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Propagation and Transplanting of an Endangered Alpine Species

ROBBINS' CINQUEFOIL

Potentilla robbinsiana (Rosaceae)

| William E Brumback, Doug M Weihrauch, and Kenneth D Kimball |

Robbins' cinquefoil (*Potentilla robbinsiana* Oakes ex Rydb. [Rosaceae]), federally listed as endangered in 1980, is found only at 2 locations, the alpine zones of Mount Washington and Franconia Ridge, New Hampshire, on land administered by the White Mountain National Forest. The goals of the species recovery plan were met, and in 2002, the plant was removed from the list of endangered and threatened plants (USFWS 2002). The recovery plan included the protection and resurgence of the natural population and the establishment and apparent success of outplanted populations. While a few other plant species have been removed from the list, mainly through discovery of new populations in the wild or taxonomic revisions, *P. robbinsiana* is the first plant on the list to recover because the populations increased at both natural and translocation sites to meet the recovery objectives.

ABSTRACT

Robbins' cinquefoil (*Potentilla robbinsiana* Oakes ex Rydb. [Rosaceae]) is best propagated for outplanting on high elevation restoration sites in New Hampshire by treating seeds to increase germination and by outplanting nondormant plants in mid-July. Best seed germination occurred by: 1) treating seeds with gibberelic acid (GA_3) before sowing in spring; or 2) sowing seeds outside in late fall thereby subjecting them to natural freezing and thawing during winter. Both methods produced seedlings, but pretreatment with GA_3 increased germination more than 2X to 86%. First-year survival on the alpine sites was 90% when actively growing transplants were moved from the sea-level nursery and ouplanted in mid-July, rather than 11% when shipped directly in early June from freezer storage. These successful techniques contributed to this plant, placed on the list of endangered and threatened species in 1980 and found only at 2 locations in the alpine zones of Mount Washington and Franconia Ridge, New Hampshire, being removed from the list in 2002. This outplanting technique may be useful for other high-elevation species.

KEY WORDS

recovery, augmentation, seed banking, seed germination, transport survival

NOMENCLATURE

ITIS (2002)

Figure 1. An outplanted *Potentilla robbinsiana* thriving on Franconia Ridge in October 2003.

THE PLANT

Potentilla robbinsiana is a slow-growing perennial herb. Mature plants form a dense 2- to 4-cm (0.8- to 1.5-in) rosette from its hairy, 3-part compound deeply toothed leaves (Figure 1). Plants form a deep central taproot, which may aid in withstanding frost heaving. *Potentilla robbinsiana* flowers soon after snowmelt, from mid-May to mid-June, making it one of the first plants to bloom in the northeastern US alpine zone. Adult plants produce from 1 to 40, 5-petalled yellow flowers on individual stems. The achenes (seeds) mature by late July and separate from the seedhead on dry, windy days. They seldom travel more than 20 cm (8 in) from the parent plant, which limits natural dispersal (Kimball and Paul 1986). Seeds are generally dormant for at least 1 winter, and germination begins the following year during June and July. Although seed viability is generally high, seedling survival is low (Iszard-Crowley and Kimball 1998).

The unique nature and rarity of *P. robbinsiana* has produced a strong interest among botanists since it was discovered in 1824 (Cogbill 1993). It is a geographically narrow endemic, with the main Monroe Flats population reaching more than 14 000 individuals within an area less than 1 ha (2.5 ac). The abundance and density at this location indicates that Monroe Flats contains distinct habitat requirements for *P. robbinsiana.* Monroe Flats, at 1550 m (5085 ft) in elevation, consists of an exposed low dome that is covered with alternating bands of relatively barren small-stoned terraces and thickly vegetated mats. All other known populations of this species are or were less than a few hundred plants.

Potentilla robbinsiana requires self-pollination for achene production (Lee 1986), and seeds are produced through agamospermy (Lee and Greene 1986). A single chromosome count showed an odd number polyploid 2n = 49, which indicated an obligate apomict (Love and Love 1965). Moreover, starch gel electrophoresis of 23 plants from Mount Washington and a single plant from the other natural population on Franconia Ridge showed virtually no isozyme heterozygosity in the species (USFWS 1991). This suggests that both populations are genetically identical and each produces offspring from seeds that are clones of the parent.

Habitat

Daily measurements from the proximate Mount Washington Observatory (2003) show winter temperatures averaging –15 °C (5 °F), a record low of –44 °C (–47 °F), winds regularly averaging above 72 km/h (45 mi/h), and peak gusts over 240 km/h (150 mi/h) each winter. The highest wind speed ever recorded on land, 372 km/h (231 mi/h), was recorded here. These winds keep Monroe Flats mostly snow- and ice-free throughout the winter, exposing the plants to abrasive blowing snow and ice, and desiccating winds. More importantly, the moist, barren soils are susceptible to frost disturbance from freeze-thaw cycles throughout much of the year. In this extreme environment of moderate solifluction (soil movement down slope) and exposed topography, *P. robbinsiana* occupies a narrow niche; it is likely a poor competitor with other species but is able to thrive in a harsh environment where few other species can survive (Cogbill 1987).

Threats

The major threat to the Mount Washington population is not weather but disturbance from hikers using a historic trail that ran through the middle of the habitat, coupled with heavy historical collection of specimens for herbaria (Cogbill 1993). Until the 1970s, only anecdotal accounts of population numbers were recorded in the literature (Cogbill 1993); the first quantitative population count of plants with a rosette diameter greater than 1.4 cm (0.55 in) was 1801 in 1973, 1547 plants of the same size-class were present in 1983; and 4575 in 1999 (USFWS 2002).

RECOVERY EFFORTS

Through a collaboration of the USDI Fish and Wildlife Service (USFWS), the USDA Forest Service, and the Appalachian Mountain Club (AMC), started in the 1980s, hiking trails were relocated, a hiker education program initiated, population studies commenced, and an experimental re-introduction and augmentation program instituted. In the 1990s, the New England Wild Flower Society (NEWFS) brought its propagation expertise to the project. NEWFS received seeds from Mount Washington plants for propagation, collected under federal permit by the AMC who also took the lead on monitoring plants in the wild. A subset of the seeds collected were seed banked in the USDA's National Center for Genetic Resources Preservation in Fort Collins, Colorado, and at NEWFS, as part of the National Collection of Endangered Plants of the Center for Plant Conservation.

The goal of the reintroduction and augmentation program is to repopulate degraded habitat at Monroe Flats and to create outlying satellite populations in case of catastrophic loss of that population. While recovery of the main population on Mount Washington was occurring because of trail relocations, closure of the species critical habitat to the public, and hiker education, early outplanting techniques were largely unsuccessful. Outplanting adult-sized plants in degraded Monroe Flats habitat and at localized sites with characteristics similar to Monroe Flats on Mount Washington and Franconia Ridge was re-initiated in the 1990s. Mortality of the plants in cultivation at near sea level in the nursery at the Garden in the Woods, the botanic garden of NEWFS, was high at times, as environmental conditions differed significantly from the alpine zone, but substantial numbers of adult-sized plants were achieved. Criteria for successful re-introduction back into the alpine habitat included the ability of nursery-grown plants to successfully achieve reproducing progeny after outplanting, without further human assistance.

Seed Collection

Seeds were collected annually by the AMC from the Monroe Flats population when achenes began to fall from mature seed heads (early to mid-July). One to two seed heads were harvested from 25 individual plants that had numerous (10+) flower stalks. Each seed head contained approximately 25 achenes, although late frosts can adversely impact seed development and viability. Seeds were separated from seed heads and counted. Immature or deformed seeds were excluded. Seeds were air dried for a few days and mailed to NEWFS in Framingham, Massachusetts, for storage and propagation.

Upon receipt at NEWFS, in late July or early August, seeds were stored in a sealed container in a refrigerator (about 2 °C [35 °F]) until sowing. Seeds to be stored in a seed bank were dried in a drying cabinet over silica gel at 15% to 20% relative humidity for 1 to 2 mo before packaging into heat-sealed foil envelopes and placement in the seed bank at –20 °C (4 °F) for long-term storage. Long-term seed banking is an effective and economical method of conserving the potential for large numbers of future plants. Seeds of most plant species must be dried to a relatively low moisture content to survive freezing temperatures in a seed bank. Normally seeds that survive the drying process are able to be successfully stored at –20 °C [4 °F]. Therefore, to ensure seed banking as an effective backup to catastrophe in the wild, germination tests on *P. robbinsiana* seeds were periodically performed on fresh, dried, or dried then seed banked (frozen) seeds.

Propagation Methods

Seeds were sown either in small (15.2 cm x 12.7 cm x 7.6 cm [6 in x 5 in x 3 in]) or large (30.5 cm x 20.3 cm x 5.1 cm [12 in x 8 in x 2 in]) flats. The medium varied but usually consisted of a mixture (by volume) of 1 or 2 parts Faffard Mix or MetroMix (a commercial seed germination medium), 2 parts granitic rice stone (for drainage) and 1 part horticultural (coarse) sand (also for drainage). Seed flats were moistened after sowing. This germination medium is typical of medium used for growing alpine plants under nursery conditions at much higher temperatures and humidity than would be encountered in the mountains. Seeds given pretreatment with gibberelic acid (GA_3) were placed on filter paper in a Petri dish in tap water solutions of either 500 or 1000 ppm GA_3 (Carolina Biological Supply, Burlington, North Carolina).

Seed flats were either sown outdoors in the nursery at NEWFS or sown indoors and moved outdoors when conditions in the greenhouse (15 to 18 °C [60 to 65 °F] minimum temperatures) became too warm. Mortality of seedlings under the temperatures and humidity of Framingham was relatively high over summer. Flats and potted plants were kept outside over winter in cold frames, under a protective blanket of Microfoam, with white 6-mil-thick plastic covering the entire frame. Microfoam is a thermal blanket under which seed flats freeze but which mitigates heaving by providing a more constant temperature. The moist conditions imposed by the Microfoam and plastic, however, caused some of the crowns to rot. We mitigated rotting by placing overturned empty plastic flats on top of the seed flats to keep an air space open between the Microfoam and the seed flats.

Seedlings were usually large enough to be individually potted during their second growing season. These transplants were potted either into plastic # 150 pots (24.2 cm^2 [3.7 in^2] base and 12.7 cm [5 in] depth) or in small Nu-pot 25s (16.1 cm^2 [2.5 in^2] base and 8.9 cm [3.5 in] depth). Growth or survival did not appear to be affected by pot size, and since the smaller pots were easier to transport and plant in the field, we eventually used only the smaller pots. Potting mixes were (by volume), 1 part regular soil (normal potting soil), 1 or 2 parts granite stone for drainage, and 1 part coarse sand (also for drainage). We did not detect any difference in germination or survival between seed flat sizes, pot sizes, or soil mixtures used in our experiments.

Germination

Initial tests of seeds in 1991 confirmed that a small proportion of fresh seeds will germinate when sown immediately under growlights and in the warm conditions of a greenhouse. In most subsequent trials, however, we sowed seeds into flats in late fall or early winter and placed the flats outside to receive exposure to the freezing and thawing of winter conditions. Germination after exposure to ambient winter conditions almost always increased. As an example, in 1 trial with seeds collected in July 1995, warm, fresh seeds sown outside in August produced germination rates ranging between 2% to 10%, averaging 6% (107 seeds sown in 12 flats) by October 1995. The same seed lot stored dry in a refrigerator and sown outside in December 1995 germinated the following spring and summer. Germination in this case varied between 3% to 21%, averaging 11% (555 seeds sown in 12 flats). Seed flats were kept outside over winter after germination, and a few more seeds often germinated after the second winter, but additional germination was rarely stimulated by a third winter.

Sowing seeds outside in late fall or early winter (October–January) was the easiest method, but soaking seeds overnight in GA_3 improved germination. In 1997 we performed germination tests on seeds collected that year in 5 different treatments varying in drying time, seed storage time, pretreatment with GA_3, and sowing date (Table 1). Germination rate was significantly different among treatments (one-way ANOVA, df 4, F-ratio = 7.56, $P = 0.001$) with a higher germination rate with treatment 1 than treatment 2, and a higher germination rate with treatment 5 than all other treatments (Tukey's mean pairwise comparison). These results suggest that pretreatment with GA_3

TABLE 1

Germination treatments and susequent rates. Means with the same letter are not significantly different.

Treatment	Drying time (d)	Seedbank storage time (mo)	Treated with gibberelic acid	Outside sow date	Seeds germinated / seeds sown by flat	Mean germination rate (s.d.)
1	78	0	No	October 1997	3 / 10 0 / 10 9 / 10 4 / 10 1 / 10 6 / 10 5 / 10	0.400 (0.306)a
2	49	6	No	April 1998	4 / 90 3 / 99 10 / 109 2 / 121 15 / 121 13 / 121 14 / 95	0.080 (0.050)b
3	49	13	No	November 1998	16 / 85 10 / 85 19 / 98	0.167 (0.043)ab
4	49	20	No	May 1999	9 / 53 11 / 53	0.189 (0.027)ab
5	49	20	Yes	May 1999	43 / 52 46 / 52	0.856 (0.041)c

before sowing greatly increased germination. Regardless of germination method used, however, seed germination varied between years, that is, seeds in some years outperformed those collected in other years using the same germination methods, reflecting differences in seed viability from year to year in the wild.

Seed-Banking Effectiveness

Seeds removed from the seed bank germinated well in 2 tests. One set of seeds, collected in July 1990, were dried over silica gel, placed in a refrigerator in a sealed container for 2 y, then re-dried and placed in a freezer until February 1995, when the seeds were sown outside. Of these 11 seeds, germination was 4 out of 6 in a flat, and 0 out of 5 in another (total germination 36%). Another test used 1991 seeds that were dried over silica gel and held in a refrigerator until January 1993 when seeds were re-dried and placed in the freezer until sowing outside in February 1995. Of these 17 seeds, 6 out of 9 germinated in 1 flat and 4 out of 8 germinated in another (total germination 59%).

Although we lack germination results for the same seed collections with freshly collected seeds to use as a comparison, and the numbers of seed-banked seeds are too low to be statistically significant, these 2 tests suggest that seeds can be successfully dried and stored in the seed bank for short time periods. Testing of older seeds from the seed bank will establish long-term seed-banking effectiveness, and it is likely that pretreatment with GA_3 will increase germination percentages.

Plant Growth

Growing an alpine species at a nursery near sea level, with the accompanying heat and humidity, usually results in high seedling mortality. Whichever methods were used to produce seedlings and transplants, because of high mortality under cultivation (which increases the longer a propagule is in cultivation), it was important to produce a transplant for outplanting in the shortest period of time. Those plants that survive in the nursery, however, often flower in the spring of the third year after germination. These nursery-grown plants are usually much larger than their counterparts in the wild (Figure 2), which need 8 to 13 y to reach blooming size in the harsh conditions of the alpine zone (Iszard-Crowley and Kimball 1998). Transplants occasionally bloom in the nursery in early May before being outplanted. We have observed that seeds produced in these flowers and released from the transplants after outplanting can result in seedlings the next growing season.

Photo by Doug Weihrauch

Figure 2. Potentilla robbinsiana #792, outplanted in 2000 at the Old Crawford Path site on Mount Washington, blooming in June 2003.

OUTPLANTING

After germination and 1 or 2 y of growth, nursery plants were transported to the remote, alpine outplant sites by foot. Care was taken to minimize jostling during transport. Suitable microhabitat sites were chosen by looking for substrate with enough soil movement to prevent vegetation except for a few well-adapted species (for example, mountain sandwort, *Minuartia groenlandica* [Caryophyllaceae], diapensia, *Diapensia lapponica* [Diapensiaceae], and bearberry willow, *Salix uva-ursi* [Salicaceae]). Outplanted *P. robbinsiana* failed to establish on areas of high solifluction, however, that supports the development of natural stone patterning (Figure 3). At suitable locations, a soil divot was removed and the plant and its surrounding potted soil matrix were placed in the hole and lightly tamped down. The location then received a heavy soaking of water. Small, numbered plastic stakes were placed near the plants to monitor the demographic fate of each individual. It was not possible to follow the fate of all plants because some stakes were lost after they were ejected by freeze thaw action in the soils, and some were removed, likely by Common Ravens. Every summer, the status of each numbered individual was recorded. In addition, a census, including wild plants and progeny from nursery-grown plants, was conducted in most years.

Bareroot Seedlings

Different outplanting techniques evolved with our parallel expansion of knowledge of this species' biology. Prior to the current research and recovery effort, outplanting was tried at approximately 20 sites (Graber and Brewer 1985). From 1986 to 1992 the AMC and the University of New Hampshire collaborated on a juvenile, bareroot outplanting technique. Because winter mortality in the natural population is impacted by freeze-thaw cycles and consequent soil movement, bareroot outplanting of dormant juvenile plants had the objective of reducing differences between potting soil and natural soil substrate around the plant. Between 1986 and 1992, 210 seedlings were outplanted on 2 different sites, but none survived so this method was discontinued. Because bareroot outplanting of juvenile-sized plants proved unsuccessful, adult-sized plants with their potting medium were used in all subsequent outplanting attempts.

Container Seedlings

For our renewed outplanting efforts from 1993 through 1998, we overwintered 2- to 3-y-old potted transplants in cold frames and then stored them in a freezer from the beginning of thaw (in Framingham approximately late February to mid-March) until outplanting. Outplanting occurred in early June, just after snowmelt in the alpine zone, and coincided with the winter dormancy break and flowering of wild populations. Our intention was to delay the break of winter dormancy and synchronize it more closely to the plants life cycle in their natural alpine habitat, and give plants the longest possible growing season before onset of winter. After some initial success this method was discarded because of poor survival rates. In 1998 we experimented with an early July outplanting. Like the previous method, plants were held in cold frames over winter, but then kept outdoors and allowed to resume growth in spring. These transplants were outplanted into alpine habitat in early July at the peak of

Photo by Doug Weihrauch

Figure 3. Stone patterning at the Old Crawford Path site on Mount Washington.

their yearly growth. Survival through the summer and subsequent winter was 92%, while percent survival of cohorts stored in the freezer and outplanted that same year was zero. Therefore, all subsequent outplanting efforts made use of transplants that were actively growing, resulting in a significantly higher survival rate than that for transplants outplanted directly from the freezer (Figure 4) (non-parametric Mann-Whitney, $U = 952.0$, $P < 0.0001$).

Much of the first-year mortality resulted from transplant shock, especially if extremes of precipitation (either drought or heavy rains) immediately followed outplanting. The remote location of the sites limited monitoring or care after outplanting. We observed that many of the transplants stored in the freezer never broke dormancy or were already dead when outplanted. First-year, annual survival of transplants stored in the freezer before outplanting was 11% and subsequent annual survival averaged 80%, while first-year survival of transplants that were actively growing when outplanted was 90% and averaged 99% for all subsequent years. After the first year, mortality appears to occur primarily due to ejection from substrate as a result of freeze-thaw fluctuations. As compared with wild plants, the plants reared in the nursery tend to have a fibrous root network, rather than a well-developed taproot to anchor plants into the soil to withstand winter frost heaving. Heaving did not, however, appear to adversely affect nursery plants any more than it affected wild plants, as the survival of transplants outplanted when they were actively growing was comparable to the 91% survival rate for adult-sized wild plants.

CONCLUSION

More than 14 000 Robbins' cinquefoil plants of various size classes are now growing on Mount Washington, primarily because of natural increases in wild plant numbers after the relocation of the hiking trail and closure to the public of the critical habitat, events prior to successful outplanting. In 1999, there were 4575 plants with a rosette diameter greater than 1.4 cm (0.6 in) as compared with 1547 plants of the same size category in 1983 (Weihrauch and Kimball 2000). Our outplanting efforts have reestablished more than 100 plants at the degraded Monroe Flats where trail activities had resulted in localized extirpation (Weihrauch and Kimball 2000; USFWS 2002). The 2 planted populations outside of Monroe Flats, designed to be back-up populations, continue to exceed the modeled minimum population size of 50 plants (Iszard-Crowley and Kimball 1998). Moreover, the 169 plants grown from seeds at NEWFS and introduced at a site near a small wild population on Franconia Ridge have now naturally increased to more than 300 individuals (Weihrauch and Kimball 2000). The greatest success for raising plants and reintroducing them to alpine sites included: 1) treating seeds with GA_3; 2) growing transplants to adult size in 1 to 3 y; and 3) allowing plants to resume growth before outplanting them in early July. The July outplantings appear to combine alpine conditions that are most benign with sufficient time for plants to

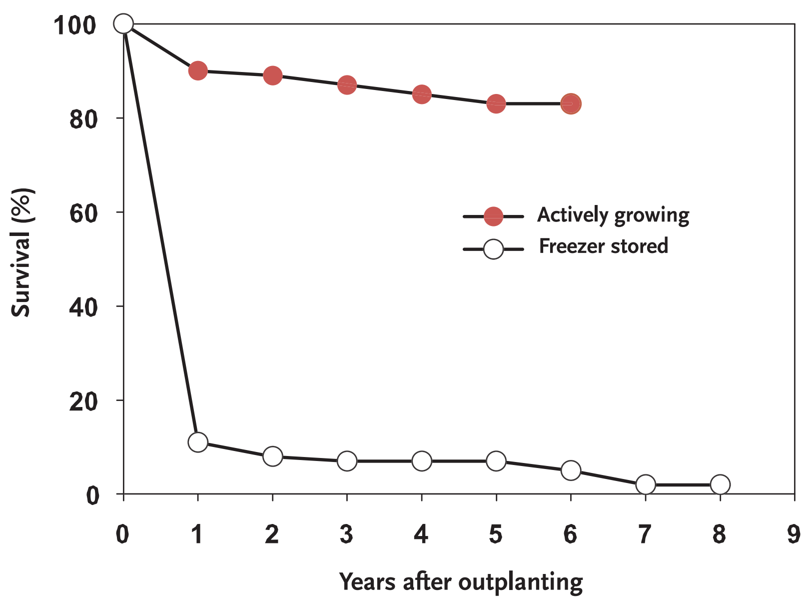

Figure 4. Comparison of cumulative seedling survival over time by outplanting method.

establish themselves before the end of the short alpine growing season.

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AUTHOR INFORMATION

William E Brumback
Conservation Director
New England Wild Flower Society
180 Hemenway Road
Framingham, MA 01701
bbrumback@newfs.org

Doug M Weihrauch
Ecologist
dweihrauch@outdoors.org

Kenneth D Kimball
Director of Research
kkimball@outdoors.org

Appalachian Mountain Club
Research Department
PO Box 298
Gorham, NH 03581