Developing biogeographically based population introduction protocols for at-risk Willamette Valley plant species:

Agrostis howellii (Howell's bentgrass)
Aster curtus (white-topped aster),
Aster vialis (wayside aster),
Delphinium leucophaeum (hot rock larkspur),
Delphinium pavonaceaum (peacock larkspur),
Erigeron decumbens var. *decumbens* (Willamette daisy),
Horkelia congesta ssp. *congesta* (shaggy horkelia),
Lomatium bradshawii (Bradshaw's desert parsley),
Lupinus sulphureus ssp. *kincaidii* (Kincaid's lupine),
Montia howellii (Howell's montia),
Sidalcea spp. (Willamette Valley checkermallows)

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Report format:

The following species are presented in alphabetical order: *Agrostis howellii* (Howell's bentgrass), *Aster curtus* (white-topped aster), *Aster vialis* (wayside aster), *Delphinium leucophaeum* (hot rock larkspur), *Delphinium pavonaceaum* (peacock larkspur), *Erigeron decumbens* var. *decumbens* (Willamette daisy), *Horkelia congesta* ssp. *congesta* (shaggy horkelia), *Lomatium bradshawii* (Bradshaw's desert parsley), *Lupinus sulphureus* ssp. *kincaidii* (Kincaid's lupine), *Montia howellii* (Howell's montia), *Sidalcea* sp. (Willamette Valley checkermallows). 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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Agrostis howellii
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Agrostis howellii (Howell's bentgrass)

Conservation status

With its extremely localized geographic distribution and inhabitation of only 20 extant sites, *Agrostis howellii* (Figure 1) is considered one of the rarest grass species in the Pacific Northwest. Accordingly, it is currently recognized as a Species of Concern by the U.S. Fish and Wildlife Service, and a Candidate for Listing as Threatened or Endangered by the State of Oregon. It is on the Oregon Natural Heritage Program 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) (ONHP 2001).

Figure 1. *Agrostis howellii*, growing near Tanner Creek in Oregon's Columbia River Gorge. (Photo by Teresa Brainard.)

The primary threats facing *Agrostis howellii* are the low number, small size, and limited geographic distribution of its extant populations--factors which cumulatively heighten the risk of overall demographic declines or extinction due to random environmental events (see "Land use threats and other limitations," below). Fortunately, adverse human impacts to *A. howellii* currently appear quite low, as most extant populations occur on steep, rocky sites that do not lend themselves to urban or agricultural development. Moreover, the majority of *A. howellii* populations are further protected by virtue of their occurrence within protected areas of the Columbia River Gorge National Scenic Area and/or on lands administered by the U.S. Forest Service and other public agencies within the Columbia Gorge (Robin Dobson, Columbia River Gorge National Scenic Area, Hood River, Oregon, personal communication). The single population known from outside the Columbia River Gorge, located far to the south in southern Linn County, Oregon, also occurs on publicly owned land managed by the Eugene District BLM (Dick Brainerd, Salix Associates, Corvallis, Oregon, personal communication) (see "Range and habitat," below), so it is similarly isolated from threats associated with private land development.

Range and habitat

Agrostis howellii was first collected by Wilhelm Suksdorf in 1885 at Bridal Veil Falls, Oregon, located at the western end of the Columbia River Gorge. The type collection for the species was made one year later by Thomas Howell in nearby Hood River, Oregon. For over a century *A. howellii* was believed to be wholly restricted to moist, shady habitats along waterfalls and streams on the south side of the Columbia River Gorge in Multnomah and Hood River Counties, Oregon. This long-held belief was recently debunked, however, with the discovery of the species nearly 100 miles to the south in the Coburg Hills of Linn County, Oregon (Simpson 1996). The occurrence of this distantly outlying population suggests the possibility that *A. howellii* might also occur on other basaltic hills (like those comprising the Coburg Hills) in the Willamette Valley, though to date there is no evidence indicating that it does.

Currently, the Oregon Natural Heritage Program has 18 population records for *Agrostis howellii* in its rare plant database, 17 of which occur in the Columbia River Gorge

(ONHP 2002). Here, habitat for *A. howellii* consists of steep, moist, mossy, basaltic canyon walls and talus slopes, in semi- or dense shade (Figure 2) (Carlbom 1967), often in the mist zone of waterfalls (Scofield 1980), at elevations ranging from 100-400 ft. (ONHP 2002). The species is reported to occur in association with *Adiantum pedatum*, *Campanula rotundifolia*, *Elymus glaucus*, *Festuca subulata*, *Montia* sp., *Tolmiea menziesii*, and various unidentified mosses (OSU herbarium label information and ONHP 2002).

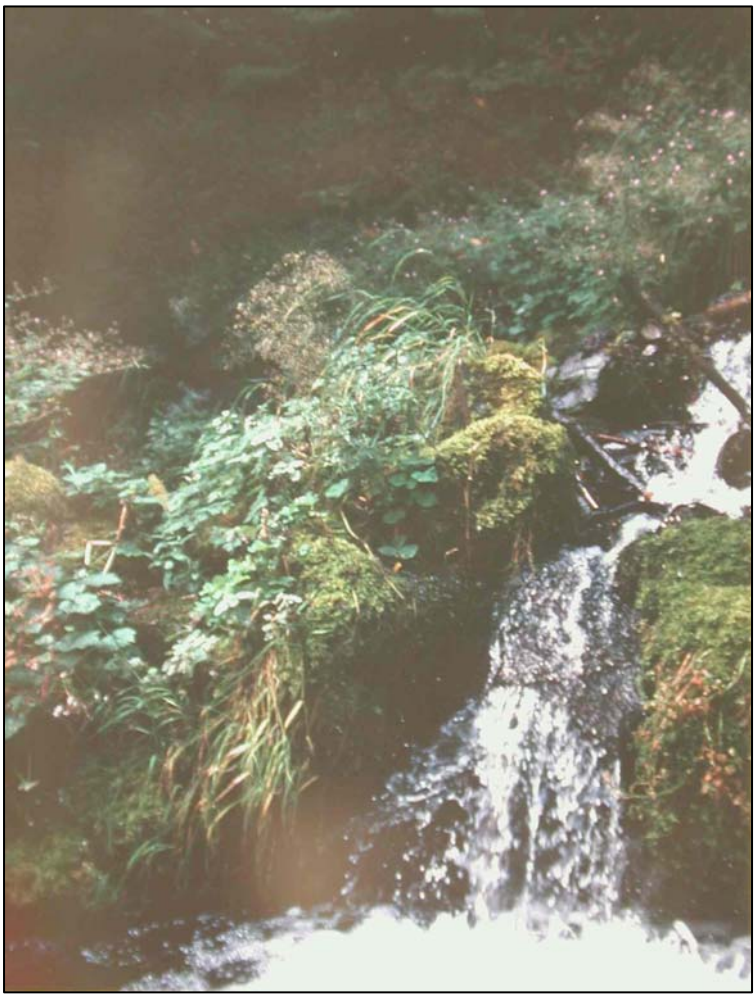

Figure 2. Most *Agrostis howellii* populations occur in shady, moist, mossy, rocky areas, typically located in the splash zone of turbulent streams and the mist zone of waterfalls in Oregon's Columbia River Gorge. (Photo by Teresa Brainard.)

In the Coburg Hills of southern Linn County, habitat occupied by *Agrostis howellii* is markedly different from that in the Columbia River Gorge. Dick Brainerd (personal communication), who discovered the Coburg Hills population, characterized the site as a broad, gently sloping swale in a moist *Acer circinatum*/*Carex deweyana* community with scattered *Fraxinus latifolia*, surrounded by mature *Pseudotsuga menziesii* forest. Other associated species include: *Athyrium filix-femina, Galium triflorum*, *Hydrophyllum tenuipes, Rubus leucodermis, Senecio jacobaea*, *Solanum dulcamara*, and *Stachys mexicana.* The elevation of the Coburg Hills site, 1480 ft., is substantially higher (over 1000 ft higher) than populations in the Columbia River Gorge.

Description of species

Agrostis howellii is a perennial grass with tufted culms, 40-70 cm tall, weakly ascending from a geniculate base. Blades are deep green, flat, lax, scabrous on both surfaces, 15-30 cm long, 3-5 mm wide, with ligules 4-6 mm long. Panicles are green, loose and spreading, 10-30 cm long, the lower branches in fascicles of 3's to 5's, the upper rays in 2's. Spikelets are pale, pedicelled, 3-5 mm long; glumes are slightly unequal, acuminate, and scabrous on the keel; lemmas are slightly shorter than the glumes, with geniculate awns (6-8 mm long) arising from near the base (Hitchcock 1905, Peck 1961, Carlbom 1967).

Agrostis howellii is readily distinguished from other congeners by its lemmas, which are awned from just above the base, the awn twisted and bent and extending beyond the glumes (Figure 3). Other *Agrostis* species possess lemmas that are either awnless or awned from midlength or above, with awns that are straight and less than the glumes (Hitchcock and Cronquist 1973).

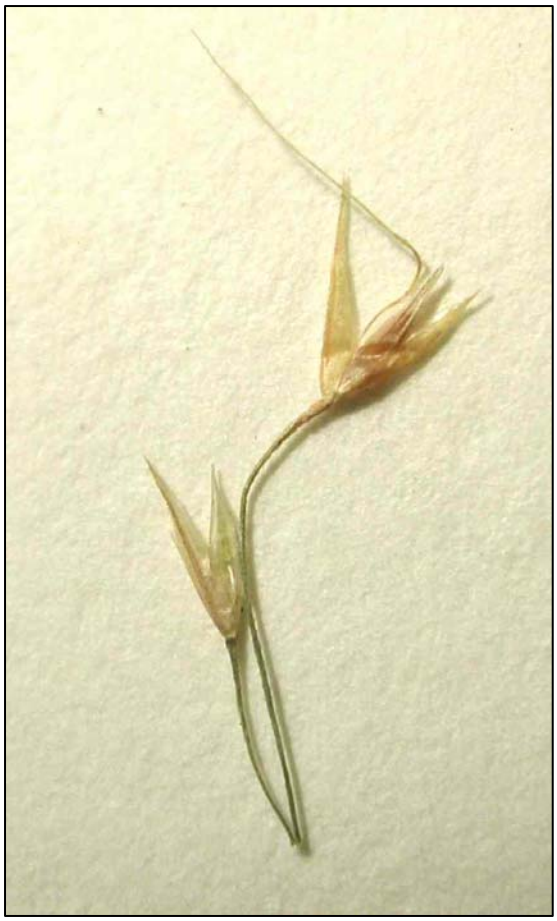

Figure 3. As exhibited by the top floret in this photograph, long, bent awns (extending beyond the glumes), attached below the mid-length of lemmas, are a diagnostic morphologic trait of *Agrostis howellii* (actual awn length= 4 mm). (Photo by Steven Gisler.)

Seed production

To date there has been no formal study or quantification of seed production in *Agrostis howellii*. The only documentation of seed production in this species is found in Carlbom (1967), who reported that *A. howellii* produces "an ample supply of viable seeds" (see "Seed germination," below). Carlbom also implied that seed production is not particularly limiting for *A. howellii*, at least compared to other environmental factors, as indicated by his statement:

> "The specialized environmental demands of this species for germination and establishment are the most important limiting factors which reduce its competiveness and therefore markedly restrict its distribution, in the present author's opinion."

Interestingly, however, Carlbom later demonstrated that *A. howellii* seeds do *not* exhibit specialized requirements for germination (see "Seed germination," below), so it is unknown why he included this reference.

Examination of pressed specimens in the OSU herbarium provide little useful data on levels of seed production in *Agrostis howellii*; although pressed specimens exhibit an abundance of nicely preserved florets, these are mostly empty and appear to have shed their caryopses prior to collection and mounting. However, if it is assumed that each floret is generally accompanied by a single seed, then herbarium specimens can still offer clues about maximum reproductive potential in this species. Floret production among all 11 pressed *A. howellii* specimens in the OSU herbarium ranges from 160-2080 per individual (mean 593.6 florets per individual, SD 535.2). Depending on the degree to which this reproductive potential is realized in nature, seed production may indeed qualify as "ample," as indicated by Carlbom.

Seed germination

Carlbom (1967) germinated seeds of *Agrostis howellii* and 21 other congeners for the purpose of obtaining common garden plant stock for biosystematic and cytological research. Although seed germination was not the central facet of Carlbom's study, he noted that seed germination in *A. howellii* was the slowest of any of the taxa for which viable seeds were available. Carlbom stated, "Although different methods were employed to germinate field-collected seeds, the first [*Agrostis howellii*] seeds did not germinate earlier than seven or eight days after imbibing distilled water." Data on levels of seed germination were not reported, nor were the "different methods" of germinating seeds described. Nevertheless, the quotation above suggests that seeds are viable and will germinate (albeit somewhat slowly in comparison to other species) under various environmental conditions following imbibition.

Vegetative reproduction

Agrostis howellii is a bunchgrass that exhibits no evidence of vegetative and/or rhizomatous expansion in its rocky habitat (Kenton Chambers, Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon, personal communication). It is unknown to what degree individuals might lend themselves to artificial division for the purpose of clonal increase in the greenhouse. The latter could prove a useful method for increasing the quantity of propagated stock for population introduction and augmentation purposes, a prospect that warrants future investigation.

Breeding system

According to Carlbom (1967), sexual self-compatibility and autogamy (self-fertilization) is common in the genus *Agrostis*, especially among the more derived, annual species, which tend to produce cleistogamous flowers. In contrast, Carlbom stated that perennial species like *A. howellii* are evolutionarily more primitive than the annual species, and although sexually self-compatible, are largely outcrossing, with chasmogamous flowers. Carlbom reported that in *A. howellii* and other more primitive species, outcrossing is favored by protandry, which accounts for one to several days of separation between pollen shed and stigma receptivity.

Hybridization

The genus *Agrostis* has been described as "a genus prone to hybridization (Tutin 1980)." This statement is supported by the work of Carlbom (1967), who observed putative hybridization between several Oregon *Agrostis* species, including *A. diegoensis* x *A. hallii*, *A. diegoensis* x *A. pallens*, and *P. humilis* x *P. thureriana*. In addition, natural (i.e., spontaneous) hybridization between *A. tenuis* and *A. stolonifera* has been documented by Stuckley and Banfield (1940) and Bradshaw (1958). Successful formation of fertile hybrids from a large number of artificial crosses between various *Agrostis* species has also been reported by Björkman (1960).

Although the genus *Agrostis* may possess a high potential for hybridization, there is no evidence of past or current hybridization in *A. howellii*. Carlbom (1967), who conducted

an in-depth biosystematic study of *A. howellii* and other western North American congeners, made no mention of ever finding evidence of hybridization involving this species, neither in the field nor in the greenhouse (whereas he *did* note putative hybridization among other *Agrosti*s species).

Interspecific hybridization in *Agrostis howellii* may be discouraged by two important factors: one ecological (pre-mating) and the other genetic (post-mating). First, although there are numerous other species of *Agrostis* within *A. howellii*'s general geographic range, realistic opportunities for interspecific gene flow may be limited by lack of fine-scale sympatry, due to *A. howellii*'s unique and isolated habitat in the Columbia River Gorge. Second, even if interspecific gene flow were to take place, successful formation of hybrids may be further discouraged by chromosomal incompatibilities arising through polyploidy. According to Carlbom (1967), *A. howellii* is among only a few tetraploid *Agrostis* species, with most other congeners exhibiting greater multiples of chromosome sets. Although polyploidy alone does not preclude the possibility for hybridization, it may nonetheless restrict the number of taxa with which *A. howellii* is capable of producing viable hybrid offspring, especially when combined with the biogeographic isolation of the species.

Cultivation

Agrostis howellii has been shown to perform well under cultivation. Carlbom (1967) cultivated *A. howellii* at Oregon State University, and reported that "established plants in the greenhouse displayed good vigor and persistence, which might suggest good competitiveness."

Although some species of *Agrostis* have been shown to host mycorrhizal fungi on their roots (Griffioen *et al*. 1994), this has not been documented in *A. howellii*, nor does it appear that the latter requires root symbionts or special soil amendments for successful cultivation. Carlbom (1967) stated,

> "…[*Agrostis howellii*] transplants and seedling plants were exposed to variable watering regimes, grown in different soil mixtures, received different fertilizer treatments and were placed under varying light and

temperature conditions, but still maintained good vigor over the two year period of study."

Carlbom's general *Agrostis* cultivation methods included use of 6-inch clay pots, steam-sterilized soil mixture (equal parts loam, peat moss and sand), and regular supplements of liquid (6-12-4) inorganic fertilizer and liquid fish fertilizer. The only specialized cultivation-related requirement of *A. howellii* noted by Carlbom was the need for "long-day light regimes" and vernalization (a winter chilling period) to stimulate flowering. This condition is not unique to *A. howellii*, however, as the majority of the other species grown by Carlbom exhibited similar flowering requirements. Unless seed production in captivity is a goal for cultivated plants, vernalization would not even be needed in order to obtain non-flowering plugs for outplanting into the wild.

Transplanting and introduction attempts

Although there have been no documented introduction attempts involving this species, Carlbom (1967) reported successfully transplanting *Agrostis howellii* from the field to garden plots at Oregon State University, where plants grew well and flowered. Here, individuals were excavated from their rocky perches in the wild and placed in polyethylene bags (after first being wrapped in moist absorbent paper) for transport back to the greenhouse. Although this information suggests *A. howellii* may tolerate being moved from the wild to a greenhouse setting, transplanting individuals from a greenhouse back into locations in the wild may prove more challenging, given the comparatively inhospitable environmental conditions offered by planting sites in the wild compared to the controlled conditions of the greenhouse or garden. However, one factor working in favor of successfully transplanting *A. howellii* into the wild is that its habitat, although steep and rocky, appears to remain moist throughout the year. As such, transplant shock resulting from moisture stress might be less of a constraint on project success than for other species occupying drier habitats.

Population monitoring

To date there has been no formal population monitoring carried out for *Agrostis howellii* (Marty Stein, Mt. Hood National Forest, Sandy, Oregon, and Robin Dobson, personal

communication). Given the physiologically discrete nature of *A. howellii* individuals and lack of rhizomatous expansion, however, there are no apparent life-history obstacles to implementation of standard population monitoring methods for this species. Here, populations could either be censused on a regular basis to obtain data on overall population trends over time, or individual plants could be mapped and monitored within fixed sampling plots to track the fates (i.e., survival, size, reproductive output, etc.) of individuals within populations. The only obvious potential hurdle to implementation of monitoring efforts for *A. howellii* is its habitat, which is steep, wet, and rocky. For instance, rocky soils could complicate installation of steel rebar or wooden plot markers, and uneven terrain could make use of meter tapes difficult. These habitat limitations, however, poses more of a logistical inconveniences than an insurmountable obstacles.

Land use threats and other limitations

The majority of *Agrostis howellii* habitat is relatively secure against imminent destruction and disturbance from development due to its extremely steep and rocky nature, and by virtue of its occurrence within the Columbia River Gorge National Scenic Area. In addition to these ecological and administrative constraints to development, most populations are afforded an additional layer of protection due to their ownership by public agencies; of the 17 known populations in the Columbia River Gorge, 12 are owned by the U.S. Forest Service, four occur on state lands, and one occurs on a mixture of state and Forest Service land (ONHP 2002). The one population found outside the Columbia River Gorge, located in the Coburg Hills of southern Linn County, is also protected from private development due to its ownership by Eugene District BLM. However, Dick Brainard (personal communication), who discovered this population, suggested potential threats at this site include timber harvest activities in the surrounding Douglas fir forest, and eventual competition arising from successional encroachment of shrubs and trees.

Given the aforementioned habitat characteristics, land use is not considered a significant threat to this species. Rather, state and federal ownership of sites would be expected to foster future conservation efforts involving population introduction and/or augmentation projects for *Agrostis howellii*.

Population introduction/augmentation strategy

Based upon the data compiled and described above for *Agrostis howellii*, there are do not appear to be any insurmountable ecological, life history, anthropogenic, or administrative barriers to the implementation of population introduction and augmentation projects for this species. All of the known extant *A. howellii* populations are publicly owned, so, pending interagency cooperation and funding availability, sites should be available both for seed collection and performance of population augmentation projects. Moreover, unoccupied habitats (i.e., wet, rocky, shady areas) are seemingly plentiful in the Columbia River Gorge, and are likewise under public ownership, so creation of new populations should also be feasible if target sites are ecologically suitable. And whereas invasive species pose serious obstacles to recovery efforts for many of the other rare Willamette Valley species treated in this manual (by reducing the quality and availability of introduction sites), noxious weed infestations are currently minimal within *A. howellii* habitats.

The biology and life history of *Agrostis howellii* likewise pose no inordinate hurdles to population introduction and augmentation projects. Viable seeds are apparently produced by the species in ample quantities and have been shown to exhibit no specialized germination requirements. In addition, *A. howellii* has demonstrated vigorous growth in greenhouse and garden settings, and exhibits no specialized requirements for propagation. Moreover, because this species has also been successfully transplanted from the wild to the greenhouse, it is likely that cultivated plugs could similarly be introduced back into the wild. However, as the latter has never been attempted, pilot studies are needed to evaluate optimal planting schedules (i.e., fall vs. spring) and plug size/age classes for introductions.

Although interspecific hybridization is a matter of concern for *Agrostis howellii*, given the propensity for hybridization among other related species in western Oregon, hybridization in *A. howellii* can probably be avoided as long as project target sites are restricted to the species' current range and habitat type (thus preventing unintentional *A. howellii* exposure to other congeners). If introductions are planned on the margins of the

species' current range, inventories should be performed to ensure the absence of other congeners prior to project implementation.

With regard to *Agrostis howellii*'s breeding system, because this species is a predominant outcrosser (due to production of protandrous, chasmogamous flowers), introduction projects should utilize as many individuals as possible, planted in proximity, to maximize wind-transferred pollen availability for seed production.

Once *Agrostis howellii* is introduced for augmentation and/or population creation purposes, its "bunching," non-rhizomatous growth habit and perennial life history should favor the use of conventional population census and/or demographic monitoring procedures to evaluate project success and population performance.

Based upon this information, the following step-down procedures are recommended for *Agrostis howellii* population introductions:

1. Select population introduction/augmentation target sites. Given the propensity of the genus *Agrostis* for interspecific hybridization, target sites for *A. howellii* introductions should be restricted to the current range and habitat type of the species, in an effort to prevent unintentional interspecific gene flow and preserve *A. howellii*'s current reproductive isolation from its congeners. Within this narrow geographical and ecological range, site selection should take place following inventories for suitable shady, moist, mossy habitats (except in the area of the Coburg Hills in the Willamette Valley, where habitat is described as a moist, lightly wooded swale). Although the habitat descriptions provided in this report (see "Range and habitat," above) may provide useful guidelines for identification of potential target sites, site selection should ultimately be supported by an intimate familiarity with local extant *A. howellii* populations, and the kinds of microsites typically occupied by species within its larger habitat type.

To ensure administrative protection of *Agrostis howellii* populations once they are introduced, public ownership of target sites should always be verified, and landowner cooperation obtained, prior to project implementation.

2. Collect *Agrostis howellii* seeds for off-site cultivation of introduction stock. Seeds of *Agrostis howellii* should be collected from the extant population(s) located nearest the introduction target site(s) to minimize undesirable mixing of gene pools and maximize conveyance of potential local adaptations (if such intraspecific variability and local adaptations exist). Based upon examination of herbarium specimens, *A. howellii* individuals can be expected to produce a maximum of 160-2080 seeds, depending on the size/age of each individual. With such high levels of fecundity, most extant populations should be capable of providing adequate seed harvests for cultivation and introduction projects in a single season. However, if only small seed source populations are available, sustainable seed collecting practices may require seed harvesting over successive years.

   As the exact timing and duration of seed maturation and shatter in *Agrostis howellii* may be difficult to accurately predict due to annual climatic variability, seed-source populations should be visited several times during the summer to track these attributes and ensure optimal seed harvest timing. One way seed capture might be optimized without the need for repeated population visits would be to enclose *A. howellii* stems within breathable polyethylene mesh bags (i.e., bridal veil or pollinator exclusion bags). Because these bags are breathable, they should allow uninhibited pollination from wind-borne pollen grains, while still serving to retain seeds as they shatter over the fruit maturation period. If it is feared bags could interfere with pollination (despite their breathability), they could still be fitted over stems after flower maturation, but before seed maturation. If bags are used for seed collection, they should probably only be used in areas that are isolated from hiking trails and roads, to minimize vandalism.

3. Cultivate *Agrostis howellii*. Once *Agrostis howellii* seeds have been harvested, previous research indicates they can be used to produce introduction stock in the greenhouse using standard cultivation techniques. The species exhibits no specialized requirements for seed germination or cultivation, though vernalization and long-day photoperiods are needed to induce flowering. However, as flowering plants are not necessarily required for introduction projects, these adaptations pose no constraints to year-round cultivation inside greenhouses or other cultivation facilities with constant heating and lighting regimes. Previous research indicates *A. howellii* seed germination levels are "normal for the genus," though data are lacking on the specific range of germination levels that can be expected.

   Once cultivated plugs are obtained in the greenhouse, it may be possible to increase their quantity through divisions, though this practice has not been attempted (or at least not documented) in this species and warrants future investigation. Because *Agrostis howellii* is self-compatible (though primarily outcrossing due to protandry), genetically identical clones derived from divisions would be expected to exhibit interfertility and seed production when introduced into the wild. As such, unlike several other species treated in this manual, *A. howellii*'s breeding system does not discourage the use of clonal propagation and introduction practices, if such are ever developed. However, *extensive* use of clones in introduction and augmentation projects may not be advisable because inbreeding depression has not yet been investigated in *A. howellii,* and clonal populations may lack the level of adaptive genetic variability inherent among sexually derived plugs.

4. Introduce cultivated plugs into target sites. Although introductions have never been attempted for *Agrostis howellii*, there is no reason to expect that such projects would not be successful, given previous transplanting and cultivation research performed on the species. Pilot studies are needed to evaluate the feasibility and success of *A. howellii* introduction projects, as well the optimal

methods (i.e., seasonal timing and plug size) for outplanting. Unlike most of the rare species treated in this manual, which occupy habitats that dry out in the summer, *A. howellii* habitat (at least in the Columbia River Gorge) appears to remain moist throughout the year, due to its proximity to shaded streams and waterfalls. As such, summer drying should not pose an inordinate restriction to spring (or possibly even summer) outplanting for this species.

Because *Agrostis howellii* is an outcrossing, wind-pollinated species, introduction projects should utilize as many cultivated plugs as possible, planted in the highest density and proximity as target sites and budgets allow, in an effort to maximize pollen availability for seed production.

5. Monitor introduced populations. Introduced or augmented *Agrostis howellii* populations should be monitored annually to evaluate project success and provide information on optimal introduction methods (see #4, above). Given the vegetatively discrete and perennial nature of *A. howellii* individuals, monitoring protocols should be relatively straightforward, either consisting of simple population censuses, or more detailed demographic monitoring of tagged and/or mapped individuals within fixed sampling plots, thus yielding data on the fates and performance of individual plants over time.

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Developing biogeographically based population introduction protocols for at-risk Willamette Valley plant species:

Aster curtus (white-topped aster)

Aster curtus (white-topped aster)

Conservation status

Exhibiting a strict ecological association with native prairie habitats, *Aster curtus* has experienced rapid declines throughout its range in the wake of expanding urban and agricultural development and the proliferation of invasive weeds. Due to this unfortunate demographic trend, this increasingly rare member of the sunflower family (Asteraceae) is recognized as a Species of Concern by the U.S. Fish and Wildlife Service, and is listed as Threatened by the State of Oregon (Figure 4). It is on the Oregon Natural Heritage Program List 1 (threatened or endangered throughout its range), and has a Natural Heritage Network Rank of G3/S2 (rare, threatened or uncommon throughout its range/imperiled in Oregon) (ONHP 2001). In Washington, home of the largest number of extant *Aster curtus* populations, the species is listed as Sensitive, though this status designation provides no administrative protection as Washington currently has no state regulatory authority for listed plants (Florence Caplow, Washington Natural Heritage Program, Olympia, Washington). *Aster curtus* has also been assigned a rank of S3 (rare or uncommon) by the Washington Natural Heritage Program (WNHP 2003).

Figure 4. *Aster curtus*. (Photo by Steven Gisler.)

On Vancouver Island, British Columbia, which defines the northernmost extent of the species' geographic range, *Aster curtus* is listed as Threatened (Schedule 1) by the newly enacted Canadian Federal Species at Risk Act (SARA). Under this act, which isn't expected to come into full force until June, 2003, *A. curtus* would be protected on all Canadian federal lands, and in the absence of a provincial protection plan in British Columbia, the species could even gain federally-mandated protection on both provincial and private lands (Laura Telford, Canadian Nature Federation, Ottawa, Ontario, personal communication).

As indicated above, the primary threats to *Aster curtus* consist of widespread destruction and fragmentation of native prairie habitat resulting from intensive agricultural and urban development, a problem facing many rare species in the Willamette Valley. Additional limitations to *A. curtus* are posed by habitat degradation caused by anthropogenic hydrologic alterations and colonization by invasive weeds (see "Land use threats and other limitations," below). Continued *A. curtus* population declines are expected in the future as development expands on private lands, which are not regulated by state or federal endangered species laws for plants. Fortunately, however, many of the largest remaining and most ecologically pristine *A. curtus* populations are located on public (primarily federal) lands in Oregon and Washington, where the species is afforded some degree of insulation from development pressures affecting private lands.

Range and habitat

Aster curtus was first described by John Lindley in 1834 from a collection made by John Scouler, who found it "abundant on undulating, dry, gravelly soils near Fort Vancouver, and low hills of the interior" (Lindley 1834). Since its initial discovery, approximately 96 populations have been identified, ranging from southwestern Vancouver Island, British Columbia (and several smaller surrounding islands), south through the Puget Trough of western Washington and into the southern Willamette Valley near Eugene, Oregon (Alverson 1983, Clampitt 1987, Gamon and Salstrom 1992, ONHP 2002, F. Caplow, personal communication). The majority of known *A. curtus* populations (those occurring in western Washington) are found primarily on gravelly, glacial outwash soils,

as originally described by Scouler. The exceptions to this edaphic generalization occur at the species' latitudinal extremes; the southernmost populations (in Oregon) are found on deep, poorly drained clayey soils, and the northernmost occurrences (in British Columbia) occupy very shallow soils overlying bedrock (Alverson 1983, Clampitt 1984, Alverson 1991, Douglas and Illingworth 1997).

In Oregon and Washington, habitat for *Aster curtus* has been described as open, grassy, seasonally moist prairie and savannah habitats (Figure 5), at elevations ranging from 90 to 525 feet (Alverson 1983). Although typically not found in forested areas, plants at one location in Washington (the DuPont population) predominantly occur in proximity to *Quercus garryana*, where the shade of the leafy canopy apparently inhibits expansion of scotch broom (ACOE 1979). Likewise, sites in British Columbia are sometimes partially shaded by canopies of *Quercus garryana* and *Arbutus menziesii* (Douglas and Illingworth 1997).

Figure 5. Wet prairie habitat occupied by *Aster curtus* near Eugene, Oregon. (Photo by Steven Gisler.)

Plant species frequently associated with *A. curtus* include various grasses (*Agrostis tenuis*, *Aira praecox*, *Anthoxanthum odoratum*, *Cynosurus echinatus*, *Dactylis glomerata*, *Deschampsia cespitosa*, *Festuca idahoensis*, and *Poa pratensis*), forbs (*Aster* [*Symphyotrichum*] *hallii*, *Camassia leichtlinii*, *Campanula rotundifolia*, *Chrysanthemum leucanthemum*, *Erigeron decumbens* var. *decumbens*, *Eriophyllum lanatum*, *Fragaria vesca* var. *crinita*, *Hypochaeris radicata*, *Juncus patens*, *J. tenuis*, *Plantago lanceolata*, *Potentilla gracilis*, *Prunella vulgaris* var. *lanceolata*, *Sidalcea campestris*, and *Viola adunca*), shrubs (*Cytisus scoparius*, *Holodiscus discolor*, and *Symphoricarpos albus*) and trees (*Arbutus menziesii*, *Fraxinus latifolia*, and *Quercus garryana*) (compiled from Alverson 1983, Clampitt 1993, ONHP 2002, and OSU herbarium specimen labels).

Description of species

Aster curtus is a perennial herb with slender creeping rhizomes and generally unbranched stems, topped by terminal clusters of flowering heads. Flowering stems are 1-3 dm tall (non-flowering stems about half as tall), and glabrous, except for the scabrous-ciliolate margins of leaves. Leaves are alternate and evenly distributed along the stem, oblanceolate and tapering to an essentially sessile base, the lowermost leaves reduced and the largest leaves (2.5-3.5 cm long) occurring along the center third of the stem. Flowers are inconspicuous, occurring in compact clusters of 5-20 small heads. Ray flowers are typically two (1-3), 1-3 mm long, and shorter than the pappus, and disk flowers are pale yellow with purple anthers. Involucres are 7-9 mm high, narrow, the bracts imbricate in several series, with a strong midrib or slight keel, chartaceous below and with spreading light green herbaceous tips (Hitchcock *et al.* 1955, Gamon and Salstrom 1992).

Aster curtus is distinguishable from other related species (most notably *A. oregonensis*) by its more compact cluster of flower heads, fewer (1-3) and smaller (shorter than the pappus) ray flowers, and the abundance of creeping rhizomes (Hitchcock and Cronquist 1973, Gamon and Salstrom 1992). Moreover, *A. curtus* is restricted to prairie habitats, while *A. oregonensis* tends to occupy more upland, woodland habitats. *Aster curtus* does

co-occur locally with *A. hallii* (=*Symphyotrichum hallii*), but the latter can be readily distinguished by its much larger, and more numerous, ray flowers (Figure 6).

Figure 6. *Aster curtus* (right) is distinguishable from sympatric *A. hallii* (= *Symphyotrichum hallii*) by its fewer, and much smaller, ray flowers. (Photo by Steven Gisler.)

Seed production

Although *Aster curtus* is capable of sexual reproduction, production of viable seeds is very low in the species. The earliest reports of seed production in *A. curtus* were made by Clampitt (1987), who estimated a mean yield of 155 seeds per *A. curtus* inflorescence, but of these, only a mean 18.3 seeds were actually filled (i.e., containing embryos). A decade later, Bigger (1999) found that fecundity can be highly variable between *Aster curtus* ramets within a single population. Here, Bigger sampled 126 ramets in one population, all with comparable sizes and numbers of capitulae, and found the number of filled seeds ranged from 8-118 per stem. Similar within-population variability was noted by Bigger at three other *A. curtus* locations.

According to Alverson (1983) and Bigger (1999), only 10-30 percent of *Aster curtus* ramets in a population are generally reproductive in any given year. In addition, among this minority of reproductive ramets, seed production can be limited by seed predators (beetle and fly larvae), which were reported to destroy as many as 12 percent of seeds (Bigger 1999). Bigger also noted that although production of viable seeds was not significantly different between two large and two small populations sampled in his study, seed predation was significantly higher in larger, denser patches of plants. Additional information on the role of breeding system in *A. curtus* seed production is discussed in "Breeding system," below.

Seed germination

Aster curtus seedlings have rarely been observed in the wild (Alverson 1983, Clampitt 1984, Giblin and Hamilton 1999), leading Alverson (1983) to conclude, "establishment by seedlings is probably an infrequent event." Nevertheless, *A. curtus* seeds are capable of germination at respectable rates. Clampitt (1984, 1987) conducted extensive germination studies and found that seeds of *A. curtus* exposed to light (16-hour photoperiod) germinated over a wide range of temperatures (10°C, 20°C, 32°C, and fluctuating 10°C/20°C). Seed germination levels ranged from 66 to 94 percent, with the highest rates exhibited at 20°C following cold stratification at 5°C for 45 days. Following removal from cold stratification, Clampitt found the time needed for 50 percent germination ranged from less than 2 days (for 20°C) to 11 days (for 10°C/20°C). According to Clampitt, although cold stratification significantly increased the rate of germination, it did not significantly affect the total number of seeds that eventually germinated. It was also noted that very few seeds germinated in the dark, leading to the conclusion that seeds in the wild that are covered by soil, litter, or dense canopy will probably not germinate.

Clark et al. (2001) also researched germination of *Aster curtus* seeds and achieved results similar to those reported above by Clampitt. Here, germination was 65.6 percent in a growth chamber, using alternating 20°C/10°C temperatures, constant light, and a germination medium of sand moistened with Hoagland's basal salt growth solution.

Interestingly, in contrast to the aforementioned findings reported by Clampitt (1984) and Clark et al. (2001), seed germination studies conducted by the Berry Botanic Garden (2002) indicated that *A. curtus* seeds require extensive cold stratification and/or scarification for germination. Here, germination rates of approximately 50 percent were observed among 3 different chilling treatments: 16 weeks cold stratification followed by 20°C, 16 weeks cold stratification followed by alternating 10°C/20°C, and excised embryos at alternating 10°C/20°C. No germination, however, was recorded among untreated (non-cold stratified) seeds, seeds that were cold stratified for only 8 weeks, and seeds that were scarified followed by 20°C.

Similar to the germination studies by The Berry Botanic Garden, Kaye and Kuykendall (2001) also investigated the influences of cold stratification and scarification on germination of *Aster curtus* seeds. In a factorial-design study, seeds were assigned two treatment levels of scarification (removal of the pappus-end of achenes with a razor blade vs. no scarification) combined with three treatment levels of cold stratification (no stratification, 4 week stratification at 4°C, and 8 week stratification at 4°C). Following exposure to treatments, seeds were then placed in an alternating 15°C/25°C growth chamber. Results showed 26 percent germination of untreated seeds (i.e., non-scarified and non-stratified seeds), and 34 and 48 percent germination for non-scarified seeds at 4 and 8 weeks of cold stratification, respectively. Maximum germination (50-58 percent) was observed among scarified seeds, regardless of subsequent cold stratification regime.

The most recent information on seed germination in *Aster curtus* was reported by Lynda Boyer (of Heritage Seedlings Inc, personal communication), who attempted to cultivate the species for use in local restoration projects (see "Cultivation," below). Boyer placed *A. curtus* seeds in Ziplock bags filled with moistened vermiculite and stratified the mixture at 1°C for 11 weeks. After stratification, the seed/vermiculite mixture was sown into flats filled with planting medium (see "Cultivation," below) and lightly covered with "a dusting of soil." Most germination occurred within 7 days. Boyer estimated germination rates at nearly 100 percent, yielding "thick and vigorous" seedlings.

Despite the respectable seed germination levels reported above, germination rates (or at least levels of seedling recruitment) appear to be much lower under natural conditions. As discussed previously, seedlings have very rarely been observed in the wild, and seedling emergence rates in outdoor seeding plots have been uniformly very low (Clampitt 1984, Clark et al.2001, Kaye et al. 2001) (see "Transplanting and introduction attempts," below).

Vegetative reproduction

It is the overwhelming consensus among *Aster curtus* researchers that population maintenance in the species is primarily achieved through vegetative expansion. *Aster curtus* is strongly clonal, with stems connected by vast, intertwined rhizomes (Clampittt 1999), ultimately capable of forming large colonies of genetically identical ramets (ACOE 1979). Alverson (1983) reported that single colonies typically cover a 1-2 square meter area, usually with 50-200 shoots. Alverson (1991) described four Oregon populations as consisting exclusively of single colonies of genetically identical clones; two populations comprised of 50 ramets each, another with 170 ramets, and the largest with 250 ramets. As discussed in "Cultivation," below, this propensity for clonal expansion via rhizomes may facilitate asexual propagation of the species using rhizome cuttings.

Breeding system

Based upon pollinator exclusion studies, Clampitt (1999) reported that *Aster curtus* requires insect pollination to produce viable seeds, with the most common insect visitors encompassing a variety of solitary bees and wasps, as well as a ringlet butterfly (*Coenonympha* sp. [Satyridae]). This finding led Clampitt to conclude that pollinator movement within and among patches is likely to strongly influence seed production, and sparked a comparative study of seed production in small versus large *A. curtus* patches (to detect possible pollen limitation). Here, despite the species' reliance upon insects for pollen transfer, Clampitt (1999) found no indication of significant pollen limitation in relation to patch size in *A. curtus*.

Giblin and Hamilton (1999) also investigated the breeding system of *Aster curtus*, and found suggestive evidence of limited self-incompatibility and inbreeding depression in the species. Their study indicated that autogamy is discouraged in *A. curtus* because individual florets are strongly protandrous, and outcrossing is encouraged because stigmas remain receptive up to 3 days after pollen shed. Autogamy is further discouraged (and outcrossing is encouraged) due to significantly greater stigma adhesion and germination of foreign pollen compared to self pollen. Despite these pre-mating barriers to self-fertilization, Giblin and Hamilton concluded that *A. curtus* is not, however, completely self-incompatible and can produce self-fertilized seeds, though not as reliably as through outcrossing. Based upon the discovery of this mixed-mating system, which would be expected to enable seed production under a variety of mating conditions, the authors concluded, "The reproductive biology of *A. curtus* apparently is not responsible for its restricted distribution…"

Hybridization

Although no attempts have been made to artificially hybridize *Aster curtus* with other relatives, Gamon and Salstrom (1992) assessed the likelihood of interspecific hybridization in this species as "very low." They stated there are no known or suspected naturally occurring hybrids in the wild, and suggested a lack of co-occurring, sexually compatible heterospecific mating partners with which *A. curtus* could successfully interbreed. *Aster curtus* is a member of the subgenus *Sericocarpus*, which consists of six species, five of which are native to North America. *Aster oregonensis* is the only other species of *Aster* in the subgenus *Sericocarpus* present in the Pacific Northwest, but this species tends to occupy woodland habitats and is not known to occur in proximity to *A. curtus*, which is restricted to open prairies (Hitchcock *et al*. 1955, K. Chambers, personal communication).

Aster curtus is known to co-occur with *A. hallii* (=*Symphyotrichum hallii*)(Figure 6) in wet prairie habitats around Eugene, Oregon, though hybridization between these sympatric species is very unlikely given very low relatedness (reflected by their recent

taxonomic segregation into separate genera) and dissimilar ploidy levels (K. Chambers, personal communication).

Cultivation

Aster curtus has been successfully cultivated by several researchers, using both seeds and rhizome cuttings, and does not appear to require soil symbionts or exhibit any other specialized propagation requirements. The earliest attempts at cultivating *A. curtus* using seeds were reported by Clampitt (1987), who noted that it was possible to successfully obtain adult plants from seeds, with cultivated individuals producing short shoots or rhizomes within two to three months after germination. *Aster curtus* was also successfully cultivated from seed by Kaye and Kuykendall (2001). Here, seedlings were potted in a medium consisting of one part peat, one part loam, and two parts pumice, and kept in a heated (20°C-25°C) greenhouse. Plants were watered twice weekly and fertilized with 20:29:20 fertilizer once weekly. Survival of one cohort of seedlings was 94 percent (n=34 plants) after six weeks, and another cohort exhibited 71 percent survival (n=8 plants) after 12 weeks.

The most recent attempts to cultivate *Aster curtus* using seeds were reported by Lynda Boyer (personal communication). As described in "Seed germination," above, seeds mixed with pre-moistened vermiculite were cold stratified for 11 weeks and then sown into flats filled with planting medium. This medium consisted of a mixture of bark, compost, peat, perlite, and Philip's pre-mix (containing crabmeal, 3 kinds of lime, micronutirents, Actino-iron, and wetting agent). After germination (typically within 7 days of sowing) and establishment in flats, seedlings were then transplanted into 5 inch x 2 3/8 inch pots. Boyer reported excellent seedling survival and growth, which she attributed to the lack of competition under cultivation.

Aster curtus has also been successfully propagated using rhizome cuttings. Giblin and Hamilton (1999) reported the use of rhizome cuttings to obtain large plants, which were grown outdoors in 10 cm plastic pots containing a standard commercial potting mix (1:1 peat:perlite), fertilized with pelleted 10:10:10 (N:P:K) slow-release fertilizer, and

irrigated as necessary. Kaye et al. (2001) also used rhizome cuttings to obtain *A. curtus* plugs, using standard propagation techniques. The authors reported high levels of plug survival in the greenhouse, and noted that, although larger rhizome cuttings (>10 cm long) yielded larger *A. curtus* plugs than smaller rhizome cuttings (3 cm long), smaller rhizomes still yielded healthy individuals that were later used in population introduction trials (see "Transplanting and introduction attempts," below).

Transplanting and introduction attempts

Perhaps facilitated by its inherently rhizomatous, vigorously clonal nature, attempts to transplant *Aster curtus* have generally proven very successful. Although Alverson (1983) experienced unsuccessful transplanting of 12 plants from Willow Creek Preserve to Cogswell Foster Preserve in Linn County, he also stated that *A. curtus* was successfully introduced into a garden setting by Kruckeberg. Gamon and Salstrom (1992) reported the occurrence of two *A. curtus* transplant attempts from areas slated for development in Washington. One attempt used heavy equipment to excavate plants and 6-8 inches of underlying soil, while the other attempt employed hand digging with shovels. Target sites were prepared by removing existing vegetation and a thin layer of soil, and in both cases transplants survived over a two-year monitoring period. A similar transplant project was carried out in West Eugene in 2000 to protect *A. curtus* from a highway and bike path construction project. Here, a small *A. curtus* colony was excavated using heavy equipment and placed into a nearby target site. Post-transplant monitoring showed continued survival of the transplanted colony over a two-year monitoring period (Thomas Kaye, Institute for Applied Ecology, Corvallis, Oregon, and Jean Battle, BLM, Eugene, Oregon, personal communication).

In addition to moving *Aster curtus* from existing sites to new locations, transplanting has also been employed as a means of establishing new populations, using plugs cultivated in the greenhouse. Kaye et al. (2001) cultivated 110 individuals in the greenhouse from rhizome cuttings and transplanted them to two sites near Eugene, Oregon (these efforts were part of a mitigation project related to the aforementioned population disturbance caused by the West Eugene bike path project). Survival at the Greenhill Road population

was measured at 75 percent after six months, and 29 of 30 original transplants survived at the Beaver Run population. Among these transplants, the authors found that individuals grown from small (3 cm) rhizome cuttings produced smaller rosettes and fewer racemes than those grown from larger (> 10 cm) rhizome cuttings, but the they were encouraged by the performance of smaller cuttings because they utilized less parent plant material to create them. Application of fertilizer and weeding treatments to a subset of *A. curtus* transplants did not significantly enhance transplant survival.

Despite the successful record of transplanting *Aster curtus*, establishing the species in the wild using broadcast seeds has proven far more problematic. Clampitt (1984) observed no seedlings at all in 17 of 18 outdoor seeding plots containing 100 seeds each. Seeding was also attempted by Clark et al. (2001) in burned and unburned plots at the Danebo Wetland near Eugene, Oregon. Here, a total of 300 seeds were sown, resulting in 3.4 and 8.4 percent establishment in burned and unburned plots, respectively. Low seedling recruitment rates were also observed by Kaye et al. (2001), who reported no successful seedling recruitment at all from direct seeding experiments in undisturbed plots at Greenhill Road (near Eugene, Oregon) and less than O.7 percent seedling recruitment in prepared bare soil plots (based on 50 seeds per plot). Seedling recruitment was reported as 6.7 and 5.5 percent in undisturbed and bare soil plots, respectively, at the nearby Beaver Run site (based on 180 seeds per plot).

Population monitoring

Monitoring of *Aster curtus* can be seriously confounded by its propensity for expansion via rhizomes. According to Alverson (1983), "seldom is the extent of a single genetic individual evident in the field, so demographic details refer to the number of shoots, rather than number of individuals." To date, monitoring of *A. curtus* has been undertaken at a number of different sites in Oregon and Washington. Consistent with ongoing monitoring of populations in Eugene, Oregon (conducted and/or sponsored by Eugene District BLM), the most effective monitoring method for larger populations appears to be measuring the frequency of occupation (i.e., presence or absence) within permanent 1 meter square sampling plots, rather than attempting to delineate individuals and/or to

count the number of shoots. In smaller colonies, counting all individual shoots might be a more feasible and ecologically meaningful population estimate.

Land use threats and other limitations

Habitat loss to urban and agricultural development, and habitat degradation by invasive species and anthropogenic hydrologic alterations, pose the greatest threats to *Aster curtus*. According to Giblin and Hamilton (1999), over 95 percent of this species' gravelly prairie habitat in western Washington has been destroyed or altered. Habitat loss is also identified as the primary threat to populations in Oregon (Siddall *et al*. 1978, Alverson 1983) and British Columbia (Douglas and Illingworth 1997).

Even when habitats are not directly destroyed by development, habitat disturbance can encourage colonization by invasive weeds. At the DuPont population in Washington, *Aster curtus* is mainly restricted to areas not occupied by scotch broom (ACOE 1979). Based upon competition experiments, Clampitt (1987) stated that competition with non-native species is probably the most important factor restricting population recruitment in *A. curtus*, more important even than low levels of seed production. Specifically, Clampitt (1993) found that habitat disturbance favors replacement of native bunchgrasses with non-native sod-forming grasses, and that *A. curtus* tends to be present only where cover of native bunchgrasses exceeds 32 percent.

Although loss of habitat has been identified as a primary threat to *Aster curtus*, it is fortunate that many of this species' extant populations occur on public lands, which provide some level of administrative security against future population declines. According to Gamon and Salstrom (1992), 19 populations in Washington are located within Fort Lewis (U.S. Army), 6 sites are managed by Washington State, and 2 occur on county lands. In Oregon, the U.S. Army Corps of Engineers owns 7 population sites, the BLM owns 1 site, The Nature Conservancy manages 2 sites, 1 site is owned by the State of Oregon, and 1 site is owned by the City of Eugene. Given the number of federal and other publicly owned sites for this species, land ownership may prove favorable to future

recovery efforts involving habitat management and population introduction/augmentation projects.

Population introduction/augmentation strategy

Based upon the biogeographical data compiled and described above for *Aster curtus*, 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. Although many *A. curtus* populations face imminent threats on private lands, and native prairie habitats have been reduced to tiny remnants of their former abundance, there are still an encouraging number of extant *A. curtus* populations that occur on public or otherwise secure landholdings. As such, pending interagency cooperation and funding availability, there should be numerous sites available for collection of seeds and/or rhizome cuttings for use in off-site cultivation projects, and open locations should also be available for population augmentation and introduction purposes. Perhaps the most serious environmental constraint to these much-needed conservation efforts is the proliferation of invasive weeds, which already pose a formidable threat to existing populations and limit the quality and availability of suitable introduction sites. However, while invasive species will continue to pose a challenge to habitat managers, there are still high quality prairie remnants remaining throughout the range of the species that can provide valuable opportunities for *A. curtus* population introduction projects.

The biology and life history of *Aster curtus* likewise pose no unavoidable hurdles to successful implementation of population introduction and augmentation projects. Although production of viable seeds is low in this species, seed germination rates among filled seeds generally range between 50-94 percent (when seeds are properly cold stratified and/or manually scarified), and *A. curtus* has been successfully cultivated from both seeds and rhizome cuttings in the greenhouse. The species exhibits no unique propagation or soil symbiont requirements. Once mature *A. curtus* plugs are obtained through off-site cultivation (which can occur as fast as 3 months from seed germination), they have demonstrated very high rates of survival when introduced into the wild. In

contrast, population introduction attempts employing direct seed sowing have proven less successful.

Although *Aster curtus* lends itself to vegetative propagation in the greenhouse and forms extensive clones in the wild, efforts should be made to maximize the frequency of genetically variable individuals when creating or augmenting populations, because preliminary evidence indicates at least some level of self-incompatibility and inbreeding depression in this species. Therefore, genetically diverse introduction stock should be used whenever possible to elevate seed production and reproductive fitness, and also ostensibly improve the odds of overall introduction success by maximizing the amount of adaptive genetic variability in the population. Interspecific hybridization does not appear to pose a serious concern for *A. curtus*, as long as introduction target sites are limited to the current range and habitat occupied by the species.

Based upon this information, the following step-down procedures are recommended for *Aster curtus* population introductions:

1. Select population introduction/augmentation target sites. Several primary factors should be considered when selecting target sites for *Aster curtus* population introduction and augmentation projects. First, target sites should obviously contain suitable habitat. However, as habitat for *A. curtus* varies throughout its range, especially in relation to soil types (i.e., poorly drained clay soils in the southern part of its range, gravelly glacial outwash soils in the central portion of its range, and shallow soil overlying basalt in the northern part of its range), habitat suitability should be evaluated based upon visits to local extant *A. curtus* populations, rather than range-wide habitat generalizations. One of the most important overriding habitat features for *A. curtus* appears to be the presence and frequency of native bunchgrasses. Based upon a review of extant populations in Washington, Clampitt (1993) suggested native bunchgrass cover below 32 percent probably indicates competitively unsuitable conditions for *A. curtus*.

Given the unfortunate demise of *Aster curtus* on private lands, inventories for suitable population introduction and augmentation sites should be focused strictly to publicly owned (or otherwise secure) lands. Selection and use of sites should be coordinated with pertinent public landowners to ensure administrative protection and management of populations following introductions.

2. Collect *Aster curtus* seeds and/or rhizome cuttings for off-site cultivation of introduction stock. Source material for off-site cultivation of *Aster curtus* should be collected from the extant population(s) located nearest to the population introduction target sites to minimize undesirable mixing of gene pools and maximize conveyance of potential local adaptations (if such intraspecific variability and adaptations exist). Given low levels of seed production documented in *A. curtus*, and the tendency for only 10-30 percent of ramets within populations to flower in any given year, seed collecting should be planned and implemented well in advance of cultivation projects to ensure adequate time (possibly several consecutive years) for the harvest of sufficient seed supplies. Based upon historic seed production estimates, individual *A. curtus* flowering heads can be expected to produce a total of 8-118 filled seeds, which in turn represent only a fraction of the total number of *unfilled* and predated achenes that are produced.

   In light of preliminary evidence of self-incompatibility and inbreeding depression documented in *Aster curtus*, efforts should be made to collect seeds and/or rhizome cuttings from as large a sample of genetically variable individuals as possible, in an effort to elevate seed production, fitness, and adaptive genetic variability within introduced populations. As described below, if rhizome cuttings are used, they can be harvested in sizes as small as 3 cm, though if available, larger (i.e., 10 cm long) cuttings have been shown to yield larger cultivated plugs.

3. Cultivate *Aster curtus*. *Aster curtus* has been successfully cultivated from both seeds and rhizome cuttings. If seeds are used for cultivation of introduction stock, previous studies suggest they should be cold stratified at 5°C for 45 days, and/or scarified at the pappus end of each achene, to maximize germination rates. Following pre-treatment, seeds should be expected to germinate within 2-11 days, at rates ranging between 50-94 percent. Seeds require light for germination, so they should not be buried during sowing or kept in a dark place during the germination period.

   If rhizome fragments are used for propagation of *Aster curtus*, previous studies suggest larger fragments produce larger plants with more stems and flowers, but these studies also report that smaller fragments (as small as 3cm) can be used successfully. The latter size may prove especially valuable in instances when source populations are very small and incapable of sustaining the harvest of larger rhizome cuttings.

4. Introduce cultivated plugs into the target site(s). *Aster curtus* introductions should be performed after the arrival of fall rains, so that soils are moist at the time of planting and plugs have ample opportunity for root system development prior to summer drying. Although previous research was unable to provide evidence that *A. curtus* is pollen-limited in the wild (even in very small patches), it may be beneficial to introduce plugs into large patches in order to improve the prospects of pollinator attraction to floral rewards (though larger patches have also been shown to attract more seed predators than smaller patches). The relative reproductive costs and benefits of different patch sizes used in introduction projects would be an interesting and valuable area of future research. Finally, the layout of introduced plugs should be designed in a manner that is consistent with subsequent population monitoring objectives (see #5, below).

5. Monitor introduced populations. Introduced *Aster curtus* plugs should be monitored annually to evaluate project success. Given the clonal nature of the species (i.e., asexual expansion via rhizomes), which can seriously complicate the definition of individuals, monitoring should probably be carried out in a way that simply estimates presence or absence, or overall *A. curtus* cover, within sampling plots. Counting the number of individual stems and/or attempting to discriminate individuals should probably only be attempted when introduced populations are very small and introduced plugs are widely spaced so that they can be reliably differentiated over time (though even in these cases stem counting may become infeasible if clones eventually become large and intertwined).

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Developing biogeographically based population introduction protocols for at-risk Willamette Valley plant species:

Aster vialis (wayside aster)

Aster vialis (wayside aster)

Conservation status

Although *Aster vialis* (Figure 7) has not yet become as uncommon or locally restricted as some of the other rare western Oregon species treated in this manual, its habitat is nevertheless becoming rapidly consumed by residential development and degraded by anthropogenic fire suppression practices and invasive species. In recognition of ongoing population declines, this rare member of the sunflower family (Asteraceae) has been listed as a Species of Concern by the U.S. Fish and Wildlife Service, and is listed as Threatened by the State of Oregon. It is on the Oregon Natural Heritage Program 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) (ONHP 2001).

Figure 7. *Aster vialis*. (Photo by Steven Gisler.)

Unlike most threatened plant species found in the Willamette Valley, *Aster vialis* does not occupy valley floor prairie habitats, but rather, occurs in drier, more upland, mixed woodland and coniferous forest habitats. These areas have been slower than agriculturally valuable valley bottom prairie habitats to suffer the adverse impacts of human occupation, though *A. vialis* is becoming increasingly constrained by habitat loss and population fragmentation associated with expanding residential development and timber harvest activities. Fortunately, the majority of extant *A. vialis* populations are now protected from development pressures due to their location on public (primarily federal) lands. However, *A. vialis* still faces threats that transcend land ownership boundaries, such as: fire suppression practices that result in excessive understory brush growth and forest canopy closure, the proliferation of invasive weeds (most notably *Rubus discolor* and *Cytisus scoparius*), low seed production and inbreeding depression, herbivory by deer, and pre-dispersal seed predation (Ho 1985, Gamon 1986, Alverson and Kuykendall 1989, Kaye and Rebischke 1995, Wogen 1998, ONHP 2002).

Range and habitat

It is an interesting coincidence that *Aster vialis* was independently discovered by two different botanists in the same year. In 1918, Frank Sipe from the University of Oregon found *A. vialis* "about 10 miles from Eugene, Oregon, on rather stony hillsides, in open woods of *Pseudotsuga*, *Arbutus* and *Castanopsis* (from Henderson 1933)." Simultaneous with Sipe's discovery, R.V. Bradshaw (1921) obtained *A. vialis* specimens on Skinner's Butte, also near Eugene, in habitat he characterized as "open woods at the summit, under the Douglas firs." After the late 1930's, repeated attempts to relocate the species were unsuccessful until its eventual discovery by Georgia Mason, former curator of the University of Oregon herbarium, who found it growing in a large patch of poison oak on Mount Pisgah, again near Eugene (Wyant 1980).

Since its rediscovery in 1980, extensive surveys have been conducted for *Aster vialis*, fortunately resulting in the identification of numerous additional populations. Currently, the Oregon Natural Heritage Program has 74 population records for *Aster vialis* in its database, spanning Douglas, Jackson, Lane, and Linn Counties, Oregon (ONHP 2002).

Recently, *A. vialis* was also recorded as far south as Del Norte County, California (Figure 8), where, according to Kenton Chambers (Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon, personal communication), the species appears to hybridize and intergrade with the closely related *A. brickellioides*, and possibly even with a third, currently unrecognized species, *A. siskiyouensis*. Given this broad geographical distribution, the Willamette Valley really represents the extreme northernmost expression of this species' geographic range.

Detailed descriptions of *Aster vialis* habitat have been provided for 5 extant populations by Ho (1985), 25 sites by Alverson and Kuykendall (1989) and 7 sites by Kaye and Rebischke (1995). Based upon these descriptions, data compiled from OSU herbarium specimen labels, and element occurrence records on file at the Oregon Natural Heritage Program (ONHP 2002), "typical habitat" for *A. vialis* consists of relatively open areas in the understory of mixed coniferous/hardwood forests, along roadsides (Figure 9), and on open slopes and prairie balds.

Figure 8. Photograph of pressed *Aster vialis* voucher specimen recently collected from California by Richard Brock (species determination by Ken Chambers), near the town of Happy Camp in Del Norte County.

In the preceding paragraph, "typical habitat" is enclosed by quotation marks because, according to K. Chambers (personal communication), it is impossible to make such a broad generalization for a species occupying such a large range of latitudes, elevations (spanning 2657 feet, see following paragraph), and habitat affinities, the latter encompassing dense coniferous forests, open deciduous woodlands, grassy balds, and exposed serpentine slopes.

Figure 9. An example of coniferous forest habitat occupied by *Aster vialis*, shown here along the edge of a roadcut near Eugene, Oregon. (Photo by Tom Kaye.)

Aster vialis occupies sites spanning a large range of elevations. Most populations occur between 150-450 meters, although one unusually high site (Buck Mountain) occurs at 960 meters. Likewise, as occupied habitats are so variable, there is a broad range of species found in association with *A. vialis*. Overstory tree species reported in association with *A. vialis* include: *Abies grandis*, *Acer macrophyllum*, *Alnus rubra*, *Arbutus menziesii*, *Chrysolepis chrysophylla*, *Cornus nuttallii*, *Corylus cornuta*, *Prunus virginiana*, *Pseudotsuga menziesii*, *Quercus garryana*, *Rhamnus purshiana*, *Thuja*

plicata, *Tsuga heterophylla*, and (in the southern portion of its range) *Quercus keloggii*. Commonly associated understory species include: *Acer circinatum*, *Achlys triphylla*, *Amelanchier alnifolia*, *Berberis nervosa*, *Ceonothus velutinus*, *Cytisus scoparius*, *Gaultheria shallon*, *Holodiscus discolor*, *Lathyrus nevadensis*, *Linnaea borealis*, *Lonicera hispidula*, *Oxalis suksdorfii*, *Polystichum munitum, Pteridium aquilinum*, *Rhus diversiloba*, *Rubus laciniatus*, *R. parviflorus*, *R. ursinus*, *Symphoricarpus alba*, *Thermopsis montanum*, and *Vancouveria hexandra*. For populations in Douglas County, Kaye (1993) reported the following additional associated species: *Arctostaphylos* sp., *Castilleja* sp., *Ceanothus integerrimus*, *Lathyrus polyphyllus, Lupinus sulphureus* ssp. *Kincaidii*, *Phlox adsurgens*, *Psoralea physodes, Sanicula crassicaulis*, *Satureja douglasii*, and *Whipplea modesta*.

With regard to the coniferous forest types occupied by *Aster vialis*, Kaye and Rebischke (1995) found Lane County populations located within a wide range of forest compositions and successional stages, including recent clear-cuts, dense second growth, and structurally diverse stands of old growth. According to Alverson and Kuykendall (1989), Kaye (1993), Kaye and Rebischke (1995) and Wogen (1998), there is evidence that the open habitat preferred by *A. vialis* was historically maintained by frequent fire return intervals, and that plant reproduction and vigor, and seedling recruitment, are inversely proportional to canopy closure. As such, successional canopy closure due to fire suppression is considered a primary threat to this species.

Description of species

Aster vialis is a perennial species with flowering stems typically 6-12 dm tall, the lowermost stem leaves reduced and scale-like, the others numerous and nearly alike, gradually reduced toward the inflorescence, elliptic to broadly lanceolate, sessile, entire or with a few irregular sharp teeth, glabrous above and glandular beneath. The flower heads are turbinate, several or many in leafy-bracteate inflorescences, with disk flowers 1-1.5 cm wide, ray flowers wanting (or with infrequent vestigial ray flowers [Kuykendall 1991]), and involucres 8-10 mm in 3-4 series, usually pale and chartaceous, well

imbricate, and sharp pointed with a strong midvein. Achenes are oblong, flattened, and villous with somewhat appressed hairs (Bradshaw 1921, Hitchcock *et al.* 1955).

According to Gamon (1986), *Aster vialis* is similar in appearance to *A. oregonensis*, but the former can be distinguished by its yellow disc flowers, lack of ray flowers (Figures 7, 8 and 10), and its smoother leaf surfaces. *Aster vialis* is also very similar to *A. brickellioides*, which tends to have somewhat smaller rayless heads, and *A. engelmannii,* which is geographically and ecologically distinct from *A. vialis*. According to K. Chambers (personal communication), *A. vialis* appears to intergrade with *A. brickellioides* in southern Oregon/northern California—a region harboring a confusing continuum of intermediate *Aster* morphotypes. As such, it can be difficult in some cases to recognize taxonomic boundaries in this confusing species group.

Seed production

Although there have been numerous reported observations of seed production in *Aster vialis* (i.e., Gamon 1986, ONHP 2002), the first effort to quantify seed production in this species was apparently undertaken by Kuykendall (1991) at the Greenhill Road population near Eugene, Oregon. Here, Kuykendall documented a mean 31.5 percent filled achene production per open-pollinated flower head (equating to a mean 6.6 achenes per head), accompanied by a mean 28.4 percent fruit damage by predators and a mean 37.1 percent fruit abortion (see "Breeding system," below, for additional study details).

Additional *Aster vialis* seed production studies were later carried out by Kaye (2002), who found that the number of ovules per flower head (ranging from 7-19) varied among four study populations (2 large populations and 2 small populations). Likewise, Kaye showed that seed production varied between populations, ranging from 0.1 percent to 4.3 percent of available ovules (equating to a mere 0.05 to 0.7 seeds per flower head). Kaye reported no evidence of a significant correlation between these reproductive parameters and population size. The achene production values documented by Kaye are markedly lower than those reported above by Kuykendall (1991), and it is unknown to what extent these differences are due to variability in fecundity between different populations,

different sampling years, and/or different methods of defining filled achenes (i.e., x-ray analysis utilized by Kaye showed that some seemingly "filled" achenes actually lack fully developed embryos). To put these seed production measurements into perspective, Kaye cited Jones (1978), who reviewed seed production in 30 perennial *Aster* species and found seed set highest (70 percent or more) in weedy species, and only 20-30 percent in most native species. As such, *A. vialis* exhibits unusually low fecundity, even compared to other native *Aster* species.

Despite low fecundity in *Aster vialis*, reported observations of seedlings and young plants in natural populations suggest that the few seeds that are produced by *A. vialis* contribute to seedling recruitment and establishment. For example, Ho (1985) reported observing "many seedlings on bare soil along roadcut," at one site, Gamon (1986) documented the presence of seedlings and younger plants at 5 of 15 visited sites, and surveys conducted by Kaye and Rebischke (1995) indicated the frequent presence of both older reproductive plants and a preponderance of plants in younger, non-reproductive age classes.

Seed germination

The earliest study of seed germination in *Aster vialis* was reported by Guerrant (1991), who consistently achieved less than 3 percent seed germination under room temperature and various cold stratification treatments. In an effort to elevate germination rates, Guerrant also tried exposing seeds to a series of experimental heat treatments. Here, seeds were exposed to three different heat levels (50°C, 75°C and 100°C) in hot water baths. Germination was mildly improved (up to 15 percent) in the mildest heat treatment (50°C), though due to variability between replicates, Guerrant suggested this trend should be viewed with skepticism. Higher temperatures (those above 50°C) reduced seed germination below levels of experimental controls.

Fortunately, subsequent *Aster vialis* seed germination trials by other researchers proved more successful, showing that seed germination rates in this rare species can actually be quite high. For example, Gasser (1999) achieved 60-80 percent germination by cold stratifying seeds for 8 weeks, though the same cold stratification treatment yielded less

than 10 percent germination when employed on seeds from a different source population. Gasser also investigated the use of embryo excision in *A. vialis*, which resulted in 100 percent germination in one seed lot, but zero percent in another. Like Gasser, Kaye (2002) also found that seed germination can be maximized by excising embryos from the fruit coat, seed coat, and inner membrane. Using this painstaking method, over half the embryos in Kaye's study germinated within 5 days, and by three weeks there was over 93 percent germination. Also like Gasser, Kaye was able to break *A. vialis* seed dormancy with cold stratification, with optimal germination (63 percent) occurring when seeds were chilled at 5°C for 12 weeks and then exposed to alternating warm temperatures (15°C/25°C). Seed germination was increased to 70 percent when cold stratified seeds were also scarified (clipped at the pappus end).

For *Aster vialis* seeds in the wild, it has been hypothesized (though not yet tested) that germination and seedling recruitment may be stimulated by light availability accompanying exposure (i.e., duff removal) through soil disturbance and/or fire (Kaye and Rebischke 1995, Wogen 1998).

Vegetative reproduction

Aster vialis is a rhizomatous species that often forms clumped ramets (Kuykendall 1991), and, according to Kaye *et al*. (1991), the species may rely primarily on vegetative reproduction for population maintenance because persistent seed banks are seemingly not produced.

Given past confusion over the frequency and accurate identification of genetically distinct individuals within populations, Erhart and Liston (2001) attempted to evaluate patterns of clonal spread in populations using DNA analysis techniques (ISSRs). Although their study provided evidence indicating extensive vegetative reproduction within populations, the authors found the data on the spatial distribution of clones confusing, with some shared haplotypes (i.e., putatitive clones) frequently interspersed among distinct haplotypes (i.e., different genets). These findings led the authors to suggest a possible "guerilla" clonal growth pattern in the species.

Breeding system

The earliest documented reference to the breeding system of *Aster vialis* was by Gamon (1986), who suggested that the species' rayless flowers might indicate a predisposition towards self-pollination. In contrast, Alverson and Kuykendall (1989) suggested that the species might be an outcrosser, despite the lack of showy ray flowers. To help resolve this compelling uncertainty, Kuykendall (1991) (also reported in Kaye et al. 1991) investigated *A. vialis*' breeding system and found the species to be highly self-incompatible and an obligate outcrosser. Kuykendall's data showed fruit set (percent total ovules converted to filled seeds) was less than 2 percent when flowers were excluded from pollinators and manually self-pollinated (Figure 10), 21 percent when manually cross-pollinated, and 31.5 percent when open-pollinated by insects.

Figure 10. Manual self-pollination of *Aster vialis*. (Photo by Wes Messinger.)

According to Alverson and Kuykendall (1989), the predominant insect visitors of *Aster vialis* are bumblebees (*Bombus vosnesenski*), the solitary bee, *Lasioglossum* sp., and skippers (*Ochlodes sylvanoides*). The authors noted:

> "In all, Andy Moldenke [Entomology Department, OSU] considered this to be a rather motley assemblage of flower visitors, a reflection of the lateness of the flowering season and the scarcity of flowers in the habitat."

Given the results reported above by Kuykendall (1991), the breeding system of *Aster vialis* is consistent with most other perennial Asters of North America, which are predominantly self-incompatible (Jones 1978). In addition to self-incompatibility, which may result in reduced seed set if compatible mating types are absent or infrequent in populations, there is some evidence that *A. vialis* may also suffer inbreeding depression, even when compatible mating types are present. Kuykendall (1991) noted that fruit set was higher (29 percent) for between-population crosses than within-population crosses (21 percent). However, it is uncertain if the reduced within-population fecundity reported by Kuykendall might, to some extent, actually reflect self-incompatibility rather than inbreeding depression *per se*, because genets and ramets can be randomly (and confusingly) dispersed within populations and impossible to differentiate using morphological markers (Erhart and Liston 2001). As such, separate "individuals" within populations used for manual cross-pollinations might actually represent genetically identical ramets, and thus lead to self-incompatibility reductions to seed production.

Hybridization

To date there have been no formal studies of hybridization in *Aster vialis*, and circumstantial evidence renders the issue unresolved. On one hand, Gamon (1986) cites Cronquist (1955), stating,

> "There appear to be some interspecific crosses within the Section Eucephalus [in which *A. vialis* belongs], producing intermediate forms. Some crosses involve those species which are thought to be most closely related to *Aster vialis*, although none involving *A. vialis* have been noted."

This statement mirrors that of Thompson (1977), who suggested that, although members of the section Eucephalus are closely related and interfertile, *A. vialis* is isolated from other close relatives in the Willamette Valley, its nearest relative located at much higher elevations in the Cascade Mountains. Thompson stated that two other *Aster* species, *A. oregonensis* and *A. brickellioides*, occur within the geographic range of *A. vialis*, but

there have been no suggestions that they are adequately sympatric for hybridization to occur between them.

In contrast to these sentiments, K. Chambers (personal communication) suggested that hybridization in *A. vialis* is *very* likely in northern California and southern Oregon, a conclusion based on his extensive review of morphological intermediacy of *Aster* specimens collected from the area. These intermediate specimens led Chambers to propose that hybridization is probably widespread between *A. vialis* and *A. brickellioides* in southern Oregon, and possibly also involving the currently unrecognized species, *A. siskiyouensis*.

In light of this evidence, it appears that *Aster vialis* may experience (or at least be susceptible to) some degree of hybridization in the extreme southern portion of its range, but hybridization is unlikely to the north, particularly the Willamette Valley, where there are no closely related sympatric species.

Cultivation

Aside from low seed production, there do not appear to be any serious obstacles to off-site cultivation of *Aster vialis*. Kaye et al. (1991) reported successfully cultivating *A. vialis* from seed in the greenhouse, and noted that cultivated plants closely resembled specimens in the wild, producing numerous flowering heads. Later, Kaye (2002) conducted a larger-scale cultivation project, and stated that "container-plant propagation from seed is an effective way of producing a large number of genetically diverse plants." Kaye found that seedlings cultivated in small, deep pots (2x2x5 inch), with organic potting soil and bi-weekly liquid fertilizer application, had high establishment rates and grew to flowering in about three months. He also reported that fertilizing plants improved flowering and branching, and reduced impacts of simulated herbivory (clipping of stems). Whereas *A. vialis* grew most rapidly in the greenhouse, Kaye also showed that the species will also grow (albeit more slowly) in an outdoor, unheated environment.

According to Kaye (2002), tissue culture methods have also been developed for *Aster vialis* by the Center for Research of Endangered Wildlife at the Cincinnati Zoo and Botanical Garden. Tissue culture protocols, described by Clark (1997), involve removing shoot tips from living plants and placing them in rooting media enriched with nutrients and plant growth hormones, with resulting rooting percentages as high as 97.5 percent. Clark stated that, after rooting, cultured plants are fragile and must be acclimatized prior to placement in potting soil. Kaye (2002) noted that one drawback of plant production using tissue culture is that the process yields lines of clones which would be unable to cross-pollinate if introduced into the wild, due to *A. vialis* sexual self-incompatibility. Kaye also suggested that the tissue culture methods are very involved and that use of seeds (especially in light of high germination rates) is an easier and more efficient method of generating new plants.

Although cultivation of *Aster vialis* can be successfully performed using seeds, it may also be possible to propagate the species using rhizome cuttings. To date this method of propagation has not been attempted (or at least not reported). If cuttings were used to propagate *A. vialis*, however, this would entail the same clonal/self-incompatibility drawbacks noted above by Kaye for propagation by tissue culture.

Transplanting and introduction attempts

According to Gamon (1986), *Aster vialis* "transplants quite easily, due largely to its fibrous root system," and notes that individuals have been successfully transplanted from the Woodsia Lane population to the Berry Botanic Garden and to the Mt. Pisgah Arboretum. Similarly, Thomas Kaye (Institute for Applied Ecology, Corvallis, Oregon, personal communication) reported very high levels of survival and establishment among container-grown *A. vialis* transplanted into the wild, in contrast to very low levels of seedling recruitment from broadcast seeds.

Population monitoring

According to BLM (2001), population monitoring for *Aster vialis* has been occurring discontinuously at various populations since 1989. The most comprehensive description of monitoring methods is described in Kaye et al. (1991), whereby all *A. vialis* individuals at seven populations were tagged, mapped, and measured (measurements included stem height, number capitula per stem, number stems per individual, and presence/absence of herbivory). Based upon this ongoing, but discontinuous monitoring, Kaye (2000) concluded, "short term demography suggests that the populations do not have dramatic swings in size." Given the inherent difficulties in monitoring clonal species like *A. vialis*, monitoring methods should probably avoid the complications of attempting to identify individuals, and instead focus on either estimation of *A. vialis* cover within fixed sampling plots, or censuses of *A. vialis* stems in different size/age classes.

Land use threats and other limitations

Although *Aster vialis* has declined from historical levels in the wake of residential development and timber harvest activities on private lands, many of the known remaining populations are located on public lands where they should be relatively insulated from these threats. According to element occurrence records provided by ONHP (2002), of the 74 known *A. vialis* occurrences, 42 are located on federal lands, 12 on county lands, 2 on state lands, and 1 on city land.

Despite this predominance of public ownership, however, several important *Aster vialis* threats largely transcend land ownership boundaries. Most notable among these threats are: fire suppression leading to excessive understory brush encroachment and canopy closure, the proliferation of invasive weeds (most notably *Rubus discolor* and *Cytisus scoparius*), low fecundity and inbreeding depression, herbivory by deer, and seed predation (see, for example, Ho 1985, Gamon 1986, Kuykendall and Alverson 1989, Kaye and Rebischke 1995, Wogen 1998).

Fortunately, public ownership of known *Aster vialis* populations may lend itself to the conservation and recovery of this species, if the aforementioned threats can eventually be ameliorated through development and implementation of habitat management and population introduction/augmentation plans. Future recovery efforts may be further encouraged by the fact that the *A. vialis* is capable of occupying a broad range of habitats and successional forest stages (Kaye and Rebischke 1995), and can be successfully cultivated for introduction/augmentation projects (Kaye 2002).

Population introduction/augmentation strategy

Based upon the biogeographical data compiled and described above for *Aster vialis*, there do not appear to be any insurmountable ecological, life history, or administrative obstacles to the successful implementation of population introduction and augmentation projects for this rare species. Most extant *A. vialis* populations are publicly owned, so, pending interagency cooperation and funding availability, sites should be available for collection of seeds and/or tissue samples (for tissue culture) for use in off-site cultivation, and suitable locations should also be available for population augmentation and introduction purposes. The primary environmental constraint to these much-needed conservation efforts is the proliferation of invasive weeds (particularly scotch broom), which already pose a serious threat to existing populations. However, given the broad range of habitats that *A. vialis* occupies, and the fact that many undisturbed and non-weedy sites still remain throughout its large geographic range, there should be ample locations available for population introduction projects.

Although low seed production in *Aster vialis* poses a significant limitation to the number of seeds that can be collected and used in a single year for off-site cultivation projects, this complication can be surmounted using sustainable seed collecting practices over multiple years prior to project implementation. Once adequate seed supplies are available, there are no apparent cultivation-related obstacles to implementation of introduction projects; seed germination is relatively high in this species (typically exceeding 60 percent following cold stratification), and the species exhibits no specialized edaphic or symbiont requirements for successful growth in cultivation.

Moreover, past research has shown this species grows quickly from seed in the greenhouse, and exhibits high survival and establishment rates when introduced into the wild.

Although *Aster vialis* exhibits clonal growth in the wild, efforts should be made to maximize the frequency of genetically different individuals in introduced or augmented populations, because previous research has shown this species is sexually self-incompatible and may exhibit inbreeding depression. Therefore, genetically diverse introduction stock should be used whenever possible to elevate seed production and reproductive fitness, and also ostensibly improve the odds of overall introduction success by enhancing the level of adaptive genetic variability harbored within populations.

One factor that should be taken into consideration during *Aster vialis* introduction projects is interspecific hybridization. Although hybridization is probably not a serious threat in the northern portion of the species' range, due to lack of sympatric heterospecific mating partners, hybridization is probably already occurring in the southern portion of the species' range near the Oregon/California border. It is unknown to what extent this hybridization might be facilitated by human habitat disturbance, which can contribute to "unnatural" species' interactions and the creation of novel microsites for hybrid establishment. Ultimately, to avoid the potentially adverse conservation implications of hybridization that could be inadvertently promoted by artificial population introduction projects, care should be taken to select introduction target sites that are isolated from other related *Aster* congeners.

Based upon this information, the following step-down procedures are recommended for *Aster vialis* population introductions:

1. Select population introduction/augmentation target sites. Several primary factors should be considered when selecting target sites for *Aster vialis* population introduction and augmentation projects. First, target sites should obviously contain suitable *A. vialis* habitat. However, as habitat for this species varies

considerably throughout its large geographic and ecological range (particularly in relation to various factors including vegetation community type, elevation, and soils), it may be difficult to make generalized predictions of habitat suitability. To assist in identification of suitable habitat, extant *A. vialis* populations in the vicinity of target sites should be visited to obtain familiarity with local habitat attributes and microsite characteristics.

Given the history of *Aster vialis* habitat destruction on private lands, and the ubiquitous threat posed by invasive species, inventories for suitable population introduction and augmentation sites should be focused strictly to publicly owned (or otherwise secure) lands that appear safe from imminent weed and successional encroachment problems. Selection and use of sites should be coordinated with pertinent public landowners to ensure administrative protection and management of populations following introductions.

2. Collect *Aster vialis* seeds and/or tissue for off-site cultivation of introduction stock. Source material for off-site cultivation of *Aster vialis* should be collected from the extant population(s) located nearest to the population introduction target sites to minimize undesirable mixing of gene pools and capitalize upon potential local adaptations (if such intraspecific variability and adaptations exist). Given the extremely low levels of seed production documented in *A. vialis*, seed collecting should be planned and implemented well in advance of introduction project dates to ensure adequate time (possibly several consecutive years) for harvest of sufficient seed supplies. Based upon historic seed production estimates, individual *A. vialis* flower heads can be expected to produce a total of only 0.05-6.6 filled seeds, which in turn represent only a small fraction of each head's total number (7-19) of available ovules.

   In light of the evidence of self-incompatibility and inbreeding depression in *Aster vialis*, efforts should also be made to collect seeds and/or tissue (for tissue culture) from as large a sample of individuals as possible, in an effort to elevate seed

production, fitness, and adaptive genetic variability within introduced populations. If the introduction target sites have several closely neighboring extant *A. vialis* populations, it may be worth considering the use of multiple local seed sources, thus further increasing the likelihood of capturing genetic variability and suitable mating partners within the introduced population.

3. Cultivate *Aster vialis*. *Aster vialis* has been successfully cultivated from both seeds and tissue culture, and it may be possible to propagate the species using rhizome cuttings. Seeds appear to be the most effective means of obtaining cultivated plugs. For optimal germination, previous studies suggest seeds should be cold stratified at 5°C for 8-12 weeks, with subsequent germination rates of 60-70 percent. Higher germination rates can be obtained using embryo excision techniques, though this is a time consuming and laborious process. *Aster vialis* has been shown to benefit from fertilization during cultivation, and can attain flowering status (from seed) after only 3 months in cultivation (slightly longer if cultivated outdoors).

   Because tissue culture produces genetically identical plants, and because *Aster vialis* has been shown to be sexually self-incompatible, such labor-intensive methods should probably only be considered as a last resort, in instances when local seed sources are completely unavailable.

4. Introduce cultivated plugs into the target site(s). *Aster vialis* introductions should be performed after the arrival of fall rains, so that soils are moist at the time of planting and plugs have ample opportunity for root system development prior to summer drying. Past introduction attempts have yielded very high rates of survival among container-grown plugs, but very low rates of seedling recruitment from broadcast seeds. Given these low recruitment rates, and considering how few seeds are produced by *A. vialis* to begin with, seeds are probably more wisely utilized for cultivation of plugs rather than direct sowing into introduction sites.

Because herbivory by deer has proven problematic at many extant *Aster vialis* populations, it may be advisable in some cases to erect deer exclusion fences or cages/tubes around introduced plugs to protect them from herbivory and assist in their establishment, at least for their first year following planting. Because of the rhizomatous nature of plants, the layout of introduced plugs should be designed in a manner that is consistent with subsequent population monitoring objectives (see #5, below).

5. Monitor introduced populations. Introduced *Aster vialis* plugs should be monitored annually to evaluate project success. Given the clonal nature of the species (i.e., asexual expansion via rhizomes), and an apparent "guerrilla" strategy of clonal expansion, both of which can complicate the definition of individuals, monitoring should either be carried out in a way that simply estimates overall *A. vialis* cover within fixed sampling plots, or censuses the number of flowering and non-flowering stems. If the definition of individuals is desired (perhaps with the goal of comparing different experimental replicates within a site), then plugs should be widely spaced, such that they remain spatially distinct over time.

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Developing biogeographically based population introduction protocols for at-risk Willamette Valley plant species:

Delphinium leucophaeum (white rock larkspur)

Delphinium leucophaeum (white rock larkspur)

Conservation status

Ranking as the rarest and most geographically restricted larkspur species in the Pacific Northwest, *Delphinium leucophaeum* (Figure 11), a member of the buttercup family (Ranunculaceae), is recognized as a Species of Concern by the U.S. Fish and Wildlife Service, and is listed as Endangered by the State of Oregon. It is on the Oregon Natural Heritage Program 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) (ONHP 2001). In Washington, *D. leucophaeum* is listed as Endangered, though this status designation provides no administrative protection because Washington currently has no state regulatory authority for listed plants (Florence Caplow, Washington Natural Heritage Program, Olympia, Washington, personal communication). *Delphinium leucophaeum* has also been assigned a rank of S1 (critically imperiled) by the Washington Natural Heritage Program (WNHP 2003).

Figure 11. *Delphinium leucophaeum.* (Photo by Steven Gisler.)

Like many of the native prairie species treated in this manual, *Delphinium leucophaeum* is primarily threatened by loss of its unique habitat to urban and agricultural development, especially in the Portland metropolitan area. Additional threats to the species include deterioration of habitat quality caused by invasive weeds, damage to plants from roadside maintenance activities, and low numbers and sizes of populations that render the species vulnerable to random demographic fluctuations (Darr 1980).

Range and habitat

Delphinium leucophaeum was first collected by Thomas Nuttal in 1834, on "open prairies and along the banks of the Wahlamet" (Goodrich 1983). Currently, the species is known from 17 extant sites, occurring in Clackamas, Marion, Multnomah, and Washington counties, Oregon, and Klickitat County, Washington. There is also a historic collection (made by Nelson in 1919) from Yamhill County, Oregon, though this site has never been successfully re-located (ONHP 2002).

According to Darr (1980), *Delphinium leucophaeum* occurs in a variety of habitat types, including: edges of *Quercus garryana* woods, dry roadside ditches, along river banks and bluffs, on moist rocky slopes, in moist lowland meadows, in dirt at cliff bases, and in open, moist areas atop basaltic shelves (Figure 12).

Species commonly associated with *Delphinium leucophaeum* include: various unidentified mosses, lichens, and liverworts, *Anthoxanthum odoratum, Arbutus menziesii*, *Aquilegia formosa, Bromus* sp., *Collinsia parviflora*, *Comandra umbellate*, *Camassia quamash*, *Cynosurus echinatus*, *Cytisus scoparius*, *Danthonia californica, Elymus glaucus*, *Eriogonum* sp. *Festuca rubra*, *Galium* sp., *Holcus lanatus*, *Holodiscus discolor*, *Hypericum perforatum*, *Plectritis congesta*, *Polypodium glycyrrhiza, Quercus garryana*, *Rhus diversiloba*, *Rosa* spp., *Rubus* sp., *Sedum* sp., and *Symphoricarpos alba* (Darr 1980, Goodrich 1983, Anonymous 1986, ONHP 2002, and OSU herbarium specimen labels). Elevations of extant sites range from 50 to 1050 ft (ONHP 2002).

Figure 12. *Delphinium leucophaeum* is frequently found along the edges of Oregon white oak woodlands, in shallow soils overlying basalt. This photograph shows occupied habitat at the Camassia Natural Area near West Linn, managed by The Nature Conservancy. (Photo by Steven Gisler.)

Goodrich (1983) analyzed soils at several extant *Delphinium leucophaeum* populations and concluded that the species occupies very distinctive substrates that are notably black in color (due to high content of organic matter), loose in texture, and very shallow (only 5-7 cm in most places). Particle size analysis of soils indicated a high percentage of sand relative to soils occupied by other Pacific Northwest *Delphinium* species, and low percentages of clay and silt. Specifically, soil analyses yielded a mean pH of 4.93, 33.47 percent organic matter, and particle sizes of 15.1 percent gravel, 58.6 percent sand, 20.0 percent silt, and 6.3 percent clay. May-June soil moisture was also higher (50.7 percent) at *D. leucophaeum* sites compared to those of the other *Delphinium* species (the latter typically ranging from 10.6-22 percent).

Given the apparent significance of edaphic factors on *Delphinium leucophaeum* distribution, Hoegler (1997) conducted soil analyses at the Camassia Natural Area and

found that the "healthiest" *D. leucophaeum* patches (those with positive increase in numbers over time) occupied sites with a mean soil depth of 7.7 cm, 54.1 percent organic matter, and 1.57 percent nitrogen. Soils occupied by declining patches tended to be slightly deeper, more organic, and contain more nitrogen, though these relationships were only weakly statistically significant.

Description of species

Delphinium leucophaeum is an erect perennial, usually leafy up to the raceme, arising from a small cluster of globose tubers each about 5-8(15) mm across. Stems are slender, 30-75 cm tall, glabrous below, villous pubescent above with curling hairs, occasionally wholly puberulent. Leaves are mostly cauline, not withering at anthesis, long-petioled, 4.5-9.0 cm wide, and the 3 (or 5) chief divisions pinnatifid or simply trifid into short acute segments. Racemes are open, especially below, 6-12 flowered, with flowers on ascending bracteate pedicels. Sepals are creamy white, ovate, umbonate, closely puberulent, 9-12 mm long, the lower petals are the same color as sepals, but upper petals are bright blue. Follicles are short, 8-10 (18) mm long, erect, with filiform and pricklelike cusps. Seeds are prismatic, 1.5-2.0 mm, truncate depressed, and all the angles distinctly winged (Ewan 1945).

Because of its white flowers, *Delphinium leucophaeum* is readily distinguishable from its many blue-purple flowered relatives. Taxonomic distinction is more difficult, however, between *D. leucophaeum* and its white-flowered relative, *D. pavonaceum* (Figure 13), which also occurs in the Willamette Valley. *Delphinium pavonaceum* is essentially parapatric with *D. leucophaeum*, insofar that its primary distribution is situated farther south in the Willamette Valley than that of *D. leucophaeum*. However, the two species' ranges may overlap somewhat in northern Marion and southern Clackamas counties. *Delphinium pavonaceum* can be distinguished from *D. leucophaeum* by its larger (11-18 mm), broadly ovate sepals, conical racemes that are wider below (due to long and spreading pedicels) and tapered above (Figure 13), and its spreading, often glandular-pubescent follicles (Darr 1980). See the following chapter of this manual for more detailed descriptions of *D. pavonaceum*.

Figure 13. *Delphinium leucophaeum* (left) can be distinguished from its look-alike relative, *D. pavonaceum* (right) by several diagnostic morphological traits. One such trait, evident in these photographs, is the difference in raceme architecture exhibited by two species. Here, *D. leucophaeum* tends to have narrow, cylindrical racemes with flowers held close to the main stem throughout its length, whereas *D. pavonaceum* typically produces more conical racemes that are wider at the base and tapered towards the top. (Photos by Steven Gisler.)

Seed production

Seed and fruit production have been extensively studied in *Delphinium leucophaeum*. The earliest of these studies was documented by Goodrich (1983), who quantified seed set in a total 247 follicles at four different *D. leucophaeum* populations in 1982, and found a mean 9.98 (SD 4.03) seeds per follicle. As each flower typically produces 3 follicles, this would equate to about 30 seeds produced per fruit. Although these seed set values seem respectable, they were the lowest of the four *Delphinium* species Goodrich studied, which otherwise ranged from 12.84-19.31 seeds per follicle. As a possible explanation for this trend, Goodrich suggested that *D. leucophaeum* seed and fruit production may have been unusually low in 1982 (the year of her study), especially at sites with shallow soils, due to drought conditions and shriveling of flowers prior to fruit development.

A decade after Goodrich, Turner (1992) reported *Delphinium leucophaeum* seed production for three populations over three years (n=127 follicles). Here, the three-year mean seed set in *D. leucophaeum* was 10.3 seeds per follicle, accompanied by a mean 13.5 total ovules per follicle (equating to a 76 percent seed:ovule ratio). Similar to the earlier findings by Goodrich, Turner found *D. leucophaeum* to exhibit the lowest seed set levels among the four *Delphinium* species studied, which otherwise ranged from 13.0-19.6 seeds per follicle.

Karoly and Webb (undated) also performed reproductive studies on *Delphinium leucophaeum*. Here, the authors measured fruit and seed production in open-pollinated *D. leucophaeum* individuals, and reported a mean 13.3 flowers, 4.2 fruits, and 66.4 seeds per plant. Mean seed set per fruit was reported as 17.4, which was markedly lower than the values reported by Goodrich (1983) and Turner (1992), possibly due to differences between years, sampling methods, and/or study sites. Reinforcing earlier suggestions by Goodrich on the importance of drought, Karoly and Webb found fruit and seed production to steadily decline over the flowering season, possibly as a result of dwindling soil moisture resources with the arrival of summer drought. Among the earliest flowers

to appear in the spring, Karoly and Webb reported 57 percent fruit set, compared to less than 5 percent among flowers appearing in later, in early summer.

Seed germination

As with seed production, above, seed germination in *Delphinium leucophaeum* has also been the subject of extensive studies by several researchers. The earliest reference to seed germination in *D. leucophaeum* was made by Darr (1980), who stated that *D. leucophaeum* seeds collected for the Berry Botanic Garden were viable, producing seedlings after two years in a pot. No specific data on germination rates or methods were reported.

More detailed seed germination information was documented by Goodrich (1983), who tested *Delphinium leucophaeum* seed germination under a variety of conditions. Seed germination in soil, under ambient outdoor environmental conditions, was tested by planting 20 seeds in small pots filled with a soil mixture of approximately equal parts peat moss, loam, and soil, with a small amount of perlite added to improve drainage. Pots were placed outdoors and resulting germination was recorded as 23 percent after two years. Goodrich also tested germination under various light and heat exposure regimes using seeds sown in moist Petri dishes (which were kept outside during winter for natural cold stratification). Results of this study showed 15 percent germination under normal diurnal light and dark conditions, 60 percent germination in complete darkness, and no germination when seeds were exposed to heat treatments (80°C, 180°C, and direct contact with a flame).

Turner (1992) also conducted an extensive study of seed germination in *Delphinium leucophaeum*, using several natural and artificial cold stratification treatments. All seeds were initially washed in 10 percent bleach solution, placed on moist filter paper in Petri dishes, and kept in the dark during the study. Resulting germination was reported as 77 percent for seeds kept at 6°C for three months (n=1366 seeds), 90 percent for seeds kept outside during a cold winter (winter of 1990-1991) (n=400 seeds), 100 percent for seeds kept outside during a relatively warm winter (winter of 1991-1992) (n=300 seeds), and

only 0.67 percent for seeds kept at 12°C (n=600 seeds). Data were not provided on the range of temperature differences between warm and cold winters.

Finally, *Delphinium leucophaeum* seed germination was also studied by Karoly and Webb (undated), who reported 88 percent germination by placing seeds between moist sheets of filter paper in glass petri dishes and cold-stratifying them in the dark at 5°C for 5 weeks. Karoly (2002) indicated that seed germination typically commences about 6 weeks following stratification, but occasionally it can take as long as 3-4 months.

Vegetative reproduction

According to Darr (1980), *Delphinium leucophaeum* shows no evidence of vegetative reproduction in the wild. These sentiments were echoed by Karoly and Webb (undated), who reported,

> "Vegetative spread does not appear to be prevalent in this species, though the tubers can be physically divided into independent plants and multiple flowering stalks are sometimes seen arising quite close to one another from the soil."

Breeding system

Valuable information on *Delphinium leucophaeum*'s breeding system has been provided by several researchers, beginning with the work of Goodrich (1983). Results of Goodrich's study indicate that *D. leucophaeum* flowers are hermaphroditic and protandrous, with a 3-carpeled gynoecium that matures two to five days following pollen release by the numerous stamens. Pollinator exclusion bags placed over racemes prior to flowering showed that the species does not produce seeds in the absence of insect vectors for pollination. Goodrich found, however, that *D. leucophaeum* is self-compatible and can produce seeds when flowers are crossed with pollen from other flowers on the same plant (i.e., geitonogamy). Seed set and germination were similar for seeds from self-pollinated flowers and those from open-pollinated flowers, suggesting that inbreeding depression is not a large problem in the species, at least not during the early life-history stages of seed production and seed germination. Goodrich found no evidence of seed production in flowers that were emasculated prior to bagging, suggesting that apomixis does not occur in the species. The primary (and only) pollinating insect ever observed by

Goodrich on *D. leucophaeum* was the bumblebee, *Bombus californicus*. Goodrich noted that the primarily bottom-to-top inflorescence foraging strategy of this bee, combined with protandry in the species, would be expected to promote outcrossing.

Breeding system research in *Delphinium leucophaeum* was also performed by Karoly and Webb (undated) at the Willamette Narrows population near the town of West Linn, Oregon, and on the adjacent Little Rock Island in the Willamette River. Comparing fruit and seed production in caged and open-pollinated plants, the authors showed that *D. leucophaeum* requires pollinator visits for seed production, thus supporting earlier findings by Goodrich (1983). Despite its reliance on pollinators, however, results of a pollen supplementation study (again by Karoly and Webb) suggest the species is not pollen-limited. In fact, fruit and seed production was actually slightly lower in plants subjected to artificial pollen addition than open-pollinated control plants. Similarly, Karoly (2002) showed that reproductive success was not correlated with patch size at the Camassia Natural Area, indicating that even small groups of plants are capable of attracting sufficient insect visitors for full seed set.

Although *Delphinium leucophaeum* has been shown to be self-compatible and capable of producing seeds through geitonogamy (Goodrich 1983, Karoly and Webb undated), Karoly and Webb showed that the majority of pollinations in their study populations were in fact *not* between flowers of the same plant, but were instead between different individuals, as reflected by a multilocus allozyme estimated outcrossing rate of 83 percent.

Hybridization

Numerous information sources suggest a high potential for interspecific hybridization in *Delphinium leucophaeum*. The earliest statement to this effect was from Ewan (1945), who suggested *D. leucophaeum* may hybridize with *D. menziesii* to form *D. pavonaceum*, another white flowered species of the Willamette Valley. A later report (Anonymous 1980) reported that *D. leucophaeum* "appears to hybridize with *D. pavonaceum* and *D. menziesii*." More recently, Chambers (2000) stated that generally similar floral structures

and absence of genetic barriers to intercrossing has led to widespread hybridization involving many different *Delphinium* species combinations, and also suggested that *D. leucophaeum* is probably derived from the blue-flowered *D. nuttallii*. These sentiments were echoed by Boyer (Heritage Seedlings Inc., Salem, Oregon, personal communication), who characterized Delphiniums as "notorious hybridizers."

Several studies have been undertaken to solidify our knowledge about hybridization in *Delphinium leucophaeum*. Goodrich (1983) investigated interspecific sexual compatibility in *D. leucophaeum* by conducting a variety of between-species crosses: *D. leucophaeum* x *D. pavonaceum* produced a mean 52.0 seeds per fruit, *D. pavonaceum* x *D. leucophaeum* produced a mean 28.5 seeds per fruit, *D. leucophaeum* x *D. nuttallii* produced a mean 30.0 seeds per fruit, and *D. nuttallii* x *D. leucophaeum* produced a mean 24.7 seeds per fruit. All combinations yielded viable seeds. The data from this study indicate that hybridization is certainly possible in *D. leucophaeum*, provided the occurrence of interspecific gene flow. Goodrich stated the latter may be discouraged, however, due to *D. leucophaeum*'s unique habitat affinity and its tendency to attract a smaller worker caste of pollinating bumblebees (*Bombus californicus*) than the larger queens that typically visit other *Delphinium* species.

Turner (1993) attempted to investigate hybridization in *Delphinium leucophaeum* and several related species using karyotype analysis techniques, but these methods did not yield the resolution needed to identify any diagnostic chromosomal features, so her results were equivocal. Karoly (2002) reported that three of his students (Beattie 1999, Lerner 1999, and Buckle 2000) were independently unable to identify any genetic markers distinguishing *Delphinium leucophaeum* from *D. nuttallii*, and that crosses between these taxa have produced fertile F1 progeny with flowers that resemble the pigmentation of *D. leucophaeum*. The latter finding indicates that alleles responsible for the white coloration in *D. leucophaeum* are dominant to the flower color alleles in *D. nuttallii*.

Cultivation

Work conducted by Goodrich (1983) and Karoly (2002) clearly show that *Delphinium leucophaeum* can be successfully cultivated in the greenhouse, and that the species does not appear to require special soil amendments or mycorrhizal symbionts for survival and growth.

Goodrich (1983) cultivated *Delphinium leucophaeum* for a comparative common garden experiment, using tubers and seeds from two different populations. No specific details on cultivation methods or success rates were given, though it was reported that an unidentified number of plants did grow and survive for at least two years (1981 through 1983), with flower production ranging from 7-26 flowers per plant, and height ranging from 27-81 cm. Seed germination methods used by Goodrich are described in "Seed germination," above. Goodrich reported that germinated *Delphinium* seeds each typically produced two, 3-5 mm cotyledons on a slender stem (2-4 cm high) during the first year. During the second year, plants usually possessed only one leaf with three small leaflets approximately the same size as the cotyledons. By the third year, two or more leaves with five leaflets or segments were common. Goodrich concluded that most *D. leucophaeum* individuals (under cultivation or in the wild), "are probably incapable of flowering until at least their fifth year, and possibly not until several years after that."

Karoly (2002), however, reported that he was successful in producing flowering *Delphinium leucophaeum* plants from seeds in as little as one year, by accelerating the growth cycle using alternating cold/dark and wet/dry treatments. Here, Karoly placed germinated seeds (see "Seed germination," above) into a combination of peat moss/perlite and coarse calcined clay, and grew them under standard greenhouse conditions. After several months the plants began to senesce, at which time watering was halted to encourage dormancy. After senescence, plants (still in their pots) were placed into dark garbage bags and then into a cold room (4-5°C) for 8 weeks, after which they were again returned to the greenhouse for their next cycle of emergence, watering, and growth. This process was then repeated, such that plants eventually experienced 3 growth cycles in a single year, after which some plants produced flowers.

Although fertilizing *Delphinium leucophaeum* in the greenhouse probably accelerates the pace of plant growth, Hoegler (1997) found that varying the frequency and concentration of fertilizer (ammonium nitrate solution) had little significant effect on *D. leucophaeum* seedling biomass.

Transplanting and introduction attempts

To date there have been no documented studies of transplanting or population introduction techniques for *Delphinium leucophaeum*, although Keith Karoly (Reed College, Portland, Oregon, personal communication) reported that *D. leucophaeum* tubers can be transplanted from one location to another fairly easily, with high rates of plant re-emergence and flowering.

Population monitoring

Monitoring of *Delphinium leucophaeum* has been performed by various workers at several extant populations. According to Karoly (undated), population monitoring at the Camassia Natural Area was initiated in 1981 and has continued annually since 1985. One report (Anonymous 1986) stated that the intent of monitoring at this site is to identify *D. leucophaeum* recruitment, mortality, and dormancy, by mapping and tracking all individuals within twelve permanent meter square plots. Based upon 5 years of monitoring, the anonymous author concluded that the *D. leucophaeum* population has fluctuated radically over time at the Camassia Natural Area, in part due to the role of plant dormancy, which caused entire "subpopulations" to temporarily disappear and others to suddenly reappear after several years. It was also stated that plants growing closely together (i.e., within 0.5 cm) were extremely difficult to distinguish from one another from year to year, and uneven topography within plots often made x-y coordinate mapping of plant locations difficult.

The confounding effects of plant dormancy and sporadic emergence on monitoring efforts were also noted by Goodrich (1983), who found that some very large *Delphinium leucophaeum* individuals bloomed profusely during one year and failed to emerge at all the next year. This pattern was also observed by Larkin and Salzer (1992), who reported

that flowering individuals within fixed demographic census plots sometimes disappeared for one or two years before re-appearing.

An additional complication to *Delphinium leucophaeum* population monitoring, according to Karoly (1997) and Hoegler (1997), is the life-history of non-reproductive plants. Here, reproductively immature plants tended to die back in late spring, at which time they became dormant under the soil. As such, these individuals are not always visible above the ground when population monitoring is typically performed for flowering individuals in early summer. This problem was also noted by Salzer (1998), who monitored the *D. leucophaeum* population located at Willamette Narrows and stated, "only flowering individuals were counted since the leaves of many non-flowering individuals had already dried up making them difficult to count." Since this population was too large to accurately census, Salzer employed long, variable length, rectangular sampling quadrats extending from linear transects. According to Salzer, this method was well-suited to accomodate the irregular boundaries of the *D. leucophaeum* population.

Lastly, in addition to the propensity of plants to disappear and reappear over time, K. Karoly (personal communication) also noted that some individuals "snake" their emerging flowering stems up through the moss layer in different positions from year to year, which can complicate re-location of individuals for monitoring purposes.

Land use threats and other limitations

According to Darr (1980), lack of secure population ownership is the primary threat to *Delphinium leucophaeum*. At the time of her report, Darr reported that the Camassia Natural Area (managed by TNC) was the only secure *D. leucophaeum* population, though some populations on private lands were reported as being fairly secure against development by virtue of their inaccessibility. Currently, ONHP (2002) records indicate that, among the 16 extant *D. leucophaeum* occurrences in Oregon, 8 occur wholly on private lands, 4 occur on county lands, 3 (including the largest known population at the Willamette Narrows) occur on a mixture of ownerships in the Willamette River Greenway, and 1 (the Camassia Natural Area) is on land owned by TNC.

In addition to anthropogenic land use threats, Darr (1980) also lists invasive species, roadside maintenance, and small population sizes among the human and ecological constraints to *Delphinium leucophaeum*.

Population introduction and augmentation strategy

Based upon the biogeographical data compiled and described above for *Delphinium leucophaeum*, there do not appear to be any insurmountable ecological, life history, or administrative obstacles to the successful implementation of population introduction and augmentation projects for this rare species. Although half of the known extant *D. leucophaeum* populations occur on private lands, most of these sites are fairly (though by no means completely) secure against development due to their steep and rocky nature (Darr 1980). Meanwhile, the remaining known extant populations of this species occur on public or otherwise administratively "friendly" ownerships, so, pending interagency cooperation and funding availability, favorable sites should be available both for collection of seeds and for population augmentation and introduction purposes. The primary environmental constraint to these much-needed conservation projects is the proliferation of invasive weeds, which already pose a serious threat to existing populations and limit the availability and quality of new introduction sites. Nevertheless, non-weedy sites still remain within this species' current range, so suitable habitat should still be available for the creation and augmentation of *D. leucophaeum* populations.

Delphinium leucophaeum has been documented as a reliable, though not particularly prolific, seed producer: the species typically yields about 4 fruits per plant, usually containing a total of about 60 seeds. Seed viability is high in the species, as are seed germination levels following 5-8 weeks of cold stratification in the dark. Following germination, *D. leucophaeum* seedlings exhibit no specialized growth or soil symbiont requirements, and can be cultivated to a reproductively mature stage within one year by exposing them to artificially accelerated cycles of growth and dormancy (see "Cultivation," above). *Delphinium leucophaeum* can also be cultivated outdoors in nursery beds, though this method may take 4-5 or more years to yield mature plants.

Currently, very little is known about the feasibility of introducing cultivated *Delphinium leucophaeum* plugs into the wild, an issue warranting future investigation. Based upon results of previous studies (see "Range and habitat," above), soils of population introduction/augmentation target sites should range between 5.5 and 7 cm in depth. Deeper soils may be correlated with poor population performance.

One factor that *should* be considered a potential complication to *Delphinium leucophaeum* introduction projects is interspecific hybridization. Evidence suggests that *D. leucophaeum* may hybridize freely with its relatives if given the opportunity for interspecific gene flow…a phenomenon currently discouraged for the most part by the species' unique habitat requirements and geographically restricted range. As such, population introduction target sites should be selected in areas strictly located within the species' current range and habitat type, and inventories should be performed to ensure the absence of other congeners within project target areas.

Based upon this information, the following step-down procedures are recommended for *Delphinium leucophaeum* population introductions:

1. Select population introduction/augmentation target sites. Several primary factors should be considered when selecting target sites for *Delphinium leucophaeum* population introduction and augmentation projects. First, target sites should obviously contain suitable *D. leucophaeum* habitat. Although the habitat information provided in this report can be used as a guide to some of the general habitat preferences exhibited this species, the suitability of potential introduction sites should ultimately be evaluated based upon an intimate familiarity with extant *D. leucophaeum* populations. In particular, extant *D. leucophaeum* populations should be visited to obtain an understanding of the specific microsites that tend to be occupied by the species within the larger habitat types in which it occurs.

   Given the history of *Delphinium leucophaeum* habitat destruction on private lands, and the ubiquitous threat posed by invasive species, inventories for suitable

population introduction and augmentation sites should be focused strictly to publicly owned (or otherwise secure) lands that appear safe from imminent weed and successional encroachment problems. Selection and use of sites should always be coordinated with pertinent public landowners to ensure administrative protection and management of populations following introductions.

2. Collect *Delphinium leucophaeum* seeds for off-site cultivation of introduction stock. Seeds used for off-site cultivation of *Delphinium leucophaeum* should be collected from the extant population(s) located nearest the population introduction target site to minimize undesirable mixing of gene pools and maximize conveyance of potential local adaptations (if such intraspecific variability and local adaptations exist). Given respectable levels of seed production reported in this species, and high levels of seed germinability, a single year should be adequate to supply enough viable seeds for cultivation projects, unless seed source populations are extremely small. Based upon historic seed production estimates, *D. leucophaeum* can be expected to produce, on average, about 4 fruits per plant, yielding a total of about 66 seeds. Perhaps due to dwindling soil moisture resources during the summer, the lowest fruits on fruiting stems tend to produce the most seeds.

3. Cultivate *Delphinium leucophaeum*. *Delphinium leucophaeum* has been successfully cultivated in greenhouse and outdoor garden settings from seeds. Although 60-100 percent germination has been reported for seeds simply left outside during the winter, a much faster and equally effective method is to artificially cold stratify seeds at 5°C for 5-8 weeks in a refrigerator. Regardless of which cold stratification method is used, seeds should be kept in a dark place to foster germination (see "Seed germination," above).

   Once *Delphinium leucophaeum* seedlings are obtained, they may be grown outdoors, in which case it may take 5 or more years for them to develop into reproductively mature plants. In contrast, flowering plants can be obtained in a

single year if the cultivated plants are exposed to artificially accelerated cycles of growth and dormancy. Although fertilizer application doubtlessly enhances the rate of seedling growth, previous studies suggest the intensity of fertilization during cultivation has little clear impact on plant size or fecundity.

4. Introduce cultivated plugs into the target site(s). *Delphinium leucophaeum* introductions should be performed sometime after the arrival of fall rains, so that soils are moist at the time of planting and plugs have ample opportunity for root system development prior to summer drying. Previous reports suggest that *D. leucophaeum* is particularly susceptible to late-season desiccation, perhaps due to the extremely shallow soils characterizing occupied habitat. As no formal attempts have been made to introduce this species from cultivation into the wild, we currently have no information about predicated survival and establishment rates for introduced *D. leucophaeum* plugs, nor information suggesting whether fall transplanting works better or worse than transplanting in late winter or early spring. Late winter planting times have been recommended for the related species, *D. pavonaceum* (see following chapter in this manual).

   Because *Delphinium leucophaeum* individuals can appear to change position over time, by "snaking" their stems up through the mossy soil in different locations from year to year (see "Population monitoring," above), plugs should be introduced with adequate spacing to allow confident identification of individuals over time. Also, as one previous researcher (Salzer 1998) identified uneven population boundaries as a potential obstacle to monitoring large populations, it may be advisable to keep monitoring objectives and population boundaries in mind when designing new populations (see #5, below).

5. Monitor introduced populations. Introduced *Delphinium leucophaeum* plugs should be monitored annually to evaluate project success. Based upon previous monitoring efforts, it appears that *D. leucophaeum* individuals may lie dormant under the soil for one or more years before re-emerging, so introduced

populations may require sustained monitoring efforts in order to develop a clear picture of overall project success. Monitoring efforts could either entail censuses of reproductive and non-reproductive plants within populations, or if more detailed information is desired (or if populations are too large to census accurately), fixed-plot sampling methods could be employed to track the fates of individuals over time. Because non-reproductive individuals typically whither away prior to emergence of flowers on reproductive plants (see "Population monitoring," above), it may be necessary to monitor populations twice during a single if vegetative individuals are to be taken into consideration.

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Developing biogeographically based population introduction protocols for at-risk Willamette Valley plant species:

Delphinium pavonaceum (peacock larkspur)

Delphinium pavonaceum (peacock larkspur)

Conservation status

With only 49 percent of its 39 historically documented populations currently known to survive (Brie-Anne McKernan, Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon, personal communication), *Delphinium pavonaceum* (Figure 14) is one of the most imperiled plant species in the native prairies of the Willamette Valley. As such, this rare member of the buttercup family Ranunculaceae) is recognized as a Species of Concern by the U.S. Fish and Wildlife Service, and is listed as Endangered by the State of Oregon. It is on the Oregon Natural Heritage Program List 1 (threatened or endangered throughout its range), and has a Natural Heritage Network Rank of G1/S1 (critically imperiled throughout its range/critically imperiled in Oregon) (ONHP 2001).

Figure 14. *Delphinium pavonaceum*. (Photo by Steven Gisler.)

As with other native species endemic to Willamette Valley grassland habitats, *Delphinium pavonaceum* has been severely impacted by habitat loss due to urban expansion and agricultural development (Darr 1980, USFWS 1995). Most extant populations are very small and occur along roadsides where they are continually threatened by road maintenance activities and herbicide application from adjacent agricultural fields (Darr 1980, USFWS 1995, Bender 1997, 1998, ONHP 2002, McKernan and Meinke 2003). Additional threats to this species include habitat degradation by invasive weeds and successional encroachment of shrubs (Finley and Ingersoll 1994), and herbivory by rodents (Goodrich 1983). The single overriding factor working in favor of this species' persistence and conservation is the location of its largest populations within the boundaries of the William Finley National Wildlife Refuge (Finley NWR) in southern Benton County. This refuge not only harbors the largest numbers of extant *D. pavonaceum* individuals (B. McKernan, personal communication), it also performs active habitat management (i.e., prescribed burning and mowing) for the benefit of the species (Jock Beall, Finley National Wildlife Refuge, Corvallis, Oregon, personal communication).

Range and habitat

The type specimen of *Delphinium pavonaceum* was collected by H.C. Gilbert in 1916 from "fields west of Corvallis" (Goodrich 1983). Although the Oregon Natural Heritage Program lists historic records for 39 populations, currently only 19 of these are known extant, distributed in Benton, Clackamas, Marion, and Polk Counties, Oregon (ONHP 2002).

Habitat for *Delphinium pavonaceum* consists of undeveloped native wet prairie communities (Figure 15) and shady edges of Oregon ash and Oregon white oak woodlands located in wildlife refuges and along roadsides and fence rows, at elevations between 150-400 ft (Darr 1980, ONHP 2002). Species typically associated with *D. pavonaceum* include: *Achillea millefolium*, *Alepocuris pratensis*, *Allium amplectens*, *Cammasia quamash*, *Delphinium menziesii*, *Deschampsia cespitosa*, *Fraxinus latifolia*, *Geum macrophyllum*, *Geranium oreganum*, *Holcus lanatus*, *Hypericum perforatum*,

Lomatium utriculatum, *Lupinus polyphyllus*, *Microsteris gracilis*, *Plectritis congesta*, *Poa pratensis*, *Potentilla gracilis*, *Rosa* spp., *Rhus diversiloba*, *Sidalcea campestris*, *S. nelsoniana*, *Spiraea douglasii*, *Symphoricarpos albus*, *Vicia* sp., and *Wyethia angustifolia* (Darr 1980, Finley and Ingersoll 1994, ONHP 2002, OSU herbarium specimen labels).

Figure 15. Native prairie habitat (being encroached upon by Oregon ash and Oregon white oak) occupied by *Delphinium pavonaceum* at Finley National Wildlife Refuge. (Photo by Steven Gisler.)

According to Goodrich (1983), Finley and Ingersoll (1994) and ONHP (2002), *Delphinium pavonaceum* often inhabits slightly higher, drier, more well-drained microsites within native wet prairies, rather than wetter depressions dominated by *Deschampsia caespitosa.* The species can, however, tolerate seasonal inundation (Goodrich 1983). Goodrich (1983) measured soil moisture at several *D. pavonaceum* populations and found the mean value for May and June to be about 30 percent by volume (as opposed to 19.53 percent for *D. nuttallii*, 50.66 percent in *D. leucophaeum*, 10.6 percent in *D. oreganum*, and about 22 percent in *D. menziesii*). Soil analyses for *D. pavonaceum* conducted by Goodrich (1983) showed a mean pH of 5.38, 11.28 percent organic matter, and particle size of: 5.8 percent gravel, 37.5 percent sand, 40.0 percent silt, and 18.7 percent clay.

Description of species

Delphinium pavonaceum is an herbaceous perennial, 30-45(90) cm tall, arising from a cluster of globose tubers. Flowering stems are erect, rather stout, with soft to hirsute pubescence. Leaves are mostly cauline, hirsute above and sparingly so below, persistent at anthesis, and extend up to the raceme as diminishing foliar bracts. Racemes are pyramidal (the lower pedicels much longer than upper ones), with large foliar bracts in the axils. Flowers are showy, with sepals that are broadly ovate, acute, 11-18 mm long, and creamy white with blue or green umbos; the lower petals are rounded distally, 8 mm wide, shallowly emarginated, and the upper petals dark blue distally. Follicles are nearly erect, 12 mm long, and exhibit viscid-pubescence (Ewan 1945, Goodrich 1983).

Delphinium pavonaceum is very similar in appearance to another rare, white-flowered *Delphinium* species in the Willamette Valley, *D. leucophaeum* (Figure 16)(described in the previous chapter of this manual). *Delphinium leucophaeum* can be distinguished from *D. pavonaceum* by its shorter sepals (9-14 mm), its columnar (non-tapering) rather than conical raceme (Figure 16), and its follicles, which are shorter (8-12 cm) and non-glandular-puberulent (Darr 1980). *Delphinium leucophaeum* has its primary range in the northern Willamette Valley, and is only believed to spatially overlap with *D. pavonaceum* in northern Marion and southern Clackamas Counties.

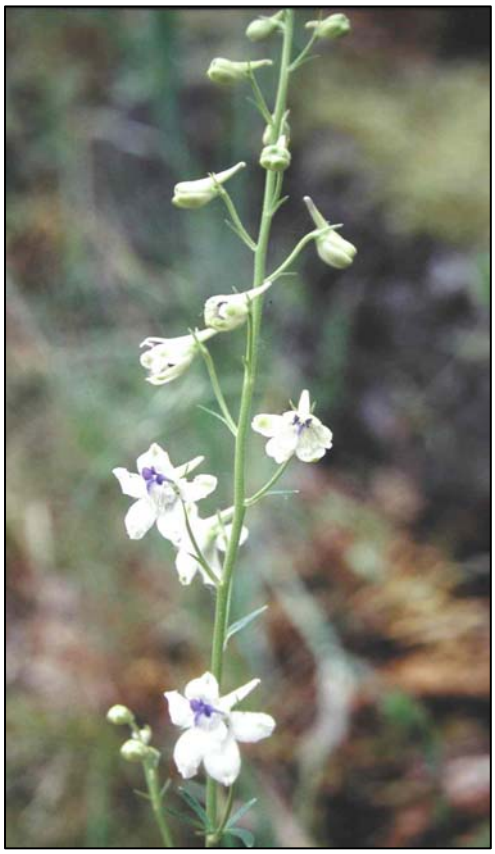

Figure 16. *Delphinium leucophaeum*, another rare, white-flowered larkspur found in the Willamette Valley.

Seed production

Based upon the work of several researchers, *Delphinium pavonaceum* appears to be a consistent seed producer. The earliest investigation of *D. pavonaceum*'s reproductive biology was performed by Goodrich (1983), who quantified seed set in 235 follicles at four different *Delphinium pavonaceum* populations in 1982. Here, Goodrich reported a mean 19.31 (SD 7.39) seeds per follicle. As each flower typically produces 3 follicles, this would equate to about 58 seeds produced per flower. According to Goodrich, *Delphinium pavonaceum* exhibited the highest levels of seed production among the four *Delphinium* species she studied, which ranged from 12.84-19.31 seeds per follicle.

Additional reproductive studies were later performed by Turner (1992), who examined *Delphinium pavonaceum* seed production in three populations over three years (n=167 follicles) and reported a three-year mean seed set of 16.3 seeds per follicle and a mean 18.6 total ovules per follicle (equating to an 88 percent seed:ovule ratio). Similar to earlier findings by Goodrich (1983), seed production in *D. pavonaceum* was the highest of the four western Oregon species studied by Turner. This group of species exhibited seed:ovule ratios ranging from 61 to 88 percent.

Page (1996) investigated reproductive potential in 9 *Delphinium pavonaceum* populations, with populations classified by habitat type (burned, unburned, and roadside habitats). Although Page did not report actual seed production values, she did estimate the number of flowers per individual to range from 1-78 across all study populations, with means ranging from 4.2 (unburned)-17.5 (roadside) flowers per individual. Page also estimated fruit production (proportion of fruits:flowers) in the three habitat classes, with means ranging from 0.75 (unburned) to 0.81 (roadside).

The most recent studies of *Delphinium pavonaceum* seed production were performed by B. McKernan (personal communication). Based upon sampling performed at four *D. pavonaceum* subpopulations at the Finley National Wildlife Refuge, McKernan estimated mean floral production at 7.09 (range 1-33) flowers per individual, mean fruit production at 4.83 (range 0-21) fruits per individual, and mean seed production at 44.59 (range 3-

104) seeds per fruit. McKernan also observed that seed production levels varied significantly between different regions of *D. pavonaceum* racemes, with the highest seed production (mean 59.78 seeds per fruit) at the bottom of racemes, and the lowest seed production (mean 30.27 seeds per fruit) at the tops of racemes. Similar spatial fecundity patterns in this species have been noted by Steve Northway (amateur botanist, Philomath, Oregon, personal communication). It is unknown to what extent this pattern is due to dwindling moisture/nutrient resources over the course of phenological development, or the result of other genetic or environmental constraints to seed production. Regardless of the cause, McKernan (personal communication) recommended that seed collecting for restoration projects should be focused on the basal portions of flowering stems, not only because of their higher levels of seed production, but also because she found these "basal" seeds to be significantly more massive (and therefore potentially of higher quality) than seeds from upper portions of flowering stems.

Seed germination

In addition to seed production, *Delphinium pavonaceum* seed germination has also been the subject of extensive research by several workers. The earliest accounts of such research are by Goodrich (1983), who investigated seed germination under a variety of natural and experimental conditions. Germination in soil, under ambient outdoor environmental conditions, was tested by planting 20 seeds (from three populations) in small pots filled with a soil mixture of approximately equal parts peat moss, loam, and potting soil with a small amount of perlite added to improve drainage. Pots were placed outdoors and resulting germination ranged from 10-30 percent for the three populations after two years of observation. Goodrich also tested germination under various light and heat exposure regimes using seeds sown in moist Petri dishes (which were kept outside during winter for natural cold stratification). Results of this study showed 7 percent germination under normal diurnal (i.e., alternating light and dark) conditions, 57 percent germination in complete darkness, and no germination when seeds were exposed to heat (80°C, 180°C, and direct candle flame).

Seed germination was also investigated by Turner (1992), using several natural and artificial cold stratification treatments. All seeds were washed in 10 percent bleach solution, placed on moist filter paper in Petri dishes, and kept in the dark during the duration of the study. Resulting germination was 96 percent for seeds kept at 6°C for three months (n=6115 seeds), 75 percent for seeds kept outside during a relatively cold winter (1990-1991) (n=2030 seeds), 50 percent for seeds kept outside during a relatively warm winter (1991-1992) (n=150 seeds), and 0 percent for seeds kept at 12°C (n=748 seeds). Turner noted that seed germination in all treatments was slow, only occurring after 70-90 days following initiation of cold stratification.

More recently, seed germination studies conducted by The Berry Botanic Garden (2002) showed 33 percent germination after 16 weeks of cold stratification followed by placement of seeds into 20°C. Germination was slightly lower (14 percent) when seeds were placed in alternating 10°/20°C following cold stratification. No seeds germinated when stratified for only 8 weeks. Similarly, Kaye (2002) reported that long-term (at least 16-week) cold stratification is required to break seed dormancy in *D. pavonaceum*.

The most recent reports of seed germination work in this species were by Lynda Boyer (Heritage Seedlings Inc., Salem, Oregon, personal communication) and S. Northway (personal communication). Boyer reported nearly 100 percent germination of seeds that were mixed with moist vermiculite in a sealed plastic bag and then placed in a 1°C refrigerator for 11 weeks. This seed/vermiculate mixture was then sown onto soil in flats, and covered with a "light dusting of soil," and cotyledons emerged 15 days later (see "Cultivation," below). Northway likewise reported very high (greater than 90 percent) germination of seeds following 80 days of cold stratification. Northway noted that germination does not occur in a single flush, but rather occurs sporadically over a two-three week period. Unlike previous reports suggesting a dark requirement for germination, Northway found that seeds will germinate in light or darkness.

Vegetative reproduction

There is no evidence that *Delphinium pavonaceum* is capable of vegetative growth, though it may be possible (as in *D. leucophaeum*, see previous chapter in this manual) to produce separate plants through manual division of the species' globose tubers. Steve Northway (personal communication) suggested that plants will sometimes send up more than one stem if the tubers are damaged by mice or other factors, but that division does not occur spontaneously.

Breeding system

To date, the most comprehensive study of *Delphinium pavonaceum*'s breeding system is that performed by Goodrich (1983), who investigated floral development, levels of autonomous selfing (autogamy), apomixis, self-incompatibility, and interspecific fertility in this and several related *Delphinium* species. Results of this work indicate that *D. pavonaceum* flowers are hermaphroditic and protandrous, with the 3-carpeled gynoecium maturing two to five days following pollen release by the numerous stamens. Pollinator exclusion bags placed over racemes showed that the species does not produce seeds in the absence of insect vectors for pollination (the same conclusion was reported by Page (1996), who also tested for autonomous selfing using bagged plants), nor does the species produce seeds through apomixis. Goodrich found, however, that *D. pavonaceum* is self-compatible and can produce seeds when flowers are crossed with pollen from other flowers on the same plant (i.e., geitonogamy). Self-pollinated and open-pollinated plants had similar levels of seed set (43-63 seeds per fruit), though the germination rate for self-fertilized seeds was only 6 percent, which is somewhat less than germination rates observed for open-pollinated seeds (10-30 percent). This preliminary data may suggest the presence of inbreeding depression in the species, an issue that may warrant future study.

In contrast to the aforementioned findings by Goodrich, S. Northway (personal communication) reported that he experienced full fruit and seed production among *Delphinium pavonaceum* plants cultivated indoors, in the absence of insect visitors.

Moreover, he also reported excellent seed germination rates (exceeding 90 percent) among self-fertilized seeds, suggesting the lack of any inbreeding depression at seed production and germination life history stages. Given these findings, there still appears to be a need for further study of both autogamy and inbreeding depression in this species, and the possible role of indoor cultivation on these factors.

According to Goodrich (1983), pollinators of *Delphinium pavonaceum* include three different bumblebee species: *Bombus californicus*, *B. appositus*, and one other unidentified species. *Delphinium* pollen was found on both the identified species, but not the unidentified species. Pollinators were observed foraging from bottoms of racemes towards the tops, which, combined with protandry, would be expected to promote outcrossing in this species. McKernan (personal communication) also reported the presence of *Bombus californicus* on *D. pavonaceum* at Finley National Wildlife Refuge (Figure 17), and noted that no other pollinators were ever observed visiting the species.

Figure 17. *Bombus californicus* visiting *Delphinium pavonaceum*. (Photo by Brie-Anne McKernan.)

Hybridization

Numerous information sources suggest a high potential for hybridization in *Delphinium pavonaceum*. Lynda Boyer (personal communication), characterized larkspurs in general as "notorious hybridizers." Ewan (1945) and Chambers (2000) state that generally similar floral structures and the absence of genetic barriers to intercrossing have led to

widespread hybridization in many different *Delphinium* combinations, and suggest that *D. pavonaceum* in particular may be particularly prone to hybridization with *D. menziesii.*

Several studies have been undertaken to solidify our knowledge about hybridization in *Delphinium pavonaceum*. Goodrich (1983) investigated interspecific sexual compatibility in *D. pavonaceum* by conducting a variety of between-species crosses: *D. pavonaceum* x *D. menziesii* produced a mean 29.8 seeds per flower, *D. menziesii* x *D. pavonaceum* produced a mean 53.0 seeds per flower, *D. pavonaceum* x *D. leucophaeum* produced a mean 28.5 seeds per flower, *D. leucophaeum* x *D. pavonaceum* produced a mean 52.0 seeds per flower, *D. oreganum* x *D. pavonaceum* produced a mean 15 seeds per flower, *D. pavonaceum* x *D. nuttallii* produced a mean 33.4 seeds per, *D. nuttallii* x *D. pavonaceum* produced a mean 50.0 seeds per flower. All combinations yielded viable seeds, with germination ranging from 4-15 percent (rates similar to conspecific *D. pavonaceum* crosses). The data from this study indicate that hybridization is certainly possible in *D. pavonaceum*, provided opportunities for interspecific gene flow. However, Goodrich pointed out that the flowering time of *D. pavonaceum* only barely overlaps with that of *D. oreganum* and *D. menziesii*, and that this phenological isolation may discourage interspecific gene flow from occurring.

Additional efforts to investigate hybridization in *Delphinium pavonaceum* were conducted by Turner (1993), who attempted to use karyotype analysis techniques to make phylogenetic and hybridization inferences. However, these techniques did not yield the resolution needed to identify any diagnostic chromosomal features, so her results were equivocal. The most recent studies into hybridization in this species were reported by Karoly (1999), who found chloroplast DNA polymorphisms within populations of *D. pavonaceum* at Champoeg State Park; a condition he suggested is "rare except in cases of hybridization."

Cultivation

Work conducted by Goodrich (1983) clearly indicates that *Delphinium pavonaceum* can be successfully cultivated in a greenhouse or garden setting, and that the species does not exhibit specialized soil amendment or soil symbiont requirements for survival and growth. Goodrich cultivated *D. pavonaceum* from 5 different population sources. Although no data were given on precise cultivation methods or survival rates, it was reported that an unidentified number of plants did grow and survive for at least two years (1981 and 1982), with flower production ranging from 3-18 flowers per plant, and height ranging from 22-60 cm. Seed germination methods used by Goodrich are described in "Seed germination," above.

During Goodrich's cultivation trials, *Delphinium pavonaceum* seedlings typically produced two 3-5 mm cotyledons on a slender stem (2-4 cm high) during the first year. During the second year, plants typically produced only single leaves comprised of three small leaflets approximately the same size as the cotyledons. By the third year, two or more leaves (each with five leaflets or segments) were likely for each individual. Based upon her initial study results, Goodrich concluded that *D. pavonaceum* probably requires at least five years to become reproductively mature.

More recent attempts to cultivate *Delphinium pavonaceum* have been conducted by Heritage Seedlings Inc., for purposes of supplying plugs for future restoration projects on county, state, and federal lands (L. Boyer, personal communication). Here, Boyer reported that most *D. pavonaceum* only developed a few true leaves during their first year, though occasionally, a few individuals did flower during their first year. Most plants senesced by late July, after which Boyer recommended they should be placed outside and watered infrequently, so that the tubers do not rot. Following vernalization during the winter, Boyer said most new leaves emerged by late January to early February. Boyer characterized second-year growth of cultivated *D. pavonaceum* as "more vigorous," than the first year, with many plants producing flowers.

Delphinium pavonaceum has also been cultivated by S. Northway (personal communication), who reported growth patterns similar to those by Boyer, above. Here, seeds were germinated after 80 days of cold stratification and then transplanted into pots filled with a standard commercial seedling mix. According to Northway, seedlings typically emerge in late January or February and can continue to growth through July if watered. At the end of the first year, plants usually produce a "BB" size bulb/tuber. By the end of the second year, 1-2 percent of plants will produce flowers, though most plants require 3-5 years to reach reproductive maturity. Northway noted that mortality of cultivated plants can be high if they get too wet (leading to damping off), or if slugs, mice, or deer gain access to plants.

As with *Delphinium leucophaeum*, it may be possible to accelerate the rate of *D. pavonaceum* growth by supplying plants with artificial, alternating growth and dormancy cycles in the greenhouse. Such methods were employed in the former species by Karoly to obtain flowering *Delphinium* plants within a single year (see previous chapter for details). Steve Northway (personal communication) reported successfully employing this method in the cultivation of *D. pavonaceum*, but he noted that it was hardly worth the effort, as each artificial vernalization period needed to be at least 80-100 days (thus, the time savings is not great), and plants sprouting in winter need artificial light supplies (thus adding expense and effort).

Transplanting and introduction attempts

To date there have been no formal studies performed to evaluate methods or success of transplanting or introducing this species, although Goodrich (1983) demonstrated that tubers can be successfully transplanted from the field to a garden setting. Lynda Boyer (personal communication) reported cultivation of *D. pavonaceum* for future introduction projects, though to date these projects have not yet been implemented. However, Boyer recommended that *D. pavonaceum* tubers should probably be outplanted in late January/early February, when soils are moist and new leaves are beginning to emerge from tubers. Boyer suggested, "these plants *should* have a high rate of success if the site is prepared well." Steve Northway (personal communication) echoed Boyer's timing

recommendation, though he cautioned that any damage to stems during this period will typically not be repaired or replaced during the same year, and may cause mortality depending upon the extent of tuber reserves to compensate for lost opportunities for photosynthesis. Northway recommended using plants cultivated in sleeves for introduction purposes, to minimize tuber damage and potting soil disturbance during planting. Northway reported successfully transplanting cultivated individuals into "wild" settings, though he noted that introduced European slugs, deer, and non-native plants often take a heavy toll on such plants. Northway also suggested that seeds might serve as effective propagules for introductions, given high rates of seedling recruitment observed on disturbed soil in the wild. He noted, however, that while recruitment seems to be encouraged by soil disturbance, colonization of disturbed soils by weeds would likely discourage seedling establishment given their slow growth and vulnerability to outcompetition by weeds.

Population monitoring

To date, monitoring of *Delphinium pavonaceum* populations appears to have been limited to sporadic censuses of flowering plants. To the author's knowledge, there have been no documented attempts to track individual plants over time, to carry out sustained population censuses over a series of consecutive years, or to encompass non-reproductive plants in population censuses.

It is probable that monitoring of *Delphinium pavonaceum* would entail many of the same complexities as those faced by other *Delphinium* species, such as those described in the previous chapter for *D. leucophaeum*. These include the tendency for non-reproductive plants to die back and/or become difficult to see during the period when mature plants are flowering in early summer, and also the tendency for individuals to become dormant, which evidently causes sporadic plant emergence between years. According to Goodrich (1983), *D. pavonaceum* may remain dormant for several years, depending on soil moisture availability. As such, Goodrich suggested that repeated years of observation may be necessary to accurately track plants over time.

Land use threats and other limitations

As with many other Willamette Valley grassland endemic species, *Delphinium pavonaceum* is threatened by urban expansion, agricultural development, herbicides, road maintenance activities, successional encroachment by shrubs and trees, and habitat colonization by invasive species (Finley and Ingersoll 1994, USFWS 1995, Bender 1997, 1998, ONHP 2002). Goodrich (1983), McKernan (personal communication), and Northway (personal communication) also reported high levels of herbivory by deer, slugs, and rodents as potential threats to some populations.

Although the majority of the 19 known extant *Delphinium pavonaceum* populations occur on public lands (i.e., state and county road right-of-ways, one state park, and two federal wildlife refuges), many of these populations continue to face serious threats to their survival. For example, within Polk County road right-of-ways, two *D. pavonaceum* populations were destroyed by herbicides over the last 10 years (in both cases herbicides were applied by adjacent private landowners), a third roadside population was destroyed in 1997 during an emergency culvert replacement project resulting from a flood event, and a fourth roadside population was disturbed in 2002 when adjacent private landowners mowed occupied habitat during the flowering period. In Benton County, one roadside population was disturbed in 2001 by intentional trespass, mowing, and uprooting of plants. And on the Finley National Wildlife Refuge, home of the largest known *D. pavonaceum* population, at least two dozen individuals were destroyed in 2002 during placement of a native prairie habitat viewing platform.

However, although publicly owned *Delphinium pavonaceum* populations continue to face a variety of threats, public land managers are simultaneously striving to conserve this rare species. For example, in recognition of the extreme vulnerability of small roadside populations, Marion County is currently attempting to cultivate individuals from seed for the creation of new populations in more secure areas. Marion, Benton, and Polk Counties are undertaking efforts to protect roadside *D. pavonaceum* populations by identifying their locations on maps and defining their boundaries with permanent metal signs. And

at the Finley National Wildlife Refuge, important efforts are being made to maintain populations and their prairie habitat with prescribed fire, mowing, and brush clearing.

Population introduction and augmentation strategy

Based upon the biogeographical data compiled and described above for *Delphinium pavonaceum*, there are no significant ecological, life history, or administrative obstacles to the successful implementation of population introduction and augmentation projects for this rare species. Most of the known extant *D. pavonaceum* populations occur on public ownerships so, pending interagency cooperation and funding availability, sites should be available for collection of seeds for use in off-site cultivation, and locations should also be available for population augmentation and introduction purposes. The only environmental constraint to these much-needed conservation projects is the proliferation of invasive weeds, which already pose a serious threat to existing populations. However, non-weedy sites still remain in many areas within this species' current range, so quality sites should still be available for introduction projects.

Delphinium pavonaceum produces ample supplies of seeds, which exhibit high levels of germination following cold stratification in the dark. Following germination, seedlings exhibit no specialized growth or symbiont requirements, and it might be possible to cultivate the species to a reproductively mature stage within one year by exposing them to artificially accelerated cycles of growth and dormancy (See "Cultivation," above). Currently there is very little known about the feasibility of introducing cultivated plugs of this species into the wild, a question requiring further investigation.

One factor that *should* be considered a potential complication to *Delphinium pavonaceum* introduction projects is interspecific hybridization. Evidence suggests that *D. pavonaceum* may hybridize freely with its relatives if given the opportunity for interspecific gene flow. As such, population introduction target sites should be selected in areas located strictly within the species' current range and habitat type, and inventories should be performed to ensure the absence of other congeners within project target areas. This concern over hybridization is echoed by Boyer (2003), who stated, "Delphiniums

are notorious hybridizers so propagation and re-introduction of both the taxa [*D. oreganum* and *D. pavonaceum*] should be thoughtfully approached."

Based upon this information, the following step-down procedures are recommended for *Delphinium pavonaceum* population introductions:

1. Select population introduction/augmentation target sites. Several primary factors should be considered when selecting target sites for *Delphinium pavonaceum* population introduction and augmentation projects. First, target sites should obviously contain suitable *D. pavonaceum* habitat. To assist in identification of suitable habitat, extant *D. pavonaceum* populations in the vicinity of target sites should be visited to obtain familiarity with potential species' adaptations to various local environmental parameters.

   Given the history of *Delphinium pavonaceum* habitat destruction on private lands, and the ubiquitous threat posed by invasive species, inventories for suitable population introduction and augmentation sites should be focused strictly to publicly owned (or otherwise secure) lands that appear safe from imminent weed and successional encroachment problems. Selection and use of sites should be coordinated with pertinent public landowners to ensure administrative protection and management of populations following introductions.

2. Collect *Delphinium pavonaceum* seeds for off-site cultivation of introduction stock. Source material for off-site cultivation of *Delphinium pavonaceum* should be collected from the extant population(s) located nearest to the population introduction target site to minimize undesirable mixing of gene pools and maximize conveyance of potential local adaptations (if such intraspecific variability and adaptations exist). Given respectable levels of seed production reported in this species, and high levels of seed germinability, a single collecting year should be adequate to supply enough viable seeds for cultivation projects, unless source populations are extremely small.

Based upon previous reproductive studies, *Delphinium pavonaceum* can be expected to produce a mean 4.8 (range 0-21) fruits per plant, and mean seed yields ranging from 44.6-57.9 (range 3-104) seeds per fruit. Perhaps due to dwindling soil moisture resources over the period of phenological development, the lowest fruits on each plant tend to produce the most seeds. As such, and because the lowest fruits have been documented to produce the most massive (and potentially highest quality) seeds, seed collecting for cultivation and restoration projects should be focused to the lower portions of fruiting stems.

3. Cultivate *Delphinium pavonaceum*. *Delphinium pavonaceum* has been successfully cultivated in greenhouse and outdoor garden settings from seeds. Although 50-75 percent germination has been reported for seeds simply left outside (in the dark) during the winter, a much faster and more effective method has been to artificially cold stratify seeds at 6°C for 12 weeks in a refrigerator, yielding 96 percent germination. Several researchers have recommended even longer (i.e., 16-week) cold stratification for optimal seed germination in this species. Regardless of the cold stratification method, seeds appear to require darkness for germination.

   Once *Delphinium pavonaceum* seedlings are obtained, they may be grown outdoors, in which case it may take 5 or more years for them to develop into reproductively mature plants. In contrast, it may be possible to obtain flowering plants in a single year if the cultivated plants are exposed to artificially accelerated cycles of growth and dormancy.

4. Introduce cultivated plugs into the target site(s). *Delphinium pavonaceum* introductions should presumably be performed after the arrival of fall rains, so that soils are moist at the time of planting and plugs have ample opportunity for root system development prior to summer drying. As no formal attempts have been made to introduce this species from cultivation into the wild, we currently

have little information about predicted survival and establishment rates for introduced *D. pavonaceum* plugs.

5. Monitor introduced populations. Introduced *Delphinium pavonaceum* plugs should be monitored annually to evaluate project success. Based upon previous monitoring efforts, *D. pavonaceum* individuals may lie dormant under the soil for several years before re-emerging, so introduced populations may require sustained monitoring efforts in order to develop a clear picture of overall project success. Monitoring efforts could either entail simple methods of population censusing of reproductive and non-reproductive plants, or more detailed methods could be employed to track the fates of individuals over time. Because non-reproductive individuals may whither away prior to emergence of flowers on reproductive plants (see "Population monitoring," above), it may be necessary to monitor populations prior to flowering if vegetative individuals are to be taken into consideration.

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Developing biogeographically based population introduction protocols for at-risk Willamette Valley plant species:

Erigeron decumbens var. *decumbens* (Willamette daisy)

Erigeron decumbens var. *decumbens* (Willamette daisy)

Conservation status

Long thought extinct, *Erigeron decumbens* var. *decumbens* (Figure 18) was rediscovered in 1980 and is currently known from fewer than 30 small native prairie sites, all occurring in the Willamette Valley of Oregon. This rare daisy is listed as Endangered by both the U.S. Fish and Wildlife Service and the State of Oregon, is on the Oregon Natural Heritage Program List 1 (threatened or endangered throughout its range), and has a Natural Heritage Network Rank of G4T1/S1 (the variety of this species is critically imperiled throughout its range/critically imperiled in Oregon) (ONHP 2001).

Figure 18. *Erigeron decumbens* var. *decumbens*. (Photo by Steven Gisler.)

Widespread loss of native Willamette Valley prairie habitat to agricultural and urban development is the primary threat to *Erigeron decumbens* var. *decumbens*. As with many other rare prairie species treated in this manual, *E. decumbens* var. *decumbens* faces the additional threats of successional encroachment of prairie habitat by trees and shrubs, competition with invasive weeds, and possible inbreeding depression arising from small population sizes (Kagan and Yamamoto 1987, Clark et al. 1993, Federal Register 1998, ONHP 2002).

Range and habitat

Herbarium collections from such notable early botanists as Louis Henderson, Thomas Howell, Morton Peck, James Nelson, Wilhelm Suksdorf, Thomas Nuttal, and J.W. Thompson collectively indicate that *Erigeron decumbens* var. *decumbens* was once fairly common and widely distributed in the wetland and upland prairies of Oregon's Willamette Valley (OSU herbarium records and ONHP 2002). Subsequent to 1934, however, the species was not collected and was believed extinct until it was finally relocated near Eugene, Oregon, in 1980 (Clark et al. 1993). Currently, the Oregon Natural Heritage Program lists historic records for 36 *Erigeron decumbens* var. *decumbens* populations, but currently only 28 of these are believed extant, distributed in Benton, Lane, Linn, and Marion Counties, Oregon (ONHP 2002). Historic populations in Clackamas, Washington and Yamhill Counties have never been relocated.

Habitat for *Erigeron decumbens* var. *decumbens* consists of undeveloped native wetland and upland prairies (Figure 19) at elevations ranging from 235-950 ft (ONHP 2002). Commonly associated prairie species include: *Achillea millefolium*, *Allium amplectens*, *Anthoxanthum odoratum*, *Aster hallii*, *A. curtus*, *Brodiaea hyacinthina*, *Bromus carinatus*, *B. japonicus*, *Carex* spp., *Camassia leichtlinii*, *Crataegus douglasii*, *Danthonia californica*, *Deschampsia caespitosa*, *Elymus glaucus*, *Eriophyllum lanatum*, *Festuca arundinaceae*, *F. roemeri*, *Fragaria virginiana*, *Fraxinus latifolia*, *Grindelia integrifolia*, *Holcus lanatus*, *Juncus* spp., *Lomatium bradshawii*, *Panicum occidentale*, *Poa nevadensis, Potentilla gracilis*, *Prunella vulgaris*, *Quercus garryana*, *Ranunculus occidentalis*, *Rosa* spp., *Saxifraga integrifolia*, *Sidalcea campestris*, and *Spiraea*

douglasii (Kagan and Yamamoto 1987, Clark et al. 1993, 1995, Federal Register 1998, ONHP 2002).

Figure 19. *Erigeron decumbens* var. *decumbens* is known to occupy both wetland and upland prairie habitats, the latter shown here at Basket Slough NWR. *Erigeron* is present as the whitish-purple flowers in the foreground, among shrubs of poison oak. (Photo by Steven Gisler.)

Description of species

Erigeron decumbens var. *decumbens* is a taprooted perennial, with decumbent stems that are often purplish at the base and 1.5-7 dm tall. Basal leaves and some or most of the cauline leaves are triple-nerved, the basal leaves up to 25 cm long and 1 cm wide, and cauline leaves becoming only gradually reduced above. Flowering heads (Figure 20) typically number from 1-20, with 20-50 purple to pale pink ray flowers (6-12 mm long, 1-2 mm wide), yellow disk corollas (2.5-4.5 mm long), and pappus consisting of 12-16 fragile bristles (Hitchcock *et al*. 1955).

Figure 20. Flowering head of *Erigeron decumbens* var. *decumbens*, exhibiting characteristic purple-tinged ray flowers. Insect visitor appears to be the native solitary bee, *Ashmeadiella* sp.(Megachilidae). (Photo by Steven Gisler.)

According to Kagan and Yamamoto (1987), *Erigeron decumbens* var. *decumbens* is the only pink-purple rayed *Erigeron* that occurs in the grassland habitats of the Willamette Valley, and is further distinguished by its gradually reduced cauline leaves, triple-nerved basal leaves, and decumbent, spreading habit.

Seed production

The earliest documented account of seed production in *Erigeron decumbens* var. *decumbens* is found in the federal status report for the species by Kagan and Yamamoto (1987). Here, the authors cited personal communication with Julie Kierstead of The Berry Botanic Garden, who indicated that most individuals produce 25-50 achenes per flower head. Later work by Clark et al. (1993, 1995a) indicated much higher levels of seed production, ranging from 157-182 achenes per head in three populations in 1993 and

160-220.9 seeds per head in 1994. However, subsequent analysis showed that less than 20 percent of these achenes were "robust," and the remaining 80 percent proved to be "empty or shriveled." Therefore, the mean number of filled seeds per head in three populations ranged only from 3.9-29.8 in 1994 (Clark et al. 1995a). Taking only these seeds into account, the number of seeds per head reported by Clark *et al*. (1993, 1995a) more closely align with those previously documented by Kagan and Yamamoto (1987).

With regard to the low production of filled seeds, Clark et al. (1995a) concluded, "…the small percentage of robust seeds produced means few seeds are available for germination and again emphasizes the need to adjust the number of seeds collected for restoration efforts." This point was poignantly demonstrated by Wilson et al. (2001b), who reported collecting 2,693 seeds in 1999 for cultivation projects, but only 5.7 percent of these seeds proved to be filled (i.e., presumably containing viable embryos). To get a better idea of how many filled seeds might actually be produced within populations (and therefore potentially be available for conservation efforts), Clark *et al*. (1995b) sampled two populations (Bald Hill in Benton County and Fisher Butte in Lane County) in 1994 and estimated filled seed production at 3,000 and 1,500 seeds per square meter of stems at the two sites, respectively.

Seed germination

Seed germination in *Erigeron decumbens* var. *decumbens* has been investigated by several researchers, with differing conclusions about levels of seed viability and optimal methods of breaking seed dormancy.

The earliest reference to seed germination research in *Erigeron decumbens* var. *decumbens* is found in Kagan and Yamamoto (1987), who stated, "some type of cold treatment is apparently necessary [for seed germination]." This conclusion was based upon personal communication with Julie Kierstead of The Berry Botanic Garden, who conducted research into germination of seeds collected in 1984. Kierstead found that no seeds germinated after 60 days without cold stratification, though exposure of seeds to ambient winter temperatures eventually resulted in 33 percent germination in early

spring. More rapid germination was achieved by chilling seeds in a refrigerator for one week and then placing them outdoors in soil. This method yielded 22 and 24 percent germination within 2 weeks of sowing, for seeds collected in 1983 and 1984, respectively.

Subsequent research showed that cold stratification can yield even higher levels of seed germination in *Erigeron decumbens* var. *decumbens*. Kaye and Kuykendall (2001) found that germination was essentially zero percent without any cold stratification, but germination increased steadily with duration of stratification, with optimal germination achieved after 12-16 weeks of chilling. Maximum germination was 45-50 percent for seeds originating from two large populations, but was only 17 percent for seeds that came from a small population. Following cold stratification at 5°C, seeds in this study were placed in growth chambers with alternating 15°C/25°C temperatures and a 16 hour photoperiod. Slightly higher germination rates were reported by The Berry Botanic Garden (2002), with 60 percent germination for seeds that were cold stratified for 8 weeks and then placed into 20°C conditions, and 78 percent for cold stratified seeds placed in alternating 10°C/20°C conditions.

An alternative approach to germinating seeds was reported by Clark *et al*. (1995a), who stated, "The germination test showed that pre-treatment cutting or seed coat scarification is essential for promoting germination of *E. decumbens* seeds." Among the variety of seed pre-treatments investigated in Clark's study, maximum seed germination (83.3 percent) was experienced by year-old seeds that were scarified (removal of pericarp and seed coat on the cotyledon end of the seed) and treated with gibberellic acid prior to placement in a germination chamber (15°C dark/30°C light). In contrast, seeds that were cold stratified (4°C for three days) instead of scarified prior to gibberellic acid treatment had 0 percent germination. Among all successful treatments, most germination occurred within one week of seed placement in germination chambers. Seed scarification was also implemented by Wilson *et al*. (2001) to germinate seeds in 2000. Here, seeds were scarified by removing the distal end of their seed coats with razor blades, and then they

were planted 3 mm below the surface of moist seedling mix. Resulting germination was 29.1 percent.

The most recent seed germination attempts in *Erigeron decumbens* var. *decumbens* were performed by Lynda Boyer (Heritage Seedlings Inc., Salem, Oregon, personal communication), who reported nearly 100 percent germination by mixing seeds with pre-moistened vermiculite inside sealed plastic bags and cold stratifying the mixture at 1°C for 13 weeks. This seed/vermiculite mixture was then sown into soil-filled flats, lightly covered with a "light dusting of soil," and most germination typically occurred within 7 days of sowing.

In addition to the germination studies that have been performed under controlled conditions, research has also been conducted on levels of seed germination/seedling recruitment under real and/or simulated field conditions. Clark *et al*. (1995a) described sowing seeds (once in early winter and again in late winter) into pots that were filled with soil from a natural population and buried outdoors, in the ground. The results of this study (reported in Clark et al. 1997) showed that although no germination was detected in the early winter pots, in the late winter pots most seeds germinated from mid-April through May, with a mean 33.5 percent seedling establishment by the end of June. These recruitment rates were much higher than those reported by Kaye et al. (2001), who sowed 3000 seeds into field plots at a restoration site near Eugene, Oregon, in November, 1999. Resulting recruitment rates were less than 0.7 percent after one year, with seedling numbers too low to allow statistical comparison between seed plot treatments. Similarly, Wilson et al. (2001) reported less than 2 percent seedling recruitment from seeds sown in burned, mowed, and unmanipulated control plots.

Vegetative reproduction

The earliest reference to vegetative reproduction in *Erigeron decumbens* var. *decumbens* is found in Kagan and Yamamoto (1987), who stated, "The plants have rhizomes and are decumbent; however, no vegetative reproduction has ever been noted in this taxon. All reproduction occurs sexually, through flowers and seeds." All subsequent references to

this species, however, indicate that the species is in fact capable of clonal growth. For example, Clark *et al*. (1995a) concluded, "*Erigeron decumbens* var. *decumbens* appears to spread vegetatively, forming large clumps. Generally, seedlings and vegetative sprouts cannot be distinguished in the field." This sentiment was later echoed in the Federal Register (1998), and most recently, Kaye (2000) concluded that the species reproduces by seeds and, "also appears to spread vegetatively over very short distances (<10 cm)."

Breeding system

Erigeron decumbens var. *decumbens* attracts numerous insects that are believed to serve as pollinators for the species. Kagan and Yamamoto (1987) and Federal Register (1998) both stated that a variety of insects have been observed visiting *E. decumbens* var. *decumbens*, including butterflies, solitary bees, bumblebees, flies, and introduced honeybees. More specifically, Clark *et al*. (1993) reported observing four different species of solitary bees (*Ceratina* sp., *Megachile* sp., *Nomada* sp., and *Halictus ligatus*), two beetle species (*Meligethes nigrescens* and *Acanthoscelides pauperculus*), and two flies (*Toxomerus marginata* and *Tachina* sp.). Additional pollinating insects were reported by Jackson (1996), who found the most common floral visitor (46.3 percent of visits) to be the native butterfly, *Phyciodes campestris*. Two species of bees in the family Halictidae accounted for 31.6 percent of visits, and a syrphid fly, *Toxomerous occidentalis*, accounted for 12.4 percent of visits. Remaining visits were performed by 4 unidentified fly species and one unidentified beetle species. Based upon pollinator flight data, Jackson estimated neighborhood area (the area in which random breeding is expected to occur) to range from 36 to 86 square meters. As seen in Figure 20, *Erigeron decumbens* var. *decumbens* is also visited by a solitary bee (*Ashmeadiella* sp.) in the Magachilidae family, adding yet another taxon to the list of potential pollinators of this species.

No information was found for *Erigeron decumbens* var. *decumbens* regarding respective levels of selfing and outcrossing, the degree of self-incompatibility, or levels of seed set in the absence of pollinators. Deborah Clark (Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon, personal communication), and Thomas Kaye

(Institute for Applied Ecology, Corvallis, Oregon, personal communication), who have experience researching this species, reported having no knowledge of any previous work in these areas. Given the preliminary evidence of low seed production and progeny performance from small source populations compared to larger ones (Kaye et al. 2001), additional information on self-compatibility and inbreeding depression would be valuable for developing future conservation and population management plans.

Hybridization

According to Kagan and Yamamoto (1987), no natural hybrids involving *Erigeron decumbens* var. *decumbens* are known or suspected, and they stated, "there are no similar species which occur within the range of this taxon, so hybridization is not a threat or concern." Similarly, Clark *et al*. (1993) reported,

> "*Erigeron decumbens* var. *decumbens* does not co-occur with any other members of the *Erigeron eatonii* complex. *Erigeron decumbens* var. *robustior* is restricted to northern California, *E. eatonii* var. *plantagineus* occurs in south-central Oregon and northeastern Calfifornia, and *E. eatonii* var. *villosus* grows in central and eastern Oregon. Naturally occurring hybridization is highly unlikely."

Cultivation

Erigeron decumbens var. *decumbens* has been successfully cultivated in the greenhouse from both seeds and vegetative cuttings. The first reference to cultivation attempts in this species is found in Clark *et al*. (1995a), who described propagation trials using two types of vegetative stock: stem cuttings (12-16 cm long) and rhizome cuttings (stems with 1.0-2.5 cm segments of rhizome tissue). Cuttings were dipped in rooting solution (Indole-3-butyric acid) and placed 1-2 cm deep into pots filled with vermiculite. After 11 weeks, surviving cuttings were moved to 10 x 10 cm pots containing sterilized potting soil and field soil, at which time many cuttings had developed extensive root systems. Results of these propagation trials indicated that rhizome cuttings were more successful than stem cuttings; only six percent of stem cuttings survived after 26 weeks, compared to 33 percent survival of rhizome cuttings.

Use of vegetative cuttings in *Erigeron decumbens* var. *decumbens* propagation is also reported in Wilson et al. (2001). Here, 114 rhizome cuttings were collected, transported in coolers from the field to the greenhouse, rinsed with mild bleach solution, and treated with a rooting hormone prior to planting. Cuttings were initially propagated in pots filled with 80 percent perlite, 20 percent peat moss, and a slow-release fertilizer. Pots containing the cuttings were placed inside a misting chamber and watered for 5 seconds every 8 minutes. After 3 months the cuttings were removed from the chamber, transplanted into commercial potting soil, and kept in the greenhouse until subsequent transplanting into field locations. Mortality of cuttings was reportedly high, with only 13.2 percent (15 plants) survival to time of transplanting.

The earliest attempt to cultivate *Erigeron decumbens* var. *decumbens* from seed is described in Clark et al. (1995a). Here, two methods were investigated: germinating seeds in Petri dishes and then transplanting them into pots filled with standard potting mix, and germinating seeds directly in potting mix, to avoid potential seedling damage during transplanting from Petri dishes. Pots for both treatments were placed in growth chambers (15°C/25°C and 12 hour photoperiod) and watered as needed. Neither method yielded successful establishment of seedlings, with most mortality attributed to fungal infection (i.e., damping off).

Fortunately, subsequent attempts to cultivate *Erigeron decumbens* var. *decumbens* from seed have been more successful. Wilson et al. (2001) reported 18.6 percent survival among seedlings that were initially grown in the greenhouse and then moved outside to await later transplanting into field locations. Even higher survival rates were reported by Kaye and Kuykendall (2001). Here, among seedling cohorts ranging in age from 4 to 8 weeks, mean survival in the greenhouse was 50-100 percent, depending on cohort age and seed source. All seedlings were cultivated in a soil mix consisting of one part peat, one part loam and two parts pumice, and plants were watered twice weekly and fertilized once a week using 20:20:20 fertilizer.

The most recent attempts to cultivate *Erigeron decumbens* var. *decumbens* were reported by L. Boyer (personal communication). As described in "Seed germination," above, seeds were mixed with pre-moistened vermiculite and cold stratified (at 1°C) inside sealed plastic bags for 13 weeks. This seed/vermiculite mixture was then sown into flats filled with a planting medium consisting of bark, compost, peat, perlite, and Philip's pre-mix (crabmeal, 3 kinds of lime, micronutrients, Actino-iron, and a wetting agent). Following establishment in flats, seedlings were transplanted into 5 inch x 2 3/8 inch pots. Boyer reported that although seedling establishment and survival were very high (nearly 100 percent), seedlings were nevertheless "not very robust" after 75 days.

Transplanting and introduction attempts

Recent attempts at introducing *Erigeron decumbens* var. *decumbens* to new locations indicate that transplanting of cultivated plants works much better than sowing seeds directly. Kaye et al. (2001) reported less than 0.7 percent seedling recruitment from 3000 seeds sown at a restoration site near Eugene, Oregon, whereas survival of individuals transplanted from the greenhouse ranged from 33 to 71 percent after 6 months. Other data from this study indicated that seedling recruitment was slightly improved by removing vegetation from seed plots prior to sowing, and transplant survival was significantly improved by fertilizer application at the time of planting (71 percent survival for fertilized transplants compared to 55 percent survival without fertilizer). Kaye *et al*. also noted that transplant survival varied in relation to seed source, with only 33 percent survival from one small population compared to 64 and 70 percent survival from two larger populations, respectively.

In another study, Wilson et al. (2001) reported less than 2 percent seedling recruitment from direct sowing of seeds into burned, mowed, and unmanipulated control plots. Cultivated seedlings and larger plants from cuttings were also transplanted into plots, but survival data was not yet available upon submission of this report.

Population monitoring

Population monitoring for *Erigeron decumbens* var. *decumbens* has been undertaken by various individuals and organizations at numerous sites, including The Nature Conservancy's Willow Creek Preserve and Fern Ridge Lake (Kagan and Yamamoto 1987), Baskett Slough National Wildlife Refuge, Bald Hill and Fisher Butte (Clark *et al*. 1995b, Finley and Ingersoll 1995, Finley 1998), and the Eugene BLM's Oxbow West site (Kaye 2000). Monitoring of these populations has generally been carried out in a consistent manner, with the establishment of permanent monitoring plots within which individuals have been censused and/or mapped, and typically measured for number of flowering stems, basal area, and plant height. Plot sizes have ranged from 0.5m x 0.5 m (Finley 1998) to 10m x 10m (Kagan and Yamamoto 1987) to 15m x 40m (Kaye 2000).

Those who have performed population monitoring of *Erigeron decumbens* var. *decumbens* have reported various noteworthy complications related to the biology and life history of the species. For instance, Kagan and Yamamoto (1987) found it difficult to determine survival and mortality between years because of irregular emergence and sporadic flowering from year to year. They suggested that some plants probably lie dormant during some years, as indicated by the sudden appearance of large plants where they were not previously recorded, and the disappearance and later re-emergence of large plants within monitoring plots. In addition, Clark et al. (1993) stated that non-reproductive individuals can be very difficult to find and monitor due to their inconspicuous nature, and that the definition of individuals can be complicated when flowering clumps overlap. These sentiments were echoed by Finley (1998), who stated, "The clonal nature of the plant makes long-term attention to separating the fates of particular individual plants problematic."

Given the problems associated with discerning physiologically distinct individuals due to overlapping clusters of stems, workers have variously defined individuals (for censusing and monitoring purposes) as any stem/stem cluster separated from its nearest neighbor by at least 5 cm (Finley 1998) or 7 cm (Kaye 2000). As a possible means of avoiding the problems of defining individuals, Finley (1998) proposed that monitoring might instead

focus only on number of reproductive stems, flower heads, and cover within fixed plots—measurements that would not necessarily rely on the definition and relocation of the same individuals from one year to the next.

Land use threats and other limitations

Like many native species endemic to Willamette Valley prairies, *Erigeron decumbens* var. *decumbens* is threatened by habitat loss due to urban and agricultural development, secondary successional encroachment of habitat by trees and brush, competition with non-native weeds, and small population sizes (Kagan and Yamamoto 1987), Clark *et al*. 1993, Federal Register 1998). According to the Federal Register (1998), habitat loss is occurring at 80 percent of remaining 84 remnants of native prairies occupied by *Erigeron decumbens* var. *decumbens* and another threatened species, *Lupinus sulphureus* ssp. *kincaidii*. Furthermore, it is stated that 24 of the 28 extant *E. decumbens* var. *decumbens* populations occur on private lands and, "without further action, are expected to be lost in the near future."

Although populations occurring on private lands are the most vulnerable to threats of development (state and federal plant protection laws do not apply to private lands), publicly owned populations are not immune from other important limitations to the species. For instance, Clark et al. (1993) identified four populations protected from development on public lands (Willow Creek, Basket Slough NWR, Bald Hill Park, and Fisher Butte RNA), but stated that even these appear to be threatened by the proliferation of non-native weeds and successional encroachment of brush and trees. Likewise, vulnerability arising from small population sizes and inbreeding depression may be a concern for the species, regardless of land ownership, especially among 17 of the 28 remaining sites that are less than 3.4 hectares in size (Federal Register 1998).

Given the predominance of privately owned populations, land ownership represents a serious obstacle to conservation and recovery of *Erigeron decumbens* var. *decumbens*. Efforts should be made to acquire privately owned sites and/or gain the voluntary cooperation of landowners in conserving the species before further habitat loss takes

place. Meanwhile, the few remaining populations on public lands should be the focus of intensive management and conservation efforts, serving both as seed sources for off-site cultivation and target sites for re-introduction and population augmentation projects.

Population introduction/augmentation strategy

Based upon the biogeographical data compiled and described above for *Erigeron decumbens* var. *decumbens*, 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. Although many *E. decumbens* var. *decumbens* populations face imminent threats on private lands, and native prairie habitats have been reduced to tiny remnants of their former abundance, there are still some extant *E. decumbens* var. *decumbens* populations occurring on public or otherwise secure landholdings. As such, pending interagency cooperation and funding availability, there should be several good sites available for collection of seeds and/or rhizome cuttings for use in off-site cultivation projects, and open locations should also be available for population augmentation and introduction purposes.

Perhaps the most serious environmental constraint to these much-needed conservation efforts is the proliferation of invasive weeds, which already pose a formidable threat to existing populations and limit the quality and availability of suitable introduction sites. However, while invasive species will continue to pose a challenge to habitat managers, there are still high quality prairie remnants remaining throughout the range of the species that can provide valuable opportunities for *E. decumbens* var. *decumbens* population introduction projects.

The biology and life history of *Erigeron decumbens* var. *decumbens* likewise pose no unavoidable hurdles to successful implementation of population introduction and augmentation projects. Although production of viable seeds is low in this species, seed germination rates among filled seeds generally range between 50-83 percent (when seeds are properly cold stratified and/or manually scarified), and *E. decumbens* var. *decumbens* has been successfully cultivated from both seeds and rhizome cuttings in the greenhouse.

Seed production and germination rates may be lower in some small populations, possibly due to inbreeding depression. The species exhibits no unique propagation or soil symbiont requirements, and seedlings and rhizome cuttings have been reported to experience fast root development (though they have simultaneously been characterized as "not very robust"). Once mature *E. decumbens* var. *decumbens* plugs are obtained through off-site cultivation, they have demonstrated fairly high rates of survival when introduced into the wild, ranging from 33 to 71 percent (the higher survival percentage apparently boosted by fertilizer application at the time of planting). In contrast, population introduction attempts employing direct seed sowing have proven less successful.

Although *Erigeron decumbens* var. *decumbens* lends itself to vegetative propagation in the greenhouse and forms clones in the wild, efforts should be made to maximize the frequency of genetically variable individuals when creating or augmenting populations, because preliminary evidence suggests the possibility of inbreeding depression in this species, and self-incompatibility has not yet been investigated. Therefore, genetically diverse introduction stock should be used whenever possible to elevate seed production and reproductive fitness, and also ostensibly improve the odds of overall introduction success by maximizing the amount of adaptive genetic variability in the population. Interspecific hybridization does not appear to pose a serious concern for *E. decumbens* var. *decumbens*, as there are no closely related, sexually compatible species in its geographic range and habitat type.

Based upon this information, the following step-down procedures are recommended for *Erigeron decumbens* var. *decumbens* population introductions:

1. Select population introduction/augmentation target sites. Several primary factors should be considered when selecting target sites for *Erigeron decumbens* var. *decumbens* population introduction and augmentation projects. First, target sites should obviously contain suitable habitat. However, habitat for *E. decumbens* var. *decumbens* can vary from poorly drained, wet prairie sites, to drier, more

upland prairie habitats. As such, although the habitat descriptions in this manual can be used to provide general habitat suitability guidelines, specific population introduction target sites should ultimately be selected based upon visits to local extant *E. decumbens* var. *decumbens* populations. Such visits should impart a better idea of the kinds of microsites occupied by individuals within their larger prairie habitat type.

Given the ongoing (and expected) decline of *Erigeron decumbens* var. *decumbens* populations on private lands, inventories for suitable population introduction and augmentation sites should be focused strictly to publicly owned (or otherwise secure) lands. Selection and use of sites should be coordinated with pertinent public landowners to ensure administrative protection and management of populations following introductions.

2. Collect *Erigeron decumbens* var. *decumbens* seeds and/or rhizome cuttings for off-site cultivation of introduction stock. Source material for off-site cultivation of *Erigeron decumbens* var. *decumbens* should be collected from the extant population(s) located nearest to the population introduction target sites to minimize undesirable mixing of gene pools and maximize conveyance of potential local adaptations (if such intraspecific variability and adaptations exist). Given low levels of viable seed production documented in *E. decumbens* var. *decumbens*, seed collecting should be planned and implemented well in advance of cultivation projects to ensure adequate time (possibly several consecutive years) for the harvest of sufficient seed supplies. Based upon historic seed production estimates, individual *Erigeron decumbens* var. *decumbens* flowering heads can be expected to produce from 4-50 viable seeds per head, which in turn represent only a small fraction of the total number of unfilled/shriveled achenes that are produced.

In light of suggestive evidence of inbreeding depression documented in *Erigeron decumbens* var. *decumbens*, and lack of data on levels of self-incompatibility in

the species, efforts should be made to collect seeds and/or rhizome cuttings from as large a sample of genetically variable individuals as possible, in an effort to elevate seed production, fitness, and adaptive genetic variability within introduced populations.

3. Cultivate *Erigeron decumbens* var. *decumbens*. *Erigeron decumbens* var. *decumbens* has been successfully cultivated from both seeds and rhizome cuttings. If seeds are used for cultivation of introduction stock, previous studies suggest they should be cold stratified at 5°C for 8-16 weeks, and/or scarified at the pappus end of each achene, to maximize germination rates. Following pre-treatment, seeds should be expected to germinate within 2-11 days, at rates typically ranging between 40-78 percent (though rates apparently can be lower in smaller population suffering from inbreeding depression). Once seeds germinate, seedling survival can be high (50-100 percent) in the greenhouse, though high mortality rates resulting from damping off have been noted by some workers.

   Erigeron decumbens var. *decumbens* can also be cultivated using rhizome fragments. Although extensive studies of these propagation techniques have not been performed, one study indicated 33 percent survival after dipping rhizome fragments in rooting hormone and placing them for 11 weeks in flats filled with vermiculite.

4. Introduce cultivated plugs into the target site(s). *Erigeron decumbens* var. *decumbens* introductions should be performed after the arrival of fall rains, so that soils are moist at the time of planting and plugs have ample opportunity for root system development prior to summer drying. Currently we have no information about the extent to which *Erigeron decumbens* var. *decumbens* may be pollen limited in small patches, or the degree to which the species may be self-incompatible. There is preliminary evidence, however, that inbreeding depression may limit seed production and progeny performance in small populations. As such, when populations are introduced into new sites, they should probably

consist of many individuals planted in large patches to help maximize opportunities for outcrossing. However, this "patch" technique may be at odds with monitoring goals, if the latter are oriented towards tracking individuals over time (since rhizomatous/clonal growth may complicate the identification of closely planted individuals) (see #5, below).

5. Monitor introduced populations. Introduced *Aster curtus* plugs should be monitored annually to evaluate project success. Given the clonal nature of the species (i.e., asexual expansion via rhizomes), which can seriously complicate the definition of individuals, monitoring should probably be carried out in a way that simply estimates presence or absence, or overall *A. curtus* cover, within sampling plots. Counting the number of individual stems and/or attempting to discriminate individuals should probably only be attempted when introduced populations are very small and introduced plugs are widely spaced so that they can be reliably differentiated over time (though even in these cases stem counting may become infeasible if clones eventually become large and intertwined).

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Developing biogeographically based population introduction protocols for at-risk Willamette Valley plant species:

Horkelia congesta ssp. *congesta* (shaggy horkelia)

Horkelia congesta ssp. *congesta* (shaggy horkelia)

Conservation status

Horkelia congesta ssp. c*ongesta* (Figure 21), a rare member of the rose family (Rosaceae) is restricted to small native wetland and upland prairie remnants in the Willamette and Umpqua River Valleys of western Oregon. The limited range of this naturally rare species has been further reduced by increasing habitat loss and degradation due to successional shrub encroachment and weed colonization. These threats prompted the U.S. Fish and Wildlife Service (USFWS) to list *Horkelia congesta* ssp. *congesta* as a Species of Concern, and the Oregon Department of Agriculture to include it on the state's Candidate list. This species is on the Oregon Natural Heritage Program List 1 (threatened or endangered throughout its range), and has a Natural Heritage Network Rank of G4/T2/S2 (the subspecies is imperiled throughout its range/imperiled in Oregon) (ORNHIC 2004).

Figure 21. *Horkelia congesta* ssp. *congesta*. (Photos by Steven Gisler.)

Range and habitat

The type specimen of *Horkelia congesta* ssp. *congesta* was collected by the northwest explorer, David Douglas, "…on the low hills of the Umpqua River [Douglas County] upon the North-west coast of America" (Hooker 1829). Since its discovery, this species has been reported from approximately 40 different locations in six western Oregon counties (Douglas, Lane, Linn, Marion, Polk, and Washington Counties). Currently, however, *H. congesta* ssp. *congesta* is known to be extant at only 26 sites in four counties (Douglas, Benton, Lane, and Linn Counties; ONHP 2002), possibly indicating that *H. congesta* ssp. *congesta* has been extirpated from the northern portion of its range.

The habitat of *Horkelia congesta* ssp. *congesta* was described by Kaye and Gisler (1993) as "grassland and oak savannah remnants in the Willamette Valley and on grassy balds in the Umpqua Valley" (Figures 22 and 23). Within Willamette Valley prairies, populations occupy a variety of microsites, ranging from slight topographic rises within wet prairies to distinctly dry uplands, and from completely open areas (i.e., grassy balds) to shady understories of oak/fir woodlands (Gisler, personal observation). Extant populations range in elevation from 275-1500 feet, with the higher elevation sites located in the southern portion of the species range (ONHP 2002).

Figure 22. Wet prairie habitat occupied by *Horkelia congesta* ssp. *congesta* at the Long Tom ACEC, owned by Eugene District BLM. Occasional overstory species include *Quercus garryana* and *Fraxinus latifolia*. (Photo by Steven Gisler.)

Figure 23. Upland prairie habitat occupied by *Horkelia congesta* ssp. *congesta* at a cemetery near Lebanon in Linn County, Oregon. *Horkelia* continues to persist in a few small areas at this cemetery, despite regular mowing of the grounds. (Photo by Steve Gisler.)

According to herbarium label and ONHP sighting report information, *Horkelia congesta* ssp. *congesta* is commonly associated with the following native and introduced species: *Agrostis tenuis*, *Allium amplectens*, *Aster hallii*, *Brodiaea congesta*, *Camassia quamash*, *Centaurium umbellatum*, *Chrysanthemum leucanthemum*, *Cynosurus echinatus*, *Cytisus scoparius*, *Dactylis glomerata*, *Danthonia californica*, *Deschampsia caespitosa*, *Daucus carota*, *Eriophyllum lanatum*, *Festuca arundinaceae*, *F. rubra*, *Fragaria virginiana*, *Fraxinus latifolia*, *Holcus lanatus*, *Hypericum perforatum*, *Lathyrus holochlorus*, *Lomatium utriculatum*, *Lotus micranthus*, *Potentilla gracilis*, *Prunella vulgaris*, *Pseudotsuga menziesii*, *Quercus garryana*, *Q. kelloggii*, *Rosa eglanteria*, *R. nutkana*, *Rubus procerus*, *R. discolor*, *Rhus diversiloba*, *Sanicula crassicaulis*, *Saxifraga oregana*, *Sidalcea campestris*, and *S. virgata* (OSU herbarium records, ONHP 2002).

Description of species

Horkelia congesta ssp. *congesta* is a taprooted perennial with erect flowering stems (usually simple but sometimes dichotomously branched) arising 1-4 dm from few-leaved rosettes. Basal leaves are 5-15 cm long, with pilose or hirsute petioles and silky-villous leaf blades, possessing 2-5 pairs of leaflets (Figure 24). Flowering heads (cymes) are congested and capitate, terminating the mostly simple or sparingly branched stems. Hypanthia are hemispheric, 3-4 mm wide, and glabrous within. Petals are creamy white, rotund, and exceed the lanceolate sepals. The 10 stamens are obscurely biseriate and anthers are broader than long (0.4-0.7 mm long) (Keck 1938).

To help discriminate *Horkelia congesta* ssp. *congesta* from two other congeners occurring within the species' range (i.e., *H. tridentata* and *H. congesta* ssp. *congesta*), Kaye (1995) conducted a morphometric analysis of 18 morphological traits. Results of this study showed stipule shape is the most useful trait for distinguishing *H. congesta* ssp. *congesta* from *H. congesta* ssp. *nemorosa*, with those of the former being markedly dissected into linear segments and those of the latter being only shallowly divided with broad segments. *Horkelia tridentata* can be distinguished by having much narrower petals (more than twice as long as wide) than *H. congesta*, and having stems almost completely red-brown pigmented, as opposed to only partial and/or weak pigmentation of stems in *H. congesta* ssp. *congesta*.

Figure 24. Pressed *Horkelia congesta* ssp. *congesta* specimen from the OSU Herbarium, showing taproot and other features.

Seed production

Although *Horkelia congesta* ssp. *congesta* seeds have been collected for several research and restoration projects (i.e., Kaye and Kuykendall 2001, Clark et al. 2001), data on seed production and pre-dispersal seed predation (if any) have apparently never been formally recorded or reported. However, in a personal communication, Thomas Kaye (Institute for Applied Ecology, Corvallis, Oregon) stated that although he had never made formal seed counts, "…seed set appears modest to okay in *Horkelia*, and I would venture to estimate that in the samples I've taken, it averages 1-3 per flower. I have seen no evidence of pre-dispersal seed predation at all, and I've looked at thousands of seeds."

The aforementioned estimate by Kaye is consistent with those subsequently made by the author in July, 2002. Based on a random sample of 25 flowers from 25 individuals at the Greenhill population west of Eugene, *Horkelia congesta* ssp. *congesta* produced a mean of 12.92 ovules per flower (range 7-19, SD 3.17) and a mean of 1.84 achenes per flower (range 0-4, SD 1.31; Gisler unpublished).

Flower production in pressed specimens of *Horkelia congesta* ssp. *congesta* in the herbarium at Oregon State University varies substantially between few and many stemmed individuals, but ranges from 100-400 flowers in typical plants producing 4-5 primary flowering stems. Therefore, if we assume an average 1-3 seeds per flower, then seed production would be expected to range from 100-1200 per plant.

Seed germination

The first documented attempts to determine methods and rates of seed germination in *Horkelia congesta* ssp. *congesta* were reported by Guerrant and Raven (1995). In this study, germination rates were 21 percent for fresh seeds placed outdoors for the winter, 23 percent for previously desiccated and frozen seeds under outdoor conditions, 10.5 percent for cold stratified seeds (5°C for six weeks). Seeds that were placed in a warm chamber (alternating 10°C and 20°C) for 6 weeks, followed by 6 weeks in a cold

chamber (alternating 5/15°C) did not germinate. Mean elapsed time for 50 percent germination ranged from 65.4 to 80 days.

To expand on the preliminary work by Guerrant and Raven (1995) and gain additional knowledge of *Horkelia congesta* ssp. *congesta* cold stratification requirements, Kaye and Kuykendall (2001) placed seven sets of 150 seeds each from two populations on germination trays and chilled them at 4°C for 0, 2, 4, 6, 8, 12, and 16 weeks. After chilling, one set from each population was placed in a growth chamber with 15/25°C alternating temperatures with eight hours of darkness at the low temperature and 16 hours of light corresponding with the high temperature. Germination rates increased with increasing duration of cold stratification, with the highest germination (57 percent and 76 percent from the two study populations, respectively) observed after 16 weeks of cold stratification. No germination was recorded from the 0 and 2 week chilling treatments.

Despite the relatively high germination rates reported above, Kaye et al. (2001) stated that seedling recruitment from scattered seeds in nature is rather low, ranging from 0.2-12 percent, depending on site conditions (see "Outplanting and transplanting," below).

Vegetative reproduction

Horkelia congesta ssp. *congesta* does not appear capable of vegetative reproduction, and reproduces solely by seeds. Although some underground caudex branching occurs in nature, leading to formation of multiple interconnected rosettes, this process does not appear to lead to spontaneous development of physiologically distinct clones (Kaye and Gisler 1993).

Breeding system

To date no studies have been conducted to identify the breeding system of *Horkelia congesta* ssp. *congesta*, though field observations indicate that insects (solitary bees and syrphid flies) are responsible for cross-pollination (Kaye and Gisler 1993). In 2002, the author (Gisler, unpublished) observed two species of solitary bees (*Halictus* sp. and

Andrena sp.) visiting *Horkelia* at the Long Tom Area of Critical Environmental Concern (ACEC) near Eugene, and one muscid fly visitor at a population located near Lebanon in Linn County. Among the bees, the only observed nectar foraging occurred between flowers of the same plant, which may have resulted in self-pollination via geitonogamy (if plants are self-compatible).

Statements by Keck (1938) suggest that many species of *Horkelia* may be predisposed to self-pollination due to inward-dehiscing anthers. Keck states, "This mode of dehiscence is particularly effective in *Horkelia*, in which a cup-shaped hypanthium is highly developed and the anthers stand just over the pistils at the time of shedding pollen." Keck reports that the inward-orientation of stamens in many species is especially pronounced in early anthesis, and that filaments may later straighten to an erect position.

Although the aforementioned information is suggestive, additional research is needed to identify the breeding system of *Horkelia congesta* ssp. *congesta*, especially with regard to self-compatibility and the relative rolls of autogamy, geitonogamy, and xenogamy. Given the small size of many extant populations, information on levels of inbreeding depression (i.e., comparative levels of fecundity and fitness between within-plant, within-population, and between-population crosses) would also be of conservation importance.

Hybridization

Although southern Oregon populations of *Horkelia congesta* ssp. *congesta* tend to exhibit intermediate morphology with *H. congesta* ssp. *nemorosa* (Keck 1938, Kaye 1995), there is no reason to believe this is due to recent hybridization events, nor is there any evidence that hybridization is currently occurring in this taxon. Although sexual compatibility with other congeners has not been investigated, hybridization in *H. congesta* ssp. *congesta* is extremely unlikely because the species is not known to co-occur with any other close relatives (i.e., potential interfertile heterospecific mating partners) at extant populations.

Cultivation

Horkelia congesta ssp. *congesta* has been successfully cultivated from seed by Kaye et al. (2001), who reported "very high survival in pots in a greenhouse" using standard potting mix, daily watering, and monthly liquid fertilizer application.

Transplanting and introduction attempts

Population creation projects utilizing both direct seeding, and transplantation of greenhouse-grown plants, were completed at three BLM-owned sites (Greenhill Road, Balboa, and Rosy) west of Eugene (Kaye 2001). At the Greenhill Road site, vegetation removal prior to sowing increased seedling emergence from 0.2 percent to three percent. At the Balboa and Rosy sites, treating plots with various soil amendments at the time sowing did not affect the mean of eight percent seedling emergence - seedling emergence ranged from six to 12 percent among four planting block.

Survival of greenhouse-grown transplants at the Greenhill site after six months was very high, exceeding 95 percent for all treatments. Survival and growth were not affected by seed source, and fertilizer did not affect survival, but did increase plant size.

In a study evaluating the effects of fire on the establishment of rare wetland prairie species, Clark et al. (2001) sowed seeds of *Horkelia congesta* ssp. *congesta* in burned and unburned plots at Danebo Wetland, Eugene, Oregon. Establishment was lower in burned plots (4.2%) than in unburned plots (7.8%), but this difference was not significant.

Population monitoring

Population monitoring at the BLM's Long Tom ACEC was initiated in 1993, and has been conducted annually since that time (Kaye and Brandt 2003). Five monitoring plots encompass nearly all of the individuals in the site, with a few scattered plants counted separately, to provide a complete census of the population. Observed population trends were positive from 1993-1996, then declined from 1997- 2002, with an increase in 2003.

At BLM's Greenhill Road site, monitoring in 1997 consisted of a complete census, using a series of 1x1 m plots, with all plants counted and measured (Kaye 1997). One hundred and eighty-one plants were present; plant measurements included diameter of rosette, height, number flowering stems, and number grazed stems. When counting plants of *H. congesta* ssp. *congesta*, it was occasionally difficult to determine if a cluster of rosettes represented one or more individuals, since some subterranean caudex branching occurs.

Land use threats and other limitations

Aside from the obvious threats of habitat loss and invasive weeds, *Horkelia congesta* ssp. *congesta* is threatened by successional changes to its grassland habitat. According to Kaye and Gisler (1993), "Succession of woody plants in the habitat is cause for concern. In the absence of fire, woody shrubs and trees may shade and outcompete many prairie plant species, resulting in a conversion of prairie/savannah habitat to woody thicket or riparian forest." Kaye (1999) also lists grazing by deer as a potential threat.

Population introduction/augmentation strategy

Based upon the biogeographical data compiled and described above for *Horkelia congesta* ssp. *congesta*, there are no significant ecological, life history, or administrative obstacles to the successful implementation of population introduction and augmentation projects for this rare species. Several large populations of this species occur in publicly owned sites; pending interagency cooperation and funding availability, these populations should be available for collection of seeds for use in reintroduction projects. Suitable unoccupied locations within publicly owned sites should also be available for population augmentation and introduction purposes.

The biology and life history of *Horkelia congesta* ssp. *congesta* pose no unavoidable hurdles to successful implementation of population introduction and augmentation projects. Adequate seed is produced, germination and cultivation protocols are available, and cultivated plants establish well. Interspecific hybridization does not appear to pose a

serious concern for *Horkelia congesta*, as long as introduction target sites are limited to the current range and habitat occupied by the species.

One potential obstacle to successful population creation is the proliferation of invasive weeds, which already pose a formidable threat to existing populations and limit the quality and availability of suitable introduction sites. However, while invasive species will continue to pose a challenge to habitat managers, there are still high quality prairie remnants remaining throughout the range of the species that can provide valuable opportunities for *Horkelia congesta* population introduction projects.

Based upon this information, the following step-down procedures are recommended for *Horkelia congesta* ssp. *congesta* population introductions:

1. Select population introduction/augmentation target sites. Several factors should be considered when selecting target sites for *Horkelia congesta* ssp. *congesta* population introduction and augmentation projects. First, target sites should contain the upland prairie or grassland habitat (described above) that is preferred by this species. To assist in identification of suitable habitat, extant populations in the vicinity of target sites should be visited to obtain familiarity with possible local microhabitat specificities. Data on associated species, soil types, and soil moisture from known extant populations in the vicinity of the target area help characterize suitable habitat, and can be used to assist with the selection of population creation sites. These data are also helpful in determining microsites within these areas that are suitable for transplanting.

   Given the history of the destruction of *Horkelia congesta* ssp. *congesta*'s prairie habitat on private lands, and the ubiquitous threat posed by invasive species, inventories for suitable population introduction and augmentation sites should be focused on publicly owned (or otherwise secure) lands that appear safe from imminent weed and successional encroachment problems. Selection and use of

sites should be coordinated with pertinent public landowners to ensure administrative protection and management of populations following introductions.

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. Collection of *Horkelia congesta* ssp. *congesta* 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. In situations involving a selected site outside the current range of the species, combined collections of seed from several populations may also be appropriate. To ensure that the genetic variation present in the extant donor population will be represented in the newly created site, seed should be collected from as many individuals as possible, located throughout the population.

   To reduce the impact to existing populations, and provide additional genetic diversity, seed for creation/augmentation projects should ideally be collected in multiple years. However, the respectable seed production, high level of germinability and ease of cultivation reported for *Horkelia congesta* ssp. *congesta* should allow an adequate supply of seeds for most population creation projects to be collected in a single year, unless source populations are extremely small.

3. Cultivate transplants. *Horkelia congesta* ssp. *congesta* transplants can be successfully cultivated from seeds. To ensure good germination, seeds should be cold stratified for 16 weeks, either artificially or under natural Willamette Valley outdoor winter conditions. Germinated seeds can be potted in standard potting soil, and grown under greenhouse or nursery conditions. Potted plants should be watered frequently, as soon as soil surface dries, and fertilized monthly.

4. Introduce cultivated plugs into the target site(s). *Horkelia congesta* ssp. *congesta* introductions should be performed after the arrival of fall rains, so that soils are

moist at the time of planting, and plugs have ample opportunity for root system development prior to summer drying. Although evidence indicating that vegetation removal increases transplant success is not conclusive, transplanting at least some stock into plots in which associated vegetation has been removed will probably improve population establishment, and will provide additional data on the specific requirements for transplanting of this species. Assuming selection of a suitable site, and careful planting of healthy stock, transplants can be expected to exhibit a high rate of emergence in the year following installation. Because seeds also establish fairly well, additional genetic diversity can be incorporated into the created population by augmenting transplants with site-sown seed.

Although there is currently no information on pollen limitation or breeding system for *Horkelia congesta* ssp. *congesta,* it may be beneficial to introduce plugs into large patches in order to improve the prospects of pollinator attraction to floral rewards. Finally, the layout of introduced plugs should be designed in a manner that is consistent with subsequent population monitoring objectives (see #5, below).

5. Monitor introduced populations. Introduced *Horkelia congesta* ssp. *congesta* plugs should be monitored annually to evaluate project success. The species is not known to spread vegetatively, so there should be few difficulties in distinguishing introduced individuals, even when they grow closely together. However, it may be helpful to place transplants into a grid or transect to facilitate relocation of individuals over time. Plugs could either be monitored through simple census techniques, or more intensive monitoring could be performed to track the fates of individuals and evaluate reproductive performance and growth.

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Developing biogeographically based population introduction protocols for at-risk Willamette Valley plant species:

Lomatium bradshawii
(Bradshaw's desert parsley)

Lomatium bradshawii (Bradshaw's lomatium)

Conservation status

Although *Lomatium bradshawii* (Rose) Math. & Const. (Figure 25) was once found throughout the extensive native prairies of the Willamette Valley (Kagan 1980), this rare Willamette Valley species is now listed as endangered by both the U.S. Fish and Wildlife Service and the State of Oregon. It is on the Oregon Natural Heritage Information Center 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). In Washington, *L. bradshawii* is listed by the State as Endangered, though this status carries no legal mandate for protection on state or other public lands (Florence Caplow, Washington Natural Heritage Program, Olympia Washington, personal communication). The species also has also been assigned a rank of S1 (critically imperiled) by the Washington Natural Heritage Program (WNHP 2003). A Recovery Plan for *L. bradshawii* was finalized in 1993 (USFWS 1993). Loss and degradation of the low elevation, seasonally wet, prairie habitat which *L. bradshawii* needs is the biggest threat to the species at this time.

Figure 25. *Lomatium bradshawii* habit. (Photo by Steve Gisler.)

Range and habitat

For many years *Lomatium bradshawii* was considered a Willamette Valley endemic, its range limited to the area between Salem and Creswell, Oregon (Kagan 1980). However, in 1994 two populations of the species were discovered in Clark County, Washington (CPC 2004). The Oregon Natural Heritage Information Center (ORNHIC 2004b) currently lists 38 occurrences of *L. bradshawii* in three populations centers located in Benton, Lane, Linn, and Marion Counties, Oregon. Most of these populations are small, ranging from about 10 to 1,000 individuals, although the largest site contains approximately 30,000 plants. The Washington populations are larger, with one site estimated to have over 70,000 individuals; unfortunately, both of these populations occur on private land and are not protected (WNHP 2004).

According to Siddall and Chambers (1978), the species was first collected in Salem by Nelson in 1916. Bradshaw collected the type specimen a few years later, in 1921, in "low swales near the high school, Eugene, Oregon." Siddall and Chambers describe *L. bradshawii*'s habitat as undisturbed sites of native Willamette Valley grassland, with associated species including: *Carex* spp., *Deschampsia caespitosa*, *Eryngium petiolatum*, *Galium cymosum, Grindelia integrifolia*, *Hordeum brachyantherum*, *Juncus* spp., *Microseris laciniata*, *Perideridia* sp., and *Poa pratensis*. Kagan (1980) further elaborates by stating that the *Lomatium* occurs on and around the small mounds created by senescent *Deschampsia caespitosa* plants, and lists

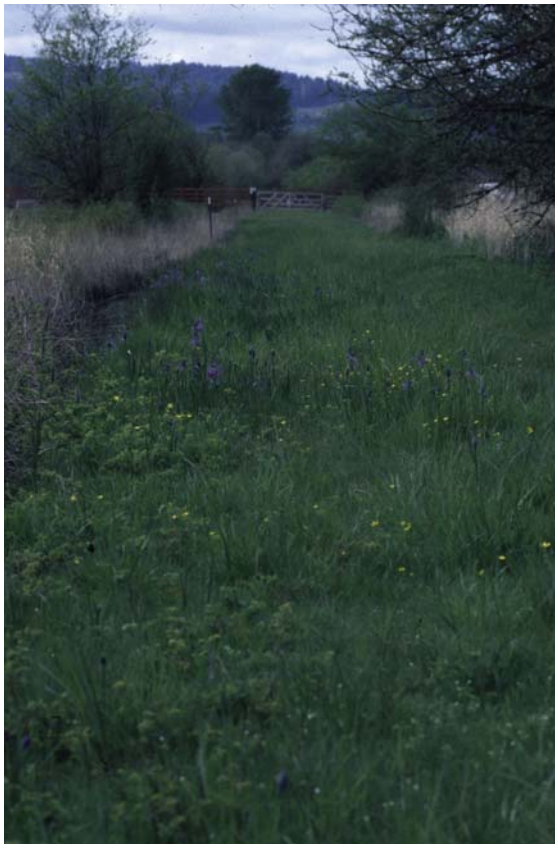

Figure 26. *Lomatium bradshawii* habitat (roadbed population at Finley NWR). (Photo by Steve Gisler.)

the following additional associates: *Carex aurea*, *C. lasiocarpa*, *C. lanuginose*, *C. obnupta*, *Danthonia californica*, *Juncus patens*, *J. acuminatus* and *Luzula campestris*. All of the populations studied by Kagan occur within 500 meters of the banks of creeks or small rivers, where soils largely consist of clay, and are shallow and poorly drained. These sites often have standing water until late spring or early summer. In populations near the Santiam River in Marion and Linn Counties, Kaye and Kirkland (1994) found that *L. bradshawii* plants occupy thin, seasonally saturated soils overlying basalt. (Habitat shown in Figure 26.)

Species description

Lomatium bradshawii is a low, erect perennial arising from a long slender taproot. Overall, the plant is glabrous and has leaves 4-12 inches long which are dissected into linear or filiform segments. *L. bradshawii* has small light yellow flowers that occur in umbels (umbellets are rarely larger than 1 cm across). Generally, only 2-5 flowers in each umbel are actually fertile. The fruit of this *Lomatium* is oblong, 1/4-1/2 inch long and glabrous with thickened, corky wings and inconspicuous dorsal ribs (Hitchcock 1961). The plant's blooming period peaks around the end of April and beginning of May, but flowers may be observed as early as the first week of April through the end of May (Kagan 1980).

Figure 27. Diagnostic bracts of *L. bradshawii*. (Photo by Steve Gisler.)

The species is distinguished from other species of *Lomatium* by its conspicuously ternately divided free involucel bracts (Figure 27). *L. utriculatum*'s range overlaps with

that of *L. bradshawii*, but its involucels are shallowly cleft and its fruit is thinly winged (WNHP 1999).

Seed production

Kaye et al. (2003) observed of unfilled seeds in mature fruits of *L. bradshawii*, but no data are available on the extent of this phenomenon. Kagan (1980) sampled seed production at six sites and found the mean number of fruits per plant to range from 7.2-18.5. He observed that seeds may be dispersed by wind or water, but they do not usually travel more than one meter from the parent plant. In another study, Kaye and Kirkland (1994a) state that the plant does not form a persistent seed bank, and average fruit production is 10.8 fruits per plant. While researching the effects of fire on *L. bradshawii*, Pendergrass et al. (1999) observed fruit production to vary in response to site, year, and burning regime, ranging between 0.3-18.0 fruits per plant.

Seed germination

Gasser (1990) performed a series of germination trials for rare Apiaceae species, including *L. bradshawii*. Using one-year-old and thirteen-year-old seed stored at the Berry Botanic Garden (BBG), Gasser cold stratified seeds for either 8 or 16 weeks, followed by germination at either a constant temperature of 68°F (20°C) or alternating temperatures of 50°F/68°F(10°C/20°C). One hundred percent germination was attained by one-year-old seeds stratified for 16 weeks, followed by a constant germination temperature of 68°F (20°C). High germination rates were also achieved for both seed ages with both the 8 and 16 week cold stratification followed by the alternating temperature regime (70-90%).

Kaye and Kuykendall (2001) investigated this cold stratification requirement further by pre-chilling *L. bradshawii* seeds for 0, 2, 4, 6, and 8 weeks at 4°C, then germinating the seeds under two alternating temperature regimes: 59°F/77°F (15°C/25°C) and 68°F/86°F (20°C/30°C). Germination percentages were the highest (50-70%) for seeds which were

cold stratified for 8 weeks, followed by lower alternating temperature regime (15°C/25°C).

The most recent *Lomatium bradshawii* seed germination attempts were performed by Lynda Boyer (Heritage Seedlings Inc., Salem, Oregon, personal communication), who reported nearly 100 percent germination by mixing seeds with pre-moistened vermiculite inside sealed plastic bags and cold stratifying the mixture at 1°C for 11 weeks. This seed/vermiculite mixture was then sown into soil-filled flats, lightly covered with a "light dusting of soil," with germination typically occurring within seven days of sowing.

Vegetative reproduction

Kaye and Kirkland (1994a) state that *L. bradshawii* is taprooted, and is not capable of vegetative reproduction.

Breeding system

Kagan (1980) found that 90% of *Lomatium* flowers are exclusively male, with hermaphroditic flowers occurring mainly on the outer umbellets of the second umbel of a plant, so fruit set is somewhat limited. Bagging experiments show that the species is completely self compatible, and suggest that plants were capable of seed production through selfing in the absence of pollinators. There was no evidence of apomixis among emasculated flowers. As in other species of *Lomatium*, flowers of *L. bradshawii* are protogynous, with the styles exerted while the stamens and the petals are still recurved inward, as in the bud. Despite extensive observations, Kagan observed very few insects near the plants. The primary insect visitors were small gnats, and these did not appear to transport any pollen. At Willow Creek a few Andrenidae bees were observed, and appeared to carry pollen, but they were infrequent. Kagan speculates that some between-umbel pollination could occur through wind dispersal of pollen. He also comments that higher fruit set among plants more distant from their nearest neighbors may indicate some inbreeding depression.

Seemingly in contrast to Kagan (1980), Kaye and Kirkland (1994a) showed through a pollinator exclusion experiment that insects are required for fruit production. Autogamy is prevented because protogyny completely separates sexual phases of flowers within an inflorescence. As such, outcrossing rates are very high within populations. Their research confirms Kagan's findings that the first umbel is all male, and that the second umbel produces some hermaphroditic flowers among the outer umbellets. Unlike Kagan, Kaye and Kirkland observed a large diversity of insect visitors, including at least 38 species of bees, flies, wasps, beetles, and others. Twenty-six of these species (primarily bees and syrphid flies) exhibited *Lomatium* pollen on their bodies. (Figure 28 shows an additional pollinator observed at Finley NWR.)

Figure 28. Pollinator (*Halictus* sp.) visiting *Lomatium bradshawii*. (Photo by Steve Gisler.)

Jackson (1996) observed 21 species of pollinator at the Fisher Butte population near Eugene. The most frequent pollinators included a large syrphid fly (*Heliophilus fasciatus*) (44 percent of visits), two species of *Andrena* (14.8 percent of visits), smaller syrphid flies (11.1 percent) and a dung fly (*Scatophaga stercoraria*) (7.4 percent). Mean

seed dispersal distance was 22.46 cm. Based upon density calculations and pollen carryover estimates, neighborhood size (the number of interbreeding individuals) was estimated using three different models from 17-95 individuals (40 m^2 to 227 m^2).

Using AFLP markers to study the genetic diversity of *Lomatium bradshawii*, Gitzendanner (1998) found high levels of genetic diversity in most of the plant's populations. He does not consider inbreeding depression to be a threat to this species, and feels that, as long as population sizes do not decrease, the long-term genetic stability of the plant looks good.

Hybridization

Siddall and Chambers (1978) site Mathias and Constance as experts on the genus; these researchers state that *Lomatium bradshawii* has no close or compatible relatives in its range and habitat, suggesting that interspecific hybridization is not likely for this species. To date, no research has been conducted regarding potential hybridization between *L. bradshawii* and other *Lomatium* species. None of the literature reviewed mentions hybridization as a possibility or concern.

Cultivation

Kaye et al. (2003) successfully propagated *Lomatium bradshawii* plants by potting germinated seeds (see "Seed germination" section above) in Oregon State University greenhouse soil mix consisting of one part peat, one part loam and two parts pumice. Seedlings were grown in cohorts in a heated greenhouse (20°C-25°C), where they were watered twice weekly and fertilized with 20:20:20 fertilizer once a week. Survival rates were high (73%-99%), and most seedling mortality occurred at the early seedling stage. Survival in the short term was not affected by pot size.

The most recent attempts to cultivate *Lomatium bradshawii* were reported by L. Boyer (personal communication). As described in the "Seed germination" section above, seeds were mixed with pre-moistened vermiculite and cold stratified (at 1°C) inside sealed

plastic bags for 11 weeks. This seed/vermiculite mixture was then sown into flats filled with a planting medium consisting of bark, compost, peat, perlite, and Philip's pre-mix (crabmeal, 3 kinds of lime, micronutrients, Actino-iron, and a wetting agent). Following establishment in flats, seedlings were transplanted into 5 inch x 2 3/8 inch pots. Boyer reported very high (nearly 100 percent) establishment and survival rates, and noted that after 60 days, "*Lomatium bradshawii* does not produce many leaves but the root is well established by this time."

Transplanting and introduction attempts

According to Kaye et al. (2003), direct seeding of *Lomatium bradshawii* is highly effective, with recruitment at one location ranging from 17 to 38% for undisturbed and bare soil plots, respectively (5 plots per treatment with 60 seeds per plot). At the second study site, seedling emergence ranged from 19-30% and was not affected by soil amendments (compost, fertilizer, mycorrhizal inoculae, and mycorrhizal inoculae mixed with compost). Several years prior to this study, Clark et al. (2001) attempted direct seeding in burned and unburned plots, located in a study grid at Danebo Wetland, Eugene, Oregon. 180 seeds were used, but there was no germination, regardless of burned or unburned treatments.

Greenhouse-cultivated *Lomatium bradshawii* plants (grown from seed) were transplanted to research plots in both the fall and spring of 2000 (Kaye et al. 2003). Seeds were collected in late June or July of 1999, and potted (in 5" or 9" pots) in mid-December of the same year. Half of the plants were outplanted in March of 2000. The rest remained in the greenhouse throughout the summer, where they were watered daily and fertilized with liquid fertilizer monthly. The fall transplants were outplanted in late October of 2000. All transplants were given 1-2 teaspoons of 17-17-17 slow release fertilizer at the time of outplanting. Two years later, approximately 40 and 75 percent of the transplants had survived at the two sites, respectively, although as of 2002 very few of the survivors were reproductive. Survival of *L bradshawii* transplants appeared to be greater for unfertilized transplants and transplants planted in the autumn. The effect of fertilizer was

not seen in the first year, but became significant in the second and third years of monitoring.

Population monitoring

Pendergrass et al. (1999) monitored three sites to determine the impacts of burning on *Lomatium bradshawii* population trends at Bureau of Land Management and Army Corps of Engineers properties. The density and abundance of reproductive plants increased in the presence of fire (burned either twice or three times) between 1988 and 1997, corroborating other evidence that fire improves population growth rate and chances for survival (Caswell and Kaye 2001). Monitoring also showed the effects of fire to be temporary, dissipating after 1 to 3 years.

Kaye and Kirkland (1994b) established permanent monitoring plots at 3 sites: Buford Park in Eugene, Finley National Wildlife Refuge (NWR) and Jackson-Frazier Wetland in Corvallis. Plots varied in shape to accommodate the distribution of plants. Plants were divided into one of seven size categories. All plants were mapped, numbered, and herbivory was recorded. First-year data showed population structures at Buford and Finley skewed towards non-reproductive plants, with only 31% and 33% of the populations reproductive, respectively. At Jackson-Frazier, 52% of the plants were reproductive. Seedlings occurred at low frequencies in all three sites, and populations were generally skewed towards smaller non-flowering plants. Plant density ranged from 1.05-28 plants per meter.

Monitoring by Drew (2000) at Oak Creek showed that grazing may increase emergence of new plants, while having no detectible effect on plant survival. Drew speculates that this pattern is possibly due to corresponding reductions in herbivory by small mammals (small mammal densities were lower in grazed plots).

Additional monitoring results are summarized by Robinson (1998). Annual monitoring has taken place at Willow Creek since 1993, showing a general downward trend in

population size from 1993-1998. Monitoring conducted from 1982-1993 at Finley NWR indicate a substantial increase in population numbers, from 41 plants to over 1700.

Conservation agreements for the two Washington populations were finalized in 1995 and 1998 (CPC 2004). As part of these agreements, both of these populations have been monitored since 1997; however, no data have been published regarding the results of this monitoring.

Land use threats and other limitations

The historical and continuing loss and degradation of Willamette Valley prairie habitat is a pressing concern for *Lomatium bradshawii*. Agricultural, commercial and residential development has almost completely eliminated the native grasslands of this area; currently less than one percent of the Willamette Valley prairie remains intact (CPC 2004). Pesticides, encroachment of woody and invasive species, herbivory and grazing are also threats to remaining *L. bradshawii* populations.

Development: The majority of Oregon's *Lomatium bradshawii* populations are located within a ten mile (16 km) radius of Eugene. The continued expansion of this city is a potential threat to the future of these sites. Even when the sites themselves are protected, the resultant changes in hydrology caused by surrounding development can alter the species' habitat (Meinke 1982, USFWS 1988, WNHP 1999, CPC 2004). Siddall and Chambers (1978) state that the majority of sites from which herbarium specimens have been collected are within areas of Salem or Eugene which are now developed for housing and agriculture.

Pesticides: Many *L. bradshawii* populations occur near roadways and other areas which are sprayed with pesticides. There is concern that these pesticides will kill the pollinators necessary for plant reproduction. Because *L. bradshawii* does not form a seed bank, any loss of pollinators (and subsequent lack of successful reproduction) could have an immediate effect on population numbers (Kaye and Kirkland 1994a).

Woody/Invasive Species: The final rule (USFWS 1988) states that one of the most significant threats to *L. bradshawii* is encroachment of its habitat by woody vegetation. Historically, Willamette valley prairies were periodically burned, either by wildfires or by fires set by Native Americans (Johannessen et al. 1971). Since European settlers arrived, fire suppression has allowed shrubs and trees to invade grassland habitat (USFWS 1988, Kaye 1993). To prevent habitat loss through successional vegetation changes, manual control of woody plants such as *Fraxinus latifolia* and *Rosa pisocarpa* has occurred as part of the conservation agreement for one of the Washington populations (CPC 2004).

Herbivory/Grazing: At a *Lomatium bradshawii* meeting, Robinson (1998) indicated that "vole herbivory at some sites is a major threat to local species viability." Studies of the effects of cattle grazing on *L. bradshawii* populations show mixed results. Grazing in the springtime, when *L. bradshawii* plants are growing and reproducing, can negatively impact the plants by biomass removal, trampling and soil disturbance (CPC 2004). However, late-season livestock grazing, after fruit maturation, led to an increase in emergence of new plants, and the density of plants with multiple umbels, although it did not alter survival rates or population structure (Drew 2000). Drew speculated that the increase in seedlings may be due to small disturbances in the soil, a reduction of shading by nearby plants, and reduced herbivory by small mammals.

Population introduction/augmentation strategy

Based upon the biogeographical data compiled and described above for *Lomatium bradshawii*, 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. Although many *L. bradshawii* populations face imminent threats, and native prairie habitat has been reduced to a small fraction of its former abundance, there are still several extant *L. bradshawii* populations that occur on public or otherwise secure landholdings. The largest known site in Oregon and another smaller site occur on private plant that has been leased to The Nature Conservancy (TNC). Three of the Oregon populations are on land designated as a

"Wetlands Special Study Area" (CPC 2004). As such, pending interagency cooperation and funding availability, there should be sites available for collection of seeds for use in off-site cultivation projects, and open locations should also be available for population augmentation and introduction purposes.

The biology and life history of *Lomatium bradshawii* likewise pose no unavoidable hurdles to successful implementation of population introduction and augmentation projects. Although the relatively low seed production of *L. bradshawii* poses a potential limitation to the number of seeds that can be collected and used in a single year for off-site cultivation projects, this complication can be overcome, if necessary, by using sustainable seed collecting practices over multiple years prior to project implementation. Seed germination was fairly high for seed that had been stored for up to 13 years at the Berry Botanic Garden. *Lomatium bradshawii* has been successfully cultivated in the greenhouse, and the species exhibits no unique propagation or soil symbiont requirements. Once adequate seed supplies are available, there are no apparent cultivation-related obstacles to implementation of introduction projects.

Based upon the information provided in this manual, the following procedures are recommended for *Lomatium bradshawii* population introductions:

1. Select population introdution/augmentation target sites. Several factors should be considered when selecting target sites for *Lomatium bradshawii* population introduction and augmentation projects. First, target sites should contain suitable prairie habitat. Although habitat descriptions have been provided in this manual, it is extremely helpful to visit extant populations and see the habitat in which the plant grows. Such visits should give a better idea of the types of microsites occupied by *L. bradshawii* individuals within their larger native grassland habitat context.

   Given the lack of long-term protection of *L. bradshawii* 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. Because grazing can have a negative impact on *L. bradshawii* populations, it is important to ensure that grazing, if allowed, is carefully monitored and scheduled for late in the season, after *L. bradshawii* fruits have matured and seed dispersal is complete.

2. Collect *Lomatium bradshawii* seeds for off-site cultivation of introduction stock. Introduction efforts involving *L. bradshawii* indicate that both direct sowing of seed and transplanting plugs are successful ways to introduce new propagules into a site. Normally, if recruitment from directly-sown seed is high, this is the best way to introduce new plants – the cost is often lower and the genetic diversity of the new population is higher. However, since there have been mixed results with the two seeding experiments conducted so far, initial introduction efforts should include both direct seed sowing and transplants. 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.

3. Cultivate *Lomatium bradshawii*. *Lomatium bradshawii* has been successfully cultivated from seed. Previous studies suggest that seeds should be cold stratified for at least eight weeks, followed by an alternating 10°/20°C (50°F/68°F) temperature treatment. Once seeds have germinated, they can be potted in 5" pots, watered daily and fertilized monthly.

4. Introduce cultivated plugs and seeds into the target site(s). *Lomatium bradshawii* propagules will probably be most likely to establish if planted in the late fall, after the arrival of the fall rains. Seed plots should have the vegetation cleared before

sowing, and no fertilizer should be used with plug transplants. Although few studies have focused on the impacts of population size on *L. bradshawii*'s ability to attract pollinators and produce viable seed and robust progeny, small populations tend to be more vulnerable to extinction through 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 *L. bradshawii* populations should be monitored annually to evaluate project success. These evaluations should take place in the late spring or early summer, when fruits are mature and it is possible to assess reproductive success. 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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Developing biogeographically based population introduction protocols for at-risk Willamette Valley plant species:

Lupinus sulphureus ssp. *kincaidii* (Kincaid's lupine)

Lupinus sulphureus ssp. *kincaidii* (Kincaid's lupine)

Conservation status

Lupinus sulphureus ssp. *kincaidii* (Figure 29) is currently listed as threatened by both the U.S. Fish and Wildlife Service and the State of Oregon. It is on the Oregon Natural Heritage Program List 1 (threatened or endangered throughout its range), and has a Natural Heritage Network Rank of G5/T2/S2 (this subspecies is imperiled throughout its range/imperiled in Oregon) (ONHP 2001). In Washington, *L. sulphureus* ssp. *kincaidii* is listed by the State as Endangered, though this status carries no legal mandate for protection on state or other public lands (Florence Caplow, Washington Natural Heritage Program, Olympia, Washington, personal communication). The species also has also been assigned a rank of S1 (critically imperiled) by the Washington Natural Heritage Program (WNP 2003).

Figure 29. *Lupinus sulphureus* ssp. *kincaidii*. (Photo by Steven Gisler.)

For the past 15 years, Kincaid's lupine has been the focus of numerous research projects. Studies investigating the genetics, breeding system, insect interactions, seed germination and seedling growth, and reintroduction and habitat restoration of this species have been completed. An extensive review of relevant literature to date is presented in Wilson et al. 2003, and abstracts and/or full text of many studies are available online at http://oregonstate.edu/~wilsomar/Papers.htm. Due to the large number of studies that have been completed, and the often varying results, specific review of individual papers will be essential to the successful planning of future reintroduction projects.

Range and habitat

The earliest collections of what is now considered *Lupinus sulphureus* var. *kincaidii* were made in the early 1900's, in the vicinities of Corvallis and Eugene, Oregon (Wilson et al. 2003). Subsequent collections have documented the occurrence of this species at 57 locations, distributed from Lewis County, Washington, south through the Willamette Valley to Douglas County, Oregon—a latitudinal span of over 400 km (USFWS 2000). The cumulative area of habitat occupied by *L. sulphureus* ssp. *kincaidii* has been estimated at 160 hectares (Kaye and Kuykendall 1993).

Lupinus sulphureus ssp. *kincaidii* is primarily restricted to undisturbed remnants of upland prairie and ecotones between grasslands and forests (Kaye and Kuykendall 1993; Figure 30). Most Willamette Valley populations occur in association with well-drained soils classified as Ultic Haploxerolls, Ultic Argixerolls, and Xeric Palehumults (Wilson et al. 2003), and in Benton County the species exhibits a strong affinity to the Dixonville soil series, and a positive association with the Witzel, Hazelhair, Briedwell, and Price soil series (A.F. Robinson, unpublished, in Wilson et al 2003). Commonly associated native plant species are those typical of intact upland prairie habitats, including: *Agoseris grandiflora*, *Arbutus menziesii*, *Balsamorhiza deltoidae*, *Brodiaea coronaria*, *Bromus carinatus*, *Calochortus tolmiei*, *Cryptantha intermedia*, *Danthonia californica*, *Delphinium menziesii*, *Elymus glaucus*, *Eriophyllum lanatum*, *Festuca idahoensis*, *F. roemeri*, *Fragaria vesca*, *F. virginiana*, *Holodiscus discolor*, *Iris tenax*, *Lomatium triternatum*, *L. utriculatum*, *Luzula comosa*, *Madia gracilis*, *Potentilla gracilis*, *Pseudotsuga menziesii*, *Pteridium aquilinium*, *Rhus diversiloba*, *Sanicula crassicaulis*, *Silene hookeri*, *Symphoricarpos mollis*, and *Whipplea modesta* (Kaye and Kuykendall 1993, Wilson et al. 1997).

Figure 30. Upland prairie habitat occupied by *Lupinus sulphureus* ssp. *kincaidii* at Baskett Slough National Wildlife Refuge. (Photo by Steven Gisler.)

Species description

Lupinus sulphureus ssp. *kincaidii* is an herbaceous perennial from a branched crown, usually with numerous unbranched stems 4-10 dm tall, with whitish or brownish stiff to silky pubescence. Basal leaves are usually persistent until after flowering, the lowermost petioles (2) 3-5 times as long as the blades, the upper cauline leaves with petioles sometimes shorter than the blades. Leaflets usually number from 7-12, and are rather narrowly oblanceolate, usually acutish, 2.5-5 cm long. The flowers are numerous but not crowded on the stem, and range in color from bluish or purple to yellowish or creamy white. The banner is distinctively ruffled (Figure 31) and not very reflexed, the upper calyx lip short, bidentate, and not concealed by the reflexed sides of the long-clawed banner. The fruit pods are not hairy, 3-4 cm long, with 1-6 pinkish-brown to black seeds. The species is distinguished from other relatives by its ruffled banner on light-colored flowers, its unbranched inflorescences, and its low growing-habit (Hitchcock 1961, Kaye and Kuykendall 1993).

Figure 31. Ruffled banners are a diagnostic trait of *Lupinus sulphureus* ssp. *kincaidii*, which assists in its delimitation from other Willamette Valley lupines. (Photo by Steve Gisler.)

Seed production

Several studies of fruit and seed production in *Lupinus sulphureus* ssp. *kincaidii*, spanning multiple sites and years, have been conducted. Seed production is characterized by high rates of pre-dispersal seed predation and possible indications of inbreeding depression and resource limitation, resulting in generally low fruit and seed set. A brief summary of the findings of these studies is provided below.

As part of a larger breeding system at a population located in Oregon State University's McDonald Forest, Kaye reported an average of 75 flowers per *Lupinus sulphureus* ssp. *kincaidii* raceme, 4.5 ovules per fruit, 4.3 percent fruit production per raceme, and 30.6 percent seed set per fruit (Kaye and Kuykendall 1993). In 1992, Kuykendall and Kaye (1993) examined seed production at four sites in Benton, Lane, Polk, and Yamhill Counties, and reported a mean 66.3-82.3 flowers per raceme, 3.4-9.9 fruits per raceme, and 0.3-1.2 seeds per fruit. As part of a larger breeding system study conducted at Fern

Ridge Reservoir in Lane County, Severns (2003a) reported a mean of 0.9 seeds per open-pollinated fruit, and a 1999 collection of seed at N. Green Oaks near Eugene yielded 0.64 seeds/pod due to heavy insect damage (Wilson et al. 2001).

Production of seeds in *Lupinus sulphureus* spp. *kincaidii* appears to be significantly limited by pre-dispersal predation and other insect-related damage. In 1990 Kaye observed 6.1 percent seed loss due to predation by bruchid beetles and weevils at a population located in OSU's McDonald Forest (Kaye and Kuykendall 1993). In 1992, Kuykendall and Kaye (1993) reported insect-related damage on 29-85 percent of fruits at four populations, and also observed high levels of floral herbivory by larvae of silvery blue butterfly (*Glaucopsyche lygdamus columbia*). Schultz (1995) also reported the presence of silvery blue butterfly larvae at three Lane County sites, and found them to damage a mean 46-61 percent of fruits. Consistent with earlier reports by Kaye and Kuykendall (1993), Schultz noted herbivory by these larvae on buds and flowers, in addition to the fruits, accounting for greater impacts to Kincaid's lupine reproduction. In addition, Schultz also discovered that predation by weevils resulted in complete seed loss in 3-36 percent of fruits.

Inbreeding depression may limit seed set and seed fitness in smaller populations of Kincaid's lupine (Severns 2003a). In this study, a mean of 1.8 seeds per fruit was produced by flowers manually outcrossed with those from a separate population, 1.1 seeds per pod by flowers manually outcrossed from the same population, and 0.9 seeds per pod by open-pollinated flowers. Severns also observed lower seed viability from within-colony crosses than between-population crosses.

Resource limitation may also limit seed set; Kuykendall and Kaye (1993) indicate that fruit set is highest in the middle portion of *L. sulphureus* ssp. *kincaidii* racemes. Flowers on the middle of racemes appear to correspond with conditions when both soil moisture and pollinators are plentiful, whereas those lower and higher on racemes may suffer from pollinator limitation and soil moisture limitation, respectively.

Seed germination

Seed germination has been well studied in *Lupinus sulphureus* ssp. *kincaidii*. The earliest research into this area was conducted by Ingersoll in 1991 (reported as unpublished data in Kaye and Kuykendall 1993). Ingersoll found that fresh seeds germinated readily when scarified, while untreated seed germination was low (56 germinants out of 300 seeds), but increased when field soil was used rather than sterile potting soil. Specifics as to germination rates of scarified seeds and those in field soil were not provided in this report.

In 1999, seeds were collected from two Lane County Kincaid's lupine populations (Fir Butte and Oxbow West) and used in an investigation of the affects of scarification, stratification, and seed source on germination (Kaye and Kuykendall 2001). For seeds collected at the Fir Butte site, untreated seed yielded nine percent germination, scarification (accomplished by rubbing seeds on fine sandpaper) alone resulted in 45 percent germination, and stratification alone (on moist filter paper for 4 and 8 weeks at 4ºC) yielded 17 and 23 percent germination, respectively. Germination was over 95 percent when scarification and stratification were combined. Seeds collected from the Oxbow West site had lower germination under all treatment regimes; only two percent of untreated seed germinated, 18 percent of scarified seed, and 31 percent of seed that had been stratified for 8 weeks. The combined scarification and stratification treatment yielded 55 percent germination. Kaye and Kuykendall also noted that radicles emerged from seeds while still in the cold treatment, indicating that warming was not needed to initiate germination.

To find an alternative to the labor-intensive hand scarification technique used by earlier researchers, Erhart (2000) investigated the use of sulfuric acid to promote germination. In this study, 50 percent of acid scarified seeds germinated, while germination of untreated was only four percent. Optimal acid soaking time was 20 minutes; treatment for greater than 60 minutes damaged the endosperm and lowered germination. Freezing seeds in liquid nitrogen for 10 minutes did not increase germination.

Leininger (2001) investigated the use of scarified and non-scarified seeds in a field-sowing experiment at Basket Slough NWR. Interestingly, non-scarified seeds had higher establishment rates (5.3 percent) than pre-scarified seeds (1.7 percent). Schultz (2001) also found poor seedling establishment in fall planted, pre-scarified seeds, compared to those planted in early spring. Schultz scarified 10,400 seeds and sowed them at two sites in Lane County in 1995. Many appeared to germinate by November the first year, but few survived the winter. Another trial showed one percent establishment of pre-scarified seeds planted in September, versus 10 percent of those planted in February. Similar poor emergence of scarified seed occurred in a later study by Severns (2003a); pre-scarification probably causes seeds to germinate shortly after sowing in fall, exposing new seedlings to winter freezing and herbivory by slugs, whereas control (non-scarified) seeds produced seedlings in spring, when conditions were more favorable.

Vegetative reproduction

Despite a well-documented ability to spread through vegetative growth, *Lupinus sulphureus* ssp. *kincaidii* does not appear to actually reproduce (i.e., form new, physiologically independent individuals) except by sexual means (Kaye and Kuykendall (1993). However, individual clones can be several centuries old (Wilson et al. 2003), and become quite large with age, producing many flowering stems. Excavations and morphological patterns suggest that plants 10 m or more apart can be interconnected by belowground stems, that clones can exceed 20 meters in diameter (USFWS 2000,Wilson et al. 2003). As part of a genetic evaluation, multiple collections taken from small populations of Kincaid's lupine at the Baskett Slough NWR where found to be genetically identical, indicating that the population consists of one or a few large clones (Liston et al. 1995).

Breeding system

Kincaid's lupine, while probably self-compatible, is almost exclusively an outcrossing species due to mechanical barriers to self-fertilization, and foraging patterns of bees on indeterminate inflorescences. However, due to clonal spread in the species, opportunities

for outcrossing may be limited and may result in a high rate of geitonogamy and potential inbreeding depression. Details of studies leading to these conclusions are briefly summarized below.

The earliest investigation into the breeding system of *Lupinus sulphureus* ssp. *kincaidii* was conducted by Kaye in 1990 (Kaye and Kuykendall 1993). In this study, Kaye observed no seed set in racemes enclosed within pollinator exclusion bags. Lack of seed set was attributed to mechanical and/or temporal barriers to self-fertilization, as genetic self-incompatibility is apparently unknown in the genus *Lupinus*. Kaye (1999) describes a piston arrangement that discourages autogamy in Kincaid's lupine flowers, whereby a string of pollen is pushed through the tip of the keel by the stigma when the pistil comes under pressure during an insect visit. Stigmas are protected from automatic self-pollination by a peristigmatic ring of hairs. Findings by Erhart (2000) suggest that this outcrossing mechanism may not entirely preclude autogamy, however, as a few viable seeds were produced by bagged flowers during his study.

A later study by Kuykendall and Kaye (1993) attempted a more thorough reproductive investigation, involving a variety of within- and between-population crossing treatments. Results were equivocal, however, as only 45 of 640 manipulated flowers developed fruit, and none produced seeds (in part due to insect damage to fruits).

In a study by Severns (2003a), mean per fruit seed set resulting from hand outcrossed pollintations was double (1.8) that of open pollination (0.9) and within-colony crosses (1.1). Seed viability was also lower from within-colony pollination (76 percent) and open pollination (78 percent) compared with outcrossing (99 percent), indicating that inbreeding depression may limit reproductive fitness in some populations. A genetic study by Liston et al. (1995) found levels of heterozygosity in populations of *Lupinus sulphureus* ssp. *kincaidii* to be consistent with those expected of an outcrossing species with high levels of gene flow occurring in the recent past.

Lupinus sulphureus ssp. *kincaidii* attracts a variety of bee visitors, including *Bombus californicus*, *B. mixtus*, *Apis mellifera*, *Andrena* sp., *Dialictus* sp., *Osmia lignaria*, and *Anthophora furcata* (Wilson et al. 2003). Kaye and Kuykendall (1993) indicated that flowers produce no nectar, so bees must visit plants for pollen. However, in subsequent studies by Schultz and Dlugosch (1999), 31 percent of flowers produced nectar, with an average 0.063 mg sugar/flower. Thus, it appears that some flowers may offer both pollen and nectar rewards to pollinators, which tend to forage from bottom to top within racemes and promote outcrossing by visiting lower female-phase flowers prior to upper male-phase flowers.

Hybridization

All available information suggests that hybridization may occur, or have the potential to occur, in *Lupinus sulphureus* ssp. *kincaidii*. Kaye and Kuykendall (1993) cite communication with Aaron Liston, who suspected the species might hybridize with *L. laxiflorus* at the Basket Butte population. A later study by Liston et al. (1995) showed isozyme evidence of past hybridization between Kincaid's lupine and sympatric *L. arbustus* at Basket Slough NWR, supporting Liston's earlier suspicions and possibly explaining the presence of morphologically intermediate individuals and sterile clones at the site. Liston also reports that hybridization is a very widespread phenomenon in the genus *Lupinus*.

Cultivation

Several researchers have cultivated plants of *Lupinus sulphureus* ssp. *kincaidii* under greenhouse conditions, indicating that it is possible to grow the species for later use in conservation projects. In a study by Kaye and Kuykendall (2001b), seedlings of *Lupinus sulphureus* ssp. *kincaidii* grown from several seed sources grew well under greenhouse conditions. After germination, seedlings were potted individually in a peat/loam/pumice soil. Plants were cultivated in a heated greenhouse (20-25°C), watered twice weekly, and fertilized with 20-20-20 liquid fertilizer once each week. Seedling mortality tended to occur in an initial flush (probably due to damping off) and a gradual die off of some

individuals, even after they appeared to become established. Survival rates of cohorts from several seeds sources, and aged 4 -11 weeks, varied from 58% - 100%.

Schultz (2001) also germinated seeds and cultivated seedlings successfully in the greenhouse. One hundred and sixty seedlings were grown for "a few weeks" before transplanting into the field as part of a restoration project.

Wilson et al. (2001) cultivated seedlings of Kincaid's lupine in the greenhouse for future use as transplants in a restoration project. In this study, 17.6% of the seeds emerged, but only 6.8 % survived until transplanting. Researchers attributed some of this seedling mortality to potassium deficiency, although treating plants for this problem produced no noticeable improvement.

The role of soil symbionts in cultivation of Kincaid's lupine is still uncertain. Like many herbaceous prairie dwellers, this species is known to host VA mycorrhizal fungi (communication with Ingham, cited in Wilson et al. 2003). However, although VA associations often benefit their host plants through increased nutrient uptake, inoculation of Kincaid's lupine seedlings with commercial mycorrhizal inoculum appeared to increase mortality among plants cultivated in 1999 by Thomas Kaye (Institute for Applied Ecology, Corvallis, Oregon, personal communication).

Transplanting and introduction attempts

To date, efforts to establish new populations of Kincaid's lupine using seeds and transplants have met with mixed success. The earliest recorded efforts to establish plants in the field were made by Ingersoll (unpublished data cited in Kaye and Kuykendall 1993). In this study, only five percent of 150 seedlings sown in April 1992 survived the first year. Direct seeding with pre-scarified seeds proved more successful, with 68 percent survival through the first growing season.

These initial seeding and transplant studies were considerably more successful than those of subsequent workers. At the Fern Ridge site, Severns (2003b) sowed 900 seeds in

1997, which resulted in 335 germinants, and survival of 213 seedlings after one year, 143 seedlings after two years, and 141 seedlings after three years. Kaye et al. (2001) reported seedling recruitment of 23.7 percent at the Isabelle site in West Eugene. At Basket Slough NWR, Leininger (2001) experienced seedling establishment rates of only 5.3 percent for non-scarified seeds and 1.7 percent for pre-scarified seeds. Schultz (2001) experienced seedling recruitment levels of one and ten percent for fall and late winter sowings of pre-scarified seeds, respectively.

The studies of Leininger (2001) and Schultz (2001) support statements by Severns (2003b), indicating that pre-scarification can cause seeds to germinate shortly after sowing in fall, exposing seedlings to winter freezing and herbivory by slugs. Thus, field sowing of seeds should probably either entail fall sowing of non-scarified seeds, or spring sowing of pre-scarified seeds, to ensure proper timing of germination.

Introduction projects involving Kincaid's lupine have not been restricted to sowing of seeds, but have also employed transplanting of cultivated individuals. Three months after transplanting at the Greenhill site near Eugene, Kaye et al. (2001) reported 70 and 87 percent survival of non-inoculated and inoculated (*Bradyrhizobium*) transplants, respectively. After three months, transplant survival at the Isabelle site near Eugene ranged from 75-95 percent, again with slightly higher survival among inoculated transplants and for fertilized transplants.

Schultz (2001) reported 60 percent survival after one month for seedlings transplanted in spring 1997, and 90 percent survival after one month for seedlings transplanted in 1998. Of the1997 transplants, nine percent survived for one year, three percent for two years, and none for three years. The 1998 transplants fared similarly, with seven percent surviving after one year, and none after two years.

Population monitoring

Kaye and Cramer (2003) documented baseline demographic monitoring at two sites in Eugene. At Fir Butte, 18 plots were established within a 216 x 288 macroplot, and at

Oxbow West, monitoring was conducted in square meter plots within a 30 m x 17 m macroplot. Plots were monitored from 1998 through 2002, with data collected on number of inflorescences and leaves, number of Fender's blue eggs on leaves, and percentage cover of *Rubus discolor*. Due to complications of clonal spread, no efforts were made at either site to identify or count individual plants. At the Fir Butte site, the mean number of flowering stems and mean leaves/plot increased each year.

Land use threats and other limitations

As with most rare plants in the Willamette Valley, Kincaid's lupine suffers from destruction of its prairie habitat. Urbanization and intensive agriculture have permanently altered many of the suitable sites for this species, and have contributed to habitat degradation of existing sites. Wilson et al. (2003) identify three major threats: habitat loss, invasion by non-natives, and elimination of disturbance regimes.

More than 95% of the prairie habitat in the Willamette valley has now been converted to farming and urban uses. Due to this loss, prairie species that were formerly wide-spread (including Kincaid's lupine) are now rare. Additionally, remaining prairie fragments have been further impacted by invasions of exotic weeds. Non-native vegetation often forms tall, dense stands around lupine plants, shading them and leading to dramatic changes in the structure of upland prairie communities. These weed invasions (especially *Arrhenatherum elatius*, and *Cytisus scoparius*) threaten many sites, as does fire suppression and the resultant succession of the species' preferred grassland habitat to woody shrubs. Prior to European settlement, intentional burning by Native Americans kept prairies open – lack of periodic fires has altered these habitats. Fortunately, several substantial populations of *Lupinus sulphureus* ssp. *kincaidii* occur on federally owned wildlife refuges, where burning and mowing have improved prairie habitat for native species; flowering of the lupine increased greatly within mowed and/or burned plots at the Basket Slough National Wildlife Refuge (Wilson et al. 2003).

Insect predation also impacts the viability of Kincaid's lupine. Kaye and Kuykendall (1993), and Schultz (1995) observed many parasites plaguing the species, including gall-

forming insects in unopened flowers and around the base of woody stems, and seed predation by weevils and bruchid beetles (see "Seed production" above).

Population introduction/augmentation strategy

Low seed production and poor transplant survival are potential obstacles to successful implementation of population introduction and augmentation projects for *Lupinus sulphureus* ssp. *kincaidii*. However, the ecological and horticultural data compiled above do not document any barriers to these types of projects that are insurmountable. Several large populations of this species occur in publicly owned sites; pending interagency cooperation and funding availability, these populations should be available for collection of seeds for use in reintroduction projects. Cultivation and transplantation protocols are available, and suitable unoccupied locations on publicly owned lands should also be available for population augmentation and introduction purposes. Although weed invasions and the succession of woody shrubs currently jeopardize both extant populations and potential reintroduction sites, management practices to improve prairie habitat are being implemented in several areas.

Although low seed production and high predation in *Lupinus sulphureus* ssp. *kincaidii* impose a limitation on the number of seeds that can be collected and used in a single year for off-site cultivation projects, using sustainable seed collecting practices over multiple years prior to project implementation will allow the collection of sufficient seed quantities. Once seed supplies are available, there are no apparent cultivation-related obstacles to implementation of introduction projects; seed germination rates are adequate (45-95%) providing scarification and stratification pre-treatments are used. Kincaid's lupine exhibits no specialized edaphic or symbiont requirements for successful growth in cultivation.

Although *Lupinus sulphureus* ssp. *kincaidii* can exist as clonal clumps of only one or a few genets, efforts should be made to maximize the frequency of genetically different individuals in introduced or augmented populations. Kincaid's lupine exhibits genetic features of a species which experienced high levels of gene flow in the recent past, and

currently has the potential for inbreeding depression. Therefore, genetically diverse introduction stock should be used whenever possible to elevate seed production and reproductive fitness, and also ostensibly improve the odds of overall introduction success by enhancing the level of adaptive genetic variability harbored within populations.

One factor that should be taken into consideration during *Lupinus sulphureus* ssp. *kincaidii* introduction projects is interspecific hybridization. Hybridization can and does occur in this genus - interspecific hybridization with existing lupines in population creation sites could potentially thwart reintroduction objectives. Ultimately, to avoid the potentially adverse conservation implications of hybridization that could be inadvertently promoted by artificial population introduction projects, care should be taken to select introduction target sites that are isolated from other related lupine congeners.

Based upon this information, the following step-down procedures are recommended for *Lupinus sulphureus* ssp. *kincaidii* population introductions:

1. Select population introduction/augmentation target sites. Several primary factors should be considered when selecting target sites for *Lupinus sulphureus* ssp. *kincaidii* population introduction and augmentation projects. First, target sites should contain the upland prairie habitat (described above) that is preferred by this species. To assist in identification of suitable habitat, extant populations of Kincaid's lupine in the vicinity of target sites should be visited to obtain familiarity with possible local microhabitat specificities. Data on associated species, soil types, and soil moisture from known extant populations in the vicinity of the target area help characterize suitable habitat, and can be used to assist with the selection of population creation sites. These data are also helpful in determining microsites within these areas that are suitable for transplanting. Inappropriate site selection is the most common cause of rare plant reintroduction failures, and may explain the varying success of previous outplanting attempts of Kincaid's lupine.

Given the history of the destruction of Willamette Valley habitat on private lands, and the ubiquitous threat posed by invasive species, inventories for suitable population introduction and augmentation sites should be focused on publicly owned (or otherwise secure) lands that appear safe from imminent weed and successional encroachment problems. Selection and use of sites should be coordinated with pertinent public landowners to ensure administrative protection and promote adaptive management of populations following introductions.

2. Collect seed for off-site cultivation of introduction stock. Source material for off-site cultivation of *Lupinus sulphureus* ssp. *kincaidii* should be collected from the extant population(s) located nearest to the population introduction target sites to minimize undesirable mixing of gene pools and capitalize upon potential local adaptations. Based upon previous seed production estimates, individual pods can only be expected to produce one seed, with less than ten pods produced per raceme. Given these low levels of seed production, and high levels of insect damage documented in Kincaid's lupine, seed collecting should be planned and implemented well in advance of introduction project dates to ensure adequate time (possibly several consecutive years) for harvest of sufficient seed supplies

   In light of the evidence for inbreeding depression in *Lupinus sulphureus* ssp. *kincaidii*, efforts should also be made to collect seeds from as many individuals as possible, in an effort to elevate seed production, fitness, and adaptive genetic variability within introduced populations. When introduction target sites have several closely neighboring extant populations, the use of multiple local seed sources will further increase the likelihood of capturing an adequate level of genetic variability within the introduced population.

3. Cultivate stock for transplanting. Assuming that an appropriate scarification/stratification regime is utilized to initiate germination, *Lupinus sulphureus* ssp. *kincaidii* can be successfully cultivated from seed under standard greenhouse conditions. Seeds should be mechanically (rubbing on fine

sandpaper) or chemically (soaking in sulfuric acid for twenty minutes) scarified, then moist stratified for eight weeks at 4ºC to promote germination. After germination, seeds should be potted into peat/loam/pumice potting mix, watered when the soil surface has dried (~twice weekly), and fertilized monthly with 20-20-20 liquid fertilizer. As damping off is a primary cause of seedling mortality, adequate greenhouse ventilation is essential to successful cultivation of this species.

4. Introduce cultivated plugs (and/or seeds) into the target site(s). *Lupinus sulphureus* ssp. *kincaidii* transplants should be planted in early spring, allowing natural rainfall to provide irrigation. As the results of adding fertilizer or other treatments at transplanting time on transplant success are equivocal, a series of treatments should be used as part of the experimental design for future reintroduction projects. Additional data on treatments that promote transplant success will be invaluable to future projects.

   Direct seeding is also a relatively successful method for creating new populations of Kincaid's lupine. Sowing seeds 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 seeds sources) and also has the advantage of being more cost-effective than the often expensive production of transplants. Seed scarification should be included in protocols for spring outplantings, but is not needed for fall sowing, as seeds are scarified naturally during the winter.

   Because of the rhizomatous nature of plants, the layout of introduced plugs should be designed in a manner that is consistent with subsequent population monitoring objectives (see #5, below).

5. Monitor introduced populations. Introduced *Lupinus sulphureus* ssp. *kincaidii* populations should be monitored annually to evaluate project success. Given the

clonal nature of the species, and the difficulty in determining the extent of individual clones, monitoring should include a census of the number of leaves and flowering stems, as well as collection of data on seed production. If the definition of individuals is desired (perhaps with the goal of comparing different experimental replicates within a site), then plugs should be widely spaced, such that they remain spatially distinct over time.

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 *Lupinus sulphureus* ssp. *kincaidii* 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 of Kincaid's lupine.

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Developing biogeographically based population introduction protocols for at-risk Willamette Valley plant species:

Montia howellii
(Howell's montia)

Montia howellii (Howell's montia)

Conservation status

Montia howellii, a small, early spring flowering annual occurring in seasonally wet soils (Figure 32), was formerly considered by U.S. Fish and Wildlife Service (USFWS) to be a Category 2 Candidate taxon - this status category is no longer recognized, and the species currently lacks any formal federal status. However, it is considered a candidate for listing as threatened or endangered by the State of Oregon, is included on the Oregon Natural Heritage Program List 4 (of conservation concern), and has a Natural Heritage Network Rank of G3/S3 (rare, uncommon or threatened but not immediately imperiled throughout its range/rare, uncommon or threatened, but not immediately imperiled in Oregon; ORNHIC 2004). *Montia howellii* is included on List 2 (rare, threatened, or endangered in California, but more common elsewhere) of the California Native Plant Society Inventory of Rare and Endangered Plants (CNPS 2004), and is not currently tracked by the Washington Natural Heritage Program.

Figure 32. Plants of *Montia howellii* flowering at Sauvie's Island. (Photo by Tom Kaye.)

Montia howellii occurs on lands owned by federal, state, county, city, and private landowners. Salstrom and Gamon (1992) identify the following public agencies as managers of sites that support populations of *M. howellii*: U.S. Fish and Wildlife Service (USFWS), Clark County, City of Port Angeles, Washington Department of Fish and Wildlife, San Juan County, Bureau of Land Management (BLM), U.S. Army Corps of Engineers, and Oregon Department of Transportation. The majority of the larger populations in Oregon and Washington are located within National Wildlife Refuges (Willapa, Ridgefield, William Finley and Sauvie's Island), providing protection from destruction of the habitat in which *M. howelli* occurs. However, these agencies are not specifically directing management activities towards the maintenance of populations of this species on their lands.

Range and habitat

Montia howellii has been reported or collected from sites in Oregon, Washington, British Columbia, and California. In Oregon, populations are located in Benton, Clackamas, Columbia, Douglas, Josephine, Lane, Lincoln, Linn, Marion, Multnomah and Tillamook Counties (ORNHIC 2004). The type specimen was collected made by Thomas Howell in 1883, "on Sauvie's Island, in the Willamette River, Oregon" (Piper 1906 reported in Kaye 1993).

Historically, *Montia howelli* occurred from Vancouver Island, British Columbia south through Washington and Oregon to northern California, although many of these sites have apparently been extirpated (Kagan 1989, Kaye 1993). This species is currently known from 30-40 sites scattered throughout its historic range, with new populations occasionally discovered during surveys, especially in California, where it was thought to be extirpated until its re-discovery in 1999 (Salstrom and Gamon 1992, Kaye 1993, Renner 2004).

Montia howellii occurs in open, non-forested lowland areas that are seasonally inundated. Sites are sparsely to moderately vegetated by low-growing mosses, grasses, and forbs, and *M. howellii* usually occurs in gaps between the other vegetation (Figure 33). Sites are typically flat or gently sloping and some resemble vernal pools. All of the Washington and Oregon sites receive periodic disturbances in the form of flooding, grazing, agriculture, grading, and/or vehicle traffic with many of them occurring along roadbeds running through more heavily vegetated pastures. Small populations have also been located on rocky balds and outcrops. Soils are heavy clay to gravel, with poorly drained subsoils, and may be moderately to heavily compacted. The species is also found on rock outcrops and on thin soil over basalt which is seasonally wet. Two sites receive spray from salt water (Salstrom and Gamon 1992, Kaye 1993).

Kaye (1991, 1993), Salstrom and Gamon (1992), Bruce Newhouse, (Salix Associates, Eugene, Oregon, personal communication), and Renner (2004) suggest that *M. howellii* may require disturbance to maintain microsites which are relatively free from competing vegetation. Regarding the role of disturbance in maintaining the population of *M. howelli* at the Long Tom ACEC, Kaye (1991), states "the restriction of the species to the roadway is so complete that it is apparent to most observers that disturbance may be necessary to maintain the habitat for the species at this site." In California, currently extant populations of *M. howellii* are restricted to disturbed sites with compacted mesic soils. Periodic disturbance of these sites (light road grading) during the plants' dormant season prevents the invasion of competing vegetation, resulting in increased plant emergence (Renner 2004).

Frequently associated species include: *Poa annua*, *Myosurus minimus*, *Montia fontana*, *Montia dichotoma*, *Matricaria matricarioides*, *Holcus lanatus, Draba verna*, *Bellis perennis*, *Plantago lanceolata*, *Centaurium umbellatum, Hypochaeris radicata, Lotus micranthus, Luzula campestris, Navaretia squarrosa, Cynosurus echinatus, Geranium* spp., *Plagiobothrys scouleri*, *Trifolium* spp., *Stellaria media*, *Cerastium viscossum*, *Capsella bursa-pastoris*, *Daucus carota*, *Rumex acetosella*, *Myosotis discolor*,

Cardamine oligosperma, *Veronica arvense*, *Orthocarpus pusillus*, *Nemophilla* spp., and *Juncus bufonius* (Kaye 1991, Salstrom and Gamon 1992).

Figure 33. Habitat of *Montia howellii* at Finley National Wildlife Refuge. (Photo by Tom Kaye.)

Species description

Montia howellii is a low-growing annual with freely branched, spreading, often nodally rooting stems 2-6 cm long. Leaves are alternate, linear-spatulate or linear-oblanceolate, 5-20 mm long and 0.5-1.5 mm broad. Flowers are borne in clusters of 3-8 along the stem from the axils of small scarious bracts opposite the leaves, the bracts often connate with the base of the leaf and resembling a sheathing stipule. Pedicels are only 1-5 mm long, usually recurved, the flowers thus much exceeded by the leaves. Petals are white, 2-5 (often wanting), equal or subequal to the sepals, which are about 1 mm long. Stamens 2

or 3, style much shorter than the ovary, the stigmas almost sessile, the 3-valved capsule equaling or slightly exceeding the sepals. Seeds are 2-3 per capsule, almost black, shining, lenticular and acute-margined, apparently smooth but very faintly depressed-reticulate, about 0.8 mm long (from Hitchcock et al. 1964)

Montia howellii often occurs with, and is somewhat similar to, *M. fontana*, but the former has spoon-shaped alternate leaves, whereas *M. fontana* has opposite leaves that are acuminate (Salstrom and Gamon 1992). Additionally, *M. fontana* is almost always erect, while *M. howellii* is smaller and always prostrate (Kagan 1989). *Montia howellii* is also known to occur with *M. dichotoma* (Salstrom and Gamon 1992, S. Gisler, personal observation), but the latter can be distinguished by its larger sepals, linear leaves, and racemose flowers that exceed the subtending leaves. Perhaps most notably, *M. howellii* is the only *Montia* which has flowers with no petals, or petals which are smaller than the small sepals; *M. fontana*, *M. dichotoma*, and *M. linearis* (another co-occurring species) all have obvious flowers (Kagan 1989).

Seed production

Although no studies have specifically quantified seed production in *M. howellii*, Kagan (1989) sampled 65 individuals in the population at the Long Tom ACEC (Area of Critical Environmental Concern) and documented a mean of 4.3 flowers produced per plant. Two to three seeds are generally produced per flower (Hitchcock et al. 1964); using these figures, each plant can be expected to produce from 8.6 to 12.9 seeds.

Most seeds are distributed near the parent plant, although water movement and animal vectors may also play a role in dispersal (Salstrom and Gamon 1992). Kaye (1993) notes that mature fruits actually snap open, and can toss seeds over one meter, confirming earlier statements by Nilsson (1971), who observed seeds being actively thrown from the plant.

Kaye (1992) found that the population of *Montia howellii* adjacent to the Long Tom ACEC forms a large, persistent seed bank. Soil samples from this site, when germinated

in an exposed cold frame at the Oregon State University greenhouses, yielded an average of 1,008 (±209.3 SE) seeds/square meter. This density of seeds in a persistent seed bank is high relative to most other accounts of seed numbers in soil; this large seed bank indicates that populations of this species are buffered against reproductive failure resulting in lack of seed production in any given year.

Seed germination

(Kaye 1992) found that seeds of *Montia howellii* require cold temperatures below 7Cº for germination. Ninety eight percent of seeds placed outside in November (with a daily temperature of approximately 4.5Cº) germinated within six weeks, and most germinated within the first week. In this study, even after cold stratification, seeds germinated better at cool temperatures than at warm temperatures. Additionally, Kaye suggests that seeds may require some after-ripening before germination, though the period of after-ripening is unknown Seedlings have been observed in naturally occurring populations in November (Kaye 1992), and winter rosettes have been observed in January and February (Kaye 1991), suggesting that seeds germinate in fall, overwinter as rosettes, and begin to initiate flowers in early spring.

Vegetative reproduction

Montia howellii is an annual species that reproduces through production of seeds (Salstrom and Gamon 1992). Kaye (1993) notes that individual large plants may root at the nodes of creeping stems, and fragments of these rooted stems may survive if severed from the parent plant and given sufficient moisture. He concludes, however, that this type of vegetative spread rarely occurs naturally, and probably contributes little to population persistence or expansion.

Breeding system

Montia howellii reproduces sexually, with the onset of reproductive maturity fluctuating according to the hydrology of individual sites. Flowering generally occurs from mid- to

late-March and early April. Considered to be largely cleistogamous, this species produces self-fertile flowers that do not require pollinators for seed set to occur (Salstrom and Gamon 1992. Most flowers normally do not open, and self-pollination takes place inside the closed flower (Nilsson 1971). Autogamy is facilitated by filaments that curve inwards and bring the anthers in direct contact with the stigmas. Flowers contain no nectaries, further decreasing the likelihood of any insect visitation. However, Kagan (1989) notes that occasional flowers, usually those with diminutive petals, open and could be available for cross-pollination, though no insects of any kind were ever observed visiting these flowers.

Hybridization

There are no known naturally or artificially occurring hybrids involving this species (Salstrom and Gamon 1992). Hybridization is expected to be extremely limited, as most flowers are cleistogamous and set seed without ever opening, and no pollinators have ever been observed visiting the species, providing no opportunities for inter-specific gene flow (or intra-specific gene flow) to occur.

Cultivation

Montia howellii has been grown from seed successfully by Kaye (1992), but no cultivation methods (beyond germination) were described in his study. Salstrom and Gamon (1992) also report cultivation (by Nelsa Buckingham), but again, no specifics as to cultivation conditions or methods are provided. Fortunately, as *M. howelli* is an annual species, site-sown seeding will most likely be the method employed for population creation or augmentation projects; the value of *ex situ* cultivation is probably limited to research projects outside the scope of this review.

Transplanting and introduction attempts

Kaye (1991) transplanted 7.5 x 7.5 cm blocks of soil containing *Montia howellii* from one portion of an extant site (at the Long Tom ACEC) to another, as part of a study on the affects of varying disturbance regimes. However, the fate of the transplants remains

unknown, as funding for monitoring of the transplant project was discontinued after the completion of transplanting.

Population monitoring

Monitoring was initiated at the Long Tom ACEC in 1989 by Kagan to determine long-term trends. Kagan (1989) established a series of randomly placed 10 x 10 cm monitoring plots along 50 m transects at the Long Tom ACEC, in burned, unburned, grazed, and ungrazed areas. Unfortunately, all transects failed to contain adequate plant numbers to allow any conclusions to be drawn regarding treatment effects. In this same study, a population size estimation method using 235 randomly placed square plots one meter in size did not consistently produce an accurate measure of the number of plants present, as populations emerged in slightly different places within the site each year.

Monitoring was continued by Kaye (1991, 1992), who established 200 20 x 20 cm microplots along transects that spanned burned and unburned roadbed. During spring monitoring he found that *M. howellii* occurred in 16 percent of the burned microplots and only six percent of those that were not burned. However, these data do not necessarily imply a relationship, since pre-burn data were not collected. Monitoring the following fall showed increases to 29 and 26 percent for unburned and burned plots, respectively.

Land use threats and other limitations

Montia howellii appears sensitive to interspecific competition; at sites where disturbances have decreased or ceased, encroaching vegetation threatens the viability of populations (Salstrom and Gamon 1992). Invasion of habitat by perennial exotic grasses appears to negatively impact populations in some sites (Kagan 1989), and colonization of sites that were potentially suitable for *M. howellii* by adjacent vegetation prevented establishment of the target species in a disturbance study at the Long Tom ACEC (Kaye 1992).

Although light disturbance prior to germination improved emergence in populations of *Montia howellii* in California, excess disturbance apparently produced a negative effect,

and Renner (2004) recommends closing roads during the plants' growing season. Closure of roads until after seed set is complete (April) is also recommended by Kaye (1992), in order to protect plants during the growing season at the Long Tom ACEC.

Because this species often prefers roadsides and the periphery of gravel parking lots, routine maintenance is a potential threat to many populations. Spraying on private timberland destroyed 250 plants in northern California (CNPS 2002), and has the potential to negatively impact other roadside populations. Site drainage, flood control, and cultivation can also damage populations (Salstrom and Gamon 1992).

Population introduction/augmentation strategy

Based upon the biogeographical data compiled and described above for *Montia howellii*, there are no significant ecological, life history, or administrative obstacles to the successful implementation of population introduction and augmentation projects for this rare species. Several large populations of this species occur in publicly owned sites; pending interagency cooperation and funding availability, these populations should be available for collection of seeds for use in reintroduction projects. Suitable unoccupied locations on publicly owned lands should also be available for population augmentation and introduction purposes.

The only environmental constraint potentially impacting future reintroduction/population creation projects is the proliferation of invasive weeds, which already pose a threat to existing populations. However, non-weedy sites still remain in many areas within this species' current range, so quality sites should still be available for introduction projects.

Montia howelli produces adequate supplies of seeds, which exhibit high levels of germination following natural or artificial cold stratification. Following germination, seedlings exhibit no specialized growth or symbiont requirements, and should establish where conditions are suitable. As some level of disturbance is needed to create and maintain a suitable seed bed for this species, a management regime that provides

adequate disturbance during dormancy, combined with restricted activities during the growing season, should be developed and implemented.

Based upon this information, the following step-down procedures are recommended for *Montia howelli* population introductions:

1. Select population introduction/augmentation target sites. Several primary factors should be considered when selecting target sites for *Montia howellii* population introduction and augmentation projects. First, target sites should contain the seasonally inundated, periodically disturbed habitat (described above) that is preferred by *M. howellii*. To assist in identification of suitable habitat, extant *M. howellii* populations in the vicinity of target sites should be visited to obtain familiarity with possible local microhabitat specificities. Data on associated species, soil types, and soil moisture from known extant populations in the vicinity of the target area help characterize suitable habitat, and can be used to assist with the selection of population creation sites. These data are also helpful in determining microsites within these areas that are suitable for transplanting.

   Given the history of the destruction of *M. howellii's* habitat on private lands, and the ubiquitous threat posed by invasive species, inventories for suitable population introduction and augmentation sites should be focused on publicly owned (or otherwise secure) lands that appear safe from imminent weed and successional encroachment problems. Selection and use of sites should be coordinated with pertinent public landowners to ensure administrative protection and management of populations following introductions.

3. Collect seeds from extant populations. In general, source material for a population creation project should utilize propagules collected from the extant population located nearest to the target site. Collection of *M. howellii* 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. In situations involving a

selected site outside the current range of the species, combined collections of seed from several populations may also be appropriate. To ensure that the genetic variation present in the extant donor population will be represented in the newly created site, seed should be collected from as many individuals as possible, located throughout the population.

To reduce the impact to existing populations, and provide additional genetic diversity, seed for creation/augmentation projects should ideally be collected in multiple years. However, the respectable seed production and high level of germinability reported for *M. howellii* should allow an adequate supply of seeds for most population creation projects to be collected in a single year, unless source populations are extremely small.

4. Direct sow seeds at the target sites. As described above, seeds of *M. howelli* require cold stratification (and potentially after-ripening) in order for germination to occur. To provide these needed treatments, seed can be sown *in-situ* immediately after collection in spring, providing a natural period of after-ripening, and allowing naturally occurring conditions during the following winter to produce the needed cold temperatures. This method eliminates the need for developing a stratification protocol, avoids the potential for unknown effects of the cultivation environment, and is easy and cost-effective. However, site-sown seeds can be lost to herbivory, unexpected climatic conditions, or habitat destruction prior to germination. To avoid these potential disadvantages, seed could also be artificially stratified prior to sowing. To promote ease in locating emerging plants for monitoring, seeds of *M. howelli* should be planted in well-marked plots.

   Because *M. howelli* produces a persistent seed bank, an alternative seed-sowing method might involve removing blocks of soil from a densely populated extant site, and introducing these into the target area. If conditions in the new site are appropriate, seeds in the seed bank should germinate and establish. This method

allows seed produced in multiple years to be included in one collection, but has several disadvantages, including an inability to easily determine the number of seeds sown, and the possibility of transplanting seeds of unwanted associated species.

5. Monitor introduced populations. Introduced populations of *M. howelli* should be monitored annually to evaluate project success. Although censusing of all plants that emerge from seeding plots will be an effective method for monitoring population creation projects of *M. howelli* in the first year after sowing, this strategy will no longer be appropriate in subsequent years. Assuming that seeds germinate, emerge and reproduce, seed dispersal and microsite-based selective germination will move plants outside of the original planting plots. Once this occurs, censusing the entire population, or developing a series of plots to evaluate presence/absence of plants throughout the site will provide an accurate picture of project success. As well as numbers of plants present, monitoring protocol should include measures of reproductive success, such as the number of flowers produced per plant and the number of seeds produced per flower. Monitoring should be continued for at least five years after sowingin order to develop a clear picture of overall project success.

6. Adaptive management. Management strategies expected to promote establishment and expansion of created populations should be developed prior to the initiation of population creation projects. Because *M. howellii* requires the periodic creation of unoccupied seed beds through regulated soil disturbance, management plans for created populations should include recommendations for an annual disturbance regime, such as light grading. When monitoring data are collected and reviewed each year, the plan should be evaluated, and adapted to meet the needs of the created population of *M. howellii*.

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Developing biogeographically based population introduction protocols for at-risk Willamette Valley plant species:

Willamette Valley checkermallows

Sidalcea nelsoniana

Sidalcea campestris

Sidalcea cusickii

Willamette Valley checkermallows:
Sidalcea nelsoniana
Sidalcea campestris
Sidalcea cusickii

Conservation status

Primarily associated with the undeveloped remnants of prairies, wetlands, and edges of woodlands and riparian areas, *Sidalcea nelsoniana* (Figure 34), *S. campestris* (Figure 35), and *S. cusickii* (Figure 36) have all dwindled to an alarming paucity of mostly small, fragmented populations. Because these three members of the mallow family (Malvaceae) are very similar with regard to their ecology, life history, anthropogenic threats, and conservation needs, they are treated together in this chapter.

Figure 34. *Sidalcea nelsoniana*. (Photo by Steven Gisler.)

Figure 35. *Sidalcea campestris*. (Photo by Steven Gisler.)

Sidalcea nelsoniana is the rarest of this group of Willamette Valley checkermallows, and has been listed as threatened by the U.S. Fish and Wildlife Service and the State of Oregon. It is on the Oregon Natural Heritage Program 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) (ONHP 2001).

Figure 36. *Sidalcea cusickii*. (Photo by Steven Gisler.)

Remaining somewhat more common than its state- and federally-listed relative, *Sidalcea campestris* is listed as a Candidate species by the State of Oregon, but lacks federal conservation status. The species is on the Oregon Natural Heritage Program List 4 (of conservation concern but not currently threatened or endangered) and has a Natural Heritage Network Rank of G4/S4 (not rare, apparently secure throughout its range and in Oregon). Although *S. campestris* has been nearly extirpated from its former distribution in the central and northern Willamette Valley (Kemp et al. 1978, Jimmy Kagan, Oregon Natural Heritage Program, Portland, Oregon, personal communication, Gisler unpublished), there are still enough populations persisting in the southern half of its range (especially Lane, Linn, Benton, Polk, and southern Marion counties), such that the species does not yet meet state and federal requirements for listing as threatened or endangered. However, such an unfortunate meeting will likely take place soon if southern *S. campestris* populations continue to follow the fate of those to the north, with populations yielding to development, successional encroachment by trees and shrubs, and roadside maintenance activities.

Sidalcea cusickii currently has no designated federal or state conservation status, though it shares the same Oregon Natural Heritage Program and Natural Heritage Network ranks

as *S. campestris*. Like the other Willamette Valley checkermallows, *S. cusickii* is primarily restricted to small, undeveloped native prairie remnants along roadsides in the southern Willamette Valley and interior valleys of the Rogue and Umpqua Rivers and their tributaries. Despite the lack of state and federal conservation status, *S. cusickii* is a very rare species occupying exceedingly vulnerable habitats. Relative to its two aforementioned congeners, the status of *S. cusickii* has been very poorly documented and this species has markedly fewer representative specimens in the OSU herbarium.

Together, the Willamette Valley checkermallows face overwhelming threats posed by widespread habitat loss to agricultural and urban development, and population displacement caused by invasive weeds and successional encroachment by trees and shrubs (Kemp et al. 1978, Siddall 1979, Scofield and Sawtelle 1985, ONRC 1986, Robinson and Parenti 1990, U.S. Fish and Wildlife Service 1993, 1998, ODA 1995, Gisler unpublished). Additional threats include pre-dispersal seed predation by weevils (U.S. Fish and Wildlife Service 1993, 1998, Gisler and Meinke 1997, 1998), and the potential threats of inbreeding depression and interspecific hybridization (Gisler 2003).

Because *Sidalcea campestris* and *S. cusickii* lack state and federal threatened and endangered species designations, there are no legal mechanisms in place for their mandated protection, regardless of the land ownership of their extant occurrences. In contrast, *S. nelsoniana* is legally protected on public lands by virtue of its formal listing as threatened, though many populations still lack protection on private lands, and most publicly owned populations continue to be threatened by lack of habitat management and other limitations that transcend land ownership boundaries (U.S. Fish and Wildlife Service 1993, 1998, Gisler and Meinke 1995).

The two overriding factors favoring the persistence and eventual recovery of Willamette Valley checkermallows are the occurrence of several large "stronghold" populations on public lands, and the very promising cultivation and re-introduction potential exhibited by all three species. *Sidalcea* recovery will ultimately hinge on the rapid implementation of large-scale habitat management and population re-introduction and enhancement

projects, such as those outlined in U.S. Fish and Wildlife Service (1998), before additional extant populations experience further declines or extirpation.

Range and habitat

Collectively, the three checkermallow species treated in this chapter span the majority of western Oregon's interior valleys, ranging from Jackson County in the south to the Puget Trough of Washington in the north.

Sidalcea nelsoniana, the rarest and most thoroughly documented of the checkermallows, is currently known from approximately 65 sites, distributed from southern Benton County, Oregon, northward through the central and western Willamette Valley, to Cowlitz and Lewis Counties, Washington (City of McMinnville 1997, U.S. Fish and Wildlife Service 1998, ONHP 2002). Additionally, this species occurs in several higher elevation Coast Range meadows that flank the western Willamette Valley in Yamhill, Washington, and Tillamook Counties, Oregon. Known populations range in elevation from 145-1,960 ft.

Although *Sidalcea campestris* and *S. cusickii* are by no means common within their respective geographic ranges, to date their populations have proven too numerous and poorly documented to reliably quantify and track in a database. Both species are limited to Oregon, and the majority of remaining populations, albeit fairly numerous (relative to some other western Oregon endemics), are extremely small, containing fewer than a hundred individuals (Gisler unpublished). The range of *Sidalcea campestris* essentially overlaps the Willamette Valley and Coast Range distribution of *S. nelsoniana*, though it is not known to extend across the Columbia River into Washington. *Sidalcea campestris* also extends beyond the range of *S. nelsoniana* into Lane County to the south, and across the Willamette Valley into the foothills of the Western Cascades.

Sidalcea cusickii has the most southerly range of the species group, extending from Jackson, Coos, and Douglas Counties in southern Oregon, northward to the southern end of Linn and Benton Counties. Interestingly, the northernmost extent of *S. cusickii*'s

distribution in Benton County precisely coincides with the southernmost extent of *S. nelsoniana*'s distribution, rendering the two species narrowly parapatric, but non-overlapping (Gisler 2003).

Habitats occupied by these three Willamette Valley checkermallow species are very similar, consisting of grasslands (wet and dry prairie), wetlands, and edges of woodlands and riparian areas, frequently existing as small habitat remnants located along roadsides (Figures 37 and 38). OSU herbarium specimen labels variously describe these habitats as: "dry prairies," "grassy fencerows," "moist, open ground and thickets," "overgrown drainage ditches," "roadside ditch with tall grasses," "wet, grassy openings along right-of-ways," "lightly wooded ash swales," and "moist flats with adobe soil." Although these species tend to occupy sites that are relatively undisturbed, such as parks and wildlife refuges and the undeveloped margins of fields and roads, all three species nevertheless appear capable of colonizing (or at least persisting within) some disturbed sites (City of McMinnville 1986, Halse and Glad 1986, Glad et al. 1994). It is uncertain, however, to what degree seedling recruitment occurs in weedy sites, and how long populations can persist under such conditions after mature plants with large, established root systems die.

Of the three species, *Sidalcea nelsoniana* tends to occupy the wettest habitats, with *S. cusickii* and *S. campestris* usually preferring slightly drier (but still seasonally wet), more upland habitats (Gisler unpublished). However, there are numerous exceptions to this generalization, and *S. campestris* co-occurs with both species (independently) in several locations, reflecting the breadth and overlap of habitat tolerances in the group (Gisler 2003).

Glad et al. (1994) reported 111 species associated with *Sidalcea nelsoniana*, about half of which were non-native. Some of the species most commonly associated with *S. nelsoniana* and the other two Willamette Valley checkermallows include: *Achillea millefolium*, *Agrostis tenuis*, *Alopecuris pratensis*, *Arrhenatherum elatius*, *Brodiaea hyacinthina*, *Carex* spp., *Cirsium* spp., *Chrysanthemum leucanthemum*, *Crataegus* spp.,

Dactylis glomerata, *Daucus carota*, *Deschampsia caespitosa*, *Equisetum arvense*, *Festuca arundinaceae*, *Fragaria virginiana*, *Fraxinus latifolia*, *Galium aparine*, *Geum macrophyllum*, *Heracleum lanatum*, *Holcus lanatus*, *Hordeum brachyantherum*, *Hypericum perforatum*, *Hypochaeris radicata*, *Juncus* spp., *Lotus corniculatus*, *Lupinus polyphyllus*, *Madia sativa*, *Parentucellia viscosa*, *Phalaris arundinaceae*, *Prunella vulagris*, *Pteridium aquilinum*, *Quercus garryana*, *Rubus* spp., *Rosa* spp., *Spiraea* douglasii, *Symphoricarpos albus*, *Vicia* spp., (Kemp et al. 1978, Siddall 1979, Halse and Glad 1986, U.S. Fish and Wildlife Service 1993, 1998, Gisler and Meinke 1995, ONHP 2002, OSU herbarium specimen label information).

Figure 37. Roadside prairie remnant occupied by *Sidalcea campestris* (linear band of white flowers along fencerow) near Lebanon in Linn County, Oregon. (Photo by Steven Gisler.)

Figure 38. *Sidalcea nelsoniana* (pink flowers) occupying a narrow strip of undeveloped prairie habitat between a county road and a grass seed field south of Philomath in Benton County, Oregon. (Photo by Steven Gisler.)

As indicated by the preceding paragraph, Willamette Valley checkermallow species are frequently associated with various trees, especially *Fraxinus latifolia* and *Quercus garryana*. These trees frequently occur as small woodlands, with checkermallows typically occupying small clearings and edges with fairly open canopies. Although *Sidalcea* species are sometimes found under closed canopies, they frequently become etiolated under such conditions and it is uncertain how long they can persist in the shade. It is likewise unknown if these shaded plants colonized habitats that were originally wooded, or if they pre-date canopy closure and simply persist in areas that have become overgrown by trees and shrubs through successional encroachment of previously open habitat. Such encroachment is considered a primary threat to the species (see "Conservation status," above).

Soils found in habitats occupied by Willamette Valley *Sidalcea* species are variable, ranging from gravelly, well drained loams, to poorly drained, hydric clay soils (City of McMinnville 1986, Glad et al. 1994). Generally, all three species are found in soils that

become saturated during the rainy season, with *Sidalcea nelsoniana* frequently becoming inundated for several weeks or longer. *Sidalcea campestris* and *S. cusickii* also grow in areas of standing water at some sites, though in general their habitats remain slightly drier throughout the year than those occupied by *S. nelsoniana* (Gisler unpublished).

Species description

Willamette Valley checkermallows are herbaceous perennials arising from stout, often somewhat rhizomatous and laterally spreading rootstocks that can form multiple crowns. All three species produce numerous, erect inflorescences ranging from 5 to 15 decimeters in height. Basal leaves are palmately lobed, with upper leaves and stem leaves becoming deeply divided. Stem and leaf pubescence traits vary between species, with *S. cusickii* typically having essentially glabrous stems and upper leaf surfaces, *S. campestris* typically having forked (or long simple and forked) stem hairs and densely hairy upper leaf surfaces, and *S. nelsoniana* usually exhibiting sparse, short simple stem and upper leaf surface hairs. All three species produce fruits that are 7-9 seeded schizocarps, with single-seeded, beaked carpels that form a ring, like the segments of an orange. Carpels separate at maturity and simply fall from the parent plant. Flowers vary considerably in size within species due to sexual dimorphism, with the larger flowers formed on hermaphroditic individuals and smaller flowers formed on female (male-sterile) individuals. Although flower color can vary dramatically within species (Gisler 2003), flower color is *usually* white to very pale pink in *S. campestris* and pink to rose in *S. nelsoniana* and *S. cusickii*. For further descriptive information see Hitchcock and Kruckeberg (1957), Peck (1961) and Halse et al. (1989).

Because of pronounced intraspecific variability, it can be very difficult to delimit Willamette Valley checkermallow species using single morphological traits. Instead, accurate identification often rests on a combination of characters. In general, *S. cusickii* can usually be discerned from its congeners by its glabrous (and typically non-glaucous) stems and leaves, dark pink flowers (though some populations contain white to pale pink flowers), sepals that are frequently widened above the base (and purple-tinged) before they taper to a point, bluntly terminated inflorescences (rather than tapered to a point)

when in bud, and by its more southerly geographic distribution (see "Range and habitat," above). *Sidalcea campestris* can usually be recognized by its white flowers (though some populations contain darker pink flowers), stem hairs that are typically long, dense, and forked (or simple and forked together), and basal leaves that are often more deeply dissected than those of the other congeners. *Sidalcea nelsoniana* typically has glaucous stems, pink flowers (though sometimes very pale pink to white in some populations in the southern portion of its range), sparse and short-simple stem and upper leaf pubescence, and a distribution entirely north of southern Benton County. *Sidalcea nelsoniana* and *S. cusickii* are the two most difficult species to distinguish from one another, though fortunately they are not known to overlap in the wild (Gisler unpublished).

Besides the checkermallows treated in this chapter, there is one other *Sidalcea* species occurring in the Willamette Valley, *S. virgata* (Figure 39). This species can be distinguished from its congeners by its more decumbent and rhizomatous habit, stellate stem pubescence, shorter flowering stems, sparser inflorescences, longer sepals that are rolled along the margins, and an earlier phenology (flowering is usually completed by early June, when the other congeners are just starting to flower) (Gisler 2003 and unpublished).

Figure 39. *Sidalcea virgata*, another species of checkermallow that occurs in the Willamette Valley. (Photo by Steven Gisler.)

Seed production

With their large floral displays and multi-carpeled fruits, Willamette Valley checkermallow species are prolific seed produces. Gisler and Meinke (1998) surveyed seed production in 20 *Sidalcea nelsoniana* populations and estimated mean seed production at 5.18 seeds per fruit (which equated to about 65 percent of available ovules). This rate was essentially equivalent to seed set levels achieved through controlled crosses using large pollen loads in the greenhouse, which yielded a mean 5.26 seeds per fruit, suggesting that individuals in the wild are neither pollen nor resource limited in terms of seed production. Analysis showed that rates of ovule conversion to filled seeds did not differ significantly between the 20 study populations, though rates of seed loss to pre-dispersal predation by weevils (ranging from 0-100 percent seed loss) did exhibit significant differences between populations.

A later study (Gisler and Meinke 2001a) showed that seed losses to pre-dispersal predators can be significantly reduced through the use of a synthetic pyrethroid insecticide applied early in the flowering season. If insecticides are utilized to reduce seed predation, then total plant seed yields can be directly estimated by multiplying seed average set rates by the number of fruits (because deductions need not be made to account for seed losses to pre-dispersal predation). Typical *Sidalcea* plants produce 30-100 or more fruits per multi-racemed inflorescence, with individuals frequently producing 10-30 or more inflorescences. As such, a typical checkermallow individual could be expected to produce as many as 1,500-15,000 seeds per year in the absence of seed predation or other reproductive constraints (i.e., herbivory, severe drought conditions, or disease). Thus, a population containing a mere 50 individuals could ostensibly produce 750,000 or more seeds in a single season.

One important caveat associated with weevil control practices, however, is that the weevil species (Figure 40) infesting Willamette Valley checkermallows are native, are specific to Pacific Northwest *Sidalcea* species, and host an unidentified species of parasitic wasp (Gisler and Meinke 1998, 2001a). As such, weevil control activities could

have unintended and undesirable consequences to the conservation status of these native organisms, which may be equally as rare, or *more* rare, than their host plants.

Figure 40. Adult weevil (*Macrorhoptus Sidalcea*) emerging from a carpel of *Sidalcea nelsoniana*. This weevil species is only known from *S. nelsoniana* and its rare coastal relative, *S. hendersonii*. Weevils dramatically reduce seed survival in some *S. nelsoniana* populations. (Photo by Steven Gisler.)

Levels of seed production in *Sidalcea campestris* and *S. cusickii* are commensurate with those stated above for *S. nelsoniana*, ranging from 60-70 percent of available ovules (Gisler 2003 and unpublished). In the wild, this species pair has an advantage over *S. nelsoniana*, insofar that they both (particularly *S. cusickii*) generally suffer much lower levels of pre-dispersal seed predation by weevils (Gisler and Meinke 1997 and unpublished).

Seed germination

With the exception of germination trails performed on *Sidalcea cusickii* by Guerrant and Raven (1995), which yielded rather low germination rates of 10.4-34.8 percent under a variety of environmental conditions, all other data suggest that seeds of Willamette Valley checkermallows exhibit high levels of viability and germination. The apparent key to seed germination in these species (a key not utilized in the *S. cusickii* trials

discussed above) is seed coat scarification. As reported by the City of McMinnville (1986)(also reported in Halse and Mishaga 1988), seed germination in *S. nelsoniana* ranged from 43-100 percent following seed coat penetration with a needle probe, whereas non-scarified seeds exhibited less than 13 percent germination. Seed germination was not significantly affected by light and dark conditions, though it was affected by level of seed maturity; lower seed germination levels were associated with immature seeds that had greenish colored seed coats at the time of collection, whereas higher germination rates were associated with mature seeds with dark brown seed coats.

The efficacy of seed coat scarification on *Sidalcea* germination was also demonstrated by Gisler (2003), who scarified seed coats with 120 grit sandpaper and reported 97 percent seed germination in *S. cusickii*, 73 percent germination in *S. campestris*, and 90 percent germination in *S. nelsoniana.* Seed germination in all three species occurred in a single flush, within 2 weeks of imbibition. This rapid pace of seed germination helps mitigate the potentially adverse impacts of mold growth, which commonly accompanies *Sidalcea* seeds after they become wet and might otherwise become problematic for more slowly germinating seeds (Gisler unpublished). Mold growth was also noted as a potential problem by the City of McMinnville (1986), but was minimized by rinsing seeds in a dilute bleach solution.

Seed scarification, accompanied by cold stratification, also yielded high germination rates during a large-scale *Sidalcea nelsoniana* cultivation project performed by Lynda Boyer (Heritage Seedlings Inc., Salem, Oregon, personal communication). According to Boyer, nearly 100 percent germination was achieved by treading and "doing the twist" on seeds, and then mixing the scarified seeds with pre-moistened vermiculite inside sealed plastic bags and cold stratifying the mixture at 1°C for 11 weeks. This seed/vermiculite mixture was then sown into soil-filled flats, lightly covered with a "light dusting of soil," and germination typically occurred within 7 days of sowing.

Vegetative reproduction

Willamette Valley *Sidalcea* species are all apparently capable of some degree of vegetative expansion via rhizomes and/or laterally spreading root systems that form multiple crowns bearing distinct clusters of flowering stems (Figure 41)(City of McMinnville 1986, U.S. Fish and Wildlife Service 1993, 1998, Glad et al. 1994, Gisler and Meinke 1998, Guerrant 1998). This tendency for rhizomatous/lateral growth has resulted in substantial confusion regarding development of appropriate monitoring methodologies and the identification of "genetic individuals" in *Sidalcea nelsoniana* (Guerrant 1998). Traditionally, this complication has been dealt with by assigning all clusters of stems within an occupied square meter of habitat as an individual (City of McMinnville 1986, Glad et al. 1994, U.S. Fish and Wildlife Service 1993, 1998), though in most cases stem clusters that appear distinct are probably indicative of physiologically (if not genetically) distinct individuals (City of McMinnville 1986).

Figure 41. Spreading rhizomes of *Sidalcea nelsoniana*, shown from the Oregon Coast Range, where the species tends to have a more rhizomatous habit than in the Willamette Valley portion of its range. (Photo by Steven Gisler.)

For the most part, the three *Sidalcea* species treated in this manual tend not to actively form new shoots *per se* through rhizomatous growth. According to the City of McMinnville (1986), "*Sidalcea nelsoniana* rhizomes are more likely to reproduce vegetatively through breaking, with the broken-off part being moved away from the parent plant, than they are by sending out long rhizomes that give rise to new plants." The only Willamette Valley checkermallow species that demonstrates a strong tendency

for developing new sprouts via long, spreading rhizomes is *S. virgata* (Gisler unpublished). It is currently unknown to what extent population maintenance in Willamette Valley checkermallows is dependent upon asexual expansion versus sexual reproduction.

One conservation benefit offered by the clonal nature of these *Sidalcea* species is that they readily lend themselves to the rapid increase of propagated through divisions. In greenhouses at OSU, the authors were able to obtain flowering individuals of all three species from divisions within two months of planting. These divisions, in turn, grew rapidly and soon lent themselves to repeated divisions, thus yielded additional propagated stock. Similarly, divisions have been successfully utilized to increase *Sidalcea* stock for on-site wetland and prairie restoration purposes by Ted Gahr (Gahr Farm, McMinneville, Oregon, personal communication) in Yamhill County. As will be discussed later, however, the use of divisions in propagation and re-introduction work should be undertaken with some restraint in order to discourage low genetic variability and skewed sex ratios in introduced or augmented populations. Instead, propagation via clonal divisions should be undertaken in concert with cultivation from seeds, the latter encouraged by high seed set and germination rates (see "Seed production" and "Seed germination," above).

Breeding system

The genus *Sidalcea* is characterized by a gynodioecious breeding system, whereby individuals can either be hermaphroditic (bearing exclusively perfect flowers with both male and female sex organs) or female (bearing exclusively male-sterile flowers). Because female flowers produce no pollen, they require insect-mediated outcrossed pollen in order to produce seeds. Although hermaphroditic flowers produce pollen, within-flower self-fertilization (auto-autogamy) is discouraged by protandry, whereby pollen dehisces 2-3 days prior to stigma emergence and receptivity. However, Willamette Valley species of *Sidalcea* are self-compatible, and self-fertilization can still occur (and probably occurs quite frequently) in hermaphroditic plants through pollen transfer between flowers of the same individual (i.e., geitonogamy) (Gisler and Meinke

1998, Gisler 2003). It is currently unknown to what extent progeny from female individuals might exhibit increased fitness over those from hermaphrodites, through greater heterozygosity (due to mandatory outcrossing). Likewise, it is unknown if progeny from females might also experience higher quality and fitness if maternal individuals allocate resources to seed production that are saved through production of smaller flowers and lack of pollen production.

Although female individuals require co-occurring hermaphroditic individuals in order to receive pollen and produce seeds, there is no evidence that female-biased population structures lead to reduced seed set caused by pollen limitation (Gisler and Meinke 1998). However, lone female individuals lacking hermaphroditic pollen sources will not produce seeds, as these species show no evidence of apomixis, or seed production in the absence of fertilization (Gisler 2003). This means that population introduction projects should include a mixture of female and hermaphroditic individuals, to ensure seed production. However, this mixture may be weighted towards female individuals without apparent undue consequences to seed yields. Most *Sidalcea nelsoniana* populations in the Willamette Valley have female-dominated sex ratios, whereas females in Coast Range populations are usually outnumbered by hermaphroditic individuals (Gisler and Meinke 1995).

Willamette Valley checkermallows produce showy floral displays and attract an impressive abundance and diversity of insect visitors. Gisler (2003) inventoried pollinators at over 25 populations of each checkermallow species in the Willamette Valley and identified over 17 species of bees, 3 species of wasps, 9 species of flies, 6 species of beetles, and 5 species of lepidopterans. Three species of bumblebees (*Bombus californicus*, *B. sitkensis*, and *B. vosnesenskii*) were the most common and active pollinators of the checkermallows, though solitary bees in the families Anthophoridae, Halictidae, and Megachilidae were also significant visitors. One interesting finding was the occurrence of *Diadasia nigrofrons* (Anthophoridae)(Figure 42) on all three *Sidalcea* species. This native bee is a specialist on *Sidalcea* in the Willamette Valley, so its status is intimately tied to the occurrence and survival of its sole host/forage species. In

contrast, given the role of Willamette Valley checkermallows as "cornucopia" species, attracting large and diverse assemblages of bees, there is little reliance of *Sidalcea* species to one particular taxon or group of pollinators, and thus little vulnerability to pollinator limitations.

Figure 42. *Diadasia nigrofrons*, a native bee specializing on species of *Sidalcea* for nectar and pollen forage in the Willamette Valley. (Photo by Steven Gisler.)

Gisler (2003) also noted that insect visitors will, if given the opportunity, transfer pollen indiscriminately between *Sidalcea* species, leading to interspecific pollen flow and the possibility for interspecific hybridization (see "Hybridization," below).

Hybridization

Because of their frequent co-occurrence in the wild, and the appearance of morphologically intermediate *Sidalcea* individuals and populations, there has long been speculation about the occurrence of hybridization between Willamette Valley checkermallow species. The risk of hybridization in this species group would seem to be high, given risk factors identified by Gisler (2003) that are believed to promote

interspecific hybridization, including: demonstrated sexual compatibility between other members of the genus (i.e., Hitchcock and Kruckeberg 1957), sympatry of potential interspecific mating partners, human-mediated dispersal of Willamette Valley checkermallow species (through horticultural, agricultural, and restoration practices), ubiquity of disturbances that promote habitat homogenization and the creation of novel microsites for hybrid colonization, outcrossing mating systems, and the ability to spread vegetatively (thus promoting hybrid establishment, even if they prove sexually sterile).

Based upon investigations of pre- and post-mating reproductive isolating mechanisms, interspecific crossing experiments, and chromosome counts, Gisler (2003) showed that *Sidalcea campestris* is largely reproductively isolated from its Willamette Valley congeners by genetic/chromosomal incompatibilities (it is a hexaploid, whereas *S. nelsoniana* and *S. cusickii* are both diploid). However, although hybridization involving *S. campestris* is therefore unlikely in the wild, it is not entirely impossible, as evidenced by a few tetraploid hybrids were successfully created through artificial crosses in the greenhouse. *Sidalcea nelsoniana* and *S. cusickii* have a very strong potential for hybridization, insofar that they share many pollinators, overlap in flowering time, and are completely sexually interfertile in the greenhouse, yielding fertile diploid hybrids. The one important obstacle to hybridization in this species pair is geographical separation; as mentioned earlier, the respective geographic ranges of these two species are currently separated by about 3 km in southern Benton County, near the southern boundary of Finley National Wildlife Refuge. This separation appears to preclude the potential for interspecific pollen flow and hybridization between *S. nelsoniana* and *S. cusickii*.

According to Gisler (2003), all three *Sidalcea* species treated in this manual share pollinators and geographic distributions with a fourth Willamette Valley checkermallow species, *S. virgata*. Hybridization is discouraged with *S. virgata*, however, by its flowering time, which begins much earlier than the other local checkermallows and generally comes to an end just as the remaining checkermallow species are beginning to flower. Moreover, the tetraploid nature of *S. virgata* serves as another obstacle to hybridization with its diploid and hexaploid relatives. Crosses between these species

yielded only occasional hybrids, and these were sterile. Nevertheless, because of their strongly clonal nature, sterile hybrids could still become established and pose a potential threat to parental species. Likewise, fertility could potentially be regained in these sterile hybrids through backcrossing and/or chromosome doubling events. Polyploidy within *S. virgata*, yielding both diploid and tetraploid cytotypes, may further influence the likelihood of hybridization if seasonal timing barriers are disrupted through random climatic events or human interference.

The main lesson from the findings by Gisler (2003) appears to be that current pre- and post-mating attributes serve to discourage hybridization among the Willamette Valley checkermallows, but great care should be taken to prevent anthropogenic dispersal of these taxa beyond their current distributions, lest hybridization potentially ensue. Likewise, *Sidalcea* species should not be grown close together for cultivation purposes, as this could promote the kind of artificially enhanced interspecific pollination events that can overcome crossing barriers and lead to hybrid formation. This danger is especially pronounced between *S. cusickii* and *S. nelsoniana*, which are fully interfertile.

Cultivation

Willamette Valley checkermallow species lend themselves to successful cultivation in the greenhouse and outdoor beds. The authors have obtained large, multi-stemmed, flowering *Sidalcea* individuals (of all three species) as rapidly as within 2 months of planting using divisions, and within 3 months using seeds, when they are supplied with ample light, warm temperatures, irrigation, and fertilization (Gisler unpublished). The authors observed no evidence that *Sidalcea* requires unique soil mixtures, symbionts, or other specialized conditions for vigorous growth under cultivation, though it was noted that these species are highly susceptible to white flies, and to a lesser extent to other greenhouse pests, including aphids and spider mites. Despite these pests, *Sidalcea* survival was extremely high under cultivation, with the major source of mortality (usually less than 5 percent) attributable to damping-off fungal infections that killed young seedlings within the first two weeks after emergence (Gisler unpublished).

Other reports of successful *Sidalcea* cultivation have come from the City of McMinnville (1986), who cultivated 1,370 seedlings and noted that although plants grew faster and larger in a warmer (22°C day/18°C night) greenhouse room than in a cooler (18°C day/16°C night) greenhouse room, those from the cooler greenhouse experienced fewer setbacks (i.e. basal leaf die-back) upon placement in outdoor cold frames. Large-scale cultivation of *S. nelsoniana* was also reported by L. Boyer (personal communication), who mixed seeds with pre-moistened vermiculite and cold stratified (at 1°C) the mixture inside sealed plastic bags for 11 weeks. This seed/vermiculite mixture was then sown into flats filled with a planting medium consisting of bark, compost, peat, perlite, and Philip's pre-mix (crabmeal, 3 kinds of lime, micronutrients, Actino-iron, and a wetting agent). Following establishment in flats, seedlings were transplanted into 5 inch x 2 3/8 inch pots, and later moved into large, outdoor beds. Boyer reported very high (nearly 100 percent) rates of seedling establishment, yielding fast-growing, "healthy and happy" plants. These cultivated individuals will be used to augment *Sidalcea nelsoniana* populations located at Baskett Slough National Wildlife Refuge (Jock Beall, Finley National Wildlife Refuge, Corvallis, Oregon, personal communication).

Cultivation of Willamette Valley checkermallows can also be successfully undertaken using large-scale "farming" techniques. One private grower, Peter Kenagy (Kenagy Family Farms near Albany, Oregon), is performing a grow-out of *Sidalcea campestris* and *S. cusickii* for purposes of supplying seeds for restoration on BLM and other public lands near Eugene. Here, thousands of seeds of both species were fall seeded using a seed drill (drilled to half an inch deep or less), into ground that was pre-fumigated to reduce competitive pressure from weeds. Although seeds were sown without any form of pre-treatment, such as scarification or stratification, excellent rates of germination and establishment were reported. Likewise, plant survival and fecundity was very high, despite the lack of any supplemental irrigation or fertilization (Peter Kenagy, Kenagy Family Farms, Albany, Oregon, personal communication).

In summary, Willamette Valley checkermallows are very easy to grow, and can be successfully cultivated under a variety of indoor and outdoor conditions. The ease of

cultivation may be one reason why these species are available in local nurseries, have already been used in numerous habitat and population restoration projects, and are present in private gardens throughout the Willamette Valley (Gisler unpublished).

Transplanting and introduction attempts

Although Willamette Valley checkermallows have no doubt been transplanted for horticultural/gardening purposes for many years, the earliest documented efforts to transplant these *Sidalcea*s for conservation-related purposes was reported by the City of McMinnville (1986). Here, to investigate the transplant potential of *Sidalcea nelsoniana*, six rhizomes from the Oregon Coast Range were transplanted to a new site in 1985, yielding "vigorous plants." Based upon this initial success, 200 rhizomes were excavated and transplanted to several new locations in 1986. After 11 years, survival of the transplanted rhizomes was reported as 87 percent (City of McMinnville 1997), though subsequently some plants were apparently lost due to inundation caused by beaver dams (Guerrant 1998).

Transplanting of *Sidalcea nelsoniana* has also been undertaken at Finley National Wildlife Refuge. Here, *S. nelsoniana* rhizomes were excavated from two sites occurring on water retention dikes slated for potentially destructive management activities, and were transplanted into more secure locations. One target location was a wet swale already occupied by *S. nelsoniana*, and another site was a former agricultural field undergoing restoration to native prairie. At one of the rhizome source sites, *S. nelsoniana* was excavated by hand, using shovels, whereas the other source site utilized a mini excavator to remove rhizomes and their surrounding sod layer. Transplanted rhizomes were watered several times during the first year to aid establishment, and survival of transplants has been "very high," with 23 of 24 still alive at one target site (J. Beall, personal communication). Interestingly, excavation of *S. nelsoniana* rhizomes with shovels apparently did not result in *complete* rhizome removal from one source site at the Refuge, as new plants were later observed re-sprouting from their former locations over subsequent years, essentially resulting in a doubling of the total number of individuals from the site.

In addition to transplanting checkermallows from one "natural" site to another, they can also be introduced from cultivation into the wild. Such methods were performed for *Sidalcea nelsoniana* by the City of McMinnville, whereby 1,370 cultivated seedlings were introduced into six new sites (City of McMinnville 1986). Eleven years after transplanting, 58 percent of introduced seedlings from 1986 survived (City of McMinnville 1997). Transplanting cultivated plugs into the wild was also reported by Gisler and Meinke (2001b). Here, 300 *Sidalcea nelsoniana* were cultivated in gallon size pots to a large, flowering size in the greenhouse, and then introduced into three sites in Yamhill County near McMinnville (Figure 43). Despite unusually droughty conditions, which severely limited soil moisture availability at the introduction sites, survival after one growing season was 100 percent at two sites, and 93 percent at the third site (this lower survival was probably due to intensive competition with dense growths of invasive weeds). Most introduced plants flowered during their first year at all three sites, and some seedling recruitment was observed during the second year (Kathy Pendergrass, U.S. Fish and Wildlife Service, Portland, Oregon, personal communication). Additional *S. nelsoniana* introductions also took place at a fourth site, located at a city park in Corvallis. Here, 125 of 130 introduced individuals survived and flowered after two years, and some new seedlings have been observed at this introduction site as well (Gisler unpublished).

Although introduction of *Sidalcea* species is probably most effectively and successfully performed using cultivated plugs, they can also be introduced using seeds. Clark et al. (2001) reported seeding of *Sidalcea campestris* at the Danebo Wetland in Eugene, Oregon. Here, 150 seeds were sown, with 0.3 percent establishment in burned plots and 1.9 percent establishment in the unburned plots. In a previous study (Clark and Wilson 2000), establishment of *S. campestris* from seed was similar, with 1.7 percent in the burned plots and 2.1 percent in unburned plots. Clark et al. (2001) also reported sowing of *S. cusickii* seeds. For this species, 120 seeds were sown, with 3.3 percent establishment in burned plots and 4.3 percent germination in unburned plots.

Figure 43. *Sidalcea nelsoniana* introduction project in Yamhill County. Plugs were planted into 10 meter x 10 meter grids, with plugs spaced 1 meter apart, to facilitate recognition and relocation of individuals over time. (Photo by Steve Gisler.)

Population monitoring

The most intensive and consistent population monitoring for *Sidalcea* has been carried out at the Walker Flat population of *S. nelsoniana*, located in the Coast Range of western Yamhill County, Oregon. Portions of this population have been monitored annually since the mid 1980's, including areas owned by the City of McMinnville and adjacent areas owned by the Salem District BLM. Because this population is so large, monitoring has typically consisted of various approaches to either detect relative changes in *S. nelsoniana* frequency within random sampling plots (Guerrant 1998), or estimate overall population size based upon random, stratified sampling within fixed grids (City of McMinnville 1997).

Although such statistically intensive sampling procedures are probably necessary to identify demographic changes in large populations like Walker Flat, most *Sidalcea* populations are fairly small, generally containing fewer than 100 individuals, and

therefore lend themselves to monitoring through simple censusing. Regular censusing has been performed for many *Sidalcea nelsoniana* populations by the City of McMinnville since 1985 (City of McMinnville 1997), providing a general picture of populations trends over time. The primary complication to direct census methods is the tendency for some *Sidalcea* individuals to exhibit vegetative (clonal) spread via rhizomes or laterally spreading root systems (see "Vegetative reproduction," above). This complication has traditionally been dealt with by considering all flowering stems within 1 square meter as a single individual, unless both female and hermaphroditic plants are present, in which case both sexes are counted as separate individuals (since individuals are either exclusively female or hermaphroditic) (City of McMinnville 1997). However, for the most part even closely spaced individuals can be recognized by the spatial distinctness of their clustered stems, a trend supported by rhizome excavations performed by the City of McMinnville (1986).

Given the aforementioned monitoring difficulties inherent among closely spaced individuals, population introductions should probably be performed in such a way that introduced plugs can be discerned and re-located over time. Therefore, if space and other environmental constraints of introduction sites allow, plugs should be widely spaced (at least one meter apart) when planted into the ground. Although such widely spaced individuals may eventually grow closer together over time, this spacing will at least initially offer several years of relatively easy monitoring to measure survival rates and transplant performance. Such measures were taken during introductions of *Sidalcea nelsoniana* in Yamhill County by the Oregon Department of Agriculture, in cooperation with the Natural Resource Conservation Service and U.S. Fish and Wildlife (Gisler and Meinke 2001b). Here, transplants were introduced into three different sites within 100 meter square grids, with each square meter occupied by a single transplanted plug (Figure 43, above). The spacing of plugs not only facilitated recognition of individuals, but the regular placement of transplants within the grid also made it easier to re-locate and track individuals using meter tapes and x-y coordinates.

Land use threats and other limitations

As briefly discussed in "Conservation status," above, Willamette Valley checkermallow species uniformly face serious threats posed by urban and agricultural development, ecological succession that results in shrub and tree encroachment of open prairie habitats, and competition with invasive weeds (Kemp et al. 1978, Siddall 1979, Scofield and Sawtelle 1985, ONRC 1986, Robinson and Parenti 1990, U.S. Fish and Wildlife Service 1993, 1998, ODA 1995, Gisler unpublished). Additional threats include pre-dispersal seed predation by weevils (U.S. Fish and Wildlife Service 1993, 1998, Gisler and Meinke 1997, 1998), and the potential threats of inbreeding depression, due to small population sizes and habitat fragmentation, and interspecific hybridization (Gisler 2003).

Fortunately, although many checkermallow populations face uncertain long-term fates, especially those on private lands that are not regulated by state and federal endangered plant laws, there are several *Sidalcea nelsoniana* and other congener populations that occur on public and other protected lands. For instance, large *S. nelsoniana* and *S. campestris* populations occur within National Wildlife Refuges, Oregon Department of Fish and Wildlife Areas, several city and county parks, and Oregon Department of Transportation lands. Both *S. campestris* and *S. cusickii* also have large populations on lands owned by the West Eugene Wetlands Program, The Nature Conservancy, and the Eugene District BLM. Therefore, while population losses on private lands still constitute one of the most pressing threats to these species, their occurrence on public lands simultaneously offers great optimism for their conservation and recovery. However, these conservation goals will hinge on actual implementation of habitat management and population enhancement projects. Without such real implementation, the threats that transcend land ownership (i.e., invasive weeds, successional encroachment, seed predation, hybridization, deer herbivory, etc.) may still pose irreversible threats to even these publicly protected populations.

Population introduction/augmentation strategy

Based upon the biogeographical data compiled and described above for the Willamette Valley checkermallows, there are no significant ecological, life history, or administrative obstacles to the successful implementation of population introduction and augmentation projects for these rare species. Many of the known extant *Sidalcea* populations occur on public ownerships so, pending interagency cooperation and funding availability, sites should be available for collection of seeds for use in off-site cultivation, and locations should also be available for population augmentation and introduction purposes. The primary environmental constraint to these much-needed conservation projects is the proliferation of invasive weeds, which already poses a serious threat to the majority of existing populations. However, non-weedy sites still remain in many "natural" areas within these species' current ranges, so high quality sites should still be available for introduction projects.

Willamette Valley *Sidalcea* species produce ample supplies of seeds (despite pre-dispersal seed predation), and seeds exhibit high levels of germination following seed coat scarification (see "Seed germination," above). Following germination, seedlings exhibit no specialized growth or symbiont requirements, and it is possible to cultivate the species to a reproductively mature stage within as little as three months in the greenhouse (see "Cultivation," above). Willamette Valley *Sidalcea* species can also be very successfully propagated by rhizome cuttings, such that propagation stock can be repeatedly divided (in the greenhouse or even in the wild) to increase the quantity of stock for population enhancement and introduction projects.

Although sowing seeds directly into field plots has not proven an effective means of establishing new checkermallow populations, cultivated plugs and rhizomes have repeatedly demonstrated very high levels of survival and establishment when placed in the field, even when placed into marginal-appearing habitats. This trend suggests that the large-scale population introduction and enhancement projects needed for recovery should both feasible and successful.

One factor that should be considered a potential complication to Willamette Valley *Sidalcea* introduction projects is interspecific hybridization. Evidence suggests that *S. nelsoniana* and *S. cusickii* are completely interfertile and are only reproductively isolated by the current geographic separation of their respective ranges (their ranges are parapatric, meeting in southern Benton County, Oregon) (Gisler 2003). Although crossing barriers exist that discourage hybridization between the other checkermallow species (i.e., *S. campestris* and *S. virgata*) in the Willamette Valley, these barriers could potentially be disrupted by anthropogenic dispersal events. As such, to avoid problems associated with hybridization, population introduction target sites should be carefully selected in areas located strictly within each species' current range and habitat type, and inventories should be performed to ensure the absence of other congeners within project target areas. Likewise, *Sidalcea* species should not be cultivated closely together for population introduction and enhancement projects, to minimize opportunities for interspecific gene flow and hybridization.

Based upon this information, the following step-down procedures are recommended for Willamette Valley checkermallow population introductions:

1. Select population introduction/enhancement target sites. Several primary factors should be considered when selecting target sites for *Sidalcea* population introduction and enhancement projects. First, target sites should obviously contain suitable *Sidalcea* habitat, which can range from wetlands and riparian areas to dry uplands and edges of coniferous forests. To assist in identification of suitable habitat, extant *Sidalcea* populations in the vicinity of target sites should be visited to obtain familiarity with potential species' adaptations to various local environmental parameters. In general, however, Willamette Valley *Sidalcea* species appear to have fairly broad ecological tolerances and have proven capable of becoming established in a variety of introduction sites. As such, although efforts should definitely be made to select the highest quality introduction sites possible, limitations posed by seemingly marginal habitat should not necessarily negate introduction attempts.

Given the history of *Sidalcea* habitat destruction on private lands, and the ubiquitous threat posed by invasive species, inventories for suitable population introduction and augmentation sites should be focused strictly to publicly owned (or otherwise secure) lands that appear safe from imminent weed and successional encroachment problems. Selection and use of sites should be coordinated with pertinent public landowners to ensure administrative protection and management of populations following introductions.

2. Collect *Sidalcea* seeds for off-site cultivation of introduction stock. Source material for off-site cultivation of Willamette Valley checkermallow species should be collected from the extant population(s) located nearest to population introduction target sites to minimize undesirable mixing of gene pools, reduce hybridization potential, and maximize conveyance of potential local adaptations (if such intraspecific variability and adaptations exist).

   Based upon previous reproductive studies, Willamette Valley checkermallows can generally be expected to produce 4-7 seeds per fruit (usually 65-70 percent of available ovules). As individual plants can produce 30-100 or more fruits per inflorescence, and 10-30 or more inflorescences per plant, total reproductive output can potentially exceed 10,000 or more seeds per plant. The primary limitation to seed production in these species, which can dramatically reduce seed yields to very low levels, is pre-dispersal seed predation by weevils (see "Seed production," above). Although *Sidalcea cusickii* and *S. campestris* suffer fewer predation losses, seed mortality often exceeds 80 percent in *S. nelsoniana*, and can even reach 100 percent in some populations (whereas some populations suffer little or no losses at all).

   Given high levels of seed production reported for these species, and high levels of seed germinability, a single collecting year should be adequate to supply enough viable seeds for most cultivation projects, unless source populations are extremely small or seed losses to pre-dispersal predation are high. In the latter case,

insecticide applications may be considered as a tool for temporarily reducing seed losses to predation, as described in Gisler and Meinke (2001a).

3. Cultivation. Willamette Valley checkermallow species lend themselves to successful and rapid cultivation in greenhouses, outdoor garden settings, and even in agricultural fields, using both seeds and rhizome cuttings. Seed germination typically exceeds 75 percent in all species following seed coat scarification. Care must be taken to collect fully ripe seeds with brown seed coats, otherwise germination of immature seeds (with greenish seed coats) will be low. Once *Sidalcea* seedlings are obtained, they can reach reproductive maturity and large size within three months in the greenhouse, or more time may be needed (up to two years) if seedlings are started and maintained outdoors. Rhizome cuttings tend to grow even more quickly than seedlings, and can reach reproductive maturity within two months in the greenhouse. Once large plants are obtained, they can be easily divided into numerous smaller divisions and used to further enhance the quantity of stock under propagation. However, to maintain genetic diversity and productive sex ratios in introduced populations, such clonal propagation techniques should be undertaken with some restraint and/or augmented with individuals derived from sexual reproduction (i.e., seeds).

   Due to the high potential for hybridization among Willamette Valley checkermallows (despite the occurrence of certain ecological and genetic crossing barriers), *Sidalcea* species should not be cultivated closely together, to minimize opportunities for interspecific pollen flow.

4. Introduce cultivated plugs into the target site(s). Willamette Valley checkermallow introductions should be performed after the arrival of fall rains, so that soils are moist at the time of planting and plugs have ample opportunity for root system development prior to summer drying. However, later season planting (even during unusually dry years) has resulted in very high transplant survival rates, a testimony to the resiliency of these species. Multiple introduction efforts

have been undertaken for *Sidalcea nelsoniana*, with uniformly high rates of survival (ranging from 85-100 percent) over several years of monitoring following introduction. Anecdotal reports and personal observation of *S. campestris* and *S. cusickii* in garden and restoration settings suggest that the other Willamette Valley checkermallow species likewise perform well when introduced as plugs.

Due to the aforementioned concerns about hybridization in Willamette Valley checkermallows, care should be taken to introduce Sidalceas into sites not already occupied by other congeners, and target sites should only be located within the current ranges of the respective species. This concern is especially pronounced for the interfertile species pair of *S. nelsoniana* and *S. cusickii*. To maintain reproductive isolation of these species, *S. nelsoniana* should not be introduced south of Finley National Wildlife Refuge in southern Benton County, and *S. cusickii* should not be introduced north of the same area.

Given potential monitoring complications related to clonal growth of introduced plugs, the design of introduction projects should keep monitoring objectives in mind and endeavor to space plugs such that they can be relocated and discerned over time, in order to evaluate project success.

5. Monitor introduced populations. Introduced *Sidalcea* plugs should be monitored annually to evaluate project success. Monitoring efforts could either entail simple methods of population censusing of reproductive and non-reproductive plants, or more intensive methods could be employed to track the fates and reproductive performance of individuals over time. In larger populations, it may be necessary to replace direct censuses with statistical sampling procedures, as described by the City of McMinnville (1997) and Guerrant (1998).

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