SEED GERMINATION OF SHORTIA GALACIFOLIA T. & G. UNDER CONTROLLED CONDITIONS[1]

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Until 1936 it was belived that *Shortia galacifolia* could not reproduce by seed because the scapes decayed and fell over before the seeds were mature (Kelsey, 1902). Observations by Ross (1936) disclosed that Shortia seeds do reach maturity and germinate, but in an unusual manner. Germination occurs in the capsule, the seeds being held against the placenta by the firm, partially opened capsule walls. In this position the seeds do not readily fall out when ripe, and are kept fairly moist because the capsule and cup-like calyx retain water. Seedlings reach the ground when the scape decays and falls. There they have little chance of survival because of predation by insects and because of the dense mass which Shortia plants form by vegetative reproduction. Attempts by Ross to germinate seeds on soil in the laboratory were unsuccessful, and he concluded that there was little possibility for the spread of Shortia plants by seed.

Ross's work suggests that seedlings of Shortia would be rare in nature, and that most of them would be near mature Shortia plants. On the contrary, several people have reported finding abundant seedlings in the field, both underneath (Crandall, 1956) and several feet from patches of mature plants (Crandall, 1956; Vivian, 1965). However, it was not determined whether these seedlings were from seeds which germinated in the capsule or on the ground. Since it is more likely that seeds are transported rather than seedlings, it appeared worthwhile to investigate the possibility of seed germination outside the capsule.

Capsules of *Shortia galacifolia* were collected on May 15, 1964, at an elevation of 1440 feet on the northwest side of

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Crossroads Mountain in Transylvania County, N.C. The capsules were open and most of them contained many seeds, but no seedlings were found in them. Immediately when picked they were wrapped in paper towels and put in plastic bags to prevent drying.

A previous experiment showed that freezing the seeds did not impair germination, so the capsules were kept frozen until the seeds could be removed and counted. When the seeds were removed from the capsules, those with any visible defect, such as mold, discoloration, or extreme flatness, were discarded. The sound seeds were then put into envelopes of 50 seeds each and refrozen until all seeds were sorted. To prevent the seeds from drying out, only about six capsules were taken from the refrigerator at a time for removal of the seeds.

Eight environmental factors were tested for their effects on germination. Temperature, moisture, and light were combined into one experiment, so there were six experiments in all. For all but the first experiment, germination was tested in an air-conditioned room with a temperature range of 20° to 24°C and a light intensity of 30 ft-c. Germination was tested in all treatments, except those involving soil, by sprinkling the seeds on moist Whatman No. 1 filter paper in a clean 9 cm petri dish. The paper was moistened once a day with distilled water from a medicine dropper.

Each treatment included six replications of 50 seeds each. The seeds were assigned to a particular treatment by numbering the envelopes of seeds and then using a table of random numbers to select the envelopes for a prearranged order of treatments. The number of seeds germinated was recorded every 4 days up to 32 days, and then once again after 50 days. The first appearance of the radical was the criterion for germination.

To determine the effect of the interaction of temperature, moisture and light, germination was tested at 10°, 20°, and 30°C, in the light and the dark, and at three moisture levels. The moisture levels were "abundant," in which the filter paper was saturated with water; "sufficient," in which it

was thoroughly moistened but not saturated; and "deficient," in which it was never thoroughly moistened. Both incandescent and fluorescent lamps provided illumination. The combinations of three temperatures, two light conditions, and three moisture levels made 18 treatments.

Two other treatments tested the effect of a changing temperature regime on germination. In one treatment seeds were placed alternately between 10°C and 20°C every 48 hours. In the other treatment seeds were moved progressively from 10°C, to 20°C, to 30°C. After 5 days at each of these temperatures, the seeds were put at 22°C for the remaining time. For both of the changing temperature treatments the seeds were kept in the light and given "sufficient" moisture.

Since Shortia seeds germinate within the capsule, it was decided to test the effect of capsule parts on germination. For example, the calyx from about 10 capsules was cut up and the pieces were sprinkled on the filter paper along with the seeds. The placenta, calyx, and ovary wall were tested for their effects in this way. To obviate the effect of moisture retention by capsule parts, a control treatment was set up in which pieces of filter paper were sprinkled in the dishes. The cumulative effect of the capsule parts was tested by two treatments: putting seeds into empty, intact capsules and putting pieces of all capsule parts with the seeds.

Three other experiments were designed to test the effect on germination of substrate type, acid, and soaking. Steam sterilized sand, sphagnum, and a mixture of two parts sandy loam, one part sand, and one part sphagnum were the substrates tested. Petri dishes were half filled with the substrate and the seeds were sown on the surface, which was kept moist. The acid solutions tried were glacial acetic acid of pH 3.5 and two leachates of pH 4.5, one of moss and the other of leaf litter collected at the site where the seeds were obtained. Soaking periods used were 1, 3, 6, and 9 days in distilled water. Seeds kept at 22°C in the light and with "sufficient" moisture were used as the control for all tests.

In the final experiment six lots of 200 seeds each were put in petri dishes and allowed to air dry for 1 to 6 days before they were transferred to moist filter paper. Duplicate sets of 100 seeds each were also air dried, then weighed and oven dried at 105°C to a constant weight. In this way moisture content of the seeds, as percentage oven-dry weight, was determined for each of the air drying periods.

Although eight tests were designed for statistical treatment of the results, for some tests the great variation in number of seeds germinated among replications or the low total number of seeds germinated made a meaningful statistical analysis impossible. Even the mean and the standard deviation cannot be given for some treatments because they indicate the possibility of a negative number of germinations, which is meaningless.

Table 1 gives the results for the effect of temperature, moisture, and light on germination. Many more seeds germinated at 20°C than at 30° or 10°C, and more germinated in the light than in the dark. No germination occurred at the "deficient" moisture level in any temperature regime. At 20°C more seeds germinated in the "abundant" moisture condition than in the "sufficient." The difference is significant at the 2.5% level using a Student's t-test (Abundant, mean number of germinations/50 seeds $= 4.33 \pm 1.51$; Sufficient, mean $= 2.17 \pm 1.33$).

The effect of the changing temperature treatments on germination is also shown in Table 1. Almost identical results were obtained for the two treatments. A constant temperature of 20°C was as good for germination and perhaps better than, the two changing temperature regimes.

The presence of the placenta, the calyx, the ovary wall, or all parts improved germination about fivefold over that of the control. An analysis of variance using the 2.5% significance level showed that there was no difference among the treatments involving capsule parts. Adding filter paper instead of capsule parts did not improve germination.

Sand and sphagnum were superior to the sandy loam mixture or filter paper as substrates for seed germination.

Table 1. Effect of Temperature, Moisture, and Light On Seed Germination of *Shortia galacifolia*

Temperature and Moisture	Number of Seeds Germinated After 50 Days (300 seeds/treatment	
	Light	Dark
10° C		
Abundant	0	0
Sufficient	0	1
Deficient	0	0
20° C		
Abundant	26	2
Sufficient	13	0
Deficient	0	0
30° C		
Abundant	1	0
Sufficient	0	0
Deficient	0	0
Changing temperature		
Alternating 10°, 20°	4	
Increasing 10° to 20° to 30° to 22°	3	
Constant 20°	13	

Using water with leaf litter in it instead of plain distilled water may have improved germination slightly, but using water with moss or glacial acetic acid added did not. Better germination was obtained for all soaking treatments than for the control, and the period of soaking had no effect.

The average moisture content of Shortia seeds before they were air dried was 12% oven-dry weight. No loss of germinative capacity occurred for seeds air-dried for up to 6 days. However, no loss of moisture occurred, because the vapor pressure deficit of the air remained low over the whole period. A separate set of 100 seeds was made up to be left until a significant amount of moisture was lost. The seeds were allowed to air-dry for about 3 days. Since no moisture loss had occurred after this time, the seeds were put in an oven at 30°C for 12 hours. Their weight was reduced 4% during this treatment, which was three times the weight lost by any of the air-dried lots of seeds. After removal from the oven, the seeds were left in the air for 20 days.

They had regained their original weight when they were put on moist filter paper to test germination. None of the seeds germinated.

For 21 treatments the number of seeds germinated was recorded every 4 days. For most of the treatments the first germination occurred between 13 and 16 days from the start. Soaking caused germination to begin 4 to 8 days earlier.

The distribution of germination over the 32 day period followed no particular pattern. A final count for all treatments was made after 50 days, and a few germinations were recorded at this time. Most germinations, however, had occurred before 32 days.

Seeds of *Shortia galacifolia* germinated in the laboratory under a wide variety of conditions, indicating that the seeds do not have to be in the capsule to germinate. The factors which appear to be most significant in influencing germination of Shortia seeds are temperature, moisture, light, and presence of capsule parts.

The optimum temperature for germination is probably around 20°C, because substantially more germination occurred at this temperature than at either 10°C or 30°C.

An abundant moisture supply is necessary for good germination. No seeds germinate when moisture is always deficient, but some may germinate when moisture is alternately available and unavailable. Although the evidence is not conclusive, it also appears that Shortia seeds lose their germinative capacity if their moisture content falls below a certain critical level, even if they later regain moisture. Soaking seems to improve germination slightly, and even 9 days of soaking does not impair germination.

Light appears to be beneficial for germination, since many more seeds germinated with 900 ft-c illumination than in the dark or with 30 ft-c.

Although any capsule part improved germination when present with the seeds, combining parts did not produce a cumulative effect. Using leachate from leaf litter to water the seeds did not improve germination as much as the

presence of capsule parts, indicating that there is some organic substance peculiar to Shortia which aids germination.

From the results of the various tests it might be predicted that the best germination of Shortia seeds would be obtained by soaking the seeds at least 1 day, using sand as a substrate with capsule parts scattered over the sand, providing an illumination of about 900 ft-c, and supplying abundant moisture. Combining the two treatments which gave the best germination, "addition of placenta" and "20°C-light-abundant moisture," was tried, but no increase in percentage of germination resulted.

Although germination of Shortia seeds in nature outside the capsule has never been observed, the results of the laboratory experiment indicate that it undoubtedly occurs. Factors which were found to be important for good germination in the laboratory are present in the natural habitat. Micro-environmental measurements made in several different stands of Shortia show that the air temperature during the growing season ranges from 13°C to 32°C, that the relative humidity never falls below 50%, and that the light intensity ranges from 20 to 1000 ft-c (Vivian, 1965). Decaying capsule parts and leaf litter are present on a sandy soil.

In 1936, Ross thought the answer to the extreme localization of Shortia was the fact, that the seeds are held tightly in the open capsule and must germinate there, thus limiting the spread by seed. Although Ross noted that some seeds fall from the capsules, he did not mention how many. The author has observed that 50% or more of the seeds may be lost within a month after the capsules open. Rain is probably the primary agent in releasing the seeds, which could easily be carried far from the mature plants by rivulets or streams. It does not seem that lack of seed dispersal can be used to explain why Shortia fails to colonize new, apparently suitable habitats. Low seedling survival may be much more important in limiting the spread of Shortia by seed. Observations suggest that the seedlings grow very slowly in nature and are quite subject to winter kill (Vivian, 1965). Seeds germinated in the laboratory produced seedlings about

2 cm high after 8 months of growth. The amount of competition that seedlings can tolerate is probably small. It seems that the problem of the restricted and discontinuous distribution of *Shortia galacifo*lia has not been solved, but that requirements of mature plants, competition, and seedling survival, perhaps along with a low percentage of germination, are more significant factors than lack of seed dispersal.

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