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# THE 120-YR PERIOD FOR DR. BEAL'S SEED VIABILITY EXPERIMENT[1]

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After 120 yr of burial in moist, well-aerated sand, 23 seeds of *Verbascum blattaria* and two seeds of a *Verbascum* sp. germinated and produced normal plants (50% germination for *Verbascum*). After a 6-wk cold treatment, a single seed of *Malva rotundifolia* germinated also, producing a normal plant (2% germination). Plants were grown to maturity in a greenhouse, and flowering was induced by exposure to a 6-wk cold treatment. Flowers were artificially pollinated to produce seed of both *Verbascum blattaria* and *Malva rotundifolia*. The *Verbascum* sp. failed to set seed. Collected seeds were subsequently germinated, producing normal plants. $F_1$ seeds of *V. blattaria* had a germination of 64%. Seeds (6%) of *M. rotundifolia* germinated after a cold treatment.

**Key words:** germination; *Malva*; seed viability; *Verbascum*; W. J. Beal.

The spring of 2000 marked the 120-yr period for the seed viability experiment initiated by Dr. William James Beal, then professor of botany and forestry at Michigan Agricultural College in East Lansing, Michigan, USA. Professor Beal initiated this study in the autumn of 1879 ''with the view of learning something more in regard to the length of time seeds of some of our most common plants would remain dormant in the soil and yet germinate when exposed to favorable conditions'' (Beal, 1886 [p. 14], 1905 [p. 140]). The design of the experiment is best described by Dr. Beal (1886, p. 14): ''I selected fifty freshly grown seeds of each of twenty-three different kinds of plants. Twenty such lots were prepared with the view of testing them at different times in the future. Each lot or set of seeds was well mixed in moderately moist sand, just as it was taken from three feet below the surface, where the land had never been plowed. The seeds of each set were well mixed with the sand and placed in a pint bottle, the bottle being filled and left uncorked and placed with the mouth slanting downward so that water could not accumulate about the seeds. These bottles were buried on a sandy knoll in a row running east and west.'' Only 21 species were included in the bottles (Table 1). Although included in the study, seeds of *Quercus velutina* (Beal, 1889), later reported as *Quercus rubra* (Beal, 1894, 1905), and *Juglans nigra* (Beal, 1889) were buried in the sand next to the bottles. Beal reported all buried acorns and nuts had decayed early in the study (Beal, 1889, 1905).

It was Dr. Beal's original intent to excavate a bottle of seeds in the autumn of every fifth year and to test for germination. The 5-yr cycle was maintained for the first 40 yr of the experiment (Beal, 1885, 1886, 1889, 1894, 1905, 1911; Darlington, 1915). The fall of 1919 was marked by an early hard frost, which prevented Dr. Darlington, who took over the experiment after Dr. Beal retired from Michigan Agricultural College in 1910, from excavating a bottle. The 40th-yr bottle was extracted in the spring of 1920 (Darlington, 1922), and all subsequent excavations have been conducted in the spring. In 1920, Dr. Darlington extended the intervals for testing to 10 yr (Darlington, 1931, 1941, 1951; Darlington and Steinbauer, 1961; Kivilaan and Bandurski, 1973, 1981). In 1990, Dr. Gustaaf de Zoeten (then Chair) and the faculty of the Department of Botany and Plant Pathology decided to extend the period to 20 yr. The 15th bottle was recovered for testing on 22 April 2000, leaving five more bottles for future testing.

## MATERIALS AND METHODS

The moist sand-seed mixture was removed from the exhumed bottle after excavation and immediately placed, without washing or sieving, into a 36 × 29 cm plastic tray containing 5 cm sterilized 1 : 1 : 1 perlite : vermiculite : Baccto (Michigan Peat, Houston, Texas, USA) soil mix. The sand mix was spread out evenly over the soil surface and lightly watered, replicating the method used in previous studies (Darlington and Steinbauer, 1961; Kivilaan and Bandurski, 1973, 1981). A second tray containing only sterile soil served as a control for the absence of viable seeds in the treated soil substratum. A third tray was planted with seed of *Verbascum thapsus*, derived from plants that germinated in the 100-yr period experiment and stored under refrigeration, for the purpose of seedling identification. All three trays were covered with a layer of cellophane wrap and placed into an EGC model M-96 growth chamber (EGC, Chagrin Falls, Ohio, USA) with a 9-h, 23°C day and a 15-h, 20°C temperature night and 60% relative humidity. Watering was done as frequently as required to keep a moist soil surface.

Seedlings were transplanted to 325-mL plastic pots, and the sand mixture was gently mixed to expose more of the substrate to light. After 102 d in the growth chamber and no subsequent germinations, the trays were given an 8-wk cold treatment under dark conditions at 4°C.

All plants were transplanted into 3.4-L pots and transferred to a greenhouse (16 h photoperiod, 23°C day and 20°C night temperature). In the autumn of 2000, three *Verbascum blattaria* (accession #20000523) were planted in the W. J. Beal Botanical Garden. The remaining *Verbascum* plants were divided into two groups of ten *V. blattaria* and one of the *Verbascum* sp. plants in order to minimize the risk of all plants dying during the cold treatment required to induce flowering of this biennial species. The first group was given an 8-wk cold treatment at 4°C in weak light. All of the *V. blattaria* survived the cold treatment and began to produce flower stalks shortly after being returned to the greenhouse. The *Verbascum* sp. died. The second group of ten

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TABLE 1. Viability of buried seeds in the Beal buried-seed experiment; results of all tests to date.

| Name of species tested | Perination | 5th year 1884 | 10th year 1889 | 15th year 1894 | 20th year 1899 | 25th year 1904 | 30th year 1909 | 35th year 1914 | 40th year 1920 | 50th year 1930 | 60th year 1940 | 70th year 1950 | 80th year 1960 | 90th year 1970 | 100th year 1980 | 120th year 2000 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Agrostemma githago* | Annual | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Amaranthus retroflexus* | Annual | 21 (42) | +[a] | 1 (2) | 4 (8) | + | + | + | 1 (2)<br>33 (66)[b] | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ambrosia artemisifolia* | Annual | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 (4) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Anthemis cotula* | Annual | 26 (52) | + | 5 (10) | 0 | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Brassica nigra* | Annual | 0 | + | 4 (8) | 9 (18) | + | + | + | 19 (38) | 4 (8) | 0 | 0 | 0 | 0 | 0 | 0 |
| *Bromus secalinus* | Annual | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Capsella bursa-pastoris* | Annual | 50 (100) | + | 21 (42) | 21 (42) | + | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Erechtites hieracifolia* | Annual | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Euphorbia maculata* | Annual | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Lepidium virginicum* | Annual | 47 (94) | + | 17 (34) | 29 (58) | + | + | + | 1 (2) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Malva rotundifolia* | Annual or Biennial | 1 (2) | 0 | 0 | 3 (6) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (2) | 1 (2) |
| *Plantago major* | Perennial | 0 | 0 | 1 (2) | 0 | 0 | 0 | 0 | 5 (10) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Polygonum hydropiper* | Annual | 3 (6) | + | 4 (8) | 1 (2) | + | ? | 0 | 0 | 2 (4) | 0 | 0 | 0 | 0 | 0 | 0 |
| *Portulaca oleracea* | Annual | 19 (38) | + | 9 (18) | 7 (14) | + | 0 | 0 | 1 (2) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Setaria glauca* | Annual | 34 (68) | + | + | 0 | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Stellaria media* | Annual | 36 (72) | + | 3 (6) | 3 (6) | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Trifolium repens* | Perennial | 2 (4) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Verbascum thapsus*[c] | Biennial | 42 (84) | ? | 18 (36) | 16 (32) | 0 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 1 (2) | 0 |
| *Oenothera biennis* | Biennial | 41 (82) | + | 6 (12) | 14 (28) | + | + | 0 | 19 (38) | 19 (38) | 12 (24) | 7 (14) | 5 (10) | 0 | 0 | 0 |
| *Rumex crispus* | Biennial | 45 (90) | ? | 7 (14) | 8 (16) | + | + | + | 9 (18) | 26 (52) | 2 (4) | 4 (8) | 1 (2) | 0 | 0 | 0 |
| *Thuja occidentalis* | Perennial | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Verbascum blattaria*[c] | Biennial | | | | | | | | | 31 (62) | 34 (68) | 37 (74) | 35 (70) | 10 (20) | 21 (42) | 23 (46) |
| *Verbascum* sp. | Biennial | | | | | | | | | | | | | | | 2 (4) |

[a] The + signs following each species indicate that one or more seeds of that species germinated for the year shown. The number indicates the number of seeds germinating, while the number in parenthesis indicates the percentage germinating.

[b] This second set of numbers represents the germination of what Darlington (1922) reported to be a second species of *Amaranthus* to appear in the Beal study. *A. graecizans*. This is an unrecognized name that has been misapplied to both *A. blitoides* and *A. albus* (Gleason and Cronquist, 1991).

[c] There is some question concerning the identification of Verbascum plants in the early period (1884–1920) as *V. thapsus* rather than *V. blattaria* (Darlington and Steinbauer, 1961).

*V. blattaria* and the remaining *Verbascum* sp. were given the 8-wk cold treatment, with all treated plants surviving and maturing to produce flower stalks. Flowers of *V. blattaria*, *Verbascum* sp., and *M. rotundifolia* were pollinated by hand in the greenhouse. Plants of *V. blattaria* were both cross-pollinated and selfed, whereas only selfing could be conducted on the single plants of *M. rotundifolia* and *Verbascum* sp. Seeds produced by controlled pollination were tested for viability.

## RESULTS

Seedlings emerged in the tray containing the 120-yr-old seed-sand mixture after 7 d and seeds continued to germinate over the next 39 d. Initially, 24 seeds germinated, producing cotyledons and young foliage characteristic of *Verbascum*. After mixing of the sand-seed mixture, one additional seedling appeared to yield a total of 25 seedlings. After the cold treatment, the trays were returned to the growth chamber. After 23 d a single seedling, which was different from all previous seedlings, emerged and produced foliage characteristic of *Malva*. No seedlings emerged in the unplanted tray containing only the sterilized soil mix.

After flowering, the plants were positively identified as: 23 *Verbascum blattaria*, 2 *Verbascum* sp., 1 *Malva rotundifolia*. The two *Verbascum* sp. plants had identical mature foliage. The leaves of the rosette were oblong, crenate, and moderately pubescent, with a mixture of both branched and glandular hairs, rarely with an occasional stellate hair. These characters were intermediate between *V. blattaria* and *V. thapsus*, making positive identification without flowers impossible. Even after flowering was induced in the remaining specimen, identification of the unknown *Verbascum* sp. was not possible. The flower stalk and floral arrangement of the *Verbascum* sp. were also intermediate to both *V. blattaria* and *V. thapsus* and did not key out to any other known *Verbascum* species. Cauline leaves were oblong, crenate, and moderately pubescent, with a mixture of both branched and glandular hairs, rarely with an occasional stellate hair. Upper cauline leaves were decurrent, with a narrow wing running to the next lower leaf node. Only one yellow flower similar to *V. blattaria* was borne in the axil of each bract. We suggest the two plants represent a putative hybrid between *V. blattaria* and *V. thapsus*. A voucher specimen (Telewski 521a) has been deposited in the Michigan State University Herbarium (MSC). The results of this study together with those of prior years are summarized in Table 1.

All crossed and selfed flowers of *V. blattaria* grown in the greenhouse and in the botanical garden yielded viable seeds. Collected seeds were subsequently germinated, producing normal plants. $F_1$ seeds of *V. blattaria* (accession #20010138) germinated without any treatment with an average germination of 64%. The *Verbascum* sp. failed to produce seeds after artificial self-pollination. Seeds produced by selfing of the single plant of *M. rotundifolia* (accession #20010331) required a cold treatment before germinating (6% germination rate). All remaining plants germinating from the sand-seed mixture extracted from the bottle representing *V. blattaria* (accession #20000523) and *M. rotundifolia* (accession #20010330) were transplanted in the W. J. Beal Botanical Garden during the spring of 2001.

## DISCUSSION

The present germination results are in general agreement with those of the 100-yr period. *Verbascum blattaria* continues to exhibit the greatest viability after long-term burial. The total number of *V. blattaria* in the present study (23) is slightly greater than the 21 reported by Kivilaan and Bandurski (1981) and could mean a plateau has been established after a period of slowly declining viability within the population of seeds. It will be interesting to see whether this plateau in viability is maintained in subsequent tests or whether a decline in viability resumes over time. Also in agreement with the 100-yr period was the germination of a single *Malva rotundifolia*, although the germination of this species in the present study did require a cold treatment after the initial germination period. *Malva* only germinated in three previous periods and it is important to note Dr. Beal's observation of 1885 with regard to this species. Only one seed of *Malva* germinated after 5 yr of burial, not requiring a cold treatment. With regard to *Malva*, Beal reported ''many empty seed-coats were seen'' in the sand mix (Beal, 1885, p. 45). This may imply that the majority of *Malva* seeds never survived beyond the first test period or possibly the seeds were devoid of embryos when they were placed in the bottle. Unfortunately, there is no indication that Beal established a base-line germination percentage for the seeds before placing the bottles in the ground. Low germination in *Malva* may be the result of poor seed set rather than loss of long-term viability.

Of interest in this study was the germination of two plants, which were tentatively identified here as a putative hybrid between *V. blattaria* and *V. thapsus*. As Kivilaan and Bandurski (1981, p. 1292) commented regarding the presence of both *V. blattaria* and *V. thapsus* (Table 1) in the Beal Seed Viability Experiment ''Although only conjecture is possible, the seeds of *V. blattaria* and *V. thapsus* appear identical to the naked eye, although the adult plants are strikingly different, and Dr. Beal could have used two seed samples, one containing *V. thapsus* and one containing *V. blattaria* to fill the 20 bottles.'' The results of the present study now indicate a hybrid *Verbascum* is also present in this study.

Although all previously published reports on this study have reported seeds germinating from the original seed mixture, no data have been published on the ability of these plants to produce viable seed. Seed of *V. thapsus* used in the present experiment (120-yr period) to verify seedling identification was produced on the original *V. thapsus* plant that germinated in the 100-yr period experiment (J. Taylor, Department of Horticulture, Michigan State University, East Lansing, Michigan, USA, personal communication). The controlled pollination experiments and subsequent germination tests of the $F_1$ seed clearly indicate that both *V. blattaria* and *M. rotundifolia* can produce normal, viable seeds after remaining dormant for 120 yr.

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