SEED PRODUCTION AND VIABILITY OF GOLDEN PAINTBRUSH (*CASTILLEJA LEVISECTA*) IN PERSISTING NATIVE LEGACY AND REINTRODUCED POPULATIONS

by

E. David Heydt

A Thesis
Submitted in partial fulfillment
Of the requirements for the degree
Master of Environmental Studies
The Evergreen State College
December 2024

This Thesis for the Master of Environmental Studies Degree

by

E. David Heydt

has been approved for

The Evergreen State College

by

____

Sarah Hamman Ph. D.

Member of Faculty

12/20/2024

____

Date

ABSTRACT

Seed Production and Viability Of Golden Paintbrush (*Castilleja levisecta*) In Persisting Native Legacy And Reintroduced Populations

E. David Heydt

The rare Washington state native plant *Castilleja levisecta* has faced difficulties in its legacy populations. While restoration populations have climbed in numbers over the past 15 years, the native legacy populations have all but disappeared. They have lost over 90% of their total numbers during this period. This may be due in part to decisions by site managers to genetically isolate native legacy populations, refusing to introduce new genetic material to those sites. This study compares two populations of *C. levisecta*, one persisting legacy population and one restoration population. Focusing on seed production and viability, this study compares and contrasts the seed count per seedpod, weight per seed, viability of seed, and germination rate of seed taken from a native legacy population and a restoration population. The findings demonstrate that there are differences in seed weight, viability, and germination rates between the native legacy and restored populations. The findings also show that a genetically isolated native legacy population is producing fewer seeds than a restored population of mixed genetic stock. These findings imply that the management driven genetic isolation is likely leading to reduced fecundity in native legacy populations via inbreeding depression. The implication of these findings is that the management strategy of genetic isolation is harmful and must be discarded in favor of genetic mixing if these native populations are to be preserved.

Table of Contents

List of Figures

List of Tables

Acknowledgements

Sarah Hamman for her mentorship and teaching

David Wilderman for his support of this study

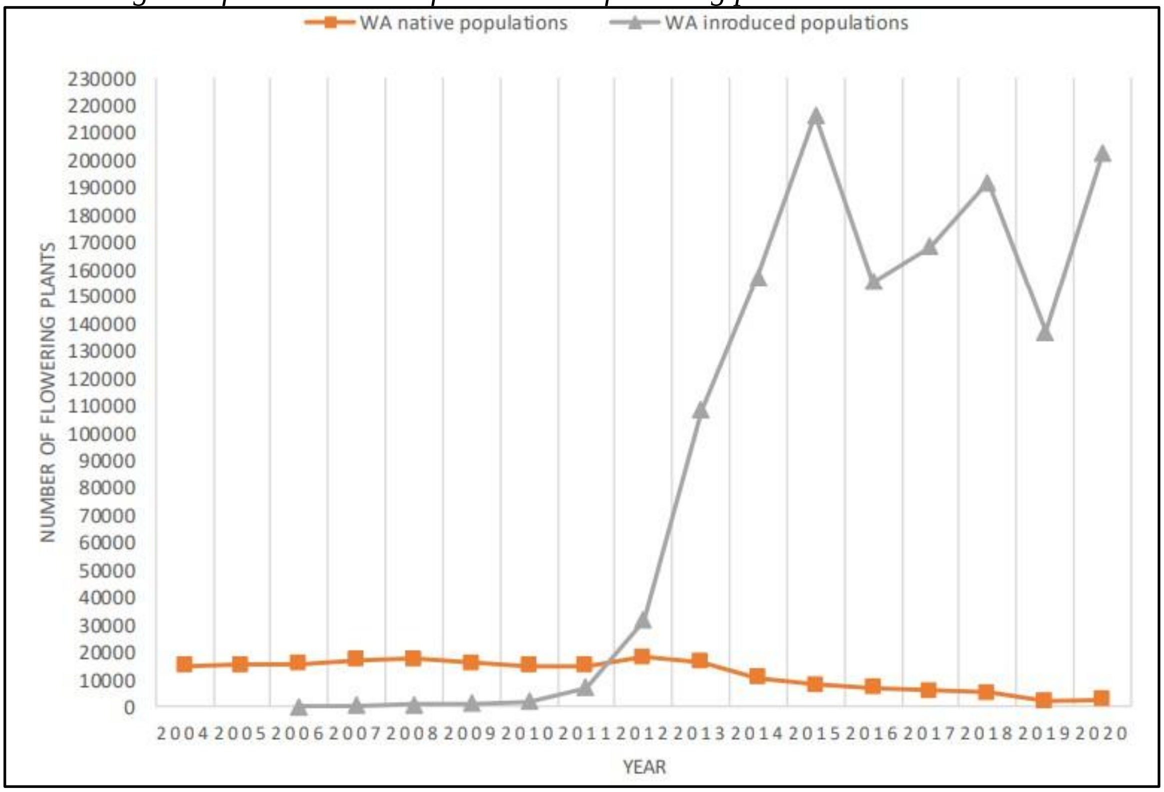
Introduction

Golden paintbrush (*Castilleja levisecta*) is a rare plant native to the Willamette Valley-Puget Trough-Georgia Basin ecoregion. The species was federally listed as threatened in 1997 and NatureServ rates it as imperiled in Washington State (ECOS 2023, NatureServ 2023). This species has received an abundance of conservation funding and effort due in part to its role as a potential host plant for *Euphydryas editha taylori*, the Whulge checkerspot or Taylor's checkerspot butterfly. This butterfly is listed as federally endangered and is the focus of a large recovery effort (Federal Register 2021, Dunwiddie et al. 2016). Thanks to intense restoration efforts of the past 25 years, the population of *C. levisecta* has increased in both total count and number of occupied sites. Due to these efforts, it has been determined that *C. levisecta* no longer meets the federal listing conditions. It was proposed that the species be delisted and protections be removed (Federal Register 2021) and very recently this delisting went into effect (Federal Register 2023).

Despite this seemingly great news about the species as a whole, the number of *C. levisecta* plants in native populations have decreased by ~90% in just the last 10 years (U.S. Fish and Wildlife Service 2019, NatureServ Explorer 2023). The decline in native populations is of great concern, particularly when the trend diverges so drastically from that of the growing introduced populations. This stark disparity between native and introduced populations is illustrated in Figure 1, while Figure 2 shows the dramatic decline in native *C. levisecta* populations rangewide.

Figure 1

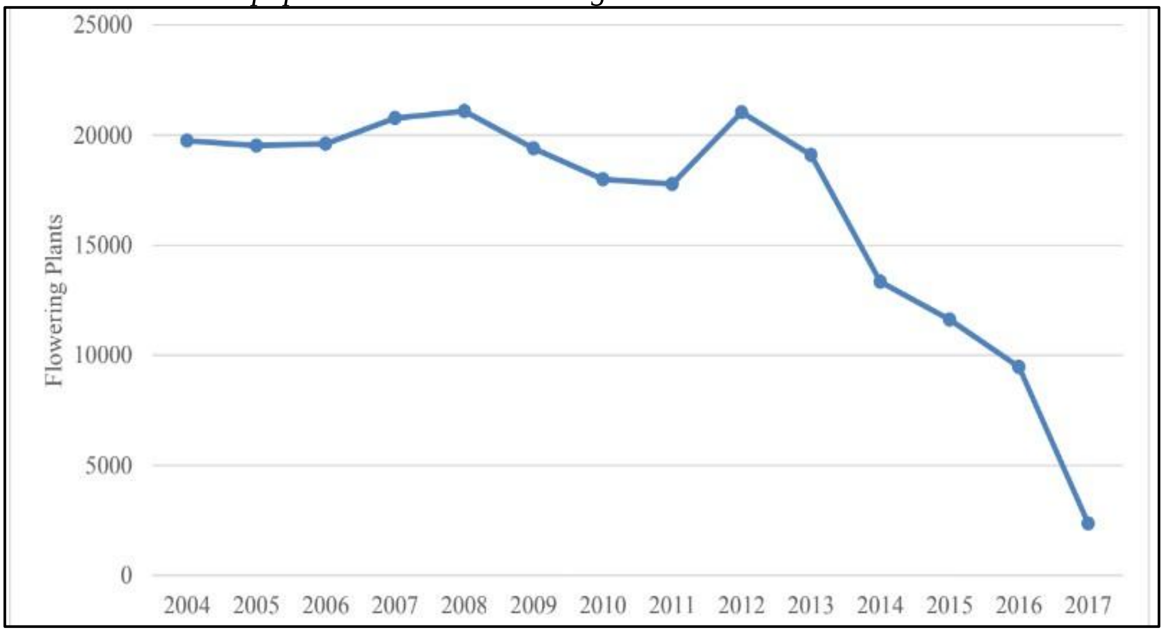
Monitoring data for abundance of C. levisecta flowering plants 2004 - 2020



Note. Number of *C. levisecta* flowering plants in native and introduced populations from 2004-2020.

Figure 2

Native C. levisecta population abundance range wide 2004-2017



Note. Number of *C. levisecta* in native populations rangewide from 2004-2017.

I have chosen to study this problem because I work as a steward of several native and introduced populations of *C. levisecta*. I have seen with my own eyes a dramatic and precipitous drop in the numbers of plants in the native populations. I have seen the reviewed data confirming these observations. The delisting of this species brings up serious concerns for the dwindling native populations and the valuable genetic resource that they represent to the conservation and restoration effort. Studies show that the relationship between genetic isolation and reduced fecundity clearly. Management decisions lead to genetic isolation (Miller and Martin 2024), the isolation leads to self and near-relative pollination of *C. levisecta* flowers due to pollinator excluding distances between sites (Figure 3), which leads to poor seed set and reduced vigor (Kaye and Lawrence 2003), leading to rate of recruitment that could fall below the death rate for the isolated population, ultimatly causing the population to collapse.

My inquiry measures and compares the amount of seed produced by *C. levisecta* plants at genetically isolated native legacy sites and genetically mixed restoration sites. I hope to shed light on possible mechanics of, or reasons for, the drastic decline in native legacy *C. levisecta* populations. Furthermore, the study details a preliminary measurement of seed viability and germination compared between the two populations. This is done with the hope of providing a pathway to additional research on the topic. My hypotheses are as follows: 1) A genetically isolated population of *Castilleja levisecta* will produce fewer seeds than a *Castilleja levisecta* population of mixed genetic stock, and 2) A genetically isolated population of *Castilleja levisecta* will produce lighter seeds with lower viability than a *Castilleja levisecta* population of mixed genetic stock.

Literature Review

Background

The focus for this literature review is the recently delisted, and formerly federally threatened prairie plant *C. levisecta*. Specifically, I review the requirements for sustainable management of the native *C. levisecta* populations. Examination of several decades of *C. levisecta* management has revealed a clear depiction of past and current states of recovery. There are some serious knowledge gaps within this topic. The rarity of this species has no doubt hindered its availability for study.

The articles and documents assessed for this review are grouped into three general categories: 1) Requirements for a healthy population of *C. levisecta*, 2) Threats to reestablishment and restoration, and 3) *C. levisecta* management strategies. The requirements for a healthy population include number of individuals, genetic heterogeneity, habitat quality, pollinator activity, and others. This section provides context for the following sections by detailing what this plant requires to thrive. Category two examines articles detailing the threats to the reestablishment and restoration of this rare native plant, such as invasive species, inbreeding, habitat loss, and hybridization. This section shows the past and current challenges the species faces in order to contextualize the effects of management efforts. The final category is that of *C. levisecta* management strategies. It lays out those which have been proposed, utilized, and evaluated. The propagation and reintroduction, habitat management, and preserve establishment practices outlined in management plans, research studies, and recovery plans are included here.

Requirements for a healthy population

The requirements for the healthy establishment and sustainable management of *C. levisecta* populations are similar to those of other plants, with several key differences. Like the vast majority of terrestrial plants, habitat type and quality are incredibly important for a population to thrive. Pollination support is also key. Unlike some plants, *C. levisecta* needs genetic heterogeneity in its populations to reproduce well, along with a large minimum population size (Caplow 2004). Finally, the species is hemiparasitic and needs a host plant connection to thrive.

High quality habitat of the appropriate type is one of the most important requirements for the health of this species. It is primarily found growing on prairies and balds (Lawrence 2006). These habitats consist of low stature plants growing in nutritionally poor soils (Crawford and Hall 1997) with bunchgrasses and forbs dominating the plant community (Franklin and Dyrness 1973). Aspects of habitat at the microsite level also play a role. High richness of native perennial forbs is correlated with survival of *C. levisecta*, as was occurrence on mounds and in swales in mounded prairie habitats (Dunwiddie and Martin 2016).

Because *C. levisecta* is a hemi-parasitic plant, the presence of the proper host plants is a key factor for the sustainability of a *C. levisecta* population. As such, its seeds must germinate near a host plant that it can form a bond with. If this fails to happen the plant can fail to grow to its full potential. *C. levisecta does* not require a host plant to live, however, when a host plant is available, *C. levisecta* will form haustoria that connect its roots to that of the host and through which nutrients, water, and other resources are taken. The host associations are extremely important, but can be very difficult to research in a laboratory, or greenhouse setting (Lawrence and Kaye 2008).

Host plants are known to include the native plants *Achillea millefolium, Danthonia californica, Erigeron speciosus, Eriophyllum lanatum, Festuca roemeri, Lupinus lepidus,* and *Solidago canadensis,* as well as others (Schmidt 2016). The host species that *C. levisecta* parasitizes can influence the amount of nutrients received, and therefore the health and performance of the plant (Schmidt 2016). *C. levisecta* derives the highest amount of nitrogen from parasitizing *E. lanatum* and *A millefolium*, while deriving the lowest amount of nitrogen from *E. speciosus*. The most carbon was derived from *S. canadensis* and the least carbon from *E. speciosus* (Delvin 2013, Schmidt 2016). Introduced species such as *Leucanthemum vulgare* can also support the *C. levisecta* as a host (Kaye 2001). The species highest recommended as host plants are *A. millefolium, D. californica,* and *E. lanatum* (Schmidt 2016).

The number of individuals in each population is also very important. It has been determined that the minimum number of mature, flowering *C. levisecta* individuals a population must have to sustain itself is 1000 (Caplow 2004). The roundness of this number indicates that it is an estimate. The number is static and does not account for factors like genetic heterogeneity. Many of the remaining *C. levisecta* populations do not reach this number of individuals (Miller and Martin 2024).

C. levisecta is an insect pollinated plant (ECOS 2003). Pollinator support is key for propagation in a sustainable management situation. Species of *Bombus* bumblebees visit the plant's flowers (Waters 2018). Those pollinators also visit other species of flowering plants, creating a network of pollination support. This network leverages the resources of an early spring flower to support the pollination of later flowers and vice versa through pollinator vitality. The protection of this network is key to the continued seed production of the *C. levisecta* plants. The foraging distance of *Bombus vosnesenskii* (a common bumblebee species in the Pacific

Northwest) is around 11.6 km (Rao and Strange 2012), suggesting that populations of *C. levisecta* must be within 11-12 km of one another in order to exchange genetic material.

Threats to reestablishment and restoration

C. levisecta faces many threats to its continued survival. Herbivory, invasive species, habitat destruction, hybridization, and inbreeding are the primary trouble. Each threat alone is a thing to be reckoned with, and the combined pressure they exert is substantial. These threats synergize to oppose the earnest restoration efforts of the *C. levisecta* conservation community. They present unique problems that inhibit *C. levisecta* from meeting its specific needs.

Herbivory is a common challenge faced by many plant species; *C. levisecta* is no different in this regard. Predation pressure from wildlife has been observed on *C. levisecta* in a field study. Greytailed voles (*Microtus canicaudus*) have been identified as a possible culprit, as have deer and rabbits (Lawrence and Kaye 2008). It is possible that other wild species feed on the plant as well. This is a knowledge gap where additional research could occur. The consumption of *C. levisecta* host plants by grazing livestock is commonplace (Matlaga 2004). Since *C. levisecta* is a hemi-parasite, there could also be impacts of herbivory on its host plant, but so far no research has evaluated this knowledge gap.

Invasive species are a major cause for concern in *C. levisecta* management. The prairie habitats of *C. levisecta* are often badly degraded. Urban development, farming, ranching of various not native animals, and exclusion of traditional, native american led fire regimes have left most *C. levisecta* habitat vulnerable to colonization by invasive species (Fleischner 1994, Crawford 1997). Invasive and non-native plants invade the remaining protected habitats on the wind itself, and on the boots of the very people who maintain and appreciate it. They are not to be taken lightly. Scotch broom (*Cytisus scoparius*) has invaded and degraded a tremendous amount of prairie habitat. The shrub fertilizes itself, chemically deters competition from other plants, and shades out *C. levisecta* habitat (Haubensak and Parker 2004). Tall oat grass (*Arrhenatherum elatius*) is also a major invasive plant in prairie habitats (Wilson and Clark 2001). This species was introduced along with several other creeping, perennial pasture grasses to support livestock cultivation for colonizers. These species spread quickly and dominantly through the prairie habitat of *C. levisecta* (Crawford 1997). The damage that has and is being done by these and other invasives is a serious challenge to the sustainable management of *C. levisecta* habitat.

Habitat destruction is the main problem *C. levisecta* is facing. Prairie habitat is greatly imperiled. It has been severely reduced since historic times. South Puget Sound prairies in particular are important for the species, and around 95% of them have been destroyed (Crawford and Hall 1997). Western Washington prairies were developed and maintained in large part by the burning practices of indigenous nations (Storm and Shebitz 2006). Colonizers forced the end of this, which has greatly affected prairie habitat quality (Vinyeta 2021). The habitats that remain are highly fractured (Crawford and Hall). As an insect pollinated species, *C. levisecta* requires close proximity to its reproductive partners, as it cannot send its pollen to travel miles on the wind. The loss of contiguous habitat makes the exchange of pollen between distant and small native populations difficult and unlikely.

Hybridization with other *Castilleja* species is an additional pressure on the survival of the species. *Castilleja hispida* in particular has the ability to interbreed with *C. levisecta* (Kaye and Blakley-Smith 2008). This causes problems for restoration efforts, as both species have been seeded and planted in close proximity in restoration areas (Kaye 2014, Clark 2015). The hybridized plants can go undetected (Clark 2015). The difficulty in detection of hybrids can lead to impure seed sources causing seed contamination (Penderson et al. 2015). Hybridization can also lead to depression in fitness of *C. levisecta* hybrids, further challenging the species (Sandlin 2018).

On the opposite end of the scale from hybridization we have the problem of inbreeding. This issue has a different cause, but can lead to negative effects similar to those of hybridization. When *C. levisecta* plants self-pollinate or mate with close relatives, their offspring may show negative effects related to inbreeding depression, while the opposite is possible if individuals that are distantly related reproduce (Kaye and Lawrence 2003). *C. levisecta* has been shown to set seed very poorly under conditions of self-pollination. When studied, the fruit set of *C. levisecta* ranged from 0.7% for self-pollinations, 33% for sibling crosses, 71% for within population crosses, and 80% for between population crosses (Evans et al. 1984). This means that genetic heterogeneity between interbreeding plants is important to sustainable management. Inbreeding in *C. levisecta* leads to reduced seed set. The reduction can leave a self-pollinated flower with less than 1% chance to set seed (Evans et al. 1984). It can also lead to reduced vigor or "inbreeding depression" (St. Clair et al. 2020). This presents a serious problem, as all the native *C. levisecta* populations are considered small for sustainable recovery purposes. They are also distant and isolated from less related populations (Caplow 2004). The management of *C. levisecta* gene pools is a topic that needs additional research, if we aim to be successful in their restoration.

Management strategies

The various needs and threats for the sustained existence of *C. levisecta* are met by the management techniques, methods, and policies used by the conservation community. To cover this topic in broad strokes, I focus on propagation and reintroduction, habitat management, and preserve establishment.

C. levisecta is an important plant in western Washington prairie restoration. As such, it has received a tremendous amount of attention regarding propagation and preservation of genetics. In vitro propagation techniques have been used (Salama et al. 2018 [1]). Cryogenic storage of living tissue is another of the methods used (Salama et al. 2018 [2]). Seed collection for nursery propagation is one common method employed (Smith and Elliot 2016). Outplanting of nursery grown *C. levisecta* plants is commonplace and a great support to the sustained survival of the species (Lawrance and Kaye 2011). Outplanting has greatly aided the reintroduction efforts bringing *C. levisecta* back to sites from which it had been extirpated. Outplanting was initially more common than the spreading of seed due to the small amount of seed available, and the complexity of the host-bonding requirement, however most restoration efforts have shifted to seeding in recent years as seed production has increased.

The physical, on the ground management of *C. levisecta* habitat is incredibly important to the species. Site preparation through weed removal and large-scale burning is an important part of the process. Preparation of outplanting habitat is often done through manual means. This includes hand pulling of many different invasive weeds, Scotch broom chief among them. Chemical methods of weed removal are also employed, and on many different species. Mowing of weeds and brush has been utilized as well (Dunn 1998). Each of these techniques manage the habitat of *C. levisecta* with the same goal, at differing scales. Controlled burns are also often used on prairie habitat as a maintenance and site prep method for *C. levisecta* (Dunwiddie 2009). Burning is a useful tool for prairie management, and therefore the restoration of *C. levisecta*.

The securing of *C. levisecta* populations is being done primarily through a system of preserves and natural areas (Caplow 2004). Some of these sites are government managed, while other sites are non-profit managed properties (U.S. Fish and Wildlife Service 2007, Kronland and Martin 2015). In Washington State, most of these sites are host to introduced populations.

Only one known wild native legacy population persists in Washington, Rocky Prairie (Miller and Martin 2024). The introduced populations source their genetics from other wild native populations, including some in British Columbia (Caplow 2004). These sites were the primary seed sources for restoration efforts. The majority of preserves with native populations do not receive input of seed from sources with new genetics. They have also been in steady decline for the last decade (U.S. Fish and Wildlife Service 2019). This is likely a problem and needs further research to be fully understood.

<u>Summary</u>

After in-depth review of available research, it is clear that the sustainable management of the native *C. levisecta* populations requires many things for successful recovery. They cannot do without an appropriately high number of individuals, genetic heterogeneity, quality prairie habitat, and direct pollinator support. Directly opposing these needs are the threats of aggressive invasive species, inbreeding depression, habitat loss, and being hybridized out of existence. Research on these threats shows that they are truly treacherous and a huge drain on recovery efforts. The assessment of techniques and policies around propagation, habitat management, and preserve establishment needs to be weighed against these needs and threats.

It is clear from the literature that *C. levisecta* needs to have genetically heterogeneous reproductive partners located within flight distance of its insect pollinators if it is to produce a healthy seed set. This need flies in the face of the preserve-based isolation policies that native *C. levisecta* populations have been subjected to by managers. Native *C. levisecta* populations are tanking, and this management policy is likely why. The situation demands that the introduction of new genetic material be made to native populations if they are to escape terminal inbreeding depression. This is known as “genetic rescue” (Ingvarsson 2001). This method has been used to support the restoration of rare plants (Willi et al. 2007, Finger et al 2011, Whiteley et al. 2014, St. Claire et al. 2020). The policy of isolation may have served to protect genetic integrity in the past, but now it must be reversed in order to achieve the goal of genetic diversity for sustained populations of this rare species.

The questions of “Are Washington's native legacy populations of *C. levisecta* (Golden paintbrush) able to survive without the introduction of new genetic material?”, and “Is it possible that native *C. levisecta* populations are becoming terminally inbred due to management driven genetic isolation?” demand an answer. In an attempt to generate data to help answer these questions, I have posited my hypotheses: 1) A genetically isolated population of *Castilleja levisecta* will produce fewer seeds than a *Castilleja levisecta* population of mixed genetic stock and 2) A genetically isolated population of *Castilleja levisecta* will produce lighter seeds with lower viability than a *Castilleja levisecta* population of mixed genetic stock.

Methods

Site Selection

Study site determination was carried out in spring of 2023. Several of the remnant prairie sites throughout the Pacific Northwest in which *C. levisecta* persist were considered for the study. The two other sites with fully legacy native *C. levisecta* populations were the San Juan Valley site on San Juan Island, and the Ebeys Landing Hill Road site on Whidbey Island. These populations proved too difficult to acquire seed from, and additionally were very small in size at 84 and 135 plants respectively (Miller and Martin 2024). This small size means they would have been unlikely to produce enough flowering individuals for an adequate sample. I ultimately chose Rocky Prairie and Mima Mound Natural Areas, both of which are owned and managed by the WA Department of Natural Resources (DNR). Rocky Prairie was in the midst of a massive *C. levisecta* population crash, like several other genetically isolated, native legacy *C. levisecta* sites. The population of *C. levisecta* at Rocky prairie has dropped by 95% in 8 years (Miller and Martin 2024). This remnant south Puget Sound prairie ecosystem had the largest population of *C. levisecta* across all genetically isolated native legacy *C. levisecta* sites. This made Rocky Prairie the best option among the genetically isolated *C. levisecta* sites for sampling a large enough number of individual *C. levisecta* plants to yield statistically significant data.

Determining a suitable comparison study site from the genetically non-isolated *C. levisecta* restoration sites was done by analyzing how other *C. levisecta* sites measured up to several qualifiers. The important factors were ecological (plant community) and environmental (soil types, land use history) similarity, as well as geographic closeness to Rocky Prairie. It was important to find a very similar site for comparison. This eliminated as much variance in environmental, climactic, and wildlife influence as possible. Additionally, it was important that the *C. levisecta* population at the selected comparison site be large enough to yield adequate samples for statistical significance. These stipulations led to the choosing of Mima Mounds Prairie as the comparison restoration study site to the native legacy site of Rocky Prairie.

Rocky Prairie is a relatively small remnant south Puget Sound prairie of 14 hectares. The primary soil type at Rocky Prairie is Spanaway-Nisqually complex (Web Soil Survey 2024). Mima Mounds Prairie is a remnant south Puget Sound prairie of 306 hectares, making it much larger than Rocky Prairie. It was determined, however, that Mima Mounds Prairie had similar enough soil, species, management, and geographic location to qualify it for comparison. The primary soil type at Mima Mounds Prairie is also Spanaway-Nisqually complex (Web Soil Survey 2024). Rocky Prairie was established by the Washington State Department of Natural Resources in the 1990s and Mima Mounds Prairie was established in 1976. Selecting a comparison site that had been under Washington State Department of Natural Resources management for a more similar duration of time was given consideration. Ultimately the duration of management was deemed to be less important than the type of management.

It was very important to find sites that had similar management treatments. The management of the native legacy site (Rocky Prairie) had included tree removal, chemical and mechanical weed management, occasional mowing and/or burning, and seeding and/or plugging of native plant species. Hand harvesting of seed had also occurred there, and the site had been used as a seed source for *C. levisecta* restoration efforts and is kept in genetic isolation. Similar to Rocky Prairie, the management of Mima Mounds Prairie had also included tree removal, chemical and mechanical weed management, periodic mowing and/or burning, as well as seeding and plugging of native plant species. Regular seed gathering took place on Mima Mounds Prairie as well. Mima Mounds Prairie had only been used as a restoration out-planting site for *C.*

levisecta restoration efforts, utilizing a range of genetic sources. Mima Mounds Prairie is located ~13 kilometers from Rocky Prairie (Figure 3). It was decided that this level of proximity would facilitate a similar profile of environmental, climactic, and wildlife influence for the two sites. This was one of the most important deciding factors in comparison site selection.

Figure 3

Map of Mima Mounds Prairie (left) and Rocky Prairie (right) site proximity

Note. Map showing the relative locations and distance apart of the two study sites.

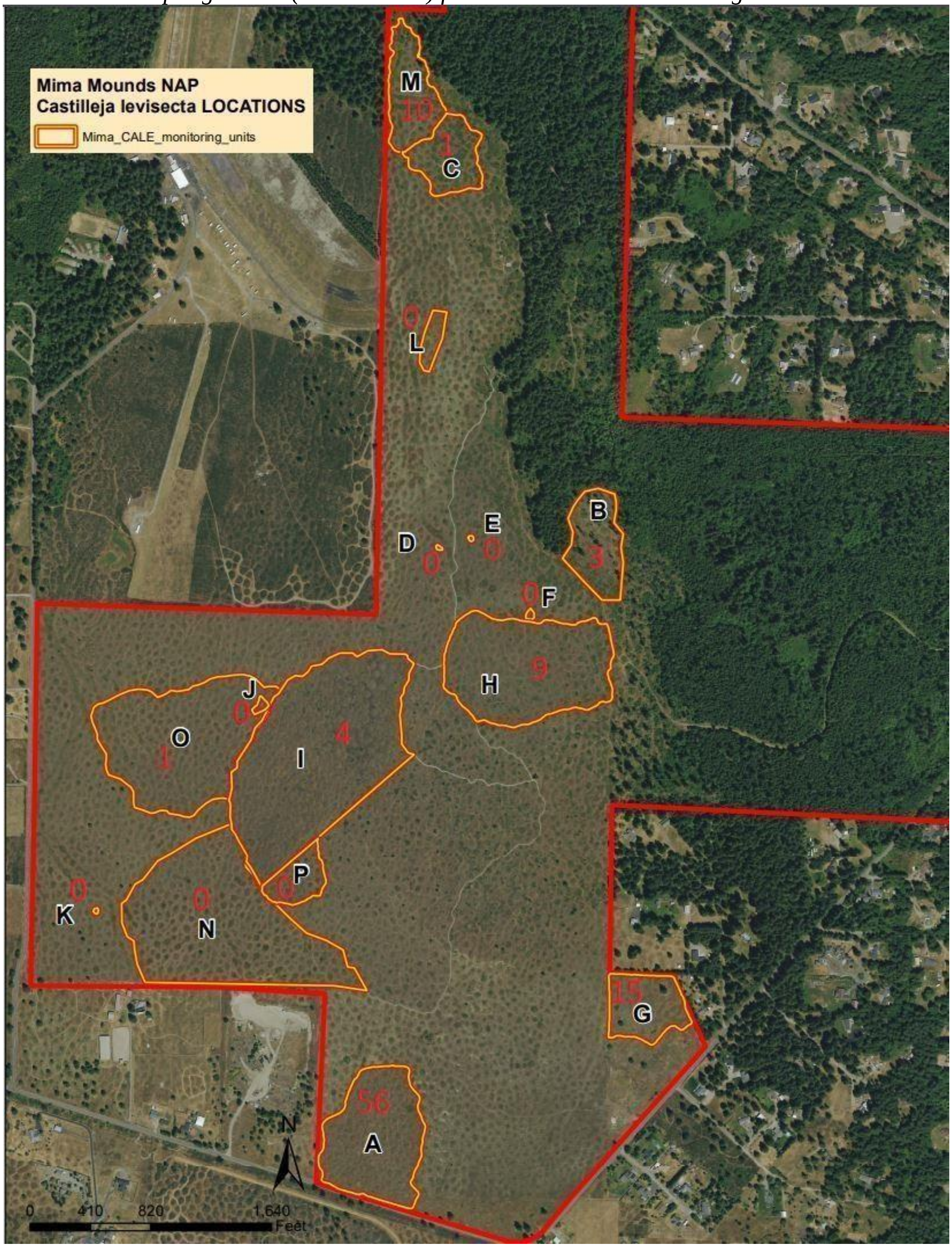
Sample Design and Collection

I collected *C. levisecta* seed from one native legacy population (Rocky Prairie), and one population that has been introduced (Mima Mounds Prairie). The goal was to sample each population 100 times if possible, with one sample being an individual seed capsule from an individual plant. This would allow for the capture of a 0.4 effect size with 0.8 power at a 95% confidence level. Plants with no seed stalks were to be excluded. In the event that the reduced population at Rocky Prairie did not have 100 fruiting samples of this rare plant, then the total number of available fruiting samples would be used. Ultimately, only 48 *C. levisecta* plants at Rocky Prairie were found to have produced seed capsules, meaning only 48 samples of genetically isolated native legacy *C. levisecta* were available and used.

For Mima Mounds Prairie, the 100 samples were taken from eight different restoration seeding units. The number of *C. levisecta* sampled per unit was a ratio equal to the ratio of said unit's *C. levisecta* population to the total *C. levisecta* population at the site, rounded to the nearest whole number. So if a unit contained 10% of the total *C. levisecta* plants present at Mima Mounds Prairie, 10% of the total samples for the Mima Mounds *C. levisecta* population were taken from that unit. This method ensured that there was a representative sample from different years and sources. This method excluded units that would have provided less than one sample using the aforementioned ratio. The sampling scheme is detailed below in Figure 4. A similar sampling scheme was developed for the six units of Rocky Prairie, but was not used due to the extremely low *C. levisecta* population at that site.

Figure 4

C. levisecta sampling counts (shown in red) for each Mima Mound seeding unit



Note. Sampling map for Mima Prairie with sample units demarcated and sample counts listed.

The *C. levisecta* plants were present in low numbers and distributed extremely unevenly within the individual units at Mima Mounds Prairie. This made randomly distributed points ineffective as a sampling tool. ArcGIS Field Maps, high visibility flagging, and naked-eye estimation were used to ensure that samples taken within each unit were as evenly distributed as possible. This included both sampling evenly throughout the unit and also attempting to take an equal number of samples from in-swale and on-mound plants at the Mima Mounds Prairie site.

C. levisecta fruits mature from late June through July, with capsules beginning to open and disperse seed in August (Evans et al. 1984). As such, sampling occurred in early July 2023 for this study. For each sample plant, I collected a random seed capsule from a random stalk. This was randomized by using a random number generator in the range of each plant's count of seed stalks, then seed capsules on that stalk. Each seed capsule was bagged individually and labeled with the date, site, sample number, and collector initials.

Seed Processing and Measurement

Seeds were kept for the duration of the research period (1 year) in a climate controlled refrigeration unit. The seed capsules were kept at 40° Fahrenheit and 40% humidity. Each seed capsule was individually dissected and the total number of seeds per capsule was counted, using a microscope to ensure accuracy. The total weight of the seed produced by each individual seed capsule was measured, using a calibrated analytical balance to ensure accuracy. These metrics were logged in a data sheet. After processing and measurement, seeds were stored in labeled paper envelopes and returned to climate controlled storage.

Data Processing and Statistical Analysis

RStudio was used to perform statistical analysis of the seed count and seed weight data. The Shapiro-Wilk normality test was used for determining normal/non-normal distribution for seed count data, therefore dictating whether parametric or nonparametric statistical tests were needed. Wilcoxon rank sum test with continuity correction was used to determine if the seed count and seed weight from native legacy and restoration populations were different by a statistically significant amount (tested against an alpha value of 0.05). Basic summary statistics were also generated for seed count and seed weight data. Rstudio was also used to generate figures used for statistical depictions.

Tetrazolium Viability Testing

Viability rate tests were run on roughly 400 sampled seeds for each site's population of *C. levisecta*. The Oregon State Seed Laboratory at Oregon State University was chosen to run the seed viability tests. The tetrazolium seed viability test is a rapid (24-48hr) biochemical viability test which determines the number of live seeds based on dehydrogenase activity in seeds. It is an International Seed Testing Association accredited test. It indicates the percentage of viable (live) and non-viable seeds in any sample regardless of its dormancy level (Oregon State University 2019). The tetrazolium estimated seed viability test has been in use since the 1940s and was selected due to its established efficacy (Vankus 1997).

The tetrazolium seed viability test uses 2,3,5-triphenyl tetrazolium chloride. The Oregon State Seed Lab procedure for the test is as follows: 1) hydration - seeds completely imbibed to activate respiration enzymes, 2) cutting/puncturing so that the tetrazolium solution can access the internal tissues of seeds, performed under the microscope for accuracy, 3) staining - the seeds are placed in a tetrazolium solution (0.1-1.0%) for 24-48 hours, and 4) evaluation of the TZ staining pattern and intensity to determine viability. Prior to testing, seeds were stored for roughly 16 months in 40° Fahrenheit refrigerated storage at 40% humidity. Due to limited availability of seed, only one viability test was run on the seeds from each population.

Seed Germination Testing

The sampled seed was tested for germination rates of each population by The Oregon State Seed Laboratory at Oregon State University. Each test used a total of 330 seeds from each of the source populations. The proportion of seeds which germinated during the test period under laboratory conditions were compared between populations. Prior to testing, seeds were stored for roughly 16 months in 40° Fahrenheit refrigerated storage at 40% humidity. Due to limited availability of seed, only one viability test was run on the seeds from each population.

Results

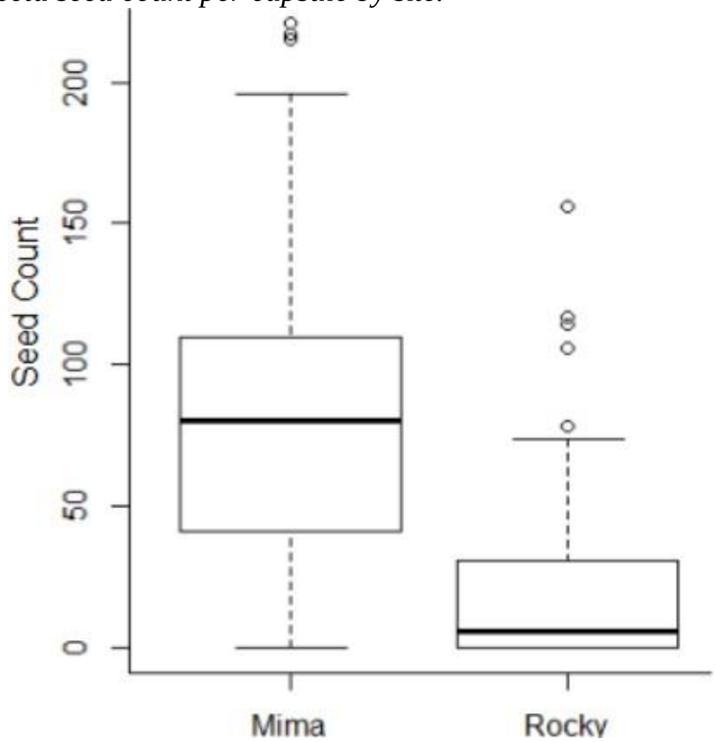
The results for this study describe the seed production and viability of *C. levisecta* from Rocky and Mima Mounds prairies in 4 distinct metrics. The metrics include seed count per capsule, seed weight per capsule, seed viability via tetrazolium testing, and seed germination rate.

Seed Count Results

The seed count per individual seed capsule at Mima Mounds Prairie ranged from 0 seeds to 221 seeds per capsule with a mean of 80.45 (± 50.20 SD) seeds per capsule. The seed count per individual seed capsule at Rocky Prairie ranged from 0 seeds to 156 seeds per capsule with a mean of 25.27 (± 37.78 SD) seeds per capsule (Figure 5).

Figure 5

C. levisecta seed count per capsule by site.

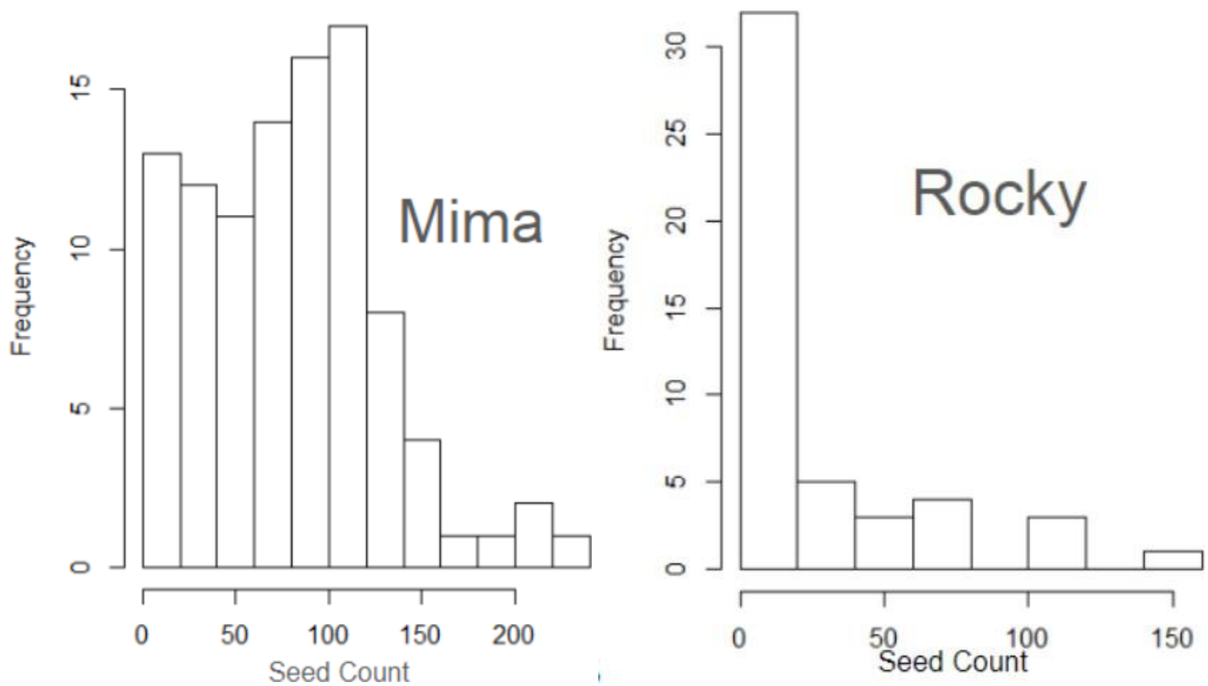


Note. Comparison of seed count data showing differences by population.

Histograms of the seed count for each population show the difference in the distribution of the number of seeds produced by each sampled capsule between the two populations (Figure 6). They show a huge difference in numbers of seeds produced per pod with Mima Mounds Prairie clearly having its pods most commonly producing around 100 seeds, while pods from Rocky Prairie most commonly produce no seeds at all. Pictured in Figure 7 are density curves of the distribution of seeds produced per capsule in each *C. levisecta* population. These curves depict 4 distinct peaks for Rocky Prairie at 0, 70,110, and 160. This contrasts with only 2 distinct peaks for Mima Mounds Prairie at 90 and 220. The comparison Table 1 provides summary statistics for seed production between the two populations.

Figure 6

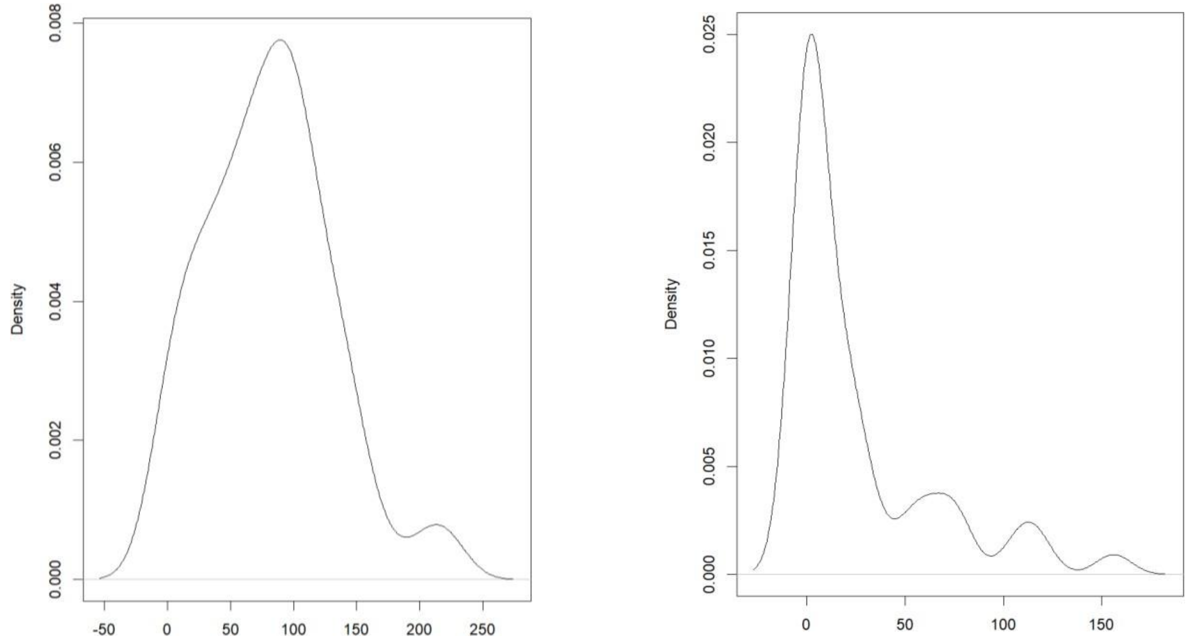
Number of seed capsules (frequency) with different seed counts at Mima (left) and Rocky (right).



Note. Comparison of the distribution of number of seeds per pod by population.

Figure 7

Seed count distribution density at Mima (left) and Rocky (right).



Note. Comparison of the distribution curve of number of seeds per pod by population.

Table 1

Mima and Rocky seed count summary statistics

	Mima	Rocky
Range	0-221	0-156
Mean	80.45	25.27
Mode	0	0
Standard Deviation	50.20	37.78
1st Quartile	41.5	0
2nd Quartile (Median)	80.5	5.5
3rd Quartile	109	29.25

Note. Comparison of summary statistics of the seed counts by population.

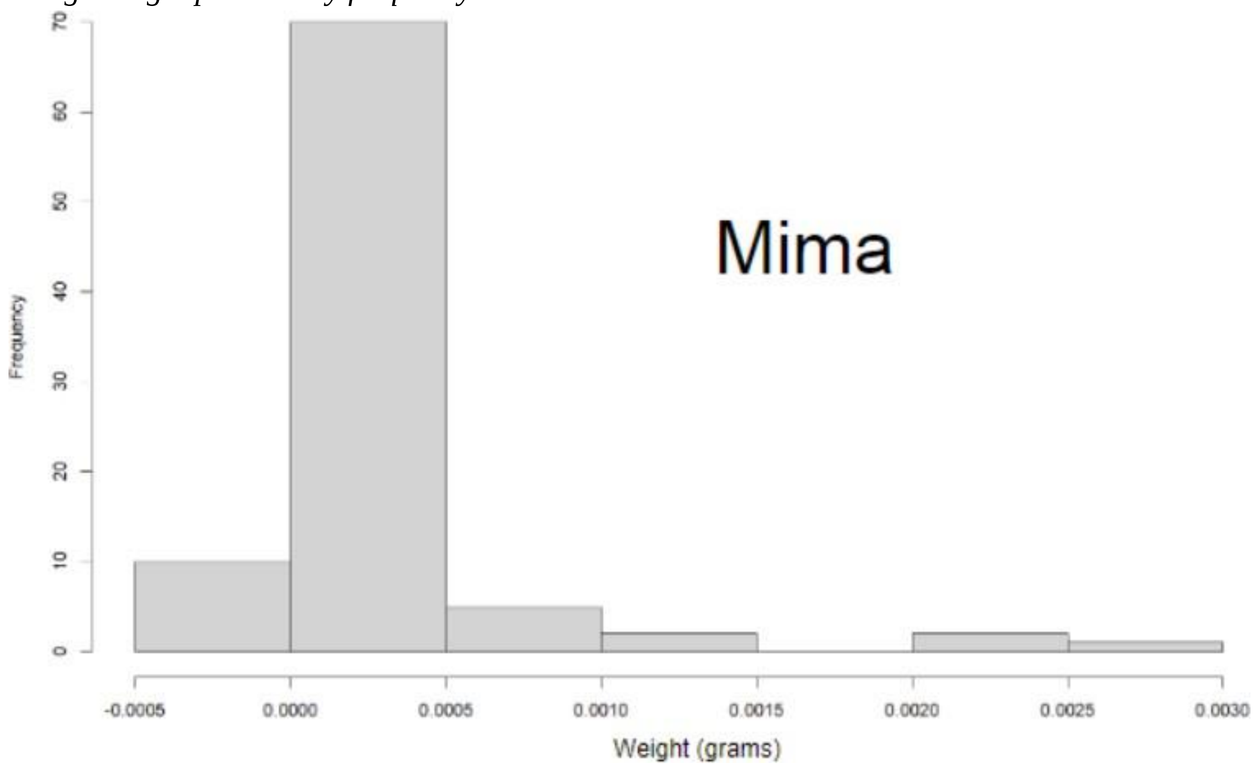
The Shapiro-Wilk normality test was used for determining normal/non-normal distribution. For Mima Prairie this test returned W = 0.97 and p-value = 0.01. For Rocky Prairie the test returned W = 0.72 and p-value = 2.75e-08. These results indicated a non-normal distribution for seed count numbers in both populations. The nonparametric Wilcoxon rank sum test with continuity correction was used to determine if the two sets of data for the native legacy and restoration populations were different by a statistically significant amount (tested against an alpha value of 0.05). This test returned W = 3932.5, p-value = 3.192e-10, indicating that the two populations are statistically significantly different in amount of seed produced.

Seed Weight Results

The seed weight per individual seed at Mima Mounds Prairie ranged from -3.56e-04g to 2.81e-03g, with a mean of 2.511e-04g per seed. The standard deviation was 0.50e-03g. The seed weight per individual seed at Rocky Prairie ranged from -4.33e-04g to 1.08e-02g with a mean of 4.37e-04g per seed. The standard deviation was 1.71e-03g. The majority of the seeds at Mima Mounds Prairie were less than 0.0005g (Figure 11), while the majority of the seeds at Rocky Prairie were less than 0.002g (Figure 12).

Figure 8

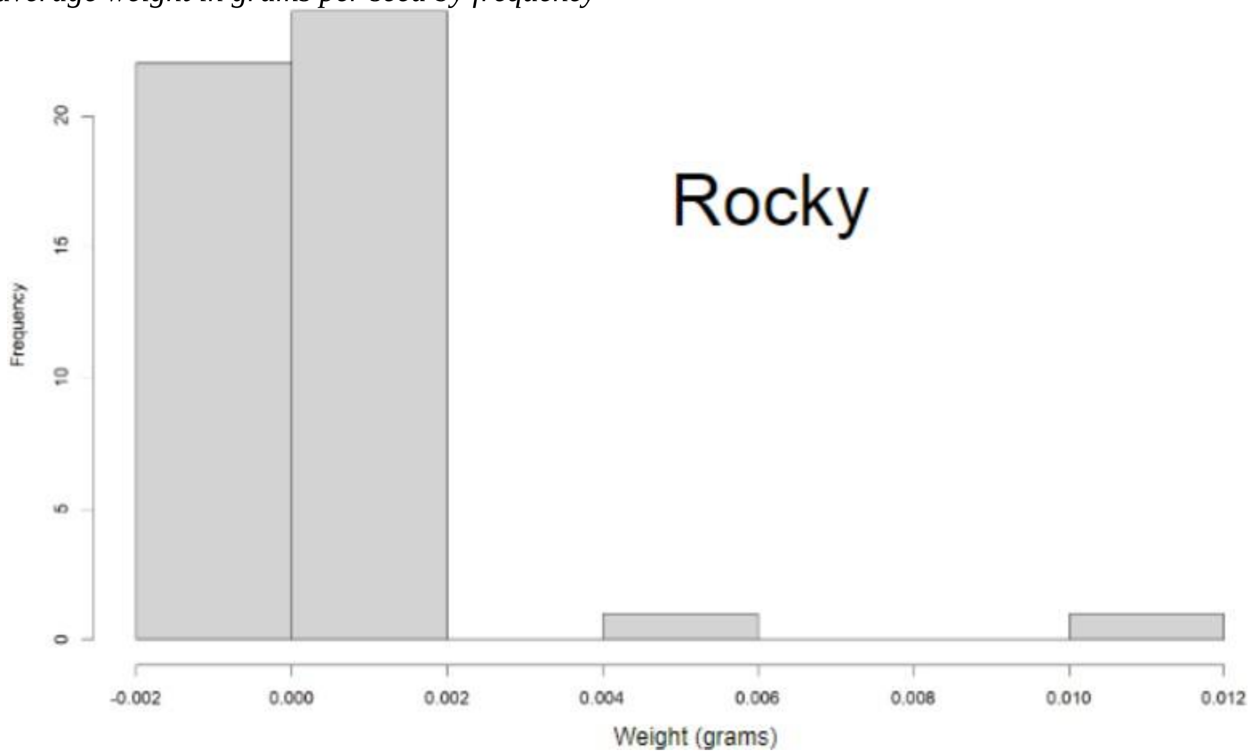
Mima average weight per seed by frequency



Note. Distribution of weight of seeds per pod at Mima Mounds Prairie.

Figure 9

Rocky average weight in grams per seed by frequency



Note. Distribution of weight of seeds per pod at Rocky Prairie.

Table 2

Mima and Rocky seed weight summary statistics

	Mima	Rocky
Range	-3.56e-04g to 2.80e-03g	-4.33e-04g to 1.08e-02g
Mean	2.51e-04g	4.37e-04g
Standard Deviation	5.03e-03g	1.71e-03g
1st Quartile	3.89e-05g	0g
2nd Quartile (Median)	7.94e-05g	5.42e-05g
3rd Quartile	2.41e-04g	1.63e-04g

Note. Comparison of summary statistics of the seed weights by population.

The Shapiro-Wilk normality test was used for determining normal/non-normal distribution for the seed weight data. For Mima Mounds Prairie this test returned W = 0.56, p-value = 4.7e-15. For Rocky Prairie the test returned W = 0.30 and p-value = 9.19e-14. These results indicated a non-normal distribution for seed weight numbers in both populations. The nonparametric Wilcoxon rank sum test with continuity correction was used to determine if the two sets of data for the native legacy and restoration populations were different by a statistically significant amount (tested against an alpha value of 0.05). This test returned W = 2642 and p-value = 0.03, indicating that the two populations are statistically significantly different in the weight of the seeds they produce.

Seed Viability and Seed Germination Results

The tetrazolium chemical test for seed viability rate returned unequal results from the two populations. Seed from *C. levisecta* growing on Rocky Prairie had a higher viability (47%) rate than that of the seed from Mima Mounds Prairie (33%). These tetrazolium viability tests were singular for each population and had no replication. The seed germination rate testing for the two populations returned very low rates. Rocky Prairie, the native legacy population, had a zero percent (0%) germination rate out of 330 seeds tested. Mima Mounds Prairie, the introduced restoration population, had a two percent (2%) germination rate out of 330 seeds tested.

Discussion

The study's findings show that a native legacy population of *C. levisecta* is producing fewer seeds than a restoration population. This has direct implications for restoration planning and preserve management, and is demonstrable reasoning to end genetic isolation of native legacy *C. levisecta* populations. The study shows that the native legacy seed has a higher average weight, though the implications there are not as clear. With Rocky Prairie performing higher on the seed viability test, there is evidence that native legacy populations may still have robust aspects. The very low germination rates returned by testing do not necessarily support or contrast against this finding. Taken together, these findings show that there are some stark differences between *C. levisecta* native legacy and restoration populations.

Seed Count

Regarding the seed production hypothesis, we can reject the null hypothesis; that a genetically isolated population of Castilleja levisecta will not produce fewer seeds than a Castilleja levisecta population of mixed genetic stock, and accept that a genetically isolated population of *C. levisecta* does in fact seem to produce fewer seeds than a *C. levisecta* population of mixed genetic stock. The data from the seed count results show that *C. levisecta* plants from the genetically isolated (by management decision) legacy native population in our study (Rocky Prairie) produce less seed than plants from the restoration population of mixed genetic stock (Mima Mounds Prairie). Their mean seed production of 25.27 per capsule for Rocky Prairie and 80.45 per capsule for Mima Mounds Prairie show that *C. levisecta* plants at the genetically mixed site are producing on average 3.18 times the number of seeds as their counterpart plants at the genetically isolated site.

These findings illuminate not only a statistically significant difference, but a massive and stark difference in seed production between these sites representing two different species management strategies. Rocky Prairie is being kept genetically isolated, while Mima Mounds Prairie is genetically mixed. The literature demonstrates that genetically isolated *C. levisecta* can suffer from inbreeding depression, and that the aspects of inbreeding depression have been documented to affect physical characteristics of *C. levisecta* plants, including their seed production (St. Clair et al. 2020). With that being the case, the difference in rate of seed production between the two populations may be due in part to those effects. The reduced level of seed production by the *C. levisecta* on Rocky Prairie is consistent with the literature on reduced seed production in genetically similar *C. levisecta* populations (Evans et al. 1984). This indicates that the management-driven lack of genetic diversity at Rocky Prairie may be contributing to the rapid and concerning decline in population at that site over the last decade. At the very least, we can be certain a *C. levisecta* plant from a population that has been genetically isolated by management actions is producing fewer seeds on average than a *C. levisecta* plant from a population of mixed genetic stock.

The seed production numbers from both populations were lower than the average seed production of 187 seeds per capsule found in the literature (Fisher et al. 2015). While this is concerning for both sites, the disparity between seed production per capsule from Rocky Prairie and from the literature is drastic. *C. levisecta* from the Rocky Prairie population is producing just 14% of what the literature would lead us to expect. This further raises the level of alarm concerning the sustainability of the native legacy population at Rocky Prairie, particularly considering that Rocky Prairie, currently with only about 300 plants, has far less than the ~1000 plants needed for a "sustainable" population, according to the literature (Caplow 2004, Miller 2024).

In addition to the difference between the two populations in terms of number of seeds produced, there is also a major difference in the distribution of the number of seeds produced per capsule for each population. The comparison shows a more evenly dispersed distribution in seed production (between 0-250 seeds/capsule) in the genetically mixed restoration population at Mima Mounds Prairie than in the genetically isolated population at Rocky Prairie (most pods had 0-5 seeds with no pods exceeding 150 seeds). The results clearly show reduced seed production at Rocky Prairie when compared to Mima Prairie.

There are possibly other unknown factors that could also be influencing the seed production of the *C. levisecta* plants at these legacy native and restoration sites. Mima Mounds Prairie is much larger than Rocky Prairie and that size difference may be a factor. Differences in micro-climate such as humidity or average wind speeds could be affecting seed production. The community of pollinators at each site may be different, leaving to variances in pollinator visitation. While any or all of these aspects could be playing a part in the great difference in seed production between the *C. levisecta* populations at the two sites, no effects from these have been demonstrated in the literature. Therefore, I believe based on the specific evidence for reduced seed production in the literature surrounding the topic of inbreeding effects on *C. levisecta,* that the genetic isolation of Rocky Prairie compared with Mima Mounds Prairie is a primary reason for the disparity in seed production.

<u>Seed Weight</u>

Regarding the seed weight hypothesis, the results were not entirely clear. On one hand, the results indicate that a genetically isolated population of *C. levisecta* will produce heavier seeds than a *C. levisecta* population of mixed genetic stock. While not a direct indicator of health or viability, weight is an interesting metric to consider when comparing seeds from different populations. It is notable that many of the lightest seeds in the study appeared to be hollow and without an embryo. The *C. levisecta* plants from a genetically isolated (by management decision) legacy native population in this study (Rocky Prairie) produce seeds that weigh roughly twice as much (4.373e-04g per seed vs. 2.511e-04g per seed) as those from a restoration population of mixed genetic stock (Mima Mounds Prairie). However, there is a worrying amount of variance in the data, several extreme outliers, and even negative seed weight measurements being returned by the analytical balance. Similarly varied measurements can be found in literature regarding seed weight (Fisher et al. 2015). The issues indicate that the instrument was unable to precisely measure the weight of these seeds. Getting accurate weight measurements for such miniscule seeds is likely a persisting problem for studies on the subject. While the mean seed weights for these two populations may indicate a relative genetic robustness amongst the Rocky Prairie stock, it is clear that these results need to be viewed as unreliable. Further investigation is needed.

Seed Viability

The tetrazolium chemical tests for seed viability returned uneven results from the two populations. Seed from *C. levisecta* growing on Rocky Prairie showed a higher viability rate (47%) than that of the seed from Mima Mounds Prairie (33%). These results do not align with the seed count results, in the sense that Rocky Prairie had lower seed production with a higher viability. These tetrazolium viability tests were singular for each population and had no replication. That said, they imply that Rocky Prairie is producing more viable seed than Mima Mounds Prairie. This, despite it producing fewer seeds than Mima Mounds Prairie on average. The literature would lead to an expectation of low viability in the isolated population, possibly due to inbreeding depression (Kaye and Lawrence 2003). That is not what we see suggested here.

Tested seed produced at the restoration site was only around 70% as viable as tested seed produced by plants at the native legacy site. The finding may be demonstrating the importance of the genetic stock at Rocky Prairie for the continued health of this species as a whole. However, considering that germination rates of around 90% have been described in the relevant literature (Fisher et al. 2015), viability rates of 33% and 47% seem low. Certainly nothing can be said for sure given that this is a single data point. Replication of sampling and testing would add depth to this superficial observation.

Germination

The singular, paired germination tests returned relatively even results from the two populations. However, seed from *C. levisecta* growing on the native legacy site (Rocky Prairie) showed a slightly lower germination rate than that of the seed from the restoration site (Mima Mounds Prairie). That said, the findings were somewhat suspect. Rocky Prairie, the native legacy population, had a zero percent (0%) germination rate out of 330 seeds tested while Mima Mounds Prairie, the introduced restoration population had a two percent (2%) germination rate out of 330 seeds tested. These results are extremely low when compared with germination rates of 90% in one study (Pederson et al. 2015). They indicate a possible problem with methodology. A 16 month cold/dry stratification (the treatment received due to storage length) may have been an inferior treatment when compared to a cold/wet stratification of similar length (Lawrence 2005). Despite the fact that these germination tests were singular for each population and had no replication, they may allude to a higher rate of germination from the population of mixed genetic stock. If the seed from these two populations were in fact only germinating at 0% and 2% in the field, it would be a disaster for recovery efforts. Replication of sampling and testing with literature suggested cold/wet stratification (Dunwidie et al. 2013) would add depth to this superficial observation.

Conclusion

To conclude, the findings of this study show that the genetically isolated population of *C. levisecta* is behaving very much as the literature on inbreeding depression in this species would suggest, and as the study hypothesized. This study shows that the genetically isolated *C. levisecta* plant population located at the Washington State Department of Natural Resources managed Rocky Prairie is clearly producing significantly fewer seeds per seed capsule than its counterpart plants from the restoration population at Mima Mounds Prairie (also managed by DNR).

No firm conclusions can be drawn from the seed weight analysis, although these preliminary data suggest that the genetically isolated population could possibly have heavier seed. Similarly, while the findings from the seed viability tests were preliminary, they showed that there are likely disparities in the viability of seed produced by the two study populations. It is possible that the legacy site with isolated genetics is producing more viable seed, although the germination rate of both populations was exceeding low. These initial investigations call for additional exploratory research into the weight, viability, and germination rate of *C. levisecta* seed from different sites, genetic groups, and management styles.

Considering this study's findings, I believe that the management style of genetic isolation is contributing to the reduced level of seed production in the *C. levisecta* plants at Rocky Prairie. It is also the suggestion of this work and its author that the reduced level of seed production in the *C. levisecta* plants at Rocky Prairie is a contributing factor in the sharp decline of that species' population at the native legacy site. As such, it is evidenced here that the management style of genetic isolation is likely a contributing cause to the rapid loss of over 90% of the *C. levisecta* population at Rocky Prairie over the last decade. It is the recommendation of this researcher that the management style of genetic isolation of Rocky Prairie *C. levisecta* stock be re-evaluated and changed to support a wider genetic diversity that exists within the species. This should be done before the native legacy *C. levisecta* population and its unique genetics are lost forever.

References

Clark, L. A. (2015). *Bee-crossed lovers and a forbidden Castilleja romance: Cross-breeding between C. hispida and endangered C. levisecta in prairie restoration sites* [Thesis]. University of Washington, Seattle WA. https://digital.lib.washington.edu:443/researchworks/handle/1773/33175

Crawford, R. C., & Hall, H. (1997). Changes in the south Puget prairie landscape. Ecology and conservation of the south Puget sound prairie landscape. The Nature Conservancy, Seattle, WA, 11-15.

Delvin, E. (2013). Restoring abandoned agricultural lands in Puget Lowland prairies: a new approach. Ph.D. dissertation, University of Washington, Seattle, WA.

Dunn, P. (1998). Prairie Habitat Restoration and Maintenance on Fort Lewis and within the South Puget Sound Prairie Landscape. The Nature Conservancy.

Dunwiddie, P., Martin, R., & Jarisch, M. (2013). Water and Fertilizer Effects on the Germination and Survival of Direct-Seeded Golden Paintbrush (Castilleja levisecta). *Ecological Restoration*, *31*, 10–12. https://doi.org/10.3368/er.31.1.10

Dunwiddie, P. W., & Martin, R. A. (2016). Microsites matter: improving the success of rare species reintroductions. PLoS One, 11(3), e0150417.

Dunwiddie, P. W., Haan, N. L., Linders, M., Bakker, J. D., Fimbel, C., & Thomas, T. B. (2016). Intertwined Fates: Opportunities and Challenges in the Linked Recovery of Two Rare Species. *Natural Areas Journal*, *36*(2), 207–215. https://doi.org/10.3375/043.036.0214

ECOS. Species Profile for golden paintbrush(Castilleja levisecta). (n.d.). Retrieved July 14, 2023, from https://ecos.fws.gov/ecp/species/7706

Evans, S., Schuller, R., and Augenstein, E. 1984. A report on Castilleja levisecta Greenm. At

Rocky Prairie, Thurston County, Washington. Unpublished report to The Nature Conservancy, Washington Field Office, Seattle, WA

Federal Registrar 2021 *Endangered and Threatened Wildlife and Plants; Removing Golden Paintbrush From the Federal List of Endangered and Threatened Plants*. (2021, June 30). Federal Register. https://www.federalregister.gov/documents/2021/06/30/2021-13882/endangered-and-threatened-wildlife-and-plants-removing-golden-paintbrush-from-the-federal-list-of

Federal Registrar 2023 *Endangered and Threatened Wildlife and Plants; Removing Golden Paintbrush From the Federal List of Endangered and Threatened Plants*. (2023, July 19). Federal Register. https://www.federalregister.gov/documents/2023/07/19/2023-14971/endangered-and-threatened-wildlife-and-plants-removing-golden-paintbrush-from-the-federal-list-of

Finger, A., Kettle, C. J., Kaiser-Bunbury, C. N., Valentin, T., Doudee, D., Matatiken, D., & Ghazoul, J. (2011). Back from the brink: Potential for genetic rescue in a critically endangered tree. Molecular Ecology, 20(18), 3773–3784. https://doi.org/10.1111/j.1365-294X.2011.05228.x

Fisher, L. L., Bakker, J. D., & Dunwiddie, P. W. (2015). An Assessment of Seed Production and Viability of Putative Castilleja levisecta× C. hispida Hybrids. Report for the Center for Natural Lands Management.

Franklin, J. F., & Dyrness, C. T. (1973). Natural Vegetation of Oregon and Washington. U.S. Government Printing Office.

Fleischner, T.L. (1994), Ecological Costs of Livestock Grazing in Western North America. Conservation Biology, 8: 629-644. https://doi.org/10.1046/j.1523-1739.1994.08030629.x

Godt, M. J. W., Caplow, F., & Hamrick, J. L. (2005). Allozyme diversity in the federally threatened golden paintbrush, Castilleja levisecta (Scrophulariaceae). *Conservation Genetics*, *6*(1), 87–99. https://doi.org/10.1007/s10592-004-7746-5

Haubensak, K. A., & Parker, I. M. (2004). Soil changes accompanying invasion of the exotic shrub Cytisus scoparius in glacial outwash prairies of western Washington [USA]. *Plant Ecology*, *175*(1), 71–79. https://doi.org/10.1023/B:VEGE.0000048088.32708.58

Ingvarsson, P. K. (2001). Restoration of genetic variation lost – the genetic rescue hypothesis. *Trends in Ecology & Evolution*, *16*(2), 62–63. https://doi.org/10.1016/S0169-5347(00)02065-6

Kaye, T. N. (2001). Restoration research for golden paintbrush (Castilleja levisecta), a threatened species. Institute for Applied Ecology. Corvallis, OR.

Kaye, T. N., & Lawrence, B. (2003). *Fitness effects of inbreeding and outbreeding on golden paintbrush (Castilleja levisecta): Implications for recovery and reintroduction*. Institute for Applied Ecology. Corvallis, OR.

Lawrence, B. A. (2005). Studies to facilitate reintroduction of golden paintbrush (Castilleja levisecta) to the Willamette Valley, Oregon. Thesis. Oregon State University

Lawrence, B. A., & Kaye, T. N. (2006). Habitat variation throughout the historic range of golden paintbrush, a Pacific Northwest prairie endemic: implications for reintroduction. Northwest Science, 80(2), 140.

Lawrence, B. A., & Kaye, T. N. (2008). Direct and Indirect Effects of Host Plants: Implications for Reintroduction of an Endangered Hemiparasitic Plant (Castilleja levisecta). *Madroño*, *55*(2), 151–158. https://doi.org/10.3120/0024-9637(2008)55[151:DAIEOH]2.0.CO;2

Lawrence, B. A., & Kaye, T. N. (2011). Reintroduction of Castilleja levisecta: Effects of Ecological Similarity, Source Population Genetics, and Habitat Quality. *Restoration Ecology*, *19*(2), 166–176. https://doi.org/10.1111/j.1526-100X.2009.00549.x

Lawrence, C. E. (n.d.). (2015) Responses of Pacific Northwest prairies to soil nutrient manipulations: Implications for restoration of Castilleja levisecta and control of invasive species. Dissertation, Oregon State University.

Maglata, David and Karyoly, Keith "Long-term grazing effects on genetic variation in Idaho fescue," Journal of Range Management 57(3), 275-279, (1 May 2004). https://doi.org/10.2111/1551-5028(2004)057[0275:LGEOGV]2.0.CO;2

Miller, J. E., & Martin, A. (2024). 2023 status and conservation assessment of golden paintbrush (Castilleja levisecta) in Washington. WADNR

NatureServe Explorer. (n.d.). Castilleja levisecta Retrieved June 14, 2023, from https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.136348/Castilleja_levisecta

Oregon State University. Viability. Seed Laboratory. (2019, October 19) https://seedlab.oregonstate.edu/testing-services/viability

Rao, S., & Strange, J. P. (2012). Bumble bee (Hymenoptera: Apidae) foraging distance and colony density associated with a late-season mass flowering crop. Environmental entomology, 41(4), 905-915.

Salama, A., Popova, E., Jones, M. P., Shukla, M. R., Fisk, N. S., & Saxena, P. K. (2018). Cryopreservation of the critically endangered golden paintbrush (Castilleja levisecta Greenm.): From nature to cryobank to nature. In Vitro Cellular & Developmental Biology - Plant, 54(1), 69–78. https://doi.org/10.1007/s11627-018-9888-z

Salama, A., Shukla, M. R., Popova, E., Fisk, N. S., Jones, M. P., & Saxena, P. K. (2018). In vitro propagation and reintroduction of golden paintbrush (Castilleja levisecta), a critically imperilled plant species. *Canadian Journal of Plant Science*, *98*(3), 762–770. https://doi.org/10.1139/cjps-2017-0207

Sandlin, I. J. (n.d.). (2008) Hybridization between Castilleja levisecta and C. hispida: Implications for Pacific Northwest Prairie Management. Oregon State University.

Schmidt, N. R., & Bakker, J. D. (2016). Parasitic plants and community composition: How Castilleja levisecta affects, and is affected by, its Community. Dissertation. Oregon State University

Smith, S., & Elliot, C. (2015). South Sound Prairies Conservation Nursery 2015 annual report. Center for Natural Lands Management Sustainability in Prisons Project.

St. Clair, A. B., Dunwiddie, P. W., Fant, J. B., Kaye, T. N., & Kramer, A. T. (2020). Mixing source populations increases genetic diversity of restored rare plant populations. *Restoration Ecology*, *28*(3), 583–593. https://doi.org/10.1111/rec.13131

Storm, L., & Shebitz, D. (2006). Evaluating the Purpose, Extent, and Ecological Restoration Applications of Indigenous Burning Practices in Southwestern Washington. Ecological Restoration, 24(4), 256–268. https://doi.org/10.3368/er.24.4.256

U.S. Fish and Wildlife Service. 2019. Species Biological Report for golden paintbrush (Castilleja levisecta). Version 1.0. Washington Fish and Wildlife Office, Lacey, Washington. 81 pp.

Vankus, V. 1997. The Tetrazolium Estimated Viability Test for Seeds of Native Plants. In: Landis, T.D.; Thompson, J. R., tech. coords. National Proceedings, Forest and Conservation Nursery Associations. Gen. Tech. Rep. PNW-GTR-419. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station: 57-62. Available at: http://www.fcnanet.org/proceedings/1997/vankus.pdf

Vinyeta, K. (2021). Under the guise of science: How the US Forest Service deployed settler colonial and racist logics to advance an unsubstantiated fire suppression agenda: Environmental Sociology: Vol 8, No 2. https://www.tandfonline.com/doi/abs/10.1080/23251042.2021.1987608

Waters, Susan M. 2018. A New Tool in Conservation of Prairies and Other Plant Communities: Plant-Pollinator Network Science. Douglasia, 42(3): 6-9.

Web Soil Survey. (2024). Soil Type Survey. (n.d.). Web Soil Survey. map. Retrieved 2024, from https://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx.

Whiteley, A. R., Fitzpatrick, S. W., Funk, W. C., & Tallmon, D. A. (2015). Genetic rescue to the rescue. Trends in Ecology & Evolution, 30(1), 42–49. https://doi.org/10.1016/j.tree.2014.10.009

Willi, Y., Kleunen, M. van, Dietrich, S., & Fischer, M. (2007). Genetic rescue persists beyond first-generation outbreeding in small populations of a rare plant. Proceedings of the Royal Society B: Biological Sciences, 274(1623), 2357–2364. https://doi.org/10.1098/rspb.2007.0768

Wilson, M. V., & Clark, D. L. (2001). Controlling invasive Arrhenatherum elatius and promoting native prairie grasses through mowing. *Applied Vegetation Science*, *4*(1), 129–138. https://doi.org/10.1111/j.1654-109X.2001.tb00243.x