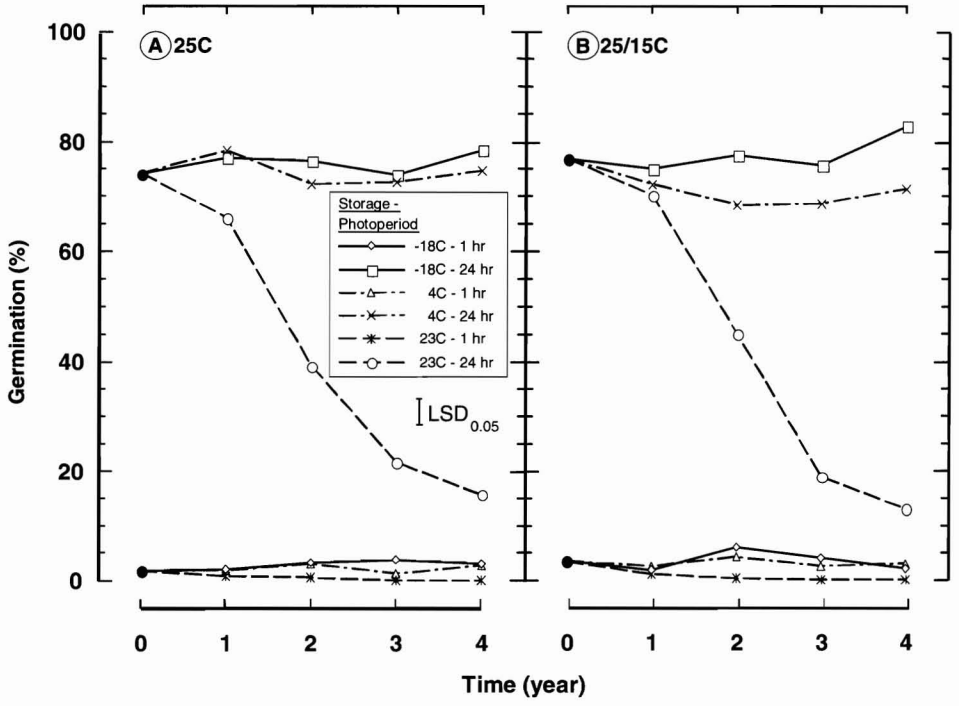

Fig. 1. Influence of storage temperatures over time on yearly, total seed germination of *K. latifolia*. Seeds were stored in a freezer [–18C (0F)], in a refrigerator [4C (39F)] or at room temperature [23C (73F)]. $LSD_{0.05} = 4.5$ for within year comparison (year 1 to 4) of one of the following three factors when two of the factors are held constant: germination temperature, storage temperature or photoperiod. (A) germinated at 25C (77F) with daily photoperiods of 1 or 24 hr. (B) germinated at 25/15C (77/59F) utilizing the same photoperiods as in (A). Legend in (A) applies to both figures. Data for the 0-hr photoperiod were omitted since germination was negligible. Time 0 data (•) represent initial viability.

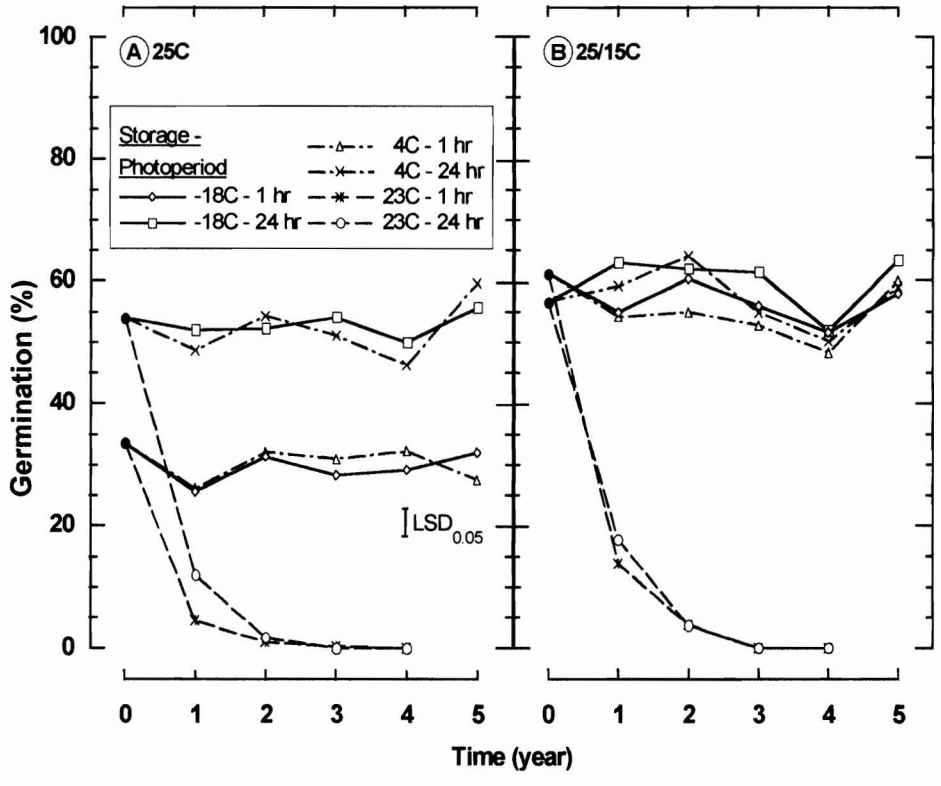

Fig. 2. Influence of storage temperatures over time on yearly, total seed germination of *L. fontanesiana*. Seeds were stored in a freezer [–18C (0F)], in a refrigerator [4C (39F)] or at room temperature [23C (73F)]. $LSD_{0.05} = 4.9$ for within year comparison (year 1 to 5) of one of the following three factors when two of the factors are held constant: germination temperature, storage temperature or photoperiod. (A) germinated at 25C (77F) with daily photoperiods of 1 or 24 hr. (B) germinated at 25/15C (77/59F) utilizing the same photoperiods as in (A). Legend in (A) applies to both figures. Data for the 0-hr photoperiod were omitted since germination was negligible. Time 0 data (•) represent initial viability.

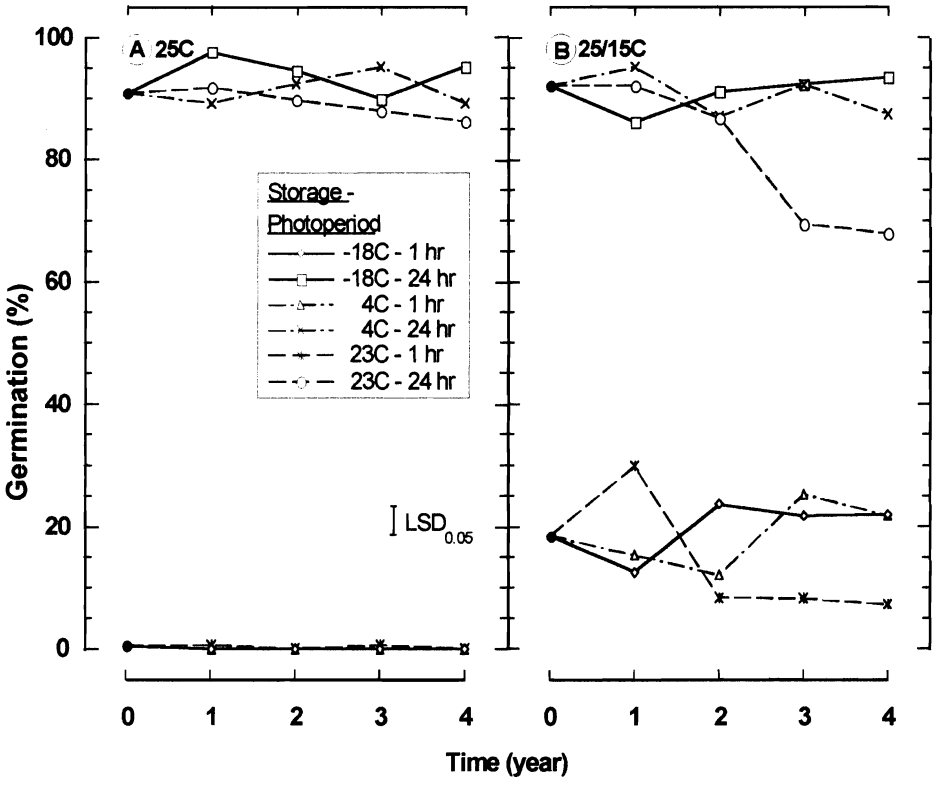

Fig. 3. Influence of storage temperatures over time on yearly, total seed germination of *R. carolinianum*. Seeds were stored in a freezer [–18C (0F)], in a refrigerator [4C (39F)] or at room temperature [23C (73F)]. $LSD_{0.05}$ = 4.8 for within year comparison (year 1 to 4) of one of the following three factors when two of the factors are held constant: germination temperature, storage temperature or photoperiod. (A) germinated at 25C (77F) with daily photoperiods of 1 or 24 hr. (B) germinated at 25/15C (77/59F) utilizing the same photoperiods as in (A). Legend in (A) applies to both figures. Data for the 0-hr photoperiod were omitted since germination was negligible. Time 0 data (•) represent initial viability.

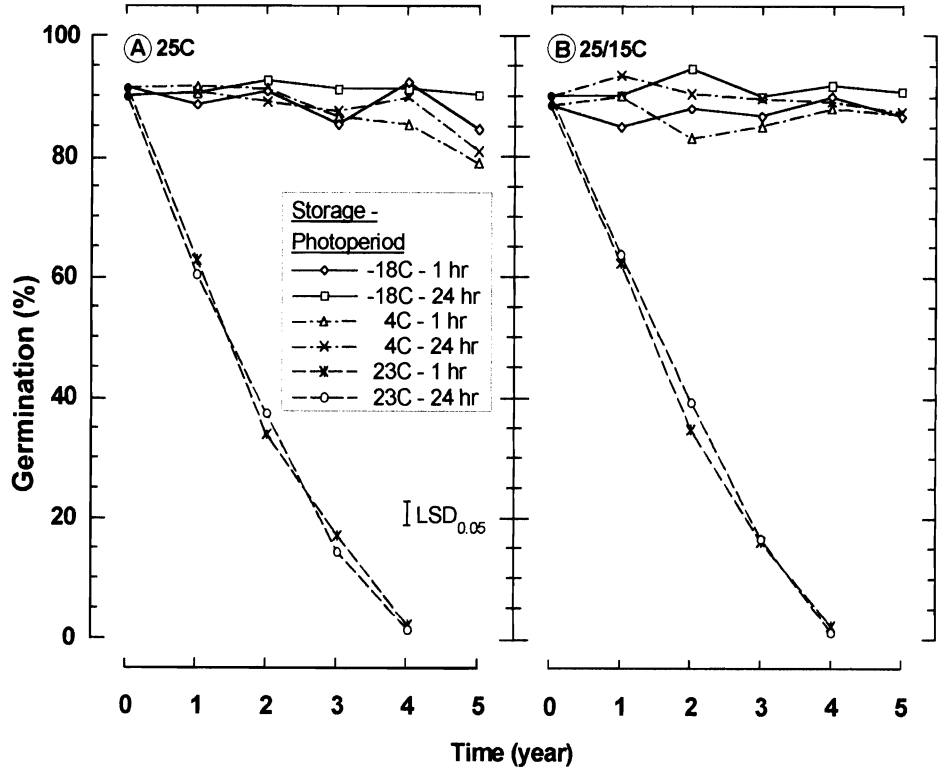

Fig. 4. Influence of storage temperatures over time on yearly, total seed germination of *R. catawbiense*. Seeds were stored in a freezer [–18C (0F)], in a refrigerator [4C (39F)] or at room temperature [23C (73F)]. $LSD_{0.05}$ = 4.1 for within year comparison (year 1 to 5) of one of the following three factors when two of the factors are held constant: germination temperature, storage temperature or photoperiod. (A) germinated at 25C (77F) with daily photoperiods of 1 or 24 hr. (B) germinated at 25/15C (77/59F) utilizing the same photoperiods as in (A). Legend in (A) applies to both figures. Data for the 0-hr photoperiod were omitted since germination was negligible. Time 0 data (•) represent initial viability.

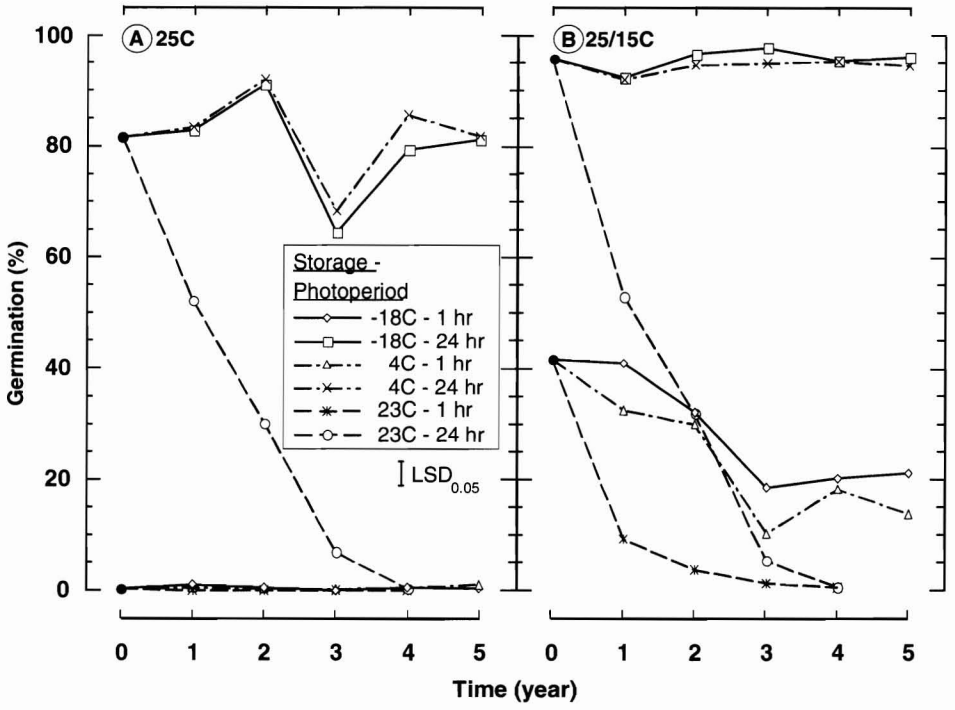

Fig. 5. Influence of storage temperatures over time on yearly, total seed germination of *R. maximum*. Seeds were stored in a freezer [–18C (0F)], in a refrigerator [4C (39F)] or at room temperature [23C (73F)]. $LSD_{0.05}$ = 4.4 for within year comparison (year 1 to 5) of one of the following three factors when two of the factors are held constant: germination temperature, storage temperature or photoperiod. (A) germinated at 25C (77F) with daily photoperiods of 1 or 24 hr. (B) germinated at 25/15C (77/59F) utilizing the same photoperiods as in (A). Legend in (A) applies to both figures. Data for the 0-hr photoperiod were omitted since germination was negligible. Time 0 data (•) represent initial viability.

and 25/15C (77/59F), respectively. The capacity for seeds of *R. carolinianum* to maintain viability when stored at 23C (73F) suggests that seeds of this species are capable of remaining viable for extended periods when stored at room temperature. Another explanation for maintenance of viability at 23C (73F) may have been related to seed moisture content. Initially, seed moisture content of *R. carolinianum* was 4% compared to 5% to 7% for the other four species (Table 1). The lower moisture content of *R. carolinianum* may explain why loss of viability at room temperature storage was less than the other four species stored under the same conditions. As noted by Hartmann et al. (10), control of seed moisture content is extremely important in terms of maintaining viability during storage. Harrington [as reported by Hartmann et al. (10)] commented that for seeds not adversely affected by low moisture conditions, each 1% decrease in seed moisture between 5% and 14% doubles the life of the seeds and each decrease of 5C (9F) between 0 and 44.5C (32 and 112F) in storage temperature also doubles seed storage life.

In the present study, a temperature of 23C (73F) was chosen to represent room temperature since we had access to a controlled-temperature facility in which temperature never deviated by ± 2C (3.6F) of the set point. Thus, temperature was maintained relatively constant. If typical room temperature conditions had been utilized, seeds would have been subjected to very wide fluctuations in temperature.

Regardless of germination temperature, seeds of *K. latifolia*, *L. fontanesiana*, *R. carolinianum*, *R. catawbiense*, and *R. maximum* required light for germination, which agrees with previous reports (2, 3, 4, 13, 15). Our findings suggest that light sensitivity of these species is not lost with storage over time as was suggested by Blazich et al. (2) for *R. catawbiense*. On the other hand, Fujii and Isikawa (9) demonstrated the need for light for seed germination of *Eragrostis ferruginea* Beauv. (lovegrass) decreased with dry storage over 16 months.

Disregarding that seed viability decreased over time for all five species stored at room temperature, the photoperiod needed to maximize germination did not change from year to year. At 25C (77F) and 25/15C (77/59F) a 1-hr daily photoperiod maximized germination of *R. catawbiense* (Fig. 4). For *L. fontanesiana*, maximum germination occurred at 25/15C (77/59F) with a daily 1-hr photoperiod, whereas at 25C (77F), constant light was required for maximum germination (Fig. 2). For *K. latifolia*, *R. carolinianum*, and *R. maximum* continuous light was necessary at both temperatures to maximize germination, although the alternating temperature of 25/15C (77/59F) partly compensated for the light requirement (Figs. 1, 3, and 5).

The responses reported herein of all five species (Figs. 1 to 5) when germinated at 25C (77F) or 25/15C (77/59F) with daily photoperiods of 0 (total darkness), 1 or 24 hr agrees with previous studies (2, 3, 4, 13, 15) which utilized freshly harvested seeds not subjected to long-term storage. Agreement between these investigations and our data suggest that temperature and light requirements for germination of *K. latifolia*, *L. fontanesiana*, *R. carolinianum*, *R. catawbiense*, and *R. maximum* do not change with dry storage.

In the present study, seed germination was defined as radicle emergence ≥ 1 mm (0.04 in). However, seed testing laboratories generally define germination as the emergence and development from the seed embryo of those essential

structures, which for the kind of seed in question, are indicative of the ability to produce a normal plant under favorable environmental conditions. Thus, what was defined herein as germination, radicle emergence, may not have been a good measure that seeds exhibiting radicle emergence were capable of developing into normal plants. It is possible that seeds stored for long periods under particular conditions might have the capacity for radicle emergence yet may have deteriorated during storage such that they would not be capable of producing normal plants. Therefore, when the final viability studies were conducted during years 4 or 5, additional seeds of each species (four petri dishes per species, each containing 25 seeds) stored at –18 or 4C (0 or 39F) were placed for germination at 25C (77F) with continuous light. Seeds of *K. latifolia* and *R. carolinianum* stored at room temperature were also included. Following radicle emergence, the seeds were left in the petri dishes to observe whether radicle emergence was followed by development of normal seedlings (i.e., seedlings with true leaves, a normal appearing primary root, etc.). For all species, with the exception of seeds of *K. latifolia* stored at room temperature, the majority of seeds exhibiting radicle emergence developed into normal appearing seedlings. Thus, freezer or refrigerated storage maintained seed viability with no adverse effect on seedling development. The same was also observed for seeds of *R. carolinianum* stored at room temperature.

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