### Soil Suitability and Site Preparation Techniques for *Castilleja Levisecta* Restoration on Whidbey Island, Washington

by

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#### Abstract

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Site selection for reintroduction of rare plants is based on a combination of practical, physical, and biological parameters. This study helps to identify soil requirements for the reintroduction of the federally threatened prairie plant, Castilleja levisecta. In 2002, baseline soil data were gathered from nine study sites: three extant C. levisecta populations and six potential reintroduction sites. That March, two experimental reintroduction sites were planted with C. levisecta. Weed competition, survivorship, and vigor of C. levisecta were tracked over the next two years. Results indicate that C. levisecta can tolerate low available nitrogen (N), phosphorous (P), and moisture. In two of the extant sites, low total N (0.04 and 0.08%), high C:N (54.08 and 34.29), form of inorganic N, low organic matter, and coarse soil texture limited plant-available N. All extant sites had low to moderate P availability (8.22, 13.60, and 8.06 mg/g). Soil moisture retention and plant availability were moderate at two sites and low at the third. Compared with extant populations, the Smith experimental site had significantly lower pH and extremely low moisture holding capacity, while soils at the Sherman experimental site were more similar to extant populations. Despite over 24 times greater mean weed biomass at the Sherman site, survival of C. levisecta was far higher than at the Smith site. At the Sherman, survival dropped from 92% after 2.5 months to 83% after one year and 31% after two years compared to 59%, 6%, and 0% survival over the same time period at the Smith site. Differences in soil characteristics appear to heavily influence C. levisecta survival and vigor. It is recommended that moisture-related soil characteristics be carefully evaluated when selecting reintroduction sites for C. levisecta.

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## CHAPTER I. INTRODUCTION

### PROBLEM OVERVIEW

In 1973 Hitchcock and Cronquist reported on the historic extent of the endangered prairie plant, *Castilleja levisecta* (Greenman), commonly known as golden paintbrush, as occurring in over 30 sites in the Puget Lowland and Willamette Valley ecoregions. By the time *C. levisecta* was listed as a federally threatened species in 1997 only 12 populations remained, five of which are located on Whidbey Island, Washington. According to goals laid out in the U.S. Fish and Wildlife Service recovery plan for the species, at least 20 stable populations of 1000 individuals or more are required for delisting (Gamon et al. 2000). Means for achieving this goal include the identification of appropriate sites for Puget Lowland prairie restoration and the subsequent reintroduction of *C. levisecta*. Among other factors, the suitability of a reintroduction site depends on edaphic conditions. A primary aim of this study is to provide a characterization of the soils of extant *C. levisecta* populations. This information may further the understanding of habitat requirements for the species and allow for improved evaluation of potential reintroduction sites.

Unfortunately, suitable prairie habitat has been lost along with *Castilleja levisecta* populations. Agricultural conversion and fire suppression are among the primary reasons for the decline and degradation of prairies in western Washington (Giles 1970, Agee 1993, Clampitt 1993, Crawford and Hall 1997). According to historical accounts, the Whidbey Island location now known as Ebey's Landing was once dominated by prairie (White 1980) and likely supported much larger populations of *C. levisecta*. However, according to studies conducted by the Washington Natural Heritage Program, less than 3% of all prairies in the Puget Sound area remain (Crawford and Hall 1997). Recovery of this endangered plant is inextricably linked to the restoration of suitable prairie habitat and establishment of a matrix of native prairie species.

Because the historic prairie soils on Whidbey Island are considered the most productive in the area, their fertility and topography have resulted in near total conversion to agricultural uses (Ness and Richins 1958). Hosting some of the earliest farms on the island, the prairies have been subjected to over 150 years of agriculture (White 1980). Human land management practices such as fire suppression have also altered the historical disturbance regime and community composition, which favored the encroachment of invasive species and conifers (Agee 1993). In addition to characterizing the soils of extant *Castilleja levisecta* populations, this study examines soils at a number of possible restoration and reintroduction sites, most of which suffer from the residual impacts of agriculture and fire suppression. This information may be used to assist in the determination of the restoration potential of these sites.

An additional step, crucial to the recovery process, is the establishment of experimental populations. The second focus of this study is a manipulative experiment designed to test different site preparation treatments in two potential restoration sites. Three types of herbicide and tillage strategies were evaluated for their ability to suppress exotic invasive plants. *Castilleja levisecta* and two other important matrix species, *Festuca roemeri* (Pavlick) and *Eriophyllum lanatum* (Pursh) were propagated and planted into the various test plots. The effects of the different treatments on native plant survival and health were assessed.

Aside from providing essential habitat for rare species like *Castilleja levisecta*, the prairies of western Washington are a significant cultural resource. Because they were intrinsically tied to the lives of indigenous peoples and were among the first lands settled by Europeans, prairies and humans share a long and culturally complex history. Evidence suggests that prairies may have become established as early as 10,000 B.P. (Ugolini and Schlichte 1973). The earliest evidence of humans living on the northwest coast is from nearly the same time period (White 1980; Kehoe 1992). For thousands of years people have had a role in the prairie landscape. In the last 40 years or so, awareness of the need to conserve and restore these imperiled ecosystems has developed. Currently, there is a strong interest in maintaining and restoring the diversity and integrity

of western Washington prairies. This study is part of a larger effort to restore prairies, and in doing so, redefine the relationship between humans and the prairie landscape.

# PRAIRIE FORMATION: GLACIAL HISTORY, CLIMATE AND THE ROLE OF ANTHTHROPOGENIC FIRE

Three major factors led to the incidence of prairies in the Puget Lowland region: glacial history and the subsequent soils that were created, regional climate, and cultural burning practices.

Prairie grasslands in western Washington developed on glacial outwash deposits that are dominated by sand and gravel and display excessive drainage (Jones 1936; Ugolini and Schlichte 1973; Kruckeberg 1991). These soils were deposited during the retreat of the Puget lobe of the Vashon Glacier approximately 14,000 B.P. (Crandell 1965). Available evidence indicates that prairies became established during the Hypsithermal Interval, a time of warmer and probably drier climate than that of the modern day. Hansen (1947) claimed that the extensive areas of well-drained glacial outwash deposits favored the establishment of xeric prairie species during this interval. In the last 4000 years, more mesic conditions have developed, resulting in a climate that favors lowland coniferous forests (Hansen 1947; Ugolini and Schlichte 1973; Franklin and Dyrness 1988). The prairies were maintained, despite this shift towards a cooler, wetter climate, primarily due to frequent prairie fires ignited by native people.

Historical evidence shows that indigenous people, who lived on and near the prairies (see Figure 1), commonly set fire to the vegetation. The case for anthropogenic burning of the prairies is further reinforced by the presence of charcoal fragments throughout the upper horizons in southern Puget prairie soils (Ugolini and Schlichte 1973). Native peoples regularly set fires to maintain open grasslands and to ensure a regular food supply from these systems (Jones 1936; Norton 1979; White 1980; Boyd 1999). For example, the Salish people spent winters along the shores of Puget Sound where salmon and shellfish were conveniently harvested. During the spring and summer, they moved to established

village areas on the prairies to gather the resources obtainable there (Haeberlin and Gunther 1930). Because the Salish valued bracken fern and camas as important food sources, and regular burnings maintain the abundance of these plants, it is likely that they set fire to the grasslands to encourage their growth (Turner and Bell 1971). The proliferation of bracken fern and camas on the prairies of Island County is some of the best evidence for prairie burning (White 1980). The continuous availability of food resources in the prairies was largely dependent on deliberate and regular burning.

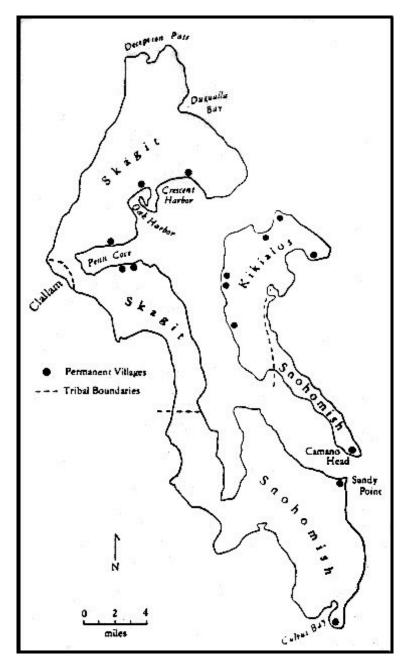


Figure 1: Salish boundaries and village sites on Whidbey Island (White 1980).

While indigenous people used fire to promote the growth of food plants, this practice in turn helped to keep the prairies clear of encroaching Douglas-fir and woody shrubs. This burning, which commonly occurred in the late summer and early fall (White 1980), was very effective in preventing tree seedling establishment on the prairies. In fact, prairies in western Washington are now considered fire-dependent communities, persisting only where fires prevent more competitive, fire-sensitive species from displacing native

vegetation. Prairies have been maintained by frequent, low-intensity ground fires that continually exclude invasive, fire-sensitive species (Agee 1993). Not only does regular burning eliminate competition from undesirable species, it increases the amount of open space available for regeneration of fire tolerant prairie species (Perdue 1997).

### CURRENT PRAIRIE DISTRIBUTION AND DESCRIPTION OF PRAIRE SOILS

Historically, native grasslands in western Washington were found in locations along the Olympic Peninsula, the San Juan Islands, Whidbey Island, along the Chehalis River to Gray's Harbor and south through the Puget Lowland to the Columbia River (Gee 1998). These grasslands are dominated primarily by *Festuca roemeri* (Roemer's fescue), *Festuca rubra* (red fescue), or *Danthonia californica* (California oatgrass) and harbor a wide variety of forbs that sometimes co-dominate with these grasses (Chappell and Crawford 1997). The Washington Natural Heritage Program (WANHP) has investigated the historic extent of native grasslands in the Puget Lowland and Willamette Valley ecoregions. Researchers from the WANHP created the following map based on the distribution of prairie soils in the northern Puget Lowland (see Figure 2):

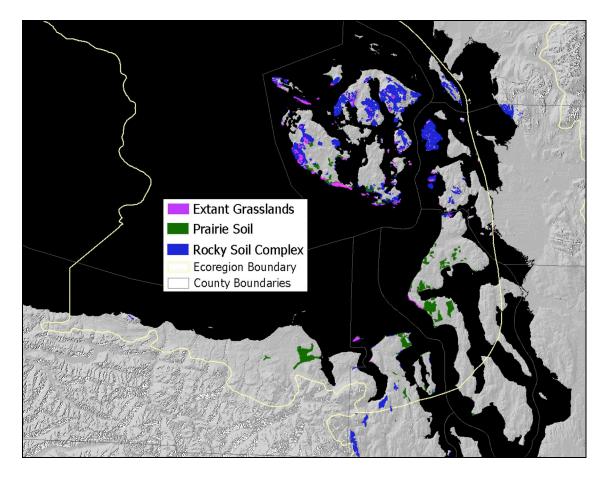


Figure 2: Pre-settlement grassland soils and extant grasslands (native, semi-native, non-native, and unsurveyed) in the northern Puget Lowland (Chappell et al. 2001).

The majority of extant prairies in western Washington are located on soils underlain by deep deposits of coarse glacial outwash. In addition to being well-drained, western Washington prairie soils are also generalized as being acidic and low in nutrients (Giles 1970; Ugolini and Schlichte 1973; Franklin and Dyrness 1988; Crawford and Hall 1997) though nitrogen tends to be higher in soils that receive less rainfall (Fowler and Wheeting 1941). Native dry and mesic grasslands currently or historically occurred almost exclusively on the following soil series: Spanaway, Nisqually, Carstairs, San Juan, Guemes, Doty, Winlock, Coupeville, Ebeys, Sequim, Sifton, Pondilla, Snakelum, Townsend, Rock Outcrop, Rock Land, Lithic Haploxerolls, Dystric Xerochrepts 70-90% slope, Rough Broken Land, or complexes involving one of these types (Chappell et al. 2001). According to Dobie-Laubenheimer (2000), soil series that are currently associated with prairie vegetation also include Wellman and Hoypus.

In southern Puget Sound, prairies occur on gravelly, well-drained soils or on other soils derived from materials with low water holding capacity. These prairie soils are shallow, sandy to gravelly loam soils collectively referred to as the Spanaway series with inclusions of Nisqually and Carstairs soils (Crawford and Hall 1997). The A1 horizon is typically a strongly acidic, gravelly sandy loam with high organic content. The soil becomes less acidic with depth until it consists of large stones in a matrix of sand and gravel below 18 inches (Lang 1961). The A horizon of the Spanaway series is medium to strongly acid with a pH between 5 and 6. It has a % N content of about 1% and a C/N ratio of approximately 13 (Ugolini and Schlichte 1973). These soil types are generalized as being low in productivity and very prone to drought.

While the major examples of western Washington prairies exist in southern Puget Sound, similar habitats are scattered over limited coastal areas of British Columbia, parts of Whidbey and the San Juan Islands, and a number of locations in the Willamette Valley in Oregon (Crawford and Hall 1997). In northern Puget Sound, open grasslands occur in dry areas within the rain shadow of the Olympic Mountains (Jones 1936). Historic prairies on Whidbey Island occur in the belt that receives the least rainfall, and during the latter part of the growing season, soils of these areas tend to dry out more than those of surrounding areas (Ness and Richins 1958). In 1958, the U.S. Department of Agriculture estimated the average annual rainfall for the Whidbey Island town of Coupeville to be 18.64 inches with a summer average of just 2.49 inches (Ness and Richins 1958; Engle et al. 2000). The region just north of Coupeville is within the rain shadow and is one of the driest areas in all of Island County. According to the Island County Precipitation Network, prairie sites have a 30-year average of between 22.5 and 25.0 inches of rain (Engle et al. 2000).

Dobie-Laubenheimer (2000) investigated historic accounts of prairies occurring on both sides of the Olympic Mountains. She determined that most of these northern prairies have disappeared and found limited published evidence of their existence. Fowler and Wheeting (1941) described the vegetative cover of these northern prairies as primarily

mixed grass and herbs. Currently, prairie species can be found on remote, inaccessible or protected areas that have been spared the impacts of agriculture and development or on sites with extremely steep slopes or shallow soils. Examples of protected areas include the Yellow Island Preserve in the San Juan Islands, the Bocker Environmental Reserve on Whidbey Island and the Trial Island Ecological Reserve in British Columbia. Remnant native vegetation also persists along inaccessible coastal bluffs, where soils are derived from sandy glacial deposits, and on shallow-soiled rocky balds with moderate to steep south or west facing slopes (Chappell et al. 1999; Dobie-Laubenheimer 2000; Chappell et al. 2001).

While some of these northern prairie remnants support similar vegetation and are underlain by similar soils to prairies in the south, others developed on different soils. Soil characteristics of balds dominated by *Festuca roemeri* in the northern Puget Lowland share the well-drained, xeric conditions typical to southern Puget prairies (Chappell et al. 2001). Dobie-Laubenheimer (2000) compared rocky balds and extant northern prairies and found similar soil characteristics and plant composition in both types of sites. However, soils of many prairie areas on Whidbey Island are not considered poor and are not underlain by coarse glacial outwash. Soils of these sites tend to be sandy loams and are well-drained and higher in productivity than the prairies of southern Puget Sound (Ness and Richins 1958; Pettibone 1979).

The soils that historically sustained prairie vegetation on Whidbey Island are a poorly developed group of soils consisting of deep, unconsolidated rock, or soft mineral deposits, in which few or no clearly expressed soil characteristics have developed. They include the San Juan, Snakelum, Townsend, Ebeys, Coupeville and Pondilla series and have developed from several different parent materials. Differences in their morphological characteristics have been brought about by differences in parent materials. The San Juan and Snakelum soils have developed from glacial outwash, the Townsend from cemented gravelly till, and the Ebeys and Coupeville soils from marine and glacial lake sediments. Some of these prairie areas may once have formed the floors of glacial lakes or the beds of brackish lagoons that drained into Puget Sound and developed into

the present black prairie soils (Ness and Richins 1958). However, this would not be a likely origin of the porous and permeable soils, such as the San Juan and Snakelum, which developed from coarse, gravelly outwash deposits and resemble the prairie soils of southern Puget Sound (NRCS 2000).

These soils range from moderately well-drained to excessively-drained. Permeability is good in these soils; roots and moisture penetrate readily to all parts of the subsoils. The soils have all developed under grass with scattered trees and shrubs; as a result they have black, deep surface layers and their organic matter content is high. Though not extensive, these prairie soils are considered the most fertile and productive in all of Island County (Ness and Richins 1958).

### LOSS OF PRAIRIE HABITAT

The severe decline of native prairie over the last 150 years has caused it to become one of the most imperiled ecosystems in western Washington. Factors contributing to the reduction and degradation of native grasslands include: fire suppression and associated conifer tree invasion, introduction of grazing, invasion of exotic species, and urban and agricultural conversion (Lang 1961; Giles 1970; Agee 1993; Clampitt 1993; Chappell and Crawford 1997; Dorner 1999; Dobie-Laubenheimer 2000). Intact prairies now occupy less than 3% of their historical range (Crawford and Hall 1997). In northern Puget Sound, the historical record indicates that prairies were far more widespread prior to European settlement than they are today. Early accounts include various descriptions of the northern Puget Prairies, especially those of Ebey's Landing on Whidbey Island. Now, only small remnants of these vast open grasslands are left (Gunther 1930; Norton 1979; del Moral and Hanson 1980; White 1980).

The arrival of European settlers in the early 1800's brought about the first of many changes to the prairie landscape and the indigenous people who maintained it. At the time of Vancouver and Whidbey's exploration, three Coast Salish tribes, the Kikiallus, Snohomish, and Skagits lived on Whidbey and Camano Islands. After the treaties of 1855 gave settlers title to western Washington, the native people gradually disappeared from Whidbey Island. However, European settlement alone did not displace them. Like native people everywhere, the Coast Salish had little resistance to exotic European diseases. Syphilis, tuberculosis, and influenza destabilized them over the first fifty years of contact, leaving a vastly weakened population, less able to resist the encroachment of settlers upon their lands. Meanwhile, the first land claims were filed on Whidbey Island in the area known as Ebeys Prairie (White 1980). The rich loamy soils of the prairies were the first to be settled and converted to agriculture. Now all but a few acres of prairie soils are farmed (Ness and Richins 1958).

As settlers converted the prairies to farmland, they prevented indigenous people from maintaining the prairies with regular fires (Lombardi 1997; Perdue 1997). Without the burning practices of native people, the surrounding forest began to encroach onto areas that had been under prairie vegetation for thousands of years. In addition, wildfire suppression allowed many exotic and fire-sensitive species to invade the prairies and radically alter their composition (Kruckeberg 1991; Agee 1993). The encroachment of woody species such as Douglas-fir and Scott's broom has further contributed to the decline of otherwise intact grasslands in southern Puget Sound (Lang 1961; Giles 1970; del Moral and Deardorff 1975; Evans et al. 1975; Dorner 1999). Furthermore, the introduction of large numbers of grazing animals and other agricultural practices tended to open the prairie community by removing the ground cover vegetation and thus creating a favorable mineral seed bed for the forest species (Ugolini and Schlichte 1973).

Much of the native grasslands that remain have been disturbed and invaded by exotic species. Recent studies have attempted to describe the species composition, environmental features, exotic species and successional status of the native-dominated prairies of Puget Sound (del Moral and Hanson 1980; Chappell and Crawford 1997; Dobie-Laubenheimer 2000). The majority of remaining grasslands are dominated or co-dominated by exotic grasses and forbs or invaded by woody species (Buschmann 1997; Chappell and Crawford 1997; Dorner 1999; Yeatts et al. 1999). Even in the remaining communities with the best conditions, non-native species are present with high

frequency. The few remaining tracts of prairie dominated by native species are comprised mostly of *Festuca roemeri* and mosses with a diverse and somewhat variable assemblage of native herbaceous species (Chappell and Crawford 1997).

### RAMIFICATIONS FOR SPECIES OF CONCERN

Because of the extreme alteration of the size and composition of the prairie landscape, remnant areas of native prairie vegetation have taken on added significance. Within the state of Washington, *Festuca roemeri* grassland ecosystems are considered a conservation priority (Rolph 1997), a priority habitat (Washington State Priority Habitat List 2003) and a known high-quality or rare plant community (WANHP 2003). *Festuca roemeri*-dominated prairies of the Puget Lowland have themselves become rare and unique ecosystems.

Many species of flora and fauna associated with these unique habitats are of conservation concern due to declines in populations, local extirpation, or close associations with declining habitat (Leonard and Hallock 1997; Rogers et al. 1997; Chappell et al. 2001). Rolph (1997) compiled a list of 31 species of concern that are found within diminishing southern Puget Sound prairie habitats. It includes 13 butterflies, 12 birds, three mammals, and three wildflower species. Federally listed species include *Polites mardon* (Mardon skipper) which is endangered within the state and a federal candidate for listing, *Thomomys mazama* (Mazama pocket gopher), both a federal and state candidate for listing, *Aster curtus* (white-top aster), a federal species of concern and state sensitive species, and *Castilleja levisecta* (golden paintbrush), a federally threatened species, listed as endangered within the state (USFWS 1997; WANHP 1997; WADFW 2003; WANHP 2003).

With the exception of *Castilleja levisecta*, these species are not currently known to inhabit the northern Puget Sound prairies. However, the present range of *Aster curtus* extends north to Vancouver Island, B.C. (Giblin 1997). In addition, the Washington Natural Heritage Program reports three prairie species that no longer exist in Island

County, but for which a historical record exists: *Agoseris elata* is state listed as sensitive, *Balsamorhiza deltoidea* is currently under state review, and *Fritillaria camschatcensis* is state listed as sensitive (WANHP 2003). Documentation of the fauna that utilize northern prairies is still incomplete. Yet the few remaining northern prairie patches do provide habitat for birds, insects and other fauna, some of which rely on grasslands for their continued survival. At least five Lepidopteron species and 75 species of birds are reported to utilize the Smith Prairie on Whidbey Island alone (Yeatts et al. 1999).

It is conceivable that many of the species declining due to habitat loss in southern prairies may benefit from the restoration and expansion of prairies in the north. It has also been argued that native-dominated examples of these vegetation types in intact landscapes are most likely to be useful in stemming the decline of species of concern. They may provide connectivity between remnant patches for wildlife dispersal and migration as well as genetically diverse seed sources. Additionally, smaller fragments of native vegetation can be useful as reference sites for future restoration or research and may benefit some species of concern (Chappell and Crawford 1997).

# REINTRODUCTION AND RESTORATION OF AN ENDANGERED WILDFLOWER: *CASTILLEJA LEVISECTA*

The subject of this study, and one of the species of greatest concern, is the rare wildflower known as *Castilleja levisecta* (golden paintbrush). *C. levisecta* is a perennial herb in the Scrophulariaceae. Like others in its genus, *C. levisecta* is considered a hemiparasite, or non-obligate parasite. This means that while *C. levisecta* produces its own chlorophyll and is capable of surviving and reproducing without a host plant, it is also able to form haustoria, or root connections, that may penetrate the roots of neighboring plants. The parasitism allows *C. levisecta* to draw water, nutrients, carbohydrates, and possibly other secondary compounds from these hosts (Mills and Kummerow 1988).

Historically, over 30 populations of *Castilleja levisecta* were reported to occupy prairies and coastal grasslands throughout Puget Sound and the Willamette Valley (Hitchcock and Cronquist 1973; Sheehan and Sprague 1984; Gamon 1995). Due primarily to habitat loss and conversion, *C. levisecta* has been extirpated from Oregon and is now restricted to 12 populations in Washington and British Columbia (Caplow 2001). The majority of the remaining populations are small both in terms of number of individuals and in total area occupied. As of 1997, only five populations contained more than 1000 plants. In the last decade, over a 50% decline in the number of plants in two of the remaining populations has occurred (USFWS 1997). Increasing concern regarding the dwindling status of *C. levisecta* resulted in its listing as a federally threatened species by the U.S. Fish and Wildlife Service in 1997. Other measures of status include G1 and S1 global and state ranking by the Washington Natural Heritage Program and listing by the state of Washington as endangered (WANHP 2003).

By 1999, the U.S. Fish and Wildlife Service released a draft recovery plan for the species. First and foremost, this plan calls for the protection and management of known populations. Additionally, recovery of *Castilleja levisecta* requires the establishment of new populations within the historic range of the species and the expansion of existing populations where possible (USFWS 2000). In a recent assessment of the viability of *C. levisecta*, the authors of the recovery plan considered it highly probable that the species will continue to decline and additional populations will become extinct over the next 10-50 years if aggressive steps are not taken to increase population sizes and establish new populations (Gamon et al. 2000).

In response to these mandates, management plans for six protected populations have been completed, new lands containing the species have been acquired, populations have been regularly monitored, and research has been conducted on host plant requirements, ecological and reproductive processes, and the effect of various restoration treatments on germination and growth (Thomas 2003). The following results from recent studies have yielded new information that will aid in the recovery of the species.

*Castilleja levisecta* is only known to reproduce by seed, though it can increase its area through vegetative spread over small distances (USFWS 2000). Because of this limitation, the careful and efficient use of source population seed has become a focus of investigation. Germination tests have yielded a variety of results. Kaye (2001) found that while fresh seeds from most Washington populations are highly viable, plants grown from different source populations differed in flowering frequency and height. Wentworth's research in 1997 demonstrated even higher germination rates, suggesting viability differences from year to year and source to source.

Other studies indicate that a host species is not necessary for germination and flowering (Wentworth 1994). However, in a nursery setting, it has been observed that plants not having a connection to a host plant may wilt faster and have a lower survival rate than those with a host (Zybas 2003). Further studies found a weak indication that host plant affected plant size but that propagation with host plants did not have a significant effect on the rate at which plants flowered (Kaye 2001).

In addition, researchers at The Institute for Applied Ecology performed experimental crosses with *Castilleja levisecta* to develop preliminary information on the breeding system of the species. Early results indicate that *C. levisecta* may be self-incompatible. This may suggest that small populations could suffer reduced seed production due to inbreeding depression and lack of compatible mating types (Kaye 2002). Most recently, in December 2002, genetic analysis was completed that will be used to determine appropriate seed sources for introduction and reintroduction of the species into its former range in southwestern Washington and the Willamette Valley, Oregon. (Thomas 2003).

Given the apparent importance of source population differences in *Castilleja levisecta* performance, identification of the environmental differences between source populations may be a crucial step in the informed reintroduction process. However, factors considered when determining appropriate seed sources for rare plant reintroduction are a subject of theoretic debate. While some restorationists believe that plant materials should be brought only from the closest, most ecologically and or genetically similar site, others

argue for the movement of plant materials from distant sources. It has also been hypothesized that locally adapted plants may have a "home-site advantage" over plants of more distant provenance and that their use in restoration projects may increase plant survival and success (Kaye 2001). Recently, researchers in California found that geographic distance of the source population to the restoration site might be less important than genetic distance and environmental similarity of the source and planting site. They concluded that genetic and environmental similarities of source populations should be considered when source materials are selected for restoration projects (Montalvo and Ellstrand 2000).

This highlights the need for site characterizations of the extant *Castilleja levisecta* populations (Caplow 2003). There is still much to be learned about the habitat requirements for *C. levisecta*. The federal recovery plan for the species indicates that *C. levisecta* occurs on generally flat grasslands, including some that are characterized by mounded topography, and on steep coastal bluffs. The coastal bluffs have a west or southwest aspect and are grass-dominated. The only extant mainland population in Washington occurs in a gravelly, glacial outwash prairie. Other populations occur or occurred on clayey soils derived from either glacial drift or glacial lake sediments. Historic populations also occurred on near-bedrock soils as well as clayey alluvial soils (USFWS 2000). The federal recovery plan points out that successful management of this species and its habitat will depend upon gathering additional information about *C. levisecta*'s habitat requirements and biology, as well as effectively monitoring populations and their response to management activities (USFWS 2000).

This study was conceived to gather just such information. Half of the existing populations in Washington are located on the northern half of Whidbey Island in northern Puget Sound. This study attempts to characterize the soils of three extant populations on Whidbey Island. As part of the next step in rare plant reintroduction (Falk et al. 1996; USFWS 2000), a second portion of this study focuses on the selection of pilot reintroduction sites and the establishment of two experimental populations on Whidbey Island.

### **RESTORATION POSSIBILITIES: CHALLENGES AND STRATEGIES**

As the importance of prairie habitat expansion has become more widely recognized, a number of potential restoration sites have become available on Whidbey Island. The Au Sable Institute, an organization committed to teaching about and conducting prairie restoration, recently purchased an important remnant of northern Puget prairie just outside of Coupeville. The Nature Conservancy has also acquired a large tract of land including prairie vegetation along the bluffs of Ebey's Landing. Increased awareness among public land managers is also leading to the consideration of restoration and preservation needs in their management plans.

Aside from the few remnant prairie patches that exist on preserves or are limited to agriculturally inaccessible terrain, soils with the potential for sustaining prairie vegetation are covered by cropland or agricultural weeds. The current challenge is therefore to reconvert selected areas of exotic grassland and farmland to prairie habitat. Crawford and Hall (1997) argue for investments in rehabilitation of exotic grasslands in order to restore the landscape structure nearer the potential indicated by soils. They further assert that while conversion of agriculture or forest sites to grassland will be costly, it will also be necessary if landscape functions of prairie species metapopulation dynamics are to be restored.

Restoring native grasslands and reconverting agricultural sites has been the subject of a number of studies in the Pacific Northwest, across the United States, and in Great Britain. Two of the major challenges facing prairie restoration in western Washington and abroad are the invasion of exotic species and the alteration of soil characteristics.

The cultivation of prairie grassland is known to change both the soil and vegetation structure, allowing disturbed areas and surrounding edges to be colonized by exotic species (Buschmann 1997). Modern agriculture has put an end to regular, low-intensity burning of the prairies, which in turn allowed for the encroachment of native woody and exotic species (Agee 1993). In addition to the alteration of fire regimes, the alteration of soil characteristics may contribute to exotic invasion.

Conversely, exotic species may have deleterious effects on native soils. Some studies have theorized that the invasion of exotic nitrogen-fixing plants potentially facilitates the spread of other exotic plants through their effects on soil nitrogen availability (Vitousek and Walker 1989; Witkowski 1991; Maron and Jefferies 1999). In 1973, Ugolini and Schlichte evaluated the differences between prairie soils that had been invaded by woody species and prairie remnants. While the vegetation differed on the sites they studied, other soil-forming factors such as parent material, topography and climate were relatively similar. They found that the soil parameters affected by differences in vegetation cover were directly or indirectly associated with organic mater. Total C, extractable C, and N were all higher in the grassland soils but C/N ratio was wider in the forested soil. The authors suggest that these differences may be explained by the higher nitrogen content of grasses, forbs and moss litter as compared to that of the woody litter that accumulates on the forest floor. pH values and clay mineralogy were apparently not affected by differences in vegetation.

Brady and Weil (1999) also describe a great loss of the active fraction of organic matter in soil when native grassland is tilled and converted to cropland. This loss may cause pronounced alterations in aggregate stability, nitrogen mineralization rates and other soil properties attributed to organic matter. In natural grasslands, the contribution of plant root biomass is of particular importance because a great proportion of the total biomass tends to accumulate as soil organic matter. So, while low intensity fire in grassland systems does remove some organic matter from the soil surface, a large proportion is conserved below ground. In comparison, typical agricultural alterations such as heavy tillage and whole plant removal and erosion significantly contribute to the loss of soil organic matter. In central Texas, it was found that long-term agricultural degradation reduced soil organic carbon to less than 25% of the content of untilled prairie soils (Potter et al. 1999).

Agriculture can also impact nutrient-poor native soils by artificially increasing their fertility or altering pH through the addition fertilizer or lime. The quantity of available phosphorus in native soil is typically low and a high proportion of that which is present is not readily available to plants. However in agricultural land in industrialized nations, large quantities of phosphate fertilizers and P-containing organic wastes have been applied to the land, and a buildup of phosphorus in the surface soil has resulted (Brady and Weil 1999). Runoff from animal holding areas where large numbers of livestock or poultry are confined is an increasingly significant source of phosphorus. When soluble sources of phosphorus, such as those in fertilizers and manures, are added to soils, they are fixed and eventually form highly insoluble compounds. Additionally, soil acidity may be increased by ammonia released from a heavy application of manure or through the nitrification of ammonium fertilizers (Brady and Weil 1999). In the British Lowlands, abandoned farmland was found to have significantly higher pH and greater concentrations of extractable phosphorus and exchangeable calcium than adjacent heathland (Pywell et al. 1995). Dorner (1999) found a possible correlation between an increase in available soil potassium and the abundance of the exotic grass Poa pratensis in prairie restoration sites in southern Puget Sound. She suggested further examination of the notion that increased soil fertility promotes exotic grass invasion and decreases the diversity of native species.

Typical management tools used in the restoration of Puget Lowland and Willamette Valley prairies include burning, mowing, selective herbicide application, revegetation with native plants, and mechanical and manual removal of exotic species (Buschmann 1997; Davenport 1997; Ewing 1997; Robohm 1997; Schuller 1997; Dunn 1998; Gee 1998; Schmidt 1998; Dorner 1999; Keeley 2000; Byler 2001; Smith and Fimble 2002; Beall 2003; Dunwiddie 2003). Combinations of invasive species control measures are often suggested or utilized in Puget Lowland prairies (Buschmann 1997; Dorner 1999). However, there are environmental implications for the use of biological controls, herbicides, large equipment, and fire, and cost and labor issues for manual control. Many of these wildland restoration strategies have been adapted from agricultural methods for controlling weeds in cropland. Tilling, disking, and herbicides are now used to clear fields of invasive vegetation and seed drills are being used to re-establish native species (Whisenant 1999). In particular, the judicious use of herbicide has proven an effective tool for invasive species management in northwest prairies (Robohm 1997). Several herbicides, including Poast<sup>™</sup>, Krenite-S<sup>™</sup>, and Roundup<sup>™</sup>, have been successful in controlling exotic grasses and many shrubs (Dunwiddie 2003). A thorough examination of appropriate herbicides, proper timing and concentration of application can maximize the effectiveness of weed control and minimize secondary impacts.

In addition to these traditional methods for restoration and invasive species control, some unconventional methods have recently been examined for restoring native prairie in weedy fields. These include: nighttime tilling, infrared soil irradiation (Fitzpatrick 2003) and soil impoverishment (Morgan 1994; Seastedt et al. 1996; Morgan and Seastedt 1999; Alpert and Maron 2000).

Efforts in the restoration of *Castilleja levisecta* habitat have included mechanical removal of Douglas-fir and Scott's broom, mechanical and chemical treatment of invasive exotic species and woody shrubs, mowing, prescribed burning, and the planting of native forbs and grasses. It has been observed that burning of native plants may encourage short-term growth. Dunwiddie et al. (2000) found that increases in *C. levisecta* populations following burning appear to result from a combination of decreased mortality rates, increased seed germination and/or seedling survival, and increased seed production by a growing number of flowering adults. However, the positive effects of burning may be temporary. Since 2000, recovery activities expanded to *C. levisecta* populations on Whidbey Island where more than 1000 seedlings of the rare plant have been installed (Thomas 2003).

### PURPOSE OF THIS STUDY

There are two primary goals of this study:

A) To assist in determining habitat requirements for Castilleja levisecta.

The first section of this study involved gathering baseline data on the soil characteristics of three extant *C. levisecta* populations and six nearby sites that may have reintroduction potential. Knowledge of these characteristics may help to determine which soil factors are important to consider when selecting locations for Puget Lowland prairie and *C. levisecta* restoration on Whidbey Island. Comparisons of soils at population sites and potential reintroduction sites may assist in the evaluation of the suitability of these sites for *C. levisecta* reintroduction. This section of the study asks two main questions:

What are the soil characteristics at these three Castilleja levisecta populations?

Are there particular soil characteristics that distinguish extant *Castilleja levisecta* populations from each other or the potential reintroduction sites, and if so, what are they?

<u>B)</u> To provide recommendations for site preparation techniques on old farm fields that limit exotic plant invasion of sites during early stages of restoration.

The second section of this study included an analysis of experimental site preparation strategies on two potential reintroduction sites. Three types of herbicide and or tilling treatments were applied in order to control the invasion of exotic species on the sites. This portion of the study asked two main questions:

How does each treatment affect the specific exotic species present on each site?

How does each treatment affect the survival of planted native species and, specifically, the vigor of planted *Castilleja levisecta*?

Limited time and resources were the main constraints of this study. Only two field seasons were available for both the installation and evaluation phases of the site

preparation experiment. Limited data from a narrow timeframe can only provide a glimpse into the actual effectiveness of the treatments employed. Secondly, a restricted budget only allowed for a few soil characteristics to be analyzed from a few sites.

# CHAPTER II. METHODS

### **SECTION A: SOIL STUDY**

### SITE DESCRIPTION AND SELECTION

The soil portion of this study focused on gathering baseline data that may assist in future *Castilleja levisecta* recovery efforts. Three types of sites were analyzed for basic physical and chemical soil characteristics: extant *C. levisecta* populations, remnant sites containing native prairie species but no *C. levisecta*, and locations experimentally planted with *C. levisecta*. The following nine sites were chosen:

SITE NAME	SITE TYPE	ABBREVIATION
Fort Casey Population	Extant Population	FCP
Ebey's Bluff Population	Extant Population	EBP
Sherman Experiment	Experimental Plants	SHE
Sherman Remnant	Remnant Vegetation	SHR
Wendy Wayne Experiment	Experimental Plants	WWE
Forbes Point Population	Extant Population	FPP
Forbes Point Remnant	Remnant Vegetation	FPR
Smith Remnant	Remnant Vegetation	SMR
Smith Experiment	Experimental Plants	SME

Table 1: Study Site Names, Types, and Abbreviations

In general, study sites were selected based on their long-term accessibility, management status and potential to represent the range of diversity of *C. levisecta* on Whidbey Island (see Figure 3 for locations).

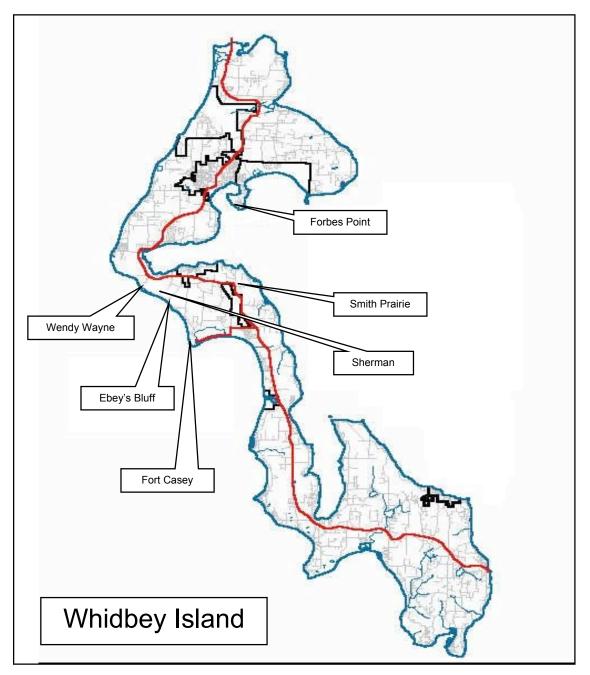


Figure 3: Map of Study Site Locations on Whidbey Island, Washington.

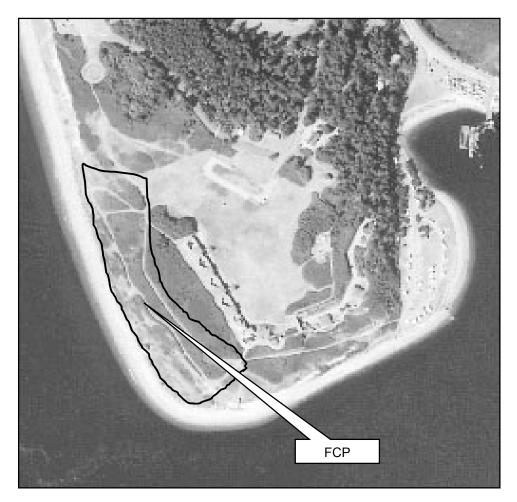


Figure 4: Aerial Photo of the Fort Casey Population (Walker and Associates 1997).

The Fort Casey Population is located along the west and southwest-facing bluffs and open terraced areas of Fort Casey State Park. The site has a mean aspect of 240° (SW) and 0-19% slope. Castilleja levisecta appears along frequently used roads, trails, and other disturbed areas with low growing vegetation or on the sandy bluff face. Currently, the park is attempting to expand the population boundaries by experimentally mowing and burning large swaths of adjacent brush. In order to decrease the vulnerability of *C. levisecta* to herbivory and human disturbance, the State Park has begun fencing small clusters and individual plants during their reproductive phase (Smith and Fimble 2002). However, the primary management of this site is for recreational use. Some officials of the Ebey's Landing National Historical Reserve have suggested that the environmental disturbance that resulted from the building of Fort Casey in the late 1890's may account for the rare plant's expansion into the terraced portions of the site. National Park Service

Naturalist Ed Schreiner has speculated that this upper area was once forested, a condition unfavorable to *C. levisecta*. Once the forests were cut, the disturbed soil may have provided an open environment for *C. levisecta* to flourish (McKinley 1993).

The soil series designation of this site is classified as Hoypus Gravelly Loamy Sand. This soil tends to exist on hilly, steep sites underlain by loose gravelly or sandy drift or glacial outwash. This soil is gravelly throughout the profile and tends to be acidic and too droughty for agricultural use because it is too loose and porous. It is characterized by excessive natural drainage, slow surface runoff, very rapid internal drainage, moderately shallow root penetration and low moisture supplying capacity. However, the Hoypus series was not originally classified as a prairie soil (Ness and Richins 1958). The soil of FCP was confirmed as having a sandy texture. Native vegetation of this soil series is described as predominantly Douglas-fir with some western hemlock, western redcedar, bigleaf maple and Pacific madrone. Understory species include salal, Oregon grape, red huckleberry, western brackenfern, blackberry, western swordfern and deer fern (NRCS 2000).

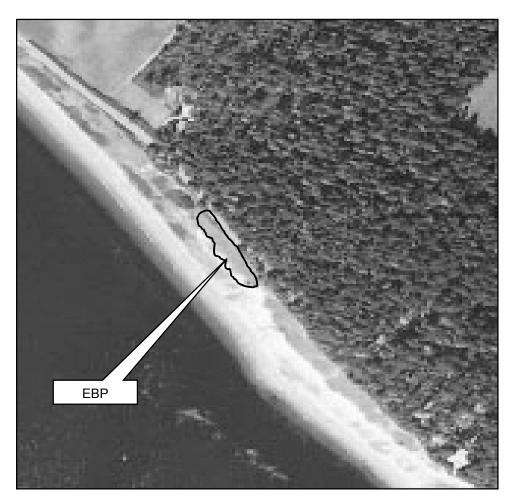


Figure 5: Aerial Photo of the Ebey's Bluff Population (Walker and Associates 1997).

The Ebey's Bluff Population is located along the extremely steep southwest-facing cliffs of Ebey's Landing. The site has a mean aspect of 240° (SW) and 8-35% slope. Erosive forces have begun to cause destabilization of the thin layer of topsoil and vegetation that cling to the bluff. Recently, in July of 2001, an unintended fire swept the southern half of the population, burning most of the aboveground vegetation. Aside from this recent anthropogenic disturbance, the Ebey's Bluff Population remains unmanaged. Vegetation on the site consists primarily of native grasses and forbs, some exotic species, and a few woody shrubs. The Nature Conservancy maintains ownership of this site. Apart from monitoring activities and some roadside littering, this site receives little regular human disturbance. The soil of EBP is classified as generic Rough Broken Land, and is part of a group of soils in Island County that have little or no agricultural value due to their steep

slopes or inaccessible locations (Ness and Richins 1958). The soil at EBP was confirmed as having a sandy texture.

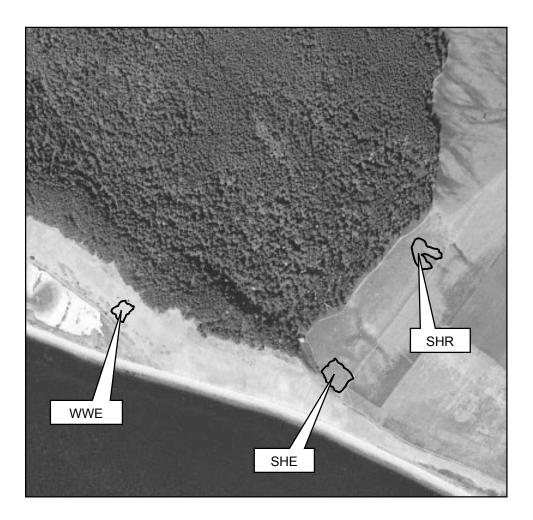


Figure 6: Aerial Photo of the Sherman Experiment, Sherman Remnant and Wendy Wayne Experiment (Walker and Associates 1997).

The Sherman Experiment is located on the sloping plain west of Coupeville and north from the Ebey's Bluff Population within the 554-acre Robert Y. Pratt Preserve. It has a mean aspect of 135° (SE) and 8-16% slope. The parcel consists of previously cultivated land within a matrix of active farms. It was last seeded in 2000 with the exotic perennial grass *Festuca arundinacea* (tall fescue). No native plants were evident on this site prior to experimental manipulation. The southeastern boundary of the site runs along an unimproved hiking trail with farm fields below. To the northwest and northeast are

fallow fields that remain indefinitely unfarmed. The southwestern boundary consists of a thin strip of shrubs, separating the site from another unimproved hiking trail and bluff face that drops to Admiralty Inlet. The Nature Conservancy maintains ownership of the Sherman Experiment site as well as the Sherman Remnant, the Wendy Wayne Experiment, and connecting lands. A primary interest of the Nature Conservancy is assessing the restoration and *Castilleja levisecta* reintroduction potential of this property.

The soil series of SHE is classified as San Juan Coarse Sandy Loam. The San Juan series exists on sloping, somewhat excessive to well-drained soils underlain by loose gravelly or sandy outwash (Ness and Richins 1958). The texture of the soil at SHE was identified as sand and is underlain with uniformly sandy, rather than gravelly outwash. This series is considered well drained with rapid permeability and slow to medium runoff. San Juan soils exist on glacial outwash terraces and plains at elevations of near sea level to about 300 feet. The native vegetation on San Juan soils is typically Douglas-fir, Oregon white oak, and lodgepole pine, with an understory of western bracken fern, Oregon grape, salal, and blackberry (NRCS 2000). The San Juan series is considered to be one of the historical prairie soils (Ness and Richins 1958; Chappell et al. 2001).

About 200 meters inland from the Sherman Experiment is the Sherman Remnant site. It has a mean aspect of 130° (SE) and 5-10% slope. It is a small U-shaped area along the sides of a natural drainage, surrounded by cultivated fields. The awkward sloping angle of the drainage has made plowing the site impractical for farmers. Because it has likely been spared cultivation, the area has maintained some of its native vegetative character. The edges of the drainage contain some *Festuca roemeri* (Roemer's fescue) and *Pteridium aqulinium* (bracken fern) in addition to exotic grasses and forbs. The bottom of the drainage fills with *Urtica dioica* (stinging nettle) in the spring. Like SHE, the soil of SHR is also classified as San Juan Coarse Sandy Loam. The texture was identified as gravelly loamy sand, underlain by loose gravelly outwash. This site is notably more gravelly than SHE. The surface soil is very rocky and shows evidence of burrowing rodent activity.

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Further up the coast and above the southern tip of Perego's Lagoon is the Wendy Wayne Experiment. It has a steep slope (8-38%) and southwest aspect (230°). While this site is steep and fairly inconspicuous from the frequently used hiking trail above, deer tracks traverse the site. Expressly because of its steep slope and inaccessibility, this portion of Ebey's Landing was never farmed. Many native prairie grasses and forbs populate this slope along with a variety of exotic invasive species. In 2000 a number of plots within this site were treated and seeded with *Castilleja levisecta*. Nursery grown plants were installed the following year. Similarly to the EBP site, the soil of WWE is designated as Rough Broken Land. The texture of this soil was confirmed as sand.

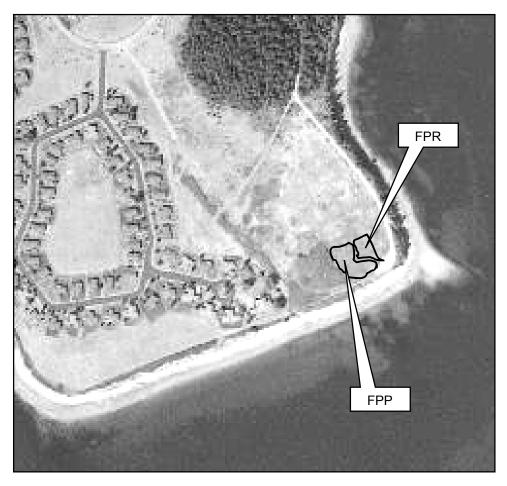


Figure 7: Aerial Photo of the Forbes Point Population and Forbes Point Remnant (Walker and Associates 1997).

Both Forbes Point sites are located on the Whidbey Naval Air Station seaplane base near the town of Oak Harbor. They have a mean aspect of 150° (SSE) and 0-6% slope. The United States Navy currently protects this *Castilleja levisecta* population from rodent herbivores with a partially buried mesh fence. In the past, the U.S. Navy has undertaken some management actions including weed control, mowing, prescribed fire and rodent control. Currently, the population remains essentially unmanaged except in some designated research plots. Vegetation within the fenced area is composed of a mixture of native and exotic grasses and forbs and a small number of encroaching shrubs (primarily *Berberis nervosa* and *Rubus ursinus*). Outside the perimeter of the fenced area is a grassy field that is mowed occasionally. The entire population extends throughout roughly half of the fenced area. The other half contains both native and exotic species, but no *Castilleja levisecta*. In addition, at least three *C. levisecta* plants were noted

growing outside the western perimeter of the containment in the mowed area. For purposes of this study, the populated half of the enclosure was designated the Forbes Point Population and the unpopulated portion of the enclosure was designated the Forbes Point Remnant.

The soils of these two sites are part of the Coveland Loam series. Coveland Loams are very gently sloping, poorly drained soils underlain by fine-textured clay till, marine or lake-laid sediments with some embedded pebbles. Textural analysis of this soil identified it as gravelly loamy sand. Although surface runoff of Coveland Loams is slow, the ground is sloped enough so that excess water is removed by overland flow. During the rainy season the soil becomes saturated, but the water stands on the surface for only a short time. Root penetration is moderately shallow, and moisture-supplying capacity is high. Typically, this soil is also high in organic matter. The surface layer is medium acid, but becomes less acid with depth. Like many of the prairie soils, the native vegetation was mainly grass, sedges, some brush and a few scattered trees. These qualities contribute to Coveland Loam being one of the most productive agricultural soils in the county (Ness and Richins 1958). While the historic extent of prairie in the Forbes Point area is unknown, it is likely that the establishment of the adjacent naval seaplane base and associated housing developments and roads have altered its size. However, though Forbes Point was likely subject to farming in the past, the establishment of the Naval Reserve may have actually served to protect theses sites from recent agricultural impacts.

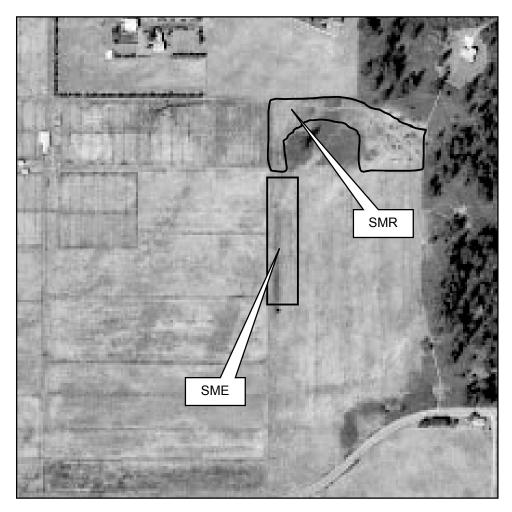


Figure 8: Aerial Photo of the Smith Remnant and Smith Experiment (Walker and Associates 1997).

The Smith Remnant and Experiment sites are located within what is known as the Smith Prairie, a few miles southeast of Coupeville. A 150-acre parcel containing this area was used by the Washington Department of Fish and Game from the late 1940's to 1995 as a place for raising pheasants. During the days of the State Game Farm, the prairie remnant was primarily used for grazing pheasants and was spared the more serious impacts of farming. According to a report from Ebey's Landing National Historical Reserve, while this land was used for agriculture it was never as productive as the other prairies, and as a result, less intensive pasture and grazing replaced market crop production and permitted the endurance of some natural open areas (Gilbert 1985). Now the Au Sable Institute owns and manages this property with the purpose of expansion and improvement of the prairie remnant. Active restoration and monitoring are part of their management regime.

The remnant itself is only about 3.5 acres in size and is a part of the property thought to never have been cultivated. It has a mean aspect of 215 ° (SW) and 0-2% slope. Situated in the far northeast corner of Au Sable's land, this small remnant harbors what is believed to be the largest and most intact examples of northern Puget lowland glacial outwash prairie. It has been referred to as one of the best examples of northern Puget prairie in existence (Byler 2001). At least 38 species of native bunch grasses and forbs have been identified on the Smith Remnant (Yeatts et al. 1999; Dobie-Laubenheimer 2000). To the east it is bordered by Douglas-fir forest, to the north by agricultural land, to the west by old pheasant enclosures, and to the south by exotic pasture grasses.

Immediately to the south of the Smith Remnant is the area designated for purposes of this study as the Smith Experiment. It has a mean aspect of 170° (SSE) and 0-2% slope. Exotic grasses and forbs dominate this area. The soil on this site contains large cobbles and the last known active cultivation of the area is thought to have occurred at least 7 years ago (Byler 2001). A dirt trail and old pheasant pens border the experimental area on the western side and a large patch of *Cirsium vulgare* (Canada thistle) lies to the east.

Soils of SMR and SME are of the Snakelum Coarse Sandy Loam series. The Snakelum series consists of deep, well-drained soils formed in glacial outwash on plains. Both Smith soils were confirmed as having gravelly sandy loam textures. Soils of the Snakelum series are used mostly for cropland and urban development. Small grains, hay and pasture are common crops. Native vegetation is thought to have been grasses and scattered Douglas-fir and western hemlock trees. The Snakelum series is considered one of the historic prairie soils (Ness and Richins 1958).

#### SAMPLING DESIGN

At each of the nine sites, five sampling points were randomly established and marked with colored flagging. All soil samples were taken from the top 15 cm of the A horizon, within a 1 m radius of the flags. The following soil characteristics were selected for

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study because of their influence on water or nutrient availability and the importance of these resources to plant growth:

- % Rock Fragments
- Bulk Density
- pH
- Soil Texture
- % Loss On Ignition
- Extractable Nitrogen  $(NH_4^+ N \text{ and } NO_3^- N)$
- CHN Content
- Extractable Phosphorous (PO<sub>4</sub> -P)
- Soil Moisture Over The 2002 Growing Season

According to James and Wells (1990) the time of year samples are collected influences the results obtained. In general, soil samples taken in late summer or early fall will probably test lower in pH and plant available phosphorus than if taken any other time of year. Highest readings would likely be measured on samples taken during the winter or early spring months. Particularly for medium or lower testing soils in phosphorus and on very acid soils, the late summer or early fall samples may more accurately reflect the plant-availability of nutrients than samples taken during the winter or early spring. Considering this seasonal variability, all samples were collected at the end of September 2002. The exception to this was seasonal moisture sampling, which occurred regularly throughout the 2002 growing season.

# DATA COLLECTION AND LAB ANALYSIS

# Bulk Density and % Rock Fragments

Bulk density samples were collected at each of the sampling points only once during the year. All samples were taken with a 5 cm diameter steel corer and slide hammer to a depth of approximately 15 cm. Samples were air dried and weighed. A 2 mm sieve was used to separate rock fragments from soil particles. Bulk density was then calculated

taking into account core volume and adjusted for rock fragments (Blake and Hartage 1986).

## Soil Texture

After bulk densities were determined, particle size analysis was performed to estimate their percent sand, silt, and clay contents (Sammis 1996). Soils were agitated for 30 seconds with approximately 500 mL DI water and 1 teaspoon of dispersing agent (Calgon<sup>™</sup> foam bath). Solutions were allowed to settle. After 40 seconds, 6 hours and 72 hours, the level of settled particles was measured as an approximation of the percent sand, silt, and clay in the soil. Volumetric percentages were converted to weight percentages and soil texture was defined with the soil texture triangle (Brady and Weil 1999).

Four 15 cm deep soil cores were taken from each sampling point, composited, and then analyzed for pH, loss on ignition, nitrate, ammonium, CHN content, and extractable phosphorous. Samples were air-dried and sieved with 2 mm.

# <u>рН</u>

pH was measured with a Hanna Instruments 8314 multimeter. 20 grams of soil were measured into 100 mL beakers. Samples were then saturated with 40 mL DI water and stirred for 10 seconds every 15 minutes for one hour. Once the suspensions settled, pH was measured (Cappo et al. 1987)

# % Loss on Ignition

Measuring the loss of weight on ignition gives an approximation of soil organic matter. Soil samples were dried overnight at 105° C. Approximately 10 g of this dried soil was measured into a tared crucible, heated to approximately 400° C for 16 hours and reweighed to determine loss of weight on ignition (Storer 1984).

# Extractable Nitrogen (NH<sub>4</sub><sup>+</sup> -N and NO<sub>3</sub><sup>-</sup>-N)

Two forms of plant available nitrogen, exchangeable nitrate (NO<sub>3</sub><sup>-</sup>) and ammonium (NH<sub>4</sub><sup>+</sup>), were measured with SSSA method 33-3. 10 grams of soil were shaken at 200 oscillations per minute with 100 mL of a 2*M* KCl solution for one hour. Suspensions were allowed to settle for approximately 30 minutes, filtered with Whatman #42 filter paper and refrigerated. Filtrates were analyzed with a Lachat colorimetric automated ion analyzer (Keeney and Nelson 1982).

## CHN Content

CHN content of the soil was measured using the Perkin-Elmer 2400 CHNS/O Analyzer. Approximately 30 mg of ground, sieved soil were analyzed using SSSA method 29-2.2.5.1 to yield total CHN content of soil (Nelson and Sommers 1996).

# Extractable Phosphorous (PO<sub>4</sub>-P)

The Bray 1 method was used to determine soil phosphorus content. 2.5 grams of soil were extracted for one minute with 25 mL of a 0.025M HCl + 0.03M NH<sub>4</sub>F solution. Samples were allowed to settle for 30 minutes, then filtered with Whatman #42 filter paper and refrigerated. Filtrates were analyzed with a Lachat colorimetric automated ion analyzer (Olsen and Sommers 1982).

#### Soil Moisture over the 2002 Growing Season

Grab samples were collected from each point at the various sites every two weeks from April through the end of August 2002 (this is the approximate growing season for grasses and forbs in this region). Soil cores were collected with a 2.5 cm diameter steel corer to a depth of approximately 15 cm (but no less than 10 cm). In cases where samples were taken from very rocky or hardpan soils, the small corer proved insufficient. In these instances, 15 cm deep samples were collected with a large, 5 cm diameter steel corer and slide hammer. Grab samples were sealed in plastic bags, transported in a cooler, and then refrigerated until lab analysis could be performed (approximately 24 hours). In the lab, samples were weighed, heated to approximately 105° C for 24 hours, reweighed, and analyzed for % gravimetric moisture.

# DATA ANALYSIS

For all the parameters measured in the different sites, initial analyses were conducted to confirm that data met the assumptions of an ANOVA. For all data that met the necessary assumptions or that could be transformed to meet the assumptions, one-way ANOVAs were run. Post-hoc multiple comparisons were performed for parameters that showed significant differences among sites, primarily Tukey HSD. In cases where data neither met ANOVA assumptions nor could be satisfactorily transformed, non-parametric analyses were conducted. Specifically, data was ranked and ANOVAs performed on the subsequent ranks. Kruskal-Wallace and Median Test statistics were generated for these data (Zar 1984). In addition, for seasonal moisture measurements, graphical interpretation of the results was further analyzed for trends. All computer-run statistical analyses were completed using SPSS for Windows software (SPSS 2002).

#### **SECTION B: SITE PREPARATION STUDY**

#### SITE SELECTION

Two experimental sites were chosen within the historic extent of prairie on central Whidbey Island, the Sherman Experimental Site and Smith Experimental Site. These sites were selected based on a variety of criteria: location within the historical extent of prairie on the island, proximity to areas of extant native prairie vegetation, soil series categorized as prairie soils (Ness and Richins 1958), accessibility, and willingness of land managers to provide future support and maintenance of sites.

# EXPERIMENTAL DESIGN

This was a randomized block design study done with replication on two experimental sites, in which herbicide treatment was randomized within three blocks for either the slope of the land (Sherman) or proximity to a *Cirsium arvense* seed source (Smith).

Treatments and blocks were both considered fixed effects in the model. Block was modeled as a fixed effect because the levels of the block were different from one another, small in number, and completely representative of the possible blocks in the study (see Figure 9).

Three treatments were employed: control (tilling only), glyphosate (Roundup-Pro <sup>™</sup>) application in addition to tilling, and oryzalin (Monterey Weed Stopper <sup>™</sup>) application in addition to tilling. All plots were tilled twice during the course of the experiment. Tilling was considered the control treatment because it constitutes the minimum action one would take when attempting to clear a site of agricultural weeds. Consequently, tilling was applied equally across all plots. Glyphosate and oryzalin applications were the treatment groups. Each herbicide has a different mode of action and therefore a different expected result.

Glyphosate is an amino acid inhibitor that prevents plants from making three essential amino acids. It is applied postemergence; after plants emerge from the soil. Once applied to the plant surface, glyphosate is then translocated within the plant. This means that the herbicide moves from the foliar application site to the target site within the plant through the plant's own transport system (OSU 2001). Glyphosate is a non-selective herbicide that is used to control annual and perennial grasses and broadleaf species. Affected plants become yellow, wilt and exhibit epinasty, progressing from new to older tissues. Glyphosate was selected for its ability to control established weeds and perennial species. A late fall/winter glyphosate application was used to target the major perennial weeds of each site, Festuca arundinacea (tall fescue) at the Sherman site and Cirsium arvense (Canada thistle) at Smith. F. arundinacea is known to invade grassland, savanna and woodland habitat and is a persistent perennial that can compete strongly with many native species. Because F. arundinacea is considered a cold season forage plant and continues to exhibit root growth during the cold months, it can translocate glyphosate while other species remain dormant and protected (Batcher 1999). This is also true of Cirsium arvense, a class C noxious weed in Washington State (2003). Though the thistle

is still in the rosette stage during this period, translocation from shoot to root continues to occur (Nuzzo 1997; Batcher 1999; William et al. 2000).

Oryzalin employs a different mode of action; it is a growth inhibitor that prevents plant cell growth. It is incorporated into the top few centimeters of soil preemergence (before plants emerge from the soil) where it contacts seeds and kills them during germination. Oryzalin was selected for its ability to control weeds in the seed bank and emerging annual weeds. Both the Smith and Sherman sites contain a substantial suite of invasive annual grasses and forbs and harbor unknown quantities of weed seed below ground.

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							* *	*			* *	*			* *	*	
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			2m			0.	0.5 m buffer		0.5 m			0.5 m					

Figure 9: Diagram of the Site Preparation Experiment.

A buffer of at least 2 m surrounded the perimeter of the experimental area. Each treatment plot was 4  $m^2$  in size. Native species were planted within the interior 1  $m^2$  of each treatment plot. All measurements were taken from this interior sampling area, allowing for 1 m of buffer space between each sampling area and the next.

On both the Smith and Sherman sites, the experimental areas were blocked to account for environmental factors that could potentially affect the experimental outcome. On the Smith site, blocks A, B and C are differentiated because block A was much closer to a source of *Cirsium arvense* propagules than block C (A=near thistle source, B=middle distance thistle source, C=far from thistle source). The Sherman site is located on a slope so blocks are differentiated to account for slope position (A=upslope, B=mid-slope, C=down slope). The upper portion of the slope was suspected to drain faster and be drier than the bottom of the slope where moisture might accumulate. At both sites the three treatments were randomly assigned within each of the blocks with four replicates of each treatment per block. There were a total of 12 replicates of each treatment at each site.

In September of 2001, both sites were plowed to a depth of approximately 15 cm to eliminate the existing exotic vegetation and to encourage germination of existing weed seeds in the soil. In January of that year a 2% solution of glyphosate was applied with a backpack sprayer to all the glyphosate plots (approximately 90 mL herbicide to 3.8 L of water per site). In February all plots were tilled to approximately 15 cm with a rototiller. In early March, the oryzalin plots were treated at a rate of 1.5 oz per 1000 square feet with a backpack sprayer and lightly raked for even distribution.

During the second week of March 2002, 360 *Festuca roemeri*, 180 *Castilleja levisecta* (with an additional 180 *F. roemeri* hosts) and 180 *Eriophyllum lanatum* were planted at each of the experimental sites (25 plants installed per meter square plot). According to Dobie-Laubenheimer's (2000) study of the vegetative composition of two prime northern Puget prairie remnants, the Kai Tai and Smith Prairies, *Festuca roemeri* and *Eriophyllum lanatum* account for the largest percentages of native species cover. All plants used in this study were propagated from locally collected seed from either Ebey's Landing or the

Smith Prairie using a mixture of native and nursery soil and employing seed stratification treatments as outlined by Drake et al. (1998). All *C. levisecta* plants were derived from the Ebey's Bluff Population and propagated by Wendy Wayne and the Rare Care Program.

# DATA COLLECTION

From this point onwards the plots were visited regularly and photographed. The following data were gathered in each plot in late May and early June; approximately 2.5 months after planting; just before many exotic species release seed:

- Total weed biomass in each plot
- Presence/absence of the primary weed species of concern for each site
- Total # of weed species in each plot
- Survival of native plantings (*Festuca roemeri*, *Eriophyllum lanatum* and *Castilleja levisecta*)
- *Castilleja levisecta* vigor (on a scale of 1-5)

In June of 2003, approximately one year after the first round of data collection, survival and vigor of planted Castilleja levisecta were measured for a second time.

# Weed Biomass

Because belowground growth accounts for much of the biomass in grassland systems, it was included with the aboveground material in biomass measurements. Hence, total weed biomass was collected by physically removing both the aboveground and belowground parts of each weed present in each plot. Soil was shaken from roots and weeds were placed in paper bags and dried at 70° C until a constant weight was achieved (approximately 96 hours). Dried soil was further separated from plant material and dry weights for each plot were taken. For the small quantities of weeds removed from the Smith site, a scale accurate to 0.1 g was used. For the larger quantities of weeds from the Sherman site, a scale accurate to 0.02 kg was used.

#### Presence/Absence

Presence/absence of the principal weed species of concern (*Festuca arundinacea* at the Sherman site and *Cirsium arvense* at Smith) were noted in each plot. Because these particular species are perennial/biennial and substantially contribute to the biomass measurements, the collection of presence/absence data was an attempt to account for skewing of biomass results. In addition, presence/absence data of the unexpected appearance of the unplanted native *Calandrinia ciliata* on the Sherman site was collected. *Calandrinia ciliata* (red maids) is a low, spreading annual of the Portulacaceae. It is a locally common native typically found in vernally moist, gravelly or compacted soils in open, low elevation grassy meadows (Pojar et al. 1994). The presence or absence of this native annual may exemplify the effects of herbicides on unknown native species in the seed bank.

#### Total # of Weed Species

The number of invasive exotic species growing in each plot was noted at the time of biomass collection. Because many of the emerging weeds were very small at this time, they were difficult to individually identify. A general count of different species was meant as an additional measurement of the effectiveness of each herbicide to control the variety of weed species present at each site.

# Survival

Survival was simply calculated by identifying the remaining number of each native species planted in the plot during spring of 2002.

#### Castilleja levisecta Vigor

Each individual plant was marked with an embossed aluminum tag at the time of planting. Vigor scores were determined by identifying each tagged plant, or the remains of the plant, and assigning it a score based on the vigor of its appearance. Each plant was placed in one of the following categories and assigned a score:

#### 1=Dead

2=Poor; alive but withered and showing substantial signs of stress

- 3=Good; green and general healthy appearance
- 4=Robust; bushy and showing signs of vigorous new growth
- 5=Abundant flowering

While this measurement was meant to be an indirect quantification of the impact of treatments on *Castilleja levisecta* health, the incidence of herbivory on both of the sites may have significantly confounded the results. Originally, the number of flowering stems of individuals was to be tallied per the recommendation of Gamon et al. (2001), but severe browsing by small mammals made this unfeasible.

# DATA ANALYSIS

Standard binary logistic regression was used to examine mortality data for the native plants installed in the plots (Sato et al. 2003). Binary logistic regression tests the effects of each herbicide treatment against the control. Estimates and Likelihood Ratio Test p-values were derived from this comparison using SPSS software (SPSS 2002).

Exact binary logistic regression was used to examine the treatment effects on presence/absence of *Festuca arundinacea* and *Calandrinia ciliata* (a native species that unexpectedly appeared after treatment) at the Sherman site and *Cirsium arvense* at the Smith site. Due to the relatively small sample size of this experiment, standard binary logistic regression proved an insufficient analytical tool because it produces unstable overestimates under these conditions. In contrast, exact logistic regression takes the number of occurrences observed, calculates all possible combinations of treatment and block assignment that could have produced that occurrence, and computes the probability that the occurrence would appear as it did for a given variable by chance (Sato et al. 2003). Estimates and Likelihood Ratio Test p-values for each presence/absence variable were computed with LogXact-4 software (LogXact 2000).

ANOVA was used to analyze the number of weed species, total weed biomass and *Castilleja levisecta* vigor scores. When significant differences existed, Tukey HSD posthoc analysis was performed to further determine which treatment/s were responsible for the differences. The Dunnet Test was used to examine how significant treatment means differed from the control. All ANOVAs were computed with SPSS software (SPSS 2002).

# CHAPTER III. RESULTS AND DISCUSSION

# SECTION A: SOIL STUDY RESULTS AND COMMENTARY

# SOIL CHARACTERISTICS ANALYZED, INTERACTIONS AND IMPLICATIONS FOR PLANT GROWTH

Bulk densities are expected to fall within the range of 0.8-1.2 g cm<sup>-3</sup> for uncultivated grassland soils, from 0.9-1.5 g cm<sup>-3</sup> for cultivated clay and silt loams and from 1.25-1.8 g cm<sup>-3</sup> for cultivated sandy loams and sands (Brady and Weil 1999). Higher than expected bulk density measurements in the known cultivated sites may indicate that soils have been weakened and compacted or that they contain a substantial portion of rock fragments. The percent of rock fragments in a given volume of soil also contributes to drainage and infiltration rates, which impact the soil moisture available to plants. Increases in bulk density typically lead to a poorer environment for root growth, reduced aeration, and reduced infiltration, drainage and water availability to plants.

Soil texture has implications for plant growth in a variety of ways. For finer-textured soils the amount of water held at field capacity tends to be greater than for coarser-textured soils. In addition to water holding capacity and drainage, soil texture affects the bulk density of soils and the accumulation of organic matter. Finer-textured soils accumulate more organic matter because the produce more plant biomass, are less well aerated, and clay-humus complexes help protect some of the soil carbon (Brady and Weil 1999).

pH levels play a role in the availability of certain nutrients to plants. In mineral soils, phosphate fixation is lowest and plant availability is highest when soil pH is maintained in the 6-7 range (Brady and Weil 1999). A low pH favors the process of ammonification over nitrification (Dahnke and Johnson 1990). Furthermore, at high pH levels ammonia volatilization may be more pronounced leading to inorganic nitrogen loss from the system. Mineralization rates are highest at a neutral pH (Brady and Weil 1999).

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Loss on ignition is a general measure of soil organic matter. Soil organic matter has numerous effects on soil health and plant growth including: increasing water availability to plants by improving soil structure and infiltration capacity, stabilizing pH, moderating soil temperature, increasing soil nutrient availability, increasing aeration and reducing erosion and surface water runoff (Brady and Weil 1999).

Organic nitrogen comprises over 95% of the nitrogen found in soil. This form of nitrogen cannot be used by plants but is gradually transformed by soil microorganisms to ammonium  $(NH_4^+)$  through mineralization. Ammonium is not leached to a great extent. Since  $NH_4^+$  is a cation, it is attracted to and held by the negatively charged soil clay. In warm, well-drained soil, ammonium transforms rapidly to nitrate  $(NO_3^-)$  through the process of nitrification. Nitrate is the principle form of nitrogen used by plants. It leaches easily, since it is an anion and is not attracted to soil clay. The nitrate form of nitrogen is a major concern in pollution (Barbarick 1996).

Plant roots take up nitrogen from the soil solution principally as nitrates and ammonium ions. However, the long-term production of these inorganic compounds depends on the total nitrogen content of the soil. The total nitrogen content of surface mineral soils normally ranges from 0.02-0.5%, a value of about 0.15% being representative of cultivated soils. Except in cases where large amounts of chemical fertilizers have been applied, inorganic nitrogen seldom accounts for more than 1-2% of the total nitrogen in the soil. Only about 1.5-3% of the organic nitrogen of a soil mineralizes annually; 2% for fine-textured soils, 3.5% for coarse textured soils (Brady and Weil 1999). In addition, nitrogen may be lost from the soil by volatilization, leaching or erosion. Considering these factors and the importance of nitrogen to plants, total N content of the soils was measured along with extractable nitrogen fractions,  $NO_3^-$  and  $NH_4^+$ .

Measuring the total CHN content of soil allows for a calculation of the soil's C/N ratio and gives a measure of total organic and inorganic nitrogen in the soil. C/N ratio is an important determinant of the amount of inorganic nitrogen in soil that is actually available for plant growth. In soils with C/N ratios of 30/1 or above, the soil microbial community actively responds to the high food supply and expresses a high demand for nitrogen, causing nitrogen depression. Little or no inorganic nitrogen is available to plants until the C/N ratio drops below 30 (Brady and Weil 1999).

Phosphorus is secondary only to nitrogen in its importance in the production of healthy plants. However, the total phosphorus level of soils is usually no more than 10-25% that of nitrogen. The concentration of phosphorus in native soils is typically low and a high proportion of that which is present is not readily available to plants (Brady and Weil 1999). Agriculture specialists from the University of Colorado indicate that in dry land production, soil phosphorus levels of 0-3 ppm constitutes low availability, 4-7 ppm is moderate, and 8 ppm or above is high (Soltanpour and Follett 1999). Environmental factors affecting plant availability of soil phosphorus include temperature and moisture. As soil moisture increases, plant uptake of phosphorus tends to increase (Fixen and Grove 1990). Chemical and physical factors affecting phosphorus availability include pH, clay content and organic matter content. Since plant roots absorb phosphorus dissolved in the soil solution, mainly as phosphate ions, PO<sub>4</sub> was the form of phosphorus measured in this study.

In addition to the essential functions of providing water to plants for growth and balancing evapotranspiration effects, soil moisture influences the decomposition of organic matter, transport of soluble nutrients, nutrient mineralization rates, and aeration (Brady and Weil 1999). Because the land in this study lies within the rain shadow of the Olympic Mountains, annual rainfall and summer droughts are the norm. Measuring soil moisture throughout the droughty growing season gives an estimate of the water available to plants during their time of greatest need.

# SOIL CHARACTERISTICS OF CASTILLEJA LEVISECTA POPULATION SITES

A cumulative range of variation for each soil characteristic was determined for the three population sites. These data suggest a rough approximation of the range of *Castilleja* 

*levisecta* tolerances for each characteristic. Table 2 contains a summary of the soil characteristics of the three population sites.

SOIL CHARACTERISTICS	POPULA	TION SITE	MEANS	CUMULATIVE RESULTS			
	FCP	EBP	FPP	MEAN	MIN	MAX	
% ROCK FRAGMENTS	6.6	10.3	20.3	12.4	1.7	29.1	
BULK DENSITY (g cm <sup>-3</sup> )	0.98	1.04	1.14	1.06	0.80	1.24	
рН	6.0	6.1	5.9	6.0	5.8	6.3	
% LOSS ON IGNITION	11.6	4.2	6.6	7.5	2.0	20.0	
% TOTAL NITROGEN	0.39	0.04	0.08	0.17	0.02	0.66	
NH₄ <sup>+</sup> NITROGEN (mg g <sup>-1</sup> )	10.54	6.26	4.90	7.23	3.50	12.10	
NO <sub>3</sub> <sup>-</sup> NITROGEN (mg g <sup>-1</sup> )	8.00	7.34	0.70	5.35	0.10	9.70	
TOTAL INORGANIC N (mg g <sup>-1</sup> )	18.54	13.60	5.60	12.58	3.70	20.30	
C/N RATIO	15.73	54.08	34.29	34.70	13.97	89.41	
PO₄ PHOSPHORUS (mg g <sup>-1</sup> )	8.06	8.22	13.60	9.96	4.24	33.80	
AVERAGE % GRAVIMETRIC MOISTURE OVER THE GROWING SEASON	15.3	3.4	15.1	11.3	2.6	19.6	
% SAND : % SILT : % CLAY SOIL TEXTURE	93:6:0 Sand	97:3:0 Sand	85:14:2 Gravelly	85:14:2 Gravelly Loamy Sand			

 Table 2: Soil Characteristics of Castilleja levisecta Population Sites

#### % Rock Fragments

The high percent of rock fragments at the FPP site was mainly due to the presence of gravel sized particles. This may serve to increase the drainage in this slightly finer textured soil. Population % rock fragment values are widely distributed over the three sites.

# **Bulk Density**

All bulk density figures are within the expected range for soils of these textures and land use histories; 0.8-1.2 g cm<sup>-3</sup> for uncultivated grassland soils (Brady and Weil 1999).

## Soil Texture

The sandy textures of FCP (93% sand) and EBP (97% sand) imply that water-holding capacities of these soils are low, aeration is good, drainage rates are high, organic matter retention is low, compactability is low, susceptibility to erosion is moderate to low, ability to store plant nutrients is poor, nitrate leaching is high, and resistance to change in pH is low. At FPP the slight clay (2%), and highest silt content (14%), contribute to a higher water-holding capacity, poorer aeration, slower drainage, slower organic matter decomposition, higher compactability, lower susceptibility to erosion and higher ability to store plant nutrients and resist changes in pH than purely sandy soils. Despite the clay content of FPP, it is still classified as loamy sand. In addition, the gravelly nature of the soil may increase the drainage of this site.

#### <u>pH</u>

pHs are all sufficient for phosphorus uptake (pH from 6-7) and are slightly acidic, which favors nitrogen mineralization (Brady and Weil 1999). All the populations have a fairly tight distribution of pH with an average cumulative pH of 6.

#### % Loss on Ignition

All the populations have higher than average amounts of organic matter in the upper 15 cm of their profiles. Typical organic matter percentages for Mollisols (as estimated from Brady and Weil, 1999) range from 1.53% to 8%. FCP is the only population that appears to have somewhat higher percentage of soil organic matter. All else being equal, soils high in clay and silt are generally higher in organic matter than sandy soils. This is the case for EBP (a sandy soil with 4.2% organic matter) but certainly not the case for FCP, also a sandy soil but with high organic matter. Additionally, the fire that burned a portion of the EBP site does not appear to have severely affected organic matter content of the site with an average of 4% LOI in the three burned locations and 4.5% LOI in the two unburned locations.

#### Nitrogen Availability (% Total N, Inorganic N, and C/N Ratio)

In general, population ammonium-N values are widely distributed. Though EBP has a low percentage of total soil nitrogen (0.04 %), it has a fairly high content of inorganic nitrogen (13.60 mg g<sup>-1</sup>), most of which is in the nitrate form. This seems unusual because many of the other measurements indicate that the soils of EBP should not be highly capable of nitrogen retention. However, the low total % N contributes to EBP's high C/N ratio (54), rendering any inorganic N present unavailable to plants. Although three of the five samples were collected from the burned half of the EBP site, the mean C/N of the burned section was 69 while the mean of the unburned section was 31. Despite this figure, a slight loss in % total C coupled with a loss in % total N may have resulted from the burn with 1.61 % C, 0.03 % N in the burned section and 1.82 % C, 0.06 % N in the unburned section. FPP has both a low % total N (0.08 %) and low inorganic N content  $(5.60 \text{ mg g}^{-1})$ . Its C/N ratio is also above 30. In this case, not only is the inorganic N unavailable to plants, but the low total amount of N on site is unlikely to be mineralized and become plant available. There is also a marked difference in the forms of inorganic N at FPP. Ammonium-N accounts for 4.90 mg g<sup>-1</sup> of the total inorganic-N content while nitrate-N accounts for only 0.70 mg g<sup>-1</sup>. It makes sense that the ammonium converted from organic nitrogen in the soil is not being leached because it may bind to the clay particles in this soil. Though none of the sites receive much rainfall during the summer

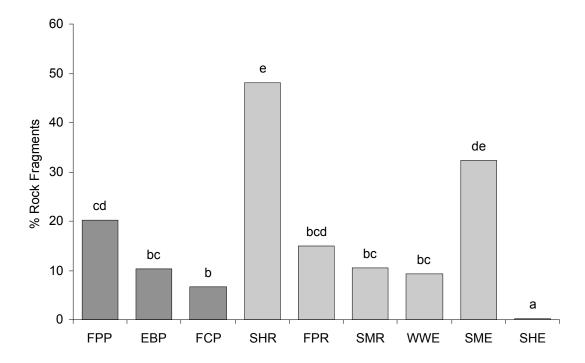
months, FPP is the least well-drained and has the lowest pH of all the populations. These conditions may favor the process of ammonification over nitrification and could account for the accumulation of ammonium-N in the soil (Fixen and Grove 1990). Nitrogen availability is quite different on the FCP site. High % total N (0.39%), high inorganic N content (18.54 mg g<sup>-1</sup>) and low C/N ratio (15.7) combine to allow sufficient N availability to plants.

#### Extractable Phosphorous (PO<sub>4</sub>-P)

All phosphorus measurements indicate that these are low to moderately fertile soils. FPP shows the highest P content of all the populations (13.60 mg g<sup>-1</sup>). While P is highest at this site, the soil also contains a small fraction of clay. According to Fixen and Grove (1990), among soils with similar pH and mineralogy, phosphorus release tends to be lowest in soils with higher clay contents. While the clay at FPP may allow it to retain more P, it is not necessarily available to plants. EBP and FCP have phosphorus values in the low range (Soltanpour and Follett 1999); 8.22 mg g<sup>-1</sup> and 8.06 mg g<sup>-1</sup>, respectively.

#### % Gravimetric Moisture

Though average gravimetric moisture over the growing season was high at FPP (15.1%), its soil texture is also higher in clay and silt than the other two populations. While clay content increases the water holding capacity of soil, it holds soil water more tightly than coarser soils, making it less available for plant uptake. High organic matter content may also contribute to higher soil moisture at FCP (11.6%). Extremely low soil moisture at EBP (3.4%) may also be related to the extremely sandy texture of this site (97% sand) and its steep slope. Very sandy soils do not hold moisture well and drain quickly. Furthermore, after accounting for the lower level of organic matter (4.2%) of EBP, the moisture availability of this site is by far the lowest.



Mean % Rock Fragment

Figure 10: Bar graph of Mean % Rock Fragments. Different letters designate groups of sites with significantly different means. Dark columns represent the extant *C. levisecta* population sites.

Figure 10 shows that mean % rock fragments on the SHE and SHR sites are significantly different from any of the extant population sites. SHE has significantly smaller % rock fragments; 27 times smaller than any population site. Though SHE has a very low fraction of rock fragments, it is a very coarse textured soil. Drainage on this site is excellent, despite the lack of larger pieces of rock. SHR has significantly greater % rock fragments; more than twice as much as any population site. In addition, much of the rock fragments were composed of large cobbles rather than the gravel-sized fractions found on other sites. The topography and fallow status of SHR may account for the high % rock fragments of that site. Dale Sherman, a descendent of one of Whidbey Island's early farming families, recalls that rocks were often removed from cultivated fields of Ebey's Landing (2001). Given this, the presence of considerable quantities of rock fragments at

this site may suggest a lack of intense farming. Farmers have also been known to dump material in the SHE drainage, which may have been a historical depository for rock cleared from nearby fields.

EBP, WWE and SMR do not have significantly different mean % of rock fragments. Despite this similarity, the sizes of rock fragments were observed to be much larger on the SMR site than on EBP and WWE and contained some cobble-sized fractions. SME and SMR, despite their proximity, have significantly different mean % rock fragments. SME has much higher % rock fragments than SMR. Also, there is quite a bit of variation in rock fragment content within the SME site. The north end of the site contained most of the large cobbles and larger rock fragments while the southern end of the site contained fewer, smaller fragments. Additionally, FPP and FPR have the same % rock fragments as would be expected by their proximity.

#### Mean Bulk Densit

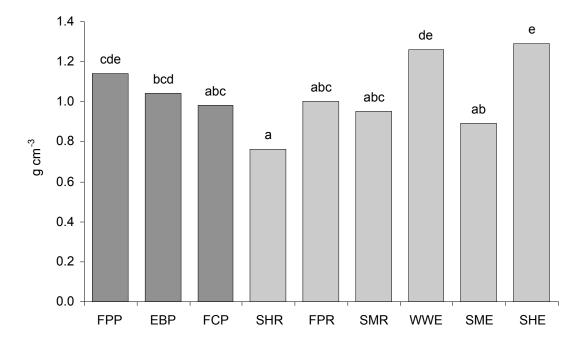


Figure 11: Bar graph of Mean Bulk Density. Different letters designate groups of sites with significantly different means. Dark columns represent the extant *C. levisecta* population sites.

Figure 11 shows that all sites share mean bulk density values with at least one extant population. All the populations have a fairly tight bulk density distribution. Values for the SHR and SHE sites are again at the extremes of the bulk density spectrum. In this case, SHR has low bulk density (0.756 g cm<sup>-3</sup>) despite its high % of rock fragments (47.9%). This suggests that the soil of SHR is composed of light, loose material and lots of organic matter (11.2% LOI) along with large rocks and cobbles. Burrowing animal activity was observed at both SHR and SMR and may have contributed to lower bulk densities at these sites. The sandy texture and low organic content (4.6% LOI) at SHE contribute to its high bulk density (1.29 g cm<sup>-3</sup>). SHE has a significantly greater bulk density than SHR.

Bulk densities of SME and SHR are a little lower than expected; they appear more like uncultivated soils, according to typical values found in Brady and Weil (Brady and Weil 1999). This supports the notion that the SHR site was not farmed. SME is known to have been cultivated, but not for at least seven years (Byler 2001). Means of SME and SMR are very similar to each other. FPP and FPR are similar as well, which would be expected given their proximity

Site	Soil Texture	% Sand	% Silt	% Clay
FPP	Gravelly Loamy Sand	85	14	2
EBP	Sand	97	3	0
FCP	Sand	94	6	0
SHR	Gravelly Loamy Sand	83	17	0
FPR	Gravelly Loamy Sand	88	12	0
SMR	Gravelly Sandy Loam	76	24	0
WWE	Sand	92	8	0
SME	Gravelly Sandy Loam	71	29	0
SHE	Sand	98	2	0

Table 3: Soil Textures Based on % Sand, % Silt, and % Clay.

No statistical analysis was performed on soil texture. However, it may be noted in Table 3 that aside from FPP, none of the soils contained discernable traces of clay (2%). Generally, the sites in this study have very coarse sandy soils and drain rapidly. SME and SMR have the highest silt contents and are loamier than the other sites. However, these sites are also very gravelly and have rapid drainage.



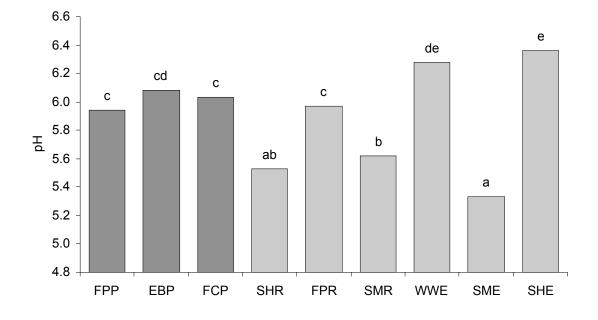
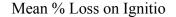


Figure 12: Bar graph of Mean pH. Different letters designate groups of sites with significantly different means. Dark columns represent the extant *C. levisecta* population sites.

Figure 12 shows that SME, SHR and SMR have significantly lower mean pHs than any of the extant populations (5.3, 5.5 and 5.6, respectively). These acidic pHs are lower than ideal for phosphorus uptake and may favor the process of ammonification over nitrogen mineralization. SHE has significantly higher mean pH than any of the extant populations (6.4). It is within the ideal range for P and N uptake. For sites in proximity to one another, WWE has the closest mean pH to EBP of all the populations; FPR has the closest mean pH to FPP; SME has significantly lower mean pH than SMR (5.3 versus 5.6); and SHR has significantly lower mean pH than SHE (5.5 versus 6.3).



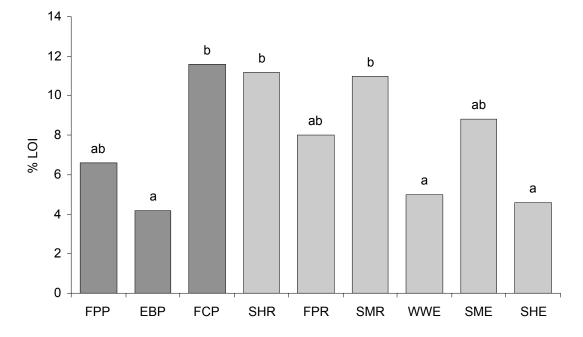
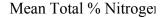


Figure 13: Bar graph of Mean % Loss on Ignition. Different letters designate groups of sites with significantly different means. Dark columns represent the extant *C. levisecta* population sites.

Figure 13 shows that all sites share mean % LOI with at least one extant population. In addition, population % LOI values are widely distributed and include both the highest and lowest values. Mean % LOI of SHE and SHR are significantly different, despite their proximity. SHR has nearly two and a half times greater organic content than SHE. Because loss of soil organic matter is a typical result of agriculture, this difference supports the idea that SHR may never have been subject to intense farming. The lowest % LOI values appear on SHE, EBP and WWE (4.6, 4.2, 5.0%, respectively). SHE has been actively farmed for some time, has a very sandy texture and has probably lost much of its organic matter to tillage effects, erosion and removal of plant biomass from the system. The EBP and WWE sites are also very sandy and have extreme slopes (57% and 38% max slope, respectively) that likely contribute to erosion and the loss of organic matter.



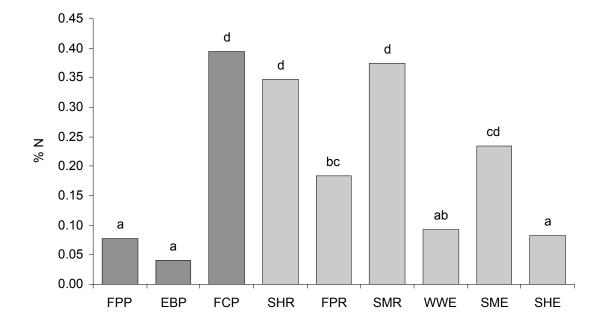


Figure 14: Bar graph of Mean % Nitrogen. Different letters designate groups of sites with significantly different means. Dark columns represent the extant *C. levisecta* population sites.

Because mean % total nitrogen is highly variable among the population sites, all other sites' means fall within the range of % N variability. Figure 14 shows that FPR is the only site that is significantly different from any population. The importance of this difference is questionable, however, because it falls between means for EBP, FPP and FCP.

Most of the mean % total nitrogen values are typical for mineral soil as noted by Brady and Weil (1999). The exceptions in this case are SME, which is high for cultivated mineral soil (0.24%) and SHE, which is low for cultivated mineral soil (0.08%). This furthers the notion that farming impacts on SME have been low and that the land may have recovered somewhat since its last cultivation. The atypically low numbers for SHE are not remarkable considering the leaching ability of the sandy soils of this site.

#### Mean Ammonium Nitroge

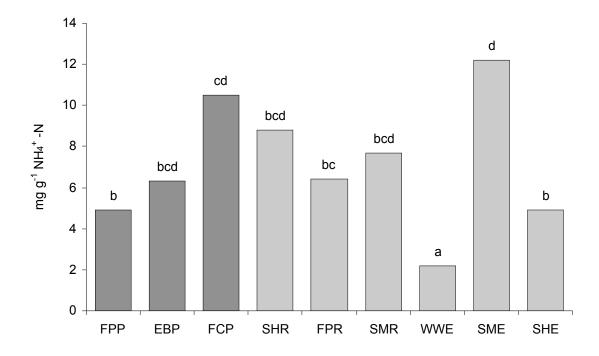


Figure 15: Bar graph of Mean Ammonium Nitrogen. Different letters designate groups of sites with significantly different means. Dark columns represent the extant *C. levisecta* population sites.

Figure 15 illustrates that only WWE has significantly lower mean ammonium-N content than any of the population sites; less than half as much. Of the sites in close proximity to each other, the means of FPP and FPR do not differ, SHE and SHR do not differ and SME and SMR do not differ. SME has the highest ammonium-N content of all the sites  $(12.16 \text{ mg g}^{-1})$  but does significantly differ from all the populations.

#### Mean Nitrate Nitrogei

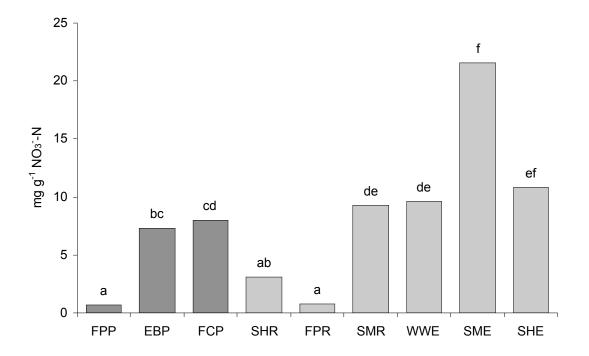
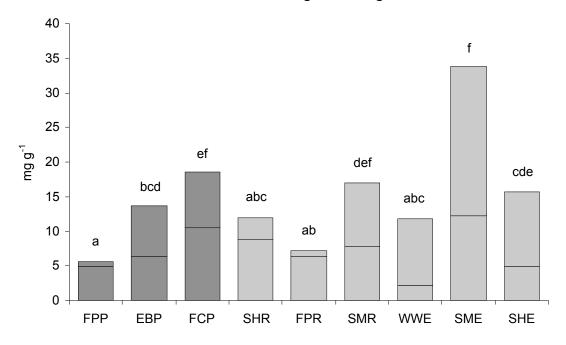


Figure 16: Bar graph of Mean Nitrate Nitrogen. Different letters designate groups of sites with significantly different means. Dark columns represent the extant *C. levisecta* population sites.

Both the Kruskal-Wallace and Median Tests show a significant difference (p=.000) among the mean nitrate-N contents of the soils on these sites. Figure 16 shows that FPR has the lowest nitrate-N content (0.46 mg g<sup>-1</sup>), followed closely by FPP (0.70 mg g<sup>-1</sup>). SME has the highest nitrate-N content (21.64 mg g<sup>-1</sup>) followed by SHE (10.78 mg g<sup>-1</sup>). According to post-hoc multiple comparisons for unequal variances (Dunnett T3), only SME and SHE have significantly higher mean nitrate-N contents than any population site. SME is nearly three times higher and SHE is 1.5 times higher than the mean of the closest population site. This result is somewhat confounding and cannot be easily explained. While these sites share a history of agricultural use and were likely subject to inorganic fertilizers, nitrate is the form of inorganic nitrogen most easily leached from soils. Both these sites are excessively drained so it is unlikely that effects of fertilization would be long-lived. In addition, the fallow SHR site has significantly lower nitrate-N content than the farmed SHE site.



Mean Total Inorganic Nitroge

Figure 17: Bar graph of Mean Total Inorganic Nitrogen. Different letters designate groups of sites with significantly different means. Dark columns represent the extant *C. levisecta* population sites. Upper half of columns indicate the contribution of nitrate N to the overall inorganic nitrogen content. Lower half of the columns indicate the contribution of ammonium N.

Both the Kruskal-Wallace and Median Tests show a significant difference among the mean total inorganic nitrogen contents of these sites. However, Figure 17 shows that no individual site is significantly different from all of the populations. SME has the overall highest mean inorganic nitrogen content (33.80 mg g<sup>-1</sup>), but is not significantly higher than all the populations.



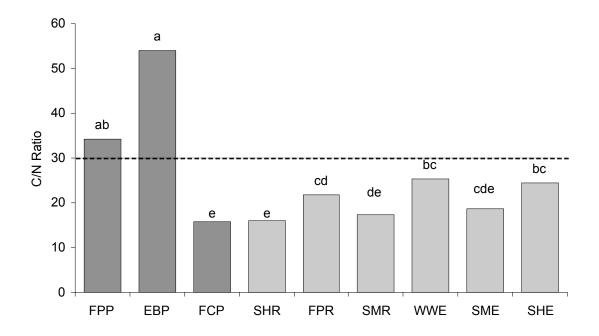


Figure 18: Bar graph of Mean C/N Ratio. Different letters designate groups of sites with significantly different means. Dark columns represent the extant *C. levisecta* population sites. The dashed horizontal line represents the cutoff point for nitrogen depression.

C/N ratios of all the population sites are widely distributed, and include both the highest and lowest means. Figure 18 shows that next to EBP and FPP, which are both above 30, WWE and SHE have the highest mean C/N ratios (near 25) and may suffer from nitrogen depression. Other sites have C/N ratios low enough to allow plant access to nitrogen. The wide variation in C/N on EBP is may be due to samples taken from within and outside the burned area of the site. Both FPR and FPP exhibit nitrogen depression and do not significantly differ in their inorganic nitrogen contents.

Mean Extractable Phosphoru

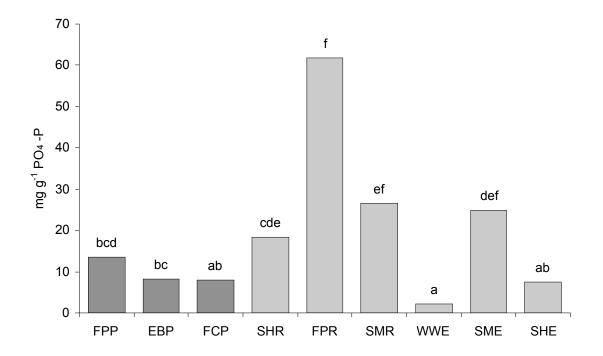


Figure 19: Bar graph of Mean Extractable Phosphorus. Different letters designate groups of sites with significantly different means. Dark columns represent the extant *C. levisecta* population sites.

Figure 19 shows that no site has significantly different phosphorus levels than all the populations. FPR, while not significantly different from all the populations, has a mean phosphorus content that is significantly higher than the closest population, FPP. In fact FPR has more than four times the phosphorus content of FPP (61.71 mg g<sup>-1</sup> as compared to 13.60 mg g<sup>-1</sup>). In addition, FPR shows a wide range in the variation of phosphorus across the site. SMR and SME both have fairly high phosphorus availability when compared to agricultural standards for dry land production (Soltanpour and Follett 1999). This may be a residual fertilizer effect from years of pheasant activity on the sites. SHR has what is considered to be moderate phosphorus availability and is significantly higher than SHE. SHE, WWE, EBP and FCP all have what is considered to be low phosphorus availability.

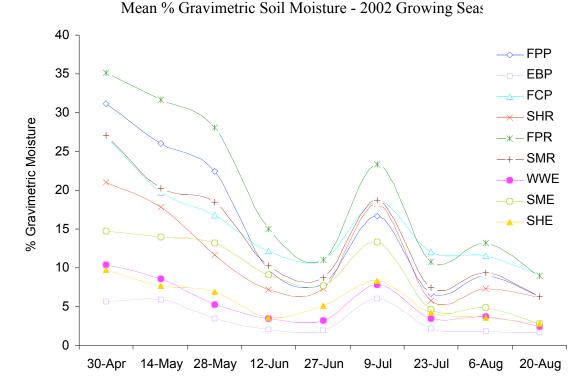


Figure 20: Line graph of Mean % Gravimetric Moisture over the 2002 growing season. Means are based on five samples per site and were collected every 2 weeks.

Figure 20 indicates a wide range in soil moisture among the different populations throughout the growing season. There is no individual site with average % gravimetric moisture over the growing season that significantly differs from all the populations. FPR both and FPP maintained high soil moisture throughout the growing season. EBP, WWE and SME showed very low soil moisture across the season, which may be attributed to factors such as slope and sandy soil textures.

## SITES SHARING SOIL CHARACTERISTICS WITH EXTANT *CASTILLEJA LEVISECTA* POPULATIONS

Table 4 tallies the number of soil characteristics that each site shares with each Castilleja *levisecta* population site. It is an attempt to provide a general picture of the similarities and differences among all the sites involved in this study. For example, in the case of the Fort Casey Population, seven of the 12 soil characteristics measured had means that were not significantly different than those of the Ebey's Bluff Population. In general, the three extant C. levisecta population sites had between five and eight characteristics with means that did not significantly differ from one another. Using this range as a rough standard, tallies for sites that shared five or more characteristics with a population site are bolded. Sites that share the most characteristics with a given population site are also bolded and marked with an asterisk. These starred sites may be appropriate sites to consider for the reintroduction of C. levisecta. It should be noted that the variation in the soil characteristics data was quite large, even among the extant populations. This may have caused differences to appear significant when the characteristics were not indeed different from one another. In light of this variation, sites with low tallies should not necessarily be ruled out for restoration and reintroduction of C. levisecta. It simply means that some potential restoration sites share many soil characteristics with particular extant C. levisecta populations. These similarities and differences may be important to consider when determining the appropriateness of a site for reintroduction.

	Fort Casey Population	Ebey's Bluff Population	Forbes Point Population
EBP	7		8
FCP		7	5
FPP	5	8	
FPR	5	6	*8
SHE	3	7	6
SHR	6	4	7
SME	6	3	3
SMR	*9	4	5
WWE	4	*8	6

Table 4: Summary of Total Shared Soil Characteristics among the Sites

### Fort Casey Population

SMR appears to share the highest number of soil characteristics with FCP. SMR significantly differs from FCP in the following three ways: lower pH (5.62 compared to 6.03), much higher phosphorus (26.46 mg g<sup>-1</sup> versus 8.06 mg g<sup>-1</sup>), and a finer, yet more gravelly soil texture. Despite these differences, substantial native prairie vegetation exists on the SMR site. The lower pH of the soil is comparable to levels found in other western Washington prairies (Ugolini and Schlichte 1973; Dorner 1999; Dobie-Laubenheimer 2000). While the soil textures differ, both are reasonably well drained.

### Ebey's Bluff Population

WWE appears to most closely resemble EBP. WWE significantly differs from EBP in the following four ways: lower ammonium-N (2.22 mg g<sup>-1</sup> versus 6.26 mg g<sup>-1</sup>), higher nitrate-N (9.64 mg g<sup>-1</sup> versus 7.34 mg g<sup>-1</sup>), lower C/N ratio (25.25 compared to 54.08), and lower phosphorus (2.08 mg g<sup>-1</sup> versus 8.22 mg g<sup>-1</sup>). While the ammonium-N and nitrate-N contents differ when measured as individual parameters, total inorganic-N

content does not significantly differ between the sites. The C/N ratio is much lower at WWE and allows for more nitrogen availability than EBP. Overall, it appears that nitrogen is more limited at EBP than WWE. Phosphorous appears less available on WWE than EBP, though both sites have generally low levels.

### Forbes Point Population

FPR seems to be most similar to FPP. FPR significantly differs from FPP in the following four ways: higher total % N (0.183% compared to 0.077%), lower C/N ratio (21.70 versus 34.29), much higher extractable phosphorous (61.71 mg g<sup>-1</sup> compared to 13.60 mg g<sup>-1</sup>), and higher average % gravimetric moisture over the growing season (19.68% compared to 15.10%). Because the total % N is higher and C/N ratio is lower, nitrogen is likely to be more available at FPR than FPP. After accounting for the higher soil moisture levels, it appears that soils of FPR are less nutrient and water limiting than FPP, even though FPP soils contain small clay and marginally higher silt fractions.

### SECTION B: SITE PREP EXPERIMENT RESULTS AND COMMENTARY

### SMITH EXPERIMENT

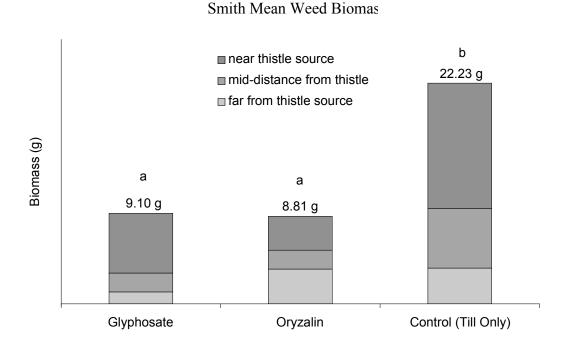
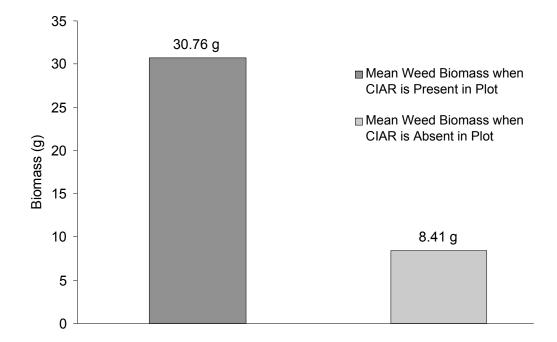


Figure 21: Bar graph of Smith Mean Weed Biomass. Stacked columns represent contribution of each block to the overall treatment mean. Different letters designate groups with significantly different means.

Figure 21 illustrates that significant differences in mean weed biomass exist among the glyphosate, oryzalin and control treatments (p=0.011). Both the glyphosate and oryzalin treatments resulted in significantly lower weed biomass than the control (approximately half as much) although they are not significantly different from each other.

### Effect of Cirsium arvense on Weed Biomass



# Figure 22: Bar graph of Effect of *Cirsium arvense* (CIAR) Presence/Absence on Weed Biomass at the Smith Experiment site.

Because simple weed biomass measurements do not distinguish between number and size of weeds, the ability of treatments to effectively control weed competition is difficult to properly estimate. Figure 22 illustrates the contribution of *Cirsium arvense* presence in a treatment plot to weed biomass measurements. Presence of *C. arvense* appears to correlate with higher weed biomass outcomes. However, according to exact binary regression results, there is no significant difference in the likelihood of *C. arvense* to be present in a given treatment (p=0.313). These observations may assist in the further interpretation weed biomass results.

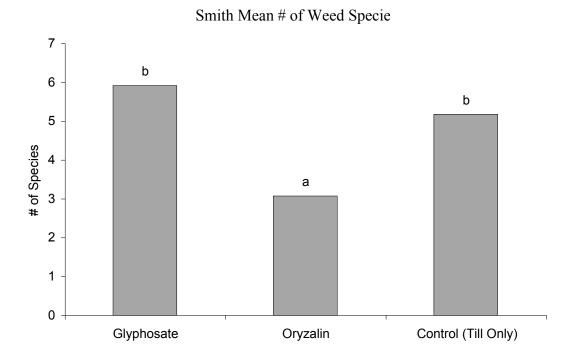


Figure 23: Bar graph of Smith Mean # of Weed Species. Different letters designate groups with significantly different means.

Significant differences in the mean number of weed species exist among the glyphosate, oryzalin and control treatments (p=0.000). Figure 23 shows that the oryzalin treatment resulted in significantly fewer weed species than the control, approximately 40% fewer. The glyphosate treatment is significantly different from the oryzalin treatment, but not from the control. It should be noted that when the glyphosate treatment was applied at this site, after the initial tilling, very few weeds species were actively growing and the ground was mostly bare. This probably influenced the relative effectiveness of the glyphosate treatment. However, since oryzalin affects seeds emerging from the seed bank, it makes sense that this treatment would result in a reduction in the number of weed species. The mean overall number of weed species was 4.6.

Smith MeanCastilleja levisecta Surviva

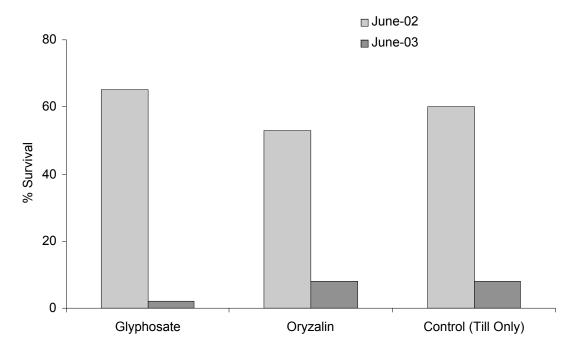


Figure 24: Bar graph of Mean *Castilleja levisecta* Survival. Light bars indicate % survival as of June 2002; dark bars indicate % survival as of June 2003.

After analyzing data collected 2.5 months after plant installation, the results of binary logistic regression show that no significant differences in the mean likelihood of *Castilleja levisecta* survival existed among the glyphosate, oryzalin and control treatments (p=0.460 for glyphosate and p= 0.570 for oryzalin). Overall survivorship for planted *C. levisecta* was approximately 59%. Similarly, no significant differences in the mean likelihood of survival of all the native plantings existed among the glyphosate, oryzalin and control treatments (p=0.184 for glyphosate and p= 1.000 for oryzalin). Overall survivorship for all planted species (*Castilleja levisecta, Festuca roemeri*, and *Eriophyllum lanatum*) was good, approximately 81%. Figure 24 shows the severe drop in *C. levisecta* survival after the first year. Overall survival of planted *Castilleja levisecta* dropped radically to 6% (only 11 of 180 plants survived). The actual significance of differences between treatments was difficult to gauge because of the extremely small number of plants surviving in each treatment.

Smith MeanCastilleja levisecta Vigor Score

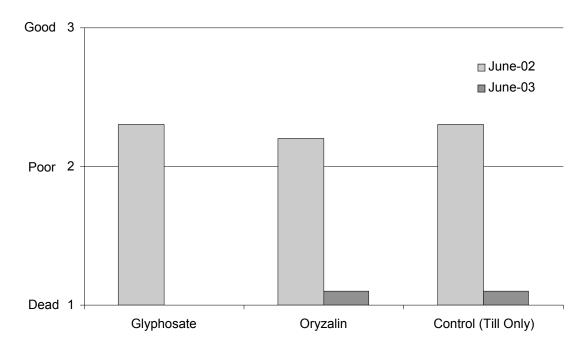
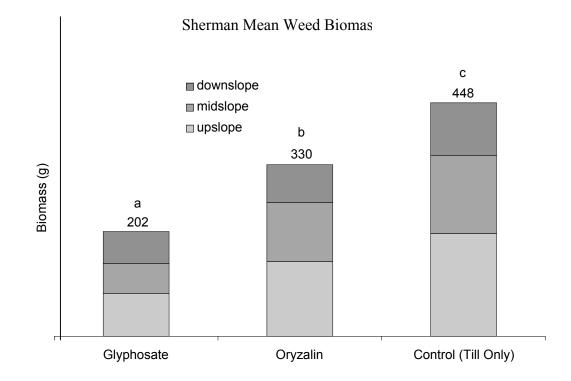


Figure 25: Bar graph of Mean *Castilleja levisecta* Vigor Score. Light bars indicate vigor as of June 2002; dark bars indicate vigor as of June 2003.

After 2.5 months, no significant differences in mean *Castilleja levisecta* vigor existed among the glyphosate, oryzalin and control treatments (p=0.951). In general, vigor of planted *C. levisecta* at this site was poor, with an overall mean vigor score of 2.2. Figure 25 shows that after one year, the overall vigor of *Castilleja levisecta* dropped to dead or just above dead in all the treatments. There were no observable or significant differences in vigor among the blocks or treatments, as most of the plants did not survive. The vigor of those that did survive was generally poor.

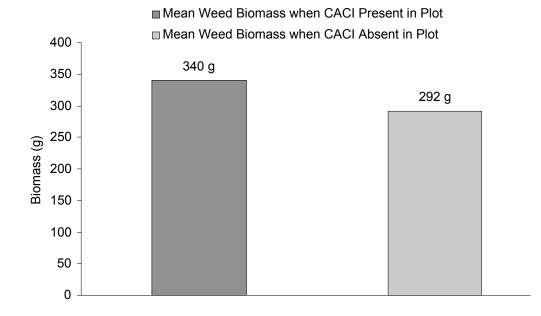
### SHERMAN EXPERIMENT



### Figure 26: Bar graph of Sherman Mean Weed Biomass. Stacked columns represent the contribution of each block to the overall treatment mean. Different letters designate groups with significantly different means.

Figure 26 shows that significant differences in mean weed biomass exist among the glyphosate, oryzalin and control treatments (p=0.000). Though a significant block effect also exists, block was modeled as a fixed effect only to account for variability due to location along the slope and is not an element of interest in this study (p=0.000). Both the glyphosate and oryzalin treatments resulted in significantly lower weed biomass than the control, 55% and 26% reduction in biomass respectively. In addition, mean weed biomass in the glyphosate treatment was significantly lower than in the oryzalin treatment.

### Effect of Calandrinia ciliata on Weed Biomass

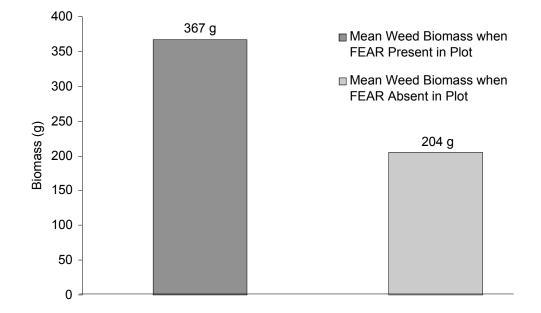


# Figure 27: Bar graph of the Effect of Presence/Absence of *Calandrinia ciliata* (CACI) on Weed Biomass at the Sherman Experiment site.

The unexpected presence of *Calandrinia ciliata* was noted in a number of plots at the Sherman site. Because *C. ciliata* was not intentionally planted and is not rare, it was weeded and considered part of the weed biomass of each plot. Figure 27 illustrates the effect of *C. ciliata* presence on weed biomass measurements. Presence of *C. ciliata* does not appear to notably contribute to higher weed biomass outcomes.

In order to gauge if *Calandrinia ciliata* presence was affected by the use of a particular herbicide, exact binary logistic regression was run. According to this test, the likelihood of *C. ciliata* to be present in the control plots was not significantly different or greater than in glyphosate treatment plots (p=0.571). *C. ciliata* was less likely to be present in the oryzalin than in the glyphosate treatment plots, but not significantly so (p=0.054). Because this p-value fails to reject the null hypothesis by such a narrow margin, a possibility exists that the use of oryzalin may indeed have a negative effect on *C. ciliata* presence. Because oryzalin is a preemergent herbicide that stops the germination of

seeds in the ground, it may inhibit *C. ciliata* seed germination in addition to the weed seeds it is intended to inhibit.



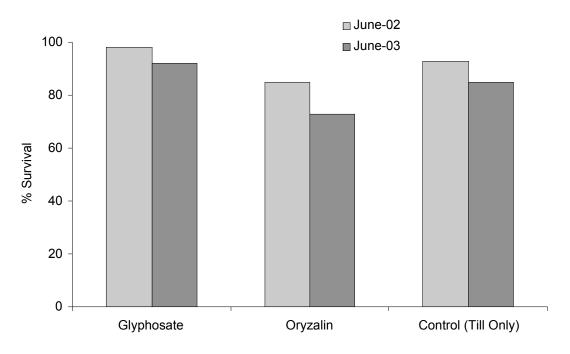
#### Effect of Festuca arundinacea on Weed Biomass

### Figure 28: Bar graph of the Effect of *Festuca arundinacea* (FEAR) Presence/Absence on Weed Biomass at the Sherman Experiment site.

Figure 28 illustrates the contribution of *Festuca arundinacea* presence to weed biomass measurements. Presence of *F. arundinacea* appears to correlate with higher weed biomass outcomes. This observation may assist in the further interpretation of weed biomass results. According to exact binary logistic regression results, there is a significant difference in the likelihood of *F. arundinacea* to be present in a given treatment (p=0.0007). The likelihood of *F. arundinacea* presence is significantly greater in the control than in glyphosate-treated plots, in fact it is more than twice as likely to occur (p=0.0138). On the other hand, there is no significant difference in the likelihood of *F. arundinacea* presence in the oryzalin-treated plots (p=1.0000). The greater likelihood of *F. arundinacea* presence in the control and the oryzalin-treated plots (p=1.0000). The greater likelihood of *F. arundinacea* presence in the control and the oryzalin-treated plots may account for their significantly higher weed biomass results.

### Total # of Weed Species

Again a block effect exists depending on a plot's location on the slope, but is not of interest to this study (p=0.019). However, no significant differences in mean number of weed species exist among the glyphosate, oryzalin and control treatments (p=0.142). The overall mean number of weed species was 10.2.

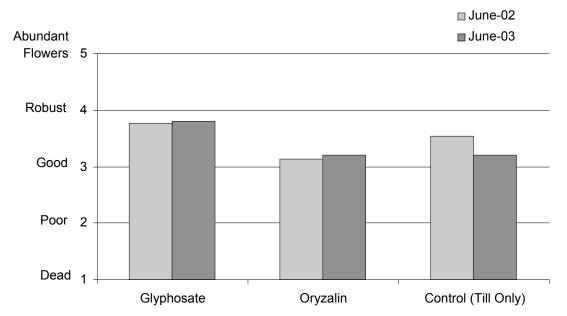


### Sherman MeanCastilleja levisecta Surviva

Figure 29: Bar graph of Mean *Castilleja levisecta* Survival. Light bars indicate % survival as of June 2002; dark bars indicate % survival as of June 2003.

Figure 29 illustrates *Castilleja levisecta* survival at 2.5 months and one year after planting. Approximately 2.5 months after installation, the results of binary logistic regression showed that no significant differences in the mean likelihood of *C. levisecta* survival existed among the glyphosate, oryzalin and control treatments (p=0.203 for glyphosate and p=0.149 for oryzalin). However the overall survival of planted *C. levisecta* was very high at this site (92%). Similarly, no significant differences in the mean likelihood of mortality of all the native plantings (*Castilleja levisecta*, *Festuca roemeri*, and *Eriophyllum lanatum*) existed among the glyphosate, oryzalin, but the overall survival

was very high (95%). Approximately one year after installation, overall survival of *C*. *levisecta* dropped slightly to 83%. Again there were no significant differences in survival among the treatments.



### Sherman MeanCastilleja levisecta Vigor Score

# Figure 30: Bar graph of Mean *Castilleja levisecta* Vigor. Light bars indicate vigor as of June 2002; dark bars indicate vigor as of June 2003.

Figure 30 shows the mean *Castilleja levisecta* vigor for each treatment at 2.5 months and one year after installation. After 2.5 months, the overall mean vigor score for *C. levisecta* planted at this site was between good and robust (3.5). After one year, the overall vigor of *C. levisecta* plants remained relatively constant (3.4). Many of the surviving plants were prolifically flowering, though widespread herbivore damage to the flowering stems was observed. The mean vigor in the glyphosate treated plots was 3.8 as compared to 3.2 in both the oryzalin and control plots. This difference was statistically significant (p=0.005) but may not be biologically significant since all treatments still fell within the same general category, 3=good.

## CHAPTER IV. CONCLUSIONS

### **SECTION A: SOIL STUDY**

A review of the results from this portion of the study suggests that there is high variation among the soil characteristics at the three Castilleja levisecta population sites. The Fort Casey Population has the most plant-hospitable soil of all the populations. Moisture and nutrients are in good supply and exist in plant-available forms. Considering the variety and quantity of vegetation on this site, the soil characteristics do not appear to limit competition from less stress-tolerant plants. An examination of the land use history of this site provides some possible reasons why the soil conditions appear less stressful than typically expected in prairies. It has been suggested that the open terraced areas of Fort Casey did not historical support prairie vegetation, but were created when the United States military cleared the area of forest during the building the fort in the late 1890's (McKinley 1993). This theory is supported by the Hoypus soil series designation that describes the primary land use of the soil of the Fort Casey Population to be timber production and the native vegetation as predominantly Douglas-fir with some western hemlock, western redcedar, bigleaf maple, and Pacific madrone (NRCS 2000). C. *levisecta* may have expanded from the sandy bluff edge into these maintained open areas since the fort was created.

The Ebey's Bluff Population and Forbes Point Population exist in less hospitable sites, with serious nitrogen limitations. Forbes Point appears to have a sufficient phosphorus supply, though the levels at Ebey's Bluff and Fort Casey are markedly lower. *Castilleja levisecta* appears to be capable of tolerating a lack of available nitrogen, low moisture during the growing season, extremely well-drained, coarse-textured soils, and a variety of organic matter contents. Ebey's Bluff is the site with the harshest environmental conditions, lowest moisture, steepest terrain, and most exposure, yet *C. levisecta* thrives on this site and is capable of withstanding these environmental hardships.

It may be interesting to note that Kaye (2001) observed differences in plant performance based on source population when propagating *Castilleja levisecta*. He found that nursery-grown plants of Ebey's Bluff provenance were significantly shorter and flowered less frequently than those of either Fort Casey or Forbes Point. Whether or not these differences are site-specific adaptations based on differential habitat stress levels has not been evaluated.

Extant populations of *Castilleja levisecta* grow under some of the most stressful conditions and on some of the harshest terrain of Whidbey Island. Historically, these areas have not been subject to the same agricultural disturbances as gentler sites, so it should not be assumed that populations exist in these locations necessarily because *C. levisecta* prefers harsh conditions. With this in mind, results of the soil study should be carefully interpreted. Though some sites may appear more suitable for *C. levisecta* reintroduction because of their apparent similarity to extant populations, the remaining populations only represent a fraction of the known range of the plant. Therefore, less similar sites may be equally if not more appropriate for reintroduction and restoration than those which appear more related to the extant populations.

This stated, results from the soil study indicate that the Smith Remnant site shares the highest number of soil characteristics with the Fort Casey Population. The most notable difference between these sites is an elevated phosphorus level at the Smith Remnant, which is more than three times higher than Fort Casey. The Wendy Wayne Experiment site appears to most closely resemble the Ebey's Bluff Population. One major difference between the sites includes a lower available phosphorus level at the Wendy Wayne Experiment, which is only a quarter of the level at Ebey's Bluff. Also, the Ebey's Bluff Population appears more nitrogen limited than the Wendy Wayne Experiment and has far higher organic matter content despite the impacts of a recent fire. Finally, as would be expected by their close proximity, soil characteristics of the Forbes Point Remnant are most analogous to those of the Forbes Point Population. Nitrogen and phosphorus availability are both significantly higher in the remnant than the population site. In

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particular, phosphorus is 4.5 times higher in the remnant. Also, the remnant had higher soil moisture throughout the 2002 growing season.

### **RECOMMENDATIONS FOR FURTHER STUDY**

Differences among all theses sites demonstrate varying levels of nutrient and water availability between the populations and potential restoration sites. Elevated phosphorus levels may be an issue in restoring the Smith Remnant and the Forbes Point Remnant, while low phosphorus levels may be an issue for the Wendy Wayne Experiment site. Experimenting with phosphorus limitation techniques, such as soil impoverishment, may be a useful step in the development of site preparation techniques for the Smith Remnant and Forbes Point Remnant sites. Previous studies indicate that any factor influencing root system size or morphology will likely affect the quantity of soil phosphorus that is available to a plant (Fixen and Grove 1990). The hemiparasitic quality of *Castilleja levisecta* may therefore allow it to take up more phosphorus than surrounding plants and allow it to compete in phosphorus-limited soils. However, inducing phosphorus depression may be difficult. According to Brady and Weil (1999), net immobilization of soluble phosphorus is most likely to occur if residues added to the soil have a C/P ratio greater than about 300, while net mineralization is likely if the ratio is below 200. Typical values for carbon rich organic material that may be added to induce phosphorus depression include conifer sawdust (C/N=600) or hardwood sawdust (C/N=400) (Brady and Weil 1999).

In addition to experimenting with methods of site preparation on potential reintroduction sites, continued work is necessary to properly characterize the *Castilleja levisecta* populations of Whidbey Island. A thorough analysis of plant community composition and structure at each population location would be a logical next step in the characterization of these sites. Further research is needed in order to determine the habitat requirements for this rare species.

### **SECTION B: SITE PREPARATION EXPERIMENT**

### SMITH EXPERIMENT

It may be concluded from the results of this study that both herbicide treatments are equally more effective at reducing weed biomass than tilling alone. Use of either herbicide reduced weed biomass by approximately 60% over the control. Control of *Cirsium arvense* should also be a high priority on this site. Its presence was correlated with substantially higher weed biomass results. Though not statistically corroborated by this study, weed control literature for C. arvense suggests spot use of glyphosate as an effective means of control. The effects of oryzalin appear to reduce the number of weed species in a plot; it resulted in approximately 40% fewer weed species than the control. Oryzalin may be a useful tool if it is suspected that a significant weed seed bank exists. The implications for use of any of these treatments on native plant survivorship and C. *levisecta* vigor are unclear. It appears that overall survivorship of all planted species was high (approximately 80%) after 2.5 months. Survivorship for Castilleja levisecta alone was lower (59%) after 2.5 months and dropped further after one year (6%). Overall vigor of planted C. levisecta was initially poor and eventually dropped to near-dead. Plantings at this site appeared to dry out and wither over time. This may be due to the late planting date (the second week of March, 2002) or unfavorable soil conditions (see Conclusions: Section C). Future native plant installation should occur earlier in the rainy season, preferably in the fall.

### SHERMAN EXPERIMENT

Results from this experiment suggest that glyphosate is more effective in reducing weed biomass than either oryzalin or tilling alone. Glyphosate treatments reduced weed biomass by nearly 55% while oryzalin treatments reduced weed biomass by more than 25% over the control. Presence of the unexpected native annual, *Calandrinia ciliata*, did not appear to significantly impact weed biomass measurements. However, its presence is a reminder to restorationists that in some sites, native seed may exist in the seed bank as

well as weed seed. Gaining knowledge of existing seed bank resources should not be overlooked in restoration. The use of preemergent herbicides such as oryzalin or methods of soil sterilization such as infrared radiation, high temperature steaming or solarization may impact these native propagules unintentionally. Control of *Festuca arundinacea* should be a high priority on this site. Its presence was correlated with substantially higher weed biomass results. Treatment with glyphosate appears to reduce the presence of *F. arundinacea*. It was 50% more likely to occur in the control than glyphosate plots. Oryzalin, however, did not appear to have any effect on *F. arundinacea* presence. The number of weed species was not affected significantly by any of the treatments. Implications for use of any of these treatments on native plant survivorship and Castilleja *levisecta* vigor are unclear. While no significant differences existed in the survival of planted species among the different treatments, it should be noted that there was an extremely high overall survival rate for all the plantings (95%) after 2.5 months and a very high rate of C. levisecta (92%) survival, which only slightly declined in the following year (83%). In addition, the overall *C. levisecta* vigor score was good to robust at this site and remained so into the following year.

## SECTION C: MANAGEMENT RECOMMENDATIONS AND INFLUENCE OF SOIL CHARACTERISTICS ON EXPERIMENTAL REINTRODUCTIONS

Generally, it appears that glyphosate is an important tool for both reducing weed biomass and controlling tenacious perennial weeds. Management recommendations for the Smith Experiment site include spot application of glyphosate on *Cirsium arvense* individuals, either in the early fall (while the plant is still in the rosette stage) or before it blooms in early June. Oryzalin may also be applied to control below ground weed sources, but not until an analysis of the seed bank is completed. Furthermore, planting time at this site may be crucial for *Castilleja levisecta* survival and success and should occur in fall rather than early spring. Finally, rodent activity and herbivory observed at this site should be considered when planting natives. Steps should be taken to protect new plants from herbivore damage. On the Sherman Experiment site, the same management suggestions apply. In addition, it is recommended that the site be mowed in the spring before *Festuca*  *arundinacea* sets seed. A second mowing may be necessary before glyphosate is applied in the fall. A second application of glyphosate may also be necessary before tilling of the site occurs. Native plants should be installed in fall rather than spring and protected from herbivory. More extensive, multiple-season site preparation is undoubtedly necessary for restoration of this site.

In general, further research into other invasive species control methods or combinations of methods should be examined. As has been noted by experienced restorationists of western Washington prairies, long-term information on weed control in specific community types is rarely available or transferable to sites with different invasive species compositions (Schuller 1997; Dunwiddie 2003). Future research may be directed towards informed trial and error or an assessment of the mechanisms at work in this particular system (Ewing 1997).

Differences in *Castilleja levisecta* vigor and survival at the Sherman and Smith experimental sites may have been affected by differences in the soil characteristics of these two sites. At both of these sites, plants from the same provenance and age were installed during the same week of the year. The same site preparation treatments, installation techniques, and methods of data collection were employed at each site. Despite this, the overall survival and vigor of planted *C. levisecta* at the Smith site (59% survival, vigor=poor) was much lower than at the Sherman site (92% survival, vigor=good to robust) at 2.5 months after installation (see Figure 31). Approximately one year after plant installation, overall *C. levisecta* survival at the Smith site dropped to 6% and the overall vigor=dead. Meanwhile, at the Sherman site, 83% of planted *C. levisecta* were surviving after one year and overall vigor= good to robust.



Figure 31: Comparative C. levisecta Vigor in June 2002 (Smith left, Sherman right).

The Sherman site has a more intensive farming history than the Smith site, and is consequently more heavily invaded by exotic species. In fact, overall mean weed biomass at the Sherman site (327 g) was an entire order of magnitude higher than at the Smith site (13 g). The mean number of weed species at the Sherman site was also more than twice that of the Smith site (10.2 versus 4.6 respectively). However, competition from neighboring weeds did not appear to result in lower survival and vigor at the Sherman site. Though widespread herbivory was noted at both sites, there was no perceptible difference in the degree of herbivore damage at either site. Additionally, the sites are located within just a few miles of each other, and rainfall patterns between the two sites do not differ (Engle, 2000). Furthermore, the Smith site has a gentler slope than the Sherman site (0-2% versus 8-16% respectively). The aspects of the two sites are comparable though Smith is slightly more south facing than Sherman (170° versus 135° respectively). After examining these factors, conditions for plant growth generally appear to be equal or more favorable at the Smith site. This suggests soil differences as a possible reason for the lower *C. levisecta* survival and vigor at the Smith Experiment site.

In terms of nutrient availability, the Smith Experiment site generally appears more favorable than the Sherman Experiment site. Smith has significantly higher inorganic nitrogen (see Figure 17) as well as significantly higher extractable phosphorus (Figure 19). However, in terms of soil moisture holding capacity and pH, Sherman is more hospitable. Though the average % gravimetric moisture was typically greater over the beginning of the growing season at the Smith site (see Figure 20), the quantity of rock in this soil as compared to the Sherman site may have actually lead to far less plant-available water in the soil (see Figure 10 and Figure 31). The percentage of rock fragments at the Smith Experiment site was over 380 times higher than at the Sherman site (30.55% compared to 0.08% respectively). This high percentage of rock means that there is a smaller volume of water-holding soil particles in a given volume of soil. High rock content serves to lower the available water for plant uptake despite the actual water content of existing soil particles. There is also far less potential for soil/root contact in such a rocky soil. Smith's slightly more south facing aspect causes the soil of this site to be even drier. Overall, it appears that water availability at the Smith Experiment site is far lower than the Sherman site. Over the summer following plant installation all planted natives, including *Castilleja levisecta*, appeared to wither and dry out at the Smith site. Lower water availability may have been a major factor contributing to the low survival and vigor of plants at Smith.

Lower pH may also have influenced the survival and vigor of *Castilleja levisecta* at the Smith site. Figure 12 shows that the Smith Experiment site has a significantly lower mean pH (5.3) than any of the extant populations. This is also notably lower than the pH at the Sherman site (6.4). While the pH of Sherman is significantly higher than any of the populations, it appears that this less acidic pH has not hindered the survival and growth of *C. levisecta*. Conversely, the more acidic pH of the Smith site may have some negative effect on *C. levisecta* survival and growth.

In summary, the Sherman Experiment site shares more soil characteristics with extant population sites than does the Smith Experiment (see Table 4). However, in the case of these two experimental sites, difference in moisture availability and pH may have more influence on the survival and vigor of *Castilleja levisecta* than nutrient related soil characteristics. If further experimental reintroduction of *C. levisecta* on the Smith site is to be conducted, fall plant installation and regular irrigation through the growing season is highly recommended. Because vigor and survival of plants at the Sherman Experiment

site were high, continued exploration of this site's reintroduction potential is also suggested. Finally, it is recommended that moisture related soil characteristics be prioritized when evaluating other potential *C. levisecta* reintroduction sites.

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# APPENDIX A

# SOIL STUDY SUMMARY STATISTICS

Soil Characteristic	Site	Ν	Mean	S.D.	S.E.	95%	o C.I.	Min	Max
						Lower	Upper		
Bulk Density (g cm <sup>-3</sup> )	FPP	5	1.14	0.10	0.05	1.01	1.27	0.99	1.24
	EBP	5	1.04	0.15	0.07	0.85	1.23	0.80	1.20
	FCP	5	0.98	0.11	0.05	0.85	1.11	0.84	1.10
	SHR	5	0.76	0.11	0.05	0.62	0.89	0.67	0.90
	FPR	5	0.99	0.07	0.03	0.91	1.08	0.88	1.05
	SMR	5	0.95	0.16	0.07	0.75	1.14	0.76	1.17
	WWE	5	1.27	0.14	0.06	1.10	1.44	1.13	1.49
	SME	5	0.89	0.13	0.06	0.74	1.05	0.75	1.06
	SHE	5	1.29	0.06	0.03	1.21	1.37	1.23	1.40
	Total	45	1.04	0.20	0.03	0.98	1.09	0.67	1.49
% Rock Fragments	FPP	5	20.28	5.41	2.42	13.56	27.00	15.90	29.10
	EBP	5	10.34	7.71	3.45	0.77	19.91	1.70	21.60
	FCP	5	6.56	5.87	2.62	-0.73	13.85	2.00	16.40
	SHR	5	47.96	7.86	3.52	38.20	57.72	36.30	55.70
	FPR	5	14.86	3.69	1.65	10.27	19.45	9.50	19.30
	SMR	5	10.48	7.09	3.17	1.67	19.29	4.70	22.70
	WWE	5	9.16	1.89	0.84	6.81	11.51	7.50	12.40
	SME	5	32.36	15.15	6.77	13.55	51.17	9.80	50.20
	SHE	5	0.24	0.43	0.19	-0.30	0.78	0.00	1.00
	Total	45	16.92	15.57	2.32	12.24	21.59	0.00	55.70
рН	FPP	5	5.94	0.10	0.04	5.82	6.06	5.84	6.10
	EBP	5	6.08	0.07	0.03	5.99	6.16	5.98	6.17
	FCP	5	6.03	0.18	0.08	5.81	6.25	5.78	6.28
	SHR	5	5.53	0.06	0.03	5.45	5.60	5.45	5.59
	FPR	5	5.97	0.14	0.06	5.79	6.15	5.82	6.15
	SMR	5	5.62	0.05	0.02	5.56	5.69	5.59	5.71
	WWE	5	6.28	0.12	0.05	6.14	6.42	6.13	6.41
	SME	5	5.33	0.09	0.04	5.21	5.45	5.21	5.47
	SHE	5	6.36	0.18	0.08	6.13	6.59	6.16	6.64
	Total	45	5.90	0.35	0.05	5.80	6.01	5.21	6.64

Soil Characteristic	Site	Ν	Mean	S.D.	S.E.	95%	5 C.I.	Min	Max
						Lower	Upper		
% Loss on Ignition	FPP	5	6.60	1.82	0.81	4.34	8.86	4.00	9.00
	EBP	5	4.20	1.64	0.73	2.16	6.24	2.00	6.00
	FCP	5	11.60	5.41	2.42	4.88	18.32	5.00	20.00
	SHR	5	11.20	0.84	0.37	10.16	12.24	10.00	12.00
	FPR	5	8.00	1.00	0.45	6.76	9.24	7.00	9.00
	SMR	5	11.00	1.22	0.55	9.48	12.52	9.00	12.00
	WWE	5	5.00	1.22	0.55	3.48	6.52	4.00	7.00
	SME	5	8.80	2.49	1.11	5.71	11.89	7.00	13.00
	SHE	5	4.60	2.61	1.17	1.36	7.84	1.00	7.00
	Total	45	7.89	3.57	0.53	6.82	8.96	1.00	20.00
% Total N	FPP	5	0.08	0.02	0.01	0.05	0.10	0.05	0.10
	EBP	5	0.04	0.02	0.01	0.01	0.07	0.02	0.07
	FCP	5	0.39	0.16	0.07	0.20	0.59	0.26	0.66
	SHR	5	0.35	0.08	0.04	0.25	0.44	0.28	0.48
	FPR	5	0.18	0.01	0.00	0.17	0.19	0.17	0.19
	SMR	5	0.37	0.11	0.05	0.23	0.51	0.18	0.44
	WWE	5	0.09	0.03	0.01	0.05	0.13	0.05	0.13
	SME	5	0.23	0.04	0.02	0.19	0.28	0.19	0.28
	SHE	5	0.08	0.02	0.01	0.06	0.10	0.06	0.10
	Total	45	0.20	0.15	0.02	0.16	0.25	0.02	0.66
C/N Ratio	FPP	5	34.29	4.24	1.90	29.03	39.55	27.77	38.08
	EBP	5	54.08	28.91	12.93	18.19	89.97	27.46	89.41
	FCP	5	15.73	1.33	0.60	14.08	17.39	13.97	17.10
	SHR	5	16.04	1.09	0.49	14.69	17.39	14.75	17.08
	FPR	5	21.70	3.35	1.50	17.55	25.85	18.01	25.79
	SMR	5	17.36	2.10	0.94	14.75	19.97	16.34	21.12
	WWE	5	25.26	6.11	2.73	17.66	32.85	20.77	35.69
	SME	5	18.65	1.17	0.52	17.19	20.11	17.24	20.06
	SHE	5	24.36	4.18	1.87	19.18	29.55	19.86	31.24
	Total	45	25.27	14.89	2.22	20.80	29.75	13.97	89.41
NH₄ <sup>+</sup> -N (mg g⁻¹)	FPP	5	4.90	1.66	0.74	2.84	6.96	3.50	7.20
	EBP	5	6.26	0.60	0.27	5.51	7.01	5.40	6.90
	FCP	5	10.54	1.23	0.55	9.01	12.07	8.70	12.10
	SHR	5	8.78	1.13	0.50	7.38	10.18	7.70	10.10
	FPR	5	6.44	4.76	2.13	0.53	12.35	3.70	14.90
	SMR	5	7.68	1.46	0.65	5.86	9.50	6.30	9.30
	WWE	5	2.22	0.75	0.34	1.29	3.15	1.20	2.90
	SME	5	12.16	5.80	2.59	4.96	19.36	7.50	22.30
	SHE	5	4.86	1.38	0.62	3.15	6.57	3.80	7.10
	Total	45	7.09	3.83	0.57	5.94	8.24	1.20	22.30

Soil Characteristic	Site	N	Mean	S.D.	S.E.	95%	6 C.I.	Min	Max
						Lower	Upper		
$NO_{3}^{-} - N \text{ (mg g}^{-1}\text{)}$	FPP	5	0.70	0.45	0.20	0.14	1.26	0.10	1.30
	EBP	5	7.34	1.12	0.50	5.94	8.74	6.30	8.70
	FCP	5	8.00	0.96	0.43	6.81	9.19	7.40	9.70
	SHR	5	3.14	2.08	0.93	0.56	5.72	0.90	5.60
	FPR	5	0.46	0.72	0.32	-0.44	1.36	0.00	1.70
	SMR	5	9.34	1.79	0.80	7.12	11.56	7.10	10.80
	WWE	5	9.64	0.76	0.34	8.70	10.58	8.80	10.40
	SME	5	21.64	5.68	2.54	14.59	28.69	16.40	31.20
	SHE	5	10.78	0.74	0.33	9.87	11.69	9.80	11.60
	Total	45	7.89	6.46	0.96	5.95	9.83	0.00	31.20
Total Inorganic N (mg g <sup>-1</sup> )	FPP	5	5.60	1.84	0.82	3.31	7.89	3.70	7.70
	EBP	5	13.60	1.53	0.68	11.70	15.50	12.20	15.30
	FCP	5	18.54	1.63	0.73	16.51	20.57	16.10	20.30
	SHR	5	11.92	3.21	1.43	7.94	15.90	8.60	15.70
	FPR	5	6.90	4.87	2.18	0.86	12.94	3.70	15.40
	SMR	5	17.02	2.29	1.02	14.18	19.86	13.60	20.00
	WWE	5	11.86	1.26	0.56	10.30	13.42	10.10	13.30
	SME	5	33.80	11.24	5.02	19.85	47.75	26.80	53.50
	SHE	5	15.64	1.45	0.65	13.84	17.44	14.20	17.50
	Total	45	14.99	8.84	1.32	12.33	17.64	3.70	53.50
P0₄ -P (mg g⁻¹)	FPP	5	13.60	11.49	5.14	-0.67	27.87	6.22	33.80
	EBP	5	8.22	2.65	1.18	4.93	11.51	4.78	11.94
	FCP	5	8.06	3.59	1.60	3.60	12.51	4.24	13.84
	SHR	5	18.46	5.14	2.30	12.07	24.84	13.27	24.80
	FPR	5	61.71	31.73	14.19	22.31	101.10	26.44	102.86
	SMR	5	26.46	2.42	1.08	23.45	29.47	22.98	29.27
	WWE	5	2.08	0.25	0.11	1.77	2.39	1.79	2.39
	SME	5	24.88	1.15	0.52	23.45	26.31	23.58	26.26
	SHE	5	7.38	0.75	0.34	6.44	8.31	6.47	8.53
	Total	45	18.98	20.10	3.00	12.94	25.02	1.79	102.86
% Gravimetric Moisture	FPP	5	15.10	1.45	0.65	13.30	16.90	12.80	16.70
	EBP	5	3.42	1.40	0.62	1.69	5.15	2.60	5.90
	FCP	5	15.30	2.74	1.23	11.90	18.70	12.30	19.60
	SHR	5	11.36	2.82	1.26	7.86	14.86	8.10	15.30
	FPR	5	19.68	2.12	0.95	17.05	22.31	17.60	22.90
	SMR	5	14.06	2.33	1.04	11.16	16.96	11.60	16.60
	WWE	5	5.30	1.16	0.52	3.85	6.75	4.40	7.30
	SME	5	9.38	1.37	0.61	7.68	11.08	7.90	10.60
	SHE	5	5.72	0.69	0.31	4.86	6.58	4.70	6.60
	Total	45	11.04	5.51	0.82	9.38	12.69	2.60	22.90

	Site	Ν	Fraction	%	Textural Classification
Texture: Particle Size	FPP	5	Sand	85	Gravelly Loamy Sand
			Silt	14	
			Clay	2	
	EBP	5	Sand	97	Sand
			Silt	3	
			Clay	0	
	FCP	5	Sand	93	Sand
			Silt	6	
			Clay	0	
	SHR	5	Sand	83	Gravelly Loamy Sand
			Silt	17	
			Clay	0	
	FPR	5	Sand	88	Gravelly Loamy Sand
			Silt	12	
			Clay	0	
	SMR	5	Sand	76	Gravelly Sandy Loam
			Silt	24	
			Clay	0	
	WWE	5	Sand	92	Sand
			Silt	8	
			Clay	0	
	SME	5	Sand	71	Gravelly Sandy Loam
			Silt	29	
			Clay	0	
	SHE	5	Sand	98	Sand
			Silt	2	
			Clay	0	

# APPENDIX B

### SITE PREPARATION EXPERIMENT SUMMARY STATISTICS

Weed Biomass (g)	Veed Biomass (g) Treatment		Mean	S.E		95% C.I.		
					L	ower	Upper	
	R		9.10	3.5	9	1.74	16.46	
	S		8.81	3.5	9	1.45	16.17	
	Т	2	22.23	3.5	91	4.86	29.59	
Cirsium arvense	Treatment	At	osent	Presen	it	Total		
Presence/Absence	R		9		3	12		
	S		11		1	12		
	Т		8	4	4	12		
	Total		28	1	8	36		
Total # Weed Species	Treatment	ſ	Mean	S.E		95% C	.l.	
					L	ower	Upper	
	R		5.92	0.41		5.08	6.75	
	S		3.08	0.41		2.25	3.92	
	Т	5.17				.331	6.00	
Total Plant Survival	Treatment		Alive	Dead		Total		
June 2002	R	246		54	54			
	S		234	6	6	300		
	Т		246			300		
	Total		726	174		900		
Castilleja levisecta	Treatment	Alive		De	ad	T	otal	
Survival		2002	2003	2002	2003	2002	2003	
	R	39	1	21	59	60	60	
	S	32	5	28	55	60	60	
	Т	36	5	24	55	60	60	
	Total	107	11	73	169	180	180	
Castilleja levisecta	Treatment	Me		S.I			N	
Vigor Score (1-5)		2002	2003	2002	2003	2002	2003	
	R	2.3	1.0	0.63	0.13	12	12	
	S	2.2	1.1	0.78	0.28	12	12	
	Т	2.3	1.1	0.82	0.45	12	12	
	Total	2.2	1.1	0.73	0.32	36	36	

### SMITH EXPERIMENT

### SHERMAN EXPERIMENT

Weed Biomass (g)	Treatment	Mean			S.E.		95% C.I.		
							Lower		Upper
	R	2	201.67		26.47		147.36		255.98
	S	Э	30.00		26.47	:	275.69		384.31
	Т	4	48.33		26.47	:	394.02		502.64
Calandrinia ciliata	Treatment	A	bsent		Present		Total		
Presence/Absence	R		1		11		12		
	S		6		6		12		
	Т		3		9		12		
	Total		10		26		36		
Festuca arundinacea	Treatment	A	bsent	I	Present		Total		
Presence/Absence	R		8		4		12		
	S		0		12		12		
	Т		1		11		12		
	Total		9		27		36		
Total # Weed Species	Treatment		Mean		S.D.		N		
	R		9.33		2.27	12			
	S	10.17			2.37				
	Т	11.00			1.86	12			
	Total		10.17		2.22	36			
Total Plant Survival	Treatment		Alive		Dead	Total			
June 2002	R		287		13		300		
	S		285	15		300			
	Т		283				300		
	Total	855			45 Dead		900	<b>T</b> . (	
<i>Castilleja levisecta</i> Survival	Treatment	2002	Alive	2003	2002	ead 200	~ ·	Tota 2002	
Survival	R			2003 55					2003
	R S	59		55 44	1	5 16		60 60	60 60
	T	51 56		44 51	9 4			60	60 60
	Total	50 160		150	4 14	9		60 180	60 180
Castilleja levisecta	Treatment	Me				30		<u>180 180</u> % C.I.	
Vigor Score (1-5)	neament	1010	an	S.E.		95% Lower			per
								υp	
		2002	2003	2002	2003	2002	2003	2002	2003
	R	2002 3.8	2003 3.8	2002 0.1	2003 1.1	2002 3.5	2003 1.6	2002 4.0	2003 6.0
	R S								
		3.8	3.8	0.1	1.1	3.5	1.6	4.0	6.0