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Thesis Title: Studies to Facilitate Reintroduction of Golden Paintbrush (*Castilleja levisecta*) to the WillametteValley, Oregon.

THESIS CHAPTER 5

Growing *Castilleja* for Restoration and the Garden

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

(Adapted version of Lawrence & Kaye (2005))

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

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

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

CASTILLEJA LEVISECTA **PROPAGATION**

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

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

Many plant species of temperate climates require a period of cold, moist conditions ("stratification") for proper germination, and golden paintbrush is no exception. We place the seeds on moistened germination paper in lidded plastic dishes, remoistening the paper as necessary throughout stratification. You could use moistened paper towels inside a plastic bag or plastic refrigerator container; maintaining proper moisture under sanitary conditions is crucial. We place the dishes in a cold, dark room at 5°C for 6–8 weeks, followed by a week or two of postchill incubation in a growth chamber set at 25°C/15°C with 12 hours of fluorescent lighting. This procedure typically results in 20 to 95 percent germination for *C. levisecta*, depending on the seed source (Figure 5-1). Seeds from some populations germinate prior to postchill incubation and can become etiolated ("stretched") if left in the dark and cold too long. Home growers may be successful using a refrigerator for the cold treatment, and placing seeds in a warm, well-lit area for post-chill incubation.

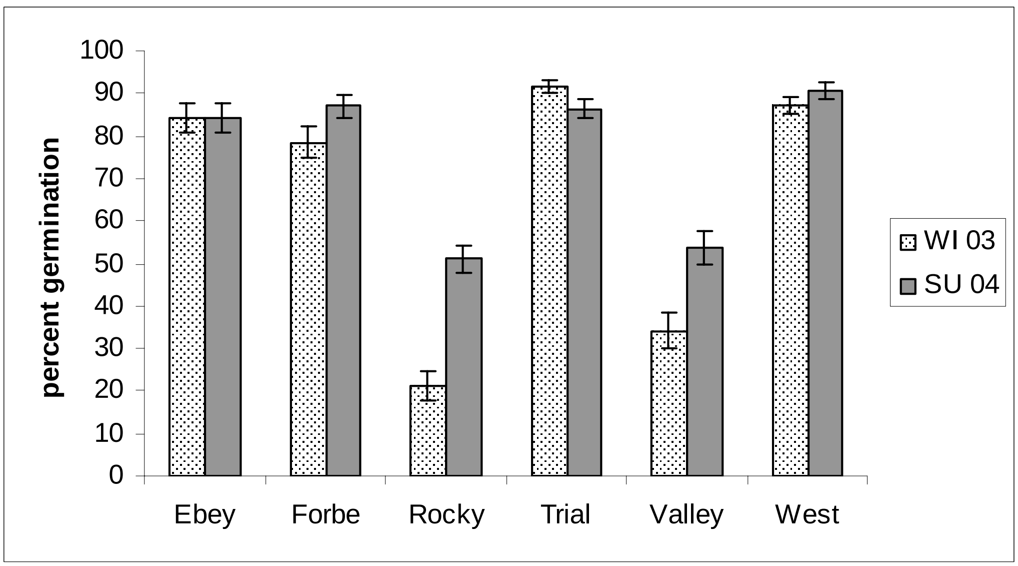

Figure 0-1. Mean percentage germination for six *C. levisecta* seed sources during two rounds of propagation, winter 2003 and summer 2004 (± 1 SE).

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

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

Figure 0-2. Categorization of *C. levisecta* seeds into three levels of viability after tetrazolium analysis. Note that two seeds in the “good” pile should actually be classified as “iffy.”

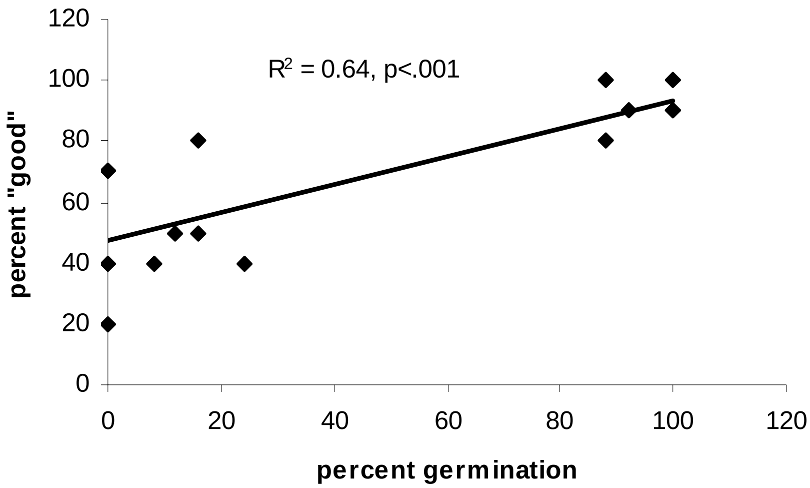

Figure 0-3. Linear relationship between the average germination of seeds from Rocky Prairie, San Juan Valley, and Trial Island vs. the percentage of seeds categorized as “good” during tetrazolium analysis.

Once the radicle (first root) and cotyledons (first leaves) have emerged, it is time to get those germinants in soil! At this point, the seedlings are very fragile and need extremely tender handling. We tried using tweezers to remove seedlings from the paper, but we believe that this technique may have damaged the slender germinants, likely only a few cells thick, as we saw high mortality within the first few weeks of growth. A less destructive approach we have used is to pick up the seedling with the tip of a plant tag or pencil and then gently place it in a small depression on the soil surface, lightly covering the radicle with fine soil. While soil-radicle contact is important, we recommend against planting too deeply or compacting the soil excessively. Each seedling is placed in one cell of a plastic cell-pack. Because this species grows in sandy glacial deposits in its native habitat, we use a well-drained soil medium amended with time-release micro and macro nutrients. Additionally, we use a liquid fertilizer (15-30-15) during watering every other week.

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

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

C. LEVISECTA EXPERIMENTAL POPULATIONS

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

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

PROPAGATION OF OTHER *CASTILLEJA* SPECIES

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

Table 0-1. Germination requirements of 22 species of *Castilleja*.

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

In general, populations and species of *Castilleja* from warmer, drier climates have shorter chilling requirements and germinate more quickly than those from high elevations with longer winters. If germination information on your species of interest is not available, estimating the number of weeks the seeds are exposed to cold temperatures (around or below 5ºC) in their natural environment may help approximate its requirement.

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

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

Paintbrushes are a wonderful addition to any garden, and can attract wildlife too. Adult butterflies use them as nectar plants, while red-flowering paintbrushes are an open invitation to hummingbirds. Bumblebees are the principal pollinators of yellow, green, and purple-flowered paintbrushes. Many species of checkerspot butterflies (*Euphydryas* spp.) use *Castilleja* as larval host plants. *Castilleja levisecta* may have been the original host plant for Taylor's checkerspot, a rare butterfly endemic to the prairies of the Pacific Northwest. As populations of golden paintbrush diminished, so have those of Taylor's checkerspot.

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

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