Factors in Storage of M. Macrophylla Seeds

by Philip G. Seitner

Summary

Freshly collected, mature *Magnolia macrophylla* seeds were submitted to trials comparing (1) dry storage to moist storage and (2) room temperature storage to refrigerator storage.

WATER: The results demonstrated a vulnerability to seed-water loss when stored dry, against which insulation afforded by retention of the outer seed coat or by storage in water-retaining containers gave only partial protection. Maximum viability was retained only by moist storage applied promptly after collection. But dry storage failed to demonstrate 100 percent kill, even after 180 days.

TEMPERATURE: Germination appeared to be unaffected or only modestly affected by storage at ordinary indoor temperature, whether under moist or dry storage, indicating that the critical storage factor was seed-water conservation.

INCIDENTAL OBSERVATIONS: The importance of cold-conditioning for germination and the adequacy of a 56-day cold-conditioning period appear to have been demonstrated. After dry storage, seeds that remained viable showed greater germination retardation than seeds stored moist, demanding a longer germination-monitoring period.

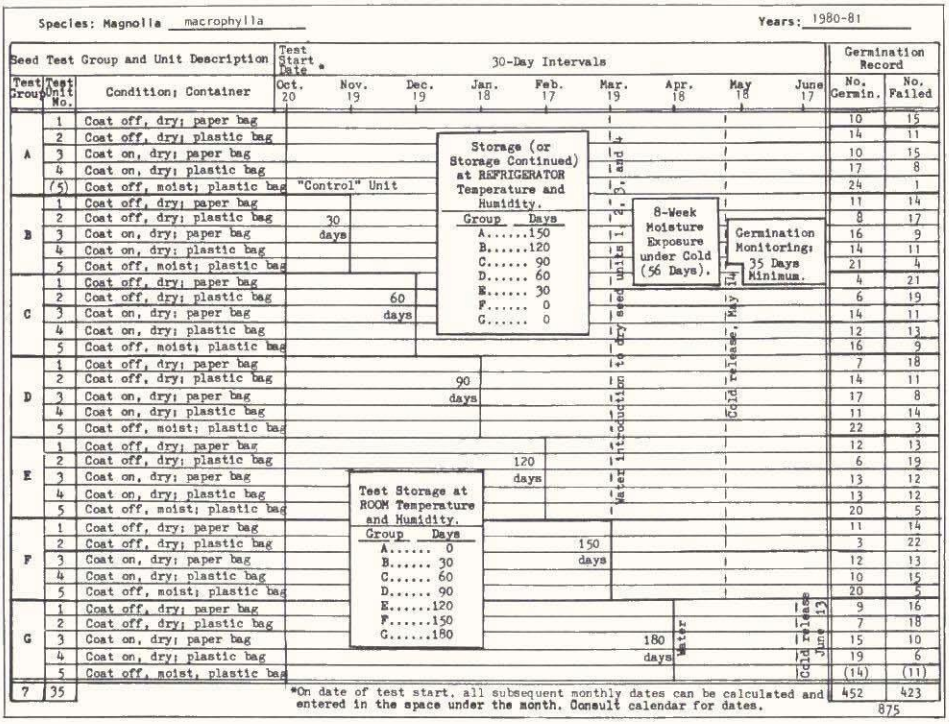
LIMITATIONS: The study was limited to a 180-day period; it did not explore the issue of storage to a second or third year. It did not explore for the minimum duration of exposure to cold to induce germination, which might conceivably be shorter than the 56-day period applied. Finally, it did not explore for a possible post-maturation period of refractoriness to the germination-conditioning effect of cold.

* *

Background and Purpose. The *Magnolia* seed embryo has long been known to have a low storage tolerance compared to that of many other plant genera. Recently, a personal experience with seeds of three *Magnolia* species and one interspecifie hybrid appeared to demonstrate that identical storage conditions had resulted in embryo death of two (*M. macrophylla* and *M. officinalis biloba*) while permitting survival of the others (*M. acuminata* and *M.* × *soulangiana*). This suggested a species difference in resistance of *Magnolia* seed embryos to potentially adverse storage conditions. The objective of the present simple study was to attempt to establish for a single *Magnolia* species the duration of survival of seed embryos stored indoors at ambient air temperature and humidity, as opposed to storage at low temperature and in a water-saturated atmosphere. It was confined to test times of up to 180 days (the spring following seed collection). The study was conceived as an initial step toward demonstrating any species difference

* *

Figure 1.

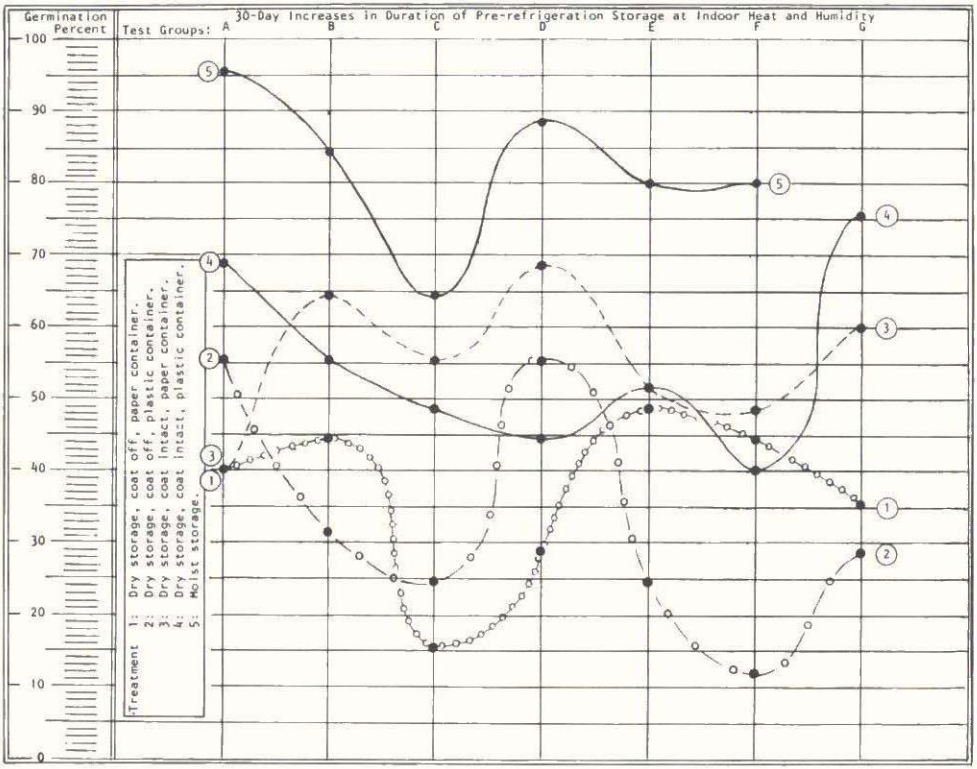


and as a possible contribution to a rational basis for seed storage.

Test Material. The test material was a quantity of *Magnolia macrophylla* seeds collected immediately after full maturation (natural release from mature fruits on the tree in October), all from the identical parent tree at North Manchester, Indiana. The species was used for this initial study primarily because circumstances offered enough seeds for adequate (25-seed) test portions; however, it was coincidentally an apt subject in view of evidence alluded to above that its seeds may be less resistant to adverse storage conditions than those of some other *Magnolia* species and therefore test results might serve as a least common denominator for this aspect of *Magnolia*.

Experimental Design (figure 1). In the following description and the figures, *moist storage* refers to seed storage in a water-saturated atmosphere preventing loss of water from the seed and satisfying any requirement of the seed for additional water during late storage and germination. That atmosphere was provided by suspension of seeds in coarse sphagnum moss kept constantly moist, not water-logged; the moss and seeds were held in a bag of thin plastic (food storage bag) which retards water vapor loss from the moss while permitting adequate oxygen and carbon dioxide passage. Moist storage was preceded by removal of the outer oil-bearing seed coat. It attempts to simulate conditions apparently ideal for survival and germination of the seed in its natural environment. *Dry storage*

Figure 2.



refers to seed storage without provision for a degree or constancy of humidity greater than that of average indoor atmosphere; this simulates conditions to which seeds are frequently exposed after collection for propagation.

The test challenge to survival of seeds stored *dry* (Treatments 1 through 4, described below) include two factors of indoor living room atmosphere, (I) low relative humidity and (II) heat, varying roughly between 18° and 22° C (65° and 72° F), emulating storage factors which *M. macrophylla* embryos appeared not to have survived after five months of exposure in the winter of 1979-1980. Seed-water extraction by low relative humidity of the atmosphere, which might accelerate with temperature increase, represented the direct survival challenge. It was suspected, however, that heat acts independently as an adverse factor, an aspect that Treatment 5 was expected to demonstrate, since the test challenge to survival of seeds stored *moist* was solely indoor heat, defined above.

Dry storage was explored further by applying to dry seed portions two storage variants apt to affect seed-water retention, (a) a water-conserving (plastic) container, as opposed to a paper container having no water-conserving property, and (b) retention of the seed's oil-bearing and probably water-conserving pulpy outer coat, as opposed to the removal of that coat. Combinations provided four different treatments with these factors: (1) outer coats removed, in a paper container; (2) outer coats removed, in a plastic container; (3) outer coats intact, in a paper container, and (4) outer coats intact, in a plastic container. These

provided a spectrum of *dry*-seed treatments from least (Treatment 1) to greatest (Treatment 4) water-conserving elements.

The test challenge as defined above was applied to seven test groups, A through G. Each group was exposed for a different *duration*, 0, 30, 60, 90, 120, 150, and 180 days. Each group in turn was divided into five subsidiary units (1 through 5) for the four conditions of *dry* storage (Units 1 through 4) and the one of *moist* storage (Unit 5). The container of each unit was indelibly labeled with the group and unit designation (for example, A-1). The total number of units was therefore 35, requiring 875 seeds for 25-seed units.

The 25-seed size of the test units was felt to be the minimum for significant results; it would have been preferable to have 50- or 100-seed units, had the seed supply and time permitted.

Following test treatment, each unit was given an eight-week germination-conditioning treatment (water introduction to dry-seed units, combined with cold storage), followed by removal to germination-inducing (ordinary indoor) temperature. (See Figure 1 and Procedure and Schedule, below.)

Control. Experience suggested continuous low temperature storage with maximum seed-water conservation as being the condition most likely to sustain *Magnolia* embryo viability for maximum duration. This was regarded as the control condition and the single unit fulfilling that condition (Unit 5 of Group A) was regarded as the control unit, that is, the unit serving as a reference to which all other units, as test units, could be related.

Procedure and Schedule (Figure 1). The starting date (October 20) was the earliest practical date on which the required number of seeds of appropriate size could be assembled. (*M. macrophylla* matures in northern Indiana and releases its seeds from late September through mid-October. Only the largest seeds were accepted and a sorting procedure was followed in an attempt to provide seed-size equivalency of all test units; procedural details were included in the protocol. Prior to October 20, the outer coats were removed from over 200 seeds; from these, 175 were selected for the fifth test units of the seven groups and held refrigerated pending a starting date. The balance of the seeds,, with coats intact, were air-dried (not exposed to sun or other heat greater than that of indoor air) to a stage of coat dehydration judged to permit their enclosure in plastic containers without risk of coat degeneration from fungal or autolytic action. Subsequently, half of these dried seeds were immersed in water only long enough to facilitate removal of the outer coat; the other half retained the dried outer coat. From these, portions were selected for test Units 1, 2, 3 and 4 of the seven groups. Because intact seed coats made uncertain the size of the seeds enclosed, an additional ten seeds with coats were added to each of Units 3 and 4, the ten smallest being removed from each unit after coat removal made size judgment possible (March 19 and April 18).

On October 20, the above preliminary steps having been made, the seeds were apportioned, packaged, and sealed from any possible insect infestation that might destroy the integrity of the seed coat. On that date, all units of Group A were placed in the refrigerator; Groups B through G were positioned in a room corner for maximum and uniform exposure to the test conditions. At the end of the first 30-day period, all test units of Group B were transferred from the room atmosphere (test conditions) to refrigeration; at the end of 60 days, Group C went into refrigeration; this procedure continued until each test group's assigned exposure period was

terminated by removal to refrigeration.

On the 150th day (March 19), all units of dry-stored seeds (except those of Group G) were introduced to moist storage. This involved brief soaking and removal of the dehydrated outer coats from all seeds of Units 3 and 4, the transfer of all seeds of Units 1 and 3 from their paper storage bags to plastic bags, and the introduction into each of Units 1, 2, 3, and 4 of each test group (a total of 28 units) moist sphagnum moss equivalent to that in Unit 5. It was carried out with care not to mix or mislabel units during coat removal and transfer — eventually requiring three evenings to complete. Group G, given a 180-day test exposure, was similarly processed on the 180th day (April 18). No antifungal agent was applied in moist storage; neither was there any reason to apply an antifungal agent to denuded seeds dry-stored (Units 1 and 2). However, in the case of dry-stored seeds bearing dehydrated outer coats, it seemed prudent to apply a pinch of Benomyl to each of Units 3 and 4. No fungal growth was visible during the study nor was there evidence of any germination failure attributable to fungal action.

Experience has indicated that an adequate germination-conditioning period for *Magnolia* (low temperature with optimum moisture and oxygen availability) is eight weeks. Therefore, on the 56th day following water introduction to dry seeds, all seeds were removed from refrigeration to germination-inducing (indoor room) temperature (May 14, except for Group G, June 13).

Germination Monitoring and Recording. After cold-release, all test units were carefully monitored for germination. Germination was regarded solely as the appearance and growth of the radicle; the mere expansion of the endosperm and separation of the hard seed coat halves without actual appearance of a viable hypocotyl was not regarded as germination.

Figure 1 depicts the study structure and bears at the right edge a record of the final tally from each test unit. A tally sheet was designed for recording the counts made on each test unit on each of the five to eight examinations at 5- or 6-day intervals; all of these original tally records and notes are retained. When no further germination occurred in two consecutive examinations, a unit was terminated, although all units were examined through the 35th day (5 weeks).

Figure 2 depicts results from each of the five test treatments as applied to the seven test groups, plotting the germination in percentage (left margin) against the treatment variance (labeled by Group letter, top margin).

Performance Observations. The most serious known aberration in performance was with Unit 5 of Group G, the seeds of which were accidentally left exposed and which dried for several hours during the second examination. Although a few of those dried seeds subsequently germinated after return to moisture, the results from the unit had to be dismissed and do not appear on Figure 2.

The remarkable depression in the response record at the 60-day exposure level (Figure 2, Group C, Treatment 5, but reflected in Treatments 1 and 3) has no known explanation; a slight delay is recalled during processing Group C at the point of water-introduction and transfer (March 19 or 20), but no event in the group's treatment would seem to account for its results being so out of line with Groups B and D. A re-run of Group C is planned for 1981-1982, if time permits.

Finally, Unit A-5, which was expected to provide a control 100 percent germination, disappointed the study when the 25th seed, after

expanding and splitting its coat, failed to sprout and decomposed.

Results and Interpretations (Figures 1 and 2). The results clearly demonstrate the importance of seed-water conservation for viability retention: Germination following Treatment 5 was consistently higher than that following Treatments 1 through 4 in all test groups, A through F. Conversely, germination following Treatments 1 and 2 was, in general, lower than that following Treatments 3 through 5. (See Figure 2).

Collective experience with *Magnolia* seed storage would have permitted accurate prediction — in principle if not degree — of this particular aspect of the results. The following results might not have been so confidently predicted.

Dry storage with the oily outer seed coat intact appears to have permitted a slightly higher rate of survival than dry storage with the coat removed: Germination following Treatments 3 and 4 was, in general, higher than that following Treatments 1 and 2.

For dry storage, the water-conserving nature of a thin plastic container (an ordinary food-storage bag) would seem to offer no or little survival advantage over storage in paper: The difference in germination between Treatments 1 and 2 and between 3 and 4 is not significant. It should be recognized, however, that a container of heavier plastic might have been more effective; this plastic was selected for testing because of some apprehension that a very thickwalled container might excessively inhibit gas exchange through the walls.

Dry storage, with no provision for water conservation and at ambient indoor heat, permitted survival of a small proportion of embryos, even after six months: None of the test groups exhibited 100 percent death with Treatment 1; germination following Treatment 1 was nearly 50 percent after 4 months (Group E) and even after 6 months exposure (Group G) was over 30 percent. This result was unexpected. The 1979-1980 dry storage, which Treatment 1 approximates, appeared to result in total germination failure and had led to the expectation that Treatment 1 would exhibit 100 percent kill at some point prior to a 6-month exposure. Thus, the earlier opinion about the 1979-1980 germination failure was invalid and requires another explanation.

Storage at low temperatures and providing the greatest insurance against seed-water loss appears not to insure 100 percent germination: Germination following Treatment 5 was less than 100 percent in all groups, despite selection of the largest and apparently most viable seeds for trial.

Nothing of the results suggests significant detriment from storage at indoor room temperatures (18°-22° C: 65°-72° F), as opposed to refrigerator storage (2° -4° C: 35° -40° F): Contrary to expectations, germination proportions did not greatly suffer from increased duration of exposure to indoor temperature. Treatment 5 best illustrates this point, because the higher temperature was applied as an independent factor in that treatment: Although survival decreased slightly after 30-day exposure (from 96 percent of Unit A-5 to 84 percent of Unit B-5), that initial decrease did not progress. Even after 150-day exposure (Unit F-5), 80 percent of the embryos survived. Furthermore, however erratic the results from the other four treatments appear in Figure 2, it must be noted that in each case the percentage of germination after the longest exposures (Groups F and G) was not considerably less than that after the more brief exposures (Groups A and B), paralleling results of Treatment 5.

Results from Treatment 5 reflect the

importance of a period of cold exposure as embryo-conditioning of *Magnolia* seed for germination: With the exception of Unit A-5, all units of this treatment (B-5 through G-5) were maintained moist and warm for six different periods (30 days through 180 days), conditions ultimately favorable for germination. Yet, in none of these units was there any evidence of germination on the date the unit was removed from room temperature and placed under refrigeration. Germination occurred only after the chilling period.

The adequacy of duration of the cold-conditioning treatment was not included as a test factor in this study. The 56-day period was selected somewhat arbitrarily but was based on experience and applied as a constant. Nevertheless, that period was varied by coincidence in Treatment 5: The range within Treatment 5 included the minimum 56 days (Group F) through 206 days (Group A). Comparing the results from Group F (80 percent) to results from Groups B (84 percent) and A (96 percent), it would not appear that chilling periods longer than 56 days offer significant advantage, that is, the period was not too brief.

One further temperature-related factor ought to be mentioned here. With respect to *M. macrophylla*, it is not clear whether there is a period after seed release from the fruit when it is physiologically refractory to the effect of cold in bringing about the changes conducive to germination. If there is no refractory period, introduction to cold would permit bringing the seed to germination eight weeks or less after collection — disregarding practicality in doing so. Any existing refractoriness and its duration could be explored by a study no more complex than the present one.

The final observation is about germination promptness/delay following cold release, of some interest because an optimum treatment would probably reduce variance in germination times within a seed group. The present trials were not designed to explore this aspect, but the results invite these observations. (The data used were derived from the tally sheets and are not shown in either Figures 1 or 2.) Most germination initiation following cold release occurred as a germination burst between the 6th and 7th days. A total of 321 seeds germinated with this degree of promptness, approximately 71 percent of the 452 total that germinated. No germination initiation occurred sooner in these trials. In most units, no germination took place after 35 days. However, if any single germinating seed were observed in a unit near or at the end of the 35-day period, that unit was retained for at least one further period before its termination. Of seeds germinating after the 6th to 11th days, the extreme was represented in separate units: In Unit A-2, one seed germinated between the 35th and 42nd days; in Unit G-1, one germinated between the 38th and 55th days. The distribution of the late-germinating seeds among the test units was interesting. The fewest occurred with Treatment 5 (14 seeds). With Treatments 3 and 4, there were 19 and 24, respectively. The greatest number occurred with Treatments 1 and 2 (36 and 38, respectively). This suggests that a larger proportion of seeds stored dry (particularly in the case of denuded seeds, Treatments 1 and 2) consistently respond more slowly than seeds stored with maximum water conservation (Treatment 5). This could easily be determined by further trials.

Recommendations. The results of this *Magnolia* seed study would seem to support the following recommendations for storage:

1. *Maturity*. Seeds should be

collected only when fully matured.

2. *Seed Coat.* Because only moist storage offers maximum viability retention, the soft outer seed coat should be removed after collection. The coat's degradation in moist storage is inconvenient or a detriment. Its removal also permits recognition of any seeds that are conspicuously abnormal (exceptionally thin or small) and unlikely to contain viable embryos.

3. *Moisture.* After collection and cleaning, seeds should be promptly deposited in a moist storage material (or planted in a prepared outside bed, if not to be stored indoors). Damp, coarse, unmilled sphagnum moss held in a thin plastic bag, with the seeds well distributed throughout, has been found satisfactory.

4. *Temperature.* The evidence at hand suggests that seeds in moist storage suffer no detriment if held at ordinary indoor temperatures for several weeks. Accordingly, there seems little advantage to refrigerator storage up to a practical date of initiating the required germination-conditioning chilling period. Moist storage at room temperature (i.e., postponement of the germination-conditioning treatment) could be a distinct advantage when the seeds are intended for distribution. The final recipient would know what treatment the seeds had received and, therefore, the treatment to be given by him at his convenience: refrigeration for several weeks. Not knowing this prior treatment places a recipient at a disadvantage; a wrong assumption may cost him loss of some or all of the seeds. One further factor: Moist storage at refrigerator temperatures is tantamount to administering cold-conditioning for germination. Its interruption after several weeks, to package and ship the seeds in their moist packing, presents the real possibility of germination beginning enroute to the recipient and the risk that the delicate sprouts will be ruined in transit.

5. *Germination Conditioning by Cold.* This should be viewed as distinct from mere storage at low temperatures. Evidence suggests that the physiological changes the embryos undergo permitting dormancy emergence are dependent on or at least influenced by chilling. Thus, if moist-stored seeds have not been refrigerated, they should be introduced to refrigeration on a date approximately two months prior to the date most practical for their germination.

6. *Dry Storage.* Seeds stored dry for several weeks prior to the conditioning treatment described above are apt to yield much lower rates of germination than seeds stored moist immediately following collection and cleaning. Because of the difficulty of storing and packaging *Magnolia* seeds to give reasonable assurance of viability, they have not been offered frequently by commercial retailers of shrub and tree seeds; purchases of such seeds have frequently been disappointing.

7. *A.M.S. Seed Counter.* The American Magnolia Society attempts to accomplish what is probably impractical for commercial seed suppliers, making available to its members seeds collected, stored, packaged, and shipped with the expertise of its members, giving reasonable assurance of viability. Here a certain responsibility is borne by seed suppliers at one end and by recipients at the other. Seeds should be supplied to the program with assurance that they are the current year's seeds (or with the stipulation of any greater age). Unless submitted promptly following collection and cleaning, they should be stored moist, but not refrigerated until transfer to the program is convenient. Regardless of how stored, the seeds should be accompanied by a brief note of storage history (dry or moist, room temperature or refrigeration).