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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with contributions by
Oregon Department of Agriculture staff
for
U.S. Fish and Wildlife Service
Grant OR-EP-2, segment 13

Acknowledgements:

We would like to thank the many people who contributed to the completion of this report. Thanks to Andy Robinson and Kathy Pendergrass (USFWS) for providing funding and encouragement (Grant no. OR-EP-2, segment 13). Kelly Amsberry, Rebecca Currin, and R.J. Meinke contributed to text completion and review, and Melissa Carr provided invaluable assistance in compiling data. Thanks also to the staff, interns and students who provided plant and habitat photos, and to Erin Amsberry Abood for assistance in final report preparation.

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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.

Literature citation: Gisler, S.D. 2004. Developing biogeographically based population introduction protocols for at-risk Willamette Valley plant species. Report to US Fish and Wildlife Service, Portland, Oregon. Native Plant Conservation Program, Oregon Department of Agriculture, Salem, Oregon.

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 gallforming 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. <u>Select population introduction/augmentation target sites</u>. 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.

Literature cited

Caplow, Florence. Botanist, Washington Natural Heritage Program, Washington Dept. of Natural Resources, PO Box 47014, Olympia, Washington, (360) 902-1793, Florence.caplow@wadnr.gov, personal communication.

Erhart, T. 2000. Population dynamics and conservation biology of *Lupinus sulphureus* ssp. *kincaidii* (Fabaceae). Senior thesis, Oregon State University, Corvallis.

Hitchcock, C.L. 1961. *Lupinus*. Pp 297-333 in C.L. Hitchcock, A. Cronquist, M. Owenby, and J.W. Thompson, eds., Vascular Plants of the Pacific Northwest, vol. 3. University of Washington Press, Seattle.

Kaye, Thomas. Director, Institute for Applied Ecology, 227 SW 6th St., Corvallis, Oregon, 97333, (541) 753-3099, kayet@peak.org, personal communication.

Kaye, T.N. 1999. Obligate insect pollination of a rare plant: *Lupinus sulphureus* ssp. *kincaidii*. Northwest Science. 73:50-52.

Kaye, T.N., J. Cramer, and A. Brandt. 2003. Seeding and transplanting rare Willamette Valley prairie plants for population restoration. Institute for Applied Ecology, Corvallis Oregon.

Kaye, T.N., and J. Cramer. 2003. Population monitoring for *Lupinus sulphureus* ssp. *kincaidii* Fir Butte and Oxbow West sites, West Eugene. Institute for Applied Ecology, Corvallis Oregon.

Kaye, T.N., and K. Kuykendall. 1993. Status report for *Lupinus sulphureus* ssp. *kincaidii*. Oregon Department of Agriculture, Salem, and U.S. Fish and Wildlife Service, Portland.

Kaye, T.N., and K. Kuykendall. 2001a. Effects of scarification, cold stratification, and source population on germination of *Lupinus sulphureus* ssp. *kincaidii*. Seed Science and Technology 29:663-668.

Kaye, T.N., and K. Kuykendall. 2001b. Germination and propagation techniques for restoring rare Pacific Northwest prairie plants. Pp. 213-224 in S.H. Reichard, P.W. Dunwiddie, J.G. Gamon, A.R. Kruckeberg, and D.L. Salstrom, eds., Conservation of Washington's native plants and ecosystems. Washington Native Plant Society, Seattle, Washington.

Kaye, T.N., K. Kuykendall, and C. Nelson. 2001. Seeding and transplanting rare Willamette valley prairie plants for population restoration. Institute for Applied Ecology, Corvallis, Oregon.

Kuykendall, K., and T.N. Kaye. 1993. *Lupinus sulphureus* ssp. *kincaidii* survey and reproduction studies. USDI Bureau of Land Management Roseburg District and Oregon Department of Agriculture, Salem, Oregon.

Liston, A., K. St. Hilaire, and M.V. Wilson. 1995. Genetic diversity in populations of Kincaid's lupine, host plant of Fender's blue butterfly. Madroño 42:309-322.

Oregon Natural Heritage Program. 2001. Rare, threatened and endangered species of Oregon. Oregon Natural Heritage Program, Portland, Oregon.

Schultz, C.B. 1995. Status of Fender's blue butterfly (*Icaricia icarioides fenderii*) in Lane County, Oregon: a year of declines. Unpublished report to the Oregon Natural Heritage Program and U.S. Fish and Wildlife Service, Portland, Oregon.

Schultz, C.B. 2001. Restoring resources for an endangered butterfly. Journal of Applied Ecology 38:1007-1019.

Schultz, C.B., and K.M. Dlugosch. 1999. Nectar and hostplant scarcity limit populations of an endangered Oregon butterfly. Oecologia 119:231-238.

Severns, P.M. 2003a. Inbreeding and small population size reduce seed set in a threatened and fragmented plant species *Lupinus sulphureus* ssp. *kincaidii* (Fabaceae). Biological Conservation 110:221-229.

Severns, P.M. 2003b. Propagation of a long-lived and threatened prairie plant, *Lupinus sulphureus* ssp. *kincaidii* (Fabaceae). Restoration Ecology 11:334-342.

Washington Natural Heritage Program. 2003. Natural Heritage Plan – List of Priorities. Washington Dept. of Natural Resources, Olympia, Washington.

Wilson, M.V., and D.L. Clark. 2001. Controlling invasive *Arrhenatherum elatius* and promoting native prairie grasses through mowing. Applied Vegatation Science. 4:129-138.

Wilson, M.V., T. Erhart, P.C. Hammond, T.N. Kaye, K. Kuykendall, A. Liston, A.F. Robinson, Jr., C.B. Schultz, and P.M. Severns. 2003. Biology of Kincaid's lupine (*Lupinus sulphureus* ssp. *kincaidii* [Smith] Phillips), a threatened species of western Oregon native prairies, USA. Natural Areas Journal 23:72-83.

Wilson, M.V., P.C. Hammond, and C.B. Schultz. 1997. The interdependence of native plants and Fender's blue butterfly. Pp. 83-87 in T.N. Kaye, A. Liston, R.M. Love, D.L. Luoma, R.J. Meinke, and M.V. Wilson, eds., Conservation and Management of Native Plants and Fungi. Native Plant Society of Oregon, Corvallis.

Wilson, M.V., S. Leininger, and D.L. Clark. 2001. Promoting and restoring Kincaid's lupine and Willamette daisy. Report submitted to U.S. Fish and Wildlife Service, Portland, Oregon.