Propagation of Rare Plants from Historic Seed Collections: Implications for Species Restoration and Herbarium Management

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Abstract

Herbaria are potentially important repositories of living seeds that could be useful for recovery of rare plant species. To examine this capacity, we tested seed germination of rare milkweed (*Asclepias*) and milkvetch (*Astragalus*) species representing different collection dates and different herbaria. These groups have contrasting seed characteristics, with greater potential for longevity in the nonpermeable hard-coated milkvetch seeds. Twelve-year-old *Asclepias lanuginosa* seeds failed to germinate. However, we achieved 45% germination from three-year-old *Asclepias meadii* seeds, but germination dropped to 0% after ages of four to five years. *Astragalus neglectus* seeds germinated from 97-, 48-, and 28-year-old herbarium specimens, and *Astragalus tennesseensis* seeds germinated from a four-year-old collection. Seedlings produced from these experiments were incorporated into *ex situ* garden populations for recovery or restoration of rare species populations. Different herbarium pest control techniques may have significant bearing on the viability of seeds stored on herbarium specimens. Microwaving can cause precipitous loss of seed viability, while deep-freezing appears to allow some seeds to remain viable. Potentially live seeds of rare species should be stored under conditions that enhance their long-term viability.

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Introduction

Herbaria can be managed not only as reservoirs of morphological and chemical data, and active enzyme and DNA material (see Ranker & Werth 1986), but also as repositories of live seeds or spores with great biosystematics potential (see Windham & Haufler 1986). Given the current endangerment and accelerated loss of vascular plant species (Falk 1987), herbarium plant collections are also valuable but nonrenewable seed resources for actual recovery of a potentially vast array of living plant species. For example, seed viability extends from 15 to 100 years in at least 12 vascular plant families (Ewart 1908), and seed longevity is estimated beyond a century for over 20 plant species, including the well-known lotus, *Nelumbo nucifera* (Roos 1986). Seeds from herbarium sheets have been germinated for chromosome studies of *Malvastrum* collected up to 46 years earlier (Hill 1983), for *Penstemon* collected up to 22 years earlier (Freeman 1983), and for studies of *Astragalus* (see Head 1957). Similarly, nearly 100-year-old fern spores have been found viable (Johnson 1985), and specimens of algae have been revived after 87 years (Lipman 1941).

The Morton Arboretum Rare Plant Program has propagated rare species from herbarium seed collections, and has incorporated these plants into living *ex situ* collections for conservation and recovery purposes. Here we summarize the sources, ages, and management of collections from which we obtained seeds, and we present the results of our propagation efforts. We used seeds from four rare species among two genera having contrasting seed characteristics. North temperature milkweeds (*Asclepias*, asclepiadaceae) are herbaceous perennial herbs with thin-coated permeable seeds that appear short-lived but are capable of long-distance wind dispersal by their attached comas (Woodson 1954). Most northern temperature milkvetches (*Astragalus*, leguminosae) are perennial herbs (Barneby 1964) with hard-coated nonpermeable seeds that have no apparent means of dispersal but have high potential for persistence in local seed banks. The mature legumes (fruits) of this group are often critical for species identification (Fernald 1950; Welsh 1960) and are thus maintained in herbaria.

Methods

Species and Seed Sources. *Asclepias lanuginosa* Nutt. (woolly milkweed) is a rare milkweed of the Great

Plains, extending eastward in dry-gravel prairies to Illinois, Wisconsin, and Iowa (Woodson 1954). Seed pods are rarely produced (but see Sorensen 1984), and are usually solitary, with 39 (±1.0 s.d.) seeds per pod (Betz 1992). A single sample from the Northern Illinois University Herbarium (DEK) was tested for germination in 1990. It consisted of seven seeds that were part of a larger collection made on August 23, 1976 (Table 1). This collection was air-dried and maintained separately from herbarium sheets under normal room temperatures.

Asclepias meadii Torr. (Mead's milkweed) is a federally listed threatened milkweed restricted to pristine prairies or haymeadows in Missouri, eastern Kansas, Iowa, and Illinois (Fig. 1); it has been extirpated from Wisconsin and Indiana (Harrison 1988). The small eastern populations rarely produce seeds, while most western populations are harvested during hay mowing, preventing seed maturation and seed dispersal (Betz 1989). Seed pods are rarely produced and are usually solitary, with 59.6 (±22.9 s.d.) seeds per pod (Betz 1992). In 1990, a sample of 44 seeds was tested for germination from three herbarium specimens collected in 1987 and maintained at the R. L. McGregor Herbarium (KANU), University of Kansas (Table 1). In 1992, 52 seeds were tested from four KANU collections, including two 1987 specimens (25 seeds) and two 1988 specimens (27 seeds) (Table 1). The August 10, 1987, KANU specimen had two pods, which were alternatively sampled in 1990 and 1992. KANU collections are dried with low-wattage driers, mounted, and stored without further treatment in cabinets under normal conditions.

Astragalus neglectus (T. & G.) Sheldon (Cooper's milkvetch) is a rare northern milkvetch of calcareous prairies, riverbanks, and exposed shale slopes (Welsh 1960); its range extends from North Dakota and Manitoba east through Ontario to New York, and south locally to southern Wisconsin, Ohio, and West Virginia (Barneby 1964). Samples ranging from 2 to 36

Table 1. Sources, ages, and germination of seeds sampled from herbarium collections.

Species	*Herbarium and Collection Date*	*Seed age yrs/Date of Germination*	*Germination Success*
Asclepias lanuginosa	DEK 23 Aug 1976	12/1989	0/7 (0%)
Asclepias meadii	KANU 10 Aug 1987	3/1990	9/15 (60%)
	23 Aug 1987	3/1990	6/23 (26.1%)
	9 Sep 1987	3/1990	5/6 (83%)
	14 Jul 1987	5/1992	0/15 (0%)
	10 Aug 1987	5/1992	0/10 (0%)
	10 Jul 1988	4/1992	0/11 (0%)
	13 Jul 1988	4/1992	0/16 (0%)
Astragalus neglectus	F 1868	121/1989	(−)
	14 Aug 1978	111/1989	(−)
	Jul 1884	105/1989	(−)
	18 Jul 1911	78/1989	(−)
	25 Jul 1933	56/1989	(−)
	7 Jul 1936	53/1989	(−)
Astragalus neglectus	NYS 1884	105/1989	(−)
	1 Sep 1926	63/1989	(−)
	7 Sep 1931	58/1989	(−)
	10 Aug 1941	48/1989	(−)
Astragalus neglectus	WIS Aug 1882	97/1989	(+)
	2 Aug 1938	51/1989	(−)
	27 Sep 1941	48/1989	(+)
	15 Aug 1947	42/1989	(−)
	29 Jul 1949	40/1989	(−)
	1 Aug 1961	28/1989	(+)
Astragalus tennesseensis	M Sep 1982	4/1986	(+)

Germination success is indicated for each *Asclepias* collection by viable/total seed number and percent germination; for each *Astragalus* collection, successful (+) or unsuccessful (−) germinations are indicated, but seed numbers are not quantified (see text). Species are ordered by herbarium source; abbreviations follow Holmgren et al. (1990): DEK = Northern Illinois University, F = Field Museum, KANU = Kansas Biological Survey, M = personal collection at Morton Arboretum, NYS = New York State Museum, WIS = University of Wisconsin at Madison.

Figure 1. Flowering ramets of Mead's milkweed (*Asclepias meadii*) in a Kansas virgin prairie. Photographed June 1992.

seeds were obtained from 16 specimens collected between 1868 and 1961 and housed at the New York State Museum (NYS), Chicago's Field Museum of Natural History (F), and the University of Wisconsin (WIS) (Table 1). These seeds were tested for germination in 1989. Because of the small samples, only a few seeds were tested from each source; remaining seeds have been stored under normal room temperatures at the Morton Arboretum. Older collections have an unknown maintenance history; but WIS specimens most recently have been protected from herbarium pests by deep-freezing.

Astragalus tennesseensis Gray (Tennessee milkvetch) is a rare southern and midwestern milkvetch of limestone cedar glades in Tennessee and Alabama and gravel prairies in northern Illinois and Indiana (Baskin et al. 1972; Webb et al. 1992). This species is short-lived and develops a seed bank (Baskin & Baskin 1989). It has been extirpated from northern Illinois and Indiana and is under consideration for federal listing. Seeds were obtained from a ramet collected in 1982 by the first author from the single Illinois population, and were tested for germination in 1986. The specimen was air-dried, unmounted, and stored under normal room temperatures.

Seed Germination and Propagation. *Asclepias* seeds do not require scarification. They were moist stratified on wet filter paper in Petri dishes at 5°C for ten weeks, and they were germinated in a calcareous silt-loam soil mixed with about one-third sand to allow drainage (Betz 1989).

Most *Astragalus* seeds can be germinated directly from herbarium sheets after scarification, although some species require stratification (Head 1957). *A. tennesseensis* seeds have a double seed coat (Baskin & Quarterman 1969) that requires scarification. Because acid treatments can kill seed embryos if over-extended, we scarified seed coats with a blade or file, allowed them to imbibe water for 24 hours, and planted them in a well-drained calcareous soil-mix (Bowles et al. 1988). A similar treatment was applied to the *A. neglectus* seeds.

Results and Discussion

Seed Germination. None of the *Asclepias lanuginosa* seeds germinated. They were apparently mature when collected, and they probably lost viability with age. Twenty (45.45%) of the Kansas *A. meadii* seeds germinated in 1990, which is similar to the 47.6% germination that Betz (1989) found for fresh seeds (Table 1). In 1992, no *A. meadii* seeds successfully germinated. One of the specimens sampled in 1992 had 60% viability in 1989. Therefore, it is possible that seeds of this species can remain viable for at least three years, but that viability drops rapidly thereafter. This conclusion is tentative, however, as the 1992 resample was from a second pod and the other four- and five-year-old seed samples were from specimens collected in July prior to hay mowing and may not have been fully mature.

No *Astragalus neglectus* seeds germinated from the F or NYS samples. However, seeds did germinate from the 1892, 1941, and 1961 WIS specimens, representing 50% of the collections sampled from this herbarium (Table 1). Although moist stratification does not appear necessary for germination, deep-freezing of the WIS seeds might have stimulated their germination, or at least did not cause seed mortality. We also obtained successful germination from the four-year-old *A. tennesseensis* seed collection (Table 1).

Propagation and Establishment of Plants. The surviving *Asclepias meadii* plants have been incorporated into a garden population that is being developed to maintain genetic material from throughout the range of the species. This population is being supplemented with propagation of wild-collected seeds when available, and from tissue culture of Illinois populations that no longer produce seeds. The garden population will be used for recovery of declining or extirpated populations, allowing representation of geographically distinct genetic material while maximizing genetic diversity and reproductive potential.

Astragalus neglectus seedlings were difficult to propagate, and the 1892 seedlings did not survive. However, individual plants from the 1941 and 1961 seed samples survived (Fig. 2) and were successfully established in dolomite prairie habitat at the Morton Arboretum. These plants flowered in 1992; the largest plant produced ten flowering ramets with 3.73 (1.5 s.d.) pods per ramet and 17.3 (4.1 s.d.) seeds per pod. These plants will be used to expand the dolomite prairie population at the Morton Arboretum and eventually for restoration of populations.

The two surviving *Astragalus tennesseensis* plants flowered in 1987, producing 246 seeds and 34 seedlings. This population was supplemented through

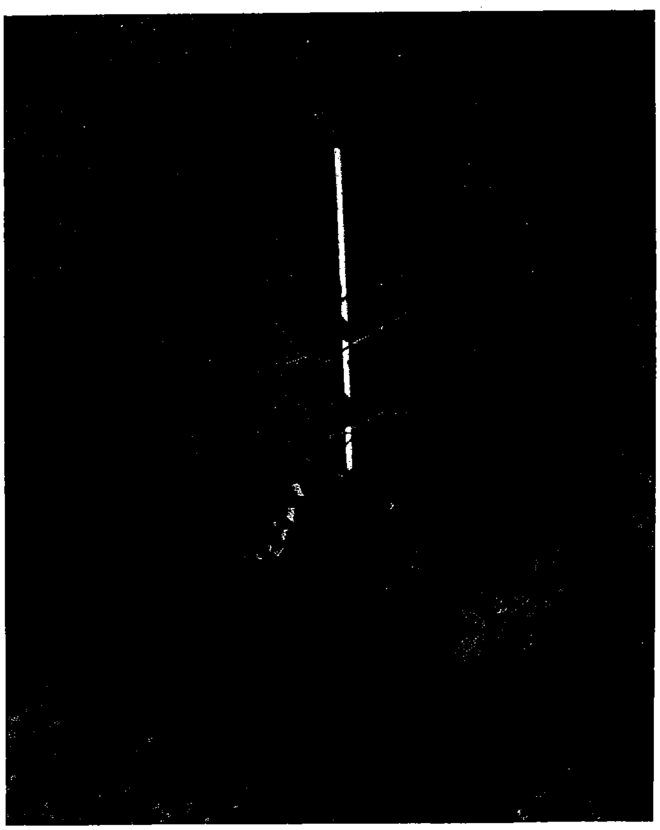

Figure 2. Juvenile Cooper's milkvetch (*Astragalus neglectus*) grown from herbarium seed collection. Photographed August 1991.

Figure 3. Tennessee milkvetch (*Astragalus tennesseensis*) introduced into restored dolomite prairie habitat at the Morton Arboretum. Photographed June 1992.

propagation of wild-collected seeds and stem cuttings, resulting in a total population of 62 plants (Bowles et al. 1988). In 1988, 30 of these plants were randomly selected and established in six restoration plots at the Wea Gravel Prairie, Tippecanoe County, Indiana, a former station for this species. In 1989, 24 (80%) of the planted cohort survived and flowered, producing 195 ramets, 191 inflorescences, 441 pods, and a mean of 16.64 seeds per pod (±6.99 s.d.). Although levels of post-dispersal seed predation are unknown, it is likely that thousands of seeds were incorporated in the soil seed bank. The remaining plants were established into a dry-gravel and dolomite prairie constructed at the Morton Arboretum in 1990 (Fig. 3). This *ex situ* population has continued to flower and produce seeds, and will be used as a source for population reintroduction in northeastern Illinois.

Implications

Use of Herbaria as Living Seed Resources. Herbarium collections that include live seeds have significant potential for recovery of rare plant species, including lost genotypes, geographic races, or entire species of plants. For self-incompatible species, or those subject to inbreeding depression, use of herbarium seed collections may be critical for restoring reproduction or genetic diversity in small or restored populations.

Museum collections are nonrenewable resources that require careful management to preserve potentially useful material (Graves & Braun 1992). Thus, the most practical direct use of seed collections for recovery actions would be the establishment of living *ex situ* populations, such as in botanic gardens, from which propagules can be derived. Such plantings should replicate natural conditions to the extent possible to promote plant survival and avoid selection for cultivars. In addition, seed collections will vary in their utility for conservation in relation to the age of the collection and the seed characteristics of individual species. Historic collections of seeds with long-term viability, such as among the Leguminosae, Malvaceae, or Scrophulariaceae, may be useful a century after their collection. In contrast, viability of milkweed seeds may decline to less than 1% after 3–6 years of storage under normal conditions. Scarification and stratification treatments may be important in reducing sample sizes by stimulating germination from ancient seeds, especially those with hard seed coats.

Herbarium Seed Collection Management. Herbarium pests are a major threat to plant collections and require extensive control, especially in southern climates (Hall 1981, 1988). However, use of microwaving, excessive heat treatments, poisons, or fumigants to kill pests also threatens live material in plant collections. Results can include precipitous loss of seed or spore viability, along with potential changes in plant morphology and chemistry (Hill 1983; Bacci et al. 1985; Windham et al. 1986). Effects of microwaving can be especially damaging to seed viability, but they vary with species. Using herbarium specimens, Philbrick (1984) found that less than 10 minutes of microwaving reduced percentage of seed germination of *Podostemum ceratophyllum*, *Abutilon theophrasti*, and *Cassia marilandica* to zero, but that *Verbascum thapsus* seeds maintained over 60% germination after 10 minutes.

For herbaria that microwave or otherwise treat specimens with live propagules, label information as to the time and intensity of the treatment would be helpful (Hill 1983). Optimum treatment would be the establishment of separate collections of rare plant seeds or spores under conditions that would not promote the loss or rapid decline of their viability. For example, seed viability for many species may be extended considerably by reducing seed moisture content to less than 10% and by storing seeds at less than 0°C (Eberhart et al. 1991). Seed samples of extirpated or endangered species could be contributed to special storage programs such as that coordinated by the Center for Plant Conservation (Falk & McMahan 1988), which would help to insure the long-term survival of rare biological material.

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