

## AN ABSTRACT OF THE THESIS OF

Beth A. Lawrence for the degree of Master of Science in Botany and Plant Pathology  
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Title: Studies to Facilitate Reintroduction of Golden Paintbrush (*Castilleja levisecta*) to  
the Willamette Valley, Oregon.

Abstract approved:

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Thomas N. Kaye

Golden paintbrush (*Castilleja levisecta* Greenman) historically inhabited the prairies of the Willamette Valley, Oregon. However, this Pacific Northwest endemic is currently restricted to eleven sites in the Puget Trough of Washington and British Columbia. Recovery criteria call for the establishment of new populations throughout the species historic range, including the Willamette Valley. In order to facilitate reintroduction to this region, we examined: (1) habitat characteristics of potential *C. levisecta* recovery sites in the Willamette Valley and compared them to remaining populations in the Puget Trough, (2) a suite of ecological and genetic factors likely to contribute to reintroduction success using common garden experiments, and (3) how the performance of this rare hemiparasite was affected by the availability of different host combinations in the greenhouse and in the field.

Potential *C. levisecta* reintroduction sites in the Willamette Valley had distinct vegetation communities and soil characteristics compared with remaining populations in the Puget Trough. This disparity was likely related to regional differences in geology, climate, ocean proximity, and land-use history. Many of the species indicative of remaining populations in the Puget Trough were native perennials, while those of potential reintroduction sites in the Willamette Valley were introduced annuals. Soil characteristics of *C. levisecta* sites were also distinct among the two ecoregions. Puget

Trough sites were located on sandy soils with generally high levels of magnesium and sulfur, while Willamette Valley sites were found on silty-clay soils with high concentrations of potassium and phosphorous. Differences in soil texture, and magnesium and potassium concentrations were associated with plant community divergence among the two regions.

Decisions regarding seed source and recovery site selection are especially difficult in portions of a species range that are uninhabited, as no reference populations exist with which to compare ecological information. Using common garden experiments, we tested hypotheses about how *C. levisecta* transplants would perform in relation to the ecological similarity between seed source and introduction site, the effective population size and genetic diversity of seed sources, and the habitat quality of the recovery site. We observed significant variation in performance measures among source populations and common garden sites. Plant community characteristics, including the abundance of non-native species and the similarity in community structure between source populations and common garden sites, helped explain the variation in these performance measures. Exotic species cover at common garden sites was associated with a reduction in performance of first year *C. levisecta* transplants. Survival of second year transplants increased with increasing similarity in plant functional groups between source and common garden sites, supporting the idea of a “home-habitat advantage.” These results indicate that high quality prairies, dominated by native perennial species with low non-native abundance, should be targeted for recovery sites. We recommend using plant material from Whidbey Island, WA, whose three populations represented in our study consistently performed well.

Rare, parasitic plants pose an interesting challenge to restoration practitioners. We examined how the performance of *C. levisecta* was affected by the availability of different host combinations in the greenhouse and in the field. *Castilleja levisecta* individuals were grown with two grass individuals (*Festuca roemerii*; Poaceae), two non-grass hosts (*Eriophyllum lanatum*; Asteraceae), one individual of each of these species, or without a host. Our greenhouse results provide little support for the complimentary diet hypothesis, which states that parasites grown with multiple host species perform better than individuals grown alone or with a single host. *Castilleja levisecta* individuals grown

with two different species performed better than those co-planted only with *F. roemerii*, but did not differ from *E. lanatum* or no-host treatments. In the field, vole activity had indirect effects on *C. levisecta* survival mediated through host species. Vole tunneling and *C. levisecta* mortality were strongly associated with host treatments including *E. lanatum*. Field survival was significantly higher among no-host *C. levisecta* individuals than those grown with *E. lanatum* or mixed host treatments. We do not suggest co-planting *C. levisecta* with *E. lanatum* in the field. Although no-host *C. levisecta* individuals had the greatest first year field survival, we suspect that perennial host plants will be beneficial to future survival. Therefore, we recommend planting *C. levisecta* in the Willamette Valley, OR with *F. roemerii*.

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Studies to Facilitate Reintroduction of Golden Paintbrush (*Castilleja levisecta*) to the  
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by  
Beth A. Lawrence

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Beth A. Lawrence, Author

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# Studies to Facilitate Reintroduction of Golden Paintbrush (*Castilleja levisecta*) to the Willamette Valley, Oregon

## Chapter 1 : Introduction

Species reintroduction is a growing component of conservation efforts worldwide. As defined by The World Conservation Union, reintroduction is “an attempt to establish a species in an area which was once part of its historical range, but from which it has been extirpated or become extinct” (IUCN 1998). Reintroduction is an exciting component of species recovery, with both theoretical and practical applications. Reintroduction efforts can serve as an “acid test for population biology” (Sarrazin & Barbault 1996), with ecological theory and population biology providing the framework within which reintroduction work is conducted, and an applied context for which to test ecological hypotheses. The science of species reintroduction is still in its infancy, and is increasingly prescribed as a method to promote species survival. Therefore, *ex situ* conservation efforts need to be practiced within an experimental framework so that we can learn from our “failures,” evaluate management options, and produce a documented outcome that can be applied to other organisms of concern in the conservation community.

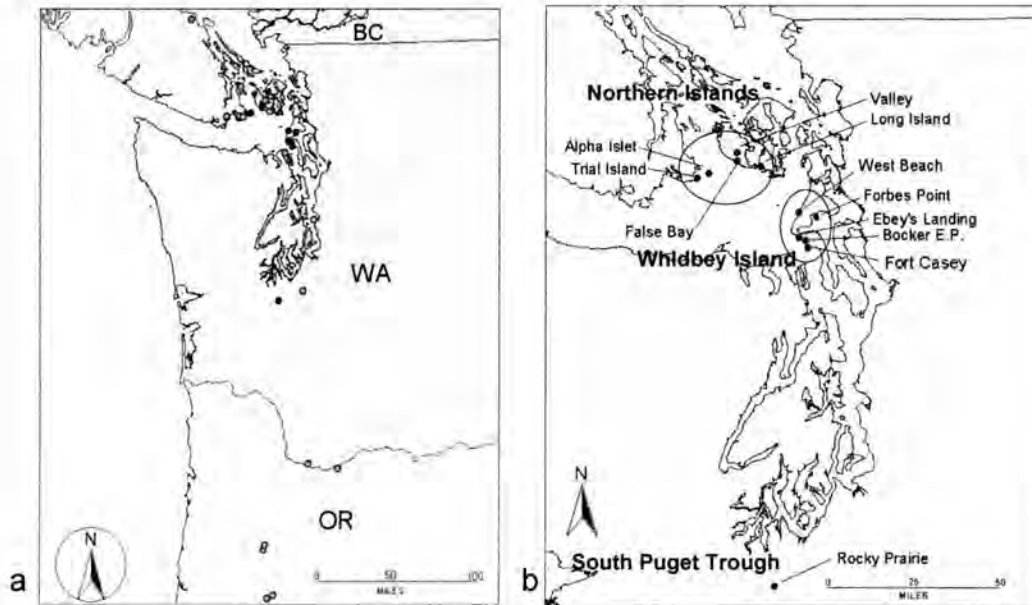
While habitat preservation and protection of threatened populations are paramount, the need for restorative action often requires off site conservation efforts to complement *in situ* efforts. Reintroduction or augmentation of populations was recommended in recovery plans for 87% of federally listed plant species to achieve recovery criteria in 1997 (Kennedy 2004). However, despite numerous efforts, few, if any, reintroduction attempts can yet be judged as true successes (Bowles & McBride 1996; Bowles et al. 2001; Guerrant 1996b; Pavlik & Espeland 1998; Pavlik et al. 1993). Although success can be defined in many ways, the primary goal of species reintroduction is to establish resilient populations, capable of self-maintenance in the face of evolutionary change. Limited understanding of the biological mechanisms associated with ecological, genetic, and horticultural dimensions during species introduction often restricts our ability to realize this goal.



Golden paintbrush (*Castilleja levisecta* Greenman) is a federally endangered hemiparasite that has been extirpated from the southern portion of its historic distribution. Reintroduction of viable populations to this region will require thoughtful consideration of appropriate seed sources and recovery sites, as well as the host needs of the species. This thesis work has been conducted in an effort to contribute to the biological understanding of species reintroduction, as well as to facilitate future reintroduction endeavors for *C. levisecta*, a Pacific Northwest prairie endemic.

## STUDY SYSTEM

Golden paintbrush is an herbaceous perennial, native to the grasslands of the Pacific Northwest. Although populations once extended from the coastal bluffs and islands of British Columbia to the Willamette Valley of Oregon, this species is completely extirpated from the southern portion of its historic range, including the state of Oregon and from southwestern Washington. At least 30 populations once inhabited the Pacific Northwest, though the species is now restricted to 11 sites in the Puget Trough of Washington and British Columbia (Figure 1-1). *Castilleja levisecta* is currently listed as a federally threatened species under the Endangered Species Act (U.S.F.W.S. 1997). Recovery criteria for the species include the existence of twenty populations, each composed of a five-year running average of 1,000 flowering individuals (U.S.F.W.S. 2000). *Castilleja levisecta* has limited capacity for natural dispersal and colonization of new sites, necessitating the creation of new populations to meet recovery goals. A strategic reintroduction plan has been prepared to support the long-term viability of the species and calls for the establishment of new populations within the species' historic range, including the Willamette Valley of Oregon (Caplow 2004).



**Figure 1-1.** (a) Distribution of extant (●) and extirpated (●) *C. levisecta* populations throughout its historic range in the Pacific Northwest. (b) A closer look at the eleven remaining populations which are concentrated in the San Juan Islands of the Puget Trough. Maps provided by Washington Department of Natural Resources.

### Pacific Northwest prairies

The prairies of the Pacific Northwest are unique plant assemblages with inextricable ties to the human inhabitants of this region. Native grasslands and oak woodlands of the Puget Trough and Willamette Valley ecoregions are found in dry environments that were historically influenced by frequent fire, the majority of which were ignited by Native Americans (Norton 1979; Smith 1949). The prairies were maintained with frequent, late summer or fall burns that promoted the growth and collection of important sources of food (e.g., *Camassia quamash*, *Pteridium aquilinum*), facilitated hunting, and provided improved forage (Boyd 1986; Norton 1979). Frequent fire favored tolerant grasses and forbs, while limiting the abundance of trees and shrubs.

Pacific Northwest prairies have largely been decimated; it's estimated that 3% of native grasslands in the Puget Trough remain (Chappell et al. 2000; Crawford & Hall 1997), while less than 1% of the prairie habitat of the Willamette Valley exists today (Noss et al. 1995). Native grasslands were converted during agricultural and urban development beginning with the arrival of pioneers in the mid 1800s (Smith

1949; Swan 1857). Increased pioneer settlement of the Pacific Northwest resulted in complete cessation of native-set fires by the mid 1840s (Boyd 1986). Remaining prairies are a mosaic of their former distribution and have been largely degraded by fire suppression, encroaching woody vegetation, and invasive species (Chappell & Crawford 1997; Chappell et al. 2000; MacDougall et al. 2004).

The prairies of the Pacific Northwest are considered one of most critically endangered ecosystems in the country (Noss et al. 1995). Many species associated with this habitat are of conservation concern due to declining populations, local extirpation, or close associations with the declining habitat (Chappell et al. 2000). In the southern Puget Sound region, 31 species of concern are associated with prairie habitats including 13 butterflies, 12 birds, three mammals, and three wildflower species (Rolph 1997). Federally listed species include Mardon skipper (*Polites mardon*), Mazama pocket gopher (*Thomomys mazama*), white-topped aster (*Aster curtus*), and golden paintbrush (*Castilleja levisecta*).

## OBJECTIVES & RATIONALE

To facilitate *C. levisecta* reintroduction in the Willamette Valley, we conducted studies investigating the (1) habitat variation throughout the historic range of *C. levisecta*, (2) appropriate seed sources and recovery sites for reintroduction of *C. levisecta* to the Willamette Valley, OR, and (3) direct and indirect effects of host use in *C. levisecta*. We also provide a description of the propagation methods we used.

### Habitat variation throughout the historic range of *C. levisecta*

Decisions regarding *C. levisecta* seed source and recovery site selection will be especially difficult in the Willamette Valley, OR, as no reference populations exist with which to compare habitat information. The species is currently restricted to the Puget Trough and has not been observed in Oregon since 1938 (Gamon 1995). Although herbarium specimens vaguely describe the locations and habitat types of historic Oregon populations, we generally believe that *C. levisecta* inhabited the upland prairies of the Willamette Valley. These prairies are grass dominated systems often associated with *Quercus garryana*-savanna occurring on well-drained, silty-clay soils on the valley

foothills (Franklin & Dyrness 1988). Potential *C. levisecta* recovery sites in the Willamette Valley appear to be ecologically distinct from remaining populations in the Puget Trough. Extant populations are found primarily on sandy, well-drained soils of glacial origin, on coastal bluffs or prairies (Chappell & Caplow 2004).

In Chapter 2 we described vegetation and soil characteristics of representative *C. levisecta* recovery sites in the Willamette Valley and compare them with those of remaining *C. levisecta* populations in the Puget Trough. We attempted to clarify *C. levisecta* habitat similarities and differences throughout the historic range of the species and provided management options to account for range-wide habitat variation during species reintroduction.

#### Selecting seed sources and recovery sites for reintroduction to the Willamette Valley, OR

Selecting appropriate seed sources and recovery sites for reintroduction to the Willamette Valley will be particularly challenging because (a) there are no reference populations in this region with which to compare ecological and genetic characteristics, and (b) potential recovery sites are geographically and ecologically distant from remaining *C. levisecta* populations. The origin of source material used to establish new plant populations is a controversial issue in conservation biology. Ecological similarity between source and introduced populations may be critical because populations may be adapted to specific habitat conditions and selective pressures (Guerrant 1996a; Huenneke 1991; Hufford & Mazer 2003). Selecting recovery sites that are ecologically similar to the seed source may improve the chances of reintroduction success. Genetic characteristics of the seed source may also be good predictors of plant fitness, as population genetic theory predicts individuals from large, genetically diverse populations will be more fit than those from small, genetically depauperate populations (Young et al. 1996). Further, much of the remaining habitat in the Willamette Valley is degraded and dominated by exotic plants, which may limit reintroduction success.

In Chapter 3, we examined a suite of ecological and genetic factors likely to contribute to *C. levisecta* reintroduction success and made recommendations on appropriate seed sources and recovery sites in the Willamette Valley. Using common garden experiments, we tested specific hypotheses about how *C. levisecta* transplants

would perform in relation to the (1) ecological and geographic distance between seed source and introduction site, (2) effective population size and genetic diversity of seed sources, and (3) habitat quality of the recovery site.

#### Direct and indirect effects of *C. levisecta* host use

Rare, parasitic plants pose an interesting challenge to restoration practitioners. In addition to the problems associated with small population size, rare parasites may also be limited by their host requirements. *Castilleja levisecta* is a facultative hemiparasite, capable of forming physical connections with root systems of several common Pacific Northwest prairie species (Kaye 2001; Wentworth 2001). Interactions between plant parasites and host species can have direct and indirect effects on both host and parasite performance, as well as their pollinators (Adler et al. 2001) and herbivores (Adler 2002; Adler 2003; Adler et al. 2001; Marko 1996; Marvier 1996). In the wild, hemiparasitic plants often parasitize several hosts simultaneously (Gibson & Watkinson 1989; Matthies 1996). The complimentary diet hypothesis proposes that generalist consumers perform better on a mixed diet relative to a homogenous diet, due to improved nutrient balance and/or dilution of toxic secondary plant compounds (Bernays et al. 1994).

Several studies have examined the host requirements of *C. levisecta* (Kaye 2001; Wayne 2004; Wentworth 2001), but clarification of its host requirements in a restoration context is necessary before large scale reintroduction efforts are pursued. In Chapter 4, we tested the complimentary diet hypothesis and examined how the performance of *C. levisecta* was affected by the availability of different host combinations in the greenhouse and in the field. *Castilleja levisecta* individuals were grown with two grass individuals (*Festuca roemerii*; Poaceae), two non-grass hosts (*Eriophyllum lanatum*; Asteraceae), one individual of each of these species, or without a host.

#### Propagation of *C. levisecta*

Mass propagation of *C. levisecta* was necessary to perform the experimental work presented in Chapters 3 and 4, and will be an important component of future

reintroduction efforts. Volunteers working with The Nature Conservancy in Washington have had difficulty growing this species in the past; we have been asked on several occasions for advice on appropriate germination methods, growing conditions, etc. The North American Rock Garden Society asked us write an article for the Rock Garden Quarterly about *Castilleja* spp. propagation methods in January 2005. In Chapter 5, we present an adapted version of this article (Lawrence & Kaye 2005) that provides detailed propagation methods for *C. levisecta*.

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## **Chapter 2 : Habitat variation throughout the historic range of golden paintbrush (*Castilleja levisecta*), a Pacific Northwest prairie endemic: Implications for reintroduction**

### **ABSTRACT**

Decisions regarding seed source and recovery site selection are especially difficult in portions of a species range that are uninhabited, as no reference populations exist with which to compare ecological information. Although golden paintbrush (*Castilleja levisecta* Greenman) historically inhabited the prairies of the Willamette Valley, Oregon, this Pacific Northwest prairie endemic is currently restricted to eleven sites in the Puget Trough of Washington and British Columbia. Recovery criteria call for the establishment of new populations throughout the species historic range, including the Willamette Valley. We described vegetation and soil characteristics of representative *C. levisecta* recovery sites in the Willamette Valley and compared them with those of remaining *C. levisecta* populations in the Puget Trough.

Potential *C. levisecta* habitat in the Willamette Valley was ecologically distant from remaining populations. This disparity was likely related to regional differences in geology, climate, ocean proximity, and land-use history. Many of the species indicative of remaining populations in the Puget Trough were native perennials, while those of potential reintroduction sites in the Willamette Valley were introduced annuals. Soil characteristics of *C. levisecta* sites were also distinct among the two ecoregions. Puget Trough sites were located on sandy soils with generally high levels of magnesium and sulfur, while Willamette Valley sites were found on silty-clay soils with high concentrations of potassium and phosphorous. Differences in soil texture, and magnesium and potassium concentrations were associated with plant community divergence among the two regions. We suggest using a plant functional group approach when comparing vegetation assemblages among Puget Trough and Willamette Valley sites, which allows comparison of taxonomically distinct communities that share ecological characteristics.

### **INTRODUCTION**

As defined by The World Conservation Union, reintroduction is “an attempt to establish a species in an area which was once part of its historical range, but from which it has been extirpated or become extinct” (IUCN 1998). Successful plant reintroduction requires evaluation of the species’ physical and biological habitat (Fiedler & Laven 1996) because selection of appropriate seed sources and recovery sites is crucial when implementing a recovery strategy (McKay et al. 2005). Habitat similarity among seed sources and prospective planting sites is important because populations may be adapted to specific habitat conditions and selective pressures (Guerrant 1996; Guerrant & Pavlik 1998; Huenneke 1991; Hufford & Mazer 2003). Choosing an introduction site that closely matches the source site (i.e., soil, vegetation, and climate) increases the likelihood that introduced plants will be genetically well-adapted to the site, and in turn, that introduction will succeed (Bowles et al. 1993; Montalvo & Ellstrand 2000; Pavlik et al. 1993).

Decisions regarding the suitability of seed sources and recovery sites can be particularly challenging in portions of a species range that are currently uninhabited, as no reference populations exist with which to compare ecological and genetic characteristics. Further, substantial changes in ecosystem function, including habitat loss, invasion, and alterations to the disturbance regime, will likely have occurred since the species was last observed (MacDougall et al. 2004). Golden paintbrush (*Castilleja levisecta*; Orobanchaceae) is a federally endangered plant endemic to the prairies of the Pacific Northwest and currently extinct in the southern portion of its historic range, which includes the Willamette Valley, Oregon. Reintroduction of *C. levisecta* to this ecoregion is a priority for its recovery (Caplow 2004; U.S.F.W.S. 2000). However, *C. levisecta* has not been observed in the Willamette Valley since 1938 and there is limited information about the location and site characteristics of historic populations (Gamon 1995).

Although there are herbarium specimens from six possible historic *C. levisecta* populations in the Willamette Valley, OR, the vegetation and soil characteristics of these populations are not well understood. Herbarium records generally did not specify the exact location or habitat characteristics of extirpated populations. Further, much of the potential habitat in the vicinity of historic locales has been converted to

agricultural use or developed commercially. Below are brief descriptions of Willamette Valley collections as outlined by Gamon (1995). *Castilleja levisecta* was first collected in Oregon in 1905 in Bonneville, Multnomah County by an unidentified collector. Potential habitat in this area was likely destroyed with construction of the Bonneville Dam beginning in 1937. There are three collections from Marion County, OR; Peck collected *C. levisecta* in 1910 from “damp open ground, Salem,” and J.C. Nelson made two 1916 collections and labeled specimens as “wet meadow, Salem,” and “wet meadow, 3 miles south of Salem.” There are four *C. levisecta* collections from Linn County, OR. A 1922 specimen from an unknown collector simply states the location as “Brownsville.” Similarly, there is also a vague 1929 collection from “Lebanon, OR.” There are two *C. levisecta* collections from Peterson Butte in 1938; E.M. Harvey collected *C. levisecta* in a prairie along a stream at the south-west base of Peterson’s Butte, and Whitaker collected the plant at “Peterson Butte Cemetery.” We recently visited Sand Ridge Cemetery which is located on the south-west flank of Peterson’s Butte and observed gravelly soils that appeared to be well-drained and several prairie species including *Camassia quamash*, *Dodecatheon hendersonii*, *Frittilaria lanceolata*, and *Danthonia californica* found. It is possible that Sand Ridge Cemetery is the same site as Whitakers’s “Peterson Butte Cemetery.”

Based on these records and our observations, it appears that *C. levisecta* inhabited the once abundant grasslands of the Willamette Valley that were maintained by fire initiated by Native Americans (Boyd 1986). Several of the specimens describe the collection sites as damp or moist, but it is not clear whether these populations occurred in wetland prairies with poor drainage, or in upland prairies associated with well-drained soils. Collections were generally made in spring (i.e., May) when soils were likely still saturated. The collections from “Bonneville” and “3 miles south of Salem” suggest that *C. levisecta* was potentially associated with riverine gravel outwashes of the Columbia and Sanitiam Rivers respectively. We believe that *C. levisecta* historically inhabited upland prairies in the Willamette Valley, because all of the remaining populations in the Puget Trough are associated with sandy, well-drained soils of glacial origin prairies (Chappell & Caplow 2004). In general, potential recovery sites in the Willamette Valley are grass-dominated systems associated with

*Quercus garryana*-savanna, commonly found on the valley foothills (Franklin & Dyrness 1988). The unglaciated soils of Willamette Valley upland prairies are generally composed of clay and silt from weathering basalt and are considered to be well-drained.

Here, we describe the variation in habitat characteristics among potential recovery sites in the Willamette Valley, OR and several extant *C. levisecta* populations located in the Puget Trough ecoregion. We examine plant communities and soil characteristics of experimental *C. levisecta* reintroduction sites and source populations used in a common garden study that was initiated to facilitate management decisions regarding seed selection and recovery site criteria in the southern portion of the species' range (Chapter 3). Our objective is to describe patterns of habitat differentiation among *C. levisecta* source populations and potential reintroduction sites in the Willamette Valley, as well as to explore the management implications of range-wide habitat variation for recovery efforts.

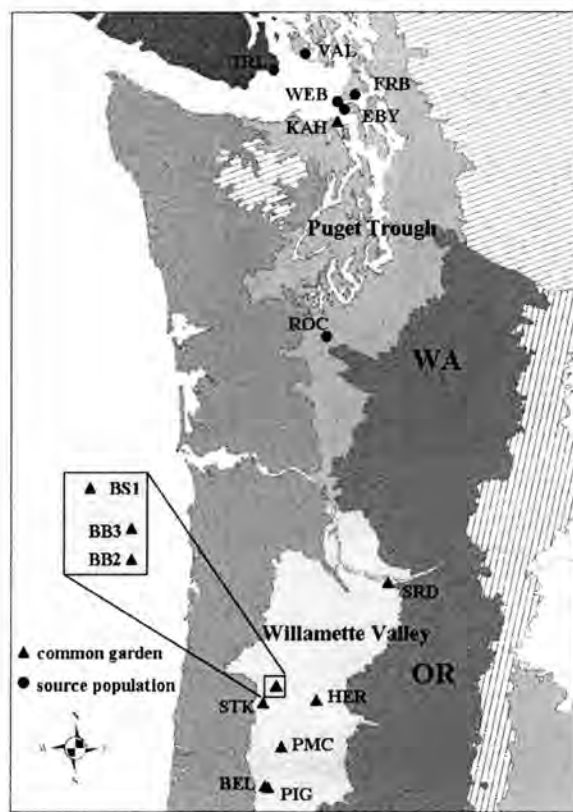
## **METHODS**

### Study sites

Six populations of *C. levisecta* in the remaining portion of the species' range were included for comparison to sites in the unoccupied, historic region in the Willamette Valley (Table 2-1). In addition, nine sites in the Willamette Valley were identified for sampling (Figure 2-1). These sites were also the locations of experimental common gardens established throughout the species historic range in 2004. One additional site (Kah Tai Prairie) near Port Townsend in the Puget Trough was also included because of its proximity to remaining populations on Whidbey Island. The ten reintroduction sites within the species' historic range encompassed a diversity of soils, vegetation, and site quality, and are representative of sites likely to be chosen for future *C. levisecta* reintroduction. We specifically targeted sites in the Willamette Valley with well-drained soils, because remaining *C. levisecta* populations in the Puget Trough are all found on sandy soils. Logistics also played an important role in site selection. Under the Endangered Species Act of 1973, listed plants are protected only on federal land. Therefore, seven of the ten potential recovery sites we characterized were located on public land.

**Table 2-1.** Code, general location, habitat type, and USGS soil series mapping unit for each *C. levisecta* extant population and reintroduction site. All Willamette Valley sites are experimental common garden sites. \* denotes a reintroduction site in the Puget Trough ecoregion.

	Code	general location	Habitat	Soil map unit
<b>Puget Trough (extant populations)</b>				
Ebey's landing	EBY	Whidbey Island, WA	coastal bluff	Rough broken land
Forbes Point	FRB	Whidbey Island, WA	coastal prairie	Coveland
Rocky Prairie	ROC	South Puget Trough, WA	mounded prairie	Spanaway-Nisqually
Trial Island	TRL	Trial Island, B.C.	coastal prairie	unavailable
West Beach	WEB	Whidbey Island, WA	coastal prairie	Bozarth
Kah Tai Prairie*	KAH	Port Townsend, WA	upland prairie	San Juan
<b>Willamette Valley (reintroduction sites)</b>				
Basket Butte 2	BB2	Baskett Slough National Wildlife Refuge, OR	upland prairie	Chehulpum
Basket Butte 3	BB3	Baskett Slough National Wildlife Refuge, OR	upland prairie	Chehulpum
Basket Slough 1	BS1	Baskett Slough National Wildlife Refuge, OR	upland prairie	Steiwer
Bell Fountain Prairie	BEL	Finley National Wildlife Refuge, OR	upland prairie	Jory
Heritage Seedling	HER	Salem, OR	restored prairie	Nekia
Pigeon Butte	PIG	Finley National Wildlife Refuge, OR	upland prairie	Dixonville
Plant Materials Center	PMC	Lewisburg, OR	agricultural field	Amity-Woodburn
Sandy River Delta	SRD	Troutdale, OR	degraded prairie	Burlington
Starck	STK	Dallas, OR	degraded prairie	Bellpine



**Figure 2-1.** *Castilleja levisecta* extant populations and reintroduction sites located in the Pacific Northwest. Source populations and one reintroduction site are situated in the Puget Trough, Washington. The nine other reintroduction sites are located in the Willamette Valley, Oregon. See Table 2-1 for site abbreviations.

#### Vegetation sampling

Plant community composition data were collected at all sites in May 2004, when phenology was optimal for observing most graminoid and forb species. Ocular estimates of percentage cover were made for each vascular plant species present within three randomly placed 5 x 5 m plots at each reintroduction site. For extant populations, we used community data collected by C. Chappell (Washington Department of Natural Resources), who placed 5 x 5 m plots in dense areas of *C. levisecta* and recorded percentage cover values of all species. Sample sizes within extant populations varied among sites ( $n = 1$  to 4), depending on the number of distinct plant communities at each site (see Chappell & Caplow 2004). We assisted with data collection at several of the source populations to calibrate estimates of species cover.

Over all, we sampled 38 plots from 14 sites to describe the variation in plant communities at current *C. levisecta* populations and potential reintroduction sites.

#### Soil sampling and characterization

We collected soil samples from each site, except the source population Trial Island, in May 2004 using an impact soil corer of known volume to 15 cm depth. Ten random samples were taken at each site and thoroughly mixed. Soil was collected from Trial Island, B.C., on April 21, 2004 by M. Fairbarns of Aruncus Consulting, who took two samples from each of four sampling sites following the methods of Chappell & Caplow (2004), and sent them to us at Oregon State University. We evaluated soils for physical (bulk density, percent sand, silt, and clay) and chemical (organic matter, pH, total organic carbon, total nitrogen, carbon to nitrogen ratio, nitrate, ammonium, potassium, phosphorus, manganese, magnesium, and sulfur) parameters. The bulk density ( $D_b$ ) of each sample was calculated as the oven-dry mass (g) of the composite sample divided by its volume ( $\text{cm}^3$ ). Trial Island soils were not evaluated for bulk density because an unknown volume of soil was collected. Two sub-samples of the bulked soil from each site were analyzed for each of the other soil parameters measured. Soil texture (% sand, silt, and clay) was calculated using the hydrometer method. The loss on ignition method was used to determine the percent organic matter content (% OM). We used a Lachat QuickChem 4200 analyzer with QuickChem 10-107-06-2-A  $\text{NH}_4$  and 10-107-04-1-A  $\text{NO}_3$  to measure ammonium ( $\text{NH}_4$ ) and nitrate ( $\text{NO}_3$ ), and Shimadzu TOC-V and Shimadzu TNM-1 to measure total organic carbon (TOC) and total nitrogen (TN). The carbon to nitrogen ratio (C:N) was calculated by dividing the average total organic carbon by the mean total nitrogen for each site. All other elemental analyses were conducted using an ICP OES-Optima 4300 DV.

#### Regional patterns in habitat characteristics

To identify differences among plant communities throughout the historic range of *C. levisecta*, we used Indicator Species Analysis to assign an indicator value to each species by combining the relative abundance and frequency of species from two



predefined groups, Puget Trough and Willamette Valley (Dufrene & Legendre 1997; McCune & Grace 2002), using PC-ORD v. 4.25 (McCune & Mefford 1999).

Indicator values range from zero (no indication) to 100 (perfect indication). We present the absolute mean cover values for the 15 species with the highest indicator value from each site from the Puget Trough and Willamette Valley ecoregions. To examine floristic similarities between the two regions, we present average cover values for species that occur at no less than half of the 14 sites.

We used Nonmetric Multidimensional Scaling (NMS) to investigate patterns of habitat differentiation among *C. levisecta* source populations and potential recovery sites (Kruskal 1964; Mather 1976). Vegetation cover values were averaged within sites, which may result in unnaturally species rich values (McCune and Grace 2002), but was necessary for site-to-site comparisons. The “slow and thorough” autopilot mode setting was used in PC-ORD v. 4.25 (McCune & Mefford 1999) to ordinate *C. levisecta* sites in plant species space. Soil variable vectors that were highly correlated ( $r^2 > 0.3$ ) with axes were overlaid on top of the ordination to help explain variation among axes.

## RESULTS

### Characterization of vegetation communities

Puget Trough indicator species were primarily perennial species, including native forbs as well as introduced weeds and grasses (Table 2-2). Native perennials that commonly occurred in low abundance ( $\leq 7\%$ ) at Puget Trough sites included *Pteridium aquilinum*, *Camassia quamash*, *Cerastium arvense*, *Berberis aquifolium*, *Rosa nutkana*, *Achillea millefolium*, *Lomatium utriculatum*, and *Frittilaria lanceolata*. *Festuca rubra*, a fine-leaved perennial grass, occurred in moderate abundance (15% - 30%) at the Whidbey Island populations (i.e., Ebey’s Landing, Forbes Point, and West Beach). However, it is unknown whether this species is native or introduced to this area (Chappell & Caplow 2004). *Poa pratensis*, an introduced grass, was present at all Puget Trough sites but was particularly abundant at Forbes Point (37.5%). Introduced perennial forb *Plantago lanceolata* was present at all sites with low to moderate abundance (0.3%- 12.5%). Other prevalent introduced species at Puget Trough sites

generally present in low abundance include perennial forbs *Hypochaeris radicata*, *Rumex acetosella*, and *Taraxicum officinale*. *Trifolium dubium* was the only annual indicator species among the Puget Trough sites.

**Table 2-2.** Puget Trough indicator species and absolute mean cover values for each site. Exotic species are indicated with “e.” The origin of *Festuca rubra* in this region is unknown. Kah Tai Prairie is a reintroduction site denoted with “\*.”

Species	Sites						
	Indicator Value	EBY	FOR	ROC	TRL	WEB	KAH*
<i>Pteridium aquilinum</i>	78.6	1.0	0.2	7.0	0.7	5.0	7.0
<i>Poa pratensis</i> <sup>e</sup>	70.1	2.0	37.5	1.5	3.1	3.0	1.3
<i>Plantago lanceolata</i> <sup>e</sup>	65.7	8.0	12.5	0.3	7.7	10.0	4.3
<i>Hypochaeris radicata</i> <sup>e</sup>	56.0	1.0	0.2	1.5	1.7	0.0	4.0
<i>Rumex acetosella</i> <sup>e</sup>	51.9	6.0	0.5	0.2	0.3	0.3	5.3
<i>Camassia quamash</i>	50.0	0.0	0.0	2.3	6.7	0.0	3.7
<i>Cerastium arvense</i>	50.0	3.0	0.0	0.2	0.3	0.0	0.0
<i>Berberis aquifolium</i>	50.0	5.0	0.7	0.0	3.7	0.0	1.0
<i>Rosa nutkana</i>	50.0	0.0	0.2	0.0	1.3	2.0	2.3
<i>Trifolium dubium</i> <sup>e</sup>	50.0	0.0	2.5	0.2	0.0	0.0	1.7
<i>Achillea millefolium</i>	44.9	1.0	1.7	0.8	0.2	0.3	0.0
<i>Lomatium utriculatum</i>	44.8	0.3	0.0	0.0	1.9	0.0	5.7
<i>Anthoxanthum odoratum</i> <sup>e</sup>	42.9	0.0	0.0	9.3	1.7	8.0	0.0
<i>Festuca rubra</i>	42.9	30.0	15.0	0.0	3.3	20.0	0.0
<i>Fritillaria lanceolata</i>	42.9	0.0	0.0	0.1	1.3	1.0	0.1
<i>Taraxacum officinale</i> <sup>e</sup>	42.9	0.0	0.7	0.8	0.0	0.3	0.1

Most species indicative of Willamette Valley reintroduction sites were exotic forbs and grasses, many of which were annuals (Table 2-3). The two native indicator species were the perennial grass *Elymus glaucus*, which occurred at most Willamette Valley sites in low abundance (0.2% - 5.7%), and perennial forb *Microseris laciniata*, which was present at three sites in low abundance (~1%). Annual non-native grasses were common at Willamette Valley sites. *Bromus mollis* and *Festuca bromoides* were prevalent, but were particularly abundant at the degraded reintroduction sites Sandy River Delta and Stark (>10%). Other exotic annual grasses common to Willamette Valley sites included *Aira caryophylla*, which was present in low abundance at most sites (<3%), and *Bromus sterilis*, which was particularly common at sites within the Basket Slough National Wildlife Refuge (0.7% - 7%). The introduced perennial grass, *Arrhenatherum elatius*, occurred at half the sites with moderate cover (8.0% - 26.3%). Common annual weeds characteristic of disturbed sites that were present at half or

more Willamette Valley sites included *Cerastium viscosum*, *Myosotis discolor*, *Sherardia arvensis*, and *Medicago lupulina* ( $\geq 3\%$ ). Frequently encountered exotic perennial forbs were *Vicia sativa*, *Cirsium vulgare*, and *Daucus carota*, which were present at most sites with low to moderate cover (0.7% - 10%).

The majority of species shared by Puget Trough and Willamette Valley sites were introduced species prevalent throughout the Pacific Northwest (Table 2-4). Several non-native perennial grasses frequently encountered in both ecoregions include *Holcus lanatus* (0.1% - 4.0%), *Dactylis glomerata* (0.1 – 11.0%), and *Poa pratensis* (0.1% - 37.5%), which was present at all but two sites. Annual exotic grasses prevalent in *C. levisecta* populations and experimental reintroduction sites were *Aira caryophyllea* and *Bromus mollis*, that generally occurred in low abundance except at a few Willamette Valley sites. *Luzula comosa* was the only native graminoid that occurred at most sites, but contributed little to total cover values (usually  $>1\%$ ). Exotic perennial forbs were the predominant functional group common among Puget Trough and Willamette Valley sites, and included the following species: *Plantago lanceolata*, *Vicia hirsuta*, *Vicia sativa*, *Cerastium viscosum*, *Hypochaeris radicata*, *Rumex acetosella*, *Daucus carota*, and *Hypericum perforatum*. Two native perennial forbs shared among the two regions were *Eriophyllum lanatum* and *Achillea millefolium*, which occurred at all but three sites with low abundance ( $<2\%$ ). In general, Puget Trough sites had greater native species richness ( $\bar{x} = 21.2 \pm 3.6$ ), and fewer exotic species ( $\bar{x} = 15.3 \pm 1.1$ ), than Willamette Valley sites ( $\bar{x} = 15.1 \pm 2.9$  and  $\bar{x} = 19.2 \pm 2.0$ , respectively).

**Table 2-3.** Willamette Valley reintroduction site indicator species and absolute mean cover values for each site. Exotic species are indicated with “e.”

Species	Indicator Value	Sites							
		BS1	BB2	BB3	BEL	HER	PIG	SRD	STK
<i>Cerastium viscosum</i> <sup>e</sup>	79.4	3.0	0.5	0.3	0.2	2.3	0.3	0.0	0.3
<i>Myosotis discolor</i> <sup>e</sup>	68.5	0.2	0.3	0.3	0.0	0.3	0.3	0.0	0.3
<i>Sherardia arvensis</i> <sup>e</sup>	61.2	0.0	3.0	2.7	3.0	0.0	0.3	0.0	0.3
<i>Arrhenatherum elatius</i> <sup>e</sup>	54.2	15.0	8.0	17.3	0.0	0.0	36.3	0.0	0.0
<i>Bromus mollis</i> <sup>e</sup>	52.8	0.0	0.7	9.0	0.0	0.0	4.8	43.3	24.3
<i>Cirsium vulgare</i> <sup>e</sup>	50.9	0.4	0.3	0.0	0.0	1.0	1.5	0.0	1.3
<i>Daucus carota</i> <sup>e</sup>	49.9	0.7	8.3	10.0	0.3	0.0	6.5	0.0	0.3
<i>Aira caryophylla</i> <sup>e</sup>	48.0	1.3	1.4	1.4	0.0	0.0	0.0	2.4	1.3
<i>Bromus sterilis</i> <sup>e</sup>	45.8	4.0	0.7	7.0	0.0	0.0	1.8	0.0	0.0
<i>Elymus glaucus</i>	45.2	0.0	5.7	3.0	3.5	0.2	3.8	0.0	0.0
<i>Medicago lupulina</i> <sup>e</sup>	41.7	2.2	0.3	0.0	0.2	0.0	0.0	0.0	3.0
<i>Vicia sativa</i> <sup>e</sup>	40.2	0.0	0.3	0.3	11.0	0.0	5.0	0.0	0.3
<i>Microseris laciniata</i>	37.5	0.0	0.0	1.0	1.0	0.0	1.1	0.0	0.0
<i>Veronica</i> spp. <sup>e</sup>	37.5	0.0	0.0	0.0	0.3	0.0	0.3	0.0	0.3
<i>Festuca bromoides</i> <sup>e</sup>	36.3	0.0	0.7	0.5	0.0	0.0	0.0	15.0	12.0

**Table 2-4.** Species and cover values (%) occurring at half or more of the 14 *C. levisecta* sites. Cover values were averaged among plots at each site. Species richness and constancy (i.e., percentage of sites where the species occurred) were calculated at the site level. Exotic species are indicated with “e.” (\* denotes an experimental common garden site in Puget Trough)

	constancy	<u>Puget Trough</u>						<u>Willamette Valley</u>							
		EBY	FOR	ROC	TRL	WEB	KAH*	BS1	BB2	BB3	BEL	HER	PIG	SRD	STK
<i>Poa pratensis</i> <sup>e</sup>	85.7	2.0	37.5	1.5	3.1	3.0	1.3	3.3	0.3	0.3	0.7	0.0	0.1	0.0	2.3
<i>Achillea millefolium</i>	71.4	1.0	1.7	0.8	0.2	0.2	0.0	0.0	0.1	0.3	1.0	0.3	1.1	0.0	0.0
<i>Plantago lanceolata</i> <sup>e</sup>	71.4	8.0	12.5	0.3	7.7	10.0	4.3	0.0	2.0	0.8	0.7	0.0	0.0	0.8	0.0
<i>Vicia hirsuta</i> <sup>e</sup>	71.4	0.3	2.5	0.0	0.8	5.0	0.0	2.0	0.3	0.8	0.3	0.0	0.3	0.0	0.3
<i>Vicia sativa</i> <sup>e</sup>	71.4	0.3	1.5	0.0	1.5	0.3	0.3	0.0	0.3	0.3	11.0	0.0	5.0	0.0	0.3
<i>Aira caryophylla</i> <sup>e</sup>	64.3	0.3	0.3	0.1	0.0	0.0	0.2	1.3	1.4	1.4	0.0	0.0	0.0	2.4	1.3
<i>Bromus mollis</i> <sup>e</sup>	64.3	0.3	0.5	0.0	0.1	0.0	0.8	0.0	0.7	9.0	0.0	0.0	4.8	43.3	24.3
<i>Cerastium viscosum</i> <sup>e</sup>	64.3	0.0	0.2	0.0	0.0	0.0	0.1	3.0	0.5	0.3	0.2	2.3	0.3	0.0	0.3
<i>Holcus lanatus</i> <sup>e</sup>	64.3	0.0	3.0	0.1	0.0	4.0	0.0	0.1	0.8	0.0	0.3	0.7	0.8	0.1	0.0
<i>Hypochaeris radicata</i> <sup>e</sup>	64.3	1.0	0.2	1.5	1.7	0.0	4.0	0.0	1.4	0.0	0.3	0.0	0.0	0.2	2.0
<i>Luzula comosa</i>	64.3	1.0	0.7	0.1	0.3	0.0	0.0	0.0	0.3	0.2	1.0	0.0	0.2	0.2	0.0
<i>Rumex acetosella</i> <sup>e</sup>	64.3	6.0	0.5	0.2	0.3	0.3	5.3	0.0	0.0	0.0	6.0	0.0	0.0	4.0	1.3
<i>Daucus carota</i> <sup>e</sup>	57.1	0.3	4.0	0.0	0.0	0.0	0.0	0.7	8.3	10.0	0.3	0.0	6.5	0.0	0.3
<i>Hypericum perforatum</i> <sup>e</sup>	57.1	0.0	0.0	1.4	0.0	0.0	0.1	0.7	0.3	0.1	0.2	0.3	0.3	0.0	0.0
<i>Dactylis glomerata</i> <sup>e</sup>	50.0	1.0	0.5	0.0	0.1	0.3	11.0	0.0	8.0	0.0	0.0	0.0	0.0	0.0	0.7
<i>Eriophyllum lanatum</i>	50.0	1.0	0.0	4.6	0.3	0.0	0.1	0.0	0.8	2.3	0.0	0.1	0.0	0.0	0.0
<i>Myosotis discolor</i> <sup>e</sup>	50.0	0.0	0.0	0.0	0.0	0.3	0.0	0.2	0.3	0.3	0.0	0.3	0.3	0.0	0.3
native species richness	-	17	12	33	31	15	19	9	21	17	19	26	20	3	6
exotic species richness	-	11	23	20	14	11	13	18	22	21	17	17	19	14	24

### Characterization of soils

Soils from Puget Trough and Willamette Valley sites were generally distinct, although they shared some qualities (Figure 2-5; Figure 2-6). Acidic soils dominate remaining *C. levisecta* populations and reintroduction sites (pH= 4.68 - 5.75), and in general, Puget Trough and Willamette Valley soils had similar levels of NH<sub>4</sub>, NO<sub>3</sub>, TN, and TOC. Rocky Prairie, Kah Tai Prairie, and Heritage Seedling had particularly high levels of NH<sub>4</sub>, NO<sub>3</sub>, and TN. The carbon to nitrogen ratio from all sites was low (6.4 – 12.6), and organic matter content was generally high (3.9 % - 15.6%). Puget Trough sites with particularly black soils, rich in organic matter (12.9% - 15.6%) included Trial Island, Rocky Prairie, and Kah Tai Prairie.

Puget Trough soils generally had higher levels of sulfur and magnesium than Willamette Valley soils. Sulfur levels were particularly high at Trial Island (434.9 µg/g) and Rocky Prairie (325.3 µg/g), and generally appear to be positively correlated with organic matter content. Sites situated on coastal bluffs and prairies had higher concentrations of magnesium (2591.0 µg/g - 3615.5 µg/g) than inland sites (1022.3 µg/g - 2454.5 µg/g). Willamette Valley sites generally had higher concentrations of potassium and phosphorous than Puget Trough sites, although there was a lot of variability among sites from each region. Soil texture most clearly distinguished the two regions. Sand was a larger component of Puget Trough soils (49.7% - 87.6%) than Willamette Valley soils (12.3% - 28.0%), excluding Sandy River Delta, which had a high sand component (64.1%). Willamette Valley sites were primarily dominated by silt (33.9% - 52.1%) and clay (27.4% - 46.3%). The sandy Puget Trough soils generally had lower bulk density (0.64 - 0.92) than the heavy clay soils of the Willamette Valley (0.81 - 1.25).

**Table 2-5.** Puget Trough mean soil values. Soil texture was abbreviated; C= clay, L= loam, S= sand. (\* denotes a reintroduction site, all other sites are remaining *C. levisecta* populations)

soil trait	Sites					
	EBY	FOR	ROC	TRL	WEB	KAH*
pH	5.75	5.51	4.88	5.60	5.32	5.42
NH <sub>4</sub> (mg/kg)	7.80	7.03	33.68	13.57	15.92	27.38
NO <sub>3</sub> (mg/kg)	7.00	0.54	10.40	3.89	0.53	7.56
TOC (µg/g)	159.05	220.99	449.69	113.29	253.72	404.33
TN (µg/g)	18.08	17.49	53.51	17.80	25.53	37.83
C:N	8.80	12.64	8.40	6.37	9.94	10.69
% OM	3.89	4.95	13.21	15.56	7.21	12.91
K (µg/g)	529.10	1180.00	457.90	1032.61	635.00	666.70
P (µg/g)	131.80	103.35	402.10	459.44	265.90	336.75
S (µg/g)	102.69	120.00	325.25	434.89	236.55	297.20
Mg (µg/g)	3615.50	2799.50	1780.50	3185.25	2591.00	3446.50
Mn (µg/g)	122.25	180.60	295.45	550.63	356.55	242.75
D <sub>b</sub> (g/cm <sup>3</sup> )	0.84	0.71	0.65	-	0.92	0.64
% sand	87.6	49.7	70.70	56.2	70.1	71.85
% silt	10.8	28.3	22.10	33.1	19.8	21.49
% clay	1.6	22.0	7.30	10.7	10.1	6.66
texture	S	SCL	SL	SL	SL	SL

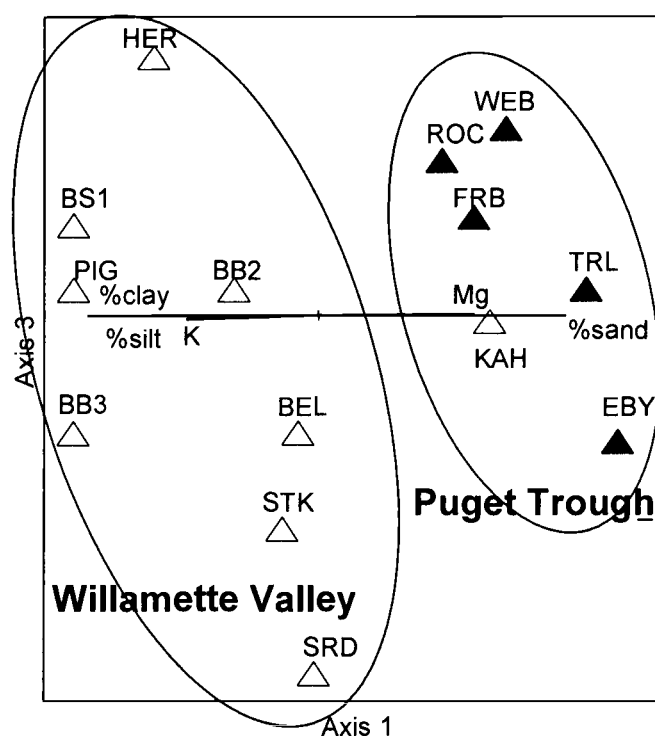
**Table 2-6.** Mean soil values for Willamette Valley reintroduction sites. Soil texture was abbreviated; C= clay, L= loam, S = sand, SI= silt

soil trait	Sites								
	BS1	BB2	BB3	BEL	HER	PIG	PMC	SRD	STK
pH	5.40	5.30	5.60	4.87	5.82	5.30	5.25	4.68	4.85
NH <sub>4</sub> (g/kg)	20.93	14.37	16.18	14.61	30.60	21.98	9.21	9.93	16.34
NO <sub>3</sub> (mg/kg)	3.29	1.74	1.90	4.24	9.79	2.87	4.50	9.91	8.49
TOC (µg/g)	299.48	188.48	210.12	297.35	276.79	206.80	122.66	151.73	193.71
TN (µg/g)	27.66	17.54	17.79	27.35	34.07	25.98	15.63	20.68	21.95
C:N	10.83	10.74	11.81	10.87	8.12	7.96	7.85	7.34	8.82
% OM	6.99	7.58	5.76	8.41	8.05	8.14	4.32	5.17	7.36
K (µg/g)	1606.50	1517.50	2496.00	1470.50	815.05	1020.65	1569.50	453.40	1419.50
P (µg/g)	165.15	107.10	126.00	260.85	629.35	430.85	448.40	480.70	348.00
S (µg/g)	159.05	137.65	131.00	128.15	155.20	128.65	65.99	167.85	119.75
Mg (µg/g)	1802.00	2147.50	2453.50	1022.25	1029.00	1350.50	2118.00	1030.80	2111.00
Mn (µg/g)	281.30	171.55	155.50	325.10	770.95	534.65	390.60	87.57	473.35
D <sub>b</sub> (g/cm <sup>3</sup> )	0.82	0.85	0.81	0.87	0.98	0.91	1.25	0.91	0.94
% sand	16.72	19.19	20.86	19.76	28.02	16.27	12.33	64.12	12.30
% silt	45.63	41.87	38.48	33.94	44.57	37.69	52.10	28.67	48.71
% clay	37.65	38.94	40.66	46.30	27.41	46.05	35.57	7.22	38.98
Texture	SICL	SICL	SICL	C	CL	C	SIC	SL	SICL



### Regional patterns in habitat characteristics

Regional divergence between Willamette Valley and Puget Trough sites was apparent along axis 1 of a 3-D NMS solution that explained 66% of the variation in the original data. The final configuration had lower stress than those found with Monte Carlo randomizations ( $p=0.020$ ). Axes 1 and 3 together explained half of the variability in community composition ( $r^2=0.24, 0.25$ , respectively), and axis 2 explained the remaining 17%. Axes 1 and 3 are displayed, as these axes accounted for the majority of variation in community composition (Figure 2-2). Sites separated regionally in the ordination space and circles were drawn around sites from the two ecoregions. Correlations of soil variables with axis 1 help explain the regional divergence in vegetation communities. Soil texture, as well as potassium and magnesium concentrations, were strongly associated with axis 1 ( $r^2=0.38-0.72$ ). Ordinations were rotated to align with percentage sand, which had the strongest relationship ( $r^2=0.72$ ), with axis 1. None of the measured soil variables were correlated with the variation in plant communities along axis 3.



**Figure 2-2.** NMS solution of sites in species space with soil variable overlays. Each point represents a site (source population =  $\blacktriangle$ , common garden sites =  $\triangle$ ). Soil variables strongly associated with vegetation community composition are indicated with vector overlays ( $r^2 > 0.3$ ). The length of the vector indicates the strength of the relationship of a variable with the ordination scores. Soil texture best explains differences in community composition with sites from the Willamette Valley and Puget Trough aggregating at opposite sides of axis 1.

## DISCUSSION

Plant communities differed considerably among sites from the northern and southern portions of *C. levisecta*'s historic range. Puget Trough and Willamette Valley sites had distinct species assemblages likely related to regional differences in geology, climate, ocean proximity, and land-use history. Although sites from these two regions did share some species in common (Table 2-4), the majority of these were invasive exotics that are widespread throughout the Pacific Northwest. *Poa pratensis*, an introduced perennial grass, was particularly common in our study, occurring at all but two sites. This species is a problematic invader of Pacific Northwest prairies because it has the capacity to recruit by tillering in dense above-ground litter layers

that result in the absence of fire (MacDougall & Turkington 2004). Nearly half of the Puget Trough indicator species were native perennials, while almost all species indicative of Willamette Valley sites were exotic, annual forbs and grasses. Puget Trough sites also had greater native species richness than those of the Willamette Valley, which in turn generally had higher numbers of exotic species. Invasion by exotic species is a major threat to the viability of remaining *C. levisecta* populations (Caplow 2004) and management of non-native species will clearly be an important component of reintroduction efforts in the Willamette Valley. The common functional groups at remaining *C. levisecta* populations (i.e., native perennials) and reintroduction sites in the Willamette Valley (i.e., introduced annuals) highlight important differences in community structure among sites from these two ecoregions.

Soil characteristics were also distinct among *C. levisecta* sites from the Puget Trough and Willamette Valley ecoregions, but did share some commonalities. All *C. levisecta* sites were acidic with abundant organic matter, and generally had low carbon to nitrogen ratios, which is characteristic of graminoid dominated soils (Brady & Weil 2002). Several soil characteristics, including texture, and levels of potassium and magnesium, were strongly associated with differences in community composition between the two ecoregions (Figure 2-2). *Castilleja levisecta* sites in the Puget Trough were found primarily on sandy coastal prairies influenced by sea spray, whose salts are often dominated by sulfates and chlorides of calcium, magnesium, and sodium (Brady & Weil 2002). The relatively high amount of magnesium at Puget Trough sites ( $\bar{x} = 2903.0 \mu\text{g/g} \pm 273.9$ ) compared to Willamette Valley sites ( $\bar{x} = 1673.8 \mu\text{g/g} \pm 201.4$ ) was likely due to their vicinity to marine environments and may influence species composition. Willamette Valley sites were typically situated in upland prairies dominated by silty-clay soils with relatively high levels of potassium ( $\bar{x} = 1374.3 \mu\text{g/g} \pm 204.2$ ). Potassium is found in high levels in most mineral soils, except those consisting primarily of quartz sand (Brady & Weil 2002). The sandy texture of Puget Trough soils was probably why we observed low levels of potassium at these sites ( $\bar{x} = 750.2 \mu\text{g/g} \pm 118.2$ ). Our soil analyses are similar to Chappell & Caplow's (2005) characterization of remaining *C. levisecta* populations,

who found Puget Trough soils to be generally high in magnesium and sand content, with low clay percentage and potassium concentrations.

Only 2.6% of pre-settlement, native dominated grasslands in the Puget Lowland are estimated to remain (Chappell et al. 2000), and less is suspected to remain in the Willamette Valley, OR. Although a mapping effort by Chappell et al. (2000) showed low *C. levisecta* co-occurrence with pre-settlement grassland soil and vegetation polygons, it was likely because the minimum map unit employed in the study was larger than the size of most of the remaining *C. levisecta* populations. This emphasizes the degree of habitat fragmentation and alteration that extant *C. levisecta* populations have been subjected to and suggests that remaining populations likely represent only a fraction of the site characteristics *C. levisecta* once inhabited. Furthermore, remaining populations appear to be relegated to habitat unsuitable for agriculture (e.g., too steep, too rocky, and/or too much sea spray influence). Efforts to reestablish species in portions of their historic range need to consider the ecological and cultural processes that once determined species occurrence (MacDougall et al. 2004).

*Castilleja levisecta* recovery site selection may be especially challenging in the southern portion of the species' historic range because prospective planting sites are ecologically distant from Puget Trough seed sources. Maximizing the ecological similarity, in terms of species composition and soil characteristics, between existing populations and prospective reintroduction sites within the Puget Trough ecoregion may be appropriate because sites share similar floras and geologic histories. Using reciprocal transplant experiments in Southern California, Montalvo & Ellstrand (2000) found that the cumulative fitness of *Lotus scoparius* decreased with increasing environmental distance, emphasizing the importance of matching seed sources with ecologically similar restoration sites within a given region. However, large differences in species composition between ecoregions make standard predictions untenable. In order to be useful at great ecological distances where floras are distinct, a different metric of ecological similarity is needed.

We suggest using a functional group approach to compare plant communities among sites from distinct ecoregions. Functional groups are useful in comparative

studies of communities, enabling the comparison of species that share ecological characteristics and play similar roles in communities, but are taxonomically distinct (Simberloff & Dayan 1991; Voigt & Perner 2004). Species could be assigned to functional groups based on life history characteristics (annual vs. perennial), origin (native vs. exotic), and habit (graminoid, forb, or woody). We predict that plant functional groups will be a useful method to measure habitat similarity and determine suitable recovery sites across large ecological and geographic distances where floristic communities differ.

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### **Chapter 3 : Common garden experiments with golden paintbrush (*Castilleja levisecta*): selecting seed sources and reintroduction sites for an endangered prairie species**

#### **ABSTRACT**

Species reintroduction is increasingly prescribed as a conservation strategy to promote the viability of endangered plants. A suite of ecological and genetic factors are likely to contribute to reintroduction success, including the ecological similarity between seed source and introduction site, the effective population size and genetic diversity of seed sources, and the habitat quality of the recovery site. We conducted common garden experiments with golden paintbrush (*Castilleja levisecta* Greenman), an endangered species endemic to the Pacific Northwest, U.S.A., and extinct in the southern portion of its historic range. We test hypotheses about how the species would perform in the southern portion of its range, as well as provide management recommendations about seed source and recovery site selection.

Ten common gardens, each comprised of *C. levisecta* individuals grown from seed collected from six of the eleven remaining source populations, were planted into field conditions similar to potential recovery sites in the Willamette Valley, OR, during two planting events in 2004. Significant variation among source populations and common gardens was observed for first year plant performance and second year survival rates. Plant community characteristics, including the abundance of non-native species and the similarity in community structure between source populations and common garden sites, helped explain the variation in these performance measures. Exotic species cover at common garden sites was associated with a reduction in performance of first year *C. levisecta* transplants. Survival of second year transplants increased with increasing similarity in plant functional groups between source and common garden sites, supporting the idea of a “home-habitat advantage.” These results indicate that high quality prairies, dominated by native perennial species with low non-native abundance, should be targeted for recovery sites. We do not recommend using genetic diversity, effective population size, or geographic distance to select seed sources for reintroduction of *C. levisecta* in the Willamette Valley. Instead, we recommend using plant material from Whidbey Island, WA, whose three populations represented in our study consistently performed well.



## INTRODUCTION

Species reintroduction is a growing component of conservation efforts worldwide. Recovery plans recommended reintroduction or population augmentation to achieve recovery criteria for 87% of the federally listed plant species in 1997 (Kennedy 2004). However, despite numerous efforts, few, if any, reintroduction attempts can yet be judged as true successes (Bowles & McBride 1996; Bowles et al. 2001; Guerrant 1996b; Pavlik & Espeland 1998; Pavlik et al. 1993). Failure to achieve success is likely a result of our poorly developed biological understanding of species reintroduction (Falk et al. 1996). Selection of biologically appropriate seed sources and recovery sites is critical when implementing a reintroduction strategy, though few studies utilize both genetic and ecological criteria during the selection process (Husband & Campbell 2004). Decisions regarding the suitability of seed sources and recovery sites can be particularly challenging in portions of a species range that are currently uninhabited, as no reference populations with which to compare ecological and genetic characteristics exist. Currently, there are no published studies that have experimentally determined appropriate seed sources and/or recovery sites for plant reintroduction in an unoccupied portion of a species historic range. In order to evaluate relevant management options for reintroduction to the southern portion of the species' historic range, we conducted common garden experiments with golden paintbrush (*Castilleja levisecta*), an endangered plant endemic to the prairies of the Pacific Northwest.

The origin of source material used to establish new plant populations is a controversial issue in conservation biology. Habitat similarity between source and introduced populations may be crucial because of ecotypic differentiation and development of co-adapted gene complexes that form in response to specific habitat conditions and selective pressures (Guerrant 1996a; Guerrant & Pavlik 1998; Huenneke 1991; Hufford & Mazer 2003). Choosing an introduction site that closely matches the source site (i.e., soil, vegetation and climate) increases the likelihood that introduced plants will be genetically well-adapted to the site, and in turn, that introduction will succeed (Bowles et al. 1993; Montalvo & Ellstrand 2000; Pavlik et

al. 1993). Numerous studies have demonstrated a fitness advantage of local transplants relative to transplants from distant sites (Gustafson et al. 2004a; Montalvo & Ellstrand 2001; Schmidt & Levin 1985; Wang et al. 1997), emphasizing the importance of the home-site advantage, or what might be more broadly termed the “home-habitat advantage.” But how local is local (McKay et al. 2005)? Geographic distance is often used by land managers as an index of ecological similarity, because local sites are likely to share similar soils, vegetation, and climate. However, in a heterogeneous landscape and at great distances, geographic distance is not necessarily a good measure of ecological distance.

Characteristics of the seed source, such as population size and genetic diversity, could be powerful tools used to predict plant performance during plant restoration. Individuals from small populations are more susceptible to inbreeding depression, genetic drift, and the disruption of plant-pollinator interactions and are predicted to be less fit than those from large populations (Ellstrand & Elam 1993; Young et al. 1996). Population size and fitness components are positively related in several fragmented, rare vascular plants (Fisher & Matthies 1998; Menges 1991; Paschke et al. 2002). Similarly, population genetic theory predicts a positive correlation between genetic diversity and fitness (Young et al. 1996), and molecular marker diversity is therefore often used to decide which populations are most suitable as restoration sources (Haig 1998; Knapp & Rice 1998). However, this relationship may be weak, as genetic markers are generally considered neutral and genetic sampling is typically small relative to the size of the genome (Reed & Frankham 2003). No consistent pattern between marker diversity and plant performance emerges among rare, fragmented species. While some studies have found significant relationships (Buza et al. 2000; Oostermeijer et al. 1994; Paschke et al. 2002), others have not (Lammi et al. 1999; Luijten et al. 2000; Ouborg & Treuren 1995).

Exotic species constitute a significant component of many regional floras and are a major threat to global diversity (Hobbs & Humphries 1995). After habitat loss, non-native species are the most prevalent threat to endangered species viability, affecting half of the imperiled species in the U.S. (Wilcove & Master 2005). Pacific Northwest native grasslands are considered one of most critically endangered ecosystems in the nation

(Noss et al. 1995), with less than 3% of pre-settlement extent remaining (Chappell et al. 2000). Many of these remaining fragments are of poor quality due to isolation, fire suppression, and invasion by tall, aggressive pasture grasses that displace native flora. In turn, these grasses may enhance habitat for small mammals (Adler & Wilson 1989), whose abundance can influence grassland dynamics and species composition (Batzli & Pitelka 1970). Competition from non-native species has hindered reintroduction efforts of several rare species including *Abronia umbellata* (Kaye 2002), *Amsinckia grandiflora* (Pavlik et al. 1993), *Stephanomeria malheurensis* (Guerrant 1996b), and *Cirsium vinaceum* (Huenneke & Thomson 1995), suggesting that site quality and plant community characteristics are likely to play an important role in *C. levisecta* recovery as well.

*Castilleja levisecta* is a federally threatened species currently restricted to 11 sites in the Pacific Northwest and is extinct in the southern portion of its historic range. Recovery criteria call for the existence of twenty populations each composed of 1,000 flowering individuals within the species' historic range (U.S.F.W.S. 2000). *Castilleja levisecta* has limited capacity for natural dispersal and colonization of new sites, necessitating *ex situ* conservation techniques to meet recovery goals. A strategic reintroduction plan has been prepared to support the long-term viability of the species and requires establishment of new populations within the historic range of *C. levisecta*, including the Willamette Valley, Oregon (Caplow 2004).

Potential restoration sites in the Willamette Valley are geographically and ecologically distant from extant populations of *C. levisecta* (Chapter 2), with no indication of which seed source is most appropriate for recovery efforts in this ecoregion. Further, the specific habitat conditions to target during recovery efforts are unknown because the species has been extinct in this region for over 60 years and herbarium records do not clearly describe habitat characteristics (Chapter 2). Therefore, we apply ecological and genetic theory to restoration ecology in order to understand which factors could promote the reestablishment of *C. levisecta* in the Willamette Valley. Our main research objective is to determine what ecological and genetic factors contribute to the success of *C. levisecta* transplants in the southern portion of its historic range by testing three specific hypotheses. We will provide management recommendations based on these results.

*Hypothesis 1: Performance of individuals decreases with ecological and/or geographic distance between source population and reintroduction site*

*Justification.* - Selecting plant materials from sites that are ecologically similar to the introduction site may be most appropriate, as individuals from populations can be adapted to specific habitat conditions (Hufford & Mazer 2003). Local adaptation has been investigated using reciprocal transplant experiments in many studies (Cheplick 1988; Gordon & Rice 1998; Helenurm 1998; Kindell et al. 1996). However, no studies that we are aware of have attempted to extrapolate this concept to rare plant reintroduction in portions of the species' range that are no longer occupied. Geographic distance is often used by restoration practitioners as an index of ecological similarity, but it was not a good surrogate for environmental distance among populations of *Lotus scoparius* (Montalvo & Ellstrand 2000). According to Hypothesis 1, the success of reintroductions can be increased by targeting recovery sites that are ecologically similar and/or geographically close to the seed source.

*Hypothesis 2: Plant performance is positively correlated with the size ( $N_e$ ) and genetic diversity of the source population.*

*Justification.* - Population genetic theory predicts that individuals from large, genetically diverse populations will be more fit than those from small, genetically depauperate populations (Young et al. 1996). Godt et al. (2005) observed a wide range of pairwise *C. levisecta* population genetic identities ( $F_{st}$  = 0.055 - 0.388), indicating that significant genetic differentiation among some remaining populations exists. Further, *C. levisecta* effective population size ( $N_e$ ) and genetic diversity were generally positively correlated (Godt et al. 2005). Hypothesis 2 suggests that recovery efforts should use seed from large and genetically diverse populations.

*Hypothesis 3: Transplant success is positively correlated with the habitat quality of the reintroduction site*

*Justification.* - Biological characteristics of the reintroduction site, such as the presence of exotic species and generalist herbivores, can prevent effective plant recovery (Fiedler & Laven 1996). Extant populations of *C. levisecta* are threatened both by exotic

encroachment and herbivore pressure (U.S.F.W.S. 2000). Potential recovery sites in the Willamette Valley represent a continuum of habitat quality, ranging from abandoned agricultural fields dominated by exotic annuals to high quality prairies composed primarily of native perennial species. Many non-native species common to Pacific Northwest prairies are aggressive competitors and can displace native flora (Thomas & Carey 1996). Hypothesis 3 suggests that high quality sites dominated by native prairie plants with low herbivore abundance should be targeted for recovery efforts.

## METHODS

### Study species

*Castilleja levisecta* (Orobanchaceae) is a short-lived (5-6 years), multi-stemmed, perennial, endemic to the native grasslands of the Pacific Northwest. It is an out-crossing species primarily pollinated by *Bombus* spp. and is known only to reproduce by seed (Kaye & Lawrence 2003; Wentworth 2001). Like other members of the genus *Castilleja*, golden paintbrush is a facultative hemi-parasite (Kaye 2001b; Wentworth 2001). Although hemi-parasites are photosynthetic and do not require a host plant, they can form haustoria, or physical connections, with other root systems to obtain nutrients, water, and possibly secondary compounds from their host (Press 1989). *Castilleja levisecta* does not require a host to reproduce in a greenhouse environment and does not appear to be host specific (Wentworth 2001). Despite its rarity, a genetic analysis of *C. levisecta* indicates that the species maintains unusually high genetic diversity compared to other endemic species and even other members of the figwort family (Godt et al. 2005).

Ten of the eleven extant *C. levisecta* populations are concentrated in the San Juan Islands of Washington and British Columbia, generally on south-west facing coastal prairies with sandy, well drained soils of glacial origin (Chappell & Caplow 2004). Only one mainland population remains (Rocky Prairie), and is distinct from others because it is characterized by mounded topography (Chappell & Caplow 2004) and receives significantly more annual precipitation (129cm) than the island populations (52- 74 cm), which are located in the rain shadow of the Olympic Mountains (WRCC 2005). Despite relatively local distribution, extant populations are geographically fragmented and isolated from one another. Pollen flow may be limited

by the inability of pollinators to fly between island populations, the geographic isolation of Rocky Prairie, and the discontinuity of appropriate nectar sources in a landscape fragmented by agriculture and development (Godt et al. 2005).

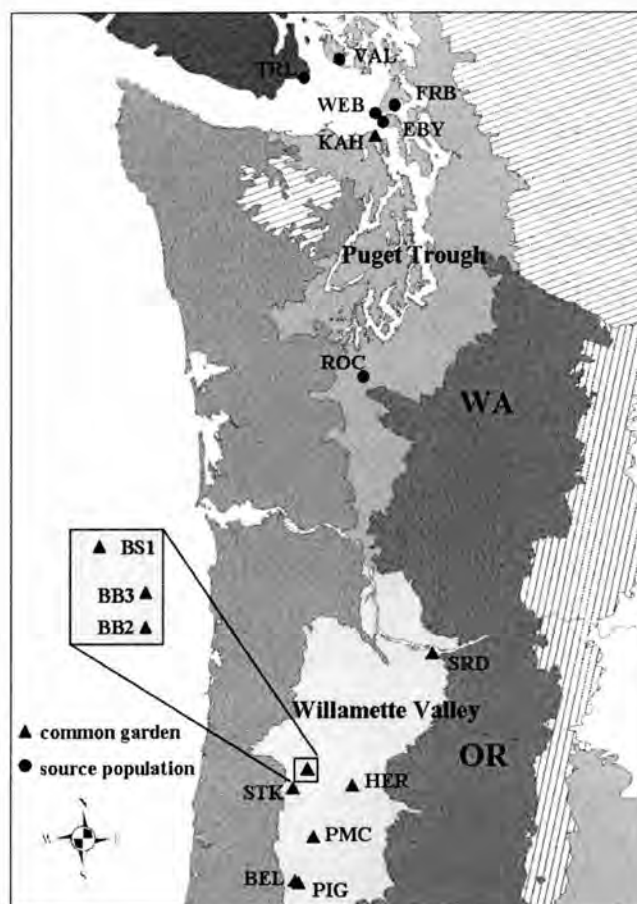
*Castilleja levisecta* was last collected in the Willamette Valley in 1938 (Gamon 1995). The vegetation communities and soil characteristics of historic populations are not well understood because herbarium records do not specify exact locations or habitat characteristics of *C. levisecta* collections (Chapter 2). We believe the species inhabited upland prairies of the Willamette Valley, which are grass-dominated systems often associated with *Quercus garryana*-savanna, on well-drained soils commonly found on the valley foothills (Franklin & Dyrness 1988). The unglaciated soils of upland prairies are generally dominated by clay and silt from weathering basalt. Willamette Valley populations were likely subjected to a wetter climate (104 - 123 cm) than the majority of the extant populations in the Puget Trough (WRCC 2005).

#### Experimental design

Ten experimental common gardens were established throughout the species historic range in 2004. Nine common gardens were planted in the Willamette Valley, OR, the reintroduction target region. One common garden site was also established in the Puget Trough, WA near extant populations (Figure 3-1). Each common garden consisted of transplants from six source populations (Table 3-1), which were selected to characterize the geographic, genetic, and ecological diversity among the remaining eleven populations. Although we specifically selected sites with well-drained soils, the common garden sites encompassed a diversity of soil types, vegetation, and site quality, and are representative of locations likely to be chosen for future *C. levisecta* reintroduction endeavors (Chapter 2). Logistics also played an important role in site selection. Under the Endangered Species Act of 1973, listed plants are protected only on federal land. Therefore, seven of the ten potential recovery sites we chose to characterize were located on public land.

**Table 3-1.** Code, general location, habitat, and USGS soil map unit for each *C. levisecta* source population and common garden. (\* denotes site without existing vegetation)

	code	general location	Habitat	Soil map unit
<b>Puget Trough (extant populations)</b>				
Ebey's landing	EBY	Whidbey Island, WA	coastal bluff	Rough broken land
Forbes Point	FRB	Whidbey Island, WA	coastal prairie	Coveland
Rocky Prairie	ROC	South Puget Trough, WA	mounded prairie	Spanaway-Nisqually
Trial Island	TRL	Trial Island, B.C.	coastal prairie	unavailable
West Beach	WEB	Whidbey Island, WA	coastal prairie	Bozarth
Kah Tai Prairie*	KAH	Port Townsend, WA	upland prairie	San Juan
<b>Willamette Valley (reintroduction sites)</b>				
Basket Butte 2	BB2	Baskett Slough National Wildlife Refuge, OR	upland prairie	Chehulpum
Basket Butte 3	BB3	Baskett Slough National Wildlife Refuge, OR	upland prairie	Chehulpum
Basket Slough 1	BS1	Baskett Slough National Wildlife Refuge, OR	upland prairie	Steiwer
Bell Fountain Prairie	BEL	Finley National Wildlife Refuge, OR	upland prairie	Jory
Heritage Seedling	HER	Salem, OR	restored prairie	Nekia
Pigeon Butte	PIG	Finley National Wildlife Refuge, OR	upland prairie	Dixonville
Plant Materials Center	PMC	Lewisburg, OR	agricultural field	Amity-Woodburn
Sandy River Delta	SRD	Troutdale, OR	degraded prairie	Burlington
Starck	STK	Dallas, OR	degraded prairie	Bellpine



**Figure 3-1.** *Castilleja levisecta* source population and common garden sites located in the Pacific Northwest. Source populations and a single common garden site were situated in the Puget Trough, Washington. All other common gardens were located in the Willamette Valley, Oregon. For site abbreviations, see Table 3-1.

Common gardens were established in 2004 during two planting events. Seed was collected from 35 maternal plants from each source population in August 2003, and propagated according to Lawrence & Kaye (2005). Between 114 and 121 transplants were planted at each common garden site on March 1-5, 2004, for a total of 1168 spring transplants. Approximately 130 additional plants were planted at seven of the ten common gardens November 10-15, 2004, for a total of 905 fall transplants. Three common garden sites were not planted in fall 2004 because of poor spring 2004



transplant performance and a limited number of plants for the fall planting event. Variable germination and differential mortality during propagation prevented a genetically balanced design, though we attempted to equalize genetic representation of source populations and maternal lines at each common garden by planting an equal number of individuals from maternal lines at all sites. Three month-old transplants were randomized by source population and planting season and planted in the center of square meters within a 20m x 15m grid at each common garden site. Transplants were planted into sites with existing vegetation and were allowed to form haustorial connections with whatever root systems they encountered. However, the Plant Materials Center site was established in an agricultural field and individuals were provided with a grass host (*Festuca roemerii*). Two and a half meter fences were erected to enclose common gardens and protect transplants from deer and elk.

#### Measurement of plant size & survival

*Castilleja levisecta* individuals were monitored during peak prairie phenology in April-July, 2004 and 2005. Spring transplants were monitored three times during the 2004 growing season. Total stem length, number of stems, and flower and fruit production were recorded during each monitoring event. Vole activity was unusually high in the Willamette Valley during the 2005 growing season, when 89% of the surviving *C. levisecta* transplants were subjected to herbivory, most likely from grey-tailed voles (*Microtus canicaudus*). Continuous response variables such as stem length and number were not reliable measures of plant performance, as herbivory stimulated resprouting and altered plant morphology (B. Lawrence, pers. obs.). Therefore, we used survival as the response variable for 2005 analyses.

#### Community composition and soil variables

Species composition and abundance, as well as soil samples were collected during May 2004 at sites according to the methods outlined in Chapter 2. Soils were evaluated for physical (bulk density, percent sand, silt, and clay) and chemical (organic matter, pH, total organic carbon, total nitrogen, carbon to nitrogen ratio, nitrate, ammonium, potassium, phosphorus, manganese, magnesium, and sulfur)

parameters. Access to the San Juan Valley source population was restricted so no habitat information was collected there. This source population and the Plant Materials Center common garden site were not included in analyses requiring habitat information.

### Statistical analyses

#### Transplant performance 2004

Because our measures of plant size and fitness (stem length, stem number, flower and fruit production) were correlated with one another, we combined them into a single index of plant performance using Principal Components Analysis (PCA) in PC-ORD v. 4.25 (McCune & Mefford 1999). The objective of PCA is to find the strongest linear correlation structure among variables and reduce them to a smaller number of synthetic variables that represent the most information in the original data set (McCune & Grace 2002). The maximum value of the three monitoring events for each parameter was used. Total stem length and stem number were log-transformed resulting in a normal, linear distribution of residuals. PCA scores from axis 1 were used as a measure of 2004 *C. levisecta* performance in further analyses.

#### Variation among sources and common gardens

We used analysis of variance (ANOVA) and binary logistic regression to test for effects of source population and common garden using S-PLUS v. 6.2 (Insightful 2000). ANOVA was used to compare mean plant performance of spring 2004 transplants (n= 1168). Binary logistic regression was used to analyze the variation in survival among source populations and common gardens for spring 2005 (n= 1168) and fall 2005 (n= 908) data. Significance was determined with drop in deviance tests using a chi-square distribution. Spring and fall transplants were analyzed separately for the 2005 analyses, as climatic variation and exposure to summer drought may have confounded observations from the two planting events. Pair-wise comparisons between source populations were corrected using Dunn-Sidak multiple comparisons.

#### Predictors of *C. levisecta* performance

In order to address our hypotheses, we developed seven predictor variables to determine if any helped explain the variation in *C. levisecta* performance and survival.

### *Ecological and geographic distance*

To address Hypothesis 1, we developed measures of ecological and geographic distance between source populations and common gardens. We estimated ecological distance with two measures, soil distance and plant functional group distance. Ecological distances were created using distance matrices in PC-ORD v. 4.25 (McCune & Mefford 1999). To estimate soil distance, or the dissimilarity of soil characteristics between sites, we used the mean value of each soil variable from all sites. Prior to calculation of a distance matrix, soil values were relativized by standard deviates that allow direct comparison of variables measured using different scales. Soil distance values were calculated using Euclidean distance because soil variables were generally linearly related.

We used plant functional groups to compare the similarity in plant community structure between sites because Puget Trough and Willamette Valley sites were floristically distinct (Chapter 2). Categorization of taxonomically diverse species into plant functional groups allows for evaluation of distinct plant communities that share ecological characteristics. Species were assigned to one of ten functional groups based on their origin (native vs. exotic), life history (annual vs. perennial), and habit (graminoid, forb, or woody). There were no annual woody species in our study system. Plant community distances were created with a Sorensen distance matrix, as recommended by McCune & Grace (2002), using the average cover value for each functional group from each site. Soil and community distances between each source population and common garden site combination were used as possible predictors of *C. levisecta* success in regression model selection.

Geographic distances between sites were calculated in Arcview 3.2 using the Bearing and Distance extension (ESRI 2000). All points were projected to Universal Transverse Mercator (UTM) with a Clarke 1866 ellipsoid and the North American Datum 1927 (NAD27). Preliminary screening of scatterplots suggested a weak, positive correlation between geographic distance and *C. levisecta* performance, which

was contradictory to the predicted relationship. Due to limited degrees of freedom and this unexpected relationship, we did not include geographic distance in the regression model building process. We report regression analyses of geographic distance and *C. levisecta* performance measures separately.

#### *Source population size and genetic diversity*

To address Hypothesis 2, we used estimates of source population size and genetic diversity as possible predictors of *C. levisecta* performance and survival. We used the observed heterozygosity of 13 allozyme markers from Godt et al. (2005) for each source population as estimates of genetic diversity. To estimate  $N_e$  for each source population, we utilized the harmonic mean of available census data between 1998 and 2003, which was log transformed to improve homoscedasticity. Census data were based on the number of flowering individuals from each population (F. Caplow unpublished data, Washington Natural Heritage Program).

#### *Habitat quality of the reintroduction site*

We used measures of common garden habitat quality, including exotic species abundance and herbivory rates, as possible predictors of *C. levisecta* performance and survival to address Hypothesis 3. Exotic species abundance was measured as the average cover of non-native plants at each common garden in 2004. Transplants planted in the spring of 2004 were subjected to various levels of herbivore pressure, including insect, small mammal, and possibly deer herbivory. Herbivory can have life-long impacts on the fitness of perennial species (Doak 1992). Therefore, we included the proportion of spring 2004 transplants subjected to herbivory in model selection for spring transplants.

#### Regression analyses

To test our hypotheses, we conducted separate regression analyses on spring 2004, spring 2005, and fall 2005 data sets. We used linear and binomial logistic regression to investigate the ability of predictors to account for variation in transplant performance and survival, respectively, using S-PLUS v. 6.2 (Insightful 2000). Mean

2004 transplant performance values (PCA scores) were used as the response variable ( $n = 45$ ) in multiple linear regression analysis. Quasi-likelihood estimation was used to fit grouped binomial logistic regression models, using the proportion of 2004 transplants surviving to 2005 from each combination of source population and common garden as the response variable, for 2005 spring ( $n = 45$ ) and fall ( $n = 30$ ) transplants. Model selection for each data set was conducted using Bayesian Information Criteria (BIC), which evaluates the parsimony of models by comparing the reduction in the sum of squares with the addition of parameters to the model (Gotelli & Ellison 2004). Models with every one, two, and three predictor combinations, as well as null and full models were screened. Models with the lowest BIC value were interpreted. Predictors screened included: soil and community distance, source population  $N_e$  and observed heterozygosity, and common garden exotic plant cover and frequency of 2004 herbivory. Predictors not included in final models are not discussed in the results.

To test Hypothesis 1 with respect to geographic distance, we examined the relationship between geographic distance and spring 2004 performance ( $n = 45$ ) using linear regression, and used grouped binomial logistic models for spring 2005 ( $n = 45$ ), and fall 2005 ( $n = 30$ ) survival.

### Functional group patterns

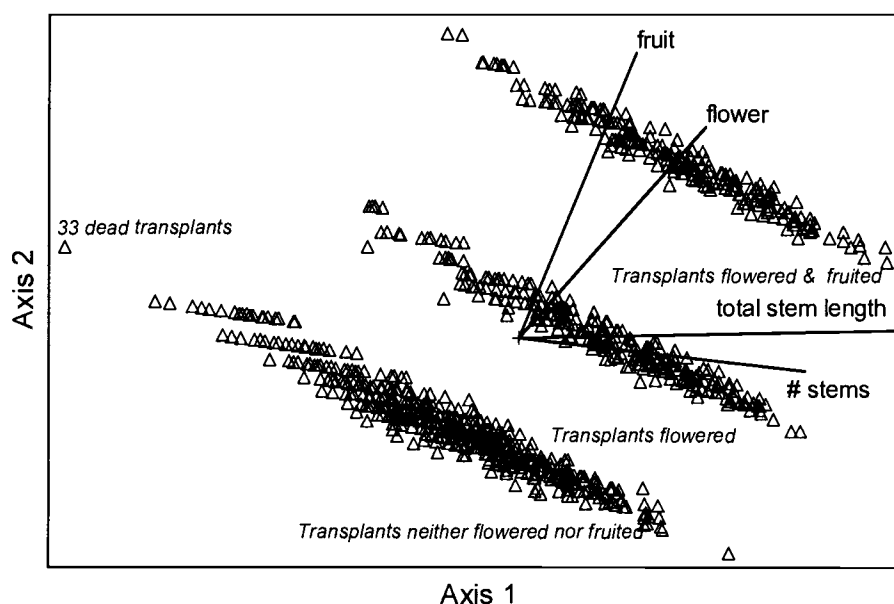
We used Nonmetric Multidimensional Scaling (NMS) to investigate similarities in plant functional groups among source populations and common garden sites and develop recommendations for recovery site selection (Kruskal 1964; Mather 1976). The “slow and thorough” autopilot mode setting was used in PC-ORD v. 4.25 to ordinate sample units in functional group space (McCune & Mefford 1999).

## **RESULTS**

### Transplant performance 2004

Comparison of eigenvalues to those produced by a random model indicated that only axis 1 contained more information than expected by chance, supporting our decision to use scores from this axis in further analyses. Axis 1 accounted for 60.1%

of the variance among performance measures and was strongly correlated with both stem length and number of stems, explaining 95.7% and 83.4% of their variation, respectively (Figure 3-2). Mortality during the first growing season was low. Thirty-three dead transplants, having values of zero for all four performance measures, are represented by the isolated point on the left end of axis 1. Table 3-2 shows the average performance measures associated with PCA scores for each source and common garden.



**Figure 3-2.** Plot of the first two principal components for 1168 *C. levisecta* transplants based on four performance variables. Each point represents a spring 2004 transplant. The solution was rotated to align the total stem length produced by transplants with axis 1. Vector overlays of the four variables were placed to depict the strength and direction of correlation with the two axes. The three bands represent different combinations of whether the transplant flowered and/or set fruit.

**Table 3-2.** Mean *C. levisecta* 2004 composite performance scores and field statistics for each source population and common garden ( $\pm 1$  SE). Source populations not sharing a common letter were significantly different ( $p \leq 0.05$ ) after Dunn-Sidak corrections.

	PCA score	stem length (cm)	# stems	% flowering	% fruiting
<b>Source population</b>					
Ebey's Landing <sup>bc</sup>	0.20 $\pm$ 0.11	82.1 $\pm$ 6.2	7.2 $\pm$ 0.3	64.6 $\pm$ 3.2	34.1 $\pm$ 3.2
Forbes Point <sup>c</sup>	0.21 $\pm$ 0.09	85.9 $\pm$ 5.5	10.4 $\pm$ 0.4	45.7 $\pm$ 3.1	19.7 $\pm$ 2.5
Rocky Prairie <sup>a</sup>	-0.75 $\pm$ 0.16	47.5 $\pm$ 6.1	6.9 $\pm$ 0.5	20.5 $\pm$ 4.5	12.0 $\pm$ 3.6
Trial Island <sup>bc</sup>	-0.02 $\pm$ 0.10	81.5 $\pm$ 5.8	10.0 $\pm$ 0.4	33.9 $\pm$ 3.0	17.9 $\pm$ 2.4
San Juan Valley <sup>ab</sup>	-0.38 $\pm$ 0.12	57.0 $\pm$ 6.2	8.4 $\pm$ 0.5	37.3 $\pm$ 4.0	11.3 $\pm$ 2.6
West Beach <sup>bc</sup>	0.13 $\pm$ 0.11	69.3 $\pm$ 5.2	8.0 $\pm$ 0.3	55.5 $\pm$ 3.4	27.8 $\pm$ 3.1
<b>Common garden</b>					
Basket Butte 2	-0.94 $\pm$ 0.08	25.2 $\pm$ 1.5	6.9 $\pm$ 0.3	18.5 $\pm$ 3.6	0 $\pm$ 0
Basket Butte 3	0.46 $\pm$ 0.12	65.0 $\pm$ 5.8	10.3 $\pm$ 0.6	19.3 $\pm$ 3.7	0 $\pm$ 0
Basket Slough 1	1.10 $\pm$ 0.13	161.7 $\pm$ 10.4	13.8 $\pm$ 0.6	71.9 $\pm$ 4.2	27.2 $\pm$ 4.2
Bell Fountain	-0.38 $\pm$ 0.15	53.2 $\pm$ 4.3	5.1 $\pm$ 0.3	44.7 $\pm$ 4.7	28.1 $\pm$ 4.2
Heritage Seedling	1.40 $\pm$ 0.14	172.6 $\pm$ 11.8	12.7 $\pm$ 0.6	76.4 $\pm$ 3.9	58.0 $\pm$ 4.5
Kah Tai Prairie	-0.78 $\pm$ 0.10	27.4 $\pm$ 2.2	6.2 $\pm$ 0.4	30.4 $\pm$ 4.3	9.6 $\pm$ 2.8
Pigeon Butte	0.29 $\pm$ 0.11	89.5 $\pm$ 6.8	8.8 $\pm$ 0.5	60.0 $\pm$ 4.5	15.0 $\pm$ 3.3
Plant Mat. Center	1.2 $\pm$ 0.11	89.3 $\pm$ 5.4	7.9 $\pm$ 0.4	89.7 $\pm$ 2.8	75.0 $\pm$ 4.0
Sandy River Delta	-0.53 $\pm$ 0.10	33.8 $\pm$ 2.6	8.9 $\pm$ 0.4	30.2 $\pm$ 4.3	6.0 $\pm$ 2.2
Starck	-0.97 $\pm$ 0.10	31.2 $\pm$ 2.3	7.1 $\pm$ 0.4	16.5 $\pm$ 3.4	0.8 $\pm$ 0.8

#### Variation among sources and common gardens

We observed differential performance among source populations ( $F_{5, 1113} = 8.29, p < 0.001$ ) and common gardens ( $F_{9, 1113} = 65.49, p < 0.001$ ) during the 2004 growing season (Table 3-2). Source populations performed similarly among common garden sites, as the interaction term between sources and common gardens was not significant ( $F_{45, 1113} = 1.01, p = 0.45$ ). Transplants from the Trial Island source population, along with the three populations located on Whidbey Island, WA (i.e., Forbes Point, Ebey's Landing, and West Beach) were the top performers in 2004 (Table 3-2).

The mean proportion of spring transplants surviving to 2005 was 0.27 (SE = 0.013). Survival varied among spring 2005 transplants from different sources ( $\text{Dev}_{5, 1113} = 33.25, p < 0.001$ ), and common gardens ( $\text{Dev}_{9, 1113} = 327.55, p < 0.001$ ), but the interaction between these two main effects was not significant ( $\text{Dev}_{45, 1113} = 52.12, p =$

0.22) (Table 3-3). Spring transplants from the Forbes Point and West Beach source populations (both located on Whidbey Island, WA) had significantly higher survival rates than Rocky Praire, San Juan Valley, and Ebey's Landing in 2005, while Trial Island had intermediate survival levels (Table 3-3). We observed little variation in fall transplant survival to 2005 which was generally high ( $\bar{x} = 0.88$ ,  $SE = 0.011$ ). Differential survival among common gardens ( $Dev_{6, 866} = 54.35$ ,  $p < 0.001$ ) was evident, but this was attributable to a single site (Pigeon Butte) that had significantly lower survival levels than all the other common gardens. Neither source populations ( $Dev_{5, 866} = 2.27$ ,  $p = 0.81$ ), nor the interaction term ( $Dev_{30, 866} = 26.61$ ,  $p = 0.64$ ) accounted for significant residual deviance.

**Table 3-3.** Average proportion of spring and fall transplants surviving in 2005 from each source population and common garden ( $\pm 1$  SE). Source populations not sharing a common letter had significantly different ( $p \leq 0.05$ ) 2005 survival rates as spring transplants after Dunn-Sidak corrections. Survival of fall transplants did not differ among sources.

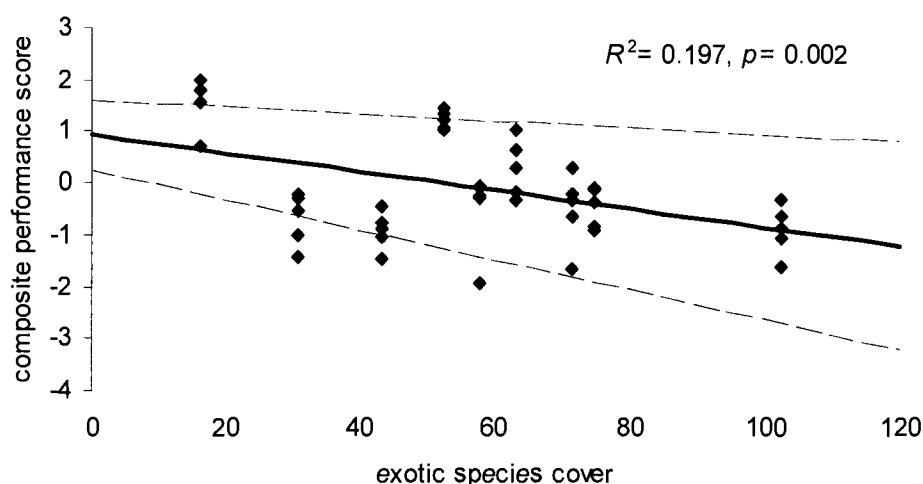
	Spring 2005	Fall 2005
<b>Source population</b>		
Ebey's Landing <sup>a</sup>	0.22 $\pm$ 0.03	0.88 $\pm$ 0.03
Forbes Point <sup>b</sup>	0.32 $\pm$ 0.03	0.87 $\pm$ 0.02
Rocky Prairie <sup>a</sup>	0.16 $\pm$ 0.04	0.84 $\pm$ 0.04
Trial Island <sup>ab</sup>	0.28 $\pm$ 0.03	0.91 $\pm$ 0.02
San Juan Valley <sup>a</sup>	0.15 $\pm$ 0.03	0.87 $\pm$ 0.03
West Beach <sup>b</sup>	0.37 $\pm$ 0.03	0.87 $\pm$ 0.03
<b>Common garden</b>		
Basket Butte 2	0.22 $\pm$ 0.04	-
Basket Butte 3	0.05 $\pm$ 0.02	0.93 $\pm$ 0.02
Basket Slough 1	0.42 $\pm$ 0.05	0.91 $\pm$ 0.03
Bell Fountain	0.14 $\pm$ 0.03	0.88 $\pm$ 0.03
Heritage Seedling	0.24 $\pm$ 0.04	0.87 $\pm$ 0.02
Kah Tai Prairie	0.30 $\pm$ 0.04	0.97 $\pm$ 0.02
Pigeon Butte	0.51 $\pm$ 0.05	0.69 $\pm$ 0.04
Plant Materials Center	0.75 $\pm$ 0.04	0.95 $\pm$ 0.02
Sandy River Delta	0.04 $\pm$ 0.02	-
Starck	0.00 $\pm$ 0.00	-

### Regression analyses

The abundance of exotic plants at common garden sites accounted for 20% of the variation in 2004 *C. levisecta* performance ( $y = 0.93 - 0.018 \times \text{exotic cover}$ ,  $R^2 =$

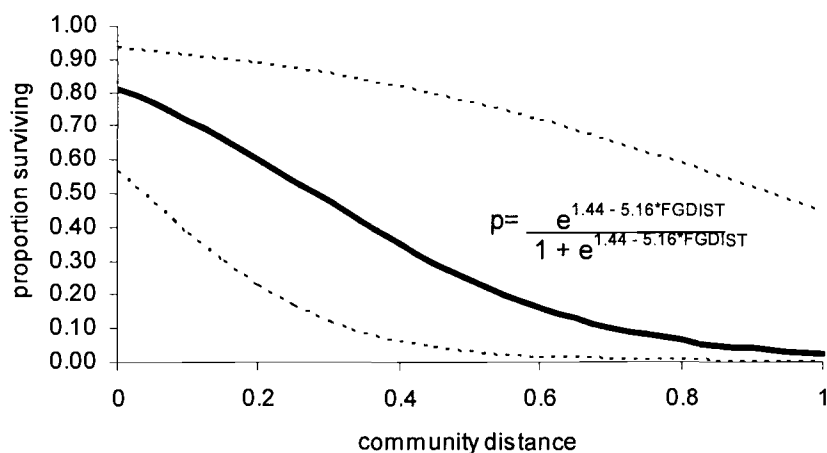


0.197). Increasing exotic species cover was associated with decreased plant performance ( $F_{1,46} = 10.56, p = 0.002$ ) (Figure 3-3).



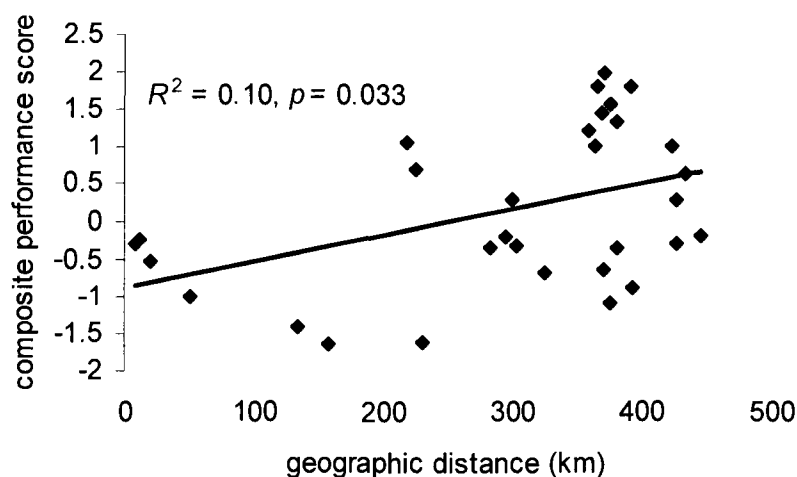
**Figure 3-3.** Scatterplot and fitted regression line of *C. levisecta* 2004 spring transplant composite performance as a function of exotic species cover at the common garden site. Dashed lines represent 95% confidence intervals.

The similarity of plant functional groups in plant communities between source populations and common gardens influenced the likelihood of *C. levisecta* spring transplant survival to 2005 ( $Dev_{1,44} = 82.8, p < 0.001$ ). Plant community distance accounted for 35.3% of the residual deviance in survival of spring transplants. The probability of survival decreased as the similarity in plant functional groups between sources and common garden sites decreased (Figure 3-4). Survival rates of fall 2005 transplants varied little; model selection indicated that the null model was better than any of the other models screened.



**Figure 3-4.** Survival of spring *C. levisecta* transplants to 2005 decreased with increasing community distance, which is a measure of the dissimilarity in plant functional group assemblage between source populations and common garden sites. Lower distance values represents greater similarity between sites. Dashed lines represent 95% confidence intervals accounting for overdispersion.

Plant performance in 2004 was weakly, but positively, correlated with the geographic distance between seed sources and common garden sites ( $F_{43,1} = 4.86$ ,  $R^2 = 0.10$ ,  $p = 0.033$ ; Figure 3-5). Geographic distance was not a good predictor of spring ( $DEV_{43,1} = 1.42$ ,  $p = 0.232$ ) or fall transplant ( $DEV_{28,1} = 3.21$ ,  $p = 0.073$ ) survival.

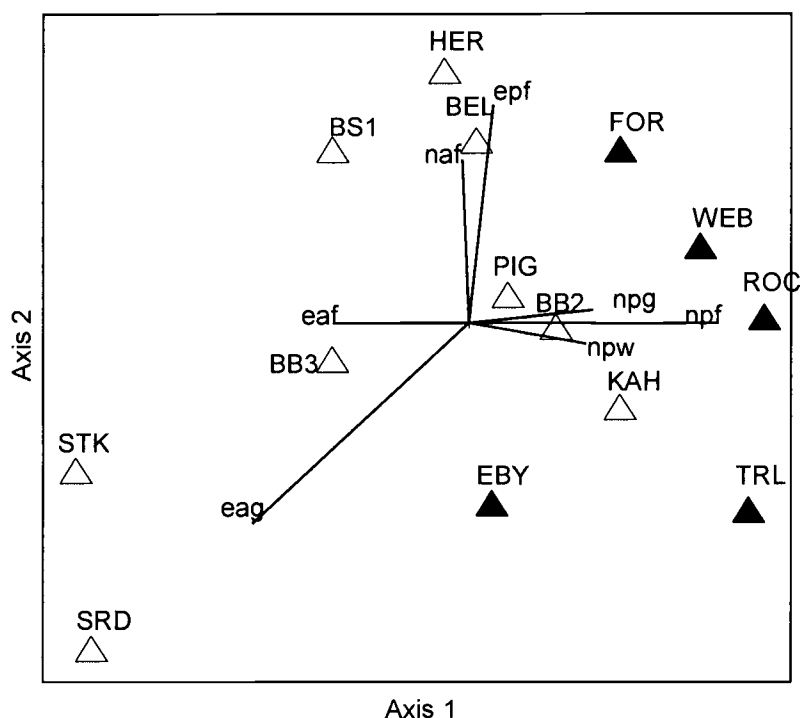


**Figure 3-5.** Scatterplot and trend line depicting a positive relationship between geographic distance and spring 2004 composite performance ( $y = -0.96 + 0.003 \times \text{geographic distance}$ ).

### Functional group patterns

A 2-D solution explained 91% of the variation in the original data set. The final configuration had lower stress than those found with Monte Carlo randomizations ( $p=0.020$ ). Most of the variation in plant functional groups was explained by axis 1 ( $r^2=0.70$ ). The ordination was rotated to align the native perennial forbs group with axis 1, which had the strongest relationship with this axis ( $r^2=0.73$ ).

Regional divergence in plant functional groups was evident among sites along axis 1 (Figure 2-3). Puget Trough sites aggregated on the right side of axis 1 which was strongly associated with native perennial forbs, grasses, and woody species ( $r^2>0.35$ ). Exotic annual forbs and grasses were associated with the left side of axis 1 ( $r^2>0.40$ ). In general, axis 1 represents a gradient from exotic annual to native perennial dominance. We observed low spring 2005 transplant survival at the common gardens Starck, Sandy River Delta, and Basket Butte 3, which were located on the left-hand side of the ordination space. Common garden sites on the right-side of axis 1, such as Pigeon Butte, Kah Tai Prairie, and Basket Butte 2, generally had a greater proportion of transplants surviving (Table 2-2). Axis 2 was strongly correlated with exotic perennials and native annual forbs ( $r^2>0.45$ ).



**Figure 3-6.** 2-D NMS solution of sample units in plant functional group space with functional group overlays. Each point represents a site (source population =▲, common garden sites=△). Vector overlays indicate the direction and strength of the relationship of plant functional groups with the axes. Axis 1 represents a gradient ranging from exotic annuals to native perennials. Vector labels are abbreviated: n= native, e= exotic, a= annual, p= perennial, g= graminoid, f= forb, w= woody. Abbreviations for source populations and common gardens are found in Table 3-1.

## DISCUSSION

### Ecological and geographic distance

*Castilleja levisecta* exhibits a “home-habitat advantage,” supporting a portion of Hypothesis 1 and suggesting that restoration sites ecologically similar to seed sources are more likely to have successful reintroductions than those that are ecologically distant. We observed higher second year transplant survival at common gardens with functional group assemblages similar to those of the transplant source population. Source populations were generally dominated by native perennial species; common gardens with similar assemblages had greater second year survival rates compared to those primarily composed of introduced annuals. Although geographic distance is often used as a measure of environmental similarity, it is not necessarily an appropriate index in this system, possibly because of the substantial differences in

habitats and great distances between extant and historic areas. Practitioners are often encouraged to use local genotypes during restoration activities, as local seed sources are more likely than non-local sources to experience similar selection pressures and be more genetically and ecologically appropriate for the recovery site (Gustafson et al. 2004b; Lesica & Allendorf 1999; McKay et al. 2005). We observed a weak, but significant, positive relationship between first year *C. levisecta* performance and the geographic distance between seed source and common garden site. This result is counter-intuitive to ecological theory and does not support the hypothesis that local sources are most appropriate for species recovery. This emphasizes the importance of using seed sources that are ecologically similar to the reintroduction site, regardless of the geographic distance involved. Matching environmental conditions between collection and restoration sites is likely to result in the use of ecologically appropriate genotypes and improve the likelihood of reintroduction success.

#### Source population $N_e$ and genetic diversity

Conservation decisions are often based on population size and levels of genetic diversity without clear evidence of the correlation between these parameters and plant performance (Reed & Frankham 2003). We did not find support for Hypothesis 2, as neither effective population size nor observed heterozygosity were correlated with *C. levisecta* fitness. The two largest *C. levisecta* populations remaining, Rocky Prairie and San Juan Valley, consistently had the lowest germination, performance measures, and survival rates in our study. Our findings support the work of others that have found individuals from small populations to be as or more viable than larger populations (Lammi et al. 1999; Ouborg & Treuren 1995), emphasizing their potential value for *ex situ* conservation. Several studies suggest that the use of neutral markers is not an effective indicator of adaptive genetic differences (Britten 1996; David 1998; Savolainen & Hedrick 1995). Molecular markers reflect patterns of historical gene flow and genetic drift, but do not necessarily lead to a better understanding of a species' adaptive potential (McKay et al. 2005). Quantitative measures of phenotypic variation are considered to be better estimates of adaptively significant genetic diversity (Storfer 1996; Young et al. 1996), and are only weakly correlated with

molecular marker diversity (Pfrender et al. 2000; Reed & Frankham 2001).

Quantification of the genetic variation underlying traits associated with morphology, physiology, and life history attributes would likely be a more effective measure of adaptively significant variation in *C. levisecta*. Further examination of population dynamics of fragmented species is necessary to elucidate the mechanisms influencing plant performance with respect to population size and genetic diversity.

#### Habitat quality

We found strong evidence to support Hypothesis 3, as components of *C. levisecta* fitness were significantly influenced by the habitat quality of the reintroduction site. This is consistent with reviews of the avian and mammalian literature which indicate that translocation success is limited to sites with high quality habitat (Griffith et al. 1989; Wolf et al. 1996). *Castilleja levisecta* performance decreased with increasing abundance of non-native plants at common garden sites during the first growing season. Exotic species pose a serious threat to both remaining populations and reintroduction efforts, possibly because *C. levisecta* is a poor competitor or because non-native species are inappropriate hosts (further discussed below). While field herbivory during the first growing season did not significantly influence *C. levisecta* performance or second year survival, the 2005 population outbreak of the grey-tailed vole (*Microtus canicaudus*) impacted virtually all *C. levisecta* transplants in the Willamette Valley and devastated the grass seed crop in the region. Voles are important grassland herbivores subject to boom-bust cycles and are capable of altering vegetation dynamics in both native and agricultural systems. The 2005 outbreak was a regional phenomenon, as *C. levisecta* populations in Washington also suffered from vole herbivory (P. Dunwiddie, pers. comm.). A mild Pacific Northwest 2004-05 winter and the cyclical nature of vole abundance likely contributed to this occurrence.

#### *C. levisecta* competitive ability & host dynamics

The competitive ability of dominant species at recovery sites appears to be an important factor contributing to *C. levisecta* performance. *Castilleja levisecta* is a

shade intolerant species, native to grasslands that were burned frequently by Native Americans (Boyd 1986; Norton 1979). Fire suppression and exotic invasion has changed the community dynamics of remaining Pacific Northwest prairies (MacDougall et al. 2004). Invasion by exotic grasses coupled with abundant litter accumulation in these prairies may result in native species being outcompeted for abiotic resources. Exotic grasses may intercept light and limit the photosynthetic capacity of native competitors (D'Antonio & Vitousek 1992). Reproductive *C. levisecta* individuals reach an average height of 30 cm, while many exotic grasses common to the region are up to five times taller (e.g., *Arrhenatherum elatius*). *Castilleja levisecta* individuals growing beneath a canopy of grass and litter often appeared chlorotic and stressed. Other observations also indicate that *C. levisecta* may be a poor competitor. Transplant performance was greatest at three common garden sites that appeared to have low above-ground biomass, including the Plant Materials Center which had no above-ground biomass other than the *Festuca roemerii* host we provided. Two of these sites had been recently managed prior to common garden establishment; Heritage Seedling was tilled and seeded with native prairie species, while Basket Slough 1 had been burned. Habitat manipulations such as fire, herbicide application, and mowing are often employed to reduce competition from exotic species and can improve the growth and reproductive output of endangered plants (Guerrant 1996b; Kaye et al. 2001; Pavlik et al. 1993).

Native and appropriate host species may be limited at sites dominated by exotic annuals. Although *C. levisecta* is capable of utilizing a diversity of host species in the greenhouse, host quality and availability may be a limiting factor in the field. Fitness and survival of *C. levisecta* transplants was lowest at sites with a high abundance of exotic annuals, possibly because annuals allocate few resources to below-ground structures and die after reproduction. Host plants can provide water and nutrients to hemiparasites during periods of critical environmental stress (Press 1989). Systems dominated by annuals likely do not have sufficient below-ground resources to support *C. levisecta* individuals, especially during summer drought conditions typical of the Pacific Northwest, when annuals usually senesce. Perennial species are essential hosts for several other parasitic plants that live in arid habitats (Coats et al.

1993; Marvier & Smith 1997; Pate et al. 1990; Sprague 1962) and may allow parasites to take advantage of deep roots and enable them to exploit nutrients and water from a large volume of soil.

We have evidence that indicates co-planting a perennial host with *C. levisecta* transplants improves second year survival. The Plant Materials Center common garden was established in an agricultural field where *C. levisecta* transplants were planted with a perennial grass (*F. roemerii*). Spring transplant survival to 2005 was particularly high ( $\bar{x} = 0.75$ ) at this site, and no *C. levisecta* individuals were observed without a live host. During experimental field studies with *C. levisecta*, Wayne (2004) found individuals co-planted with *F. roemerii* were more successful than those not provided with a host. Host plants were generally not provided in our study, allowing plants to make haustorial connections with existing root systems. Haustorial connections were evident between *C. levisecta* and nearby roots during excavation of some transplants, including *Arrhenatherum elatius*, a non-native perennial grass. Successful conservation and restoration of parasitic plants necessitates the management of thoughtfully selected host populations (Marvier & Smith 1997). We further investigated *C. levisecta* host requirements and preference in a companion study (Chapter 4).

#### Summer drought as a selective force

Summer drought appears to be a strong selective force resulting in differential transplant mortality. Natural populations of *C. levisecta* emerge in early March and become senescent in late summer in response to summer drought conditions (Caplow 2004). Survival was very high for first year transplants, and generally did not vary among sources or planting sites. At the time of monitoring in 2004, spring transplants had yet to experience a summer drought (nor had fall transplants when sampled in 2005). Survival rates for first year transplants were high (spring 2004  $\bar{x} = 0.99$ , fall 2005  $\bar{x} = 0.88$ ), but dropped dramatically the second growing season (spring 2005  $\bar{x} = 0.22$ ). Others working with *C. levisecta* have also observed high second year mortality rates in the field (Swenerton 2003; Wayne 2004). Second year survival rates were greater for transplants that performed well during their first growing season



compared with those that performed poorly (B. Lawrence, unpublished data), indicating that first year performance measures may be indicative of future survival.

### Management Implications

Plant community composition of the reintroduction site will likely play a critical role in future *C. levisecta* recovery efforts. Our work suggests that high quality prairies should be targeted for reintroduction in the Willamette Valley, OR. Non-native plant cover at common garden sites was an important factor associated with reduced transplant vigor, suggesting that competitive interactions with exotic species are likely to play an important role in *C. levisecta*'s re-establishment. Native perennial communities are likely to support more host species that are appropriate for *C. levisecta* than those dominated by exotic annuals. Sites dominated by invasive species, particularly invasive annuals, should be avoided, while sites that are functionally similar to source populations, in particular those with high native perennial abundance, should be targeted. Although our results did not indicate that soil similarity influenced *C. levisecta* performance, we believe that sites in the Willamette Valley with relatively well drained soils are most appropriate since all remaining *C. levisecta* populations are found on well-drained sandy soils.

Active site management will be a critical component of *C. levisecta* reintroduction success. Steps to limit exotic abundance and the accumulation of biomass, as well as ameliorate native community composition and structure, should be taken at reintroduction sites. Clearly herbivore management, including voles as well as other mammals known to graze *C. levisecta* (i.e., deer, rabbits), will be an important component of recovery efforts and may coincide with habitat management objectives. Prescribed burns during times of *C. levisecta* dormancy have been used to manage vole populations at extant populations (P. Dunwiddie, pers. comm.). Fire consumes litter and reduces cover leaving voles more susceptible to predation and may also alleviate competition for light resources. Further, evidence suggests that *C. levisecta* responds favorably to fire in Washington prairies (Dunwiddie et al. 2000). Fencing may also be used to deter herbivores. Two and a half meter tall fences effectively excluded large mammals from our common garden experiments, and wire cages dug

into the ground successfully prevented rabbit herbivory at a site on Whidbey Island (B. Lawrence, pers. obs.). A regional study aiming to promote native abundance in degraded Pacific Northwest prairies is currently investigating the effectiveness of management techniques that include burning, mowing, grass-specific herbicide use, and seeding with native species. Results from this work will likely have relevant implications for prairie habitat management during *C. levisecta* recovery (T.N. Kaye, pers. comm.).

We do not recommend using genetic diversity, effective population size, or geographic distance as indices of appropriate *C. levisecta* seed sources for recovery efforts in the Willamette Valley. Instead we suggest that plant material from Whidbey Island populations be utilized, as these populations consistently had high germination rates, vigorous greenhouse growth, and superior field performance. Five of the eleven remaining populations of *C. levisecta* are found on Whidbey Island, three of which were represented in this study. Whidbey Island populations are relatively close together (within 15 km of each other), are considered to be ecologically similar (Chappell & Caplow 2004), and evidence suggests that recent gene flow has occurred among these populations (Godt et al. 2005). While the remaining *C. levisecta* populations on Whidbey Island are currently fragmented, these populations are considered a single genetic/geographical group and were probably less isolated from each other in the past (Chappell & Caplow 2004).

The merits of using single versus multiple seed sources during restoration activities is heavily debated in the conservation literature (Guerrant 1996a; Kaye 2001a). Recovery efforts utilizing plant material from a single source may restrict ecologically relevant genetic variation that is necessary for evolutionary resilience, and may also result in restored populations with cross-incompatible mating types (DeMauro 1993). Meanwhile, use of mixed seed sources can result in outbreeding depression, which is a growing concern in the restoration community (Hufford & Mazer 2003). We believe however that the use of multiple Whidbey Island sources for recovery efforts in the Willamette Valley is justified. We observed heterosis in the F1 during a preliminary analysis of *C. levisecta*'s breeding system, with increased size, flowering rate, and seed set with increasing genetic distance of the cross, ranging

from self, sibling, intra-population to inter-population crosses (Kaye & Lawrence 2003). Based on these results, we would predict that reintroductions composed of genetic material from multiple source populations would have greater fitness and be more likely to succeed than those created from a single source, at least in the short term. Although outbreeding depression may not be detected until the F2 or further generations, this phenomenon is theoretically restricted to crosses between ecologically and genetically distinct populations. Currently, we believe that the putative Whidbey Island meta-population is the most appropriate seed source for *C. levisecta* recovery efforts in the Willamette Valley, OR.

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## Chapter 4 : Direct and indirect effects of host plants: Implications for reintroduction of an endangered hemiparasitic plant (*Castilleja levisecta*)

### ABSTRACT

Rare, parasitic plants pose an interesting challenge to restoration practitioners. In addition to the problems associated with small population size, rare parasites may also be limited by their host requirements. We examined how the performance of *Castilleja levisecta* (Scrophulariaceae), a rare hemiparasite, was affected by the availability of different host combinations in the greenhouse and in the field. *Castilleja levisecta* individuals were grown with two grass individuals (*Festuca roemerii*; Poaceae), two non-grass individuals (*Eriophyllum lanatum*; Asteraceae), one individual of each of these species, or without a host. Our greenhouse results provide little support for the complimentary diet hypothesis, which states that parasites grown with multiple host species perform better than individuals grown alone or with a single host. *Castilleja levisecta* individuals grown with two different species performed better than those co-planted only with *F. roemerii*, but did not differ from *E. lanatum* or no-host treatments. In the field, vole activity had indirect effects on *C. levisecta* survival mediated through host species. Vole tunneling and *C. levisecta* mortality were strongly associated with host treatments including *E. lanatum*. Field survival was significantly higher among no-host *C. levisecta* individuals than those grown with *E. lanatum* or mixed host treatments.

Our results emphasize the importance of basing conservation decisions on experimental research conducted under conditions similar to those of the intended application. Our greenhouse results were a poor predictor of field performance. Increases of *C. levisecta* seed are possible in a horticultural setting with any of the host treatments we tested if adequate water and nutrients are supplied. However, we do not suggest co-planting *C. levisecta* with *E. lanatum* in the field. Although no-host *C. levisecta* individuals had the greatest first year field survival, we suspect that perennial host plants will be beneficial to future survival. Therefore, we recommend planting *C. levisecta* in the Willamette Valley, OR with *F. roemerii*.

### INTRODUCTION

Parasitic plants are an important component of many plant communities because they are capable of altering productivity (Marvier 1998b; Matthies 1997), competitive interactions (Gibson & Watkinson 1991; Matthies 1996), and community structure (Gibson & Watkinson 1992; Press 1998). Although many parasitic plants are considered agricultural pests, some are of conservation concern (Marvier & Smith 1997). Rare parasitic species pose an interesting challenge to restoration practitioners. In addition to the diversity of obstacles during typical reintroductions, rare parasites may also be limited by their host requirements. Uncertainties associated with the availability and quality of hosts at restoration sites, as well as parasite host specificity, are likely to impede recovery of parasitic plants (Marvier & Smith 1997). Successful management of rare parasites necessitates consideration of their unique biology. We conducted greenhouse and field experiments with *Castilleja levisecta*, a hemiparasite endemic to the prairies of the Pacific Northwest, to evaluate its host preferences and support recovery actions in the southern portion of its' historic range.

Although facultative hemiparasites are photosynthetic and do not require a host plant, they often form haustoria, or physical connections, with other root systems to obtain nutrients, water, and secondary compounds from their host (Kuijt 1969; Press 1989). In natural systems, unattached mature facultative parasites are rare, and attachment to a host generally stimulates the parasite's growth (Kuijt 1969). Most members of the genus *Castilleja* are considered generalist hemiparasites, capable of parasitizing multiple host species (Dobbins & Kuijt 1973; Heckard 1962). However, the degree to which a host stimulates hemiparasite fitness varies considerably among host species (Chuang & Heckard 1971; Gibson & Watkinson 1992; Marvier 1998b; Matthies 1996, 1997; Seel & Press 1993). Interactions between plant parasites and host species can have direct and indirect effects on both host and parasite performance, as well as their pollinators (Adler et al. 2001) and herbivores (Adler 2002; Adler 2003; Adler et al. 2001; Marko 1996; Marvier 1996). For example, parasitic plants can acquire secondary compounds from host species (Govier et al. 1967; Schneider & Stermitz 1990; Stermitz & Harris 1987), which in turn can alter species interactions. Acquisition of alkaloids from the host *Lupinus albus* directly

reduced insect herbivory of *Castilleja indivisa*, and indirectly increased pollination (Adler et al. 2001).

In the field, hemiparasitic plants often parasitize several hosts simultaneously (Gibson & Watkinson 1989; Matthies 1996). The complimentary diet hypothesis proposes that generalist consumers perform better on a mixed diet relative to a homogenous diet, due to improved nutrient balance and/or dilution of toxic secondary plant compounds (Bernays et al. 1994). Many taxa benefit from multiple food sources, including insects (Bernays et al. 1994), gastropods (Pennings et al. 1993), reptiles (Bjorndal 1991), and hemiparasitic plants (Marvier 1998a). Therefore, we believe that providing multiple host species for rare parasites may increase fitness components compared to single or no-host plantings and facilitate recovery.

*Castilleja levisecta* is a federally listed hemiparasite currently restricted to eleven sites in the Pacific Northwest and extinct in the southern portion of its historic range including the Willamette Valley, Oregon. Recovery criteria for *C. levisecta* call for the existence of twenty populations composed of 1,000 flowering individuals (U.S.F.W.S. 2000). However, the species has limited capacity for natural dispersal and colonization of new sites, necessitating *ex situ* conservation techniques to meet recovery goals. A strategic reintroduction plan has been prepared to support the long-term viability of *C. levisecta* and calls for the establishment of new populations within its historic range (Caplow 2004). Although several studies have investigated *C. levisecta* host use, clarification of its host requirements in a restoration context is necessary before large scale reintroduction efforts are pursued.

While *C. levisecta* does not require a host to reproduce in a greenhouse environment and does not appear to be host specific (Wentworth 2001), evidence suggests co-planting *C. levisecta* in the field with a perennial host increases reproductive output (Wayne 2004), and possibly survival rates (B. Lawrence personal observation). Greenhouse observations indicate that *C. levisecta* can form haustorial connections with several perennial prairie species (e.g., *Leucanthemum vulgare*, *Eriophyllum lanatum*, *Festuca roemerii*, and *Fragaria vesca*), and with itself when grown alone (Kaye 2001; Wentworth 2001). Field experiments indicate that outplanting *C. levisecta* with *F. roemerii* increases the number of inflorescences

produced compared to no-host controls, although host presence did not effect field survival rates (Wayne 2004). In addition, the species is frequently eaten by small mammals (Caplow 2004; Wayne 2004), but we are not aware of any previous evaluations of host-mediated effects of herbivory on *C. levisecta*.

Here, we present theoretical and practical explorations of *C. levisecta* host use during greenhouse and field studies in the Willamette Valley, OR. We test the hypothesis that *C. levisecta* individuals grown with multiple host species perform better than individuals grown alone, or with a single host species, and that host-interactions affect herbivory under field conditions. We compare our greenhouse and field results and provide recommendations for future *C. levisecta* recovery efforts.

## METHODS

### Study species

*Castilleja levisecta* (Orobanchaceae) is a short-lived (5-6 years), multi-stemmed, perennial endemic to the native grasslands of the Western Pacific Northwest. It is an out-crossing species primarily pollinated by *Bombus* spp. and is known only to reproduce by seed (Kaye & Lawrence 2003; Wentworth 2001). The eleven remaining *C. levisecta* populations are concentrated in the San Juan Islands of the Puget Trough eco-region, and are found on sandy, well drained soils of glacial origin (Chappell & Caplow 2004). Only one inland prairie population remains in Thurston County, WA, while the ten island populations are generally located on southwest facing coastal prairies that are dominated by graminoids and forbs (Chappell & Caplow 2004). Despite the species' rarity, its remaining populations maintain unusually high levels of genetic diversity compared with other endemic species and even other members of the figwort family (Godt et al. 2005).

*Castilleja levisecta* was last collected in the Willamette Valley, OR in 1938 (Gamon 1995). All that remains of the historic populations of this region are herbarium records, which do not describe the specific location or habitat of these populations. Based on information from herbarium specimens and site visits, we believe that *C. levisecta* once inhabited upland prairies in the Willamette Valley. These grass dominated systems are often associated with *Quercus garryana*-savanna

that occur on well-drained soils commonly found on the valley foothills (Franklin & Dyrness 1988). The unglaciated soils of these foothills are dominated by clay and silt from weathering basalt and are generally well-drained.

### Greenhouse experiment

To test for differences in *C. levisecta* performance in different host combinations, we randomly assigned individuals to one of four host treatments, including no-host (control), two *E. lanatum* individuals, two *F. roemerii* individuals, or one individual of each of these host species ("mixed hosts"). We used plant material from two *C. levisecta* source populations, Ebey's Landing and Forbe's Point, located on Whidbey Island, WA. Twenty host treatment replicates were conducted for each source population, for a total of 40 replicates per host treatment (n= 160).

*Eriophyllum lanatum* and *F. roemerii* were used as host plants because *C. levisecta* forms haustorial connections with them (Wayne 2004, B. Lawrence pers. obs.). Additionally, these species are native perennials common to the prairies of the Pacific Northwest and are likely to be present at reintroduction sites.

*Castilleja levisecta* seed capsules were collected from source populations in August 2003 to provide seeds for this experiment. Seeds were moist-cold stratified on filter paper for six weeks at 5 °C, then transferred to a warm room for one week with light and temperature on a 12 hour cycle (15 °C/25 °C). On 1 December 2003, germinants were planted into cell flats in a well-drained medium (Sunshine Mix #4 Aggregate Plus) amended with slow release micro- and macro- nutrients. Plants were grown in a greenhouse with 400 watt high pressure sodium lights and temperature fluctuating every 12 hours (12 °C/18 °C).

Host treatments were randomly assigned on 28 January 2004 and were transplanted into gallon pots. *Castilleja levisecta* individuals and potential hosts were planted in a triangle with all plants 10 cm apart (Figure 4-1); *C. levisecta* individuals assigned the no-host treatment were planted in the center of the pot. *Eriophyllum lanatum* plants were rooted cuttings from Willamette Valley genetic stock provided by Heritage Seedling Co., Salem, OR. We used *F. roemerii* individuals grown from Willamette Valley seed that were a year old when paired with *C. levisecta*. We

attempted to equalize above- and below-ground biomass among provided hosts. Plants were randomized on greenhouse benches and fertilized biweekly with a liquid 15-30-15 fertilizer. Plants were moved to a shade-house in June 2004 and received supplemental water throughout the summer. We recorded the total stem length, stem number, and flower number on each *C. levisecta* individual on 10 May 2004, approximately 15 weeks after potting the parasites and hosts together.

#### Field experiment

To test our host and herbivore hypotheses under field conditions, we transplanted the same potted plants with hosts used in the greenhouse study to an upland prairie on 1 December 2004. Our study site was located at Pigeon Butte, Finley National Wildlife Refuge, OR, in habitat likely to be used for future *C. levisecta* recovery efforts in the Willamette Valley. The site had a high diversity of native perennials and abundant invasive non-native pasture grasses (e.g., *Festuca arundinacea* and *Arrenatherum elatius*). Pigeon Butte is situated on the shoulder of a butte at 150 m elevation and is dominated by Typic Argixerolls of the Dixonville series that are well-drained, silty-clay-loam soils, formed from weathered basalt. Average annual precipitation is approximately 115 cm, with average annual minimum and maximum temperatures of 5°C and 17°C, respectively (WRCC 2005).

We randomly planted host-parasite replicates into square meters within a 10m x 15m grid. A balanced design could not be executed in the field because many of the greenhouse plants died during a malfunction of the automatic watering system in summer 2004. However, at least 22 replicates of each of the four host treatments were transplanted into the field (n= 121). *Castilleja levisecta* individuals at the time of outplanting were mostly dormant, with little above-ground biomass.

Field transplants were monitored on 23 May 2005. Vole abundance was unusually high in the Willamette Valley, OR during the 2005 growing season. All surviving *C. levisecta* individuals at Pigeon Butte were subjected to some herbivory, most likely from grey-tailed voles (*Microtus canicaudus*). Stem length and number were not reliable measures of *C. levisecta* performance, as herbivory appeared to stimulate resprouting and alter plant morphology (B. Lawrence, pers. obs.) Therefore, we measured *C. levisecta* survival as the response variable for the field component of

our study. Vole tunneling was also very frequent, indicating herbivore pressure occurred in the root zone as well as above ground. Tunnels were unevenly distributed throughout the study area, so we measured herbivore pressure by voles as presence or absence of their tunnels within 15cm of the transplant root crown.

#### Statistical analyses

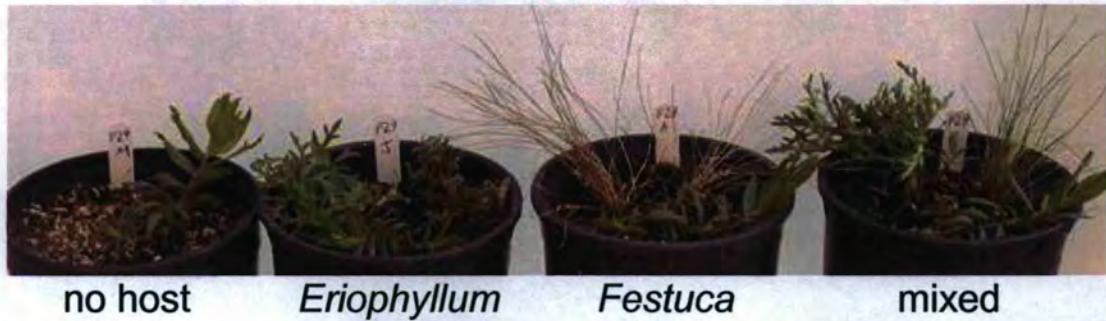
Because our measures of plant size and fitness (stem length, stem and flower number) were correlated with one another, we combined them into a single index of plant performance for each greenhouse plant using Principal Components Analysis (PCA) in PC-ORD v.4.25 (McCune & Mefford 1999). The objective of PCA is to find the strongest linear correlation structure among variables and reduce them to a smaller number of synthetic variables that represent the most information in the original data set (McCune & Grace 2002). Prior to PCA, total stem length and stem number were log transformed to improve homoscedasticity.

PCA scores from axis 1 were used as the response variable in analysis of variance (ANOVA) to determine if performance differed among host treatments in the greenhouse. Source population and maternal effects were used as blocking factors in this analysis because differential growth among populations and individuals from different maternal lines has been observed in *C. levisecta* (Kaye 2001). This and all further data analyses were conducted in S-PLUS v. 6.2 (Insightful 2000). The significance of all pair-wise host treatment comparisons were tested using Dunn-Sidak corrections.

We tested for differences in *C. levisecta* field survival and vole tunnel presence among host treatments using binary logistic regression analyses. We used drop in deviance tests with a chi-square distribution to test for significance. Dunn-Sidak corrections were used to correct for multiple pair-wise comparisons between host treatments. We tested the linear relationship between vole tunnel presence and plant survival by host treatment using linear regression. Finally, we calculated an odds ratio to compare the odds of *C. levisecta* survival when co-planted with *E. lanatum* vs. the odds of survival when not planted with this species.

## **RESULTS**

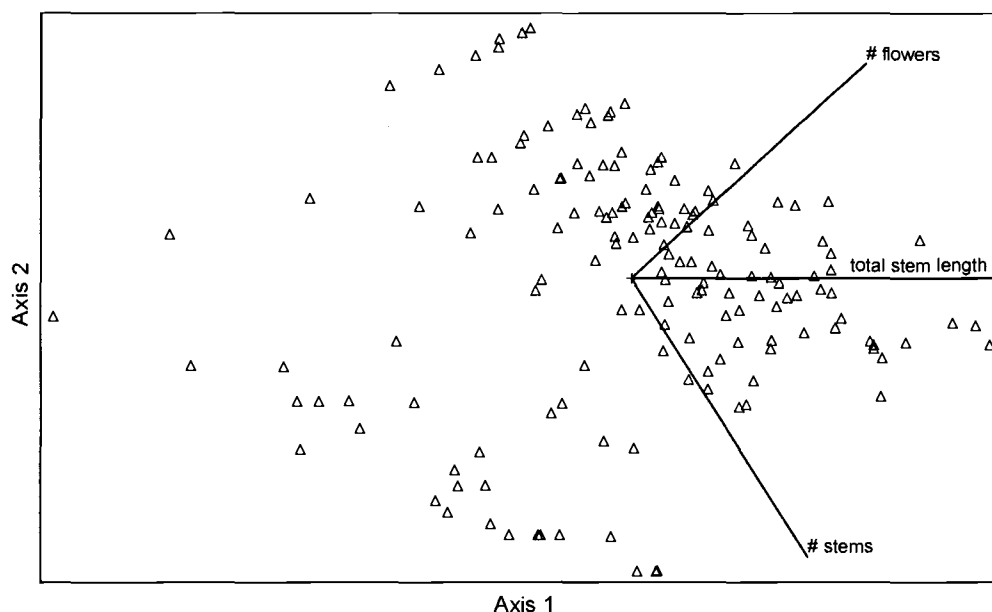




**Figure 4-1.** Host treatments one week after potting *C. levisecta* and hosts together.

#### Greenhouse experiment

The first PCA component accounted for 59.9% of the variation in stem length, stem number, and flower number. Comparison of eigenvalues to those produced by a random model indicated that only the first axis contained more information than expected by chance ( $p = 0.020$ ), supporting our decision to use scores from this axis in further analyses. The solution was rotated to align stem length with axis 1 (Figure 4-2), because this axis was most highly correlated stem length ( $r^2 = 0.93$ ). Axis 1 also accounted for substantial variation in flower and stem number ( $r^2 = 0.50, 0.38$ , respectively). Axis 2 is primarily a function of flower and stem number ( $r^2 = 0.46, 0.59$ , respectively). Large, flowering plants are located in the upper right-hand region of the ordination space, while small, non-reproductive plants with few stems are located in the lower-left portion of the ordination. The component loadings (eigenvectors) for the first principal component are: stem length (0.718), stem number (0.457), and flower number (0.526). The average performance measures associated with PCA scores for each host treatment are presented in Table 4-1.

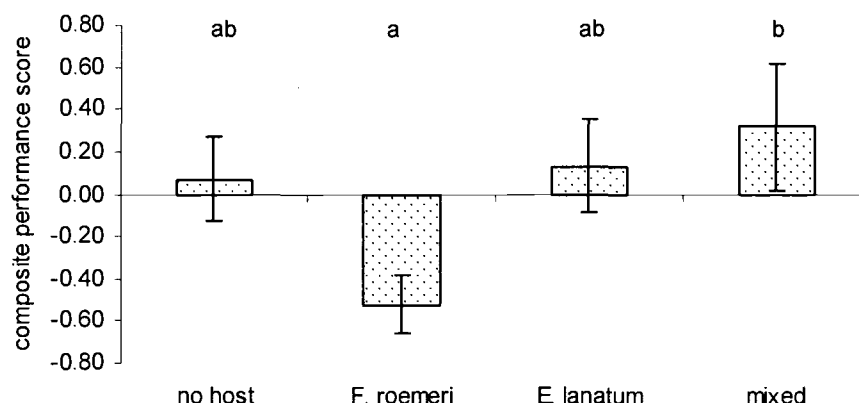


**Figure 4-2.** Plot of the first two principal components for 159 greenhouse grown *C. levisecta* individuals based on three measurements of performance. Each point represents a *C. levisecta* individual. Vector overlays of the performance measures depict the strength and direction of correlation with the two axes.

**Table 4-1.** Mean ( $\pm 1$  SE) *C. levisecta* composite performance (PCA) scores and stem length, flower number and stem number for plants grown in the greenhouse under each host treatment.

host treatment	PCA score	stem length (cm)	# flowers	# stems
<i>F. roemerii</i>	$-0.52 \pm 0.14$	$63.7 \pm 4.4$	$29.4 \pm 3.3$	$5.33 \pm 0.51$
no host	$0.07 \pm 0.20$	$80.7 \pm 6.4$	$36.9 \pm 4.5$	$7.24 \pm 0.60$
<i>E. lanatum</i>	$0.13 \pm 0.22$	$89.0 \pm 8.1$	$34.5 \pm 3.8$	$7.03 \pm 0.81$
mixed	$0.32 \pm 0.30$	$92.6 \pm 10.4$	$36.7 \pm 5.2$	$7.88 \pm 0.77$

*Castilleja levisecta* performance differed significantly among host treatments in the greenhouse ( $F_{3, 122} = 3.22, p = 0.025$ ) (Figure 4-3). Performance differed among maternal lines ( $F_{32, 122} = 2.03, p = 0.003$ ), but overall, *C. levisecta* individuals from the two source populations performed similarly ( $F_{1, 122} = 0.013, p = 0.91$ ). *Castilleja levisecta* individuals grown with mixed hosts were significantly larger on average than those grown with *F. roemerii*, but did not differ from those grown without a host or with *E. lanatum* (Figure 4-3).

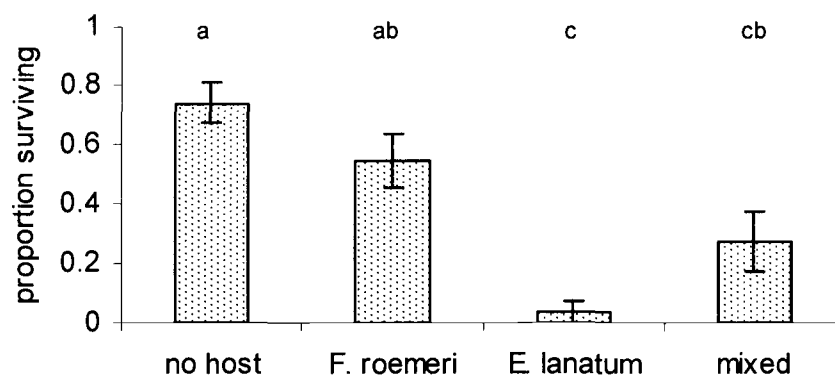


**Figure 4-3.** *Castilleja levisecta* greenhouse performance differed among host treatments ( $\pm 1$  SE). Host treatments not sharing a common letter were significantly different ( $p \leq 0.05$ ) after Dunn-Sidak corrections.

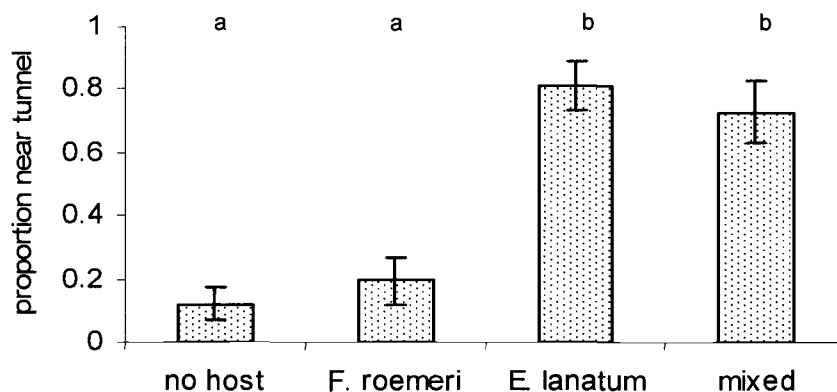
#### Field experiment

Field survival of *C. levisecta* differed significantly among host treatments ( $DEV_{3, 83} = 44.65, p < 0.001$ ), which accounted for 26.8% of the residual deviance in the model. Neither source population ( $DEV_{1, 83} = 0.089, p = 0.77$ ) nor maternal line ( $DEV_{32, 83} = 34.43, p = 0.40$ ) accounted for significant residual deviance. No-host *C. levisecta* individuals survived significantly more often than those planted with either *E. lanatum* or mixed hosts, but their survival did not differ from plants with *F. roemerii* hosts (Figure 4-4). Also, *C. levisecta* planted with *F. roemerii* hosts had significantly higher survival than those planted with *E. lanatum*.

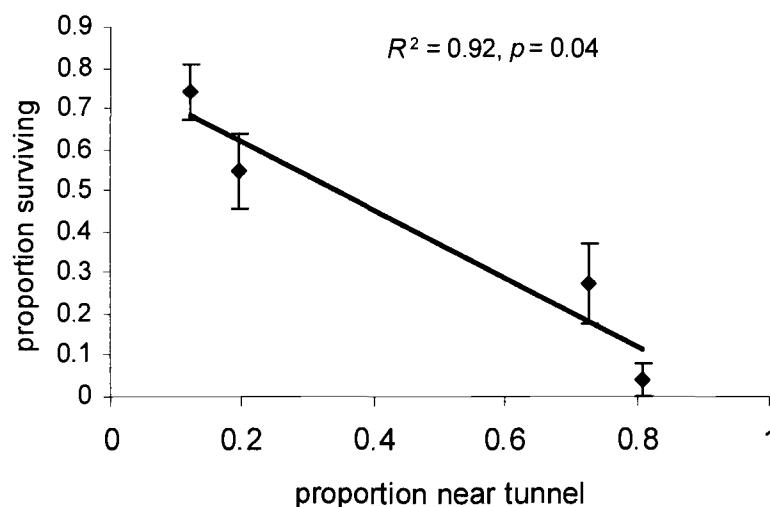
The presence of rodent tunnels near host transplants was significantly different among host treatments ( $DEV_{3, 117} = 50.17, p = 0.40$ ). *Castilleja levisecta* individuals co-planted with *F. roemerii* or without a host had significantly fewer rodent holes near their root crowns compared to those planted with either *E. lanatum* or mixed hosts (Figure 4-5). In addition, we measured a strong inverse relationship between *C. levisecta* survival and the presence of tunneling within the vicinity of the root crown ( $F_{1,2} = 23.07, p = 0.04, R^2 = 0.92$ ) (Figure 4-6). The odds of a *C. levisecta* transplant surviving in the field when planted without an *E. lanatum* host was 11.25 (95% C.I. = 4.29, 28.78) times greater than when co-planted with an *E. lanatum* host.



**Figure 4-4.** *Castilleja levisecta* field survival by host treatment ( $\pm 1$  SE). Host treatments not sharing a common letter were significantly different ( $p \leq 0.05$ ) after Dunn-Sidak corrections.



**Figure 4-5.** Proportion of *C. levisecta* transplants located within 15cm of rodent tunnels ( $\pm 1$  SE). Host treatments not sharing a common letter were significantly different ( $p \leq 0.05$ ) after Dunn-Sidak corrections.



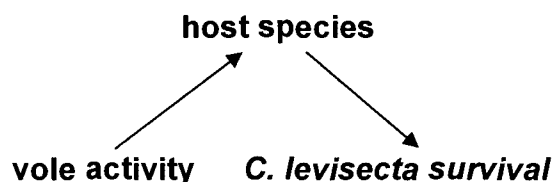
**Figure 4-6.** Scatterplot and trendline of the average proportion of *C. levisecta* transplants within 15 cm of a vole tunnel and average transplant survival for each host treatment.

## DISCUSSION

Our results provide little support for the complimentary diet hypothesis that predicts individuals with multiple nutritional sources will perform better than those set on a limited diet. In fact, no-host controls performed as well or better than *C. levisecta* individuals co-planted with multiple host species in both greenhouse and field environments. Multiple host species may provide complimentary resources for *C. levisecta* individuals by improving nutrient balance or possibly diluting any harmful secondary compounds obtained from either host. However, the mechanism responsible for improved *C. levisecta* greenhouse performance when grown with mixed hosts compared with *F. roemerii* is unclear. Marvier (1998a) observed that half of the *C. wightii* individuals grown with mixed hosts failed to establish functional connections with one of the host species. We did not look for haustoria between *C. levisecta* and associated hosts or conduct chemical analyses of plant tissues, because such destructive sampling would have precluded our field study. We believe our greenhouse observation that *F. roemerii* is a poor host in pots may be explained by root competition: *F. roemerii* host treatment replicates were generally more root bound than other host treatments (B. Lawrence, pers. obs.). This is consistent with our previous work that found *C. levisecta* grown in pots were smaller and flowered less frequently

in the second growing season when potted with *F. roemerii* than with *E. lanatum* (Kaye 2001).

*Castilleja levisecta* survival in the field did not support the complimentary diet hypothesis as the mixed host treatment was indirectly and negatively affected by herbivore activity.



**Figure 4-7.** Schematic of the indirect effect of vole activity on *C. levisecta* survival mediated by host species.

Vole activity had strong indirect effects on *C. levisecta* field survival mediated through host species (Figure 4-7). Populations of the grey-tailed vole (*Microtus canicaudus*) exploded in the Willamette Valley during the 2005 field season due to a mild winter in 2004-05, increasing herbivore pressure on *C. levisecta* transplants and devastating the region's grass seed crop. In fact, nine Oregon counties were declared agricultural disaster areas by the U.S. Department of Agriculture due to large crop losses (A.P. 2005). While herbivory was evident on all surviving *C. levisecta* individuals at Pigeon Butte, vole tunneling and field mortality were strongly associated with host treatments that included *E. lanatum*, whose roots appear to be particularly palatable to voles. *C. levisecta* plants co-planted with two *E. lanatum* individuals had higher mortality and were associated with greater tunneling activity than those co-planted with a single *E. lanatum* individual (mixed host), although these effects were not strictly additive. The mechanism contributing to high mortality of *C. levisecta* individuals associated with *E. lanatum* is unclear, but root system disturbance, direct grazing of *C. levisecta* roots, or the indirect effect of reduced host vigor/survival likely contributed to this observation. Meanwhile, no-host *C. levisecta* individuals and those planted with *F. roemerii* generally had much higher survival rates and less rodent tunneling. We believe this indicates that voles did not just target potting soil or areas with low root density to tunnel in, but were specifically attracted



to *E. lanatum*. Foliage and roots of the genus *Eriophyllum* contain sesquiterpene lactones (Bohlmann et al. 1981), which occur in the majority of tribes in the Asteraceae family (Seaman 1982). This group of compounds is extremely diverse; some act as toxins and feeding deterrents to a variety of invertebrate and vertebrate species, while others exhibit anti-fungal, anti-bacterial, anti-tumourgenic, or anti-inflammatory properties and may be desirable to herbivores (Picman 1986). The chemical attributes of *E. lanatum* likely contributed to increased vole tunneling in its' vicinity.

Results from our greenhouse and field studies suggest that co-planting *C. levisecta* with a host may not be necessary. Although our findings were likely context dependant, no-host controls performed as well or better than all other host treatments in both greenhouse and field environments. Host plants can provide water and nutrients to hemi-parasites during periods of critical environmental stress (Kuijt 1969; Press 1989). However, given ideal growing conditions with ample water, nutrients, and light, *C. levisecta* individuals produced abundant biomass and had high reproductive output without host plants. In our field study, no-hosts *C. levisecta* individuals had the highest proportion surviving ( $\bar{x} = 0.78 \pm 0.07$ ). However, at the time of monitoring in 2005, these plants had yet to experience summer drought conditions typical of the Pacific Northwest. Natural populations of *C. levisecta* emerge in early March and senesce in late summer in response to dry conditions (Caplow 2004). Other work suggests that summer drought is a strong selective force resulting in differential *C. levisecta* transplant mortality, as field survival is typically high the first growing season, but drops dramatically the second growing season (B. Lawrence pers. obs.; Swenerton 2003; Wayne 2004). We have some evidence that indicates co-planting a perennial host with *C. levisecta* transplants improves second year survival (Chapter 3). During common garden experiments with *C. levisecta*, transplants were not provided with a host except at a site established in an agricultural field, where individuals were co-planted with a perennial grass (*F. roemerii*). Second year survival of transplants was particularly high at this site ( $\bar{x} = 0.75 \pm 0.04$ ), compared to the average proportion surviving at the other nine common gardens ( $\bar{x} = 0.21 \pm 0.06$ ) or Pigeon Butte ( $\bar{x} = 0.51 \pm 0.05$ ). This suggests that co-planting a

perennial host with *C. levisecta* in the field is beneficial, and may allow the parasite to take advantage of host roots that enable them to exploit nutrients and water from a larger volume of soil during periods of environmental stress.

#### Management implications

Our results emphasize the importance of basing conservation decisions on experimental research conducted under conditions similar to those of the intended application. Simple extrapolation of greenhouse results to natural systems can oversimplify the complex biotic interactions that species are exposed to in the field, and worse, suggest inappropriate management actions. Our greenhouse results suggested that *F. roemerii* was a poor host for *C. levisecta*, but our field studies showed that this species is superior to *E. lanatum* for promoting plant survival at reintroduction sites when voles were present. If we had limited our experiments to the greenhouse we could have made recommendations that would have resulted in poor *C. levisecta* field-establishment.

It may be useful to grow *C. levisecta* in pots in a horticultural setting to mass produce seeds for recovery efforts. Pollinators are essential to fruit and seed production in *C. levisecta* because the species is an obligate outcrosser (Kaye and Lawrence 2003). We observed abundant seed set in our shade-house environment where pollinators (i.e. *Bombus* spp.) were present. If seed increases of *C. levisecta* in a horticultural setting is a management objective, we recommend not using a host if plants are provided with adequate water and nutrients or co-planting with *E. lanatum*. In the field however, we do not recommend co-planting *C. levisecta* with *E. lanatum*, as this host species was associated with vole tunneling and high *C. levisecta* mortality rates. First year field survival was highest for no-host *C. levisecta* individuals and those planted with *F. roemerii*. We suspect co-planting *C. levisecta* with a perennial host will increase future field performance and recommend using *F. roemerii* as a host for Willamette Valley *C. levisecta* recovery efforts. This grass was once a dominant species of upland prairies in the region, is relatively easy to grow, and was not linked to destructive herbivore activity.

Although the 2005 field season was an anomalous year in terms of vole abundance, herbivore management, including that of deer, elk, and rabbits, will clearly



be an integral part of *C. levisecta* recovery efforts. Herbivore management may actually involve the same actions as prairie habitat management (e.g., mowing or burning, fencing) and improve growing conditions for *C. levisecta*. Prescribed burns during times of *C. levisecta* dormancy have been used to manage vole populations at extant populations (P. Dunwiddie, pers. comm.). Fire consumes litter which reduces cover and leaves voles susceptible to predation while also reducing competition for light resources. Further, mounting evidence suggests that *C. levisecta* responds favorably to fire in Washington prairie (Dunwiddie et al. 2000). Fencing may also be used to deter herbivores; two and a half meter fences effectively excluded large mammals from our common garden experiments in the Willamette Valley, while wire cages dug into the ground successfully prevented rabbit herbivory at an extant population on Whidbey Island.

Further investigation of the host dynamics of *C. levisecta* may increase our capacity to successfully reintroduce this species to the southern portion of its historic range. Although the mycorrhizal status of this species has not been investigated, many hemiparasites in the Scrophulariaceae are considered non-mycorrhizal (Harley & Harley 1987). However, the mycorrhizal status of the host plant can influence the performance of the hemiparasite. Several studies have shown that hemiparasites attached to mycorrhizal hosts have greater biomass and flower production than those growing with non-mycorrhizal hosts (Davies & Graves 1998; Salonen et al. 2001). Native perennial species other than *F. roemerii*, especially showy angiosperms that can attract pollinators, may also be appropriate hosts for *C. levisecta*. Leguminous hosts are commonly better hemiparasite hosts than grass species because of their capacity to fix nitrogen (Adler 2003; Gibson & Watkinson 1991; Matthies 1997; Seel & Press 1993). Additionally, alkaloid uptake from leguminous hosts can confer hemiparasite resistance to herbivory (Adler 2002), and increase pollinator visitation (Adler et al. 2001). We suggest that mycorrhizal inoculation of *C. levisecta* host plants and new potential host species should be examined experimentally in the field to improve the success of large-scale reintroductions of this endangered species.

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## **Chapter 5 : Growing *Castilleja* for Restoration and the Garden**

(Adapted version of Lawrence & Kaye (2005))

They have been described as “nearly impossible to cultivate in a garden” (Art 1990), with “many problems associated with growing them from seed” (Borland 1994). *Castilleja* species are historically a notorious, even mysterious, group of plants to propagate, eluding growers for years. These false allegations arise principally because of the parasitic nature of the genus. Species of *Castilleja* are hemiparasites, benefiting from but not requiring a companion host species in order to successfully establish themselves in a garden setting. Nevertheless, successful germination and seedling establishment do not require the presence of a host species. We have successfully grown more than 3000 individuals of golden paintbrush (*Castilleja levisecta*), an endangered perennial endemic to the Pacific Northwest, for restoration purposes. To facilitate propagation of common *Castilleja* species by wildflower enthusiasts, we here share our experiences growing this rare beauty.

*Castilleja* is a charismatic and colorful genus commonly known as “Indian paintbrush” or “painted cup,” and is a member of the figwort family (Scrophulariaceae). There are approximately 200 species, the majority found in western North America. Paintbrushes are hemiparasites (“half-parasites”), attaching themselves to the root systems of other plants via structures called haustoria (physical connections between roots) to obtain water and nutrients. Hemiparasites can grow successfully without a host, but greenhouse studies have shown that providing a host plant results in larger plants that are more likely to flower (Kaye 2001). Other hemiparasitic members of the figwort family include *Orthocarpus* (owl-clover), *Pedicularis* (lousewort), and *Cordylanthus* (bird’s beak).

*Castilleja levisecta* is a federally listed threatened species with only 11 populations remaining within its historic range. Although it once grew from the coastal bluffs and islands of British Columbia to the Willamette Valley of Oregon, it is thought to have been completely extirpated from the state of Oregon and from southwestern Washington. Golden paintbrush has limited capacity for natural dispersal and colonization of new sites, necessitating a strategic reintroduction plan to support its long-term viability (Caplow 2004). We have established experimental populations throughout the Willamette Valley in an effort to determine which seed sources and habitat types are appropriate for large-scale reintroduction endeavors.

### **CASTILLEJA LEVISECTA PROPAGATION**

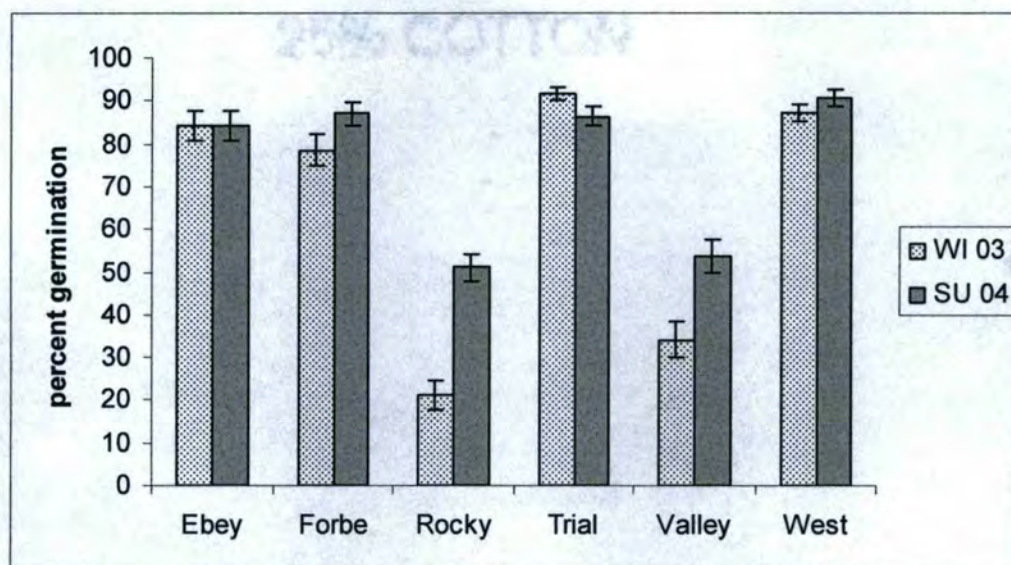
Our success with this plant is attributable to careful attention to seed collection, germination, and seedling establishment. The techniques are tailored to the production of several hundred individuals at a time but can be altered to accommodate smaller-scale production. Although our experience with growing *Castilleja* is limited to a single species, we believe that our techniques may be useful for other species. As members of the U.S. Fish and Wildlife Service's 'recovery team' for this species, we observe the guidelines developed by this group and laws governing endangered species, and urge gardeners not to collect any material (including seeds) of this and other federally listed endangered plants. There are many other *Castilleja* species to grow in gardens.

Successful propagation begins with timely seed collection. Mature paintbrush capsules hold many small seeds – in *C. levisecta*, up to 300. It is easiest to collect the entire capsule and remove the seeds later. We collect ripened capsules late in the growing season (August or September) from a large number of individuals when capsules begin to split at the tip and the seed is easily shaken out. Place the capsules in dry envelopes until further processing. Accurately labeling the envelopes with seed collection information (species, date, location, etc.) is important. We clean our seed under a dissecting microscope, but it can also be done on a light table or in a well-lit room. Under magnification, paintbrush seeds are remarkable! A reticulated membrane, reminiscent of a sponge, encloses the embryo. The function of this unique seed coat is not clear, but may facilitate uptake of water by the embryo. Separate the seeds from debris and store them in a dry envelope in a freezer until sowing.

Many plant species of temperate climates require a period of cold, moist conditions ("stratification") for proper germination, and golden paintbrush is no exception. We place the seeds on moistened germination paper in lidded plastic dishes, remoistening the paper as necessary throughout stratification. You could use moistened paper towels inside a plastic bag or plastic refrigerator container; maintaining proper moisture under sanitary conditions is crucial. We place the dishes in a cold, dark room at 5°C for 6–8 weeks, followed by a week or two of postchill



incubation in a growth chamber set at 25°C/15°C with 12 hours of fluorescent lighting. This procedure typically results in 20 to 95 percent germination for *C. levisecta*, depending on the seed source (Figure 5-1). Seeds from some populations germinate prior to postchill incubation and can become etiolated ("stretched") if left in the dark and cold too long. Home growers may be successful using a refrigerator for the cold treatment, and placing seeds in a warm, well-lit area for post-chill incubation.



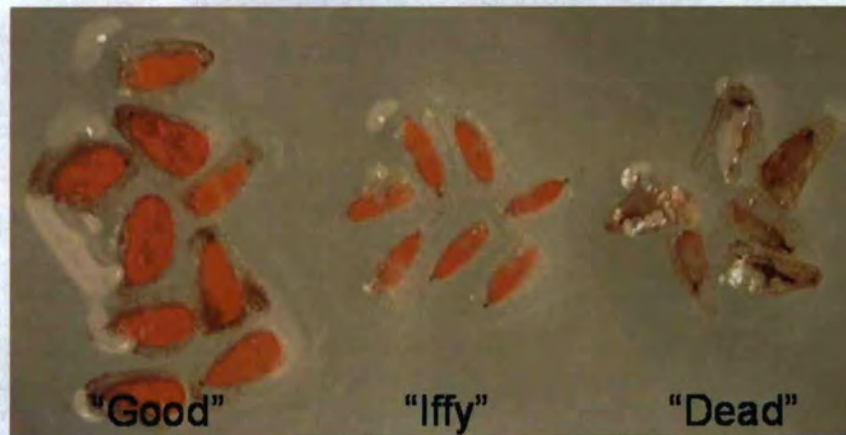
**Figure 5-1.** Mean percentage germination for six *C. levisecta* seed sources during two rounds of propagation, winter 2003 and summer 2004 ( $\pm 1$  SE).

Seed viability is critical to successful germination. Viable *Castilleja* seeds have a robust embryo, visible with the microscope, within the seed coat. Small, shriveled embryos likely indicate nonviable seeds. The viability of *C. levisecta* seeds varies with source population and maternal plant. The genetic diversity of remaining plants within small populations may play a role in this variation. Also, *C. levisecta* seed viability may decline with storage time. Research by Jane Wentworth at the University of Washington showed that seeds stored dry at 5°C for three years did not germinate, while seeds stored for two years had 13 percent germination, and one-year-old seeds had 47 percent germination. However, seeds stored at the Berry Botanic Garden for more than three years have shown high viability, with germination rates up to 90 percent. These seeds were stored in a low-temperature and humidity-controlled

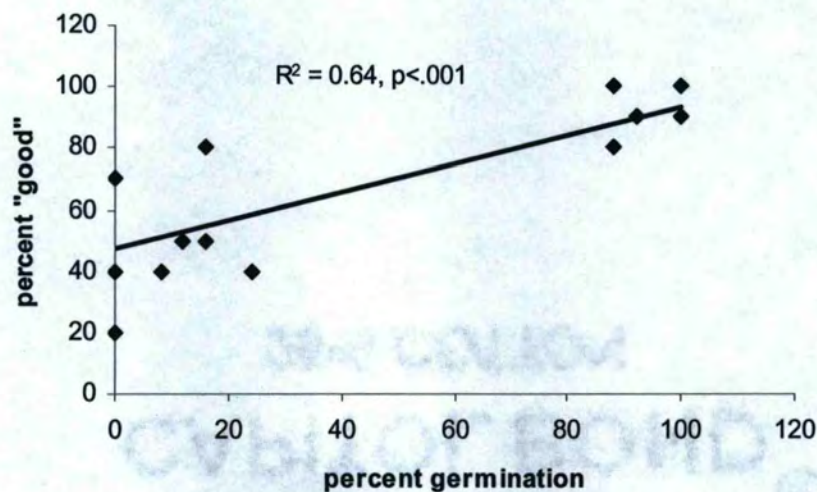


seed vault, where thousands of rare and endangered plant seeds from the Pacific North West are kept for future conservation efforts.

During *C. levisecta* propagation for our common garden experiment, two populations, Rocky Prairie and San Juan Valley, had particularly low germination in our 2003 winter propagation round (Figure 5-1). We conducted a tetrazolium analysis on seeds from these two populations, as well as seeds from Trial Island, in order to determine their viability. After tetrazolium treatment, we categorized seeds into three groups (i.e., good, iffy, and dead) based on embryo size and stain richness, which is an indicator of metabolic activity (Figure 5-2). Seeds categorized as “good” appeared to have large, healthy embryos, while “iffy” seeds were smaller and lighter in color. Dead seeds did not appear to have a living embryo. To determine if tetrazolium was a good method of assessing *C. levisecta* seed viability, we tested the linear relationship between the percentage of seeds germinating and the percentage of seeds we categorized as “good” (Figure 5-3). While the relationship between these two variables was significantly linear, at the low end of the scale the percentage of seeds categorized as “good” was higher than the percentage that actually germinated. This indicated that these seeds would potentially benefit from a longer period of cold stratification. Therefore, for the second round of propagation, we cold stratified seeds from these two populations for six extra weeks, which resulted in elevated germination rates (Figure 5-1). However, germination was still significantly lower than the other seed sources, indicating that low seed viability was probably genetically mediated as well.



**Figure 5-2.** Categorization of *C. levisecta* seeds into three levels of viability after tetrazolium analysis. Note that two seeds in the “good” pile should actually be classified as “iffy.”



**Figure 5-3.** Linear relationship between the average germination of seeds from Rocky Prairie, San Juan Valley, and Trial Island vs. the percentage of seeds categorized as “good” during tetrazolium analysis.

Once the radicle (first root) and cotyledons (first leaves) have emerged, it is time to get those germinants in soil! At this point, the seedlings are very fragile and need extremely tender handling. We tried using tweezers to remove seedlings from the paper, but we believe that this technique may have damaged the slender germinants, likely only a few cells thick, as we saw high mortality within the first few

weeks of growth. A less destructive approach we have used is to pick up the seedling with the tip of a plant tag or pencil and then gently place it in a small depression on the soil surface, lightly covering the radicle with fine soil. While soil-radicle contact is important, we recommend against planting too deeply or compacting the soil excessively. Each seedling is placed in one cell of a plastic cell-pack. Because this species grows in sandy glacial deposits in its native habitat, we use a well-drained soil medium amended with time-release micro and macro nutrients. Additionally, we use a liquid fertilizer (15-30-15) during watering every other week.

Maintaining an adequate moisture level is critical during the first few weeks of growth. We flood-water our flats from below during that period so the soil becomes fully saturated and the seedlings are not injured by overhead spray. After four to six weeks, the golden paintbrush seedlings have three to eight pairs of true leaves and an established root system, and are ready to be transplanted from their cells to larger four inch or gallon pots.

This is the right time to provide a host plant for *Castilleja* species. We have planted *C. levisecta* with several different host plants with varying success. Oregon sunshine (*Eriophyllum lanatum*), a composite, proved to be a better companion in the greenhouse than Roemer's fescue (*Festuca roemerii*), or planting without a host (Kaye 2001). Other species have worked too, including *Potentilla gracilis* and *Sidalcea* spp. One cultivator has successfully grown this species with shrubs including *Symphoricarpos albus* and *Spiraea japonica*. We have been successful using host seedlings or rooted cuttings and planting them within a few inches of the paintbrush root crown. Overall, golden paintbrush plants that have a companion are larger and more likely to flower than those without. However, with adequate fertilizer, water, and light, we have produced flowering *C. levisecta* individuals without a host within six weeks in a shadehouse (summer) and eight weeks in a greenhouse (winter).

### ***C. LEVISECTA* EXPERIMENTAL POPULATIONS**

Nine golden paintbrush experimental populations or "common gardens" have been established in remnant prairies and restoration sites throughout the Willamette Valley. Each common garden is composed of plants grown from seed from six of the remaining

populations. Plants were grown in a greenhouse for three months prior to outplanting in March or November 2004. With the help of friends and volunteers, we planted a total of more than 2000 individuals into grids at each site. The transplants will likely form haustorial connections with whatever root system they encounter, as this species is not particularly picky about what kind of plant it parasitizes. We monitor each transplant and record information about its size and fecundity. We are also characterizing each site by examining the soil and vegetation community. Unfortunately, golden paintbrush is not only appealing to the human eye, but also to the palate of many wildlife species, including deer, elk, voles, and other rodents. Consequently, most of the common gardens have been fenced to keep out large herbivores.

Field mortality was very low overall during the first growing season – an exciting result in itself! Data from the 2004 growing season indicate plants from two populations in Washington produce significantly larger offspring that are more likely to flower than plants from other populations. This may be related to the high genetic diversity of these populations. Likewise, several common garden sites stand out as initial “winners,” where the plants were larger and more likely to flower (regardless of the seed source). The plant communities of these successful sites are largely composed of native prairie species growing on relatively well-drained soils, while sites with lower transplant success are dominated by exotic grasses. The size of the plant at the time of planting is important too. Larger starts become larger plants after several months in the field. By following these plants in 2005, we hope to determine how the success of golden paintbrush transplants relates to where they came from, similarity of the environment of the common garden site to the source population, and the planting season.

## **PROPAGATION OF OTHER *CASTILLEJA* SPECIES**

Perhaps the most challenging aspect of paintbrush propagation is how to stimulate germination. Despite common belief, *Castilleja* seeds do not require root exudates from a host plant to stimulate germination, as is the case for strict parasites. Germination variability, however, is the rule. Many species require moist, cold stratification, while others will readily germinate given moist soil. The requirements of 22 different species of *Castilleja* are summarized in Table 1. Notice that different researchers may report

different germination requirements for the same species (such as *C. linariifolia*), indicating that requirements may vary among seed sources.

**Table 5-1.** Germination requirements of 22 species of *Castilleja*.

Species	Requirements for maximum germination	Reference
<i>C. affinis</i>	None	(Borland 1994)
<i>C. ambigua</i>	2 weeks moist chill at 5°C for	(Young 2002)
<i>C. applegatei</i>	3 to 6 months moist chill at 2°C, depending on source	(Meyer & Carlson 2004)
<i>C. chromosa</i>	3 to 6 months moist chill at 2°C, depending on source	(Meyer & Carlson 2004)
<i>C. chromosa</i>	1 to 3 months moist chill at 2-5°C	(Borland 1994)
<i>C. cusickii</i>	5 months outdoor chill, or 3 months cold moist at 2°C	(Luna et al. 2004)
<i>C. exilis</i>	1 month moist chill at 2°C	(Meyer & Carlson 2004)
<i>C. flava</i>	3 to 6 months moist chill at 2°C, depending on source	(Meyer & Carlson 2004)
<i>C. foliolosa</i>	None	(Borland 1994)
<i>C. hololeuca</i>	None	(Borland 1994)
<i>C. indivisa</i>	None	(Borland 1994)
<i>C. integra</i>	None	(Borland 1994)
<i>C. lanata</i>	None	(Borland 1994)
<i>C. latebracteata</i>	None	(Borland 1994)
<i>C. levisecta</i>	1.5 to 3 months moist chill at 5°C, depending on source	(Kaye 2001)
<i>C. linariifolia</i>	2 months moist chill at 2°C	(Heckard 1968)
<i>C. linariifolia</i>	1 month moist chill	(Butler & Frieswyk 2001)
<i>C. linariifolia</i>	1 to 4 months moist chill at 2°C, depending on source	(Meyer & Carlson 2004)
<i>C. linariifolia</i>	None	(Borland 1994)
<i>C. miniata</i>	3 months moist chill at 2-5°C	(Borland 1994)
<i>C. purpurea</i>	None	(Borland 1994)
<i>C. rhexifolia</i>	2 months moist chill at 2°C	(Wick & Luna 2004)
<i>C. rhexifolia</i>	3 to 6 months moist chill at 2°C, depending on source	(Meyer & Carlson 2004)
<i>C. sessiliflora</i>	None or 1 month moist chill	(Borland 1994)
<i>C. subinclusa</i>	3 weeks or longer moist chill in peat	(Young 2002)
<i>C. tenuis</i>	3 months moist chill at 2-5°C	(Bartow 2003)
<i>C. wightii</i>	2 to 3 weeks moist chill	(Young 2002)

In general, populations and species of *Castilleja* from warmer, drier climates have shorter chilling requirements and germinate more quickly than those from high elevations with longer winters. If germination information on your species of interest is not



available, estimating the number of weeks the seeds are exposed to cold temperatures (around or below 5°C) in their natural environment may help approximate its requirement.

*Castilleja* species occupy diverse habitats throughout western North America, especially coastal prairies, subalpine rocky outcrops, and arid grasslands. Thus, growing requirements are likely to vary substantially among species. However, several general propagation methods appear in the literature. Well-drained soils, such as Sunshine Mix #4 Aggregate Plus or Fafard Growing Mix #2, have been used for several different paintbrush species. Heavy fertilizing, especially during the establishment phase, is critical. Several growers have amended their soil media with Osmocote (13-13-13) slow-release macronutrients, as well as with Micromax micro nutrients, to get high establishment rates. Additional biweekly fertilizing is suggested until the paintbrushes establish haustorial connections with host plants. Maintaining a moist, warm, light environment during establishment is essential, but everything in moderation, of course. During a heat wave of temperatures above 104° F last summer, we watched 2250 seedlings shrivel and die before our eyes! *Castilleja* seedlings are infamous for their high transpiration rates, which may be attributable to their hemi-parasitic nature. Misting or flood-watering from below is suggested during the first month of growth.

Choosing a host can be the most exciting part of *Castilleja* propagation. Paintbrushes are generally not highly host-specific, though they vary in their degree of parasitism. Composites, grasses, and legumes are their most common host types. In addition to supplementing the paintbrush with water and nutrients, some host plants (e.g., *Lupinus* spp.) play a role in attracting pollinators and may provide secondary compounds that protect the plant from herbivores (Adler 2003). The most appropriate host plants are native species found in the same habitat. Seedlings or rooted cuttings of the host plant should be planted within a few centimeters of the paintbrush seedlings about 6 weeks after germination. Allow the plants to grow together for 6–8 weeks before planting them out to ensure that haustorial connections have been made. Host plants can out-compete the paintbrush for resources, so keep the host in check by trimming it periodically if necessary.

Paintbrushes are a wonderful addition to any garden, and can attract wildlife too. Adult butterflies use them as nectar plants, while red-flowering paintbrushes are an open

invitation to hummingbirds. Bumblebees are the principal pollinators of yellow, green, and purple-flowered paintbrushes. Many species of checkerspot butterflies (*Euphydryas* spp.) use *Castilleja* as larval host plants. *Castilleja levisecta* may have been the original host plant for Taylor's checkerspot, a rare butterfly endemic to the prairies of the Pacific Northwest. As populations of golden paintbrush diminished, so have those of Taylor's checkerspot.

There are currently eight species of *Castilleja* listed as threatened or endangered under the Endangered Species Act. Gardeners can play an important role in *Castilleja* conservation by developing propagation protocols of more common species, which, in turn, can help inform restoration efforts of rare species.

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## Chapter 6 : Conclusions & management recommendations

"Here is the means to end the great extinction spasm.  
The next century will, I believe, be the era of restoration in ecology"  
-E. O. Wilson

Although *C. levisecta* historically grew in the prairies of the Willamette Valley, Oregon, the species has not been seen in the region in over sixty years. Reintroduction of viable *C. levisecta* populations to the southern portion of the its historic range is a primary goal of the species reintroduction plan (Caplow 2004). The purpose of this chapter is to synthesize our findings and provide recommendations for restoring populations of *C. levisecta* to the upland prairies of the Willamette Valley, based on the results of our primary research topics: (a) habitat variation throughout the historic range of *C. levisecta* (Chapter 2) (b) seed source and site selection criteria (Chapter 3), and (c) direct and indirect effects of *C. levisecta* host use (Chapter 4). Our recommendations should only be applied to upland sites because that is the population of inference.

### **HABITAT VARIATION THROUGHOUT THE HISTORIC RANGE OF *C. LEVISECTA***

Habitat characteristics throughout the historic range of *C. levisecta* vary significantly among remaining populations in the Puget Trough and potential reintroduction sites that we examined in the Willamette Valley, OR. *Castilleja levisecta* sites from these ecoregions had distinct species composition and soil characteristics likely because of regional differences in geology, climate, land-use history, and proximity to marine environments. Many of the species indicative of remaining populations in the Puget Trough were native perennials, while those of potential reintroduction sites in the Willamette Valley were introduced annuals. Soil characteristics of *C. levisecta* sites were also distinct among the two ecoregions. Puget Trough sites were located on sandy soils with generally high levels of magnesium and sulfur, while Willamette Valley sites used in this study were found on silty-clay soils with high concentrations of potassium and phosphorous. Differences in soil texture

and magnesium and potassium concentrations were associated with plant community divergence among the two regions.

Matching the ecological characteristics (i.e., vegetation, soils) between seed source and prospective planting site is encouraged during species translocation (McKay et al. 2005; Montalvo & Ellstrand 2000), because populations may be adapted to specific habitat conditions and selective forces. However, if vegetation communities of seed sources and reintroduction sites do not overlap, using similarity measures based on species composition is inappropriate. We suggest using functional groups to compare plant communities between the Puget Trough and Willamette Valley because the *C. levisecta* sites we characterized from these two ecoregions had distinct species assemblages.

### ***C. LEVISECTA* SEED SELECTION FOR WILLAMETTE VALLEY REINTRODUCTION**

Common garden experiments revealed that neither effective population size, genetic diversity, nor geographic distance were effective predictors of *C. levisecta* performance in the Willamette Valley. The largest *C. levisecta* populations in our study (i.e., San Juan Valley and Rocky Prairie) consistently performed poorly, while small populations performed as well or better than large populations. The measure of genetic diversity we used was based on allozyme markers, which may not be an appropriate measure of adaptively significant variation (Britten 1996; David 1998; Savolainen & Hedrick 1995). Quantification of the genetic variation underlying traits associated with morphology, physiology, and life history attributes would likely be a more effective measure of adaptively significant genetic variation in *C. levisecta*. Although minimizing the geographic distance between seed source and reintroduction site is often recommended (McKay et al. 2005), this measure may not be appropriate when geographic and ecological distances are large, as they are in our study system. Geographic distance was positively correlated with *C. levisecta* performance in 2004, which is contrary to ecological theory and not particularly useful for managers.

We suggest that individuals from Whidbey Island populations be utilized for *C. levisecta* recovery efforts in the Willamette Valley, as the three populations represented in

our study (i.e., Forbes Point, West Beach, and Ebey's Landing) consistently had high germination rates, vigorous greenhouse growth, and superior field performance. Although the merits of using single versus multiple seed sources during restoration activities are debated in the conservation arena (Kaye 2001), we think that mixing Whidbey Island seeds during recovery efforts in the Willamette Valley is justified. We observed heterosis in the F1 during a preliminary analysis of *C. levisecta*'s breeding system, with increased size, flowering rate, and seed set with increasing genetic distance of the cross, ranging from self, sibling, intra-population to inter-population crosses (Kaye & Lawrence 2003). Based on these results, we would predict that reintroductions composed of genetic material from multiple seed sources would have greater fitness and be more likely to succeed than those created from a single source, at least in the short term. Although outbreeding depression may not be detected until the F2 or further generations, this phenomenon is theoretically restricted to crosses between ecologically and genetically distinct populations. While the remaining *C. levisecta* populations on Whidbey Island are currently fragmented, these populations are considered a single genetic/geographical group and were likely less fragmented in the past (Chappell & Caplow 2004). Currently, this putative meta-population appears to be the most appropriate seed source for *C. levisecta* recovery efforts in the Willamette Valley.

### ***C. LEVISECTA* RECOVERY SITE SELECTION FOR WILLAMETTE VALLEY REINTRODUCTION**

Habitat quality and plant community composition of the reintroduction site are likely to play an important role in the success of future *C. levisecta* recovery efforts. Our work suggests that high quality prairies should be targeted for reintroduction sites in the Willamette Valley. Non-native plant cover at common garden sites was associated with reduced *C. levisecta* vigor in 2004. We also found support for the "home-habitat hypothesis" based on the similarity of functional group assemblage among *C. levisecta* seed sources and reintroduction sites. Remaining *C. levisecta* populations were generally dominated by native perennial species, while experimental reintroduction sites in the Willamette Valley represented a diversity of community types ranging from exotic annuals to native perennials. *Castilleja levisecta* survival at experimental sites increased with increasing similarity to source population functional

group assemblage. These results suggest that the species composition of recovery sites will be important to *C. levisecta* re-establishment in the Willamette Valley. Sites dominated by invasive species, particularly invasive annuals, should be avoided, while sites that are functionally similar to source populations, in particular those with high native perennial abundance, should be targeted. Now that species lists from remaining Puget Trough populations have been developed (Chappell & Caplow 2004), site selection in the Willamette Valley should attempt to match species composition from remaining populations if possible. Although our results did not indicate that soil similarity influenced *C. levisecta* performance, we believe that sites in the Willamette Valley with well-drained soils are most appropriate since all remaining *C. levisecta* populations are found on well drained sandy soils.

Active site management will be an important component of *C. levisecta* reintroduction success. Steps to limit exotic invasion and the accumulation of biomass, as well as to improve native community composition and structure, should be taken at reintroduction sites. A regional study aiming to promote native abundance in degraded Pacific Northwest prairies is currently investigating the effectiveness of management techniques that include burning, mowing, grass-specific herbicide use, and seeding with native species. Results from this work will likely have relevant implications for prairie habitat management during *C. levisecta* recovery (Kaye pers. comm.). Although the 2005 field season was an anomalous year in terms of vole abundance, herbivore management, including that of deer, elk, and rabbits, will clearly be an integral part of *C. levisecta* recovery efforts. Herbivore management may actually coincide with that of prairie habitat and improve growing conditions for *C. levisecta*. Prescribed burns during times of *C. levisecta* dormancy have been used to manage vole populations at extant populations. Fire consumes litter which reduces cover and leaves voles susceptible to predation while also alleviating competition for light resources. Further, evidence suggests that *C. levisecta* responds favorably to fire in Washington prairies (Dunwiddie et al. 2000). Fencing may also be used to deter herbivores; two and a half meter fences effectively excluded large mammals from our common garden experiments in the Willamette Valley, while wire cages dug into the

ground successfully prevented rabbit herbivory at Ft. Casey, a remaining population found on Whidbey Island (Caplow pers. comm.).

### **DIRECT AND INDIRECT EFFECTS OF *C. LEVISECTA* HOST USE**

Results from our host experiment emphasize the importance of basing conservation decisions on experimental research conducted under conditions similar to those of the intended application. Extrapolation of greenhouse results to natural systems can oversimplify the complex dynamics that species are exposed to in the field. Our greenhouse results suggest that *C. levisecta* is not host specific and can be grown successfully with any of the host treatments we used. Seed increases of *C. levisecta* are possible in a horticultural setting, but co-planting a host is not necessary if plants are provided with adequate water and nutrients. Pollinators are essential to fruit production, as *C. levisecta* is an obligate outcrossing species. We observed abundant seed set in a shade-house environment where pollinators (i.e., *Bombus* spp.) were present.

We do not recommend co-planting *C. levisecta* with *E. lanatum* in the field, as this host species was associated with vole tunneling and high *C. levisecta* mortality rates. First year field survival was highest for host-less *C. levisecta* individuals and those planted with *F. roemerii*. We suspect co-planting *C. levisecta* with a perennial host will increase future field performance and recommend using *F. roemerii* as a host for Willamette Valley *C. levisecta* recovery efforts. This grass was once a dominant species of upland prairies in the region, is relatively easy to grow, and was not associated with destructive herbivore activity in our field study. Other native perennial species may be appropriate hosts as well. Further host studies should investigate the influence of mycorrhizal and/or leguminous hosts on *C. levisecta* fitness components in the field.

### **OTHER CONSIDERATIONS**

The ultimate goal of plant reintroduction is to establish resilient populations, capable of self-maintenance in the face of evolutionary change. While we have presented information that may increase the fitness and survival of founding *C.*

*levisecta* individuals in the Willamette Valley, seedling recruitment will be necessary for population persistence and viability. Despite prolific seed production, seedling recruitment is limited at remaining *C. levisecta* populations (Wentworth 1994) as well as when seeds are experimentally sown (Pearson & Dunwiddie 2002; Wayne 2004). Wentworth (1994) suggested that *C. levisecta* recruitment may be limited by the competitive effects of vegetative cover and litter. Dense cover may reduce the area available for seed to soil contact necessary for successful germination and establishment. Observations during our 2005 field season support this. Although we attempted to collect all fruiting stems and seeds from experimental populations in order to prevent establishment of individuals of unknown genetic composition, we observed nine seedlings at the Plant Materials Center common garden site. This site was established in an agricultural field with no vegetation other than the *F. roemeri* host we provided. A thin layer (approximately 2 – 5 cm) of western hemlock mulch was laid down to reduce weed abundance. Although we did not detect seedlings at other common garden sites (likely obscured by vegetation), this observation indicates that recruitment of *C. levisecta* individuals is possible in the Willamette Valley where there is minimal above-ground biomass.

Habitat manipulation such as burning or thatch removal may increase the availability of suitable microsites for seedling establishment in the field. The influence of burning, soil scarification, and control treatments are being investigated in ongoing seeding experiments at Rocky Prairie and two nearby prairies (P. Dunwiddie, pers. comm.). Seedling recruitment has been observed in all three treatments up to three years after seeding. Preliminary analysis suggests that seedling recruitment is variable, but greatest in plots where biomass has been removed.

## RECOMMENDATIONS

- 1) Use plant functional groups to compare vegetation communities among Puget Trough and Willamette Valley sites, because the species composition of these ecoregions is distinct.

- 2) Use seed from Whidbey Island populations for recovery efforts in the Willamette Valley. These populations consistently performed well during our studies.
- 3) Target high quality reintroduction sites that are dominated by native perennial species and have a low abundance of exotic annuals. Avoid sites that are known to have high abundances of generalist herbivores (i.e., deer, voles).
- 4) Choose reintroduction sites that share similar vegetation and soil characteristics with the seed source, regardless of the geographic distance between the two sites.
- 5) Actively manage sites to reduce biomass accumulation (i.e., burn or mow when *C. levisecta* is dormant) and promote native plant abundance.
- 6) Outplant *C. levisecta* to the field with a native perennial host that does not attract herbivores, such as *Festuca roemerii*. Do not plant in the field with *Eriophyllum lanatum*.
- 7) Incorporate herbivore management into *C. levisecta* site recovery plans (i.e., fence). Mammalian herbivory appears to be the rule rather than the exception.

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## APPENDICES

**Table A-1.** *Castilleja levisecta* experimental common garden locations; established 2004. As of fall 2005, three garden categories exist: all plants removed (removed), all plants remain (remain), or only Whidbey Island source populations remain (WI). Number of plant remaining at each site does not account for plant mortality.

common garden	UTM_E	UTM_N	general location	status	# plants remaining
BB2	10479457	4978750	Basket Slough NWR, OR	WI	54
BB3	10479457	4978839	Basket Slough NWR, OR	WI	112
BS1	10479272	4979246	Basket Slough NWR, OR	remain	277
BEL	10472370	4916733	Finley NWR, OR	remain	235
HER	10504648	4970377	Heritage Seedling, OR	remain	299
KAH	10516971	5329020	Port Townsend, WA	remain	214
PIG	10474641	4915973	Finley NWR, OR	WI	120
PMC	10483011	4941089	Plant Materials Center, OR	removed	0
SRD	10549063	5043458	Sandy River Delta NFS, OR	removed	0
STK	10471524	4968370	Starck Family Property, OR	removed	0

**Table A-2. Directions to Kah Tai Prairie**

Common Garden: Kah Tai Prairie

General Location: Port Townsend, WA

Directions from Corvallis:

N on I-5 to Olympia, WA

Take exit 104- follow signs to 101 N (to Olympic Peninsula)

Take 20E to Port Townsend

Once in town, take left at 2nd light onto Kearny

Right onto Blaine St.

First left into Port Townsend Public Golf course

Prairie within chainlink fence on either side of road

Park in clubhouse lot

Plot location:

Transplants planted into two 11x7m grids- one on east side of road ("up") and one on west side of road ("down")

Located in southern most portion of prairie, near chain link fence bordering the golf course (see sketch map)

Corners of each grid marked with 3' rebar- galvanized nails also placed next to rebar posts if they are removed

Origin corners: upper plot (east side of road)= SW; lower plot (west side of road)= NE

Transplants located with x, y coordinates within grid.

At this site, *C. levisecta* individuals planted into 1m x 0.5m rectangles; first transplant at Kah Tai at 0.5m, 0m, next one at 1.5m, 0m

**Table A-3. Directions to Heritage Seedling**

Common Garden: Heritage Seedling

General Location: East of Salem, OR

Directions from Corvallis:

N on I-5 towards Salem

Take exit #223 onto 22E

Take 22E to exit 5 (Joseph St.)

Make right onto Joseph St.

Drive about 1 mi.

Before fork in road (Salem/Adamsville), see driveway on right

Make sharp right into driveway, drive past barn on left and park at end of road

Plot is in fenced area on the right

Plot location:

Transplants planted into 15x20m grids within fenced area

Corners of each grid marked with 3' rebar- galvanized nails also placed next to rebar posts if they are removed

Origin corner: SW

Transplants located with x, y coordinates within grid.

At this site, *C. levisecta* individuals planted into 1m x 1m rectangles; first transplant at 0.5m, 0.5m

**Table A-4. Directions to Pigeon Butte and Bell Fountain Prairie**

Common Garden: Pigeon Butte and Bell Fountain Prairie

General Location: Finley NWR, south of Corvallis

Directions from Corvallis:

**To Pigeon Butte:**

Take 99S about 10 miles

Make right onto Bruce Rd. (gas station/country store on corner)

Drive about 1 mile past wetland observation areas

Gated gravel road on right (gate combo: 1776)- lock behind you!

Drive about 1 mile (past red barn on right), veer left upslope towards quarry- park here

**To Bell Fountain Prairie from Pigeon Butte:**

Make right onto Bruce Rd. from gated road

Drive about 3+ miles til hit Bell Fountain Road, make right

Drive about 2.5 miles north

Bell Fountain is clearing on right hand side of road (before oak savanna restoration sign)

Park in front of gate

Plot location:

**Pigeon Butte: (see sketch map)**

From quarry, walk west

Follow abandoned road west downslope

Cut north through blackberries toward open prairie with oak trees on your right

Head toward 2 oaks in the opening, plot about 150m from abandoned road

Pigeon Butte common garden origin is in SW corner of plot, 15mx20m plot- first transplant at 0.5m, 0.5m

Aspect 300 NW, slope about 6 degrees

**Pigeon Butte Host Experiment plot:**

Host plot adjacent to common garden plot on north side, but shifted west about 7m to avoid blackberry patch

12mx 9m grid with origin in SW corner

**Table A-4.** (continued)

**Bell Fountain Prairie:**

Plot is fenced, east of large oak tree

Origin is in NW corner, 15mx20m plot- first transplant at 0.5m, 0.5m

aspect 310 NW, with slope about 7 degrees

Pigeon Butte and Bell Fountain common garden plants planted into 15x20m grids

**Table A-5.** Directions to Basket Slough 1, Basket Butte 2, and Basket Butte 3

Common Garden: Basket Slough 1 (BS1), Basket Butte 2 (BB2), and Basket Butte 3 (BB3)

General Location: Basket Slough NWR, west of Salem

Directions from Corvallis:

Take 99W north

Go past junction with Hwy 22

**To BB2 & BB3:**

Go about 1.5 miles past 22, then take left on Coville Rd.

Follow gravel road to parking lot at base of Baskett Butte; BB2 and BB3 are on butte

**To BS1:**

Go about 3 miles past 22, then take left on Smithfield Rd.

Go about 2.5 miles and take left into gated road into refuge (gate combo: 1007)

Take gravel road to turn around area

Plot location:

**BB2:** climb paved path, veer left at both path intersections, going toward viewing platform

plot is on right side of path, about 50m from trail, the northeast corner of plot near large poison oak patch

BB2 origin is in SE corner, aspect= 310 NW, slope about 8 degrees

**BB3:** From BB2, walk downslope on north side of draw, plot is on grassy knoll that is open all the way through oaks,

about 100m northwest of BB2

BB3 origin is in SE corner, aspect= 265W, slope about 8 degrees

**BS1:** walk south towards lower edge of oaks through abandoned field

plot within fenced area about 3/8 mi. from parking area

BS1 origin is in SE corner, aspect= 284W, slope about 10 degrees

\*\* Note, BS1 is on lower western flank of Baskett Butte. You can walk upslope through oaks to get to BB sites

**Table A-5. (continued)**

Transplants planted into 15x20m grids

Corners of each grid marked with 3' rebar- galvanized nails also placed next to rebar posts if they are removed

Transplants located with x, y coordinates within grid.

At these sites, *C. levisecta* individuals planted into 1m x 1m rectangles; first transplant at 0.5m, 0.5m



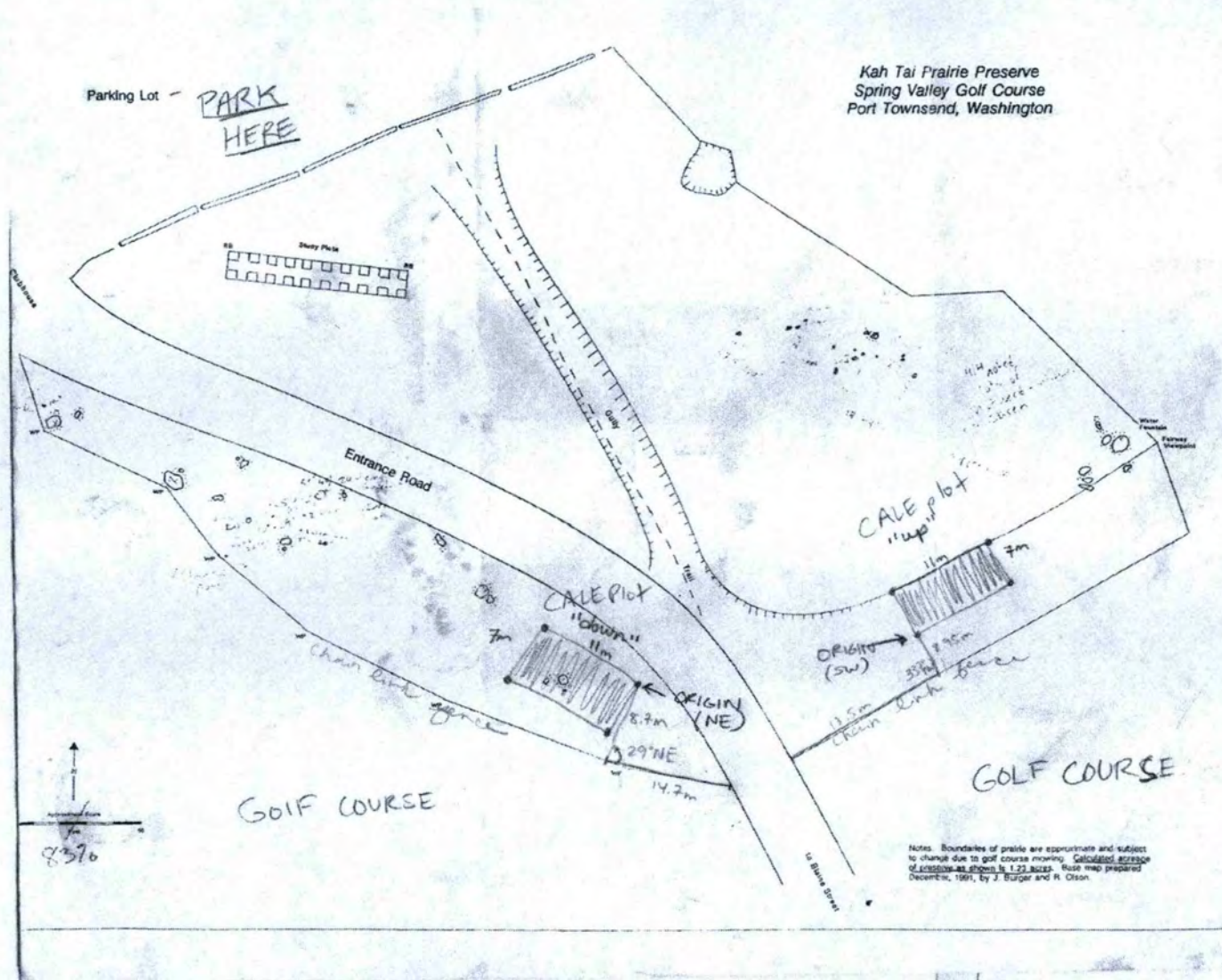


Figure A-1. Sketch map of Kah Tai plot locations.



CALE: Pigeon Butte common garden

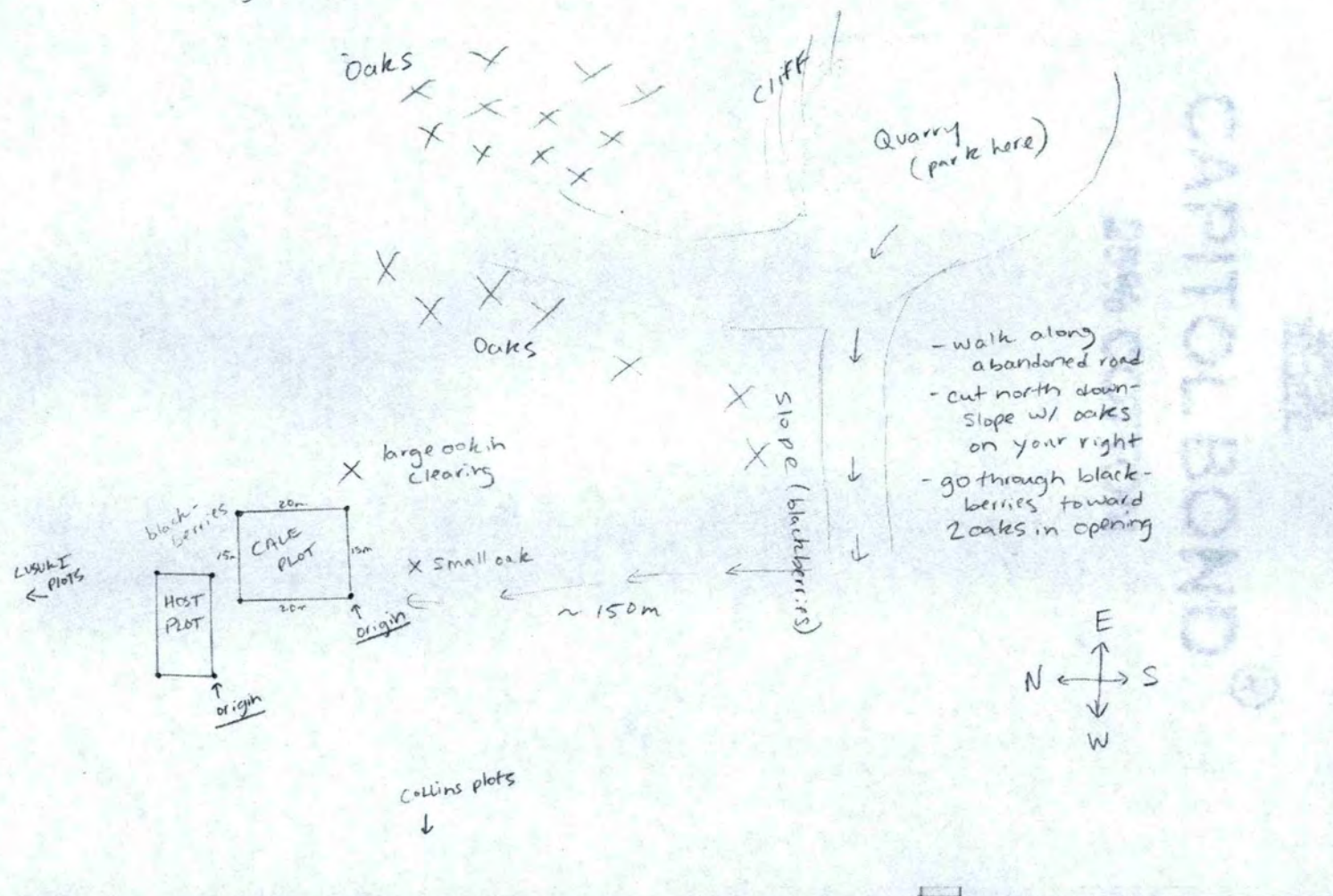
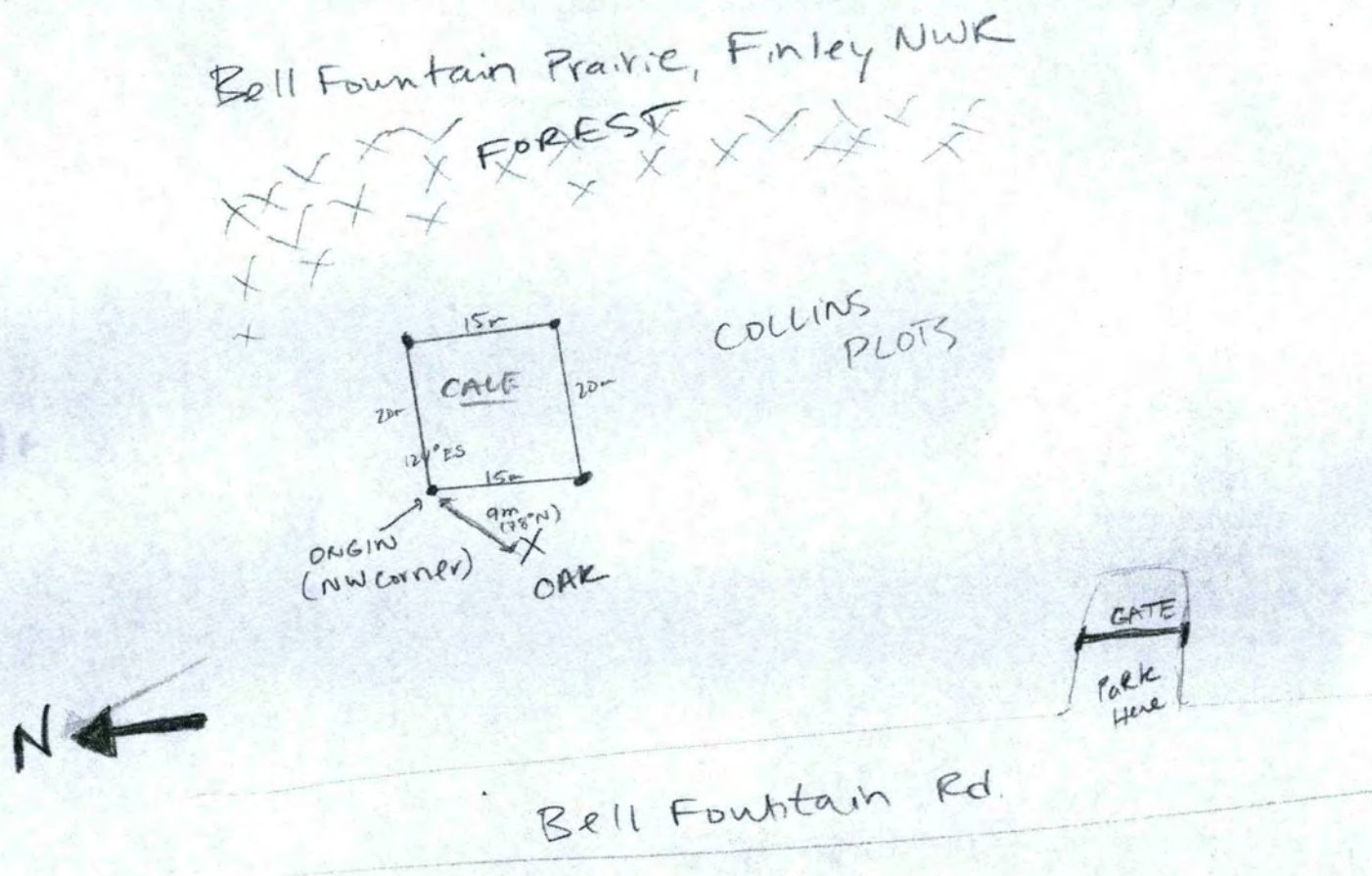


Figure A-2. Sketch map of plot location to Pigeon Butte, in Finley National Wildlife Refuge, OR.





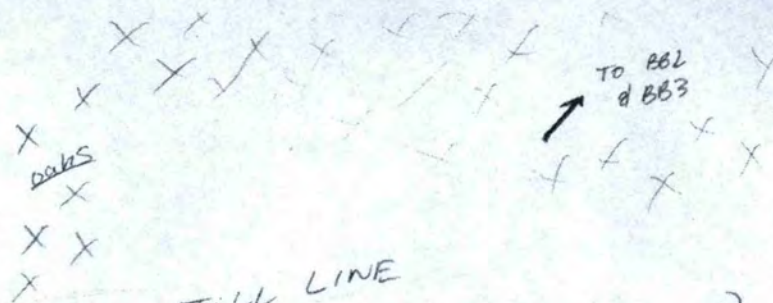
**Figure A-3.** Sketch map of Bell Fountain Prairie plot location in Finley National Wildlife Refuge, OR.



Basket Slough #1 ('area 1')

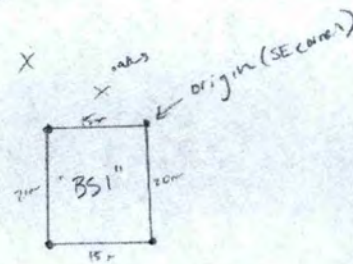
PARK  
in turn-  
around

Abandoned  
ag. field



TILL LINE

thicket  
draw



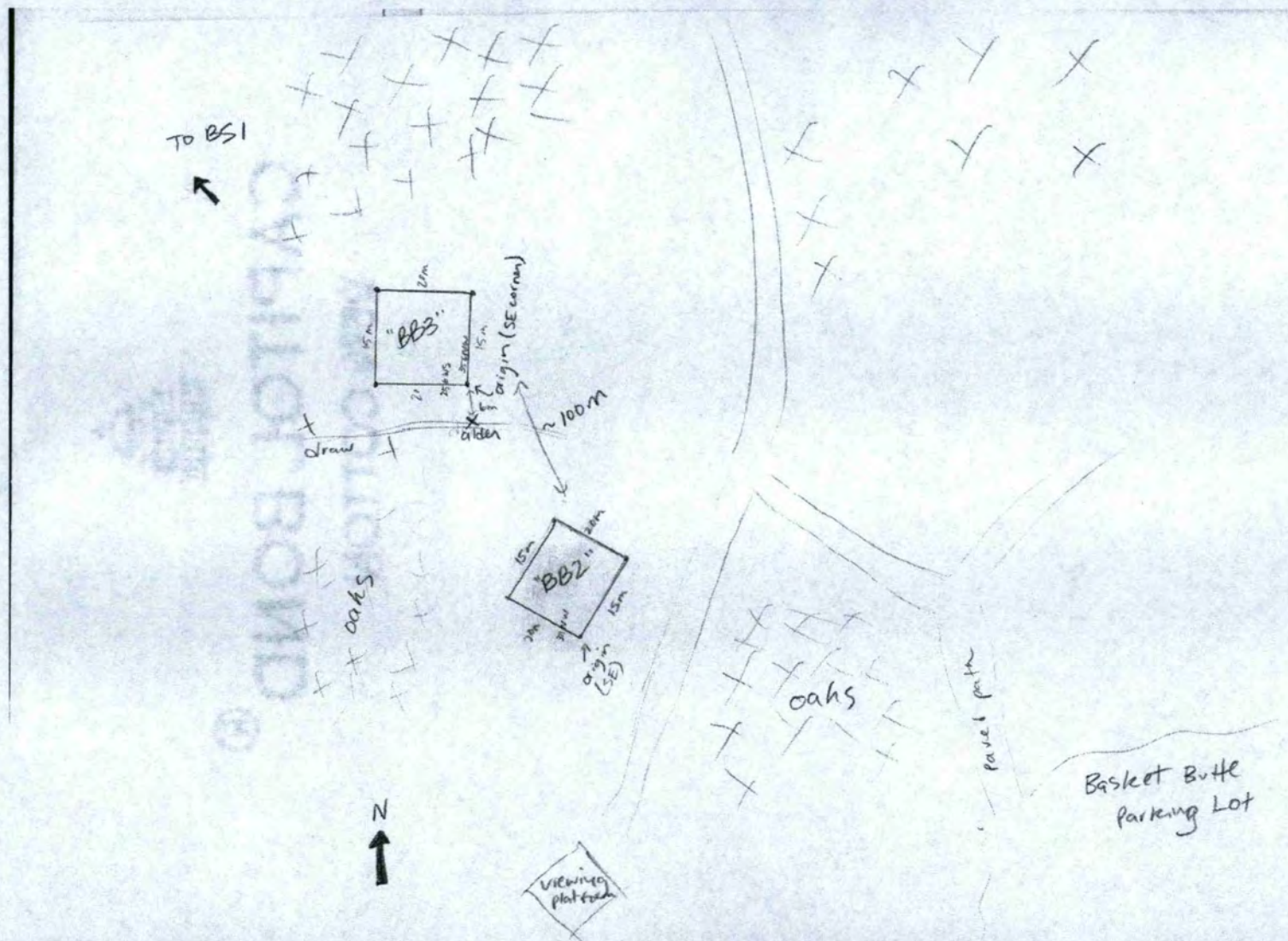
path that's sometimes mowed

N

H<sub>2</sub>O

Figure A-4. Sketch map of Basket Slough 1 plot within Basket Slough National Wildlife Refuge, OR.





**Figure A-5.** Sketch map of plot locations of Basket Butte 2 and Basket Butte 3 within Finley National Wildlife Refuge, OR.