REINTRODUCTION OF GOLDEN PAINTBRUSH TO OREGON: 2012 ANNUAL REPORT

2012

Report to the US Fish & Wildlife Service

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Institute for Applied Ecology

PREFACE

The Golden Paintbrush Recovery Project is coordinated by the Institute for Applied Ecology (IAE) and is funded by the US Fish and Wildlife Service and other sources. IAE is a non-profit organization whose mission is conservation of native ecosystems through restoration, research and education. IAE provides services to public and private agencies and individuals through development and communication of information on ecosystems, species, and effective management strategies. Restoration of habitats, with a concentration on rare and invasive species, is a primary focus. IAE conducts its work through partnerships with a diverse group of agencies, organizations and the private sector. IAE aims to link its community with native habitats through education and outreach.

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Cover photograph: Golden paintbrush (*Castilleja levisecta*) with pollinating bumblebee at a reintroduction site at Finley National Wildlife Refuge, Oregon. *Photo by TN Kaye.*

SUGGESTED CITATION

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REPORT TO THE US FISH AND WILDLIFE SERVICE

INTRODUCTION

Castilleja levisecta Greenm. (golden paintbrush) is listed as Threatened by the US Fish and Wildlife Service. The species once occupied prairies and grasslands throughout the Puget Trough and Willamette Valley. Habitat destruction and alteration over the past century have resulted in substantial declines in native vegetation in this ecoregion, and several species of prairie habitats are now listed by state and federal agencies as threatened or endangered (USFWS 2010). All remaining populations of *C. levisecta* occur in Washington and British Columbia, the species is considered to be extirpated in Oregon (USFWS 2000). The Recovery Plan for *C. levisecta* (USFWS 2000) identifies population reintroduction and development of propagation methods as high priority actions to meet recovery objectives.

Figure 1. *Castilleja levisecta* (golden paintbrush) in a reintroduction plot at Finley National Wildlife Refuge.

Castilleja levisecta (Figure 1) is an herbaceous perennial that reproduces by seed. Like most paintbrushes (Heckard 1962), this species is a hemiparasite – its roots penetrate the roots of neighboring plant species and derive nutrients, carbohydrates, and possibly other secondary compounds from these hosts.

This project supports the recovery of *C. levisecta* through full-scale reintroduction to prairie sites in Oregon's Willamette Valley. This is a three-year project that begins with trial plantings at several locations to identify suitable locations for the species' growth, followed by monitoring and additional plantings. This interim report summarizes information from the first full year of work. A Reintroduction Plan for this species identifies population establishment in the Willamette Valley as a conservation need and provides guidance for the reintroduction process (Caplow 2004).

Experimental introductions have been implemented at Mima Mounds Natural Area Preserve and Glacial Heritage Preserve in the South Puget Sound (Pearson and Dunwiddie 2003, 2006), Whidbey Island (Swinerton 2003, Wayne 2004), and a variety of sites in Oregon's Willamette Valley (Lawrence and Kaye 2009). More recently, larger scale introductions have been implemented at six locations in the South Puget Sound region (Dunwiddie 2009, 2011). These efforts and others suggest that the success of C. *levisecta* plantings is sensitive to site conditions such as soil chemistry (Dunwiddie 2009), soil moisture (Sprenger 2008), abundance of invasive plants and community composition (Lawrence and Kaye 2009), host plants and herbivory (Lawrence and Kaye 2008), and fire as a site preparation technique (Dunwiddie et al. 2001, Dunwiddie 2009). These factors can all vary among sites as well as within sites, making site selection and microsite identification a challenge during reintroduction. This project relies on trial plantings to measure site suitability directly through growth of C. *levisecta* at several sites, followed by additional larger scale plantings of seeds and/or plugs at promising sites. This report updates the 2011 annual report with activities and findings during 2012.

GOALS AND OBJECTIVES

The goal of this project is to contribute to recovery of golden paintbrush by reintroducing the species to the Willamette Valley, Oregon, an historic portion of the species' range. The species was last seen in this region in 1938 (Kaye, from review of herbarium records). Golden paintbrush occurs in upland prairies in Washington and British Columbia (Chappell and Caplow 2004, Caplow and Chappell 2005, Lawrence and Kaye 2006), and formerly occupied upland and possibly wetland prairies in Oregon.

This project has three primary objectives:

1) Increase plant materials for introduction and relieve wild populations from seed collection pressure through a seed production program that emphasizes genetic diversity.

2) Establish five new populations of golden paintbrush in the Willamette Valley.

3) Improve introduction protocols by examining factors that affect the success of reintroduced plants.

METHODS

Seed increase

Seed production was conducted at the Natural Resource Conservation Service, Corvallis Plant Materials Center. Seeds from four source populations were planted together into a single seed production bed. The four source populations include Ebey's Landing, Naas, and Fort Casey (all on Whidbey Island) and

Rocky Prairie (South Puget Sound) (Table 1). The Whidbey Island sources were identified for use in the Willamette Valley because of their superior performance in previous experimental plants (Lawrence and Kaye 2009). Rocky Prairie plants had low germination, survival, and growth in previous trials, but represent the closest remaining seed source to the Willamette Valley. Therefore, they were included in the production field to ensure that their genotype is represented in future plant materials used in restoration while emphasizing the more successful types from Whidbey Island. This compromise approach was discussed and approved by the Golden Paintbrush Recovery Team prior to implementation. Since C. *levisecta* is a hemiparasite, host plants of *Festuca ammobia* (sand fescue) were planted with the paintbrushes while they were initially cultivated in cones and remained with them in the production field (Figure 2). This species of fescue was used because it did not create pollen crossing complications on the farm where other fescues were under production. Seed germination and cultivation procedures generally followed Lawrence and Kaye (2005).

Seeding

2010 Seeding

In 2010 we seeded C. *levisecta* at a total of 10 plots at Baskett Slough NWR (three sites), Finley NWR (three sites), Beazell County Park (two sites), and Bald Hill City Park (two sites) (Appendix). At most sites, 40 g of seeds were sown in fall in areas that had been burned a few weeks earlier. Seeding was conducted into plots that were 5 x 40 m (200 m^2) in size. At an estimated 8000 seeds/g for C. *levisecta*, we seeded at a rate of approximately 1600 seeds/m^2. Plots were sampled in June and July 2011 to determine establishment rates.

2011 Seeding

We seeded at several additional sites in 2011 (see Appendix) at the same rate as 2010, about1600 seeds/m^2 (0.2 g/ m^2). Plots were sampled in May and June 2012 to determine establishment and flowering rates

Table 1. Seed sources and representation of *Castilleja levisecta* in the mixed-population seed production bed.

Seed source	Number of plants in production bed	Percentage of plants in production bed
Ebey's Landing	600	32%
Naas	528	29%
Fort Casey	507	27%
Rocky Prairie	216	12%
Total	1,851	

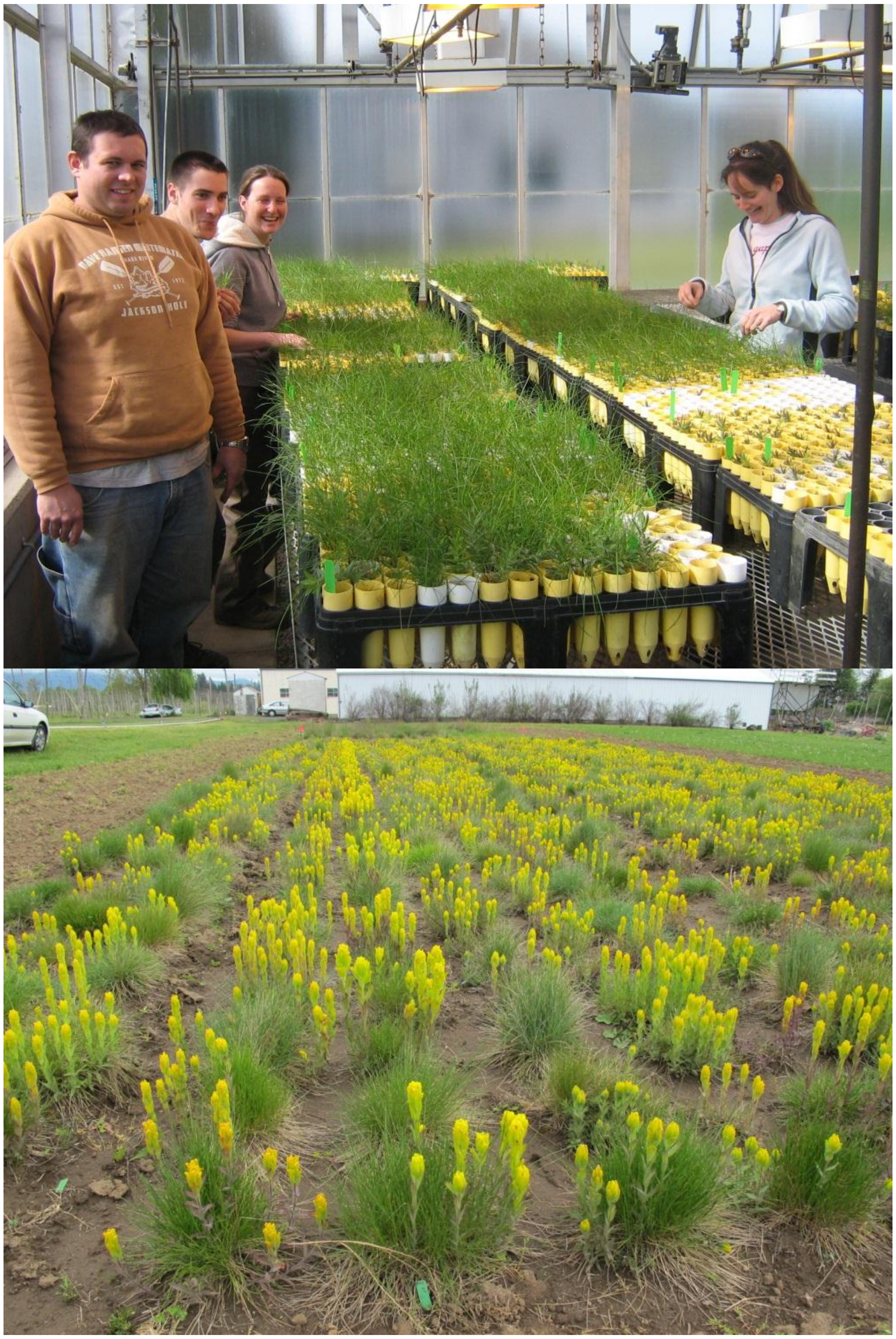


Figure 2. *Castilleja levisecta* plants in plug production at the Corvallis Plant Materials Center (top) and in a seed production bed (bottom).

Plug planting

Previous plantings in the Willamette Valley (Lawrence and Kaye 2009) have emphasized upland, well drained soils because populations in Washington occur in this type of habitat. However, we included some wetland sites in the 2010 plantings because precise information on the locations and habitats of historic populations in the Willamette Valley is lacking. Some historic herbarium specimens mention that the plants were in 'damp' or 'moist' open ground (WILLU, M.E. Peck specimens 3462 and 3463, collected in 1910 from Salem, Oregon), suggesting that wetland prairies may have been habitat for the species prior to its extirpation from Oregon.

Plantings were conducted in April, 2010, 2011 and 2012 at a wide range of locations in the Willamette Valley (Figures 3 and 4). All 2010-planting sites were re-visited and all plants were monitored in June 2010, 2011 and 2012 to document plant survival, growth and reproduction. Vole and deer herbivory have been identified as a cause of plant mortality in this species (Lawrence and Kaye 2008), so evidence of herbivory was recorded as well. Spring of 2010, 2011 and 2012 had unusually high precipitation, with higher than normal rainfall and many rainy days. Therefore, some of the wetland sites were wetter than average with water standing above the soil surface well into spring, at least during and after heavy rains.

Individuals were planted with dibbles that created holes in the soil the same shape and dimensions as the conetainers the plants were grown in. Plantings were conducted in the same lay-out at all sites with the exception of the "Collins plots" at Bellfountain Road, where plantings targeted research plots with varying treatment histories and current plant communities. At all other sites, planting plots were 8 m x 38 m in size, with plants on 2-m centers. This was accomplished by planting in five rows, each 2-m apart, with plants placed every other meter (Figure 5). A total of 97 to 100 paintbrushes were planted at each site (Table 2). Plot corners were marked with rebar topped with large square plastic caps or square cement blocks pounded low to allow mowing and other equipment to pass over without damaging the plot markers (Figure 6). At most sites, a 6-foot fence post was used to mark the northwest plot corner in addition to the rebar. In 2010 Individuals from each source population were randomly assigned a position within the plots. Rocky Prairie plants were typically smaller than those from Whidbey Island sources at the time of planting (Figure 7). Plants used in 2011 and 2012 were mixed accession, developed from multiple source populations. Sketch maps of the planting layout with landmarks at each site are included in Appendix.

At the Bellfountain Road site, we planted a second set of C. *levisecta* into plots used for a previous experiment on prairie restoration methods that concluded in 2009 (the Collins project) and resulted in a replicated set of experimental treatment units with differing treatment histories and plant communities (Stanley, Kaye and Dunwiddie 2008). We planted 5 individuals from the Naas source population into each of these 20 treatment units, for a total of 100 plants. This allowed us to measure differences in plants established into a variety of upland prairie types. Also, a previous experimental planting at this site has shown promise with many plants surviving over 5 years, so this additional planting gave us the opportunity to more thoroughly examine the suitability of this site.

All plants placed at sites as plugs in 2010 were revisited in May and June 2011 and 2012, at which time we recorded survival, flowering, number of stems, length of every stem, and evidence of herbivory. In 2011 we also sampled the associated vegetation around plants at the 2010 planting sites in upland habitats, including Baskett Butte Upper, Pearcy-Schoener, Lupine Meadows North, Field 29, Bellfountain, and Bellfountain Collins. At each C. *levisecta* plant location, we recorded the identity of every vascular plant present within 10-cm.

2010 Bioassays

To determine which sites might best support *C. levisecta* in future reintroductions, we planted 100 (or nearly so) plants at ten locations in the Willamette Valley in 2010 (Table 2, Figure 3). In this way we used *Castilleja levisecta* as a phytometer to bioassay each site and rank sites for future large scale planting, in a similar manner as Dunwiddie (2009) and as recommended in Kaye (2009). All planting sites were selected to be prairie habitat (see Figure 4 for site photos) in public ownership or under conservation easement. Federally owned sites included two locations at the Baskett Slough National Wildlife Refuge, three sites at Finley National Wildlife Refuge, and one at the Finley National Wildlife Refuge's Oak Creek Unit near Lebanon, Oregon. One site, Kingston Prairie, was a preserve owned by The Nature Conservancy. Two sites were located on property owned by the Greenbelt Land Trust at Lupine Meadows, a site with a management plan that identifies *C. levisecta* reintroduction as a priority (Greenbelt Land Trust, 2008). Finally, the Pearcy-Schoener site was located on private property soon to be under conservation easement held by Benton County and managed for Threatened and Endangered species conservation.

Table 2. *Castilleja levisecta* 2010 planting locations, habitat types, and number of individuals from each source population.

Site	Habitat	Ebey's	Fort Casey	Naas	Rocky Prairie	Totals
Baskett Butte Lower[1]	mix	20	20	37	22	99
Baskett Butte Upper[1]	upland	20	22	37	18	97
Bellfountain Rd[2]	upland	20	20	40	20	100
Bellfountain Rd, Collins[2]	upland	N/A	N/A	100	N/A	100
Finley Field 29[2]	mix	22	23	35	20	100
Kingston Prairie	wet	25	25	25	25	100
Lupine Meadows North	upland	25	26	25	24	100
Lupine Meadows South	wet	25	25	25	25	100
Oak Creek Unit[2]	wet	33	22	28	15	98
Pearcy-Schoener	upland	25	26	25	24	100
Totals	10	215	209	377	193	994

[1]Baskett Slough National Wildlife Refuge, USFWS

[2]Finley National Wildlife Refuge, USFWS

[3]Ancilary unit of Finley Wildlife Refuge, USFWS, near Lebanon, Oregon

2011 Plantings with hosts at high-ranked sites

Following the results of the 2010 bioassays at ten locations, five sites were selected for large-scale (>500 plugs) plantings in 2011 (Table 3). Plantings were conducted in April 2011. Plugs grown in 6-in conetainers were planted on 1-m centers in rows 39 m long, spaced 1 m apart. The number of rows varied with the number of plants placed at each site. These plants were also assigned host-plant treatments (Table 4).

Table 3. *Castilleja levisecta* locations selected for planting in 2011, habitat types, and number of individuals from each source population.

Site	Habitat	2010 bioassay site-rank	2011 plugs planted*	2012 plugs planted*
Oak Creek Unit, Finley NWR	wetland	5.0	547	
Bellfountain Rd, Finley NWR	upland	5.6-7.3	786	982
Finley Field 29, Finley NWR (two plots)	upland and wetland	6.3	1076	1000
Lupine Meadows North, GLT	upland	7.33	717	1000
Pearcy-Schoener, Benton Co. Parks	upland	4.7	635	1000
Beazell, Benton County	upland			592
Field 14Z, Baskett Slough NWR	upland			1000
Total			3761	5574

* Planted with hosts, see Tables 4 and 5 for details and table x for host species codes

Table 4. 2011 plantings by host. Analysis of hosts only conducted on experimental plantings, non-exp plants omitted from analysis but counted for total establishment.

	ACMI	DACA	DECE	ERLA	FERO	KOCR	PRVU	SIVI	No Host	non-exp	Total
Bellfountain	57	59		31	75	60	59	49	60	336	786
Field 29 dry	49	30		16	45	45	39	30	30	292	576
Field 29 wet	40	30	58	16		41	39	30	30	216	500
Lupine Meadows N.	59	59		1	90	61	60	60	60	267	717
Pearcy-Schoener	59	60			60	91	60	60	60	185	635
Oak Creek	60	38	131	16		23	26	63	162	28	547

2012 Plantings with hosts

Based on the success of plug plantings in upland sites in 2010 and 2011, six upland sites were selected for large-scale (up to 1000) plantings in 2012 (Table 3). Plantings were conducted in April 2012. Plugs grown in 6-in conetainers were planted on 1-m centers in rows 49 m long, spaced 1 m apart. The number of rows varied with the number of plants placed at each site. These plants were also assigned host-plant treatments (Table 5). Establishment rates and host effects well be assessed in 2013.

Table 5. 2012 plantings by host. Analysis of hosts only conducted on experimental plantings, non-exp plants omitted from analysis but counted for total establishment.

	ACMI	DACA	ELTR	ERLA	FERO	KOCR	PLCO	PRVU	RAOC	SIVI	No Host	non-exp	Total
Baskett Slough, 14Z	52	63	58	29	78	77	69	85	69	82	100	238	1000
Beazell	10	24	43		60	45		39	14	70	64	223	592
Bellfountain	53	64	57	28	77	76	69	85	68	83	100	220	980
Field 29	53	63	57	29	78	77	69	85	68	84	100	237	1000
Lupine Meadows N.	53	64	57		77	76	69	84	69	84	100	267	1000
Pearcy-Schoener	53	64	57		78	76	70	84	68	84	100	266	1000

Wetland vs. upland effects

To determine if plant survival from 2010 plantings was affected by soil hydrology, we compared plant survival in wetlands to uplands with a two sample t-test, assuming unequal variances.

Host plant and community effects

Greenhouse performance with hosts

All plants grown in 2011 and 2012 in the greenhouse as plugs were assigned a host plant treatment and were co-planted in the 6-in container with one of several potential host species, including six forbs and five grasses, or with no host (Table 6). Plants were grown at the Corvallis PMC greenhouse facility for four months. In March 2011 and May 2012, to examine the effects of different host plant species on C. *levisecta* survival and growth, we measured, a subset of 30 C. *levisecta* individuals from each host-treatment were for plant size (total stem production , cm). Insufficient *Eriophyllum lanatum* survival in the conetainers due to a rot in 2011 caused us to omit this species from the measurement for that year.

Field performance with planted hosts

Large-scale field plantings were also conducted at the sites listed in Table 3 as described above. Each plug planted at the field sites was randomly assigned a location and host plant. *Festuca roemeri* was used as a host only at upland sites, and *Deschampsia cespitosa* only at wetland sites. In May 2012, we sampled all sites with the exception of Oak Creek where we found no surviving C. *levisecta*. To test for the effects of different host species on survival and flowering we used a generalized linear mixed effects model with the lme4 package (Bates 2011) in R version 2.14.0 (2011), with C. *levisecta* survival and flowering as the response variables, host species as the fixed effect and site as a blocking factor (random effect). We used multiple linear regression R version 2.14.0 (2011) to test for the effects of host identity and host size on the total size and number of flowering stems of the planted C. *levisecta*. Field 29 dry at Finley NWR was planted into an area that had been herbicided, community composition was very different and performance of both C. *levisecta* and host species differed significantly from all other sites.

Field 29 dry was therefore omitted from all analysis that used site as a blocking factor because the conditions at that plot are dissimilar to all the rest.

Table 6. Host plants grown with *Castilleja levisecta* in conetainers prior to outplanting to field sites listed in Table 3.

host	code	type	Year Planted
Achillea millefolium	ACMI	forb	2011, 2012
Danthonia californica	DACA	grass	2011, 2012
Deschampsia cespitosa	DECE	grass	2011
Eriophyllum lanatum	ERLA	forb	2011, 2012
Festuca roemeri	FERO	grass	2011, 2012
Koeleria cristata	KOCR	grass	2011, 2012
Prunella vulgaris	PRVU	forb	2011, 2012
Sidalcea virgata	SIVI	forb	2011, 2012
Elymus trachycaulus	ELTR	grass	2012
Plectritis congesta	PLCO	forb	2012
Ranuculus occidental	RAOC	forb	2012
no host control		n/a	2011, 2012

Community diversity

To test for biodiversity effects on C. *levisecta*, we used logistic regression to test for effects perennial and annual plant species richness on plant survival from 2010 to 2011. We used a generalized linear mixed effects model with the lme4 package (Bates 2011) in R version 2.14.0 (2011), with plant survival as the response variable, richness of annuals and perennials as fixed effects, and site as a blocking factor (random effect). Wald confidence intervals (95%) around the logistic regression curves were calculated using the covariance matrix of the fixed effects coefficients (Bates 2011). Once again, we performed this analysis with only the upland sites. We included only upland sites in this analysis because mortality at wetland sites was very high. Sites included were Baskett Upper, Pearcy-Schoener, Bellfountain, Bellfountain Collins, and Lupine Meadows. At Lupine Meadows a portion of the plot was in wetland habitat and plants in that area were omitted from the analysis (n=70 after dropping wet planting sites).

Community composition

We tested for differences in plant community composition at locations where C. *levisecta* planted in 2010 survived or died in 2011 with Multi-Response Permutation Procedure (MRPP). Once again, we performed this analysis with only the upland sites. MRPP is a multivariate procedure that avoids the assumption of normal distributions and equal variances of the response variables which are not met when analyzing some community data (McCune and Grace, 2002), including ours due to the presence-absence (0, 1) nature of our data. We used PC-ORD statistical software, version 5.0 (McCune and Mefford, 2006) with Sørensen's distance measure and 10,000 permutations to test the hypothesis that differences between sites where C. *levisecta* survived or died were no larger than expected by chance ($\alpha < 0.05$). Separate analyses were conducted for each area because floristic composition of the plant communities differed among the study areas. Species occurring in ≤5% of the plots (i.e., 2 or fewer plots per site) were excluded from the analyses to reduce noise and improve the correlation structure of the data set, as suggested by McCune and Grace (2002) and McCune and Mefford (1999).

Indicator species

MRPP tests for differences between groups in community composition, but does not identify how these groups differ. Therefore, we used Indicator Species Analysis (ISA) to quantify how individual species were distributed among treatment groups and identify those species that were significant indicators of particular treatments. We conducted this evaluation with individual taxa as well as species pooled into eight potential functional groups based on annual vs. perennial, native vs. invasive, and grass vs. forb. As above, we included only upland sites in this analysis. ISA combines the abundance and relative frequency of each individual species within a particular group and assigns an indicator value (Dufrêne and Legendre, 1997). Our data did not include abundance of each species, so the analysis assumed equal abundance of each species present. A perfect indicator species always occurs in one group and never occurs in others. Indicator values range from 0 to 100, where zero means that a species is not associated with any particular group and 100 corresponds to exclusive frequent and abundant membership by a species to a particular group. We performed a Monte Carlo randomization with 10,000 permutations to determine if an indicator value for a given species was larger than expected by chance ($\alpha < 0.05$) using PC-ORD (McCune and Mefford, 1999). Species occurring in less than 5% of the plots were excluded from the analysis.

Measuring haustorial connections and biomass

To further evaluate the effect of host species on C. *levisecta* growth we selected ten 6-inch conetainers from each host-treatment to measure haustorial connections. First, to complement quantitative analysis of haustorial connections, we photographed roots using a handheld digital microscope camera. All soil was then cleaned away from the roots using water; the host species was separated from the C. *levisecta* using hair detangler to carefully tease apart roots. The above ground plant material was cut from the root, dried at 70°C for 24 hours then weighed on a Mettler balance to the nearest 0.001g. Using a dissecting scope, all haustoria were counted in 1-cm sections along both the host and C. *levisecta* roots. Haustoria were categorized as either connected or un-connected. Because of direct connections between root systems, complete separation of root systems was not possible therefore host and C. *levisecta* roots were dried and weighed together using the same methods as aboveground biomass.

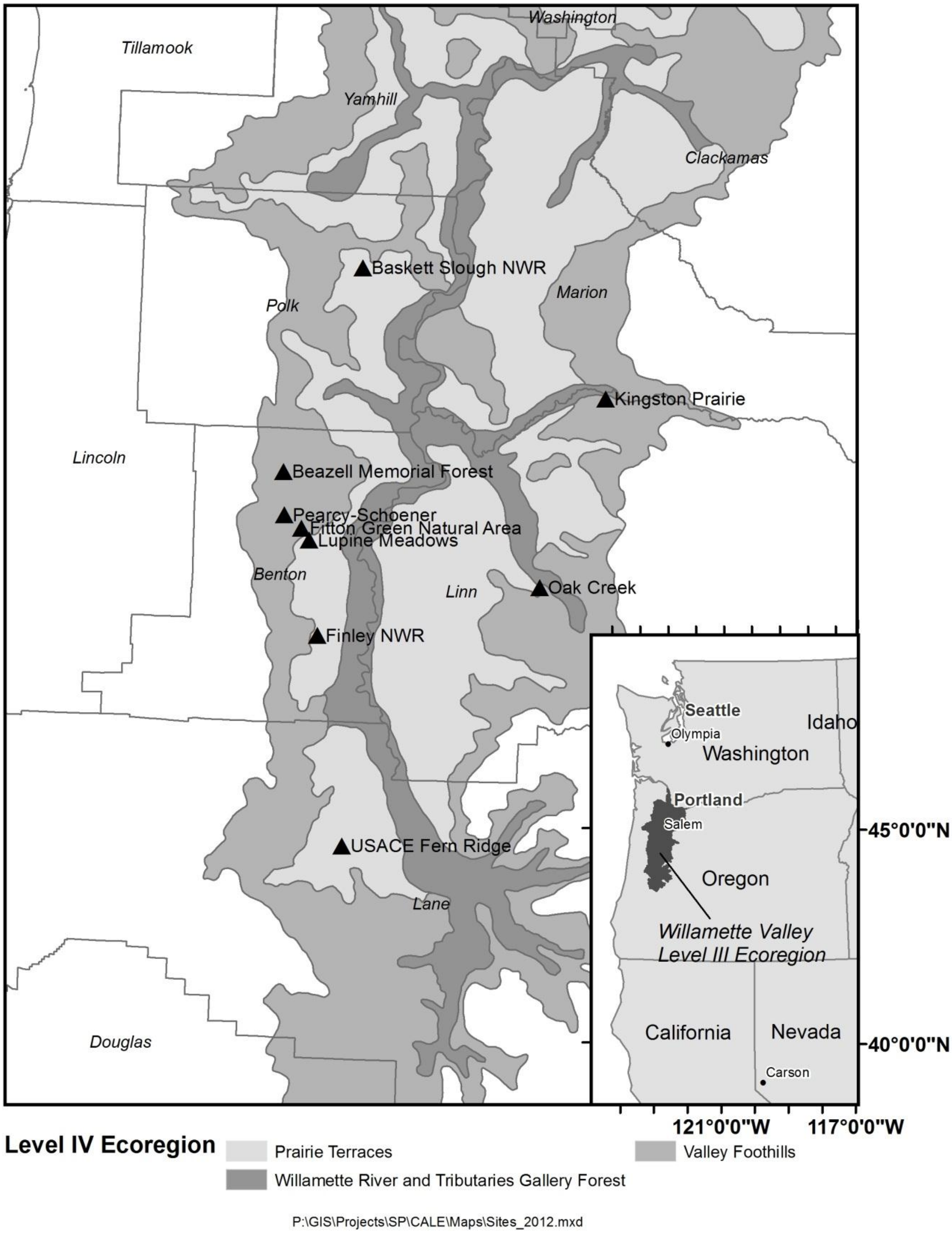


Figure 3. Sites planted with *C. levisecta* in 2010-12.

Figure 4. Site photos for the ten locations planted with *Castilleja levisecta*, 2010.

Baskett Butte Upper, Baskett Slough Wildlife Refuge, USFWS, Polk County, Oregon. Upland prairie.

Baskett Butte Lower, Baskett Slough National Wildlife Refuge. Mix of wet and upland.

Field 29, Finley Wildlife Refuge, USFWS, Benton County, Oregon. Mix of wet and upland.

Bellfountain, Finley Wildlife Refuge, USFWS, Benton County, Oregon. Upland prairie.

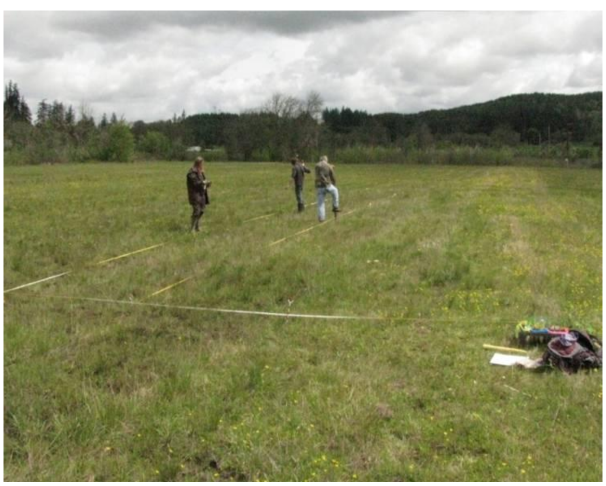
Collins Plots, Bellfountain, Finley Wildlife Refuge, USFWS, Benton County, Oregon. Upland prairie.

Lupine Meadows South, Greenbelt Land Trust, Benton County, Oregon. Wet prairie.

Lupine Meadows North, Greenbelt Land Trust, Benton County, Oregon. Upland prairie.

Pearcy-Schoener, private, Benton County, Oregon. Upland prairie.

Kingston Prairie Preserve, TNC, Marion County, Oregon. Wet prairie.



Oak Creek , USFWS, Willamette Valley Refuges, near Lebanon, Oregon. Wet prairie.

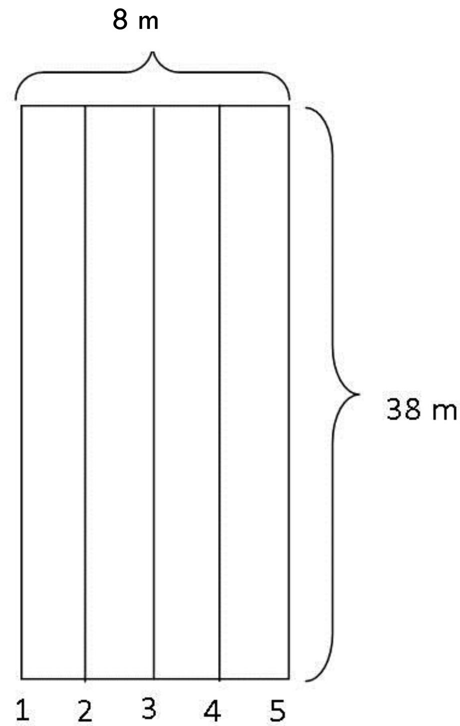


Figure 5. Typical planting design in 2010 for *Castilleja levisecta* resulting in plants spaced on 2-m centers. Five rows, each 2-m apart, were planted at every other meter with a total of 20 plants per row and 100 plants per plot. Plot corners were marked with rebar posts topped with large square caps or cement blocks pounded close to the soil surface to allow mowing and other equipment to pass over without damaging the posts. Plants from each of four source populations were randomly placed. Plug plantings in 2011 and 2012 followed a similar layout with 38 m and 49m lines respectively, spaced 1-m apart, and planted every meter, with a variable number of rows to accommodate different numbers of plants.

Figure 6. Plastic (left) and cement (right) plot corner markers with adjacent fence post.

Rocky Prairie transplants. On average, these were the smallest transplants of the four source populations.

Flowering transplants from Fort Casey source population. Representative of average size of transplants from Ebey's Landing, Naas and Fort Casey.

Figure 7. Plants in conetainers immediately prior to planting. Note that plants grown from seed from Rocky Prairie (left) were generally smaller than those from other sites (right).

RESULTS

Seed increase

In 2010, yield from the seed increase field was 1.4 lbs (0.64 kg, roughly 5 million seeds). In 2011 and 2012, the harvest increased to 3.2 pounds (1.4 kg), a very strong yield. Because of the successful seed production from this field and the very positive early results from seeding in 2010-11, the field will be continued to support additional reintroductions.

Overview of plant establishment for recovery 2010-12

As of 2012, nine populations of C. *levisecta* have been established in Oregon for the purpose of recovery (Table 7). The recovery target for Oregon is five populations with at least 1000 flowering plants each (USFWS 2000, 2010). Three of the nine reintroduced populations are on the Finley National Wildlife Refuge and are separated from one another by at least 1 km. At the remaining sites, the populations are spread in plots across various habitat patches, but all plots are within 1 km of one another. The number of flowering plants at each site varied from 0 at Spires and Fitton Green to 2,688 at Beazell Memorial Forest, which was the only site with over 1000 flowering plants (Table 7). Most sites have low numbers (100-350) of flowering individuals but relatively high numbers of vegetative plants (over 1000 at five sites). In all, there are currently 18,827 C. *levisecta* plants growing at restoration sites in Oregon, 14,908 vegetative and 3,919 flowering.

Summary of establishment in 2011

2010 Seeding

Plant establishment from seed varied substantially among sites in 2011. Seeding was most successful at upland sites, including Baskett Butte, Beazell and Field 29 (dry area) at Finley NWR. Establishment at those sites ranged from 108 to 1015 plants. Seeding completely or nearly failed at wetland sites at Baskett Slough and Finely Field 29. Some sites that were planted in 2010 were not relocated due to heavy vegetation growth, or were visited too late in the season for a reliable count. In total, <u>2,959 plants established</u> in plots seeded in 2010 (Appendix).

2010 Planting and Bioassay

In 2010, we planted 994 C. *levisecta* at ten locations in the Willamette Valley. In 2011, establishment of these plants differed substantially from site to site (Table 8), totaling 305 overall (see Appendix). Plantings in 2011 emphasized those sites with the highest mean rank. Kingston Prairie, which was the top ranked site from 2010, received no plantings in 2011 in part because early season observations at that site suggested the plants had low survival over the winter, and the ranking was inaccurate over the long term.

Table 7. Summary of C. *levisecta* plant establishment in Oregon as of 2012.

Site name	Location (ownership)	Permanent protection?	Population size
Finley NWR - Bellfountain Road	Benton County, OR (Federal: US Fish & Wildlife Service)	Yes	216 (951 vegetative) plants
Finley NWR - Field 29 (Woodpecker Loop)			346 (4689 vegetative) plants
Finley NWR - Pigeon Butte			24 (2075 vegetative) plants
Baskett Slough NWR	Polk County, OR (Federal: US Fish & Wildlife Service)	Yes	142 (1225 vegetative) plants
Beazell Forest	Benton County, OR (County: Benton Co. Parks & Recreation)	Yes	2688 (3168 vegetative) plants
Lupine Meadows	Benton County, OR (Private/NGO: Greenbelt Land Trust)	Conservation Easement	186 (396 vegetative) plants
Pearcy-Schoener	Benton County, OR (County: Benton Co. Parks & Recreation)	Conservation Easement	317 (153 vegetative) plants
Spires	Lane County, OR (Federal: US Army Corps of Engineers)	Yes	0 (2034 vegetative) plants
Fitton Green	Benton County, OR (County: Benton Co. Parks & Recreation)	Yes	0 (217 vegetative) plants

Table 8. *Castilleja levisecta* survival in wetland vs. upland sites planted in 2010 and resampled in 2011. Note that sites ranked differently than in 2010.

Site	habitat	planted (plugs) 2010	survived 2011	survival	Rank
Baskett Butte Upper	upland	98	27	27.6%	5
Pearcy-Schoener	upland	100	56	56.0%	8
Bellfountain Rd	upland	100	60	60.0%	9
Bellfountain Rd, Collins	upland	100	72	72.0%	10
Lupine Meadows North	upland	100	55	55.0%	7
Baskett Butte Lower	wetland	98	5	5.1%	4
Oak Creek Unit	wetland	98	0	0.0%	1.5
Finley Field 29	wetland	100	28	28.0%	6
Lupine Meadows South	wetland	100	0	0.0%	1.5
Kingston Prairie	wetland	100	2	2.0%	3

Summary of establishment in 2012

After two years of seeding and planting vegetative plugs of C. *levisecta* there are 18,844 individuals, (3,928 of which are reproductive) at ten upland prairie sites in the Willamette Valley (Table 9). The majority are these are the result of seeding efforts but greenhouse grown individuals flower with greater frequency.

Table 9. 2012 Establishment of C. *levisecta* from 2010 and 2011 seed and plug plantings.

Site	Seed			Plug			Total		
	Rep.	Veg.	Total	Rep.	Veg	Total	Rep.	Veg.	Total
ACOE, Fern Ridge Res.	0	2,034	2,034				0	2,034	2,034
Baskett Slough NWR 14Z	0	132	132				0	132	132
Baskett Slough NWR Upper	140	1,079	1,219	2	14	16	142	1,093	1,235
Benton County Preserve, Pearcy-Schoener				317	153	470	317	153	470
Benton County, Beazell	2,688	3,168	5,856				2,688	3,168	5,856
Benton County, Fitton Green	0	217	217				0	217	217
Finley NWR, Bellfountain	1	671	672	215	280	495	216	951	1,167
Finley NWR, Field 29	43	4,566	4,609	312	131	443	355	4,697	5,052
Finley NWR, Pigeon Butte	24	2,075	2,099				24	2,075	2,099
Lupine Meadows North	2	183	185	184	213	397	186	396	582
Grand Total	2,898	14,125	17,023	1,030	791	1,821	3,928	14,916	18,844

2010 Seeding

Establishment of C. *levisecta* plants from seeds planted in 2010 was highly variable between sites (Table 9). The two plots at Beazell were the most successful with 5,856 individuals, a 487% increase over the 2011 count. The number of individuals at Baskett Butte and Finley NWR decreased from 2011 sampling. In 2012, we counted a total of 6,662 individuals in six plots at three sites that were seeded in 2010 (Table 10).

Table 10. Summary of reproductive (Rep.) and vegetative (veg.) establishment by seed at each plot.

				2011			2012	
Plot	Year Seeded	Amount (g)	Rep.	Veg.	Total	Rep.	Veg.	Total
ACOE, Fern Ridge Res., Spires 1	2011	40				0	669	669
ACOE, Fern Ridge Res., Spires 2	2011	40				0	831	831
ACOE, Fern Ridge Res., Spires 3	2011	40				0	534	534
Baskett Slough NWR Upper 1b	2011	40				0	910	910
Baskett Slough NWR Upper East	2010	40	41	974	1,015	105	125	230
Baskett Slough NWR Upper West	2010	40	95	13	108	35	44	79
Baskett Slough NWR, Field 14Z north	2011	80				0	76	76
Baskett Slough NWR, Field 14Z south	2011	40				0	56	56
Benton County, Beazell Annex	2010	40	33	435	468	1,329	1,045	2,374
Benton County, Beazell North	2010	40	41	694	735	1,359	2,123	3,482
Benton County, Fitton Green 1	2011	40				0	68	68
Benton County, Fitton Green 2	2011	40				0	122	122
Benton County, Fitton Green 3	2011	40				0	27	27
Finley NWR, Bald Top 1 (by tree)	2011	40				0	411	411
Finley NWR, Bald Top 2	2011	40				3	849	852
Finley NWR, Bellfountain Prairie 4	2011	40				1	671	672
Finley NWR, Field 29 hill top 2	2011	40				6	1,871	1,877
Finley NWR, Field 29 lower 2	2010	40	2	0	2	9	8	17
Finley NWR, Field 29 north slope 1	2010	40	0	631	631	25	391	416
Finley NWR, Field 29 north slope 2	2011	40				0	1,036	1,036
Finley NWR, Field 8N	2011	80				0	2,035	2,035
Finley NWR, Pigeon Butte upper	2010	40				24	40	64
Lupine Meadows North 3	2011	40				0	80	80
Lupine Meadows North 4	2011	40				2	103	105
Total from 2010 seeding			212	2,747	2,959	2,886	3,776	6,662
Total from 2011 seeding						12	10,349	10,361
Total from all seedings			212	2,747	2,959	2,898	14,125	17,023

2010 Planting

A total of 260 of the 994 plants planted in 2010 survived till 2012, all in upland sites. The greatest levels of survival and flowering occurred at Pearcy-Schoener, and at dry sites in Finley NWR and Lupine Meadows North (Appendix).

2011 Seeding

Twenty plots were seeded in 2011 with either 40 or 80 grams of C. *levisecta* seeds. We counted flowering and vegetative individuals at 17 of those plots in 2011. Seeding was most successful at Finley NWR and Fern Ridge Reservoir. The highest establishment rate occurred at Field 29 dry, Finley NWR a site that had been treated with a broad-spectrum herbicide prior to planting and appeared to have lower competition from other species and likely greater incidence of seed-soil contact. Across all sites, we counted 10,349 vegetative individuals and 12 flowering individuals one year after seeding Appendix.

2011 Planting

Survival at upland sites ranged from 38% at Field 29 in Finley NWR to 63% at Pearcy-Schoener. We included one wet site, Oak Creek, in our 2011 planting, mortality in 2012 at this site was 100%. Flowering rates ranged from 41% to 73% of surviving individuals. Establishment from 2011 plantings totaled 1,560 individuals in 2012.

2012 Seeding and Planting

Plant establishment from planting and seeding in 2012 will be measured 2013.

Wetland vs. upland effects

Survival was very poor in wetland sites planted in 2010 (Table 8). On average, only 7% of C. *levisecta* survived one year in wetland habitats, compared to 54% in uplands. This difference was statistically significant (t=5.21, df=7, p=0.001, Figure 8).

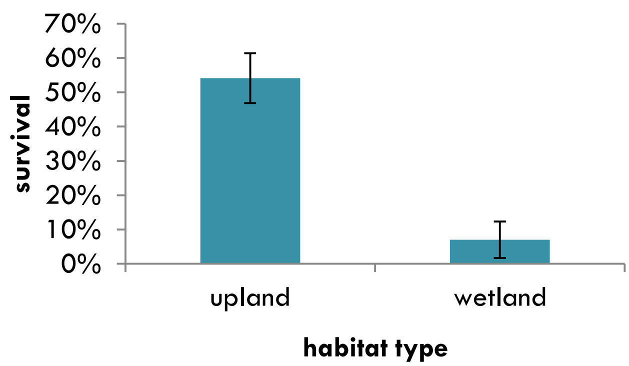
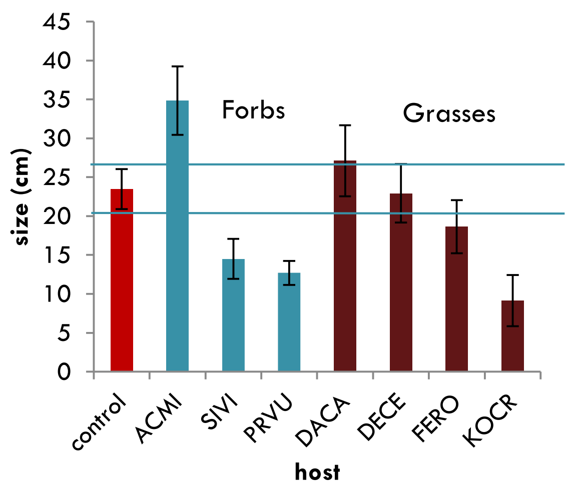


Figure 8. Comparison of C. *levisecta* survival in upland and wetland sites

Host plant and community effects

Greenhouse performance with hosts

When grown in a greenhouse with companion plants, C. *levisecta* size varied significantly among potential host species and between years (Figure 9). Plant size, measured as total stem length (sum of the length of all stems produced on a single plant) averaged 23 cm in 2011 and 30 cm in 2012 in control pots with no hosts. In 2011,for forbs, C. *levisecta* grown with *Achillea millefolium* were significantly larger (35 cm) than control plants, while plants grown with *Sidalcea virgata* and *Prunella vulgaris* were smaller (14 cm and 13 cm, respectively). Plants grown with the grasses *Danthonia californica*, *Deschampsia cespitosa*, and *Festuca roemeri* did not differ from controls. *Koeleria cristata* yielded C. *levisecta* plants that were only 9 cm. These trends were not repeated in 2012; *S. virgata* was the only species, grass or forb that produced C. *levisecta* plants larger than those planted without a companion plant. It is likely that these results reflect differences in growing conditions or even competition for resources between species in the conetainers rather than a direct effect of host species identity on C. *levisecta* size.



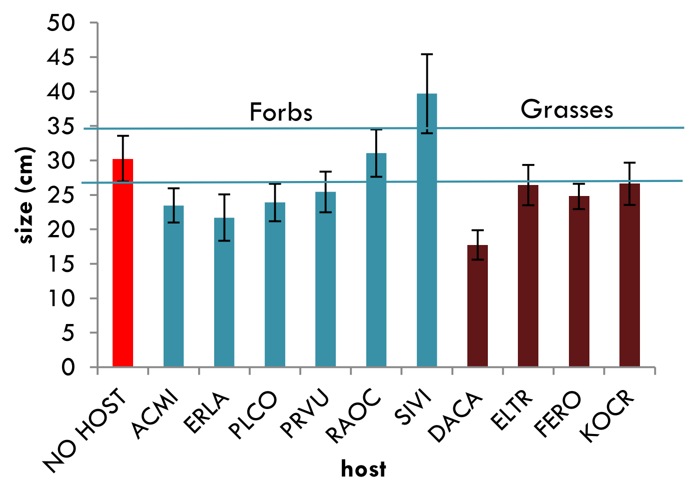


Figure 9. Total stem length of C. *levisecta* grown in a greenhouse with a range of host plants in 2011 (left) and 2012 (right). Error bars are 95% confidence intervals and the horizontal lines are drawn to facilitate comparison of C. *levisecta* mean size when grown with hosts to the no-host controls. See Table 6 for explanation of host species codes.

Field performance with planted hosts – one host only

At the Field 29 dry plot at Finley NWR, C. *levisecta* individuals that flowered produced on average 0.74 more flowering stems than C. *levisecta* at all other sites (95% CI 0.27 to 1.23, p=0.0026 two sample t-test). The size and number of flowering stems produced were highly variable but the range of values at Field 29 dry was much greater than at all other sites. Maximum size for C. *levisecta* at all sites except Field 29 dry was 275 cm (No Host at Pearcy-Schoener); the maximum size at Field 29 dry was almost 2.5 times greater, 667 cm (grown and planted with *Eriophyllum lanatum*). Likewise, the highest average flower stems produced by C. *levisecta* at all sites except Field 29 dry was 10 (No host, ACMI, DACA and

KOCR at Pearcy-Schoener) whereas the maximum numbers of flowers at Field 29 dry was 24 (ACMI and ERLA).

In the absence of an established plant community (Field 29 dry only), the probability of C. *levisecta* survival and flowering differed by host (p<0.0001, ANOVA f-test). The predicted probability of survival ranged from 25% for *S. virgata* to 95% for *F. roemeri* (Figure 10). C. *levisecta* planted without a host had a 70% probability of survival. The predicted probability of surviving individuals flowering ranged from only 9% for *P. virgata* to 89% for *E. lanatum*. C. *levisecta* planted without a host had a 62% probability of flowering (Figure 10).

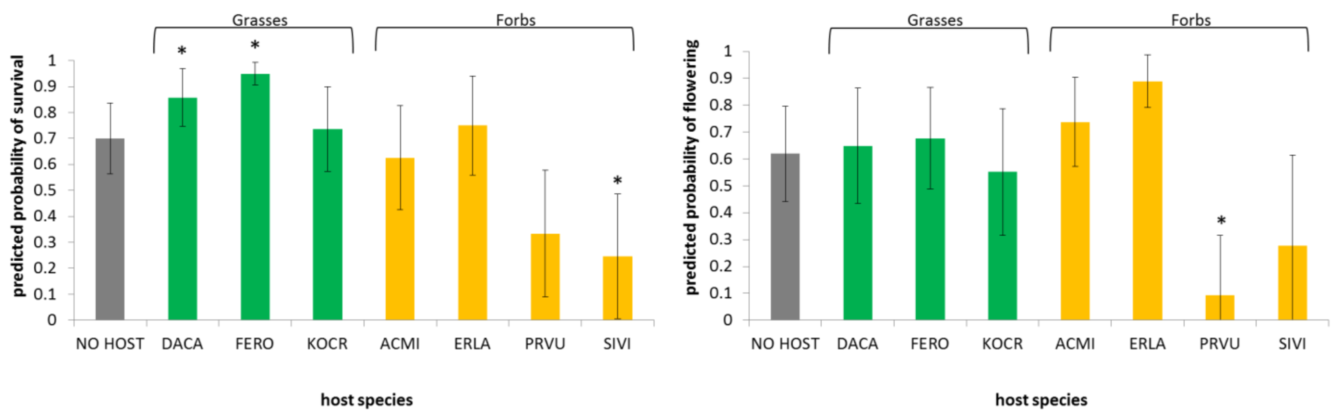


Figure 10. Results of logistic regression for the probability of the C. *levisecta* surviving (left) and flowering (right) when planted with hosts at Field 29 dry. Host=fixed effect, Site=random effect. Error bars indicate 95% Confidence Interval for the estimate. Statistically significant (*: $p<0.05$, +:$0.05<p<0.1$) effects are noted above bars. Green bars represent grass host species, yellow bars represent forb host species.

Field performance with planted hosts – one host plus surrounding vegetation

At upland sites that were not pre-treated with a broad spectrum herbicide prior to planting, sites at which there was an established plant community and a range of potential hosts present at the time of planting (all except Field 29 dry), the probability of survival in the first year ranged from 37% for *Sidalcea virgata* to 74% for *Danthonia californica* , and 81% for plants not grown originally with a host (Figure 11). The probability that surviving individuals flowered at the same sites ranged from 22% for *Prunella vulgaris* to 63% for *Achillea millefollium.*

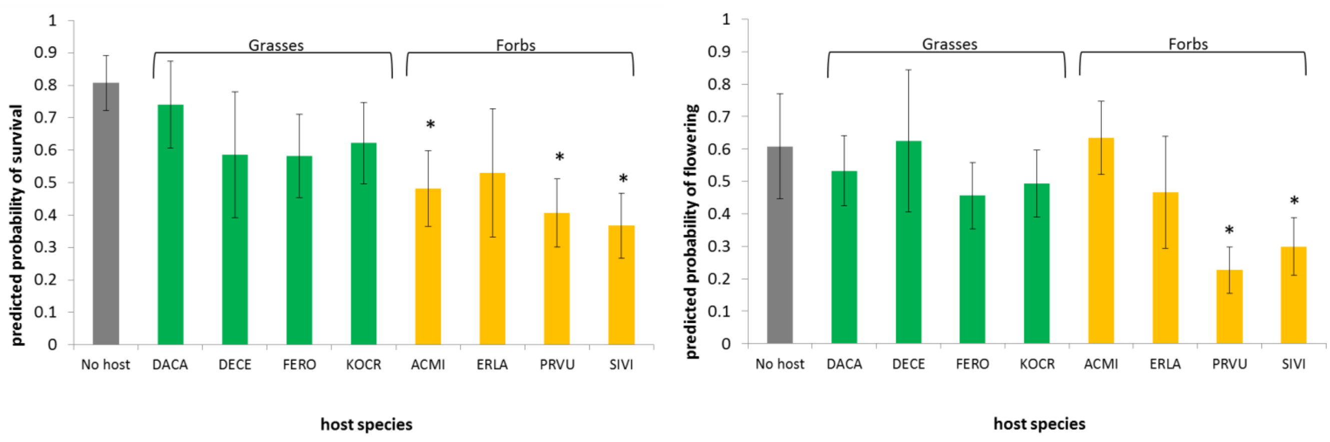


Figure 11. Results of logistic regression for the probability of the C. *levisecta* surviving (left) and flowering (right) when planted with hosts at all sites except Field 29 dry. Host=fixed effect, Site=random effect. Error bars indicate 95% Confidence Interval for the estimate. Statistically significant (*: $p<0.05$, +:$0.05<p<0.1$) effects are noted above bars. Green bars represent grass host species, yellow bars represent forb host species

Effect of host size

Where the surrounding plant community was cleared away with herbicide prior to planting (Field 29 dry), C. *levisecta* size was positively correlated with foliar area of *A. millefolium* ($p<0.0001$, $R^2=0.67$ Figure 12) and *Eriophyllum lanatum* ($p=0.028$, $R^2=0.45$, Figure 13). C. *levisecta* size was not significantly correlated with the size of other hosts at that site. At the remaining sites, C. *levisecta* size was not correlated with host size with the exception of a weak negative correlation with *Sidalcea virgata* at Lupine Meadows North ($p=0.07$, $R^2=0.6$, Figure 14). There was additional evidence that host area was correlated with C. *levisecta* size and flowering stems at several sites but in most cases, host size only accounted for a small portion of the variability in C. *levisecta* size (Tables 11 and 12).

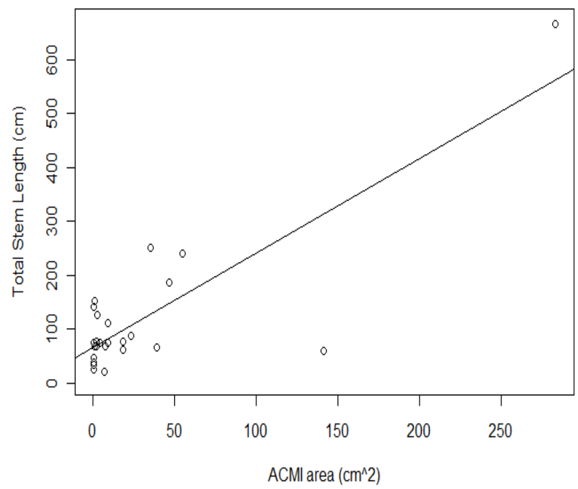


Figure 12. Correlation of *C. levisecta* size with *A. millefolium* at Field 29 dry.

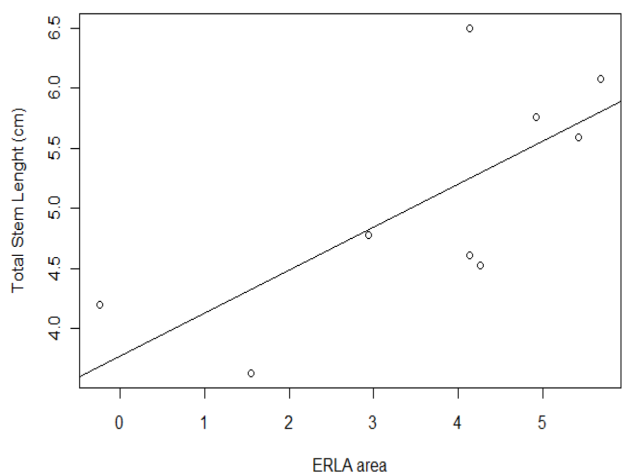


Figure 13. Correlation of *C. levisecta* size with *E. lanatum* at Field 29 dry. Both variables are presented on a log scale.

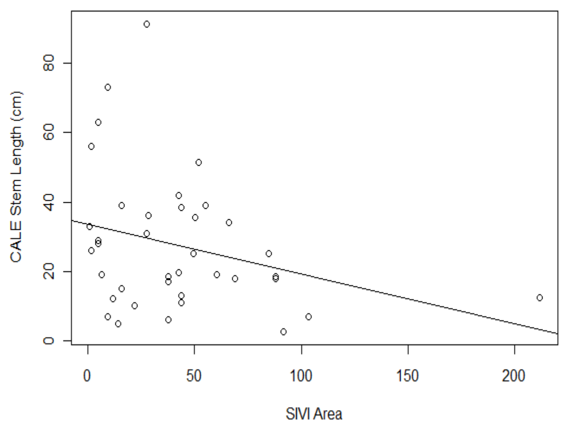


Figure 14. Correlation of C. *levisecta* size with *S. virgata* area at Lupine Meadows North.

Table 11. Results of linear regressions with *p*<0.1 on the effect of host elliptical (foliar) area on C. *levisecta* size (sum of stem lengths). No results reported for Bellfountain because p>0.1 for all regressions. n/a indicates species that were not planted at the indicated site, results with an asterisk (*) on the slope required log transformation of both the response (C. *levisecta* area) and explanatory variable (host area) to meet assumptions of the test.

	Field 29 wet			Lupine Meadows N.			Pearcy-Schoener			Field 29 dry		
Host	**slope**	**p-value**	**r2**	**slope**	**p-value**	**r2**	**slope**	**p-value**	**r2**	**slope**	**p-value**	**r2**
ACMI										1.756	<0.0001	0.669
DACA							0.27	0.067	0.044	0.15*	0.06	0.1
DECE					n/a			n/a			n/a	
ERLA		n/a			n/a			n/a		0.357*	0.028	0.45
FERO		n/a		0.5	0.03	0.05	0.527	0.0038	0.14	0.35	0.09	0.045
KOCR												
PRVU												
SIVI	0.3	0.044	0.28	-0.143	0.07	0.6						

Table 12. Results of linear regressions with $p<0.1$ on the effect of host elliptical area on *C. levisecta* flowering (no. flowering stems). For omitted sites, $p>0.1$ for all regressions, n/a indicates species that were not planted at the indicated site, or where none of the surviving *C. levisecta* flowered. Results with an asterisk (*) on the slope required log transformation of the explanatory variable (host area) to meet assumptions of the test.

	Field 29 wet			Pearcy-Schoener		
Host	slope	p-value	r2	slope	p-value	r2
ACMI						
DACA				0.0184	0.01	0.127
DECE	-0.025	0.0446	0.205		n/a	
ERLA		n/a			n/a	
FERO		n/a				
KOCR		n/a		-0.648*	0.0008	0.202
PRVU						
SIVI						

Community diversity

Species richness within 10-cm of *C. levisecta* had a significant effect on plant survival at most sites individually and overall (Figure 15), indicating that the performance of *C. levisecta* may depend on the nature of the plant community at the local microsite scale. Perennial species richness had a positive effect on survival at Pearcy-Schoener ($p<0.0001$), Bellfountain ($p=0.012$), Bellfountain Collins ($p=0.009$), and Baskett Upper ($p=0.06$), but no effect was detected at Lupine Meadows North ($p=0.45$). At all sites overall, the effect was very strong and significant ($p<0.00001$) (Figure 15, left panels). The narrow range in perennial richness observed at Baskett Upper and Lupine Meadows North (Figure 14, top left panel) may explain the weak and non-significant relationships at those sites.

Annual species richness was also significantly associated with *C. levisecta* overall ($p=0.0003$) as well as at Pearcy-Schoener ($p=0.001$) and Baskett Upper ($p=0.005$) individually (Figure 15, right panels). No effect of annual richness was detected at the remaining sites ($p=0.335$-0.96) individually, again possibly because of the narrow range in annual richness observed at those sites (Figure 15, top right panel).

Community composition

Community composition differed significantly between locations where *C. levisecta* survived and where it died from 2010 to 2011 at Pearcy-Schoener ($p<0.00001$), Bellfountain ($p=0.023$), and Bellfountain Collins ($p=0.006$) when evaluated with MRPP (Table 13).

Indicator species

ISA found statistically significant indicator species of *C. levisecta* fate at all sites (Table 14). A total of seven perennial forbs were indicators of plant survival, but these species differed among locations. For example, *Achillea millefolium* was an indicator of survival at Bellfountain and Baskett Butte, while *Prunella vulgaris* was frequently associated with survival at Bellfountain and Bellfountain Collins. In contrast, all of the eight indicators of mortality identified across three sites were annuals (Table 14). When evaluated as functional groups, all groups with perennials (perennial invasive grasses, perennial native grasses, perennial invasive forbs, and perennial native forbs) were significant indicators of C. *levisecta* at one or more sites. Annuals were again indicators of mortality, with annual native forbs and/or annual invasive grasses associated with loss of C. *levisecta* at four sites (Table 14).

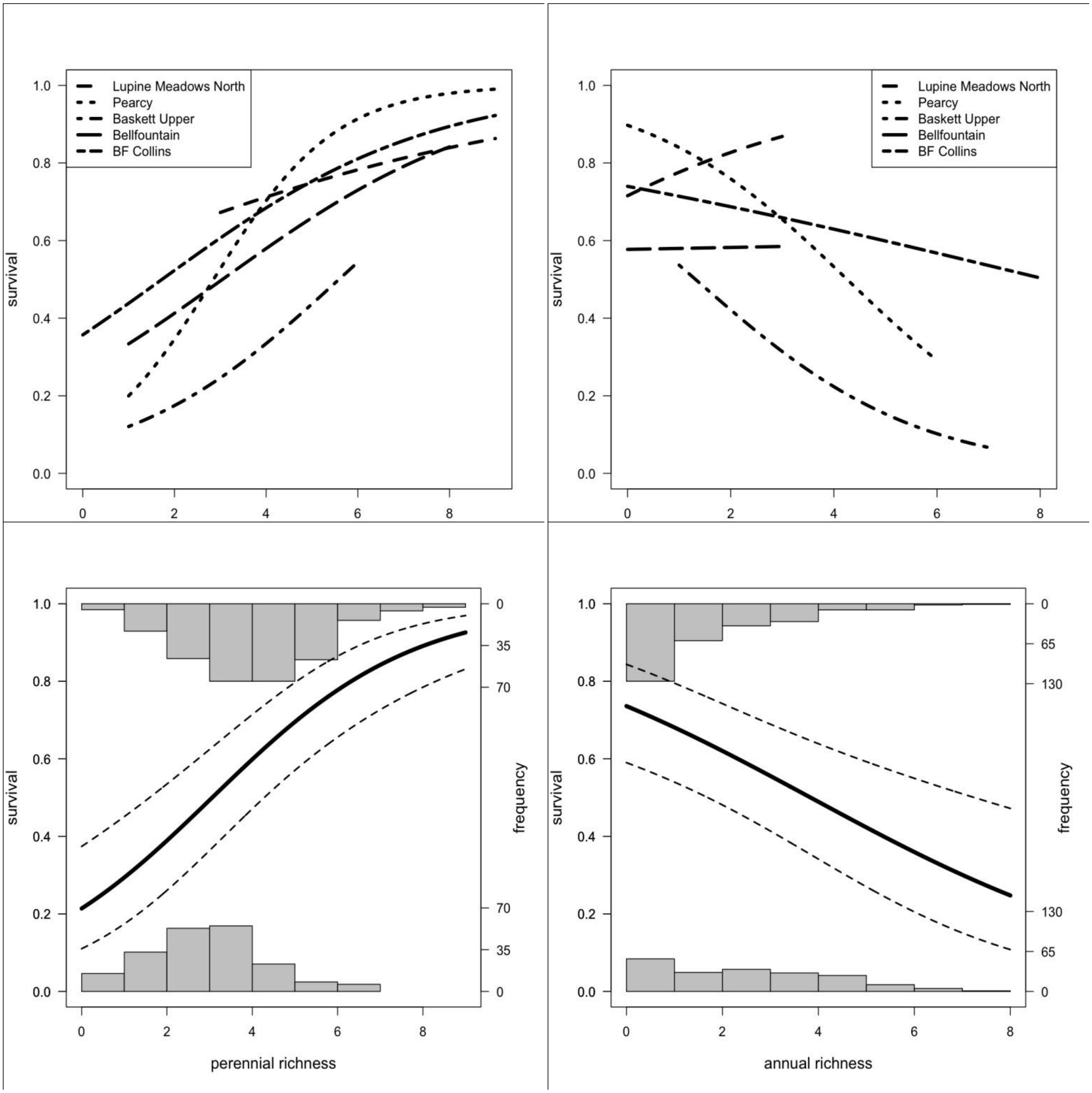


Figure 15. Estimated survival of *Castilleja levisecta* associated with plant diversity adjacent to planting locations. Survival is significantly positively associated with perennial plant species richness (left panels) and negatively associated with annual richness (right panels). Top panels show logistic regression curves for each site and bottom panels show the relationships (with 95% confidence intervals) with all sites combined using site as a blocking factor. Histograms in the bottom panels indicate the frequency of species richness values for both survival (top axis) and mortality (bottom axis).

Table 13. MRPP results for tests of community differences by *Castilleja levisecta* fate at each site showing the chance-corrected within-group agreement (A) and significance.

	site									
	Bellfountain, Collins (n=100)		Bellfountain, large meadow (n=100)		Pearcy-Schoener (n=100)		Baskett Butte, Saddle (n=97)		Lupine Meadows, North (n=70)	
MRPP	A	*p*	A	*p*	A	*p*	A	*p*	A	*p*
	0.0203	**0.006**	0.016	**0.026**	0.056	**<0.00001**	0.005	0.1786597	0.007	0.2298

Table 14a. Indicator values and significance for *Castilleja levisecta* fate for each species with $p < 0.05$ at each site. Indicator values (IV) are scaled from 0 to 100. A perfect indicator species (indicator value of 100) is always present in a group and never occurs in other groups. Indicators of survival were all perennial species while indicators of mortality were annuals. Functional groups represent the cumulative frequency within each plot of annual vs. perennial, native vs. invasive, and grass vs. forb species.

	site									
	Bellfountain, Collins (n=100)		Bellfountain, large meadow (n=100)		Pearcy-Schoener (n=100)		Baskett Butte, Saddle (n=97)		Lupine Meadows, North (n=70)	
species	**IV**	***p***	**IV**	***p***	**IV**	***p***	**IV**	***p***	**IV**	***p***
survival										
Achillea millefolium			18.6	0.048			14.8	0.004		
Agrostis stolonifera					26.8	0.014				
Fragaria virginiana					51	0.008				
Hypericum perforatum							14.2	0.048		
Hypochaeris radicata	23.8	0.019							22.6	0.049
Prunella vulgaris	22.2	0.002	24.8	0.042						
Veronica sp.	22.3	0.044								
mortality										
Bromus tectorum					12.5	0.039				
Geranium columbinum					31.8	0.022				
Madia glomerata					17.8	0.031				
Medicago lupulina					17.2	0.004				
Myosotis discolor	31.3	0.029								
Sherardia arvensis					33.3	0.001				
Taeniatherum caput-medusae							18.6	0.025		
Vicia sativa	21.6	0.021								

Table 14. Continued.

	site									
	Bellfountain, Collins (n=100)		Bellfountain, large meadow (n=100)		Pearcy-Schoener (n=100)		Baskett Butte, Saddle (n=97)		Lupine Meadows, North (n=70)	
species	**IV**	***p***	**IV**	***p***	**IV**	***p***	**IV**	***p***	**IV**	***p***
functional group										
survival										
Perennial invasive grasses					50.3	0.005				
Perennial native grasses			36.3	0.011	30.2	0.08				
Perennial invasive forbs	28.4	0.047								
Perennial native forbs	45.8	0.015			52.1	0.058				
mortality										
Annual invasive grasses					30.8	0.022	53.9	0.085		
Annual native forbs	44.1	0.017								

Haustorial connections and biomass

Above ground biomass of C. *levisecta* ranged from 0.23 g when grown with *S. virgata* to 0.74 g for C. *levisecta* planted without a host (Figure 16) and differed significantly between species ($p<0.0001$, ANOVA f-test).

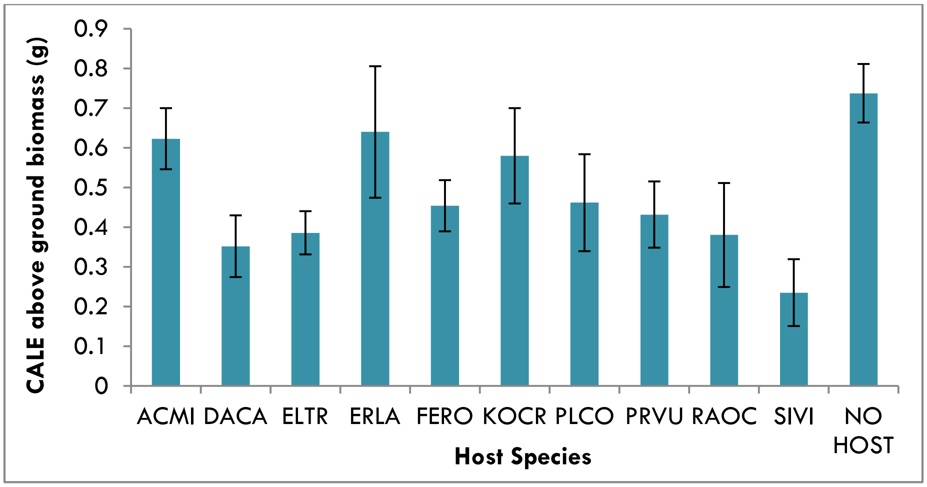


Figure 16. Average weight of C. *levisecta* for each host treatment, error bars indicate 95% confidence interval for the estimated mean.

The average number of connected haustoria in each paintbrush-host pair ranged from 12 haustoria on the roots of C. *levisecta* planted without a host to 679 haustoria on root pairs of C. *levisecta* planted with *Koelaria cristata* (Figure 17).

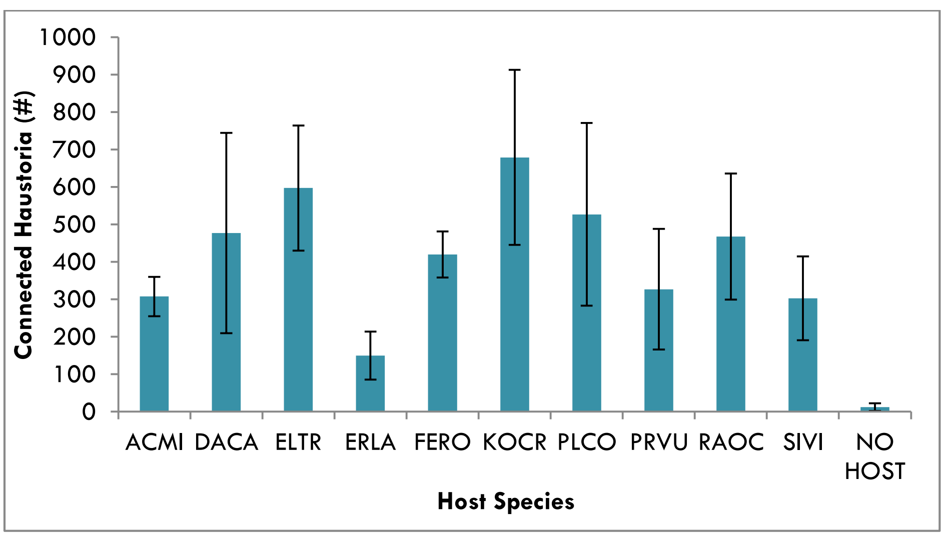


Figure 17. Average number of connected haustoria on roots of C. *levisecta*-host pairs. Error bars indicate 95% confidence interval of the estimated mean.

Implications for conservation and management

Planting and seeding can work

Both planting plugs and seeding appear to be viable methods of establishing C. *levisecta* populations in the Willamette Valley. Plug planting results in larger plants overall after one year than seeding, but is more labor intensive because the plants must be grown in a greenhouse, transported to the site, and planting, whereas seeding involves a single seed production bed followed by broadcast or drilling. Seeding on the other hand requires a large amount of seed for plant establishment. Our overall average seedling establishment rate from seed at sites where the plants established at all was approximately 0.02%. Therefore, seeding is a viable option only if large quantities of seed are available. Fortunately this is currently the case in Oregon as a result of the seed production beds.

Upland habitats should be targeted

Plug plantings and seedings in uplands were much more successful than in wetlands. Wetlands appear to be inappropriate habitat for C. *levisecta* in Oregon, despite indications from historic collections that wetlands might have been a common habitat for the species. It is possible that populations in the Willamette Valley represented a different ecotype from those in Washington, and successful reintroduction of the species to this region may require adapting to the available plant materials. Alternatively, notes on historic specimens may not have been intended to describe the habitat as wetland, and ecotype differences around soil moisture preferences did not exist. Either way, our plantings in wetlands failed completely or nearly so at every site. Even in upland sites with some moist areas the plants died frequently in the more hydric zones.

Plant diversity of neighbors may control survival

Perennial plant diversity improved C. *levisecta* survival at the microsite scale, while annual plant diversity reduced plant success. <u>This is one of the most significant findings of this project to date</u>. As perennial diversity within 10-cm of the planting site increased, average one year survival of C. *levisecta* also increased, from about 20% survival with no perennial neighbors to 90% on average with 9 species of neighbors. Annuals reduced survival from 75% with no annual neighbors to 25% with 8 neighbors.

These results strongly suggest that the number and quality of hosts affects C. *levisecta* performance in the wild. Perennials may be superior because they have larger root systems that persist throughout the entire year, while annuals in general have smaller, ephemeral roots systems.

In addition, perennial host diversity may improve C. *levisecta* survival because having multiple hosts available increases the chance that one of them will be highly compatible with the parasite. Also, large numbers of hosts can allow the parasite to draw compounds and resources from different hosts improving the species' overall diet, or draw the same resources from multiple sources widening the space from which the parasite can draw resources (Joshi et al. 2000). These explanations of the benefits of biodiversity to ecosystem processes are termed sampling effect and complementarity, respectively (Fargione et al. 2007). Our results from pot experiments demonstrate that host quality may vary substantially for C. *levisecta*. Also, other hemiparasites have been shown to benefit from a mixed diet resulting from connections to more than one species of host plant (e.g., Marvier 1998). If host quality varies and multiple connections are beneficial for C. *levisecta*, then both sampling effects and complementarity may be operating simultaneously in this system.

We suggest that new planting sites emphasize locations and microsites with high perennial plant diversity to improve survival of C. *levisecta*. Seeding with diverse natives may be improve microsite scale diversity and increase the success of C. *levisecta* reintroductions in Oregon prairies.

Plant communities within uplands matter

Even within uplands, the plant community surrounding C. *levisecta* differed among sites where the plant survived or died in 2011. Some species were clear indicators of survival while others were indicators of mortality, and these differences were consistent with the effects of perennial vs. annual diversity. In general, survival indicators were perennials and species associated with mortality were annuals. The list of perennial indicators included grasses and forbs, as well as natives and invasive species. Such a wide diversity is consistent with the observation that C. *levisecta* may be a generalist hemiparasite (Lawrence and Kaye 2008), capable of attaching to and drawing resources from a wide range of vascular plants. These results are consistent with the recommendation that C. *levisecta* be planted into microsites with high perennial richness and low annual diversity, and that seeding with a diversity of natives may improve conditions for reintroduced and wild populations of this threatened species.

Effect of host species depends on competitive environment

When grown in the absence of other vegetation, C. *levisecta* plants were significantly affected by the host they were planted with. Grass species tended to promote survival of C. *levisecta*, while species in the Aster family tended to improve flowering when compared to those planted without a host. In contract, when C. *levisecta* was planted with a host into existing prairie vegetation, forb hosts tended to reduce C. levisecta survival and flowering – with the exception of *Achillea millefolium* which tended to increase flowering of C. *levisecta*. Explanations for this difference include A) competition from surrounding vegetation limited the growth of the planted hosts, B) new parasitic connections between C. *levisecta* and the surrounding plants were costly to C. *levisecta* and reduced its growth, and/or C) parasitic connections between C. *levisecta* and the surrounding plants confounded our ability to detect effects of the planted host.

Adaptive management works

Our adaptive management approach using small plantings at many sites as bioassays to rank sites for future plantings has provided for an efficient method of improving planting success as the project moves forward. Coupling the lessons learned about the preference of the species for upland sites with the importance of perennial diversity for plant survival may help us improve our planting success in 2012 to maximize the likelihood of project success in the near term.

2013 Tasks

- Harvest additional year of seed production from seed increase field.
- Select sites for additional planting.
- Plant 1000 individuals at each of five sites.

- Monitor all 2010-12 plantings in May-June.
- Update project report.

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APPENDIX

Summary of *Castilleja levisecta* planting and seeding quantities and locations in Oregon, 2010-11, with number of established plants from 2010 plantings (1-yr old).

	planted (plugs)			seeded (grams)		Established plants	
site	2010	2011	2012	2010	2011	2011	2012
ACOE, Fern Ridge Res., Spires 1					40	--	669
ACOE, Fern Ridge Res., Spires 2					40	--	831
ACOE, Fern Ridge Res., Spires 3					40	--	534
Baskett Slough NWR finger meadow					40	--	--
Baskett Slough NWR lower 1	98					5	
Baskett Slough NWR lower 2				40		--	
Baskett Slough NWR Upper 1	98					27	16
Baskett Slough NWR Upper 1b					40	--	910
Baskett Slough NWR Upper East				40		1015	230
Baskett Slough NWR Upper West				40		108	79
Baskett Slough NWR, Field 14Z north					80	--	76
Baskett Slough NWR, Field 14Z south					40	--	56
Baskett Slough, NWR, Field 14Z south 2			1000				
Benton County Preserve, Pearcy 1	100					56	71
Benton County Preserve, Pearcy 2		635				--	399
Benton County Preserve, Pearcy 3			1000				
Benton County, Beazell Annex				20		468	2374
Benton County, Beazell Middle 1					40		
Benton County, Beazell Middle 2					40		
Benton County, Beazell Middle 3			592				
Benton County, Beazell North				40		735	3482
Benton County, Fitton Green 1					40	--	68
Benton County, Fitton Green 2					40	--	122
Benton County, Fitton Green 3					40	--	27
City of Corvallis, Bald Hill Park Lower				40		--	--
City of Corvallis, Bald Hill Park upper				20		--	--
Finley NWR, Bald Top 1 (by tree)					40	--	411
Finley NWR, Bald Top 2					40	--	852
Finley NWR, Bellfountain Prairie 2	100					60	50
Finley NWR, Bellfountain Prairie 3		786				--	376
Finley NWR, Bellfountain Prairie 4					40	--	672
Finley NWR, Bellfountain Prairie 5			982				
Finley NWR, Bellfountain Prairie Collins	100					72	69
Finley NWR, Field 29 hill top 1		576				--	219
Finley NWR, Field 29 hill top 2					40	--	1877
Finley NWR, Field 29 hill top 3			200				
Finley NWR, Field 29 low slope 3		500				--	215
Finley NWR, Field 29 lower 1	100					28	8
Finley NWR, Field 29 lower 2				40		2	17
Finley NWR, Field 29 lower 3			800				
Finley NWR, Field 29 north slope 1				40		631	416

	planted (plugs)			seeded (grams)		Established plants	
site	2010	2011	2012	2010	2011	2011	2012
Finley NWR, Field 29 north slope 2					40	--	1036
Finley NWR, Field 8N					80	--	2035
Finley NWR, Pigeon Butte upper				40		--	64
Lupine Meadows North 1	100					55	46
Lupine Meadows North 2		717				--	351
Lupine Meadows North 3					40	--	80
Lupine Meadows North 4					40	--	105
Lupine Meadows North 5			1000				
Lupine Meadows South	100					0	0
Oak Creek, USFWS WVRC	98					0	0
Oak Creek, USFWS WVRC		547				--	
TNC Preserve, Kingston Prairie	100					2	
total	994	3761	5574	360	880	3264	18843