Appendix L: Plant Material Collection and Plant Introduction Protocols

1.1 Introduction

To mitigate for impacts to Covered Species of plants resulting from one or more Covered Activities (including habitat restoration and enhancement activities), Benton County and Cooperators will, in addition to habitat enhancement, increase covered plant abundance through population introduction, augmentation, and relocation.

- Introduction is the establishment of a new population in suitable habitat.
- Augmentation is addition of more individuals to an existing wild population.
- Relocation involves movement of individuals from an existing wild population to a new site or different existing population.

Augmentation of existing populations by adding individuals will be given highest priority, where possible and appropriate, because it increases the viability of existing populations and targets plantings to areas where the habitat is known to be appropriate. Introductions into areas currently unoccupied by the Covered Species will be used to recreate a lost population at a suitable site. Relocations of existing populations will occur in circumstances where the covered plant species will be permanently impacted, and may be used as a method of population introduction or augmentation.

The following protocols[4] outline how plant introduction, relocation and augmentation (hereinafter "Plant Introductions") activities will occur at Prairie Conservation Areas (PCAs). The entity conducting work (collection, transportation, storage, cultivation, etc.), must comply with existing state and federal regulations, and possess any required permits.

1.2 General Protocols

Plant introductions will be accomplished by collecting seeds from covered plant species and then planting them directly or cultivating plugs from the seeds, or both, depending upon the species.

[4] Protocols based in part on USFWS Programmatic Formal Consultation on Western Oregon Prairie Restoration Activities, August 14, 2008.

To ensure plant introductions serve to ultimately benefit and not harm the species, such mitigation activities will follow these guidelines. In addition, this work will be performed under the supervision of a qualified specialist. If during the course of the Permit, other treatment options other than those listed in this HCP become available or are identified through the adaptive management process, Benton County will present these options to the USFWS and ODA, and the parties will decide whether the options should be incorporated into these protocols.

1.2.0 Target Site Selection

Inappropriate site selection is the most common cause of rare plant introduction failure. To improve the success of plant introductions, target sites shall include habitat appropriate for the Covered Species. Factors to consider include geographic distance from the site of origin, soil type, aspect, elevation, hydrology, and plant community. All target sites shall be in areas within the species' current range and habitat type. The risk of hybridization with closely related species shall be reduced by prioritizing sites with no closely related species (species in the same genus) present. Seeds and other plant materials used in introductions should originate from genetically diverse sources (largest sample sizes possible) and should be from populations as near to the target site as possible, with priority to sites within the same Recovery Zone as defined in the Recovery Plan (USFWS 2010). All sites will be surveyed for presence of listed and/or covered plant and butterfly species using the HCP site survey protocols prior to initiation of plant introduction projects.

1.2.1 Plant Material Collection

Seeds and rhizomes of existing covered plant species populations may be collected and used for habitat restoration and enhancement projects. A qualified specialist will determine the number of propagules (seeds and rhizomes) needed for plant introduction objectives based on the number of individuals needed for mitigation or other restoration objectives.

The collection limits for each covered plant species in any single year shall be as follows (From USFWS 2008i):

Species	Populations under 50 individuals	Populations between 50-500 individuals	Populations of >500 individuals	Any population to be permanently impacted by a Covered Activity
Bradshaw's lomatium	50% of seeds	15% of seeds	25% seeds	100% of seeds and plants
Kincaid's lupine	50 % of seeds	15% of seeds	25% of seeds	100% of seeds and plants
Nelson's checkermallow	50% of seeds, 2% of rhizome biomass	15% seeds, 2% of rhizome biomass	25% of seeds, 2% of rhizome biomass	100% of seeds, plants and rhizome biomass
Peacock larkspur	50% of seeds	15% of seeds	25% of seeds	100% of seeds and plants
Willamette daisy	50% of seeds, 2% of rhizome biomass	15% of seeds, 2% of rhizome biomass	25% of seeds, 2% of rhizome biomass	100% of seeds, plants and rhizome biomass

Seeds

Persons collecting seeds may gather loose seeds or seed pods, capsules, or heads. Seeds pods, capsules, or heads may be removed by hand or by using cutting devices. Mesh bags may be tied over stems with developing fruits to capture seeds, a technique especially useful for species whose seeds disperse when seed pods snap open, such as Kincaid's lupine. Collectors should avoid damage to the plants by minimizing trampling, removing as little tissue as possible from the plants during seed collection (unless the plants are already senescent), and removing seeds in a manner that does not result in plants being pulled from the ground. Loose seed from the plant or the ground may be gathered by hand or with hand-held harvesting devices, such as flails or hoppers, a method most appropriate when collecting seeds from large populations. In general and as possible, collections should be made from twenty or more individuals and avoid obtaining a large proportion of seeds from any single individual to minimize genetic drift from uneven sampling.

1.2.1.0 Rhizomes

Rhizomes from mature plants shall be exposed by careful hand digging to avoid harm to the plants or exposing plant roots. Any exposed rhizomes shall be reburied. Rhizomes shall be taken from throughout the population to maximize genetic diversity.

1.2.1.1 Relocated Plants

Where the entire plant will be relocated, care shall be taken to avoid damage to any parts of the plants, including the roots.

1.2.2 Transport

Seeds shall be cleaned by hand, sieve, or blower as appropriate to the species prior to transfer to storage containers. Rhizomes shall be stored in cool moist conditions until transferred to potting medium or to the new site. Transport will be completed as quickly as possible. During transport, propagules shall be protected from temperature and moisture extremes.

Containers will be labeled with name of plant, place of collection, and date of collection. Propagules from individual plants may be placed in separate containers, if appropriate.

1.2.3 Storage

Propagules will be cleaned and properly stored prior to cultivation or outplanting. Diseased propagules will be removed and discarded. Seeds shall be thoroughly dried before long-term storage.

Seeds shall be stored in airtight and moisture proof containers to maintain their viability. A drying agent, such as silica gel, dry wood ash, diatomaceous earth, dry charcoal, lime, or paper may be used to help absorb moisture in the container. Seed

material may only be stored for up to two years before cultivating or outplanting, unless placed in a cold-storage facility.

Rhizomes shall be stored under cool, moist conditions with a suitable medium to keep them alive and viable until cultivation.

Plants to be relocated shall be stored under cool, moist conditions with sufficient soil and water to keep them alive and viable until transplantation.

1.2.4 Cultivation

Propagules will be grown in a greenhouse or nursery facility where genetic contamination of any produced seeds through cross pollination will be prevented, unless intentional to increase genetic diversity. Mixing of source populations through captive breeding may be conducted when the source population(s) are small or genetic evidence suggests inbreeding depression, genetic drift or other issues may cause progeny to have low fitness. Suitable growing medium, soils, fertilizer, or other chemical additives will be used, as necessary, to prevent algal, fungal, or insect infestations.

Seed and rhizome material and their F1 progeny may be cultivated for plant enhancement activities. Under greenhouse conditions, propagules and their progeny from F1 and F2 generations may be used for introduction and augmentation into prairie habitat. Only F1 generation will be used for subsequent propagation. F2 generation propagules and plant plugs may be outplanted, but further greenhouse or agricultural generation is discouraged unless necessary to produce sufficient propagules for successful establishment of individuals.

1.2.5 Outplanting

Field personnel shall take measures to avoid trampling any Covered Species. Dead and living vegetation, except for listed or Covered Species present, may be cleared away from the immediate planting site to expose the soil. Existing rhizomes of Covered Species will not be disturbed. Any site preparation activities will minimize negative environmental impacts and follow the habitat management guidelines in Appendix J: Prairie Habitat Vegetation Management Guidelines.

Seeds may be sown by either hand-broadcasting or no-till drill. Drilling may be used if soil is dry enough to support vehicle weight without substantial soil compaction and no covered or other listed species are present. Harrowing may be used if no other method is feasible and harrow equipment is operated at least 2 m (6 ft) from existing listed or covered plant species.

Rhizomes or plugs, and if possible, relocated plants, will be planted when soils are saturated by rain – generally November through April (see below for specific timeframes

for the various covered plant species) or when irrigation can be ensured and plants will not be exposed to intense heat. Also, the growing cycles of introduced covered plants should match those growing in the field. Soil will be excavated to the depth and width of the plug or rhizome. Plugs will be inserted directly into the soil or amended soils containing mulch or fertilizer so the rim of the plug is level with the surrounding soil. To reduce desiccation, a small amount of native soil may be added over the plug.

Equipment used during plantings should be cleaned prior to use and disturbance at the target site shall be minimized to avoid spreading non-native plant species.

1.2.6 Timing of Planting or Seeding

Plant introduction projects shall be planned so collection and planting occur at the appropriate time of year. For example, rhizome collection should be targeted for the period when plants are dormant, or when donor-plants will not be killed by the collection procedure. Outplanting of seeds and/or cultivated plugs shall occur within the correct time frame (described for each species below). Relocations shall take place either soon after plants begin growing for the year or after the peak growing season, preferably during cool and moist conditions.

1.2.7 Monitoring

Plant introduction projects will be monitored to determine plant establishment and difference in planting methods (to inform adaptive management). Propagules will be planted in a manner that facilitates subsequent monitoring. To assist with post-planting monitoring, mapped grids, metal tags, or flags will be used to indicate planted areas.

1.3 Species Specific Protocols for Cultivation and Introduction Using Seeds or Cultivated Plant Materials

Research into factors that affect introduction of these species was conducted by Kaye and Brandt (2004) for Bradshaw's Lomatium, Willamette daisy, and Kincaid's lupine. General review of propagation and reintroduction protocols for Covered Plant Species is available in Gisler (2004). Recommendations provided here are largely derived from these sources.

1.3.0 Bradshaw's lomatium

1.3.0.0 Target sites

Plant introduction projects at PCAs will occur in wet prairies. Optimal microhabitats include small depressions or seasonal channels with open, exposed soils (USFWS 1993a) and broad, flat areas of soils with wetland hydrology.

1.3.0.1 Collection

Seeds may be collected for off-site cultivation. Bradshaw's lomatium seeds may be collected by hand off the exposed terminal ends of the flower structure (umbels), and usually are mature in June.

1.3.0.2 Cultivation

Direct seeding and transplanting plugs have both resulted in successful introductions, and both methods may be used in introduction efforts. To break seed dormancy for cultivation in a greenhouse, Bradshaw's lomatium seeds need cold stratification- moist conditions at ~5° C (40°F) for at least eight weeks, followed by warm conditions such as alternating 10°/20°C (50°F/68°F). Once seeds have germinated, they may be potted with a standard soil mix, watered daily, and fertilized bi-weekly.

1.3.0.3 Outplanting

Direct seeding into field sites may be accomplished in the late fall, when seeds can be sown on the ground, either directly on the soil surface, or into areas prepared by raking or light tilling, or other activity that creates bare soil. Seed burial is not necessary for this species. Any soil preparation will avoid impacts to existing Covered Species.

Field planting of cultivated plugs may be conducted in spring or fall when the soil is moist. Fertilizer is not recommended for this species except during fall plantings in areas with little competing vegetation.

1.3.1 Kincaid's lupine

1.3.1.0 Target sites

Plantings will prioritize sites with grassland vegetation with a diversity of forb species, and near Fender's blue butterfly populations and associated butterfly nectar plants. Soils for the lupine are typically well drained but the species does not appear to prefer any single or small group of soil series. Instead, Kincaid's lupine tends to occur on a variety of upland soils and grows poorly in wetland soils. Sites with minimal encroachment of trees and shrubs may be preferred.

1.3.1.1 Collection

Seeds will be collected for off-site cultivation or direct seeding at target sites. Seed is best collected by tying mesh bags over developing fruit clusters and harvesting the bags after the seed pods have snapped open. Bagging is best done in early June and seeds are generally mature in July. Precise dates vary from year to year and site to site.

1.3.1.2 Cultivation

Kincaid's lupine can be successfully cultivated from seed. Seed dormancy may be broken by scarifiying seed (abrading the seed coat) followed by cold stratification at

~5ºC (40ºF) for 4 to 8 weeks. After these procedures, seed will germinate under warm conditions, such as alternating temperatures of 10°/20°C (50°/68° F), either on germination paper or in pots with a suitable soil mix, watered when the soil surface has dried (~twice weekly), and fertilized monthly with 20-20-20 liquid fertilizer. Survival rates of Kincaid's lupine seedlings grown from several seed sources vary from 58% - 100%. Plant health and subsequent growth after planting may be improved by adding nodulating bacteria (e.g., *Bradyrhizobium lupinii*) to the germinating seed or during potting.

1.3.1.3 Outplanting

Direct seeding may use either scarified or non-scarified seeds in the fall and winter, although seedings in winter may be most successful if seeds are scarified. Non-scarified seeds should be direct-seeded at project sites from October to January; while scarified seeds may be planted October through March. Seeds should be sown without fertilizer onto soil, raked ground, or lightly tilled soils, either on the soil surface or buried to a depth of 0.25-1.0 cm [1/8 to ½ in]). Invasive species will be cleared to the extent practicable prior to seeding.

Field planting of cultivated plugs may occur in late fall, late winter, or early spring. Plugs can be planted by hand into pre-excavated soil pits suitable to accommodate the plug along with soil amendments, if necessary (including mix of planting or native soils). Nitrogen fertilizer should not be used, but phosphorus and micronutrient fertilizers may provide the species with advantages over non-leguminous competing vegetation.

1.3.2 Nelson's checkermallow

1.3.2.0 Target site

Planting sites should contain at least one of the following: remnant native wet prairies, wetlands, ash swales, riparian areas, or small clearings with hydric soils and edges with fairly open canopy. None of the areas should have persistent flooding into later spring, although saturated soils during the raining season (inundation for several weeks or longer) or flooded soils mid-November through mid-April is acceptable (Gisler 2004, Bartels & Wilson 2003).

1.3.2.1 Collection

Seeds and rhizome cuttings will be collected for off-site cultivation of plugs needed for seed increase, plant increase, and introductions. Seed capsules or loose seeds may be collected. Seeds generally mature in July-August. A maximum of two 8 cm (3 in) long rhizomes segments per plant may be collected, or up to 2% of a single plant.

1.3.2.2 Cultivation

Nelson's checkermallow can be cultivated using both seeds and rhizome cuttings. Some seed may need to be cold stratified for 8-12 weeks at ~5° C (~40° F) to break

dormancy, followed by exposure to warm conditions such as room temperature. Seeds can be germinated in flats; transferred to pots containing appropriate soil such as bark, compost, peat, vermiculite, and Phillips Pre-mix (Gisler 2004); then transferred to larger outdoor beds before introduction to a target site.

Large plants can be divided to generate more individuals for planting, although plantings should ensure that genetic diversity is maximized by, for example, including individuals derived from sexual reproduction. Large, reproductively mature individuals are possible within two-three months of planting using divisions, and within three-five months using seeds, when they are supplied with ample light, warm temperatures, irrigation, and fertilization.

Rhizomes can be cultivated under greenhouse conditions or in field beds. No special soil mixtures, symbionts, or special growing conditions are necessary to achieve growth so long as pest infestations are prevented (Gisler 2004).

To minimize the risk of hybridization, different *Sidalcea* species should not be cultivated closely together.

1.3.2.3 Outplanting

Planting greenhouse-grown container stock has proven most effective to date, but direct seeding may be a useful technique at some sites and if ample seeds are available, such as through a seed increase program. Plugs can be transplanted by hand into pre-excavated soil pits suitable to accommodate the plug and soil amendments (including mix of planting or native soils) after the arrival of the fall rains and before June.

Plantings will not occur at sites south of the natural southern range limit of the species, which is approximately McFarland Road in southern Benton County.

1.3.3 Peacock larkspur

1.3.3.0 Target Site

Sites for peacock larkspur introduction shall contain appropriate habitat. Peacock larkspur habitat includes native wet and upland prairie communities (often the slightly higher, drier, more well-drained microsites within and adjacent to wetlands), shady Oregon ash and Oregon white oak woodland edges (forest clearings), native prairie grasslands, and in floodplains on well-drained mounds (Finley and Ingersoll 1994). Previous research has found peacock larkspur to occur at elevations ranging from 46 to 122 m (150 to 400 ft) (Gisler 2004), in shallow, slightly acidic soils (5.38 pH), with low organic matter (11.28%), and mostly sand and silt soil particles (Goodrich 1983). Additional evaluations could broaden these observations. Peacock larkspur tolerates seasonal inundation.

1.3.3.1 Collection

Seeds for cultivation and restoration projects will be collected initially from wild populations. Fruits on the lower portions of fruiting stems tend to produce the greatest numbers of seed per fruit. Seeds generally mature in June.

1.3.3.2 Cultivation

Peacock larkspur can be successfully cultivated in a greenhouse or in outdoor beds, and does not require specialized soil amendments or soil symbionts. The larkspur can be cultivated using seeds. Seeds may need to be cold stratified in a refrigerator at ~5°C [~40°F] for 12-16 weeks to break dormancy, after which they may be placed in pots with standard sterilized potting mixture, and watered and fertilized as needed.

1.3.3.3 Outplanting

Peacock larkspur seeds, plugs or tubers may be planted upon arrival of fall rains. To date there have been no published studies evaluating methods or success of direct seeding, transplanting or introducing this species, although tubers have been successfully transplanted. Once planted, seedlings require up to five years or more to become reproductively mature, although some individuals appear to grow and flower rapidly.

Hybridization with other *Delphinium* species is a concern. To minimize the risk of hybridization, different *Delphinium* species will not be cultivated closely together, and outplanting sites should be checked for the presence of other *Delphinium* species and plantings should occur no closer than 100 m from any resident populations of different species.

1.3.4 Willamette daisy

1.3.4.0 Target Site

Willamette daisy is found in both wetland and upland habitats in the Willamette Valley, including bottomland grasslands consisting of flat, open, seasonally flooded prairie especially those with some bare soil and little litter layer between the large bunches of grasses (Kagan & Yamamoto 1987), and upland prairie sites having moderate to well-drained soils and a mix of native bunchgrasses such as *Festuca roemeri* (Roemer's fescue), *Bromus carinatus* (California brome), and *Elymus glaucus* (blue wild rye) (Clark 2000).

1.3.4.1 Collection

Both seeds and rhizome cuttings may be collected for off-site cultivation of plugs for use in plant introduction projects. Seed heads or loose seed may be gathered. Seeds generally mature in mid to late July. Rhizomes of approximately 2.5 cm (1 in) length may be harvested from individual plants, but this should be performed only on larger individuals and no more than two rhizome segments should collected per plant.

1.3.4.2 Cultivation

To maximize germination rates, seeds need to be cold stratified at ~5°C (~41ºF) for 10-16 weeks followed by alternating 10°/20°C (50°/68° F) temperatures. Seeds may also be scarified at the pappus end or removed from the achene to break dormancy, but this procedure is very labor intensive. Germination rates of 40-78% can be expected within 2-11 days of placement in warm conditions, although germination rates of seeds collected from smaller populations may be low, possibly due to inbreeding depression. After germination, seeds may be planted in pots containing a standard commercial potting mix.

Cultivation of rhizomes may occur under greenhouse conditions or in outside beds. Rhizomes may be dipped in a rooting hormone to stimulate root development and planted 1-2 cm (0.5 - 1.0 in) deep in soil-filled pots. Plants should be rooted within 8-11 weeks, and may be transferred to larger pots or beds.

1.3.4.3 Outplanting

Outplanting of container plants and direct seeding maybe used as techniques to establish plants at field sites. Previous experiments have shown that direct seeding results in relatively low rates (~1%) of plant establishment, so large numbers of seeds may be needed to support this technique, which may require a seed increase program to produce the necessary quantity of seeds. Seeding should be conducted in the fall to provide seeds with a sufficient cold period to stimulate germination.

Plugs may be planted when soils are moist, generally between October and May. Fertilizer should not be used during outplanting. Plugs should be planted primarily in high quality, native prairies with minimal non-native plant cover. To prevent inbreeding depression, individuals should be planted in large patches to maximize opportunities for outcrossing. A 33% survival rate may be expected using rhizome cuttings, although this will vary from year to year, site to site, and among source populations of Willamette daisy.