

Supplementation of the autumn buttercup population in Utah, USA, using *in vitro* propagated plants

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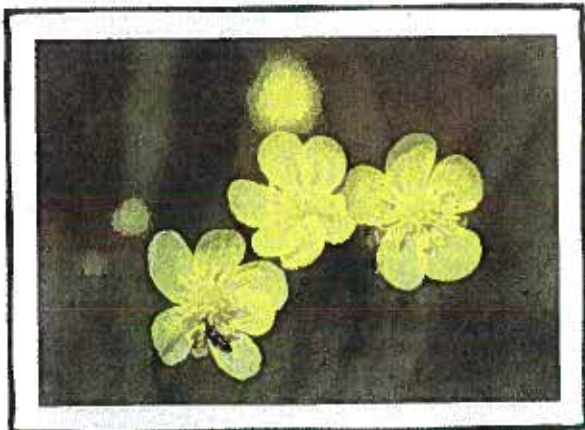
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Introduction

The autumn buttercup, *Ranunculus aestivalis* (*Ranunculus acriformis* var. *aestivalis* L. Benson), is a wet meadow species limited to one or two small populations located in the Sevier River Valley, 1,963 m elevation, in southwestern Utah. First collected in 1894, it was formally described in 1948. A 1975 report to Congress indicated that the species was thought to be extinct (Ripley, 1975). After being re-discovered in 1982, decline in numbers from approximately 500 to 22 contributed to its listing in 1989 (54 FR 30550). It also has a G1 ranking, and is listed as endangered by the IUCN and as an S1 species in Utah. In 1989, The Nature Conservancy purchased 44 acres containing the last known location of the autumn buttercup. The current known population on the TNC preserve has stabilized within the past few years to approximately 18 individuals. An additional population has been documented approximately 11.3 km north of the preserve, but is not well described since it occurs on private land. This project may establish up to 300 plants, taking an important step in increasing the current population to 1,000 plants and providing the knowledge to



Close-up of Autumn buttercup flowers

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Plants



Autumn buttercup plants *in vitro*

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establish new sites as described in the recovery plan (USFWS 1991).

Goals

- **Goal 1:** To augment or supplement autumn buttercup (*Ranunculus aestivalis*) at The Nature Conservancy Sevier River Valley Preserve, utilizing plants propagated through tissue cultures initiated from parent seed collected within this population.
- **Goal 2:** To increase numbers of individuals in the current known population, in order to maintain the genetic diversity of the population and fulfill recovery goals, which aspire to 1,000 individuals at this site.
- **Goal 3:** To document methods used and monitor program progress for established plants over a 5-year period following the initial planting.

Success Indicators

- **Indicator 1:** Successful germination of a high proportion of the available seed at the Center for Conservation and Research of Endangered Wildlife (CREW).
- **Indicator 2:** Initiation of sterile, propagating shoot cultures and rooting of those cultures *in vitro*, with the production of at least five individuals each of at least 60 genetic lines at CREW.
- **Indicator 3:** Successful transport of plants, *in vitro*, from CREW to The Arboretum at Flagstaff.
- **Indicator 4:** Acclimation of the plants to soil and ambient conditions at The Arboretum at Flagstaff (TAF).
- **Indicator 5:** Identification of suitable locations for the outplanting at the Sevier River Valley Preserve.
- **Indicator 6:** Successful transport of plants from Flagstaff to the Preserve.
- **Indicator 7:** Survival of outplanted individuals at the Preserve in the weeks immediately following the outplanting.
- **Indicator 8:** Survival of the plants in subsequent years of monitoring.

Project Summary

Feasibility Stage: Partners, such as the CREW at the Cincinnati Zoo & Botanical Garden, TAF, Utah Valley State College (UVSC), The Nature Conservancy (TNC), and U.S. Fish & Wildlife Service (USFWS), existed with the expertise to propagate plants using tissue culture methods, acclimate and grow to a transplanting size, facilitate and do research, provide funding and guidance under the Endangered Species Act and provide long-term conservation.

- **Habitat:** The autumn buttercup is a wet meadow species, which grows on

small rises (hummocks) in the transition zone between a moist *Carex* (sedge) spring-fed meadow and upland meadow.

- Species: autumn buttercup (*Ranunculus aestivalis*) (L. Benson) Van Buren & Harper (Ranunculaceae).
- Socio-political and economic issues: At the time of listing in 1989, the Autumn buttercup was thought to be extirpated from its type locality due to intense agricultural activities, primarily livestock grazing of wet meadows (54 FR 30550).



Outplanting autumn buttercup

© Linda Whitham

Today's greatest impediment to recovery on TNC lands is low numbers. Purported threats such as grazing have been removed and competition with other plants is being controlled through ecological burns and clipping. Active livestock grazing continues on surrounding private lands. The status of potential existing plants on other private lands is unknown.

Implementation: Each step in the implementation of this project required different expertise and protocols. Work at CREW began with the receipt of 60 seeds from TAF from the population at the Sevier Valley Preserve. These required stratification for germination, and, in some cases, several rounds of stratification, as the seeds did not germinate synchronously. The seeds were germinated aseptically and a separate tissue culture line was initiated from each individual seedling. Because of the labor involved in maintaining culture lines, only four to six shoot cultures of each line were maintained at each subculture. Individual shoots were transferred to a separate medium for rooting. Once the *in vitro* plants were of sufficient size and roots were obtained, they were ready for transport to TAF by overnight shipping. From October 2006 to May 2007, TAF received and acclimated 241 plantlets from test tubes to soil pots. In June of 2007, 136 of the largest plants, representing 35 lines of culture, were transported to the Sevier Valley Preserve to be planted. TAF kept 105 plants for future use. Between June and October, 2007, more plantlets were sent to TAF from CREW, for a total of 239 individuals in the greenhouse at TAF over the winter of 2007 - 2008, representing 52 genetic lines. Suitable planting sites were identified at the Preserve by researchers from UVSC. The habitat had been previously described and this description was used to identify the most desirable sites for re-introduction. Two somewhat different micro-habitats were selected, one a more arid site than the other, but both are presently or have historically been occupied with autumn buttercups.

The plants were transported from Flagstaff to the Preserve (approximately 4 hrs) in an enclosed truck. UVSC researchers designed experimental treatments that

would identify the impact of root competition in the planting experiment. All plants were planted in holes dug 1 m apart. At both planting sites, half of the holes were lined with paper to decrease root competition during the first few weeks of growth. Researchers and volunteers from UVSC and TNC carried out the experimental site preparation and planting activities during June, 2007. Once the planting was completed and each individual tagged, measurements were recorded for number of leaves, average vegetative diameter, and general condition for each of the 138 plants included in the design. Following the initial planting, the plants received water once each week for the next six weeks. The monitoring continued each week and data stated above were recorded. UVSC researchers returned to the site early in September 2007, to record data for the growing season. Of the original 138 plants included in the study, 128 had survived the summer (92.8% survivorship). Another group of plants is being prepared at CREW and Flagstaff for outplanting in 2008.

Post-release monitoring: The site will be monitored at least once a year for the next five years to determine survival and measure growth. Results of such post-release monitoring activities. As stated above, survivorship for the first summer following out-planting is over 92%. In addition, the majority (nearly 90%) of the individuals surviving were in good or fair condition and are expected to re-emerge in spring of 2008.

Major difficulties faced

- The entire seed lot does not germinate synchronously. This required additional rounds of stratification in order to induce additional germination.
- Maintaining the large number of different genetic lines in tissue culture. This required maintaining only a few individuals of each line.
- Transport of the large number of plants as *in vitro* cultures 3,219 km from CREW (Ohio) to TAF (Arizona). This required careful packing and overnight shipping.
- Occasional plant die-off during acclimation. This was related to insufficient root development in vitro and insect pests in the greenhouse.
- Physically transporting water from the source to the planting sites for the initial weekly waterings. This was made easier by the fact that there was water naturally occurring on the site.

Major lessons learned

- Although tissue culture is a clonal propagation process, with careful monitoring it is feasible to maintain small numbers of many genetic lines, in order to provide genetic diversity for outplanting projects.
- Having a larger number of plants to work with, in this case provided by tissue culture propagation, contributes to a greater likelihood of success.
- The initial size of the plants is important for success with this species.
- Outplanting sites and the timing of planting need to be carefully determined by having an understanding of the habitat requirements for the species.
- Strong coordination of all parties is important.
- What can be accomplished by bringing together people with various expertise

and resources to address a specific goal - in this case the goal of increasing the numbers of the autumn buttercup.

Success of project

Highly Successful	Successful	Partially Successful	Failure
√			

Reasons for success/failure:

- The project brought together groups with several areas of expertise and resources: Site protection and source of seeds (TNC), tissue culture propagation expertise and facilities (CREW), facilities and expertise for acclimation and growth to planting size (TAF), expertise on the species' habitat, site characteristics and general biology (UVSC), labor for planting and monitoring (UVSC, TNC), funding and coordination of the project (USFWS).
- These groups were well coordinated and each fulfilled their tasks and responsibilities for the project.
- The plants had a high degree of survival initially and through the first summer, increasing the number of plants *in situ* approximately six-fold. More plants will be added in 2008.

References

- Ripley, L. (1975) Report on endangered and threatened species of the United States. House Document 94-51: 1 - 200.
- USFWS (1991) Autumn Buttercup (*Ranunculus acriformis* var. *aestivalis*) Recovery Plan. Denver, Colorado: U.S. Fish & Wildlife Service. p.20.

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Close-up of Autumn buttercup flowers
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Autumn buttercup plants *in vitro*
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Goals

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