Developing biogeographically based population introduction protocols for at-risk plant species of the interior valleys of southwestern Oregon:

Fritillaria gentneri (Gentner's fritillary)
Limnanthes floccosa ssp. *bellingeriana* (Bellinger's meadowfoam)
Limnanthes floccosa ssp. *grandiflora* (big-flowered wooly meadowfoam)
Limnanthes floccosa ssp. *pumila* (dwarf wooly meadowfoam)
Limnanthes gracilis var. *gracilis* (slender meadowfoam)
Lomatium cookii (Cook's desert parsley)
Perideridia erythrorhiza (red-rooted yampah)
Plagiobothrys hirtus (rough popcorn flower)
Ranunculus austro-oreganus (southern Oregon buttercup)

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The following species are presented in alphabetical order: *Fritillaria gentneri* (Gentner's fritillary), *Limnanthes floccosa* ssp. *bellingeriana* (Bellinger's meadowfoam), *Limnanthes floccosa* ssp. *grandiflora* (big-flowered wooly meadowfoam), *Limnanthes floccosa* ssp. *pumila* (dwarf wooly meadowfoam), *Limnanthes gracilis* var. *gracilis* (slender meadowfoam), *Lomatium cookii* (Cook's desert parsley), *Perideridia erythrorhiza* (red-rooted yampah), *Plagiobothrys hirtus* (rough popcorn flower), and *Ranunculus austro-oreganus* (southern Oregon buttercup). Each species' section consists of segments covering Conservation Status, Range and Habitat, Species Description, Seed Production, Seed Germination, Vegetative Reproduction, Breeding System, Hybridization, Cultivation, Transplanting and Introduction Attempts, Population Monitoring, and Land Use Threats and other Limitations, followed by a final segment outlining a specific Population Introduction/Augmentation Strategy.

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Developing biogeographically based population introduction protocols for at-risk plant species of the interior valleys of southwestern Oregon:

Plagiobothrys hirtus
(rough popcorn flower)

Plagiobothrys hirtus. Photo by M. Carr.

Plagiobothrys hirtus (rough popcorn flower)

Conservation status

The rough popcorn flower (*Plagiobothrys hirtus* Greene Johnst.) was collected, although infrequently, in the Umpqua Valley of Douglas County from 1887 to 1961. However, by 1978, no extant populations were known (Siddall and Chambers 1978). Surveys in the early 1980s rediscovered several populations, all within the Umpqua Valley drainage. All extant populations are small, having been impacted by the conversion of wetlands to agricultural lands, and more recently by rapid urban and industrial development in the Sutherlin area (Figure 26).

Figure 26. Rapid development of wetland habitat in the Sutherlin area has destroyed several populations of *P. hirtus.* Photo by K. Amsberry.

Land use patterns since the time of European settlement have greatly influenced vegetation patterns throughout the West, and habitat destruction has been of particular importance to the loss of vernal pool and seasonal wetland species. Conversion of wetlands to agricultural fields was identified as a major contributor to the extinction of vernal pool species as early as 1941 (Hoover 1941), and researchers currently estimate that 60-90% of pools extant at the time of European settlement have now been destroyed, along with the endemic plant and animal species associated with them (Keeley and Zedler 1998). In the Umpqua Valley, conversion of wetlands to agricultural lands through hydrological alterations has drastically reduced the number of seasonal wetlands that can support the rough popcorn flower. Even within areas that have escaped wholesale destruction due to development or agriculture, changes in land management practices in neighboring wetlands have altered the nature of remaining pools. Draining of adjacent land has affected pool depth and size, reducing the suitability of these habitats for the rough popcorn flower.

In addition to being subject to filling and draining, wetlands have also been modified to the point of unsuitability for the popcorn flower by other land management practices. Fire suppression since the time of European settlement has drastically altered vegetation successional patterns in seasonal wetlands (Johannessen et al. 1971). Increasing shade due to canopy closure over pools previously kept open by fire has reduced suitability for the popcorn flower, and encroachment of competing native and exotic wetland vegetation has decreased the vigor and viability of existing populations of this rare plant. Sustained heavy grazing, especially by sheep, has also probably contributed to the destruction of plants, and has damaged wetland integrity and stability.

Only one population of *P. hirtus,* owned by Oregon Department of Transportation (ODOT), occurs on publicly owned land (Amsberry and Meinke 1997). This population, adjacent to Interstate 5, is administratively protected under state law, and is managed by ODOT for conservation of the popcorn flower (Figure 27). Plants in this site are vigorous, and the population appears to be self-sustaining. However, the potential for destruction due to highway-related chemical spills or accidental herbicide spraying puts this population in a continually precarious position. Plants in this site extend throughout ODOT's roadside ditch and onto adjacent privately owned land. This privately owned parcel contains a significant portion of this *P. hirtus* population, and perhaps more importantly, the water source that creates the favorable habitat for the entire population. This parcel is currently for sale, making the future viability of this population even more uncertain.

Figure 27. The population at the Yoncalla site is managed by ODOT to insure its protection. Bright green recently emergent plants can be seen in the center of the photo. Photo by K. Amsberry.

Only one population (consisting of several patches) of *P. hirtus* is truly secure. The William Oerding Popcorn Swale Preserve is owned and managed by The Nature Conservancy (TNC) specifically for the conservation of the popcorn flower. TNC periodically removes invasive exotics, monitors plants annually, and collects seed for storage and future research projects.

Less than one dozen populations of *P. hirtus* are currently extant, with three of these declining rapidly. In response to this anthropogenic destruction and decline, this species is listed as endangered by the U.S. Fish and Wildlife Service, the Oregon Department of Agriculture, is on the Oregon Natural Heritage Information Center (ORNHIC)'s List 1 (threatened with extinction or presumed to be extinct throughout its entire range), and has a Natural Heritage Network Rank of G1/S1 (critically imperiled throughout its range/critically imperiled in Oregon; ORNHIC 2004). A recovery plan specifying recovery goals and providing information on the proposed implementation of recovery actions for the rough popcorn flower was released in 2003 (USFWS 2003).

In addition to the extant naturally occurring populations mentioned above, *P. hirtus* occurs in three created populations on the North Bank Habitat Management Area (NBHMA) east of Wilbur. These populations were created in 1998-2001 by planting greenhouse-grown plants from Oregon State University (OSU) into selected sites. These transplants, grown from seeds collected at naturally occurring sites, have persisted and increased, with the most successful population currently consisting of approximately 10,000 plants. Although continued monitoring will be necessary to determine the ultimate fate of these created populations, their initial success indicates that population creation in protected habitat will be an important component of recovery for this species.

Range and habitat

The rough popcorn flower is found only in the Umpqua River drainage in Douglas County, Oregon, at sites ranging from 102-232 m (330-750 ft) in elevation. Extant, naturally occurring populations of this species occur along the Sutherlin Creek drainage from Sutherlin to Wilbur, adjacent to Calapooya Creek west of Sutherlin, and in roadside ditches near Yoncalla Creek just north of Rice Hill. The northern-most reported site is near Yoncalla, and the southern-most at Wilbur. Until recently, all known sites were east of I-5, but in 1998, a site was discovered at the junction of Stearns Lane and Highway 138, approximately 0.5 miles west of the highway. The eastern-most extant population is just east of Plat K Road outside Sutherlin. Historic collections have been made farther east near Nonpareil, but recent surveys (1998-1999), although limited due to private ownership of most land in this area, did not locate any populations in this area. Collections from outside this area of Douglas County are probably misidentified collections of the morphologically similar *P. figuratus* (Chambers 1978).

The rough popcorn flower occurs only in seasonal wetlands where it remains submerged under standing water from late fall through spring. The majority of extant and extirpated sites occur on the Conser soil series - deep, poorly drained soils present in depressions in alluvial stream terraces. An apparent water table is at its uppermost limit within these soils from November to May. *Plagiobothrys hirtus* also appears on the Brand soil series - poorly drained soils in low stream terraces with apparent water tables at or near the soil surface from November to May (Natural Resources Conservation Service 1997, Soil Survey Division 2000). Several other soil series are occasionally associated with *P. hirtus*; most are poorly drained flood plain soils.

The rough popcorn flower often occurs in dense, monospecific groups in the deepest portion of the shallow pools in which it resides. Associated species occurring along the immediate periphery of rough popcorn flower populations are typical of sedge/grass-dominated open marsh. Native herbaceous associates include green-sheathed sedge (*Carex feta),* clustered sedge (*C. arcta),* one-sided sedge (*C. unilateralis*), common rush (*Juncus effusus*)*,* pointed rush (*J. oxymeris),* tapered rush (*J. acuminatus*), western mannagrass *(Glyceria occidentalis),* sloughgrass (*Beckmannia syzigachne*), tufted hairgrass (*Deschampsia caespitosa*), and Leichtlin's camas (*Camassia leichtlinii*). Annuals present in these sites include skullcap speedwell (*Veronica scutellata*)*,* Willamette downingia (*Downingia yina*), and Douglas' meadow-foam (*Limnanthes douglasii*). Most sites are moderately to highly disturbed due to agricultural and development activities - consequently, they suffer from infestations of exotic weeds, including teasel (*Dipsacus sylvestris),* Himalayan blackberry (*Rubus discolor)*, pennyroyal (*Mentha pulegium*) and knapweed (*Centarea* sp).

Native oaks (*Quercus garryana*) and ash (*Fraxinus latifolia*), as well as introduced *Pyrus* sp., exist on the perimeters of some pools, but the rough popcorn flower does not occur in the shaded understorys of these sites. Both circumstantial and experimental evidence suggest that shading diminishes the vigor and reproductive capacity of the popcorn flower, and reduces seedling recruitment and establishment (Amsberry and Meinke 1999). Before European settlement, sites were probably kept open by periodic burning due to fires, either purposefully set by Native Americans, or occurring naturally from lightning strikes (Johanessen et al. 1971).

Description of species

The rough popcorn flower is an herbaceous plant which can be 50-60 cm tall and perennial, or considerably smaller and annual, depending on environmental conditions (Amsberry and Meinke 1998). The upper stems are distinctly hairy with hairs perpendicular to the stem, and the bright green, simple linear leaves have hairy margins. Flowering stems are spreading, with paired coiled inflorescences bearing white, five-petaled flowers with yellow centers (fornices). Large plants can consist of over 50 flowering stems, and each stem produces 10-100 flowers. As in most members of the Boraginaceae, anthers are included and epipetalous. Each flower can produce four tan-colored to black nutlets; due to fruit abortion or lack of pollination, calyces with fewer than four nutlets are often observed (Figure 28).

Figure 28. Nutlets and flower of *P. hirtus*. Photo by K. Amsberry.

Rough popcorn flower and fragrant popcorn flower (*Plagiobothrys figuratus*), the other species of popcorn flower found throughout western Oregon, are both members of the subgenus *Allocarya*, (Abrams 1951, Peck 1961) and are quite similar in appearance. Rough popcorn flower is the larger of the two, growing to 60 cm in height (*P. figuratus* generally reaches only 15-45 cm), with stouter stems (4-5 mm wide in comparison to approximately 2 mm in *P. figuratus*), and often larger flowers. Nutlets, the basis for taxonomic differentiation within *Plagiobothrys*, are remarkably similar in the two species, although the attachment scar is generally basal in *P. hirtus*, and lateral in *P. figuratus*. In the field, the two taxa are readily discernable by the distinctly spreading (rather than appressed) pubescence, large size, and facultatively perennial nature of *P. hirtus*, which easily distinguish it from *P. figuratus*, as well as other species (*P. scouleri, P. nothofulvus*) that may be present in our area. Seedlings of rough popcorn flower germinate in fall and overwinter as submerged rosettes; this aquatic juvenile stage is similar in appearance to the rosettes of many species of wetland plants, and is very difficult to identify.

Seed production

Plagiobothrys hirtus, like most borages*,* can potentially produce four nutlets per flower (Smith 1977). Based on a study consisting of evaluations at five naturally occurring populations of *P. hirtus*, seed production in this species is fairly prolific, with a mean of 73% (± 15%) of ovules producing mature nutlets during the peak reproduction period (Amsberry and Meinke 2001). In this study, the time of peak seed production varied among sites, with plants in wetter sites flowering and maturing seeds later than those in sites which dried more quickly. In most sites, copious numbers of mature seeds were observed from mid-June through early September, but plants in a few wetter habitats delayed seed maturation until the beginning of August. Later studies also documented a similar high nutlet/ovule ratio, with means of 78% - 85% of ovules producing mature nutlets in a series of sites over multiple years (Amsberry and Meinke 2003).

The number of seeds produced by individual plants is largely controlled by the number of flowers produced, and not too surprisingly, large plants produce more flowers. As plants of *P. hirtus* can be perennial, growing to 70 cm tall, with dozens of flowering stems and hundred of flowers, or can be diminutive annuals with only a few flowers (Amsberry 2001), the number of seeds produced per plant varies greatly. In a multi-year evaluation of the reproductive capacity of transplants and naturally occurring recruits at three created populations on the BLM's North Bank Habitat Management Area, mean per plant seed production varied from 208 to 8610 (Amsberry and Meinke 2003). Cultivated plants in the greenhouse or garden also produce large amounts of seed, providing that pollinators are available, or plants are manually pollinated (Amsberry, unpublished).

Seed germination

In a series of studies to evaluate the potential for inbreeding depression in *P. hirtus*, the germination rates of selfed and open-pollinated seeds were compared (Amsberry and Meinke 2001). Open-pollinated seed collected from two field sites and selfed seed produced in the OSU greenhouses were placed in petri dishes on very wet filter paper under ambient conditions in the lab (without additional light, at approximately 21°C). Seeds were not given any pre-treatments. Radicals began to emerge within two days, with 30% of open-pollinated seed, and 60% of greenhouse-grown selfed seed germinating by the end of three weeks (Figure 29).

Figure 29. As well as germinating easily in the green-house, seeds of *P. hirtus* germinate prolifically in suitable field conditions. Photo by K. Amsberry.

Due to the potential confounding effect of the environment in which the seeds matured (in the greenhouse for the selfed seed, and the potentially lower resources of the field for the open-pollinated seed), a second germination test was completed using only seed matured in the field. Flowers of both species were manually selfed, then bagged in pollination bags to prevent further naturally occurring transfer of pollen. In this second study, completed under similar conditions to previous germination tests, open-pollinated *P. hirtus* seeds germinated much better (a total of 67%of seeds germinated by the end of the study period) than selfed seeds from the same site (a total of 17% germination), indicating that inbreeding depression occurs at this stage. To evaluate possible causes for the contradictory results of the two trials, seeds of greenhouse- and field-grown plants were weighed. Seeds of greenhouse-grown *P. hirtus* seeds were heavier than seeds matured in the field – the higher resource conditions in the greenhouse probably influenced stock plants to produce larger and potentially more viable seeds. This increased viability more than compensated for the effects of inbreeding depression in the first set of germination trials.

A subsequent study looking at intraspecific variation within *P. hirtus* also included germination tests (Amsberry and Meinke 2002). In this study, open-pollinated seeds from several populations, collected in multiple years, were placed in flats of soil (bark, peat moss, pumice, dolomite lime and a wetting agent) in the greenhouse and watered thoroughly. Seeds were watered each day, and grown under a 12 hour cycle of artificial illumination. Germination rates varied significantly among sites and years, and ranged from 50%-100%, with an overall mean of 71% of seeds germinating within three weeks.

Germination trials for *Plagiobothrys hirtus* seeds were also conducted at the Berry Botanic Garden (Center for Plant Conservation 2004). Seeds were first subjected to either eight weeks of cold stratification or no cold stratification. Seeds were then placed in either constant 68°F (20°C) or alternating 50°/68°F (10°/20°C) temperature regimes. Sixty-seven % of seeds that were cold stratified and then placed in constant temperatures geminated, while only 27% of seeds that were cold stratified and then placed in alternating temperatures germinated. When seeds were not cold stratified, 0% of seeds germinated under constant temperatures while 67% germinated under alternating temperatures.

Vegetative reproduction

Plants of *P. hirtus* are capable of reproducing by developing adventitious roots at stem nodes (Amsberry 2001; Figure 30). In some sites, plants root readily during the growing season, spreading into large vegetative mats made of many interconnected rooted rosettes. During the subsequent winter, connecting internodes rot away, leaving a series of independent, but genetically identical individuals.

Figure 30. Adventitious roots form at the stem nodes of *P. hirtus* - the tendency to produce these roots varies among populations. Photo by K. Amsberry.

However, the tendency to produce adventitious roots varies among cohorts of plants grown under controlled conditions in the greenhouse (from seed collected in different sites), and is presumably genetically controlled. In a study evaluating intraspecific variation among populations of *P. hirtus*, the percentage of plants producing adventitious roots after cultivation in individual pots on open benches in the greenhouse for three months varied from 0% (Popcorn Swale population), to 93% (Yoncalla population) (Amsberry and Meinke 2002).

The ability of *P. hirtus* to asexually reproduce allows large numbers of plants to be produced in the greenhouse from stem cuttings. To propagate transplants for a population augmentation project at two Douglas County populations, cuttings were taken from stock plants grown from seed collected at these two sites. Cuttings (each containing several stem nodes) were placed in individual pots of peat/pumice soil, where they were either watered daily or placed in water-filled trays, and were fertilized with liquid fertilizer as needed. Although plants grown from seed collected at the Popcorn Swale site did not spontaneously produce adventitious roots when grown in pots on dry greenhouse benches (see above), cuttings taken from plants of this genotype quickly rooted under these optimal greenhouse conditions (Amsberry 2001). Treating cuttings with powdered rooting hormone prior to placing in soil did not effect survival, as 99% of both treated and untreated cuttings grew (Amsberry unpublished).

Breeding system

In a 2001 study, the breeding system of *Plagiobothrys hirtus* was evaluated by comparing the seed set (percent of total ovules converted to filled nutlets) of selfed and outcrossed plants (Amsberry and Meinke 2001). To determine if pollen transfer by insects is required for fertilization, unmanipulated plants, presumably grown without access to pollinators (in the greenhouse), were also included in the seed set comparison.

Without pollinator access in the greenhouse, plants set little or no seed, with less than one percent of ovules producing nutlets. Manual self or outcrossed pollinations yielded more seed, with about 30% of ovules developing into mature seeds - pollen source did not affect seed set. Open-pollinated plants in the field yielded the greatest numbers of seeds, with 78-85% of ovules yielding fruits. (The lower seed set of study plants in the greenhouse relative to open-pollinated field grown plants was attributed to the potentially greater efficiency, and repetition, of insect visits - the very small flowers and adnate anthers of *P. hirtus* make manual pollination difficult.) The similar seed set rates for artificial selfed and outcrossed pollinations also indicate that inbreeding depression is not present at this stage. (See "Germination" section, above, for a discussion of potential inbreeding depression at the germination stage.)

In the field (at four Douglas County sites), plants of *P. hirtus* were visited frequently by a variety of insect pollinators, including species of *Bombus* (Figure 31), as well as members of Halictadae, Megachilidae, and Syrphidae (Amsberry and Meinke 2001). Additionally, a diurnal moth restricted to wet prairie habitat (*Ctenucha rubroscapus*) was observed visiting plants in all field sites throughout the flowering period. As many pollinators were observed going to multiple flowers on a single plant, and *P. hirtus* is self-compatible, seeds in the field are probably produced from a mix of selfed and outcrossed pollinations. As insect vectors are required to mediate both outcrossed and selfed pollination, maintaining an adequate pollinator fauna is critical to viability of this species.

Figure 31. Many pollinators visit the showy flowers of *P. hirtus*. Photo by K. Amsberry.

Hybridization

The potential for hybridization of both naturally occurring and transplanted plants of *P. hirtus* with sympatric congeners (especially *P. figuratus*) has been a cause for concern (Amsberry and Meinke 2002). Morphological intermediates between the two are occasionally observed (Amsberry unpublished), and the two species are considered to be closely related (Chambers 1989). Although studies to evaluate the potential for hybridization have been undertaken (Amsberry and Meinke 2002), the time-consuming and difficult nature of completing artificial crosses of these species makes completing conclusive studies difficult. In this preliminary research, seed was produced from artificial crosses between the two species, but key morphological characters of the developing progeny were consistently similar to the maternal parent – crosses may have been contaminated with self pollen. As *P. figuratus* occurs throughout Douglas County, evaluating the potential for hybridization between the rare and common congeners will be an important topic for future research.

Cultivation

Plagiobothrys hirtus is easily cultivated under greenhouse or outdoor conditions, and transplants for several population creation projects have been produced at the OSU greenhouses (Figure 32). For a 1996 augmentation project, plants were propagated by taking cuttings (see "Vegetative reproduction" above). Rooted plantlets were potted in pots of peat/pumice/bark potting mix, and placed in water filled trays in a cool (50° F/60° F) greenhouse (Amsberry 2001).

As seeds germinate easily without pre-treatment, growing plants from seed has also proved to be an efficient and economical way to produce transplants (Amsberry and Meinke 1998). Once seeds germinated, and the first true leaves began to develop, emerging seedlings were transplanted to 4" wide plastic pots filled with a moisture retentive peat/bark/potting mix. In this study, plants were grown on open greenhouse benches with daily watering, fertilized twice monthly with 20-20-20 soluble fertilizer, and were frequently checked for aphids and sprayed with Safer's © insecticidal soap as needed. Mortality of transplants developing from seeds or cuttings was less than one percent, with large plants produced within three to six months (Amsberry unpublished).

Similar procedures were used to cultivate transplants at the Oregon State Correctional Institute in 2002 (Amsberry and Meinke 2003). Seeds were placed in flats in the greenhouse, and were transplanted into pots of a bark/pumice/coir soil as they germinated. Plants were grown in a cool greenhouse (45°F/58°F), and fertilized weekly with 20-10-20 liquid fertilizer.

Figure 32. Plants grew well under cool greenhouse conditions at OSCI (left), and at OSU (right). Photos by K.Amsberry.

Transplanting and introduction attempts

Plants of *P. hirtus* have been successfully transplanted to both augment existing populations and create new ones. In 1996, 480 greenhouse grown plants were transplanted into two Douglas County sites that already supported populations of this species (Amsberry 2001). In this study, plants were planted in six plots within three soil moisture zones at each of the two sites, and were randomly assigned one of four treatments: weeded, fertilized, given both treatments, or left untreated as controls. Transplant success was evaluated by monitoring survival and fecundity. The soil moisture zone in which plants were transplanted affected success; transplants were most successful in the wettest zones (Table 1).

Table 1. Number of surviving plants (out of 80 transplanted) in each zone after one year (from Amsberry 2001).

	Popcorn Swale	Yoncalla
Xeric	0	8
Mesic	0	32
Hydric	3	56

Weeding plots before transplanting improved establishment in this study, while fertilization (in the greenhouse prior to transport to the study site) increased plant size in the first year, but did not affect establishment. Additionally, this study characterized successful transplant microsites based on associated species composition; as the proportion of vegetation comprised of wetland species increased, the proportion of transplants surviving also increased.

Figure 33. Plants were transplanted into standing water to avoid the need for supplemental watering. Photo by K. Amsberry.

Beginning in 1998, greenhouse-grown transplants were transplanted into a total of three sites on the BLM's NBHMA (Amsberry and Meinke 1998, Amsberry and Meinke 2003; Figure 33). Initially, in March 1998, two pre-selected wetland sites were prepared by scarifying the substrate and removing a portion of the existing vegetation (mostly *Juncus*), thereby creating shallow pools among the remaining vegetation. In April, 1000 plants were installed in standing water that had subsequently collected in these created pools. Three hundred plants were placed in monitoring plots encompassing both sunny and shady microsites, with the remaining plants transplanted into optimal habitat within the sites.

Mortality of the monitored transplants was less than 5% throughout the first growing season after transplanting. Transplants grew quickly, spread clonally, and bloomed prolifically. Large amounts of seed were produced and dispersed (see "Seed production" above). Although plants in the sunny portions of both sites established and reproduced successfully, those in the shaded sites fared more poorly. Although a few plants continued to persist in these shaded plots, their numbers declined each year, and fecundity was low. Additional plants were added to one of these sites in 1999 (Westgate), and a third population was created at another site on the NBHMA in 2003. Both of the original two populations have continued to grow and reproduce; the larger population currently consists of 5,000 – 10,000 plants, and the smaller one of approximately 1,000 – 5,000. The third site has not been as successful, although further monitoring is needed to determine the ultimate fate of this population.

Population monitoring

Monitoring of transplanted plants of *P. hirtus* was included in both population creation/augmentation projects described above (in "Transplanting and introduction attempts"). In the 1996 population augmentation project, plants were placed in individually marked m^2 plots and monitored for stem length, number of ramets, number of inflorescences produced, number of flowers per inflorescence, and number of seeds per flower (Amsberry 2001). By the end of the first growing season, surviving plants had rooted at the nodes and developed into mats of vegetation, making identification of individual transplants difficult. By the following summer, seedlings and overwintering ramets existed within and nearby many plots, and monitoring of individual plants was discontinued.

At the NBHMA, 15 plants were placed in each irregularly shaped plot. (Plots were irregularly shaped to encompass the maximum amount of optimal wetland habitat available.) Again, due to the clonal, spreading nature of P. hirtus, determining individual plant survival quickly became difficult. Once plants had expanded to the point that they could no longer be tracked as individual transplants, the number of rooted stems (ramets), in each plot, as well as number of flowers, seed/ovule ratios, and number of flowers per plant were counted. Eventually, monitoring was modified to include a once per year census of all plants (seedlings and ramets) present in the site, and an evaluation of reproductive effort.

Additional plants were added to one population on the NBHMA in 1999. In an attempt to overcome previous difficulties in monitoring *P. hirtus*, 16 plants were placed in each m^2 plot, and each plot was evaluated for percent cover of the target species. Plants quickly filled the plots, with some plots exhibiting 90% cover of *P. hirtus* by the end of the first growing season. This monitoring method allowed for efficient evaluation of transplant survival and growth, and eliminated the need to count individual plants.

Land use threats and other limitations

Despite the negative effects of agriculture, the most devastating threat to the popcorn flower has occurred in recent years due to the rapid population increase, and subsequent urban expansion, in the Sutherlin area. This rapid growth rate, in a city built almost entirely within the historic drainage of Sutherlin creek, has resulted in the filling and draining of wetlands for residential and commercial development at an unprecedented pace. Four populations of the rough popcorn flower within the boundaries of Sutherlin have been lost to residential development within the last five years.

Other than habitat destruction, competitive exclusion from native and non-native wetland vegetation most likely represents the most significant ongoing threat to the rough popcorn flower. *Mentha puligeum* (exotic) and *Juncus* (native) compete directly with the popcorn flower and appear to reduce plant size, fecundity, and especially seedling establishment.

Severely invasive exotics, such as *Dipsacus sylvestris* and *Centaurea* sp., can become monocultures in wetlands, eliminating native plants and reducing wetland functions. Research has shown that transplants of the rough popcorn flower establish better with vegetation removal (Amsberry 2001), and other studies have demonstrated increased transplant success and seedling recruitment of native plants in relation to vegetation removal (Carlsen et al. 2000, Pendergrass et al. 1999).

Population introduction and augmentation strategy

Based on this information, the following step-down procedures are recommended for *Plagiobothrys hirtus* population introductions:

1. Select population introduction/augmentation target sites. Several factors should be considered when selecting target sites for *Plagiobothrys hirtus* population introduction and augmentation projects. First, target sites should contain the seasonal wetlands (described above) that are preferred by this species. Data on associated species, soil classification, and soil moisture from known extant populations are available, and a model correlating vegetation composition with transplant success for this species has been developed. This information can be used to help select among available sites, and can determine the microsites within wetlands that are most likely to support transplants of *P. hirtus*. Inappropriate site selection is the most common cause of rare plant reintroduction failures – care in selecting suitable sites during the initial phases of planning will promote project success.

   Due to the history of the destruction of seasonal wetlands on privately owned land in Douglas County, inventories for suitable population introduction and augmentation sites should be focused on publicly owned (or otherwise secure) lands. Existing and proposed management in these sites should be specified prior to project commencement to insure that planned activities are compatible with popcorn flower growth and reproduction. As weed infestations can reduce transplant success and impact wetland quality, sites with minimal cover of weedy species should be selected if available. As hybridization of transplants with pre-existing plants of related species is a potential concern, sites without populations of *P. figuratus* should be selected if possible. Selection and use of sites should be coordinated with pertinent public landowners to ensure administrative protection and promote adaptive management of created populations.

2. Collect seeds for off-site cultivation of introduction stock. In general, source material for a population creation project should utilize propagules collected from the extant population located nearest to the target site. As ecological differentiation among populations of *P. hirtus* has been documented, collection of seed from the site in closest geographic (and/or ecological) proximity to the target site maximizes conveyance of potentially important local adaptations and increases the likelihood of successful population establishment. As this species occurs in three watersheds, source material for population creation projects within any one watershed should be collected from the nearest site within the same watershed. However, as administrative considerations may require the selection of a site outside the current range of the species, combined collections of seed from multiple sites may be appropriate in these situations.

   To ensure that the genetic variation present in the extant donor population(s) will be represented in the newly created site, seed should be collected from as many individuals as possible, located throughout the population. Achieving the maximum genetic variation possible will be especially important in created populations of *P. hirtus*, as inbreeding depression may occur in this species. As *P. hirtus* produces abundant seed in most sites, seeds germinate readily, and plants are easy to cultivate, one year of collection should yield an adequate amount of seed for most population creation projects.

3. Cultivate stock for transplanting. Assuming that seeds are placed in suitably wet conditions, *P. hirtus* can be successfully cultivated from seed under standard greenhouse conditions. Seeds should be placed in Petri dishes on very wet filter

paper, or in seedling flats of wet soil. After germination, seeds should be potted into individual pots of peat/loam/pumice potting mix, and watered daily or placed in water-filled trays. Plants should be fertilized twice monthly with 20-20-20 liquid fertilizer, checked frequently for pests, and treated immediately should aphid infestations begin to develop. To prevent transplant shock, plants should be grown continuously in a cool greenhouse (50° F/60° F), or if grown under warmer conditions, should be moved to a cool greenhouse, or a sheltered yard, for the two weeks prior to transplanting.

4. Introduce cultivated plugs into the target site(s). *Plagiobothrys hirtus* transplants should be planted in spring (March to May), when sufficient water is present in seasonal wetlands to promote establishment and growth. If plants must be planted later, during the active growing season, supplemental water should be supplied at the time of transplanting and again the following week. As removing adjacent vegetation prior to planting increases transplant establishment, weeding should be included in the transplant protocol if feasible.

   As populations of *P. hirtus* generally consist of dense monocultures, transplant arrays that mimic this configuration are probably most likely to develop into successful populations. Placing plants in large, dense groups throughout the available suitable habitat allows plants to develop quickly, preventing weed infestations, and promoting the attraction of pollinators. However, because of the rhizomatous nature of plants of this species, this type of planting array may make monitoring difficult.

   Alternatively, to allow for evaluations of differential survival in response to experimental treatments, or to differences in microhabitat, plugs can be planted in a widely spaced array (if a sufficient amount of suitable habitat is available), prolonging the ability to identify specific individuals. The layout of introduced plugs should be designed in a manner that is consistent with subsequent population monitoring objectives (see #5, below).

Although direct seeding of *P. hirtus* into field sites has not been attempted, the high germination rates of seed of this species suggest that sowing seed might also be a successful component of population creation projects. Sowing seed has the potential to incorporate more genetic variability into new populations than does creating populations solely from cultivated plugs (which are often grown from limited seed sources) and also has the advantage of being more cost-effective than the often expensive production of transplants.

5. Monitor introduced populations. Introduced populations should be monitored annually to evaluate project success. Although germinating seedlings are easily observed after rains begin in the fall, first year seedlings can become quite large and bloom prolifically during the growing season, making the differentiation of seedlings from overwintering rosettes difficult beyond the first growing season.

   Due to the clonal nature of *P. hirtus*, and the difficulty in determining the extent of individual clones, monitoring should include a census of the number of flowering stems within the entire created population, as well as collection of data on flower and seed production. Evaluating percent cover also provides adequate data on plant growth and survival, but should be combined with an assessment reproductive effort.

6. Develop an adaptive management strategy. Management strategies expected to promote establishment and expansion of created populations should be developed prior to the initiation of population creation projects. Because *P. hirtus* grown and reproduces more prolifically when adjacent vegetation is removed, management plans for created populations should include recommendations for periodic burning or mowing. When monitoring data are collected and reviewed each year, these plans should be evaluated, and adapted to meet the needs of the created population.

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Developing biogeographically based population introduction protocols for at-risk plant species of the interior valleys of southwestern Oregon:

Ranunculus austro-oreganus (southern Oregon buttercup)

Ranunculus austro-oreganus. Photo by Medford District BLM staff
http://www.or.blm.gov/EE/EESites/tableroc.htm)

Ranunculus austro-oreganus
(southern Oregon buttercup)

Conservation status

Ranunculus austro-oregana Benson (Figure 34) is an attractive herbaceous perennial in the Ranunculaceae family, known in only a few sites in Jackson County in southwest Oregon (Figure 1). It was designated as a Candidate for federal listing by the U.S. Fish and Wildlife Service in 1980 (USFWS 1980), but the plant was subsequently removed from the Candidate list. Currently, *R. austro-oreganus* is a State of Oregon Species of Concern. It is on the Oregon Natural Heritage Information Center (ORNHIC) List 1 (threatened or endangered throughout its range) and has a Natural Heritage Network Rank of G2/S2 (imperiled throughout its range/imperiled in Oregon) (ORNHIC 2004a).

Figure 34. Herbarium speciman of *Ranunculus austro-oreganus*. Photo by R. Currin.

As with many rare species, habitat degradation and loss are the primary threats facing *Ranunculus austro-oreganus.* All known populations occur in central Jackson County, near the cities of Medford and Ashland (Figure 35). Agricultural and urban development are potential threats. Many of the sites are lightly to heavily grazed, and spraying may also have a negative impact on the populations (Meinke 1982, ORNHIC 2004b). Non-native invasive weeds have also severely altered *R. austro-oreganus* habitat in some areas. There has also been evidence of off-road vehicular

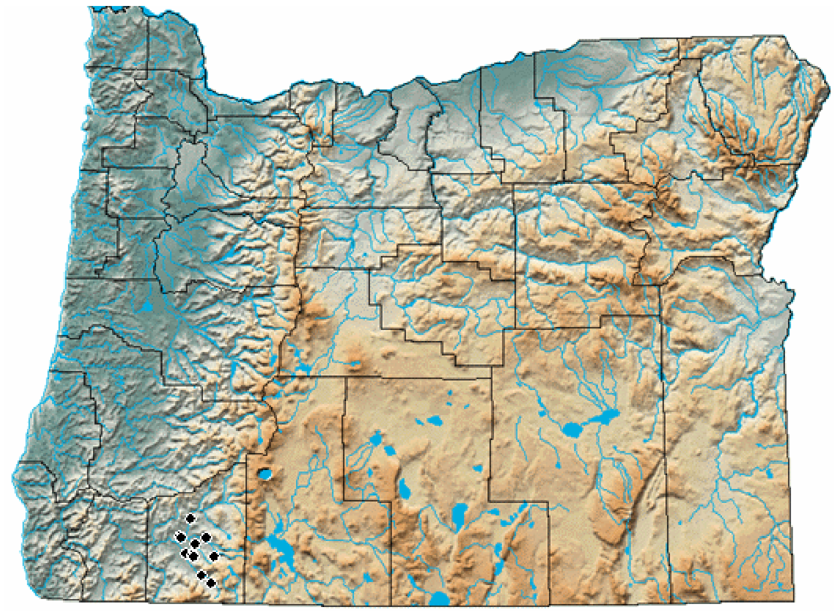

Figure 35. Partial distribution of *Ranunculus austro-oreganus* sites. Map courtesy of the Oregon Flora Project.

traffic use in several of the sites. In addition, *R. austro-oreganus* is closely related to the more widespread *Ranunculus occidentalis*, and a recent study indicates that *R. austro-oreganus* hybridizes with its congener (Jessup 2001). There is some concern that continuing interspecific mingling could lead to *R. austro-oreganus* being absorbed back into the *Ranunculus occidentalis* complex (Steve Jessup, Southern Oregon University, Ashland, Oregon, personal communication).

Range and habitat

According to the Oregon Natural Heritage Information Center database, there are fifty-three populations of *Ranunculus austro-oreganus*. However, four of these populations have not having been visited in over 50 years, and their current status is uncertain. Thirty percent of the populations consist of less than 100 plants, and were considered to be in poor condition when last observed. In addition, another 19 percent of the populations have an unknown number of plants (ORNHIC 2004b).

Ranunculus austro-oreganus species is frequently found on dry grassy slopes and fields under scattered oak (*Quercus garryana*) trees (Figure 36), although one population is

Figure 36. Oak savanna at Lower Table Rock. Photo courtesy of the Medford District BLM (http://www.or.blm.gov/EE/EESites/tableroc.htm)

located in damper ground, and several of the populations are located near water. Elevations range from 1200 to 2400 feet. *R. austro-oreganus* usually grows in loam soils (Meinke 1982, ORNHIC 2004b); however, populations have been reported to occur in clay soil or in gravelly areas. The species tolerates a variety of light conditions, with populations occupying sites with conditions ranging from full sun to partial or filtered shade.

Plants associated with *Ranunculus austro-oreganus* include A*chillea millefolium, Acnatherum lemmonii, Agoseris heterophylla, Amelanchier alnifolia, Arbutus menziesii, Arctostaphylos viscida, Berberis aquifolium, Berberis nervosa, Bromus hordeaceus, Bromus japonica, Bromus tectorum, Calochortus tolmei, Camassia quamash, Ceanothus cuneatus, Cercocarpus betuloides, Clarkia gracilis, Claytonia perfoliata, Cryptantha intermedia, Crystopteris fragilis, Cynoglossum grande, Cynosorus echinatus, Danthonia californica, Dodecatheon hendersonii, Eriophyllum lanatum, Galium aparine, Galium triflorum, Garrya viscida, Festuca californica, Festuca idahoensis, Fraxinus latifolia, Fritillaria recurva, Horkelia daucifolia, Iris chrysophylla, Koeleria cirstata, Ligusticum californicum, Lomatium triternatum, Lomatium urtriculatum, Lonicera hispidula,* *Lupinus polyphyllus, Melica subulata, Ozmorhiza chilensis, Pinus ponderosa, Plagiobothrys nothofulvus, Poa bulbosa, Poa secunda, Pseudotsuga menziesii, Quercus garryana, Quercus kelloggii, Sanicula crassicaulis, Senecio integerrimus, Sisyrinchium douglasii, Sitanion hustrix, Symphoricarpos albus, Taeniatherum caput-medusae, Tonella tenella, Toxicodendron diversilobum, Toriis arvensis, Tragopogon dubius, Vulpia myuros* and *Zigadenus venenosus* (ORNHIC 2004b).

Description of species

Ranunculus austro-oreganus (Figure 37) is a perennial herb with one to several slender stems (2-4 dm high) arising from a bulbous or thickened base. Lower cauline leaves are sparse and and 3-lobed, with each part again 2 to 3-lobed and sometimes toothed. The leaf blade is 3-4 cm long with loosely appressed hairs on the upper surface and densely silky pubescence below. Petioles are 5-10 cm long with thinly spreading hairs. The five sepals are reflexed, villous and 4.5 cm long. Petals are five as well, oblong, bright to pale yellow on top and bronzy or coppery-red dorsally. Achenes are 4 mm long, straight except for a tiny hook on the end of the beak, and occur 6-10 in a hemispheroid cluster. The plant blooms in April and May (Benson 1954, Peck 1961). The dark purplish-red coloring of the petals distinguishes this species from varieties of the closely related *Ranunculus occidentalis* (Figure 38) and *Ranunculus californicus* (Figure 39) (Meinke 1980, Southworth 1998). Frank Lang (Southern Oregon University, Ashland, Oregon, personal communication) states that the two species may also be distinguished by the mature fruit

Figure 37. Herbarium speciman of *Ranunculus austro-oreganus* flower and achenes. Note the dark underside of the petals. Photo by R. Currin.

Figure 38. *Ranunculus occidentalis* flower. Photo courtesy of Calflora http://www.calflora.net/bloomingplants/westernbuttercup.html)

Figure 39. *Ranunculus californicus* flowers. Photo courtesy of http://www.nuc.berkeley.edu/students/asztalos/)

morphology: the *R. occidentalis* beaks are smaller (<1.4 mm) with a curved style, whereas the beak of *R. austro-oreganus* is larger (>1.6 mm) and straight.

Seed production

Aside from Benson (1954) mentioning that *Ranunculus austro-oreganus* produces 6-10 achenes per cluster, there is no information available about the number and viability of seeds produced by individual *Ranunculus austro-oreganus* plants. Seeds of the closely related *R. occidentalis* germinated easily at Heritage Seedlings, Inc. (Lynda Boyer, Heritage Seedlings, Inc., Salem, Oregon, personal communication), and there is no reason to believe that *R. austro-oreganus* seeds are not similarly viable.

Seed germination

No seed germination research has been conducted with *Ranunculus austro-oreganus*. However, as previously mentioned, Lynda Boyer (personal communication) has germinated the closely related *Ranunculus occidentalis* seeds. Seeds sown in outdoor garden beds in mid-October germinated by mid-November. There appears to be a short dormancy period required for the seeds. When seeds were sown in a greenhouse flat immediately after collection, there was no germination. Neither did seeds germinate after a two week cold stratification period. Boyer did not do further greenhouse germination studies, but estimated that *R. occidentalis* seeds required about a four week cold/moisture stratification period in order to germinate.

Vegetative reproduction

There are no data available regarding vegetative reproduction in *Ranunculus austro-oreganus*. Plants have stems which are erect or suberect, and Benson (1954) makes a point of mentioning that the stems do not root. It is not known whether or not older plants form clumps which can be divided.

Breeding system

No research has been conducted investigating the breeding system of *Ranunculus austro-oreganus*. It is not known whether this species is self-compatible. Because of its proclivity for hybridization, it is clear that *R. austro-oreganus* is able to outcross. The yellow and red flowers and nectary scales of *R. austro-oreganus* suggest that this species is insect pollinated.

Hybridization

In a study conducted at Southern Oregon University, Jessup (2001) states that *Ranunculus austro-oreganus* "appears to form a hybrid complex with members of the *Ranunculus occidentalis* complex." An attempt was made to analyze the hybrid complex using PCR; however, the DNA fingerprinting procedure results were inconclusive, due to the fact that the researchers were unable to obtain purified DNA. As a result, Jessup compiled circumstantial evidence to suggest that *R. austro-oreganus* hybridizes with members of the *R. occidentalis* complex. The genus *Ranunculus* forms a polyploid series, and polyploids are often the basis for hybrid complexes due to the ability of their chromosomes to pair within sets during meiosis (thereby preventing the meiotic abortion of gametes common in diploid hybrids). In addition, several Old World *Ranunculus* species groups are known to consist of hybrid swarms (Whittemore 1997). Finally, Jessup noted that the distribution of the *R. austro-oreganus – R. occidentalis* complex closely echoes a frequently observed hybrid complex distribution pattern, with relictual distributions of parental diploids and more widespread distributions of the polyploid products.

Cultivation

There have been no attempts to cultivate *Ranunculus austro-oreganus* in the greenhouse or nursery. Horticultural references provide little information about the cultivation of any *Ranunculus* species, other than stating that they tend to self-sow easily (Hartmann et al. 1990, Taylor and Hamblin 1963). Some cultivation work has been done by Lynda Boyer of Heritage Seedlings, Inc. (personal communication) with *Ranunculus occidentalis*, the closest relative to *R. austro-oreganus*. Boyer tilled under a bed at the nursery and sowed the seeds directly on the soil in mid-October. By mid-November there were a huge number of seedlings in the bed, and the following spring most of the plants flowered without any additional water. (*Ranunculus occidentalis*, like *R. austro-oreganus*, begins flowering in April and has mature fruit by late May or early June. Seeds germinate in the fall and first year plants usually flower and set seed.)

Transplanting and introduction attempts

No transplanting and introduction studies have been attempted with *Ranunculus austro-oreganus*. As mentioned in the "Cultivation" section, Lynda Boyer of Heritage Seedlings, Inc., Salem, Oregon (personal communication) did successfully grow *Ranunculus occidentalis* by sowing seeds directly on tilled ground in mid-October. Based on her work with this related species, Boyer suggested that on-site seed sowing should be a suitable method for population creation; she also stated that planting plugs in the fall should work quite well.

*Ranunculus occidentali*s was also used in a *Quercus garryana* meadow restoration project conducted in the San Juan Islands (Dunwiddie 2002 in Bein 2003). The study found that outplanting plugs of *R. occidentalis* was the most effective method of establishing native vegetation (as compared to direct sowing of seed or transplanting plants salvaged from building sites).

Population monitoring

There has been no formal monitoring of *Ranunculus austro-oreganus* populations. As previously mentioned, many of the locations have not been visited in over ten years – for some of the populations it has been over 50 years. If monitoring were to occur, efforts would be confused by the issue of hybridization, and the fact that many of the populations are a mixture of *R. austro-oreganus*, *R. occidentalis* and intermediates which display traits of both parent species (ORNHIC 2004b).

Land use threats and other limitations

Although *Ranunculus austro-oreganus* is not federally listed at this time, many of the extant populations are facing a variety of current and potential threats. Agricultural, recreational and urban development are of increasing concern to the well-being of these sites. Grazing, exotic weed infestations and fire also impact *R. austro-oreganus* and its surrounding habitat. Recreational uses, both legitimate and unauthorized, have already damaged several *R. austro-oreganus* populations, and the possibility of future damage has not been eliminated. The issue of hybridization has not been thoroughly explored, but it may pose a threat to remaining populations as well.

Thirty-one of the 53 *R. austro-oreganus* populations occur on public land. Twenty-six of these are located within the Bureau of Land Management's Medford District, in the Ashland or Butte Falls Resource Areas. Two populations are on state land – one in the Denman Wildlife Management Area, and one at the Touvelle State Recreation Site. One population is located on Jackson County land, at the Jackson County Sports Park. Of the 24 populations which occur on private land, two are located within The Nature

Conservancy's Whetstone Savanna Preserve. These are the only two populations on protected private land that is being managed for rare plant species. Unfortunately, many of the populations, whether located on public or private land, are not protected from the impacts of the threats outlined below.

Hybridization: At least 13 of the 53 *Ranunculus austro-oreganus* populations listed by ORNHIC are actually a mixture of *R. austro-oreganus*, *R. occidentalis* and their intermediate hybrids (ORNHIC 2004b). Several observers stated concern that some of the *R. austro-oreganus* populations might be reabsorbed into the *R. occidentalis* complex completely.

Development: Several of the *R. austro-oreganus* populations are at immediate risk of damage due to development. The population at Upper Table Rock is adjacent to the trailhead parking area. Initial development of the parking lot very probably eliminated a part of the original population, and any further development in the parking area will likely affect the site's hydrology and could adversely impact what remains of this population (Jessup 2001). The ORNHIC records list trail construction and maintenance as immediate threats to the population at Lower Table Rock. One of the *R. austro-oreganus* sites is adjacent to the Jackson County Sports Park, and there is a good chance that individuals were lost in the development of that complex as well (ORNHIC 2004b). Certainly any further development of that land could have a negative impact on remaining plants.

Grazing: Grazing is listed as a threat to over a third of the *R. austro-oreganus* populations.   Field observations suggest that grazing negatively impacts *R. austro-oreganus* both directly (trampling, herbivory) and indirectly (as a disturbance factor which encourages annual weeds rather than perennial grasses). In areas previously overgrazed, *R. austro-oreganus* plants were observed to occur mostly at the edges of the suitable habitat. In contrast, sites which did not have a history of overgrazing had plants throughout the healthy openings (ORNHIC 2004b).

Unauthorized Off-Road Vehicle (ORV) Use: As is the case with many rare plant populations occurring on public land, there is a growing threat from off-road vehicular use in unauthorized areas. At least three of the *R. austro-oreganus* sites show signs of such use (ORNHIC 2004b).

Vegetative Succession: Although *R. austro-oreganus* can be found underneath scattered oaks, and it is able to tolerate partial or filtered shade, the encroachment of shrubs and trees into the more open and grassy habitat favored by this plant could result in the shading out of extant sites (Jessup 2001).

Exotic Invasive Plants: *Ranunculus austro-oreganus* is not immune to the threat of competition from exotic weeds. Observers cited non-native invasives as a concern at several of the sites (ORNHIC 2004b). Given that many of these observations are over ten years old, it is most likely that this issue has continued to increase in importance.

Fire: Although no studies has been conducted investigating the impacts of fire on *R. austro-oreganus*, concerns have been raised about potential harmful effects of a low burning ground fire on this species (ORNHIC 2004b). More research needs to be done in order to determine what, if any, impacts to the plant might occur as a result of fire.

Population introduction/augmentation strategy

Based on the biogeographical data compiled and described above for *Ranunculus austro-oreganus*, there do not appear to be any insurmountable ecological, life history, anthropogenic, or administrative obstacles to the successful implementation of population introduction and augmentation projects for this rare species. It is important to remember, however, that the available information is quite limited, and that much of the data actually pertains to *R. occidentalis,* rather than *R. austro-oreganus*. Although many *R. austro-oreganus* populations face threats on both private and public lands, there are still extant *R. austro-oregonus* populations occurring on public, relatively secure landholdings. As such, pending interagency cooperation and funding availability, there are several good sites available for collection of seeds for use in either off-site cultivation or direct sowing projects, and open locations should also be available for population augmentation and introduction purposes.

Ranunculus austro-oreganus appears to produce a sufficient number of achenes. These propogules (if they follow the pattern of *R. occidentalis*) should germinate readily after being cold-stratified for a period of time. Following germination, it should be possible to cultivate the species in the greenhouse. There is no information to indicate that seedlings would exhibit specialized growth or symbiont requirements. Once suitable transplant sites are located, both seeds (fall sowing) and transplants (early spring planting) should establish easily. It is recommended that both methods of propagation be used, since transplants of *R. occidentalis* have been successfully used in restoration projects, and seeds of this species have been successfully sown. In both cases, previous work suggests that preparing the ground before outplanting is helpful (Lynda Boyer, personal communication; Dunwiddie 2002 in Bein 2003). Additionally, sowing seed increases genetic diversity in a population, which is especially important when establishing a new population, rather than augmenting an existing one (Guerrant 1996). Whenever possible, genetically diverse introduction stock should be used to improve the odds of overall introduction success by maximizing the amount of adaptive genetic variability in the populations.

Based upon the information already provided in this manual, the following procedures are recommended for *Ranunculus austro-oreganus* population introductions:

1. <u>Select population introdution/augmentation target sites.</u> Several factors should be considered when selecting target sites for *R. austro-oreganus* population introduction and augmentation projects. Obviously, target sites should contain suitable habitat – in this case it appears that elevation, moisture levels, soil type and associated vegetation are helpful indicators of suitable locations. Although habitat descriptions have been provided in this manual, it is extremely helpful to visit extant populations and actually see the habitat in which the plant grows.

Such visits should give a better idea of the types of microsites occupied by *R. austro-oreganus* individuals within their larger habitat context.

Given the lack of long-term protection of *R. austro-oreganus* on private lands, inventories for suitable sites should focus on publicly owned or otherwise secure lands. Selection and use of sites should be coordinated with public landowners or agencies to ensure administrative protection and management of populations following introductions. Initial field observations have indicated that grazing has a negative impact on *R. austro-oreganus* populations, so it would also be important to ensure that no grazing is allowed to occur on the on the proposed target sites.

2. Collect *Ranunculus austro-oreganus* seeds for off-site cultivation of introduction stock and direct sowing. Ideally, introduction efforts would include the transplanting of plugs, for rapid reproduction capacity, and seeds, to increase genetic diversity within the new or augmented population. Source material for off-site cultivation should be collected from the extant population(s) located nearest to the introduction target sites to maximize conveyance of potential local adaptations. When collecting seed, an effort should be made to collect seeds from as large a sample of genetically variable individuals as possible, in an effort to elevate seed production, fitness and adaptive genetic variability within the introduced population. Finally, it is important that seed is either collected from populations which do not have *R. occidentalis* co-occurring, or that great care is taken to collect true *R. austro-oreganus* seeds, and not the seeds of hybrid intermediates.

3. Cultivate *Ranunculus austro-oreganus*. Ideally, cultivation studies of *R. austro-oreganus* need to be conducted to determine the germination and cultivation protocol for this species. However, as mentioned in the "Seed Germination" section above, it is likely that the seeds need to be moisture-cold stratified for about four weeks in order to germinate. Germinated seeds should be placed in

small (4”) pots with a standard planting medium and watered as needed. There is no indication that fertilizer is needed.

4. Introduce propagules into the target site(s). *Ranunculus austro-oreganus* seeds should be sown in the fall, after the arrival of fall rains. Although no studies have been done comparing the success of seed sown with or without ground preparation, the ground should most likely be tilled beforehand in order to reduce the competing vegetation. Plugs could probably be outplanted in either the fall or the spring. Lynda Boyer of Heritage Seedlings, Inc. (personal communication) suggested outplanting *R. oreganus* plugs in the spring. Fall planting, after the rains have arrived, would ensure that soils are moist at the time of planting and plugs have ample opportunity for root system development. If resources permit, introduction efforts might include outplanting plugs in both the spring and the fall. In general, small populations tend to be more vulnerable to such factors as stochastic events and inbreeding depression. Therefore, it is recommended that introduced populations consist of many individuals planted in large clusters.

5. Monitor introduced populations. Introduced *R. austro-oreganus* populations should be monitored annually to evaluate project success. These evaluations should take place in the spring, when flowers and fruits are available for the accurate identification of *R. austro-oreganus*. Monitoring should, at least in the first several years, consist of demographic monitoring of individuals in order to yield data on the survival and performance of individual plants over time.

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