Parasitic plants and community composition: how *Castilleja levisecta* affects, and is affected by, its community

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A dissertation

submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2016

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Program Authorized to Offer Degree:

School of Environmental and Forest Sciences

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Abstract

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Parasitic plants are native to many ecosystems around the world. Their effects on their environment are not always negative, and in some cases their presence can increase diversity in an ecosystem. We used *Castilleja levisecta*, a parasitic angiosperm native to the Pacific Northwest, to investigate both the effects of the parasite on the community, and the host plants' (community's) effect on the parasite. First, we examined host plant effects on the parasite by outplanting *C. levisecta* in host-parasite pairs, using eleven different host species, and monitored a variety of growth and reproductive traits. Second, we investigated the mechanism behind host effects by adding stable isotopes of carbon and nitrogen to host plants parasitized by *C. levisecta,* and tracked movement of these elements into the parasite. Finally, we used an existing study of

prairie restoration methods to statistically test the effect of *Castilleja* plant density on the surrounding plant community.

We found that the identity of the parasite's host plant did make a difference in parasite performance, in survival, growth, and reproduction. We also found that *C. levisecta* received differing levels of nutrition (measured in heavy isotope levels) from some host species. However, when looking at the reverse effect, we found inconclusive evidence of the *Castilleja's* influence on the community. In some cases, the parasite did affect community composition, but not in a consistent pattern. In summary, the relationship between this parasite and the surrounding plant community is complex: the community has influence on, and is sometimes influenced by, *Castilleja levisecta*.

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Acknowledgements

I am grateful to my advisor, Jon Bakker, for his continued patience, support, and mentorship throughout the past five years. I am also thankful to my committee members, Kern Ewing, Sarah Hamman, Peter Dunwiddie, Soo-Hyung Kim, Dick Olmstead, and Janneke Hille Ris Lambers, who each inspired me and provided valuable comments over the course of my degree. I am forever indebted to my past and current labmates, who all helped me on my path in various ways. Especially Eric Delvin, whose epic research provided the data used in Chapter 4, Rachel Mitchell, who is hilarious and gave me someone to aspire to, and Loretta Fisher, who will remain my lifelong friend. Also of tremendous service were my lab assistants: Del Brummet, Will Mooreston, Ben Saari, and Rae Mecka. I could not have done all this without your help. I would also like to thank the Center for Natural Lands Management and the Center for Urban Horticulture for their contribution of time and resources, and I am grateful for the great help and pleasing demeanor of Mark Roth on those cold out-planting days. I am thankful to have received support from the University of Washington's Royalty Research Fund, the United States Fish and Wildlife Service, and the Washington State Federation of Garden Clubs (WSFGC) Scholarship.

Finally, I would like to thank my family. My parents, Cathy and Dave, have always supported me and were a pillar of strength through the ups and downs of this process. My brother, Andrew, pushed me to achieve by his own achievements, and continues to put my brain to the test. And lastly, my dear husband Chris Jones. I could not have done this without you. Many worlds we've come since we first left home.

Note to the reader: Chapters 2-4 are intended as separate manuscripts for publication. Therefore, each includes an abstract, appendices, and a stand-alone references section, and "we" language is used in place of "I." Target journals include Northwest Science (Chapter 2), New Phytologist (Chapter 3), and Ecological Processes (Chapter 4).

Chapter 1: An introduction to research on *Castilleja levisecta* and Pacific Northwest prairies

Parasitic angiosperms occupy a unique niche in the ecosystems they inhabit (Press and Phoenix 2005). They are present in systems throughout the world and are regarded as agricultural pests as well as ecosystem engineers (Watson 2009). Research at present is embarking on a better understanding of the latter role, and recent studies have shown potential for parasitic plants to be used in the restoration of degraded ecosystems (e.g. Westbury *et al.* 2006).

This potential for restoration is of special interest when the parasite itself is a threatened species, as is the case with *Castilleja levisecta*. This root hemiparasite has been extirpated in most of its native range from British Columbia to Oregon (Caplow 2004). Much of this loss has been linked to habitat loss, as Pacific Northwest prairies now occupy less than 10% of their historical area (Crawford and Hall 1997). In the process of restoration of this individual species it is possible that restorative effects will also be observed in the greater plant community.

We conducted three experiments to examine the potential for restoration benefits to *Castilleja levisecta* and Pacific Northwest prairies. In the first, *C. levisecta* was planted in the field with one of eleven known or potential host species. For two years we monitored each parasite for survival, growth, and reproduction, and used these data to determine differences between host species contributions to *Castilleja*. In the second study, seven host species were planted with *C. levisecta* and labelled with heavy isotopes of carbon and nitrogen. We allowed the isotopes to travel from the host to the parasite, then sampled *Castilleja* to establish differences in relative nutrition received from each host species. The third study used community composition data from a multi-year prairie experiment to test the effects of parasite density on the prairie

community. We used PERMANOVA to test this effect with four years of data from four sites in western Washington.

Chapter 2: Host species influence on growth and reproduction of threatened hemiparasite *Castilleja levisecta*

Abstract

Restoration of a threatened species requires an understanding of the life history and resources necessary to facilitate the establishment and reproduction of new populations. Pacific Northwest native *Castilleja levisecta* (Orobanchaceae) is a facultative hemiparasite: it prefers but does not require a host to complete its life cycle, and it is able to sequester carbon via photosynthesis. The aim of this study was to elucidate the most useful host species for growth and reproduction of this parasite. We used host-parasite pairs in a two-year field study to compare the effects of a variety of host species. Our data show that host identity affects survival, and growth and reproductive traits of *Castilleja levisecta*. Furthermore, the two host species widely cited in the literature, *Eriophyllum lanatum* and *Festuca roemeri*, were not always the top performers in our group of hosts, and in many cases *Achillea millefolium* was among the top treatments for *Castilleja* performance. Thus, *Achillea* and other novel hosts should be considered for use in preservation and reintroduction of *Castilleja levisecta*.

Introduction

Parasitic plants make up nearly 1% of angiosperms in the world and are present in nearly every biome (Nickrent *et al.* 1998, Press and Phoneix 2005). Some parasitic plants are detrimental to crops, (i.e. *Striga*), while others appear to have positive and often complex influences on their communities (Bao *et al.* 2015, Fisher *et al.* 2013). This influence is not limited to the plants they parasitize and can extend to higher trophic levels, affecting invertebrates as well as other non-host species (Hartley *et al.* 2015). As a result, parasitic angiosperms often have effects on their communities that are greater than expected given their biomass, and have been proposed as

keystone species and ecosystem engineers (Rowntree *et al.* 2014, Watson 2009). In this light, it is crucial to understand the role of parasites in their native systems and the interactions with which they directly and indirectly influence the community around them.

Castilleja levisecta (golden paintbrush) is a federally threatened parasite native to Washington, Oregon, and British Columbia. This species, as with all plants in the genus *Castilleja*, attaches to hosts using parasitic root structures, called haustoria, which facilitate a xylem-xylem connection between the parasite and its host (Kuijt 1969, shown in Figure 2.1). As a facultative hemiparasite, *Castilleja* is able to survive and reproduce without a host and is also able to sequester its own carbon via photosynthesis (Wentworth 2001). Despite this ability to live without host resources, many studies have shown considerable increases in hemiparasite growth and reproductive traits in the presence of a host plant, and in some cases a large proportion of the parasite's total carbon is host-derived (Pageau *et al.* 1998).

Host species identity appears to play a major role in the survival and overall performance of *Castilleja levisecta* (Delvin 2013, Lawrence and Kaye 2008). Several studies of host influence have been conducted, mainly comparing the host quality of *Eriophyllum lanatum* and *Festuca roemeri* (e.g Lawrence and Kaye 2008). However, little has been done to investigate the effects of host identity on *Castilleja levisecta* using a wide range of hosts. And while functional group may sometimes be a predictor of host quality (Lawrence and Kaye 2011), studies in other systems have shown that species-level differences, not functional groups, are better predictors of hemiparasite performance based on host identity (Demey *et al.* 2015, Rowntree *et al.* 2014). If we are to truly understand the nature of *Castilleja*'s relationship with different hosts for the

ultimate goal of restoration, we are obliged to examine numerous diverse host species and their effects on parasite growth and reproduction.

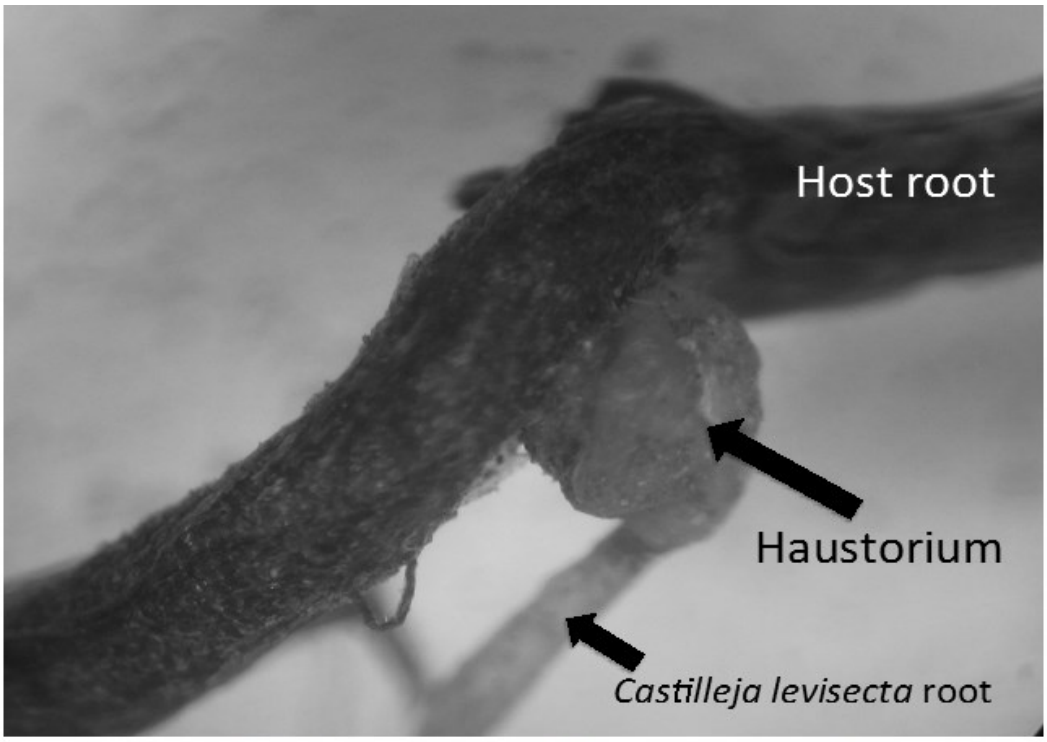

Figure 2.1 *Castilleja levisecta* parasitizing *Rumex acetosella*. Darker root is *R. acetosella* and lighter root (with haustorium) is *C. levisecta.*

Recent studies (e.g. Kaye *et al*. 2011) have begun to explore the possibility of host species beyond *Eriophyllum lanatum* and *Festuca roemeri*. Ecologists monitoring extant *C. levisecta* populations have also noticed some individuals thriving in the absence of these two known hosts (Dunwiddie and Bakker, pers. comm.). This suggests that *C. levisecta* may be able to parasitize a wide variety of hosts, which is consistent with our understanding of the genus *Castilleja* as generalist parasites (Dobbins and Kuijt 1973, Heckard 1962).

One study found that *C. levisecta* grown with *Achillea millefolium* were significantly larger than controls and those grown with other hosts, including *Festuca roemeri* (Kaye *et al*. 2011). This

study also tested *Danthonia californica* as a possible host, but results were not significantly different from no-host controls. Personal observations by researchers working with *C. levisecta* have led to speculation that *Rosa nutkana*, *Symphoricarpos albus*, and *Erigeron speciosus* may also be viable hosts (Dunwiddie and Bakker, pers. comm.). Research also suggests that *C. levisecta* has higher survival when planted with a perennial host species (Lawrence 2005), and that in some cases, plantings with known host *Eriophyllum lanatum* have decreased *C. levisecta* survival compared to controls with no host (Lawrence and Kaye 2008).

Other species of hemiparasitic *Castilleja* have been known to acquire defensive compounds from host plants (especially composites and legumes) through haustorial connections (Stermitz and Harris 1987, Marko and Stermitz 1997). This type of transfer could increase the ability of *C. levisecta* to withstand herbivory and decrease mortality in out-planting sites. Adler (2003) also found that *Castilleja indivisa* parasitizing a lupine species gained reproductive advantages (increased seed production and increased visitation by pollinators) over those parasitizing a graminoid species.

In light of this species' potential to parasitize a wide range of host species, and the mixed and sometimes conflicting results gleaned from literature review, we tested a variety of known and novel host species to assess their effect on the survival, growth, and reproduction of *Castilleja levisecta*. We also tested over a two-year period in order to measure changes in these effects over time. This improves our understanding of not only the breadth of hosts parasitized by this species, but also our knowledge of potential new directions for recovery and preservation of

current populations. To this end, the objective of this study was to identify the host species that enable *Castilleja levisecta* to survive, grow, and ultimately reproduce most effectively.

Methods

This experiment used what we refer to as "companion plantings." This method pairs a single parasite individual with a single host individual in a field planting. Control pairings contained two *Castilleja levisecta* (CALE) individuals to account for competition but remove parasitism.

Parasites and host plants were grown from seed (except for woody species) in a greenhouse setting for 4 months prior to planting. Seeds were germinated according to established methods in growth chambers then transplanted into greenhouse plug trays for establishment. Woody species were propagated vegetatively by cuttings obtained from the Union Bay Natural Area (Seattle, WA). Cuttings were dipped in rooting hormone prior to striking and trays were placed on a mist bench for 1-2 weeks for root establishment, followed by normal greenhouse growth with the seeded species. All individual plants were grown in separate plugs prior to outplanting in the field for ease of transport (as in Schmidt 1998).

Companion pairs were planted at Glacial Heritage Preserve (Littlerock, WA), a former agricultural site that is currently the location of restoration and preservation efforts of Pacific Northwest prairies. We used twenty replicates of each unique host-parasite pairing. The pairs were planted in the fall of 2012, host and parasite in the same hole, with roots touching. All pairs were measured in the spring and fall of 2013 and 2014. They were set up on a grid with one meter between each pair to discourage CALE from attaching to individuals other than the host

being tested, and ordering of pairs was randomized. All pairs were weeded in the spring of 2013 and 2014.

Table 2.1 Host species used in companion plantings. Basis for inclusion key: 1 - known host of *C. levisecta*; 2 - genus parasitized by other *Castilleja* species; 3 - observed near robust *C. levisecta* in field.

Species	Species abbreviation	Family	Functional Group	Basis for inclusion
Achillea millefolium	ACMI	Asteraceae	Forb	1
Danthonia californica	DACA	Poaceae	Grass	1
Deschampsia caespitosa	DECA	Poaceae	Grass	1
Erigeron speciosus	ERSP	Asteraceae	Forb	1
Eriophyllum lanatum	ERLA	Asteraceae	Forb	1
Festuca roemeri	FERO	Poaceae	Grass	1
Lupinus lepidus	LULE	Fabaceae	Legume	1
Lupinus littoralis	LULI	Fabaceae	Legume	2
Rosa nutkana	RONU	Rosaceae	Shrub	3
Solidago canadensis	SOCA	Asteraceae	Forb	1
Symphoricarpos albus	SYAL	Caprifoliaceae	Shrub	3

Host species used in the study were chosen for a variety of reasons, detailed in Table 1 above, and represent a diversity of families and functional groups. ERLA and FERO have been documented extensively in the literature as hosts of *C. levisecta* (e.g. Lawrence and Kaye 2008). Additional hosts (ACMI, DACA, DECA, ERSP, LULE, SOCA) have been tested in our greenhouse facilities, where we found haustorial connections between these species and *C. levisecta* (Schmidt, unpub. data). The two woody species (RONU and SYAL) used in this study are found in Pacific Northwest prairies and have been noted in proximity to robust *C. levisecta* populations where few other potential hosts are present. Lastly, in an attempt to widen the pool of potential hosts, an additional *Lupinus* species (LULI) was added to observe potential differences in host suitability with two nitrogen-fixing hosts.

Measured variables

Measurements were conducted in spring and fall of 2013 and 2014 (Table 2.2). Survival was counted positively when both the host plant and the parasite survived. Then, using those pairs that survived, we measured additional variables of the *Castilleja* individuals. In the spring, we began by measuring the number of flowering stems as a metric of potential reproductive capacity. However, deer browse occurred on the site, leading us to additionally measure browsed stems and the number of surviving plants browsed to assess changes in reproductive potential and overall growth. Another growth trait, height, was determined by measuring the length of the longest stem. In the fall we returned to the site and measured the final reproductive output for that growing season: number of fruiting stems (stems that had at least one seed capsule) and the total number of seed capsules per plant.

Table 2.2 Variables and seasonality of *Castilleja levisecta* measured in companion experiments.

Measurement	Measurement season	Trait category	Model type
Survival (y/n)	Spring	Survival	Binomial
Number of flowering stems	Spring	Reproductive	Poisson
Number of browsed stems	Spring	Growth/Reproductive	Poisson
Plants browsed (y/n)	Spring	Growth/Reproductive	Binomial
Height	Spring	Growth	Poisson
Number of fruiting stems	Fall	Reproductive	Poisson
Number of seed capsules	Fall	Reproductive	Poisson

Statistical Analyses

For each year, each trait was compared with host identity using generalized linear models (glm) and linear models (height only – both years) to test for differences between host groups and the control (two parasites with no host). Survival and number of browsed plants were tested with a glm using a binomial distribution, while all other variables (except height) were tested with a glm using a poisson distribution. We tested the effect of host treatment on each response variable, followed by pairwise comparisons among hosts. Traits other than survival used data only from those host-parasite pairs that survived in that year. This should be noted when interpreting comparisons in performance between hosts, as some hosts may have a significant effect on surviving *Castilleja* but a low percentage of overall survival, and some *Castilleja* survived even when the host died. Analyses were conducted using R statistical software (version 3.1.3, Appendix A). For a qualitative analysis, we also ranked the mean values of each trait by

host species to create a table that visually represented the performance of *Castilleja* with each host.

Results

Survival

Our companion experiment resulted in a range of variation of CALE survival. Survival ranged from 11% (*Solidago canadensis* group in 2014) to 74% (*Deschampsia caespitosa* in 2013), (Figure 2.2). Survival in the LULE treatment group was zero in both years, so it was removed from the remainder of the analyses. At the end of the experiment, DACA and DECA treatment groups had the highest survival, followed by ERLA, FERO, ACMI, LULI, and the no host control group. The lowest survival was in the RONU, SYAL, ERSP, and SOCA treatment groups.

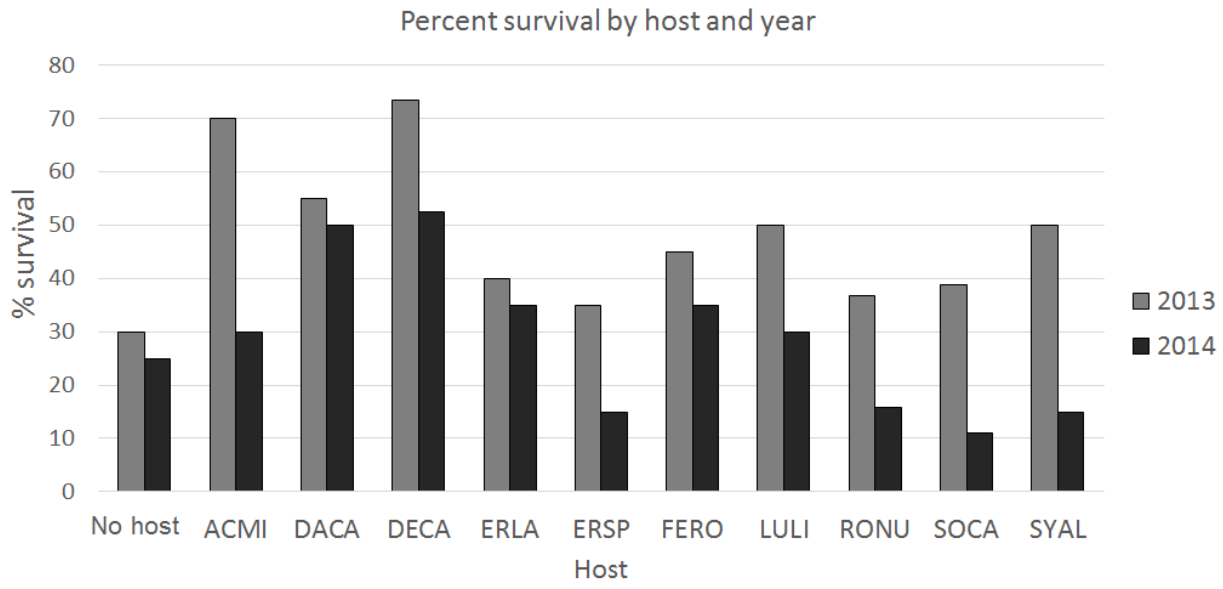

Figure 2.2 Percent survival of *Castilleja*-host pairs over two years of study. Survival was only counted when both plants in the pair were alive at the time of sampling. See Table 2.1 for full species names.

Growth

There were significant differences in all growth traits in all years, with one exception: percentage of plants browsed per treatment in 2014 was not significant (Table S2.1). For measures of height in 2013, CALE was tallest in the ACMI and DECA treatment groups and shortest in SOCA, FERO, and ERLA groups (Figure 2.3). However, in 2014, ERLA, ACMI, and LULI treatments had the tallest CALE, while RONU, SOCA, and the no host control had the shortest. Overall height values were higher in 2014 than 2013.

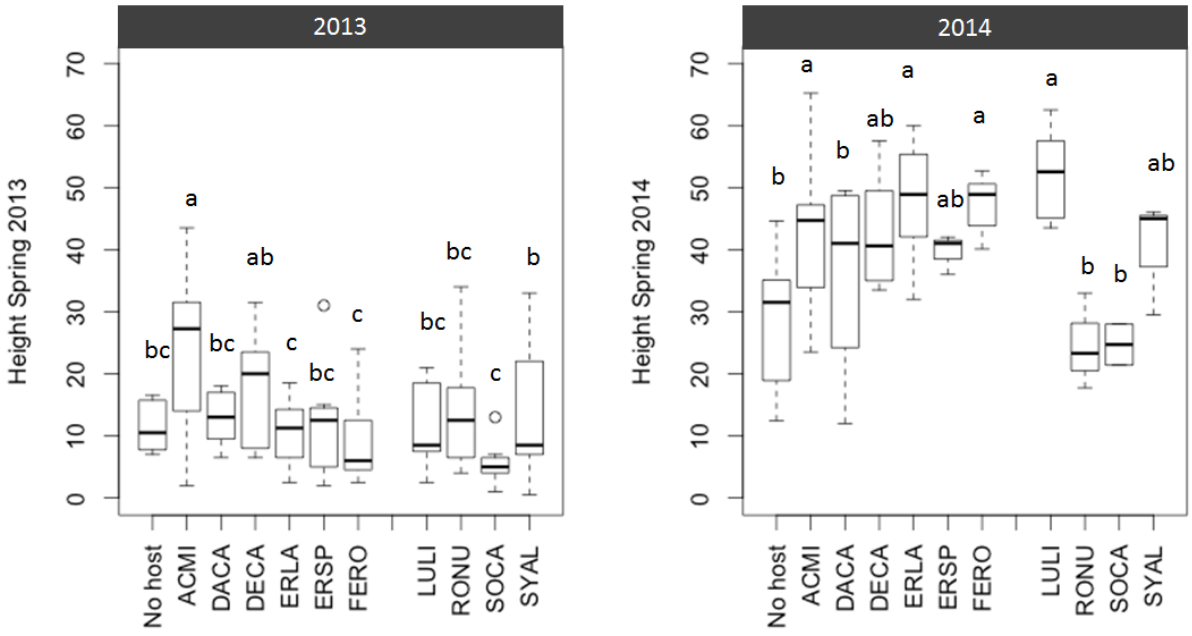

Figure 2.3 Height of *Castilleja* in 2013 and 2014 by host species. Data were only included from live pairs. Dotted lines indicate the nominal range of the data inferred from the upper and lower quartiles (box edges). Points that fall outside this range are shown as open circles. See Table 2.1 for full species names.

Number of browsed stems and percentage of browsed plants per treatment also varied among the treatment groups (Table S2.1). Treatment groups with the highest number of browsed stems in

2013 were ACMI, ERSP, and the no host control, while the lowest numbers were found in the LULI, RONU, and SOCA groups (Figure S2.2). However, in 2014, the ERSP group dropped to one of the lowest numbers of browsed stems, along with RONU and SYAL. The no host control, DACA, and ACMI groups had among the highest percentages of live plants browsed in both 2013 and 2014 (Figure 2.4). DECA and ERLA had a relatively lower percentage of plants browsed in 2013 but increased significantly in 2014, while ERSP had relatively high browse in 2013 and lower in 2014. LULI, RONU, SOCA, and SYAL treatment groups remained in the lower relative percentages of browse in both years. Overall percent of plants browsed increased from 2013 to 2014 (Figure 2.4).

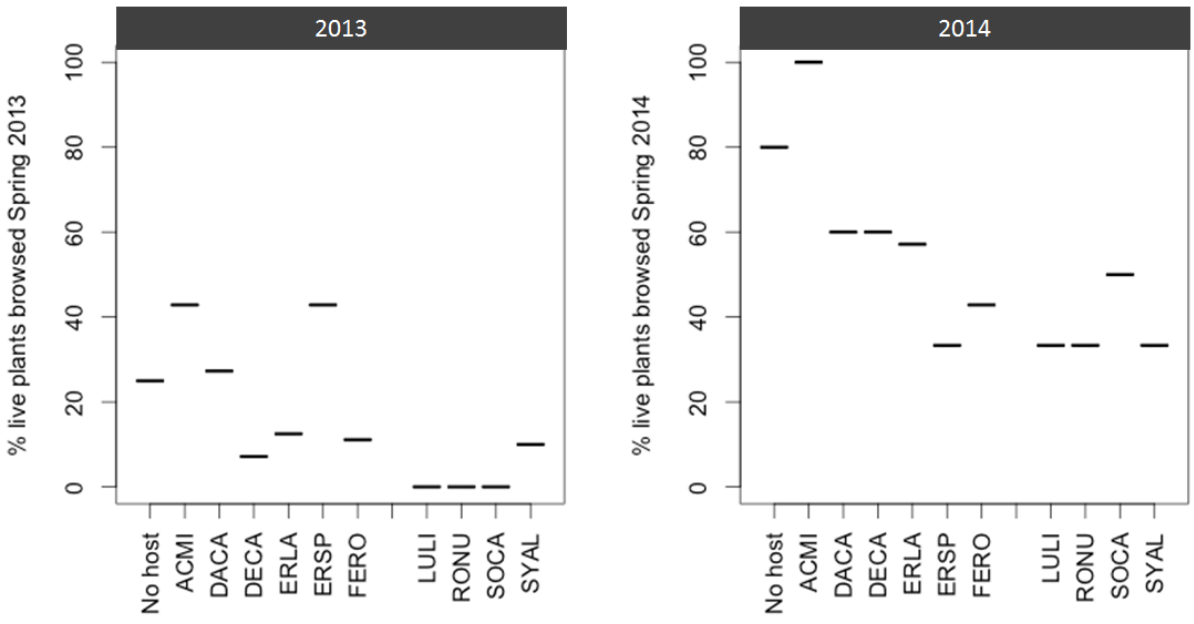

Figure 2.4 Percent of live *Castilleja* plants that were browsed in each treatment group. While a generalized liner model showed that number of browsed plants differed by host, only marginal ($p>0.05$) differences appeared in pairwise comparisons. Data were only included from live pairs. See Table 2.1 for full species names.

Reproduction

Flowering stems were significantly different based on host identity (Table S2.1). In 2013, The ACMI and DECA groups had the highest number of flowering stems, and the no host control had the lowest (Figure S2.1). *Castilleja* in the DECA treatment remained with relatively high numbers of flowering stems in 2014, and the ERLA group rose to the top performer for this trait. The control group remained in the lower relative numbers, joined by the RONU and SOCA groups.

Fruiting stems showed a similar response to host treatment: the ERLA group had lower numbers in 2013 but rose to be one of the top performers in 2014 (Figure S2.3). ACMI was a top performer in both years, as was DACA. One difference from flowering stems was the SYAL treatment, which was the top group in 2013 and remained among the top performers in 2014.

Seed capsule output, the trait that most directly assessed reproduction, showed a wide range of values (Figure 2.5). *Castilleja* in the SYAL and ACMI groups produced more capsules in the first year of the experiment, while the ERSP had the lowest (and the no host control had no capsules in 2013). In 2014, the DACA and ERLA groups had the most seed capsules, while the SOCA and control groups had the least. For the most part, treatment groups had similar or increased average capsule outputs in 2014, with the exception of SOCA and SYAL, which decreased their average capsule number in the second year of the study.

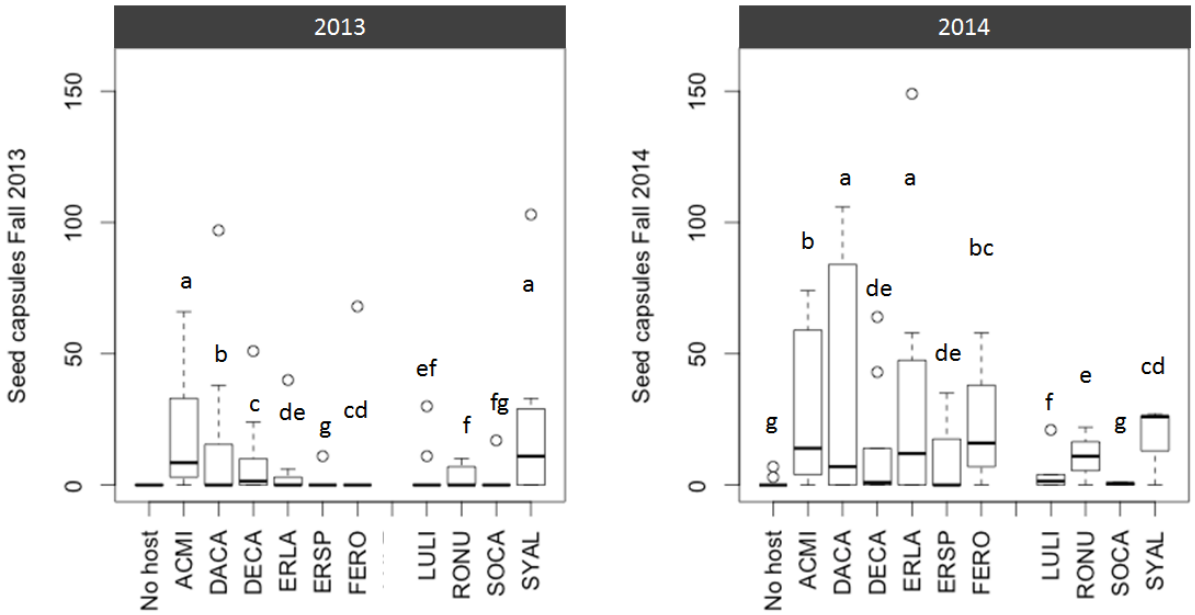

Figure 2.5 Number of *Castilleja* seed capsules in 2013 and 2014 by host species. Data were only included from live pairs. See Table 2.1 for full species names and Figure 2.3 for plot description.

Combining capsule production and survival over both years of the study, we find that the highest producers are those parasitizing ACMI and DACA (Figure 2.6). Despite relatively high mortality in the second year of the study (Figure 2.2), the *Castilleja* in the ACMI treatment that survived produced large quantities of seed capsules.

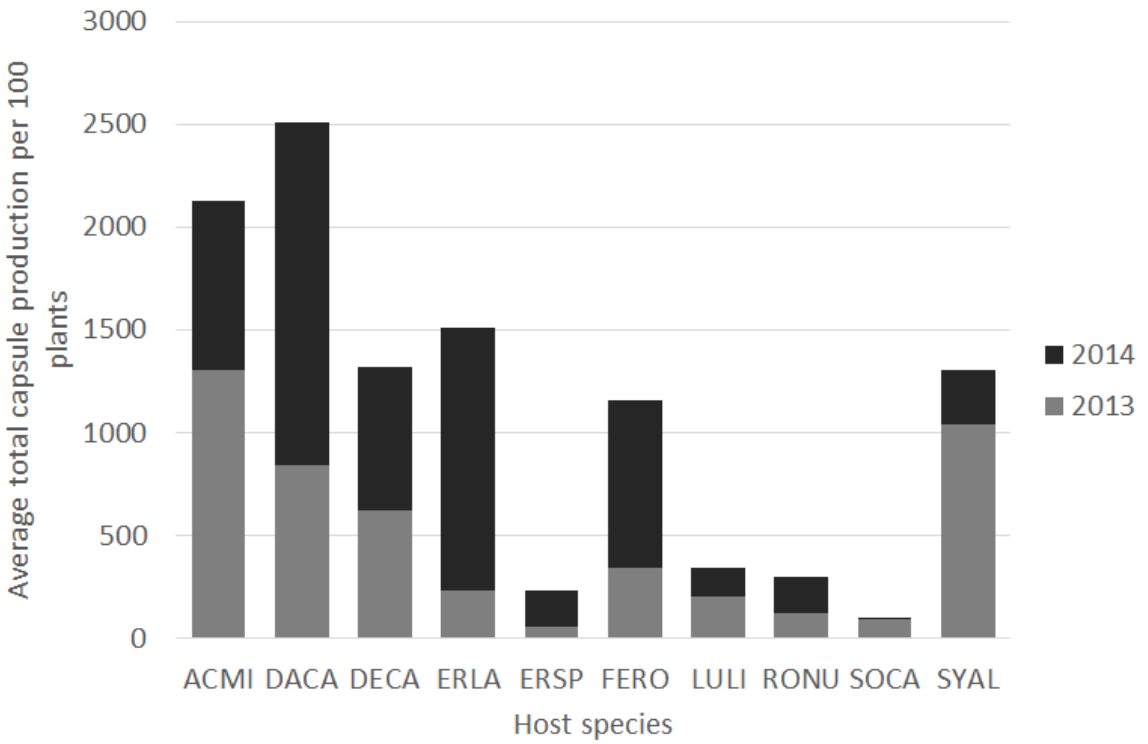

Figure 2.6 Sum of 2013 and 2014 total seed capsule averages in *Castilleja* plants, by host. *Castilleja* in the no host treatment did not have any capsule production in 2013, and no significant production in 2014. See Table 2.1 for full species names.

Overall trends

In order to look at all traits simultaneously, we ranked each host species in its performance as a host and calculated an overall average rank for each measurement year (Tables 2.3 and 2.4). ACMI was included in the top three host groups in both years, while SYAL and DECA did well in 2013 but were overtaken by FERO and ERLA in 2014. It should also be noted that many of the traits measured are related to one another, and caution should be used when interpreting the results together rather than each trait individually.

Table 2.3 Comparative rankings of *Castilleja* performance with each host species in 2013 (year 1). Ratings are based on the rankings of each variable in positive relation to performance (highest = 1, lowest = 11). Ranks 1-3 are **bolded** and ranks 9-11 are grayed. See Table 2.1 for full species names.

2013	Survival	Flowering stems	Browsed stems	Plants browsed	Height	Fruiting stems	Seed capsules	Average rank
ACMI	**2**	**1**	**1**	10.5	**1**	**2**	**2**	**2.79**
DACA	**3**	7	4	9	5	**3**	**3**	4.86
DECA	**1**	**3**	8	4	**2**	4.5	4	**3.79**
ERLA	7	9.5	5	6.5	10	7	6	7.29
ERSP	10	5	**2**	10.5	6	8	10	7.36
FERO	7	9.5	6	6.5	9	6	5	7.00
LULI	4.5	6	10	**2**	7	10	8	6.79
RONU	9	4	10	**2**	4	4.5	7	5.79
SOCA	7	11	10	**2**	11	9	9	8.43
SYAL	4.5	**2**	7	5	**3**	**1**	**1**	**3.36**
No host	11	8	**3**	8	8	11	11	8.57

Table 2.4 Comparative rankings of *Castilleja* performance with each host species in 2014 (year 2). Ratings are based on the rankings of each variable in positive relation to performance (highest = 1, lowest = 11). Ranks 1-3 are **bolded** and ranks 9-11 are grayed. See Table 2.1 for full species names.

2014	Survival	Flowering stems	Browsed stems	Plants browsed	Height	Fruiting stems	Seed capsules	Average rank
ACMI	5.5	6	**1**	11	4	**1**	**3**	**4.50**
DACA	**2**	7	**3**	8	8	4	**1**	4.71
DECA	**1**	**2**	6	7	5	6	6	4.71
ERLA	**3**	**1**	**2**	9	**2**	**2**	**2**	**3.00**
ERSP	8.5	5	11	**3**	7	8	7	7.07
FERO	4	4	5	6	**3**	**3**	4	**4.14**
LULI	5.5	8	8.5	**3**	**1**	8	9	6.14
RONU	8.5	9	8.5	**3**	10	8	8	7.86
SOCA	8.5	11	7	**3**	11	10	10	8.64
SYAL	8.5	**3**	10	**3**	6	5	5	5.79
No host	7	10	4	10	9	11	11	8.86

Discussion

Survival

Survival was somewhat low in general, although our methods tended to overestimate mortality. Because only pairs in which host and *Castilleja* survived were counted, it is possible that multiple pairs with living *Castilleja* were not counted due to host plant mortality. In our observations over the course of the experiment, some treatments (such as ACMI) had a large drop in host survival after the first year, with many *Castilleja* still alive. Conversely, groups such as SOCA had more host survival combined with *Castilleja* mortality. This may point to outside environmental factors influencing host-parasite interactions, or host plant mortality in some cases may have been due to the strength of parasitism reducing the host plants' ability to survive. Similarly, high host survival rates could be a sign of a poor host, as it may not be adversely affected by parasitism. This could also be due to differences in host species' ability to resist parasitism, as has been documented with European hemiparasite *Rhinanthus minor* (Cameron *et al.* 2006). In that case, rather than being a selective difference mitigated by the parasite, the host's resistance created a barrier to parasitism and greatly reduced nutrient acquisition by the parasite. This mechanism may be present in the grass species we studied, as Poaceae is known to have resistance to parasitism but the strength of resistance varies by species (Rümer *et al.* 2007).

Growth

Castilleja in the ACMI treatment were consistently high performers in height, but had a relatively high percentage of live plants browsed. While they also had a relatively high number

of browsed stems, this could be considered a positive: that there were a high number of stems available to be browsed means that the plants had a high level of growth (possibly due to host resources) to begin with. This native forb is prevalent in Pacific Northwest prairies and can be easily seeded into restoration and preservation areas. It has recently been suggested that clonally spreading species may be easier targets for parasitism (Demey *et al.* 2015), and this may be one of the underlying mechanisms for ACMI's high performance as a host since it spreads clonally. Root structure in general may play a large part in the ability of parasites to form and maintain haustoria, and future studies of this nature should consider categorizing root structures of each host species in addition to other measures.

The ERLA, DECA, and DACA host groups also performed relatively well: they were among the tallest average *Castilleja* in 2014 and also had more browsed stems in 2014 (Figures 2.3 and S2.2). However, all of these treatment groups had more than 50% of the live *Castilleja* plants browsed in 2014. ERLA is a known host of *Castilleja* (Lawrence and Kaye 2008), but DACA and DECA have not been widely studied as hosts for this species.

Number of browsed stems and percent of plants browsed fluctuated considerably between the two years of measurement (Figures 2.4 and S2.2). *Castilleja* in the LULI, RONU, and SOCA treatments were not browsed at all in 2013, but all three groups had over one quarter of their live *Castilleja* browsed in 2014. It is possible that different host-derived compounds are acquired or are more important in different growth stages of CALE, as has been observed with *Striga hermonthica* (Aflakpui *et al.* 2005, Pageau 1998). Despite the increase in browse in 2014, these

three host groups remain among the lowest relative percentages of live plants browsed per treatment.

The hosts associated with lower *Castilleja* browse may have conferred extra-nutritional benefits to the CALE parasitizing them, such as defensive compounds dissolved in xylem sap, or protection from herbivory by mechanical means. Lupines are known in some cases to produce bitter alkaloids which limit herbivory, and these compounds are sometimes passed on to parasites (Adler 2002, Adler 2003). For example, several studies show that other species of *Castilleja* receive quinolizidine alkaloids when parasitizing various species of lupines, but lack these compounds when using other species as hosts (Stermitz and Harris 1987, Stermitz and Pomeroy 1986, Adler and Wink 2001). This may be the case with the LULI in our study. However, our other lupine species, LULE, had no surviving pairs in either year of the study. Thus, these effects may vary significantly by lupine species. The RONU hosts may have limited herbivory by spine production, making an attempt to eat the shorter *Castilleja* plants a painful proposition. In the case of SOCA, it is unclear whether the abundant foliage hid *Castilleja* from the sights of herbivores, or a defensive compound of some kind was provided through parasitism.

Reproductive traits

An additional metric of value for restoration and conservation is the overall output of seed from each host treatment. This helps to determine a population's ability to persist and replenish plants following mortality. To this end, we plotted the sum of seed capsule averages (per all plants, including those that died) in 2013 and 2014 in each treatment group to show the contribution of seed capsules by *Castilleja* in that host treatment over two years (Figure 2.6). While there may

be variation in the number of seeds in each capsule, one study has shown that the number of seeds per capsule is not significantly different with different host treatments in this *Castilleja* species (Fisher *et al.* 2015). Over the span of two years, living *Castilleja* plants produced the most seed when parasitizing ACMI and DACA: each species averaged an output equivalent to over 2000 seed capsules per 100 plants (Figure 2.6).

Still producing the equivalent of over 1000 seed capsules per 100 plants were the DECA, FERO, SYAL, and ERLA groups, and with an average of around 180 seeds per capsule (Fisher *et al.* 2015), this equates to over 180,000 seeds. Of these four, SYAL is especially exciting since woody species have not previously been described as hosts for this species of *Castilleja*. The mechanism for this high seed yield due to SYAL parasitism is unclear, but root structure and lack of defense against root parasites may be factors involved. The large stature of SYAL may also play a role in protection, or in an increased nutrient flow to the parasite. It should be noted, however, that the high performance of *Castilleja* with SYAL is markedly decreased in 2014 compared to 2013. It is possible that as the SYAL matured, its roots became more resistant to parasitism or the plant grew large enough to shade *Castilleja,* thereby decreasing the parasite's ability to sequester carbon.

The lowest showings for seed capsule production were the ERSP, LULI, RONU, and SOCA groups, all with the equivalent of fewer than 500 seed capsules per 100 plants (Figure 2.5). These species may be beneficial to *Castilleja* in some growth traits (such as LULI limiting herbivory), but their benefits do not appear to extend to the end goal of reproduction. ERSP and SOCA are closely related and may have a mechanism for defense against parasitism as suggested

above (Cameron *et al.* 2006). RONU, a woody species, has not previously been tested as a host and its thicker roots may not be compatible with *Castilleja's* method of parasitism. LULI, while seeming to confer some benefits to *Castilleja,* does not seem to be adding nutrition that assists in reproduction. Whether this is due to active resistance on the part of the host, or a physiological trait within the roots that limits haustorial attachment is unknown.

Overall patterns

Changes in top performing groups over the two years of the study suggest that host influence may fluctuate over time or based on additional factors (Tables 2.3 and 2.4). *Castilleja* may be benefitted by different hosts more or less depending on life stage or environmental changes. This has been documented in a study using *Castilleja wightii* in which the parasite had greater growth and reproduction when parasitizing multiple hosts than with a single host species (Marvier 1998). The host species with the highest management value is a subjective measure based on which traits one is concerned with. If long-term survival is the main objective, DACA and DECA may be the "best" hosts. If higher overall seed output is the goal, DACA and ACMI may be superior choices.

Despite the overall high rankings of ACMI, ERLA, and FERO, it appears that some hosts are higher performers for *Castilleja's* growth traits (e.g. DECA), while others confer greater benefits for reproduction (e.g. DACA). One implication for management is that an ecosystem with a diversity of native prairie species may be the ideal habitat for preservation and reintroduction of this species. It also demonstrates that more work is needed to elucidate the mechanisms of differing benefits based on host species identity.

Management implications

The native habitat of this species, Pacific Northwest prairies, has decreased considerably in area since European settlement, leading to a plethora of native prairie species becoming rare, threatened, or endangered. One currently endangered species is a rare butterfly, Taylor's checkerspot (*Euphydryas editha taylori*), which lays eggs on *C. levisecta* leaves so that larvae can feed on plant tissues when they hatch (Haan, unpub. data). This connection combined with plant community interactions through parasitism make *C. levisecta* a crucial member of these prairie communities. These in conjunction with a federal mandate for recovery create the need for a more nuanced investigation of the contributions of host species to *C. levisecta* recovery.

This study sheds light on the presence and possible use of novel hosts for propagation, restoration, and conservation of *C. levisecta*. Neither DACA nor DECA have been previously studied as hosts in this depth, and our results indicate they may be wise choices to add as host plants for *C. levisecta*. In addition, DACA and ACMI appear to be the best choices when goals include high seed set, although it is not known whether the relatively high numbers of seed capsules correlate with actual higher numbers of viable seeds. The issue of host plant mortality could be overcome in seed increase beds by seeding greater numbers of ACMI seed, or reseeding yearly to ensure adequate host plants for each *Castilleja*.

In addition to selecting specific hosts to improve specific traits in *C. levisecta*, it may be beneficial (as previously mentioned) to provide multiple host species to give the parasite a mixed diet (Marvier 1998). A recent study also found that higher microsite richness of native perennial

forbs was strongly correlated with *C. levisecta* survival and flowering (Dunwiddie and Martin 2016). This indicates that a higher number of potential hosts (native plants) broadens the diet available to *C. levisecta.* Its increased performance with greater richness may imply that the parasite gains benefit from multiple hosts at once.

Limitations

There were several limitations in this study that we hope will be overcome with future experiments. Perhaps the greatest was the lack of distinction between host and parasite mortality. If we had measured this factor (rather than one or both dying being uniformly recorded as the death of the pair), we may have been better able to draw conclusions about survival. Additionally, we would always benefit from more host species to test and larger sample sizes. Mortality in the field will often shrink sample sizes to less than optimal, and starting with larger numbers may curtail this issue. We also had issues with deer browse, which may have confounded our analyses. In the future, excluding deer from the site may be an appropriate course of action.

Conclusions

This experiment shows the range of responses of this threatened parasite to a variety of different hosts. *Castilleja* appears to gain at least some benefits from a wide range of hosts and hosts differ in the quantity and quality of their benefits, and for the most part having any host produced more positive results than having no host.

The lack of a single consistent high-performing host species across all traits in the two sampling years indicates that host contributions to *Castilleja's* growth and reproduction may be variable and potentially compounded by environmental factors. *Castilleja levisecta* seems to receive growth and reproductive benefits from a range of host species, thus addition of a wide range of native grasses, forbs, and possibly woody shrubs may be advisable in restoration and conservation settings for optimal performance.

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Supplemental Materials

Table S2.1 Glm/lm outcomes for companion plantings. Asterisk (*) denotes statistically significant p-values. Deviance values were used for survival and Spring browsed plants, all others used sum of squares (SS) values.

Variable	df	Deviance/SS	P
Survival 2013	11	-41.64	<0.0001
Spring flowering stems 2013	10	-60.49	< 0.0001
Spring browsed stems 2013	10	-87.67	< 0.0001
Spring browsed plants 2013	10	-19.78	0.0314
Height 2013	10	-2308.1	0.0007
Fall fruiting stems 2013	9	-56.91	< 0.0001
Fall total capsules 2013	9	-447.36	< 0.0001
Survival 2014	11	-31.71	0.0009
Spring flowering stems 2014	10	-118.64	< 0.0001
Spring browsed stems 2014	10	-97.54	< 0.0001
Spring browsed plants 2014	10	-13.15	0.2154
Height 2014	10	-4417	0.0002
Fall fruiting stems 2014	10	-82.76	<0.0001
Fall total capsules 2014	10	-703.68	< 0.0001

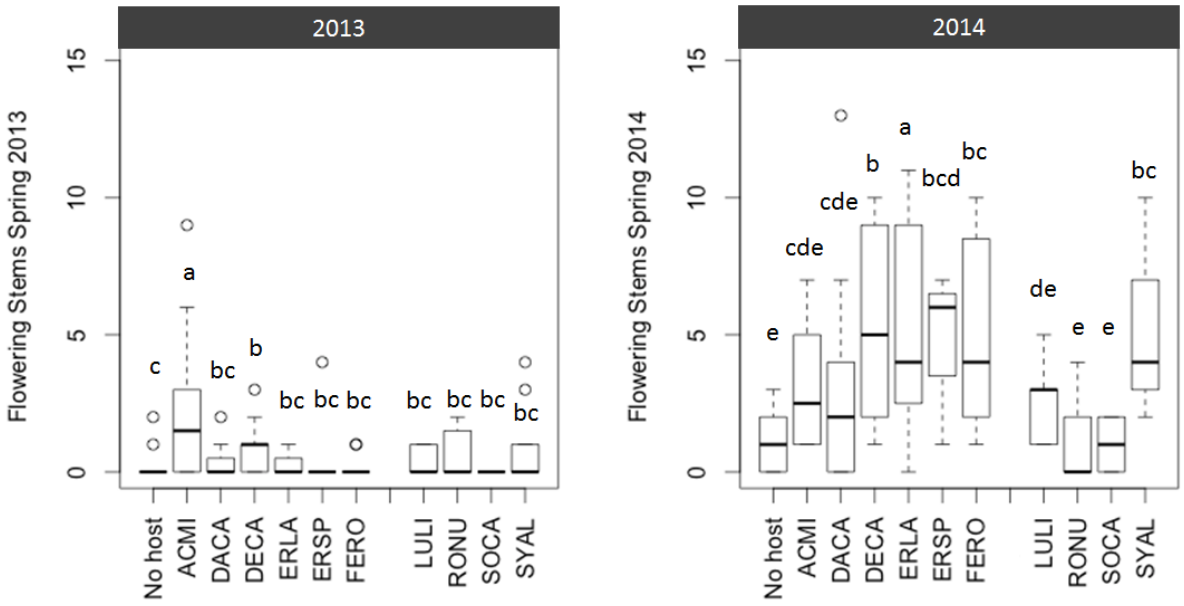

Figure S2.1 Flowering stems of *Castilleja* in 2013 and 2014 by host species. Circles indicate outliers, some of which were removed to improve scaling (DECA, y = 25, and ERLA, y = 46). Data were only included from live pairs. See Table 2.1 for full species names and Figure 2.3 for plot description.

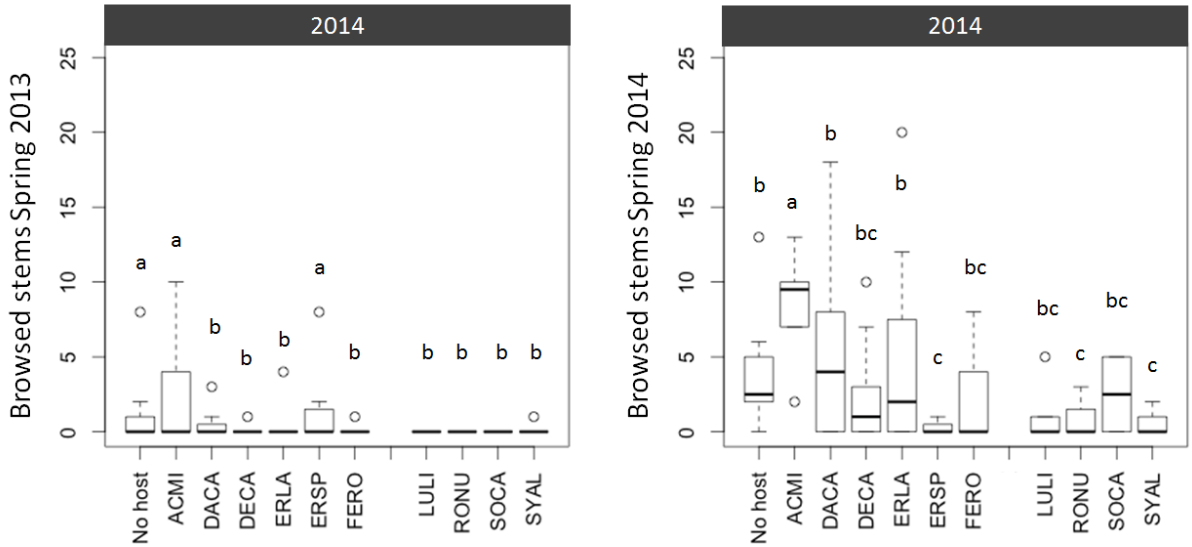

Figure S2.2 Browsed stems of *Castilleja* in 2013 and 2014 by host species. Data were only included from live pairs. See Table 2.1 for full species names and Figure 2.3 for plot description.

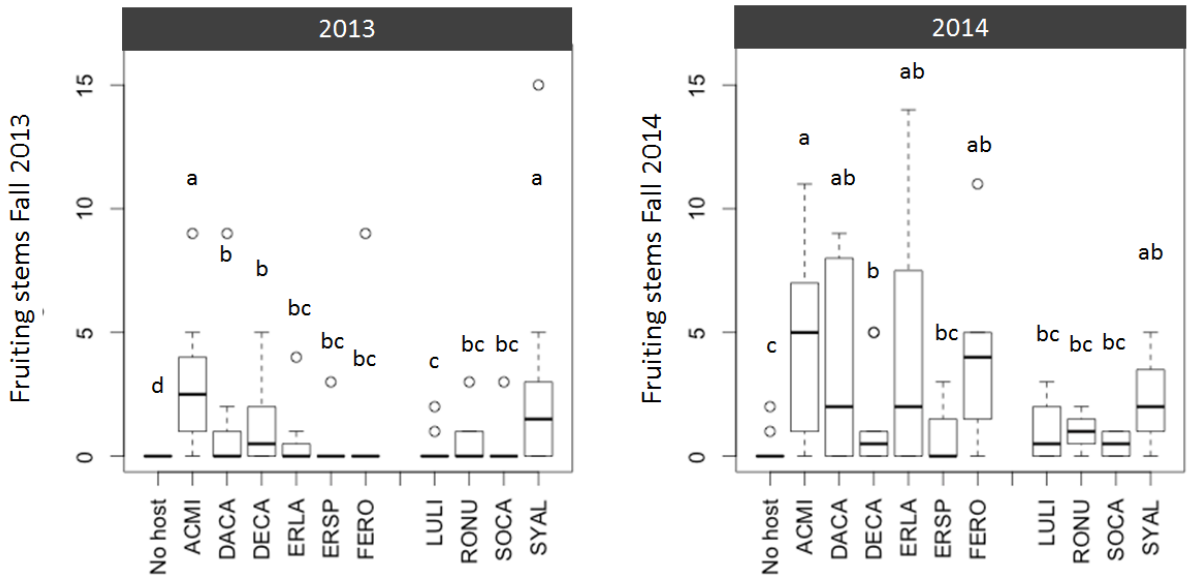

Figure S2.3 Fruiting stems of *Castilleja* in 2013 and 2014 by host species. Circles indicate outliers. Data were only included from live pairs. See Table 2.1 for full species names and Figure 2.3 for plot description.

Chapter 3: Differences in parasites' host-derived nutrients shown by stable isotopes of carbon and nitrogen

Abstract

Parasitic plants are widespread and present in a variety of ecosystems across the globe. The mechanisms by which they receive differential advantage from different hosts is not well understood. In order to test differing nutrition as a mechanism for host suitability, we grew hemiparasite *Castilleja levisecta* with one of seven host species and added stable isotopes to the host plants. By measuring the elevated levels of the isotopes (^{13}C and ^{15}N) in the parasite, we determined that nutrient acquisition does vary by host species, especially in the case of carbon. Thus, we show that one mechanism for differential host suitability is the difference in key nutrients gained from each host.

Introduction

Parasitic angiosperms have multiple functions within ecosystems, from invasive agricultural pests like *Striga* to ecosystem engineers such as *Rhinanthus*. The complexity of their interactions with their plant communities has been studied to some extent, but we lack a more sophisticated understanding of the specifics within host-parasite interactions of non-pest species. While it is of critical importance to investigate the mechanisms of problem parasites for the purpose of resistance or eradication, it still remains important to study those parasites that are not pests, or even may be contributing positive influences to a system.

In particular, the differences in host plants' contribution to many parasites' growth is not well understood. Hemiparasites are often selective in the host species they parasitize, in that their attachment to hosts near them is often non-random (Suetsugu *et al.* 2008). Many parasitic plants

appear to have "preferred" hosts which give a greater advantage to the parasite than other available hosts (Alder 2003, Li *et al.* 2013). These hosts may confer added benefits to the parasite in the form of nutrients, water, or other compounds (e.g. defensive alkaloids to reduce herbivory, Stermitz and Harris 1987, Marko and Stermitz 1997).

In our study, we looked at a possible mechanism for differences in parasite performance due to host: differences in acquired nutrients. Specifically, we chose to investigate carbon and nitrogen. These two macronutrients are essential for plant growth, and both can be limited in certain environments.

Soil nitrogen is limiting in many systems for all plants including parasites. In some cases, it appears that it may be more advantageous for parasites to gain nitrogen primarily from host plants heterotrophically than to invest in larger root systems to gain nitrogen autotrophically (Li *et al.* 2013). While hemiparasites are able to photosynthesize to sequester carbon without host assistance, they can sometimes be shaded out by surrounding vegetation and may acquire significant percentages of their carbon from host plants (Tesitel *et al.* 2010). While xylem sap generally has lower concentrations of carbon than phloem sap, other xylem-tapping hemiparasites show host-derived carbon to make up between 5 and 62% of carbon in their tissues, (Marshall and Ehleringer 1990, Schulze *et al.* 1991, Pate *et al.* 1991, Marshall *et al.* 1994). One study using *Castilleja linariifolia* found hetertrophic carbon gain to average 40% of total parasite carbon (Ducharme and Ehleringer 1996). The acquired carbon from host xylem sap may come from carbon attached to nitrogen-based solutes, amino acids, and organic acids (Marshall and Ehleringer 1990, Marshall *et al.* 1994).

The acquisition of carbon and nitrogen (and other nutrients) may also be linked and regulated by the parasites' rate of photosynthesis (Tesitel *et al.* 2015). We looked at a range of host species to determine if there were, in fact, significant differences in parasites' heterotrophic nutrient gain due to host identity.

Methods

Study species

We have investigated these host-parasite interactions using *Castilleja levisecta*, a perennial hemiparasite native to the prairies of the Pacific Northwest. As a hemiparasite, *Castilleja* produces chlorophyll and has the capability to sequester its own carbon via photosynthesis. It is also a facultative parasite: it does not require a host to grow and reproduce. An additional benefit to using *C. levisecta* is its status as a federally threatened species. Once ranging from British Columbia to the Willamette Valley in southern Oregon, this species was reduced at one time to just 11 populations in the world. As recovery efforts continue, a better understanding of the mechanisms and specific of host compatibility will serve to guide land managers and conservationists to hone recovery efforts of this species.

Method of nutrient measurement

We chose to use stable isotopes of carbon and nitrogen as labels to measure nutrient movement between hosts and parasites. Stable isotopes are non-radioactive atoms of elements with an additional neutron compared to the most abundant form of that element. In the case of carbon, ^{12}C is the most abundant form, and ^{13}C is the isotope used for labelling. For nitrogen, ^{14}N is the

most abundant form, and ^{15}N is used for labelling. These labels are often used to track nutrient exchanges between organisms at different trophic levels, such as insects, fungi, and plants (e.g. Leroy *et al.* 2011).

Lag time pilot

An initial test with a single host species was carried out to determine an appropriate lag period for sampling. *Castilleja* and host species *Eriophyllum lanatum* were grown from seed and planted in pairs in containers. Seedlings were planted with roots overlapping to increase the likelihood of haustoria formation. Pairs were grown in the greenhouse for five weeks prior to labelling. At the time of labelling, all host plants were labelled with ^{13}C-rich carbon and ^{15}N-rich nitrogen (methods consistent with labelling methods in full experiment below). Groups of pairs were destructively sampled every three days: plants separated and washed, then dried in a 65-degree F drying oven. Each plant was ground and sent to the UC Davis Stable Isotope Facility for analysis of isotope content. Using this method, we determined that a 30-day lag time would yield the most accurate results. For full lag pilot details see Appendix 3.1.

Full experiment

The final experiment used seven host species: *Achillea millefolium* (ACMI), *Danthonia californica* (DACA), *Eriophyllum lanatum* (ERLA), *Erigeron speciosus* (ERSP), *Festuca roemeri* (FERO), *Lupinus lepidus* (LULE), and *Solidago missouriensis* (SOMI). Basis for host species inclusion is outlined in Table 3.1 below. As with the lag time pilot study above, plants were grown in host-parasite pairs for 5 weeks prior to labelling. All plants were grown in a greenhouse setting in a seedling soil mix (Sunshine #1) with minimal fertilizer. Pairs were

grown in greenhouse plug trays and watered regularly to ensure survival. Each host-parasite pair was replicated 26 times. Some mortality occurred during the experiment, and the lowest final treatment group size was 18 host-parasite pairs.

Table 3.1 Host species used in companion plantings. Basis for inclusion key: 1 - known host of *C. levisecta*; 2 - genus parasitized by other *Castilleja* species; 3 - observed near robust *C. levisecta* in field).

Species	Family	Functional Group	Basis for inclusion
Achillea millefolium	Asteraceae	Forb	1
Danthonia californica	Poaceae	Grass	1
Erigeron speciosus	Asteraceae	Forb	1
Eriophyllum lanatum	Asteraceae	Forb	1,3
Festuca roemeri	Poaceae	Grass	1
Lupinus lepidus	Fabaceae	Legume	1,2
Solidago canadensis	Asteraceae	Forb	1

Isotope labelling

Carbon was added via $^{13}CO_2$ (purchased from Sigma-Aldrich) injected into bags covering host plants as in Philip & Simard (2008), (see Figure 3.1). Injected bags were left on host plants for a five-hour pulse period, then removed in a windy environment to avoid contamination. Nitrogen was added by immersing cut leaves of the host plant in a 30 mM ammonium sulfate ($(^{15}NH_4)_2SO_4$) solution (purchased from Sigma-Aldrich) as in Aflakpui *et al*. (2005), (see Figure 3.1). Leaves were left in the emersion for 24 hours, after which the solution was removed. All pairs were given a thirty-day lag period before sampling to allow transfer from host to parasite. Once the lag period ended, host and parasite were separated, rinsed, and dried. Whole plant *Castilleja* individuals were then ground to powder and a sample (1.0 - 2.5 mg) of each sent out for testing.

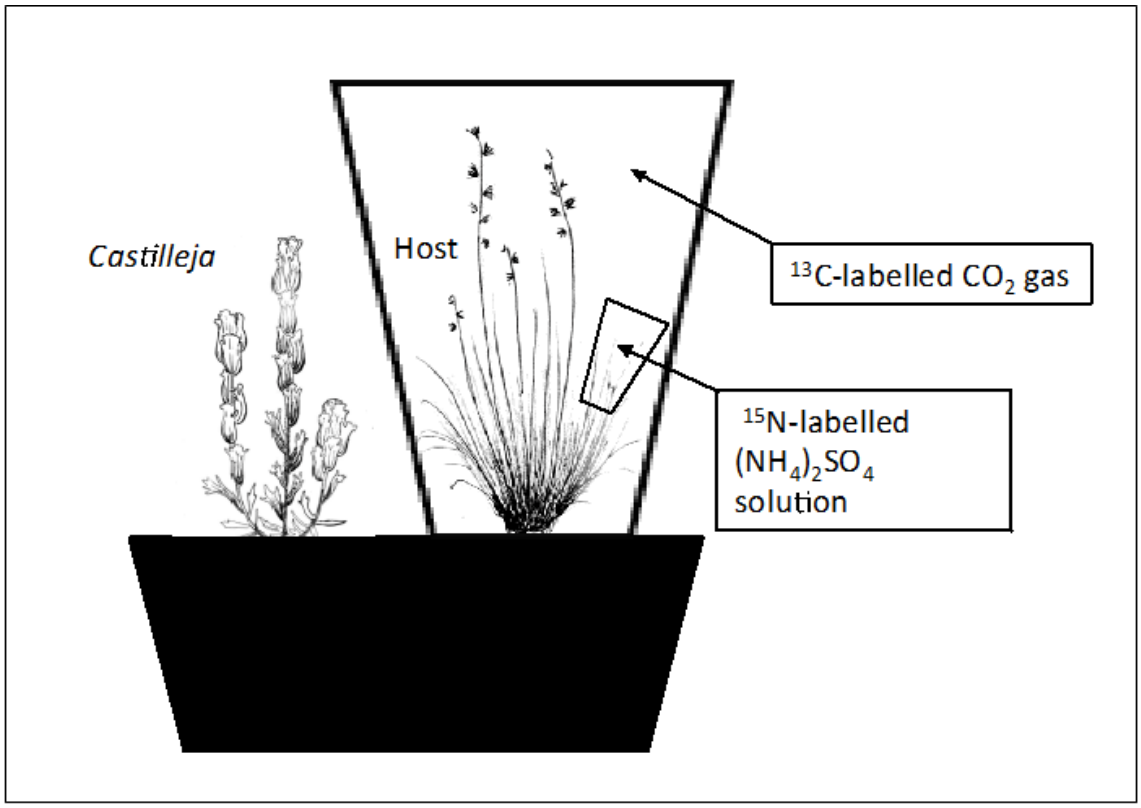

Figure 3.1 Schematic showing labeled carbon and nitrogen addition to host plant parasitized by *Castilleja levisecta*.

Samples were sent to the UC Davis Isotope Lab for analysis. Data were returned as δ (del) values of carbon and nitrogen. Del is defined by the following equation, where R is the ratio of the heavy to the light isotope, and the standard is an accepted, known substance used for comparison:

$$\text{Del value (in permil)} = (R_{sample}/R_{standard} - 1) * 1000$$

The standards used for comparison were VPDB (Vienna Pee Dee Belemnite) for carbon and air for nitrogen.

Analysis

Values of each host group were compared for del ^{13}C and del ^{15}N using a Kruskal-Wallis test (alpha = 0.05) in R statistical software (version 3.2.2, Appendix B). This test was used because the data did not fit the assumptions of normal distribution needed for an ANOVA test. Pairwise comparisons were then made between each treatment group to determine specific differences in host-derived nutrition using a post-hoc Nemenyi test (alpha = 0.05). We also compared the del values of carbon and nitrogen within host treatments and overall in the study.

Results

Outcomes from the Kruskal-Wallis test are shown in Table 3.2 below. Both carbon and nitrogen analyses had significant results.

Table 3.2 Chi-squared, degrees of freedom, and p-values for Kruskal-Wallis tests of host identity as a predictor of del values of ^{13}C and ^{15}N in *Castilleja.*

	Chi-squared	**df**	**p-value**
Carbon	51.112	6	$p < 0.00001$
Nitrogen	57.164	6	$p < 0.00001$

Carbon

Host identity was a significant predictor of del ^{13}C (Table 3.2). Some host groups differed in the amount of labelled carbon found in the paired *Castilleja* (Figure 3.2). *Solidago* was the highest performing host group, with the highest del ^{13}C value (the highest portion of *Castilleja* biomass was host-derived). This group differed significantly from all other treatment groups. All other

host groups were similar to each other, with the exception of the lowest performing group: *Erigeron*.

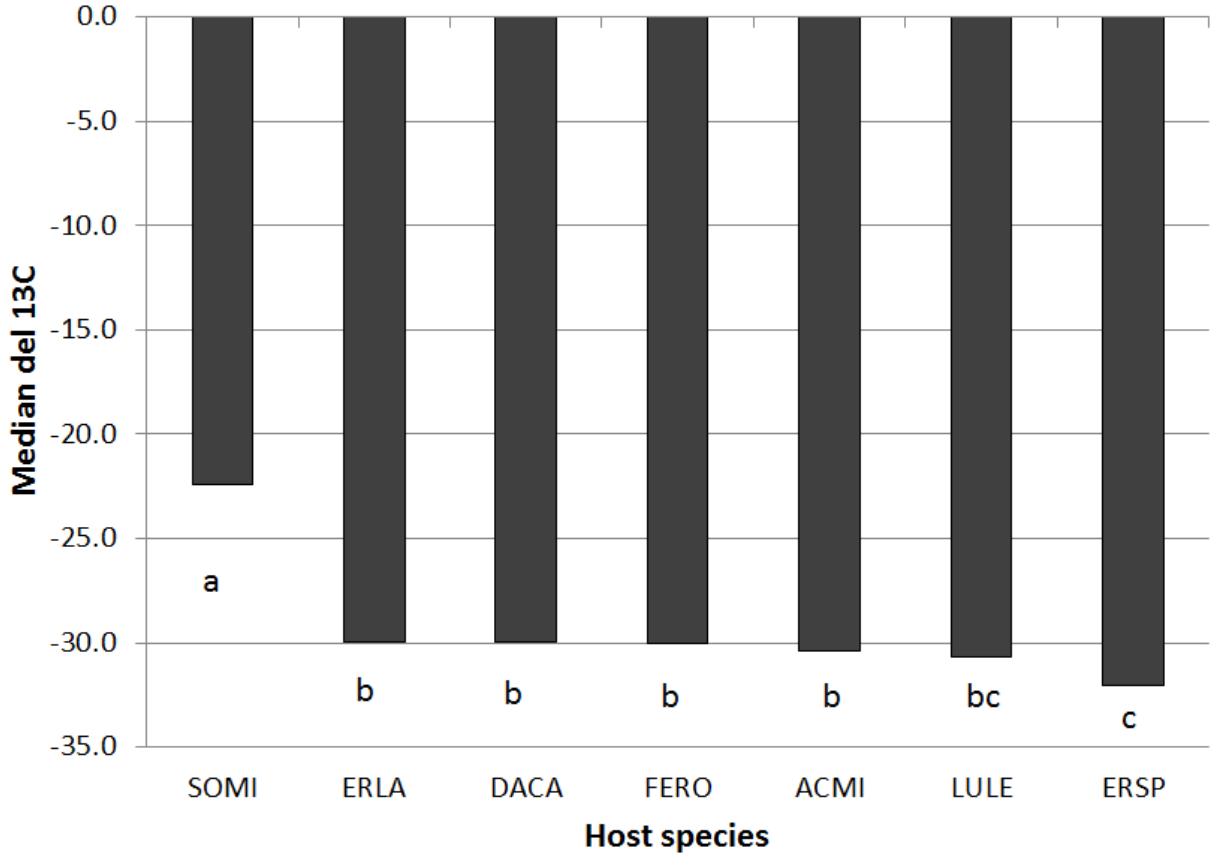

Figure 3.2 Median del values of ^{13}C in *Castilleja* by host species. Letters represent grouping by significant differences ($p<0.05$).

Nitrogen

Host identity was also a significant predictor of del ^{15}N (Table 3.2). As with carbon, many groups' del 15N values were significantly different from others, and a wide range of values was seen among the groups (Figure 3.3). Highest values were present in those *Castilleja* parasitizing *Eriophyllum,* and *Achillea*, while the lowest value was seen in the *Erigeron* treatment group.

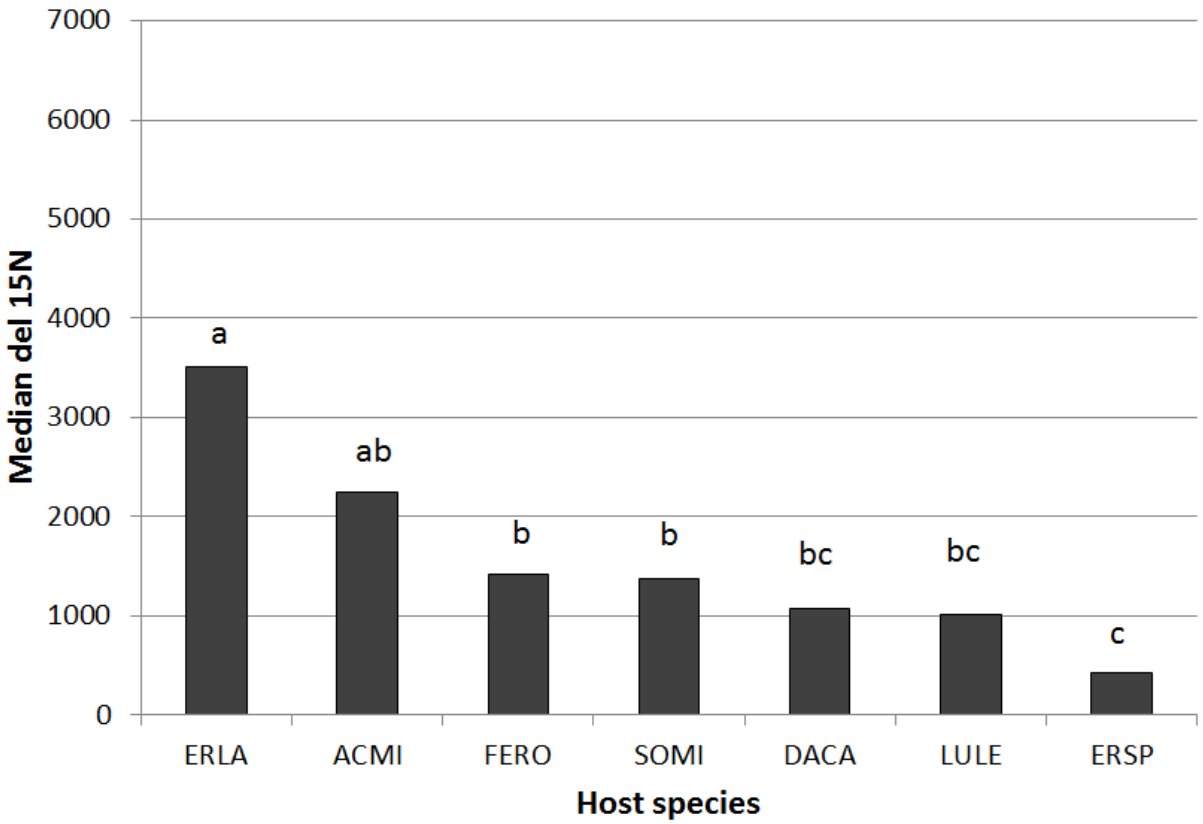

Figure 3.3 Median del values of ^{15}N in *Castilleja* by host species. Letters represent grouping by significant differences ($p<0.05$).

Discussion

Carbon

Globally (excluding dry climates) most C3 plants' del ^{13}C values range between 28 and 32 (Kohn 2010). Based on this, there did not appear to be large quantities of host-derived carbon present in *Castilleja* overall (Figure 3.2). There are two likely reasons for this. The first is that the actual product acquired from the host (xylem sap) does not contain large quantities of carbon to begin with. If this is the case, the parasite would need to obtain large quantities of xylem sap in order to get significant amounts of carbon from its host. Looking at our lag pilot data (Figure

S3.2), the del ^{13}C in *Castilleja* peaks at a far lower value than the host plant. This indicates that, while there may be some quantity of carbon acquisition from hosts, *Castilleja* does not seem to gain large amounts of carbon from host plants. The second possible reason is that there is not a great enough need, and thus not a large enough "pull" from the parasite for carbon. Since *Castilleja* is a hemiparasite and can sequester its own carbon, it does not rely on its host for a significant portion of its overall carbon. Our test environment was well-lit (greenhouse) and all plants were early enough in their life cycles that they did not compete with each other for light. Had we used older pairs in which hosts shaded out the parasites, perhaps we would have seen greater amounts of host-derived carbon in the parasites. Studies on hemiparasites in the same family (Orobanchaceae) found that shading increased the proportion of parasite biomass that was host-derived (Tesitel *et al.* 2011).

Nitrogen

Nitrogen acquisition differed greatly between host treatments (Figure 3.3). The standard used for ^{15}N comparison is atmospheric nitrogen (del = 0), and due to low levels of nitrogen isotope discrimination by plants, the expectation is that base (unlabeled) del ^{15}N values are near zero (Evans and Ehleringer 1994). Compared to this baseline, all treatments in our study showed high levels of host-derived nitrogen in the parasites. Earlier studies have shown *Eriophyllum* to be a good host for this parasite (Delvin 2013), and the nitrogen input shown in Figure 3.3 may point to a mechanism. Pacific Northwest prairie remnants are often found in nitrogen-limited soils (Dunwiddie and Bakker 2011), so additional inputs of host nitrogen could have great benefits to the parasite. An additional note is the high level of nitrogen gained from the host *Achillea*.

Additional studies in this lab (Schmidt, unpub. data) have found *Achillea* to be a highly suitable host for this species, and greater inputs of nitrogen could lead to this advantage.

Care should be taken with interpretation of ^{15}N input from the *Lupinus* species. The measured level of nitrogen input does not include additional nitrogen gained from bacteria-mediated atmospheric N-fixation, which would not have been labelled using our methods. With this in mind, we are unable to fully estimate the true amount of nitrogen being sequestered by *Castilleja* parasitizing lupine hosts. The additional nitrogen supplied by this host could increase *Castilleja*'s competitive ability and allow it greater access to sunlight by means of increased height.

Study limitations

One caveat to this study is the possibility that the results could have been skewed due to the lag time and isotope labelling methodologies applied. The pilot study to determine lag time utilized a single host species (*Eriophyllum*), while the full experiment employed multiple host species with varying leaf sizes. This may be important for the nitrogen isotope results, as cut leaves were the method of application of the labelled compound (see methods). While an attempt was made during labelling to ensure an adequate area of tissue was cut on each species, it was difficult to cut large areas of grass leaves, especially those as thin as *Festuca*. It is yet unclear whether this significantly affected our results (by differential host uptake of carbon or nitrogen), but should be addressed in future experiments of this nature. It is also possible that some degree of host benefit was related to host species' root structure or number of haustorial connection, which were not categorized in this study.

Implications

This study points to mechanisms behind host preference by parasites and differential parasite performance related to host species. For *Castilleja levisecta* specifically, there are implications for management due to its status as a threatened species. In sites where competition with taller-statured species is likely, *C. levisecta* may have less access to sunlight and thus become carbon limited. In this case land managers may wish to seed or plant *C. levisecta* with *Solidago missouriensis* to increase heterotrophic carbon acquisition opportunities for the parasite. Similarly, if soil is nitrogen-limited, it may be prudent to plant or seed with *Eriophyllum lanatum*. Some parasitic plants have shown improved performance with mixed-host diets (Marvier 1998), so an optimal host regime may even be a mix of *S. missouriensis* and *E. lanatum*.

On a broader scale, this research adds to the growing body of knowledge investigating the mechanisms of host selectivity in parasitic angiosperms. It adds to the conclusions of Demey and colleagues' 2015 study showing differential performance of two other hemiparasites (*Rhinanthus* and *Pedicularis*) based on host identity. Their study showed differences within rather than among host functional groups, aligning with our host-derived nutrition results. Rather than relying on the broad categorization of functional groups to define host quality, we need to investigate at a finer scale to identify optimal host species. With this as a platform, we can continue to investigate the intricacies of host-parasite relationships.

Conclusions

This study sheds light on the mechanisms behind host suitability to parasitic plants and the role that nutrition plays in these relationships. Future studies may seek to elucidate the precise fate of host-derived nutrients in parasites (leaves, stems, roots), or to track additional host-derived resources such as water or specific defensive compounds. Gaps in knowledge are also present in the study of this mechanism on additional parasitic angiosperms. It is our hope that experimentation in this line of inquiry continues for both widespread and rare or endangered parasitic angiosperms.

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Appendix 3.1: Lag time pilot experiment

Timing of parasitic nutrient uptake can vary between species and within species at different life stages. In the study above we looked into the nutrient transfer of many host species to a specific parasite: *Castilleja levisecta*. In order to prepare to test this transfer using multiple host species for *Castilleja levisecta*, we first needed to determine an appropriate lag time (time between isotope labelling of hosts and sampling of *Castilleja*).

An initial test with a single host species was carried out to determine an appropriate lag period for sampling. *Castilleja* and host species *Eriophyllum lanatum* were grown from seed and planted in pairs in containers. Seedlings were planted with roots overlapping to increase the likelihood of haustorium formation. Pairs were grown in the greenhouse for five weeks prior to labelling. At the time of labelling, all host plants were labelled with ^{13}C-rich carbon and ^{15}N-rich nitrogen.

Carbon was added via $^{13}CO_2$ injected into bags covering host plants as in Philip & Simard (2008). Injected bags were left on host plants for a five-hour pulse period, then removed in a windy environment to avoid contamination. Nitrogen was added by immersing cut leaves of the host plant in a 30 mM ammonium sulfate solution ($(^{15}NH_4)_2SO_4$) as in Aflakpui *et al*. (2005). Leaves were left in the emersion for 24 hours, then the solution was removed.

Groups of pairs were destructively sampled every three days: plants separated and washed, then dried in a 65-degree F drying oven. Each plant was ground to powder and sent to the UC Davis Stable Isotope Facility for analysis of isotope content. Data was returned as δ (del) values of

carbon and nitrogen. Del is defined by the following equation, where R is the ratio of the heavy to the light isotope, and the standard is an accepted, known substance used for comparison:

$$\text{Del value (in permil)} = (R_{sample}/R_{standard} - 1) * 1000$$

Del ^{15}N values of the host plants decrease over time as *Castilleja*'s values increase (Figure S3.1). There is an early bump in host del ^{13}C values followed by a smaller bump in *Castilleja*'s values around 12 days following labelling (Figure S3.2). However, after this initial bump the values of del ^{13}C in *Castilleja* continue to fluctuate. The scale in each of the graphs should also be noted, such that despite the seemingly smaller slope in the rise of *Castilleja*'s del ^{15}N, there is a much greater increase in labelled nitrogen over time than there is carbon. This in itself is somewhat expected due to *Castilleja*'s status as a hemiparasite: it is able to photosynthesize and sequester carbon without the aid of a host. This fact coupled with the full sun conditions of the experiment perhaps led to less need for host-derived carbon in this setting.

Based on the steady increase in del 15N of *Castilleja* through the duration of the pilot and despite the minor uptick in del 13C around the midpoint of the experiment, we chose to use the longest tested lag time, 30 days, for the full experiment. The nitrogen transfer appeared to be the stronger signal to indicate the highest level of xylem sap transfer.

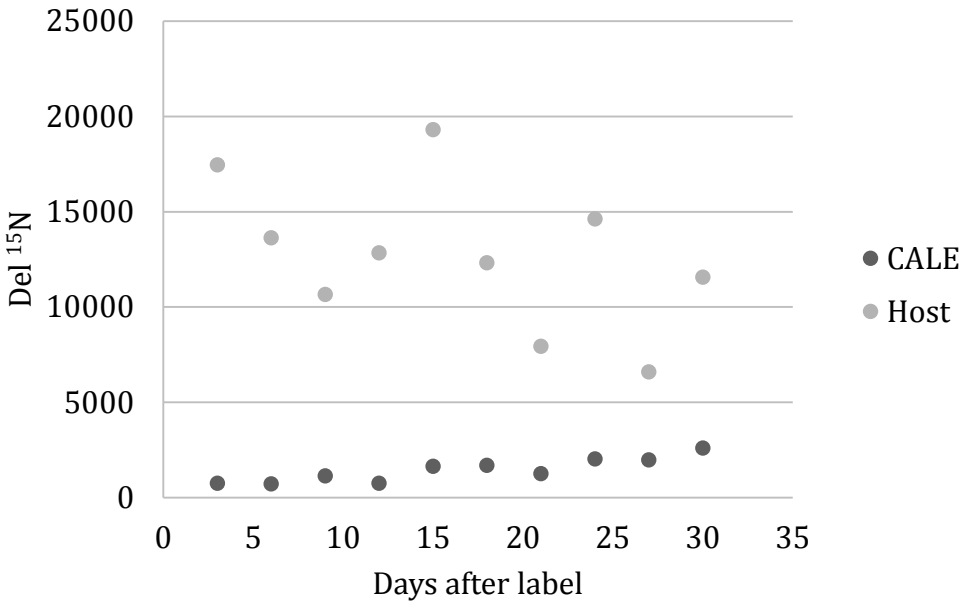

Figure S3.1 Del ^{15}N values of *Castilleja* (CALE) and host species in days following label addition to host plant.

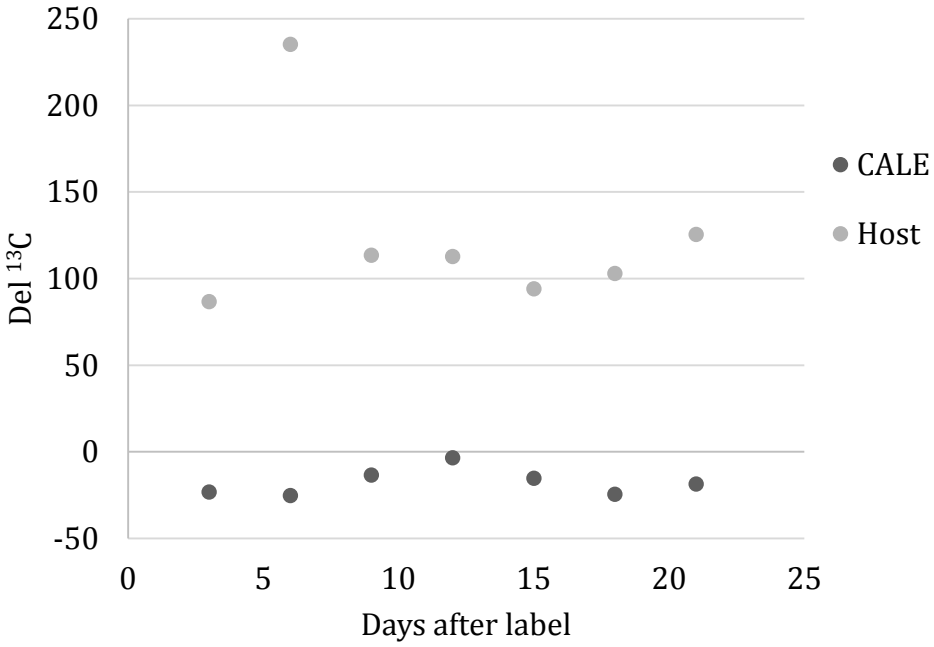

Figure S3.2 Del ^{13}C values of *Castilleja* (CALE) and host species in days following label addition to host plant.

Chapter 4: Do all parasitic plants work as ecosystem engineers? A case study in Pacific Northwest prairies

Abstract

Parasitic plants inhabit a unique niche in their native ecosystems, which some ecologists argue makes them ecosystem engineers. Several case studies have shown parasitic plants affect the composition of the communities they inhabit. We examined this effect at varying spatial and temporal scales using Pacific Northwest prairies as a test system, and hemiparasitic *Castilleja* as our ecosystem engineer. Unlike previous studies, we find conflicting evidence for this community effect, whether due to a weak signal or masking by other community change factors such as local weather. Our results suggest that examining this type of ecosystem effect on a larger spatial and temporal scale may add nuance to our understanding of parasitic plants' roles in their native systems, and that parasites may not always have a strong effect.

Introduction

Parasitic angiosperms have long been studied for their negative effects on crop species and silviculture (e.g. Spallek *et al.* 2013). *Striga* (witchweed) and *Arceuthobium* (dwarf mistletoes) are two examples of heavily studied parasites. *Striga* parasitizes food crops such as corn (Mumera and Below 1993), and dwarf mistletoes grow on and parasitize large trees such as *Pinus* (Stanton 2006). Much of our initial knowledge of mechanisms of parasitism was gleaned from the study of these devastating parasites.

More recently, scientists have begun investigating the potential for positive effects of parasites on their native systems. These positive impacts range from facilitation of greater species richness (Bao *et al.* 2015) to increased nitrogen transformation in soil (Demey *et al.* 2014).

Several studies show that parasitic angiosperms may play an important role in community assembly and composition (Pywell *et al.* 2004, Grewell 2008, Decleer *et al.* 2013). There are many species which may facilitate nutrient cycling in their native ecosystems by acquiring nutrients from their host(s) and redistributing them through nutrient-rich litter (Quested *et al.* 2005, Press 1998). Hemiparasitic Orobanchaceae are also thought to alter competition between hosts and non-hosts, thus causing changes in community composition (Phoenix and Press 2005). In some cases, the parasite's negative impact on host biomass may sometimes be outweighed by their positive impact on the total plant community biomass (Fisher *et al.* 2013).

In Europe, the addition of hemiparasitic *Rhinanthus* species into degraded landscapes has been used in grassland restoration to increase species richness and diversity (Pywell *et al.* 2004, Westbury *et al.* 2004, Bullock and Pywell 2005). Overall, studies are beginning to show that parasitic plants can have significant direct and indirect effects on the surrounding plant community (Watson 2009).

In a recent study, Bao *et al*. (2015) showed an increase in species richness on plots with hemiparasite *Pedicularis kansuensis* present. Their study used a degraded grassland site in the Qinghai-Tibet Plateau and set up plots with and without *P. kansuensis* through removal of the parasite from control sites. After one year of this treatment, they measured species richness in each plot and found greater richness in those plots with the parasite present.

We examined this effect to determine the variability it shows over various spatial and temporal scales. Our objective was to answer the following questions:

1. Is there an effect of parasites on plant community similar to recent findings present in an untested system?
2. Is this effect consistent at all sites in all years?
3. If present, does this effect grow stronger or weaker over time?

Methods

Study species and site

Castilleja levisecta Greenm. and *C. hispida* Benth. are short-lived perennials native to the prairies of the Pacific Northwest. Both species are facultative hemiparasites: they procure resources from other host plants but they are able to survive and reproduce without a host present, and they have the ability to sequester carbon via photosynthesis. These species, as with all plants in the genus *Castilleja*, attach to their hosts using parasitic root structures, called haustoria, which facilitate a xylem-xylem connection between the parasite and its host (Kuijt 1969). Both species have small, nondescript flowers covered by showy bracts; *C. levisecta*'s are a bright yellow and *C. hispida* most often red-orange but occasionally yellow.

The prairies of the Pacific Northwest represent a unique ecosystem: they were historically maintained with intentional burning for centuries by native peoples (Boyd 1999) and are home to a variety of rare and endangered species (Altman 2011, Fazzino *et al*. 2011, Schultz *et al*. 2011, Stinson 2005). Since European settlement, these ecosystems have declined from covering over 73,000 ha to fewer than 7,000 ha, due in large part to fire suppression, development, and agriculture (Franklin and Dyrness 1988, Crawford and Hall 1997, Dunwiddie 2002).

Abandoned agricultural fields show promise as sites for prairie restoration in the Pacific Northwest, as native prairies historically occupied many of these areas. We chose four sites in western Washington that had recently been restored to native grasslands from abandoned agricultural fields: Ebey's Landing, Glacial Heritage, Smith Prairie, and West Rocky. Each site was divided into plots (25 - 40 m^2) which were treated with different combinations of soil pretreatments (burn, solarize, herbicide) and native seed mixes (grass-rich, forb-rich, mixed) as part of a larger restoration experiment in 2010. Each plot contained 4 – 6 quadrats that were one meter squared. We chose to use a subset of these data (the burn/forb-rich combination) as they had the greatest range of *Castilleja* densities. Each year in the spring all quadrats were monitored for percent cover of every species present (composition) as well as density of *Castilleja* (measured as total number of plants per quadrat). Densities were monitored from 2011 until 2013 in every site except West Rocky (2011 and 2012 only), and composition was monitored from 2012 until 2014 for all sites except West Rocky (2012 and 2013 only).

Analysis

We used PERMANOVA (with a Bray-Curtis distance measure and alpha of 0.05) to compare *Castilleja* density in one year to community composition in subsequent years:

Composition (year t+1, t+2, etc.) ~ Density (year t)

This year-offset was used to explore whether the effect of the parasites grew stronger or weaker over time, and allowed at least one-year lag time for the community to show effects, as was the case in the Bao *et al.* (2015) study. Prior to PERMANOVA, all composition data were

transformed using the "wisconsin" function, in which species are first standardized by maxima and then sites by site totals (Oksanen *et al.* 2015).

Following PERMANOVA, ordisurf plots were created to visualize differences in community composition based on *Castilleja* density. Ordisurf plots (from the "vegan" statistics package) show the similarity between plots using multivariate community composition data represented in two dimensions overlaid with *Castilleja* density at each plot (NMDS). These plots were obtained using a Bray-Curtis distance measure. The position of the bubbles represents the similarity or difference in community data between plots and the size of the bubbles shows the relative density of *Castilleja* at each plot. The lines are a fitted contour of *Castilleja* densities. Using this function, we create a visual representation of the similarities between plant communities in addition to *Castilleja* density data from which we may be able to see a pattern.

We additionally used PERMANOVA (with an Euclidean distance measure, alpha = 0.05) to test species richness in a similar manner:

Richness (year t+1, t+2, etc.) ~ Density (year t)

This provided a total of six analyses per site (except West Rocky):

2011 density x 2012 composition/richness

2011 density x 2013 composition/richness

2011 density x 2014 composition/richness

2012 density x 2013 composition/richness

2012 density x 2014 composition/richness

2013 density x 2014 composition/richness

These tests were replicated with each community measure (multivariate community composition or species richness), for a total of 12 separate analyses per site.

We also visually analyzed the possible time lag effect of *Castilleja* influence by graphing the number of years between measurements (e.g. *Castilleja* density measured in 2011 and species richness in 2014 = 3-year difference) and the associated proportion of variation explained by *Castilleja* density. This helped us to explore the possibility of *Castilleja*'s impact growing stronger or weaker over time. Similarly, we visually represented the median density in each analysis compared to the R^2 value for that PERMANOVA. We used this method to examine the possibility that the signal of parasites' effect on the community was more visible on sites with greater ranges of *Castilleja* densities. The purpose of these multiple analyses was to ensure that if there were community changes due to parasite abundance, they were found and included in the study. Analyses were conducted using R statistical software (version 3.1.3, Appendix C).

Results

Community composition

PERMANOVA results comparing *Castilleja* density to community composition were variable between different sites and different combinations of years (Table 4.1). *Castilleja* density had a significant effect on composition in all combinations of years at the Ebey's Landing site, but only two combinations of years at Glacial Heritage and none of the combinations at the other two sites. At Glacial Heritage the 2011 *Castilleja* density did not show a significant effect on community until three years later, but the 2012 density only had a significant effect the following year.

Table 4.1 R^2 and p values of PERMANOVA community composition comparisons with *Castilleja* density (total plants per quadrat). Shaded values indicate a p-value < 0.05.

Site	*Castilleja* density year, composition year	R^2	P-value
Ebey's Landing	2011, 2012	0.14	0.001
	2011, 2013	0.12	0.011
	2011, 2014	0.14	0.001
	2012, 2013	0.20	0.001
	2012, 2014	0.12	0.004
	2013, 2014	0.12	0.007
Glacial Heritage	2011, 2012	0.04	0.219
	2011, 2013	0.05	0.158
	2011, 2014	0.07	0.041
	2012, 2013	0.07	0.005
	2012, 2014	0.05	0.108
	2013, 2014	0.06	0.052
Smith Prairie	2011, 2012	0.03	0.887
	2011, 2013	0.04	0.641
	2011, 2014	0.02	0.943
	2012, 2013	0.05	0.462
	2012, 2014	0.02	0.965
	2013, 2014	0.03	0.85
West Rocky	2011, 2012	0.05	0.078
	2011, 2013	0.03	0.331
	2012, 2013	0.02	0.831

Ordisurf plots made from density data largely showed no relationship between *Castilleja* density and community composition. For example, plots of the 2011 density and 2012 composition PERMANOVA models showed some grouping by *Castilleja* density in the plots, but most were not well grouped (Figure 4.1).

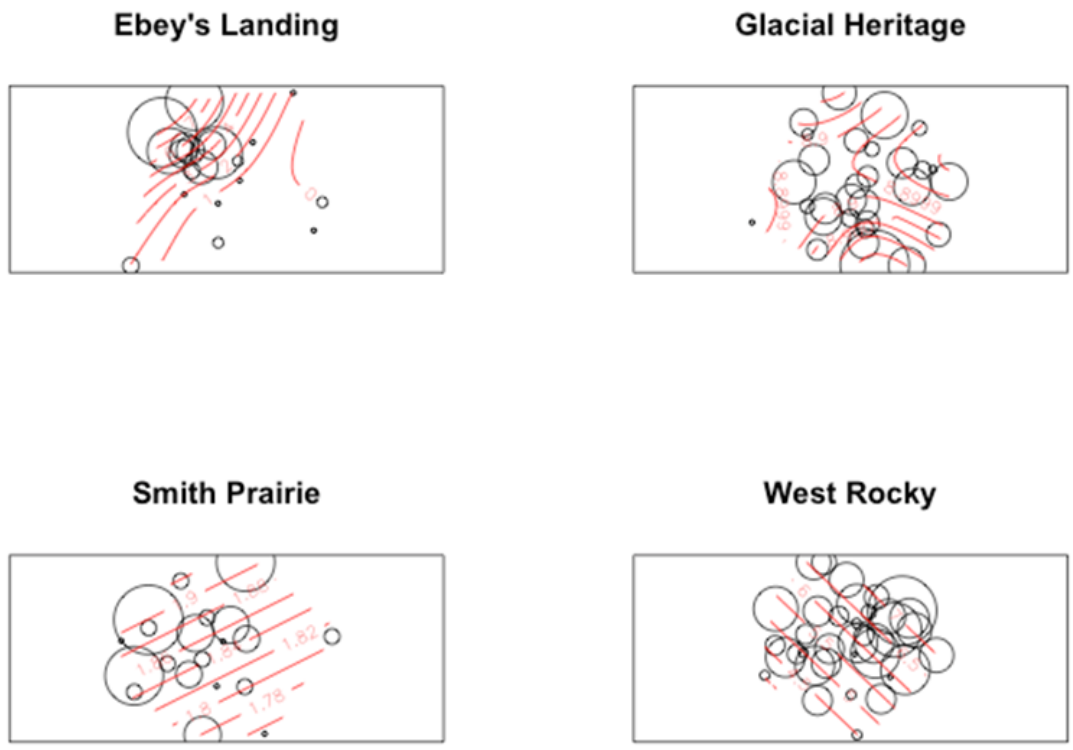

Figure 4.1 Ordisurf plots of *Castilleja* plant density (bubble size) and community composition (bubble placement) from PERMANOVA analysis of 2011 density and 2012 composition at each site. Density ranges (bubble sizes) are as follows: EL (0-11), GH (2-22), SP (0-6), and WR (1-14).

Species richness

The results of PERMANOVA exploring the effect of *Castilleja* density on species richness showed minimal instances of significance (Table 4.2). Glacial Heritage, Smith Prairie, and West Rocky sites had no significant effects of parasite density in any combination of years. At the Ebey's Landing site, *Castilleja* density in 2011 significantly affected species richness in all of the following years, while 2012 density only had a significant effect on 2013 richness.

Table 4.2 R^2 and p values of PERMANOVA species richness comparisons with *Castilleja* density (total plants per quadrat). Shaded values indicate a p-value < 0.05.

Site	*Castilleja* denisty year, species richness year	R^2	P-value
Ebey's Landing	2011, 2012	0.23	0.037
	2011, 2013	0.26	0.024
	2011, 2014	0.25	0.03
	2012, 2013	0.27	0.012
	2012, 2014	0.11	0.146
	2013, 2014	0.1	0.193
Glacial Heritage	2011, 2012	0.03	0.35
	2011, 2013	0.01	0.56
	2011, 2014	0.02	0.499
	2012, 2013	0.08	0.133
	2012, 2014	0.12	0.06
	2013, 2014	0.12	0.067
Smith Prairie	2011, 2012	0.13	0.132
	2011, 2013	0.01	0.39
	2011, 2014	0.02	0.521
	2012, 2013	0.02	0.627
	2012, 2014	0.02	0.575
	2013, 2014	0.05	0.326
West Rocky	2011, 2012	0.02	0.429
	2011, 2013	0.01	0.468
	2012, 2013	0.01	0.562

Lag time

There appears to be no discernable pattern in the proportions of variation in richness explained by *Castilleja* density at each site based on the number of years between density measurements and species richness measurements (Figure 4.2). That is, the possible lag time between increased parasite density and the amount of variation explained by parasite abundance did not seem to be consistent on either individual sites or a combination of all sites' data.

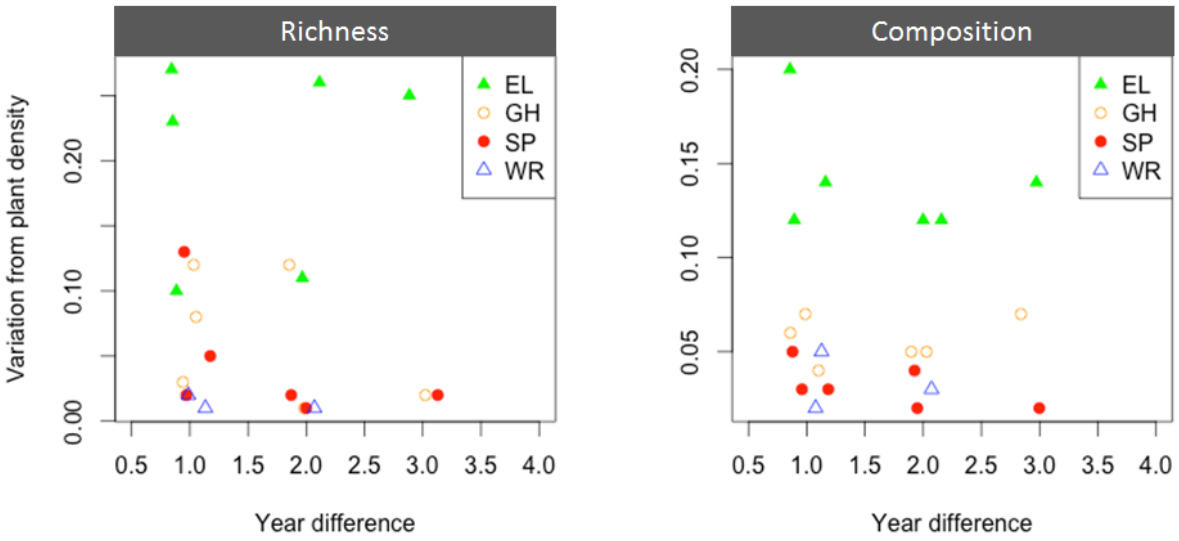

Figure 4.2 Years between measurement of density (e.g. 2011) and richness/composition (e.g. 2014), and variation in species richness and composition attributed to *Castilleja* density. Overlapping values were spaced using "jitter" function. Filled markers indicate northern sites (Ebey's Landing and Smith Prairie), hollow markers indicate southern sites (Glacial Heritage and West Rocky).

It is also interesting to note that in the 2011:2012 comparisons, species richness increased with *Castilleja* density at every site except for Ebey's Landing, the only statistically significant of the four (Figure 4.3). This trend was present in other significant Ebey's Landing comparisons, but marginally significant comparisons at Glacial Heritage showed the opposite trend (Figure 4.4).

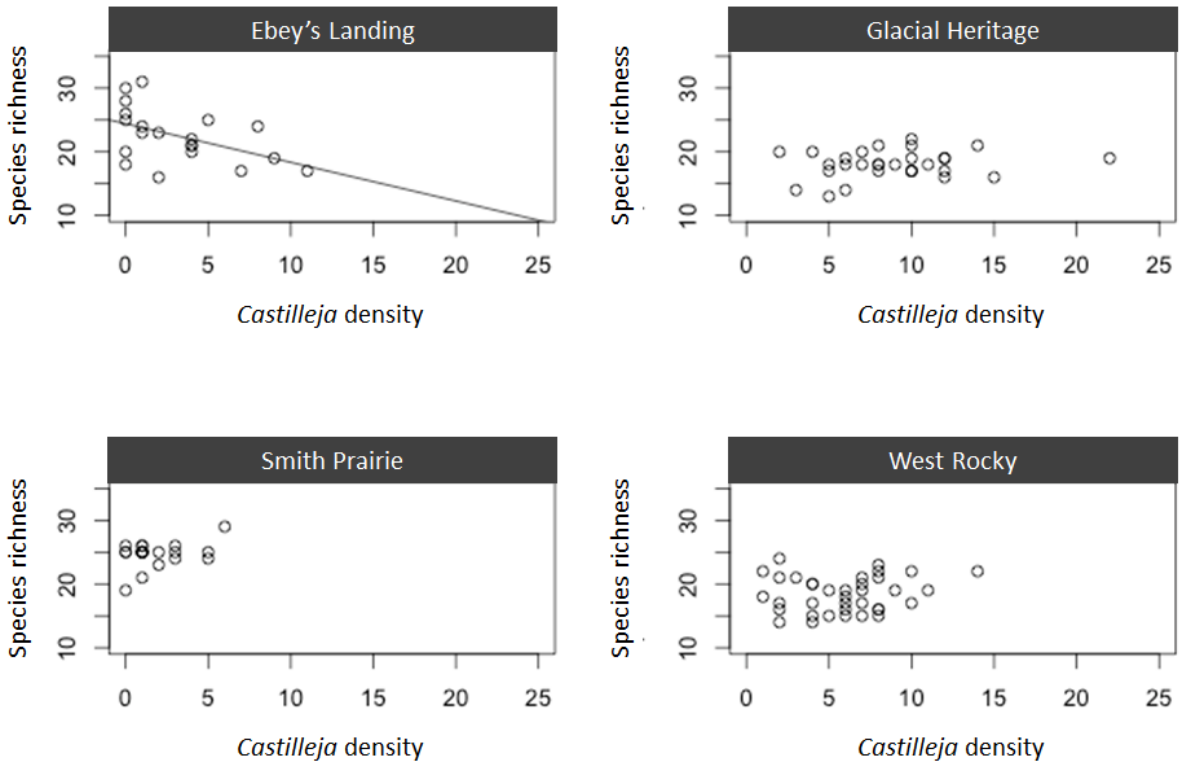

Figure 4.3 Density of *Castilleja* in 2011 and quadrat species richness in 2012 at each site. It should be noted that these are actual quadrat-level values, not averages, and only the Ebey's Landing comparison was statistically significant.

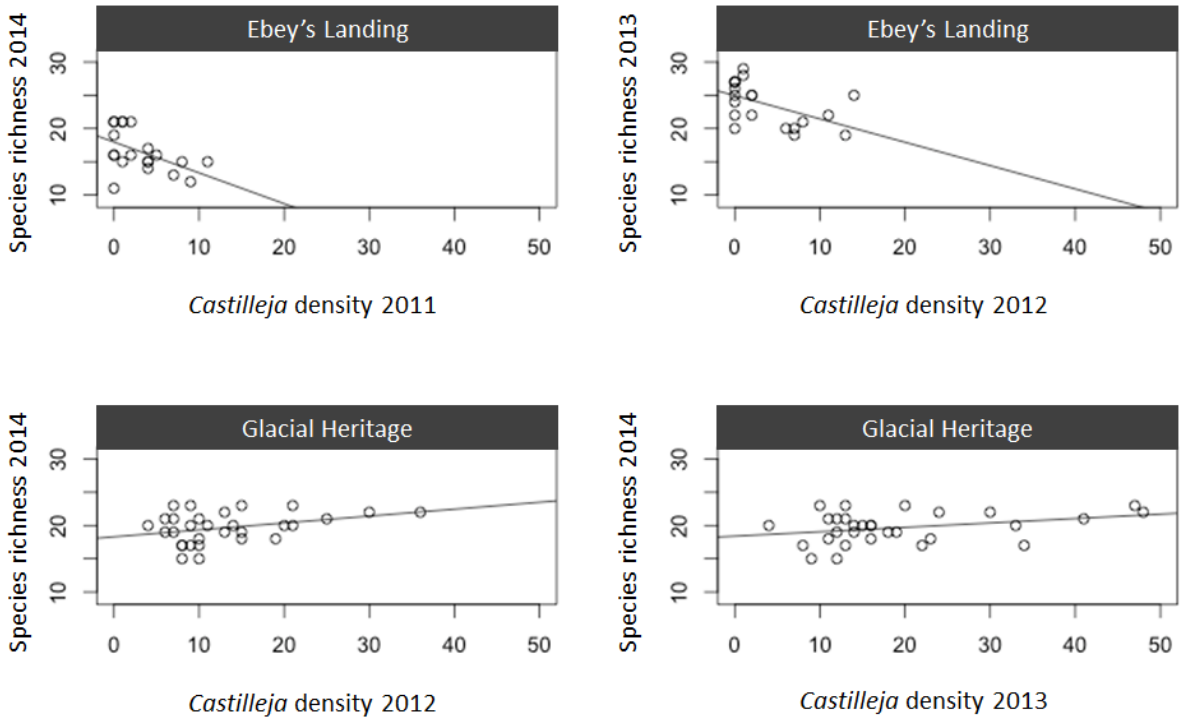

Figure 4.4 Density of *Castilleja* and species richness in significant ($p<0.05$, top) and marginally significant ($p<0.07$) analyses. It should be noted that these are actual quadrat-level values, not averages.

Discussion

While a single site had significant results of parasite density effects on community composition and richness, the majority of our site and year combinations were not significant. The one other site that showed significant results had inconsistency in lag time. Our results did not indicate a clear lag time present throughout the sites, although the data from Ebey's Landing suggests that in some cases the effect of a parasite on the community can be present for several years. The overall results indicate two possibilities:

1) The effect of parasites on composition and richness in this system varies spatially and temporally
2) There are significant effects of parasites on most sites but they are sometimes masked by other factors

Changes in significance by year and site may be due to actual significance of this effect being outweighed by other factors such as weather, herbivory, or another site-specific variable. While the sites chosen were paired in geographic location, there were still potential differences in microclimate at each site in each year. This may have been one cause of the stochastic results we observed. Additionally, other factors such as herbivory may have varied site to site.

Differences in initial site conditions may also play a role in these inconsistencies. Although all sites used in this study were abandoned agricultural fields, they varied in their usage history and previous crops on site. While we endeavored to use site pretreatments to ensure as much uniformity as possible among sites, it is possible that historical usage still had an effect on our results. In the South Sound sites, Glacial Heritage soils are categorized as Nisqually loamy fine sand, while West Rocky soils are Spanaway-Nisqually complex (NRCS online database). Nisqually loamy fine sand soils in the area are mostly used for agricultural production, while the Spanaway-Nisqually complex is present at many of the poorer prairie sites left as remnants. In the North Sound, soil at both sites is characterized as San Juan sandy loam, but Smith Prairie's soil has more gravel and holds less moisture than Ebey's Landing (Delvin 2013). In addition, *Castilleja levisecta* is known to be more successful in soils that are deeper and more productive (Dunwiddie *et al.* 2016, Delvin 2013).

The range of variation of the actual parasite densities (summarized in Figure S4.1) may also have influenced our results. It should be noted that while Glacial Heritage has the highest median density values, it does not always show the greatest R^2 values to match, and in the case of community composition it is the lower of the median densities from that site which show the greatest R^2 (Tables 4.1 and 4.2).

Despite this variability and potential for site-specific differences, it seems that in several cases there is an effect of parasitic plant density on community composition and richness. Two mechanisms have been suggested for this effect.

The first is an alteration of nutrient cycling by parasites. When nutrients are obtained from the host plant, they are incorporated into parasite tissues, including parasite leaves. When leaves are dropped in summer, this nutrient-rich litter becomes incorporated into the soil and the nutrients become available to other plants (Fisher *et al.* 2013). By this mechanism, nutrients originating in host species tissues can be redistributed to soil and from there to non-host plants in the community.

The second mechanism is through host suppression. As parasites take nutrients from their hosts, hosts become less able to grow and thrive, thus reducing their competitive ability in relation to non-host species. In cases where host species are dominant or abundant in the plant community this can cause significant reduction in plant biomass overall, while also potentially increasing species richness (Demey *et al.* 2015). In our study, *Castilleja* density had negative or non-significant effects on species richness, depending on the site and year (e.g. Figures 4.3 and 4.4),

although two marginally significant effects had a positive effect (see Glacial Heritage in Table 4.2, Figure 4.4).

It should be noted that our comparisons with other studies are not direct, since we used the range of parasite density already present in place of a parasite removal for comparison (as in Bao *et al.* 2016 and Demey *et al.* 2015). In removal experiments it is possible that removal plots retain the effect of the parasites following removal for some period of time, which has the potential to skew results. Our method provides a natural gradient of variation in parasite density, but does not look at the direct comparison of communities with none and with an abundance of parasites.

Despite the mixed results indicating parasite effects on the plant community, the several significant results lead us to believe that this effect does exist in this system. However, it may not be as strong as that shown in Bao 2015. As we begin to study ecosystems over larger spatial and temporal scales, (as in Grace *et al.* 2016), our understanding of more general ecological patterns is improved.

Conclusions

To answer our original research questions, 1) We did in some cases find an effect of parasites on plant communities, but not in a majority of our samples. Overall our results were dissimilar to the Bao 2015 study. 2) The effect was not consistent across sites or years, and there was much inconsistency among sites concerning which years had significant results. 3) The effect did not seem to have a pattern of strengthening or weakening over time. Results seemed more stochastic among different years and did not appear to be linked to a specific lag time.

While our data did not conclusively support the case for community changes due to parasitism across the board, they do point to the need for further research in this area. Studies that explore this effect over larger spatial and temporal scales may shed more light on the nuances of these parasite-community interactions. For example, if we had used only the Ebey's Landing or a single year's data in our study, we may have come to different conclusions. Larger and longer studies are needed to more fully understand the complexities of the relationship between parasitic plants and their communities.

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Supplemental materials

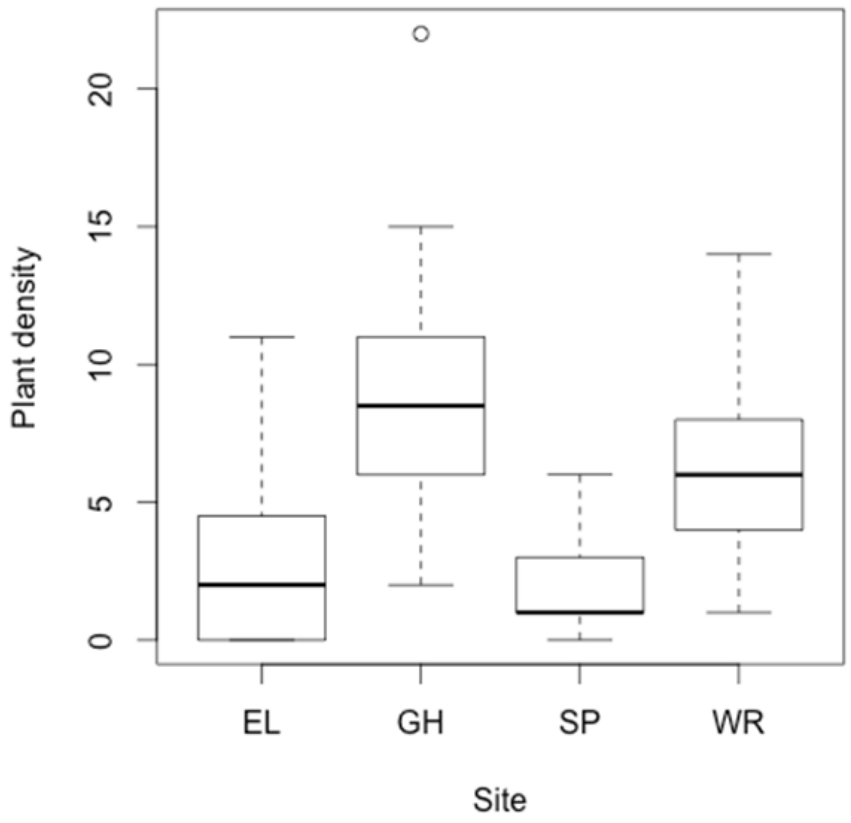

Figure S4.1 *Castilleja* density in each quadrat by site in 2011. Dotted lines indicate the nominal range of the data inferred from the upper and lower quartiles (box edges). Points that fall outside this range are shown as open circles.

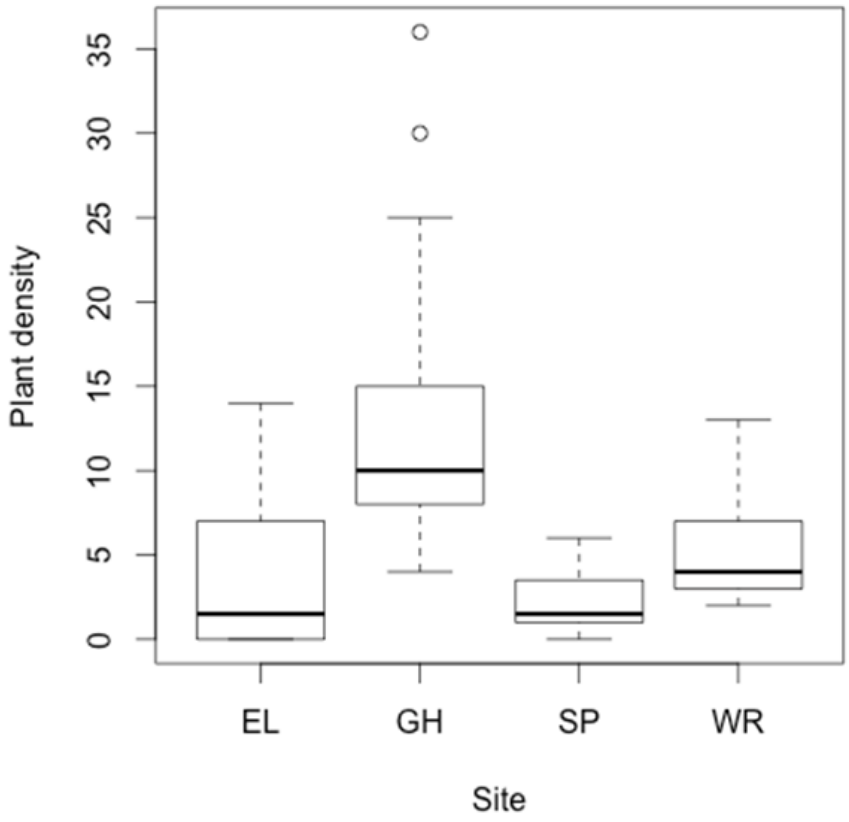

Figure S4.2 *Castilleja* density in each quadrat by site in 2012. See Figure S4.1 for plot description.

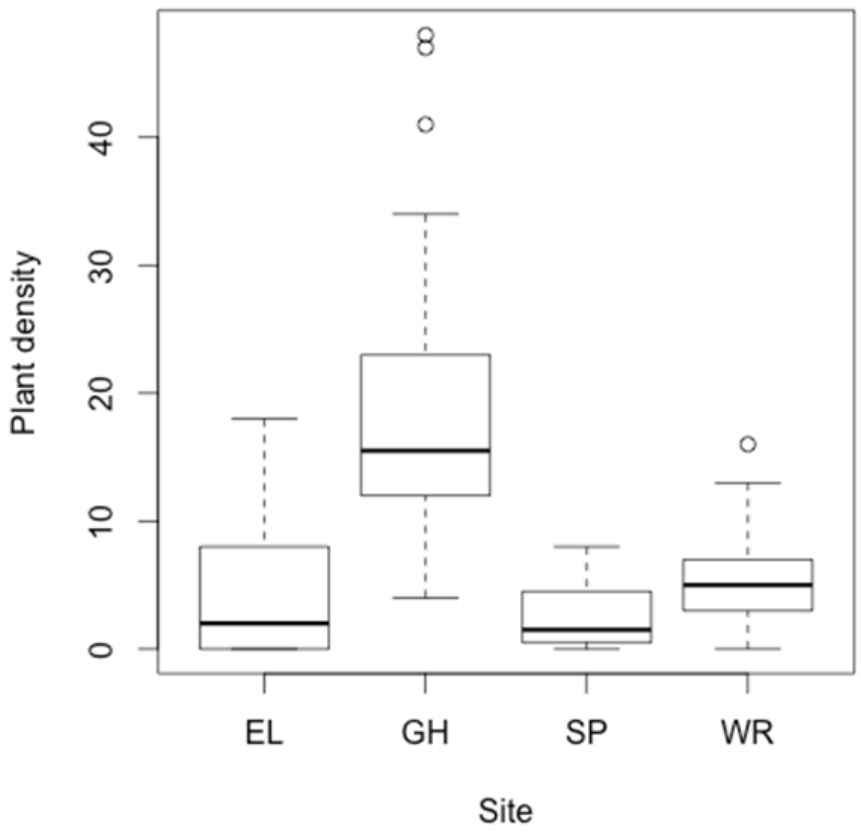

Figure S4.3 *Castilleja* density in each quadrat by site in 2013. See Figure S4.1 for plot description.

Chapter 5: Synthesis

Over the course of research for the previous chapters, we found clear differences in parasite survival, growth, and reproduction related to host identity (Chapter 2). Host species' contributions to these traits in *Castilleja levisecta* fluctuated over the two years of monitoring, and we found that total seed capsule numbers were highest in *C. levisecta* planted with *Achillea millefolium* and *Danthonia californica* (Figure 2.6). This metric is among the most important in restoration and conservation goals, as it creates greater opportunity for new or recovering populations to become sustainable. These results contribute valuable novel hosts to the tools used in management of this threatened species.

Clear differences were also present in the nutrition derived from different host species by *C. levisecta* (Chapter 3). Nitrogen was more variable than carbon, and the highest contributor to parasite tissue nitrogen was *Eriophyllum lanatum* (Figure 3.3). This may indicate one mechanism by which a host can benefit *Castilleja*, but when compared to results from chapter 1, it may be one of several indicators of host quality.

When results from chapters two and three are combined (using the species or genera that overlapped between the two experiments), the top hosts for overall *Castilleja* performance are *Achillea millefolium, Danthonia californica,* and *Eriophyllum lanatum* (Table 5.1). LULE and LULI were combined using LULI data from Chapter 2 (no LULI pairs survived and thus were not included in that analysis), and the LULE data from Chapter 3. It should be noted that of these three, only *Eriophyllum* is used as a common host in the literature. The other two are rarely, if ever, cited in the literature as hosts of this *Castilleja* species. These new suggestions

for host species can inform land management practices in the reintroduction and conservation of this threatened species.

Table 5.1 Comparative rankings of *Castilleja* performance with each host species in chapters 2 and 3. Ratings are based on each variable in positive relation to performance (best performance = 1, worst performance = 7). Ranks 1-2 are **bolded** and ranks 6-7 are grayed. See Tables 2.1 and 3.1 for full species names.

	ACMI	DACA	ERLA	ERSP	FERO	LULI/ LULE	SOCA/ SOMI
Survival 2013	**1**	**2**	5	7	5	3	5
Flowering stems 13	**1**	4	5.5	**2**	5.5	3	7
Browsed stems 13	**1**	3	4	**2**	5	6.5	6.5
Plants browsed	6.5	5	3.5	6.5	3.5	**1.5**	**1.5**
Height 2013	**1**	**2**	6	**3**	5	4	7
Fruiting stems 13	**1**	**2**	4	5	3	7	6
Seed capsules 13	**1**	**2**	4	7	3	5	6
Survival 14	4.5	**1**	**2**	6.5	3	4.5	6.5
Flowering stems 14	4	5	**1**	3	**2**	6	7
Browsed stems 14	**1**	3	2	7	4	6	5
Plants browsed	7	5	6	**2**	4	**2**	**2**
Height 14	4	6	**2**	5	3	**1**	7
Fruiting stems 14	**1**	4	**2**	5.5	3	5.5	7
Seed capsules 14	3	**1**	**2**	5	4	6	7
Carbon (isotope)	5	**3**	**2**	7	4	6	**1**
Nitrogen (isotope)	**2**	5	**1**	7	3	6	4
Average Rank	**2.8**	**3.3**	**3.3**	5.0	3.8	4.6	5.3

While we found ample evidence for surrounding plants' effects on *Castilleja*, there was less evidence for the reverse effect of the parasite on the community (Chapter 4). Studies in other systems have shown significant effects of parasites on community composition and richness, but some had limited spatial and temporal scale of monitoring (e.g. Bao *et al.* 2015). Our analysis did find significant effects of parasites in some cases, but no clear pattern at a larger scale of space and time (Chapter 4).

Together, these studies demonstrate the complexity of parasite-community relationships and point to areas of research for future studies. Given the generalist nature of many hemiparasites, it is likely that investigation of a variety of hosts could yield valuable insights into parasitic relationships. More research should be done to uncover the mechanisms behind host quality, including not just carbon and nitrogen, but additional macronutrients, water, and defensive compounds. This would give a more holistic view of host contributions to this parasite. Finally, there is a need for more long-term studies than span a greater geographical area to elucidate a generalized theory of parasite community effects. Collaborative studies such as the Nutrient Network (including Grace *et al.* 2016) point to a method of achieving this goal.

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Appendix A: 'R' code used for Chapter 2 analyses

```
### Load packages
library(plyr)
library(lsmeans)
library(ggplot2)

field.data<-read.csv(file.choose(), header=TRUE)
# choose 'Cleaned_Field_Companion_Data151126.csv'; 260 x 33

### Take out '2 CALE' triplets (omit; not mortality)
fd.all <- field.data[field.data$Notes_Spr13 == "0", ] # 256 x 33
fd.all.summary <- ddply(fd.all, .(Host.ID), summarize, N = length(ID.Number))

### Analyze 2013 and 2014 survival (focusing only on those plantings where both the
CALE and host survived)
fd.all$Live_Spr13 <- ifelse(fd.all$CALE.alive_Spr13 == 1 & fd.all$Host.alive_Spr13 == 1, 1, 0)
sum(fd.all$Live_Spr13) # 109 observations
fd.all$Live_Spr14 <- ifelse(fd.all$CALE.alive_Spr14 == 1 & fd.all$Host.alive_Spr14 == 1, 1, 0)
sum(fd.all$Live_Spr14) # 67 observations

Surv.13 <- glm(Live_Spr13 ~ Host.ID, data = fd.all, family = binomial)
summary(Surv.13)
anova(Surv.13, update(Surv.13, ~ . - Host.ID), test="Chisq") # host effect significant
lsmeans(Surv.13, "Host.ID", contr = "trt.vs.ctrl", adjust = "none") # ACMI, DECA, (DACA)
lsmeans(Surv.13, specs = pairwise ~ Host.ID, adjust = "none")

Surv.14 <- glm(Live_Spr14 ~ Host.ID, data = fd.all, family = binomial)
summary(Surv.14)
anova(Surv.14, update(Surv.14, ~ . - Host.ID), test="Chisq") # host effect significant
lsmeans(Surv.14, "Host.ID", contr = "trt.vs.ctrl", adjust = "none") # DECA, (DACA)
lsmeans(Surv.14, specs = pairwise ~ Host.ID, adjust = "none")

fd.survival <- ddply(fd.all, .(Host.ID), summarize,
                     N = length(Live_Spr13),
                     N.live13 = sum(Live_Spr13),
                     prop.live13 = N.live13 / N,
                     N.live14 = sum(Live_Spr14),
                     prop.live14 = N.live14 / N)

### Focus on live plants in 2013 (those where both CALE and host are alive)
fd.live13 <- fd.all[fd.all$Live_Spr13 == 1, ] # 109 x 35

fd.live13$Flowering.stems_Spr13<-as.numeric(fd.live13$Flowering.stems_Spr13)
fd.live13$Browsed.stems_Spr13<-as.numeric(fd.live13$Browsed.stems_Spr13)
fd.live13$CALE.height_Spr13<-as.numeric(fd.live13$CALE.height_Spr13)
```

```
fd.live13$Fruiting.Stems_Fall13<-as.numeric(fd.live13$Fruiting.Stems_Fall13)
fd.live13$Total.pods_Fall13<-as.numeric(fd.live13$Total.pods_Fall13)
fd.live13$Browsed.Plants_Spr13 <- ifelse(fd.live13$Browsed.stems_Spr13 > 0, 1, 0)

fd.live13.summary <- ddply(fd.live13, .(Host.ID), summarize,
                Flowering.stems = mean(Flowering.stems_Spr13),
                Flowering.stems = mean(Flowering.stems_Fall13),
                Browsed.stems = mean(Browsed.stems_Spr13),
                Browsed.Plants = sum(Browsed.Plants_Spr13),
                CALE.height = mean(CALE.height_Spr13),
                Fruiting.Stems = mean(Fruiting.Stems_Fall13),
                Total.pods = mean(Total.pods_Fall13),
                N.live = sum(Live_Spr13))
fd.live13.summary <- merge(fd.live13.summary, fd.all.summary, by = "Host.ID", all.y =
FALSE)

### glm
Spr.flow.stem.13 <- glm(Flowering.stems_Spr13 ~ Host.ID, data=fd.live13, family=poisson)
summary(Spr.flow.stem.13)
anova(Spr.flow.stem.13, update(Spr.flow.stem.13, ~ . - Host.ID), test = "Chisq") #significant
lsmeans(Spr.flow.stem.13, "Host.ID", contr = "trt.vs.ctrl", adjust = "none") # ACMI, DECA,
(SYAL)
lsmeans(Spr.flow.stem.13, specs = pairwise ~ Host.ID, adjust = "none")

Spr.brow.stem.13<-glm(Browsed.stems_Spr13 ~ Host.ID, data=fd.live13, family=poisson)
summary(Spr.brow.stem.13)
anova(Spr.brow.stem.13, update(Spr.brow.stem.13, ~ . - Host.ID), test = "Chisq") #significant
lsmeans(Spr.brow.stem.13, "Host.ID", contr = "trt.vs.ctrl", adjust = "none") # DECA, FERO,
SYAL, (ACMI)
lsmeans(Spr.brow.stem.13, specs = pairwise ~ Host.ID, adjust = "none")

Browsed.plants.13<-glm(Browsed.Plants_Spr13 ~ Host.ID, data=fd.live13, family=binomial)
summary(Browsed.plants.13)
anova(Browsed.plants.13, update(Browsed.plants.13, ~ . - Host.ID), test = "Chisq") #significant
lsmeans(Browsed.plants.13, "Host.ID", contr = "trt.vs.ctrl", adjust = "none") # NS
lsmeans(Browsed.plants.13, specs = pairwise ~ Host.ID, adjust = "none")

CALEheight.13 <- lm(CALE.height_Spr13 ~ Host.ID, data=fd.live13)
summary(CALEheight.13)
anova(CALEheight.13, update(CALEheight.13, ~ . - Host.ID)) #significant
lsmeans(CALEheight.13, "Host.ID", contr = "trt.vs.ctrl", adjust = "none") # ACMI, DECA
lsmeans(CALEheight.13, specs = pairwise ~ Host.ID, adjust = "none")

fruit.stem.13 <- glm(Fruiting.Stems_Fall13 ~ Host.ID, data=fd.live13, family=poisson)
summary(fruit.stem.13)
#need to drop control because none had fruiting stems
```

```
fruit.stem.13 <- glm(Fruiting.Stems_Fall13 ~ Host.ID, data=fd.live13[fd.live13$Host.ID != "aa No Host",], family=poisson)
summary(fruit.stem.13)
anova(fruit.stem.13, update(fruit.stem.13, ~ . - Host.ID), test = "Chisq") #significant
lsmeans(fruit.stem.13, specs = pairwise ~ Host.ID, adjust = "none")

seedpods.13 <- glm(Total.pods_Fall13 ~ Host.ID, data = fd.live13, family = poisson)
summary(seedpods.13)
#need to drop control because none had fruiting stems
seedpods.13 <- glm(Total.pods_Fall13 ~ Host.ID, data=fd.live13[fd.live13$Host.ID != "aa No Host",], family=poisson)
summary(seedpods.13)
anova(seedpods.13, update(seedpods.13, ~ . - Host.ID), test = "Chisq") #significant
lsmeans(seedpods.13, specs = pairwise ~ Host.ID, adjust = "none")

### Focus on live plants in 2014 (those where both CALE and host are alive)
fd.live14 <- fd.all[fd.all$Live_Spr14 == 1, ] # 67 x 35

fd.live14$Flowering.stems_Spr14<-as.numeric(fd.live14$Flowering.stems_Spr14)
fd.live14$Browsed.stems_Spr14<-as.numeric(fd.live14$Browsed.stems_Spr14)
fd.live14$CALE.height_Spr14<-as.numeric(fd.live14$CALE.height_Spr14)
fd.live14$Fruiting.Stems_Fall14<-as.numeric(fd.live14$Fruiting.Stems_Fall14)
fd.live14$Total.pods_Fall14<-as.numeric(fd.live14$Total.pods_Fall14)
fd.live14$Browsed.Plants_Spr14 <- ifelse(fd.live14$Browsed.stems_Spr14 > 0, 1, 0)

fd.live14.summary <- ddply(fd.live14, .(Host.ID), summarize,
                    Flowering.stems = mean(Flowering.stems_Spr14),
                    Flowering.stems = mean(Flowering.stems_Fall14),
                    Browsed.stems = mean(Browsed.stems_Spr14),
                    Browsed.Plants = sum(Browsed.Plants_Spr14),
                    CALE.height = mean(CALE.height_Spr14),
                    Fruiting.Stems = mean(Fruiting.Stems_Fall14),
                    Total.pods = mean(Total.pods_Fall14),
                    N.live = sum(Live_Spr14))
fd.live14.summary <- merge(fd.live14.summary, fd.all.summary, by = "Host.ID", all.y = FALSE)

### glm
Spr.flow.stem.14 <- glm(Flowering.stems_Spr14 ~ Host.ID, data=fd.live14, family=poisson)
summary(Spr.flow.stem.14)
anova(Spr.flow.stem.14, update(Spr.flow.stem.14, ~ . - Host.ID), test = "Chisq") #significant
lsmeans(Spr.flow.stem.14, "Host.ID", contr = "trt.vs.ctrl", adjust = "none") # ERLA, DECA, FERO, SYAL, ERSP, DACA, ACMI, LULI
lsmeans(Spr.flow.stem.14, specs = pairwise ~ Host.ID, adjust = "none")

Spr.brow.stem.14<-glm(Browsed.stems_Spr14 ~ Host.ID, data=fd.live14, family=poisson)
```

```
summary(Spr.brow.stem.14)
anova(Spr.brow.stem.14, update(Spr.brow.stem.14, ~ . - Host.ID), test = "Chisq") #significant
lsmeans(Spr.brow.stem.14, "Host.ID", contr = "trt.vs.ctrl", adjust = "none") # ACMI, LULI,
ERSP, RONU, SYAL, (DECA)
lsmeans(Spr.brow.stem.14, specs = pairwise ~ Host.ID, adjust = "none")

Browsed.plants.14<-glm(Browsed.Plants_Spr14 ~ Host.ID, data=fd.live14, family=binomial)
summary(Browsed.plants.14)
anova(Browsed.plants.14, update(Browsed.plants.14, ~ . - Host.ID), test = "Chisq") #NS
lsmeans(Browsed.plants.14, specs = pairwise ~ Host.ID, adjust = "none")

CALEheight.14 <- lm(CALE.height_Spr14~ Host.ID, data=fd.live14)
summary(CALEheight.14)
anova(CALEheight.14, update(CALEheight.14, ~ . - Host.ID)) #significant
lsmeans(CALEheight.14, "Host.ID", contr = "trt.vs.ctrl", adjust = "none") # LULI, ERLA,
FERO, DECA, ACMI, (DACA), (SYAL)
lsmeans(CALEheight.14, specs = pairwise ~ Host.ID, adjust = "none")

fruit.stem.14 <- glm(Fruiting.Stems_Fall14 ~ Host.ID, data=fd.live14, family=poisson)
summary(fruit.stem.14)
anova(fruit.stem.14, update(fruit.stem.14, ~ . - Host.ID), test = "Chisq") #significant
lsmeans(fruit.stem.14, "Host.ID", contr = "trt.vs.ctrl", adjust = "none") # ACMI, ERLA, FERO,
DACA, SYAL, DECA, (LULI)
lsmeans(fruit.stem.14, specs = pairwise ~ Host.ID, adjust = "none")

seedpods.14 <- glm(Total.pods_Fall14 ~ Host.ID, data = fd.live14, family = poisson)
summary(seedpods.14)
anova(seedpods.14, update(seedpods.14, ~ . - Host.ID), test = "Chisq") #significant
lsmeans(seedpods.14, "Host.ID", contr = "trt.vs.ctrl", adjust = "none") # ERLA, DACA, ACMI,
FERO, SYAL, DECA, ERSP, RONU, LULI
lsmeans(seedpods.14, specs = pairwise ~ Host.ID, adjust = "none")


#### Combine survival and seed production in both years

fd.live13.summary$s13xcap13 <- fd.live13.summary$Total.pods * (fd.live13.summary$N.live /
fd.live13.summary$N) * 100
fd.live14.summary$s14xcap14 <- fd.live14.summary$Total.pods * (fd.live14.summary$N.live /
fd.live14.summary$N) * 100

fd.summary <- merge(x = fd.live13.summary[, c("Host.ID", "s13xcap13")],
                    y = fd.live14.summary[, c("Host.ID", "s14xcap14")])
fd.summary$Total <- fd.summary$s13xcap13 + fd.summary$s14xcap14


write.table(fd.summary, "/users/christopherjones/fd.summary.txt", sep="\t")
```

```
fd.live13.summary$Perc.browsed<-(fd.live13.summary$Browsed.Plants /
fd.live13.summary$N.live) * 100

fd.live14.summary$Perc.browsed<-(fd.live14.summary$Browsed.Plants /
fd.live14.summary$N.live) * 100
```

graphs

```
par(mfrow=c(1,2))
```

flowering stems

```
plot(Flowering.stems_Spr13 ~ Host.ID, data=fd.live13, las=3, xlab="", ylab="Flowering Stems
Spring 2013", ylim = c(0, 15))
plot(Flowering.stems_Spr14 ~ Host.ID, data=fd.live14, las=3, xlab="", ylab="Flowering Stems
Spring 2014", ylim = c(0, 15))
```

browsed stems

```
plot(Browsed.stems_Spr13 ~ Host.ID, data=fd.live13, las=3, xlab="", ylab="Browsed Stems
Spring 2013", ylim = c(0, 25))
plot(Browsed.stems_Spr14 ~ Host.ID, data=fd.live14, las=3, xlab="", ylab="Browsed Stems
Spring 2014", ylim = c(0, 25))
```

height

```
plot(CALE.height_Spr13 ~ Host.ID, data=fd.live13, las=3, xlab="", ylab="Height Spring 2013",
ylim = c(0, 70))
plot(CALE.height_Spr14 ~ Host.ID, data=fd.live14, las=3, xlab="", ylab="Height Spring 2014",
ylim = c(0, 70))
```

fruiting stems

```
plot(Fruiting.Stems_Fall13 ~ Host.ID, data=fd.live13, las=3, xlab="", ylab="Fruiting Stems Fall
2013", ylim = c(0, 16))
plot(Fruiting.Stems_Fall14 ~ Host.ID, data=fd.live14, las=3, xlab="", ylab="Fruiting Stems Fall
2014", ylim = c(0, 16))
```

seed capsule number

```
plot(Total.pods_Fall13 ~ Host.ID, data=fd.live13, las=3, xlab="", ylab="Seed capsules Fall
2013", ylim = c(0, 160))
plot(Total.pods_Fall14 ~ Host.ID, data=fd.live14, las=3, xlab="", ylab="Seed capsules Fall
2014", ylim = c(0, 160))
```

percent of live plants browsed

```
plot(fd.live13.summary$Host.ID, fd.live13.summary$Perc.browsed, las=3, xlab="", ylab="% live plants browsed Spring 2013", ylim = c(0, 100))
plot(fd.live14.summary$Host.ID, fd.live14.summary$Perc.browsed, las=3, xlab="", ylab="% live plants browsed Spring 2014", ylim = c(0, 100))
```

Appendix B: 'R' code used for Chapter 3 analyses

```
iso <- read.csv(file.choose(), header = TRUE)  #Use 'Iso_Data_Final'
iso$Host<-as.factor(iso$Host)
iso$del.C<-as.numeric(iso$del.C)
iso$del.N<-as.numeric(iso$del.N)

library(PMCMR)
diff.N <- kruskal.test(del.N ~ Host, data=iso)
diff.N
posthoc.kruskal.nemenyi.test(del.N ~ Host, data = iso)

diff.C <- kruskal.test(del.C ~ Host, data=iso)
diff.C
posthoc.kruskal.nemenyi.test(del.C ~ Host, data = iso)

library(lsmeans)

diff.N <- aov(del.N ~ Host, data=iso)
summary(diff.N)
lsmeans(diff.N, specs = pairwise ~ Host, adjust = "none")
diff.C <- aov(del.C ~ Host, data=iso)
summary(diff.C)
lsmeans(diff.C, specs = pairwise ~ Host, adjust = "none")

### plot all C vs N

plot(iso$del.C, iso$del.N, main = "All groups", xlab = "del 13C", ylab = "del 15N")

### plot each host group C vs N

iso.ACMI<-iso[iso$Host == "ACMI",]
iso.DACA<-iso[iso$Host == "DACA",]
iso.ERLA<-iso[iso$Host == "ERLA",]
iso.ERSP<-iso[iso$Host == "ERSP",]
iso.FERO<-iso[iso$Host == "FERO",]
iso.LULE<-iso[iso$Host == "LULE",]
iso.SOMI<-iso[iso$Host == "SOMI",]

par(mfrow=c(1,1))
plot(iso.ACMI$del.C, iso.ACMI$del.N, main = "ACMI group", xlab = "del 13C", ylab = "del
15N")
plot(iso.DACA$del.C, iso.DACA$del.N, main = "DACA group", xlab = "del 13C", ylab = "del
15N")
plot(iso.ERLA$del.C, iso.ERLA$del.N, main = "ERLA group", xlab = "del 13C", ylab = "del
15N")
```

```
plot(iso.ERSP$del.C, iso.ERSP$del.N, main = "ERSP group", xlab = "del 13C", ylab = "del
15N")
plot(iso.FERO$del.C, iso.FERO$del.N, main = "FERO group", xlab = "del 13C", ylab = "del
15N")
plot(iso.LULE$del.C, iso.LULE$del.N, main = "LULE group", xlab = "del 13C", ylab = "del
15N")
plot(iso.SOMI$del.C, iso.SOMI$del.N, main = "SOMI group", xlab = "del 13C", ylab = "del
15N")
```

Appendix C: 'R' code used for Chapter 4 analyses

```
comp <- read.csv(file.choose(), header = TRUE)
comp[is.na(comp)] <- 0
comp$AGR_sp <- comp$AGCA + comp$AGCA_AGST + comp$AGR_sp
comp$AGCA <- NULL
comp$AGCA_AGST <- NULL
comp$AIR_sp <- comp$AICA + comp$AIPR
comp$AICA <- NULL
comp$AIPR <- NULL
comp$SOSP <- comp$SOSP + comp$SOMI_ERSP_SOSP
comp$SOMI_ERSP_SOSP <- NULL
comp$VER_sp <- comp$VER_sp + comp$VEAR + comp$VEHE + comp$VEOF
comp$VEAR <- NULL
comp$VEHE <- NULL
comp$VEOF <- NULL
comp$VUL_sp <- comp$VUBR + comp$VUMY
comp$VUBR <- NULL
comp$VUMY <- NULL
comp$LUP_sps <- NULL
comp$RUB_sps <- NULL
comp$VITE <- comp$VITE + comp$VITE_VIDI
comp$VITE_VIDI <- NULL
spp <- colnames(comp)[10:ncol(comp)]

EL.12 <- comp[comp$SiteAbbrev == "EL" & comp$SampleYear == 2012, ]
EL.13 <- comp[comp$SiteAbbrev == "EL" & comp$SampleYear == 2013, ]
EL.14 <- comp[comp$SiteAbbrev == "EL" & comp$SampleYear == 2014, ]

SP.12 <- comp[comp$SiteAbbrev == "SP" & comp$SampleYear == 2012, ]
SP.13 <- comp[comp$SiteAbbrev == "SP" & comp$SampleYear == 2013, ]
SP.14 <- comp[comp$SiteAbbrev == "SP" & comp$SampleYear == 2014, ]

WR.12 <- comp[comp$SiteAbbrev == "WR" & comp$SampleYear == 2012, ]
WR.13 <- comp[comp$SiteAbbrev == "WR" & comp$SampleYear == 2013, ]

GH.12 <- comp[comp$SiteAbbrev == "GH" & comp$SampleYear == 2012, ]
GH.13 <- comp[comp$SiteAbbrev == "GH" & comp$SampleYear == 2013, ]
GH.14 <- comp[comp$SiteAbbrev == "GH" & comp$SampleYear == 2014, ]
```

Density

```
density<-read.csv(file.choose(), header = TRUE)
density[is.na(density)] <- 0
density$CA_TotalPlants <- density$CALE_TotalPlants + density$CAHI_TotalPlants

EL.dens.11 <- density[density$SiteAbbrev == "EL" & density$SampleYear == 2011, ]
```

```
EL.dens.11$CA_TotalPlants <- EL.dens.11$CALE_TotalPlants+EL.dens.11$CAHI_TotalPlants

EL.dens.12 <- density[density$SiteAbbrev == "EL" & density$SampleYear == 2012, ]
EL.dens.12$CA_TotalPlants <- EL.dens.12$CALE_TotalPlants+EL.dens.12$CAHI_TotalPlants

EL.dens.13 <- density[density$SiteAbbrev == "EL" & density$SampleYear == 2013, ]
EL.dens.13$CA_TotalPlants <- EL.dens.13$CALE_TotalPlants+EL.dens.13$CAHI_TotalPlants

SP.dens.11 <- density[density$SiteAbbrev == "SP" & density$SampleYear == 2011, ]
SP.dens.11$CA_TotalPlants <- SP.dens.11$CALE_TotalPlants+SP.dens.11$CAHI_TotalPlants

SP.dens.12 <- density[density$SiteAbbrev == "SP" & density$SampleYear == 2012, ]
SP.dens.12$CA_TotalPlants <- SP.dens.12$CALE_TotalPlants+SP.dens.12$CAHI_TotalPlants

SP.dens.13 <- density[density$SiteAbbrev == "SP" & density$SampleYear == 2013, ]
SP.dens.13$CA_TotalPlants <- SP.dens.13$CALE_TotalPlants+SP.dens.13$CAHI_TotalPlants

WR.dens.11 <- density[density$SiteAbbrev == "WR" & density$SampleYear == 2011, ]
WR.dens.11$CA_TotalPlants <- WR.dens.11$CALE_TotalPlants +
WR.dens.11$CAHI_TotalPlants

WR.dens.12 <- density[density$SiteAbbrev == "WR" & density$SampleYear == 2012, ]
WR.dens.12$CA_TotalPlants <- WR.dens.12$CALE_TotalPlants +
WR.dens.12$CAHI_TotalPlants
```

Richness

```
GH.dens.11$Rich12 <- rowSums(GH.12[,10:140] > 0)
GH.dens.11$Rich13 <- rowSums(GH.13[,10:140] > 0)
GH.dens.11$Rich14 <- rowSums(GH.14[,10:140] > 0)

GH.dens.12$Rich12 <- rowSums(GH.12[,10:140] > 0)
GH.dens.12$Rich13 <- rowSums(GH.13[,10:140] > 0)
GH.dens.12$Rich14 <- rowSums(GH.14[,10:140] > 0)

GH.dens.13$Rich12 <- rowSums(GH.12[,10:140] > 0)
GH.dens.13$Rich13 <- rowSums(GH.13[,10:140] > 0)
GH.dens.13$Rich14 <- rowSums(GH.14[,10:140] > 0)

EL.dens.11$Rich12 <- rowSums(EL.12[,10:140] > 0)
EL.dens.11$Rich13 <- rowSums(EL.13[,10:140] > 0)
EL.dens.11$Rich14 <- rowSums(EL.14[,10:140] > 0)

EL.dens.12$Rich12 <- rowSums(EL.12[,10:140] > 0)
EL.dens.12$Rich13 <- rowSums(EL.13[,10:140] > 0)
EL.dens.12$Rich14 <- rowSums(EL.14[,10:140] > 0)
```

```
EL.dens.13$Rich12 <- rowSums(EL.12[,10:140] > 0)
EL.dens.13$Rich13 <- rowSums(EL.13[,10:140] > 0)
EL.dens.13$Rich14 <- rowSums(EL.14[,10:140] > 0)

SP.dens.11$Rich12 <- rowSums(SP.12[,10:140] > 0)
SP.dens.11$Rich13 <- rowSums(SP.13[,10:140] > 0)
SP.dens.11$Rich14 <- rowSums(SP.14[,10:140] > 0)

SP.dens.12$Rich12 <- rowSums(SP.12[,10:140] > 0)
SP.dens.12$Rich13 <- rowSums(SP.13[,10:140] > 0)
SP.dens.12$Rich14 <- rowSums(SP.14[,10:140] > 0)

SP.dens.13$Rich12 <- rowSums(SP.12[,10:140] > 0)
SP.dens.13$Rich13 <- rowSums(SP.13[,10:140] > 0)
SP.dens.13$Rich14 <- rowSums(SP.14[,10:140] > 0)

WR.dens.11$Rich12 <- rowSums(WR.12[,10:140] > 0)
WR.dens.11$Rich13 <- rowSums(WR.13[,10:140] > 0)

WR.dens.12$Rich12 <- rowSums(WR.12[,10:140] > 0)
WR.dens.12$Rich13 <- rowSums(WR.13[,10:140] > 0)
```

Plot number as factor

```
GH.dens.11$Plot.Number<-as.factor(GH.dens.11$PlotNumber)
GH.dens.12$Plot.Number<-as.factor(GH.dens.12$PlotNumber)
GH.dens.13$Plot.Number<-as.factor(GH.dens.13$PlotNumber)

EL.dens.11$Plot.Number<-as.factor(EL.dens.11$PlotNumber)
EL.dens.12$Plot.Number<-as.factor(EL.dens.12$PlotNumber)
EL.dens.13$Plot.Number<-as.factor(EL.dens.13$PlotNumber)

SP.dens.11$Plot.Number<-as.factor(SP.dens.11$PlotNumber)
SP.dens.12$Plot.Number<-as.factor(SP.dens.12$PlotNumber)
SP.dens.13$Plot.Number<-as.factor(SP.dens.13$PlotNumber)

WR.dens.11$Plot.Number<-as.factor(WR.dens.11$PlotNumber)
WR.dens.12$Plot.Number<-as.factor(WR.dens.12$PlotNumber)
```

MULTIVARIATE

Wisconsin

```
library(vegan)
```

```
EL.wcomp12<-wisconsin(EL.12[,10:140])
EL.wcomp13<-wisconsin(EL.13[,10:140])
EL.wcomp14<-wisconsin(EL.14[,10:140])

GH.wcomp12<-wisconsin(GH.12[,10:140])
GH.wcomp13<-wisconsin(GH.13[,10:140])
GH.wcomp14<-wisconsin(GH.14[,10:140])

SP.wcomp12<-wisconsin(SP.12[,10:140])
SP.wcomp13<-wisconsin(SP.13[,10:140])
SP.wcomp14<-wisconsin(SP.14[,10:140])

WR.wcomp12<-wisconsin(WR.12[,10:140])
WR.wcomp13<-wisconsin(WR.13[,10:140])
```

PERMANOVA

 # total plants

```
GH.1112<-adonis(GH.wcomp12 ~ GH.dens.11$CA_TotalPlants, permutations=999,
method="bray"); GH.1112
GH.1113<-adonis(GH.wcomp13 ~ GH.dens.11$CA_TotalPlants, permutations=999,
method="bray"); GH.1113
GH.1114<-adonis(GH.wcomp14 ~ GH.dens.11$CA_TotalPlants, permutations=999,
method="bray"); GH.1114
GH.1213<-adonis(GH.wcomp13 ~ GH.dens.12$CA_TotalPlants, permutations=999,
method="bray"); GH.1213
GH.1214<-adonis(GH.wcomp14 ~ GH.dens.12$CA_TotalPlants, permutations=999,
method="bray"); GH.1214
GH.1314<-adonis(GH.wcomp14 ~ GH.dens.13$CA_TotalPlants, permutations=999,
method="bray"); GH.1314

EL.1112<-adonis(EL.wcomp12 ~ EL.dens.11$CA_TotalPlants, permutations=999,
method="bray"); EL.1112
EL.1113<-adonis(EL.wcomp13 ~ EL.dens.11$CA_TotalPlants, permutations=999,
method="bray"); EL.1113
EL.1114<-adonis(EL.wcomp14 ~ EL.dens.11$CA_TotalPlants, permutations=999,
method="bray"); EL.1114
EL.1213<-adonis(EL.wcomp13 ~ EL.dens.12$CA_TotalPlants, permutations=999,
method="bray"); EL.1213
EL.1214<-adonis(EL.wcomp14 ~ EL.dens.12$CA_TotalPlants, permutations=999,
method="bray"); EL.1214
EL.1314<-adonis(EL.wcomp14 ~ EL.dens.13$CA_TotalPlants, permutations=999,
method="bray"); EL.1314
```

```
SP.1112<-adonis(SP.wcomp12 ~ SP.dens.11$CA_TotalPlants, permutations=999,
method="bray"); SP.1112
SP.1113<-adonis(SP.wcomp13 ~ SP.dens.11$CA_TotalPlants, permutations=999,
method="bray"); SP.1113
SP.1114<-adonis(SP.wcomp14 ~ SP.dens.11$CA_TotalPlants, permutations=999,
method="bray"); SP.1114
SP.1213<-adonis(SP.wcomp13 ~ SP.dens.12$CA_TotalPlants, permutations=999,
method="bray"); SP.1213
SP.1214<-adonis(SP.wcomp14 ~ SP.dens.12$CA_TotalPlants, permutations=999,
method="bray"); SP.1214
SP.1314<-adonis(SP.wcomp14 ~ SP.dens.13$CA_TotalPlants, permutations=999,
method="bray"); SP.1314

WR.1112<-adonis(WR.wcomp12 ~ WR.dens.11$CA_TotalPlants, permutations=999,
method="bray"); WR.1112
WR.1113<-adonis(WR.wcomp13 ~ WR.dens.11$CA_TotalPlants, permutations=999,
method="bray"); WR.1113
WR.1213<-adonis(WR.wcomp13 ~ WR.dens.12$CA_TotalPlants, permutations=999,
method="bray"); WR.1213
```

Richness

```
GH.rich1112<-adonis(GH.dens.11$Rich12 ~ GH.dens.11$CA_TotalPlants, permutations=999,
method="euc"); GH.rich1112
GH.rich1113<-adonis(GH.dens.11$Rich13 ~ GH.dens.11$CA_TotalPlants, permutations=999,
method="euc"); GH.rich1113
GH.rich1114<-adonis(GH.dens.11$Rich14 ~ GH.dens.11$CA_TotalPlants, permutations=999,
method="euc"); GH.rich1114
GH.rich1213<-adonis(GH.dens.12$Rich13 ~ GH.dens.12$CA_TotalPlants, permutations=999,
method="euc"); GH.rich1213
GH.rich1214<-adonis(GH.dens.12$Rich14 ~ GH.dens.12$CA_TotalPlants, permutations=999,
method="euc"); GH.rich1214
GH.rich1314<-adonis(GH.dens.13$Rich14 ~ GH.dens.13$CA_TotalPlants, permutations=999,
method="euc"); GH.rich1314


EL.rich1112<-adonis(EL.dens.11$Rich12 ~ EL.dens.11$CA_TotalPlants, permutations=999,
method="euc"); EL.rich1112
EL.rich1113<-adonis(EL.dens.11$Rich13 ~ EL.dens.11$CA_TotalPlants, permutations=999,
method="euc"); EL.rich1113
EL.rich1114<-adonis(EL.dens.11$Rich14 ~ EL.dens.11$CA_TotalPlants, permutations=999,
method="euc"); EL.rich1114
EL.rich1213<-adonis(EL.dens.12$Rich13 ~ EL.dens.12$CA_TotalPlants, permutations=999,
method="euc"); EL.rich1213
EL.rich1214<-adonis(EL.dens.12$Rich14 ~ EL.dens.12$CA_TotalPlants, permutations=999,
method="euc"); EL.rich1214
```

```
EL.rich1314<-adonis(EL.dens.13$Rich14 ~ EL.dens.13$CA_TotalPlants, permutations=999,
method="euc"); EL.rich1314


SP.rich1112<-adonis(SP.dens.11$Rich12 ~ SP.dens.11$CA_TotalPlants, permutations=999,
method="euc"); SP.rich1112
SP.rich1113<-adonis(SP.dens.11$Rich13 ~ SP.dens.11$CA_TotalPlants, permutations=999,
method="euc"); SP.rich1113
SP.rich1114<-adonis(SP.dens.11$Rich14 ~ SP.dens.11$CA_TotalPlants, permutations=999,
method="euc"); SP.rich1114
SP.rich1213<-adonis(SP.dens.12$Rich13 ~ SP.dens.12$CA_TotalPlants, permutations=999,
method="euc"); SP.rich1213
SP.rich1214<-adonis(SP.dens.12$Rich14 ~ SP.dens.12$CA_TotalPlants, permutations=999,
method="euc"); SP.rich1214
SP.rich1314<-adonis(SP.dens.13$Rich14 ~ SP.dens.13$CA_TotalPlants, permutations=999,
method="euc"); SP.rich1314


WR.rich1112<-adonis(WR.dens.11$Rich12 ~ WR.dens.11$CA_TotalPlants, permutations=999,
method="euc"); WR.rich1112
WR.rich1113<-adonis(WR.dens.11$Rich13 ~ WR.dens.11$CA_TotalPlants, permutations=999,
method="euc"); WR.rich1113
WR.rich1213<-adonis(WR.dens.12$Rich13 ~ WR.dens.12$CA_TotalPlants, permutations=999,
method="euc"); WR.rich1213
```

Graphs

 # Ordisurf

```
par(mfrow=c(1,1))

EL.test12<-metaMDS(EL.wcomp12, autotransform=FALSE, wascores=FALSE, distance =
“bray”, k = 2, trymax = 100)
EL.test13<-metaMDS(EL.wcomp13, autotransform=FALSE, wascores=FALSE, distance =
“bray”, k = 2, trymax = 100)
EL.test14<-metaMDS(EL.wcomp14, autotransform=FALSE, wascores=FALSE, distance =
“bray”, k = 2, trymax = 100)

GH.test12<-metaMDS(GH.wcomp12, autotransform=FALSE, wascores=FALSE, distance =
“bray”, k = 2, trymax = 100)
SP.test12<-metaMDS(SP.wcomp12, autotransform=FALSE, wascores=FALSE, distance =
“bray”, k = 2, trymax = 100)
WR.test12<-metaMDS(WR.wcomp12, autotransform=FALSE, wascores=FALSE, distance =
“bray”, k = 2, trymax = 100)
```

```
ordisurf(EL.test12, EL.dens.11$CA_TotalPlants, bubble=6, xlab="", xaxt="n", ylab="",
yaxt="n", main = "Ebey's Landing")
ordisurf(GH.test12, GH.dens.11$CA_TotalPlants, bubble=6, xlab="", xaxt="n", ylab="",
yaxt="n", main = "Glacial Heritage")
ordisurf(SP.test12, SP.dens.11$CA_TotalPlants, bubble=6, xlab="", xaxt="n", ylab="",
yaxt="n", main = "Smith Prairie")
ordisurf(WR.test12, WR.dens.11$CA_TotalPlants, bubble=6, xlab="", xaxt="n", ylab="",
yaxt="n", main = "West Rocky")

ordisurf(EL.test13, EL.dens.11$CA_TotalPlants, bubble=6, xlab="", xaxt="n", ylab="",
yaxt="n", main = "CALE density 11 x comm 2013")
ordisurf(EL.test14, EL.dens.11$CA_TotalPlants, bubble=6, xlab="", xaxt="n", ylab="",
yaxt="n", main = "CALE density 11 x comm 2014")
ordisurf(EL.test13, EL.dens.12$CA_TotalPlants, bubble=6, xlab="", xaxt="n", ylab="",
yaxt="n", main = "CALE density 12 x comm 2013")
```

 # R2 vs median plant density

```
R2.plot<-read.csv(file.choose(), header=TRUE)
R2.plot$Site<-as.factor(R2.plot$Site)
```

 # First try years between sampling just for fun

```
par(mfrow=c(1,2))

plot(R2.plot$Year.Diff, R2.plot$prop.rich, type="p", col="white", xlim = c(0.5,4), xlab="Year
difference", ylab="Variation from plant density")
points(jitter(R2.plot$Year.Diff[R2.plot$Site=="EL"]), R2.plot$prop.rich[R2.plot$Site=="EL"],
col="green", pch=17)
points(jitter(R2.plot$Year.Diff[R2.plot$Site=="GH"]), R2.plot$prop.rich[R2.plot$Site=="GH"],
col="orange", pch=1)
points(jitter(R2.plot$Year.Diff[R2.plot$Site=="SP"]), R2.plot$prop.rich[R2.plot$Site=="SP"],
col="red", pch=19)
points(jitter(R2.plot$Year.Diff[R2.plot$Site=="WR"]),
R2.plot$prop.rich[R2.plot$Site=="WR"], col="blue", pch=2)

legend(x="topright", pch=c(17, 1, 19, 2), legend = c("EL", "GH", "SP", "WR"), col=c("green",
"orange", "red", "blue"))

plot(R2.plot$Year.Diff, R2.plot$prop.comp, type="p", col="white", xlim = c(0.5,4), xlab="Year
difference", ylab = "")
points(jitter(R2.plot$Year.Diff[R2.plot$Site=="EL"]), R2.plot$prop.comp[R2.plot$Site=="EL"],
col="green", pch=17)
points(jitter(R2.plot$Year.Diff[R2.plot$Site=="GH"]),
R2.plot$prop.comp[R2.plot$Site=="GH"], col="orange", pch=1)
```

```
points(jitter(R2.plot$Year.Diff[R2.plot$Site=="SP"]), R2.plot$prop.comp[R2.plot$Site=="SP"],
col="red", pch=19)
points(jitter(R2.plot$Year.Diff[R2.plot$Site=="WR"]),
R2.plot$prop.comp[R2.plot$Site=="WR"], col="blue", pch=2)

legend(x="topright", pch=c(17, 1, 19, 2), legend = c("EL", "GH", "SP", "WR"), col=c("green",
"orange", "red", "blue"))

par(mfrow=c(1,2))

# Now look at variation in richness compared to median density

plot(R2.plot$Med.plants, R2.plot$prop.rich, type="p", col="white", xlim = c(0.5,16),
xlab="Median plant density", ylab="R2 value")
points(R2.plot$Med.plants[R2.plot$Site=="EL"], R2.plot$prop.rich[R2.plot$Site=="EL"],
col="green", pch=17)
points(R2.plot$Med.plants[R2.plot$Site=="GH"], R2.plot$prop.rich[R2.plot$Site=="GH"],
col="orange", pch=1)
points(R2.plot$Med.plants[R2.plot$Site=="SP"], R2.plot$prop.rich[R2.plot$Site=="SP"],
col="red", pch=19)
points(R2.plot$Med.plants[R2.plot$Site=="WR"], R2.plot$prop.rich[R2.plot$Site=="WR"],
col="blue", pch=2)

legend(x="topright", pch=c(17, 1, 19, 2), legend = c("EL", "GH", "SP", "WR"), col=c("green",
"orange", "red", "blue"))


# And variation in composition compared to median density

plot(R2.plot$Med.plants, R2.plot$prop.comp, type="p", col="white", xlim = c(0.5,16),
xlab="Median plant density", ylab="")
points(R2.plot$Med.plants[R2.plot$Site=="EL"], R2.plot$prop.comp[R2.plot$Site=="EL"],
col="green", pch=17)
points(R2.plot$Med.plants[R2.plot$Site=="GH"], R2.plot$prop.comp[R2.plot$Site=="GH"],
col="orange", pch=1)
points(R2.plot$Med.plants[R2.plot$Site=="SP"], R2.plot$prop.comp[R2.plot$Site=="SP"],
col="red", pch=19)
points(R2.plot$Med.plants[R2.plot$Site=="WR"], R2.plot$prop.comp[R2.plot$Site=="WR"],
col="blue", pch=2)
legend(x="topright", pch=c(17, 1, 19, 2), legend = c("EL", "GH", "SP", "WR"), col=c("green",
"orange", "red", "blue"))

### Show density range in each site in each year

par(mfrow=c(1,1))
```

```
plot(CA_TotalPlants[density$SampleYear=="2011"] ~
SiteAbbrev[density$SampleYear=="2011"], data = density, xlab="Site", ylab="Plant density")
plot(CA_TotalPlants[density$SampleYear=="2012"] ~
SiteAbbrev[density$SampleYear=="2012"], data = density, xlab="Site", ylab="Plant density")
plot(CA_TotalPlants[density$SampleYear=="2013"] ~
SiteAbbrev[density$SampleYear=="2013"], data = density, xlab="Site", ylab="Plant density")
```

density and richness

```
par(mfrow=c(2,2))
plot(Rich12 ~ CA_TotalPlants, data = EL.dens.11, xlab = "Castilleja density", ylab = "Species
richness", xlim = c(0, 25), ylim = c(10, 35))
plot(Rich12 ~ CA_TotalPlants, data = GH.dens.11, xlab = "Castilleja density", ylab = "Species
richness", xlim = c(0, 25), ylim = c(10, 35))
plot(Rich12 ~ CA_TotalPlants, data = SP.dens.11, xlab = "Castilleja density", ylab = "Species
richness", xlim = c(0, 25), ylim = c(10, 35))
plot(Rich12 ~ CA_TotalPlants, data = WR.dens.11, xlab = "Castilleja density", ylab = "Species
richness", xlim = c(0, 25), ylim = c(10, 35))

plot(Rich13 ~ CA_TotalPlants, data = EL.dens.11, xlab = "CALE density", ylab = "Species
richness")
plot(Rich13 ~ CA_TotalPlants, data = GH.dens.11, xlab = "CALE density", ylab = "Species
richness")
plot(Rich13 ~ CA_TotalPlants, data = SP.dens.11, xlab = "CALE density", ylab = "Species
richness")
plot(Rich13 ~ CA_TotalPlants, data = WR.dens.11, xlab = "CALE density", ylab = "Species
richness")

plot(Rich14 ~ CA_TotalPlants, data = EL.dens.11, xlab = "CALE density", ylab = "Species
richness")
plot(Rich14 ~ CA_TotalPlants, data = GH.dens.11, xlab = "CALE density", ylab = "Species
richness")
plot(Rich14 ~ CA_TotalPlants, data = SP.dens.11, xlab = "CALE density", ylab = "Species
richness")

plot(Rich13 ~ CA_TotalPlants, data = EL.dens.12, xlab = "CALE density", ylab = "Species
richness")
plot(Rich13 ~ CA_TotalPlants, data = GH.dens.12, xlab = "CALE density", ylab = "Species
richness")
plot(Rich13 ~ CA_TotalPlants, data = SP.dens.12, xlab = "CALE density", ylab = "Species
richness")
plot(Rich13 ~ CA_TotalPlants, data = WR.dens.12, xlab = "CALE density", ylab = "Species
richness")

plot(Rich14 ~ CA_TotalPlants, data = EL.dens.12, xlab = "CALE density", ylab = "Species
richness")
```

```
plot(Rich14 ~ CA_TotalPlants, data = GH.dens.12, xlab = "CALE density", ylab = "Species
richness")
plot(Rich14 ~ CA_TotalPlants, data = SP.dens.12, xlab = "CALE density", ylab = "Species
richness")
plot(Rich14 ~ CA_TotalPlants, data = EL.dens.13, xlab = "CALE density", ylab = "Species
richness")
plot(Rich14 ~ CA_TotalPlants, data = GH.dens.13, xlab = "CALE density", ylab = "Species
richness")
plot(Rich14 ~ CA_TotalPlants, data = SP.dens.13, xlab = "CALE density", ylab = "Species
richness")

plot(Rich14 ~ CA_TotalPlants, data = EL.dens.11, xlab = "CALE density 2011", ylab = "Species
richness 2014")
plot(Rich13 ~ CA_TotalPlants, data = EL.dens.12, xlab = "CALE density 2012", ylab = "Species
richness 2013")
plot(Rich14 ~ CA_TotalPlants, data = GH.dens.12, xlab = "CALE density 2012", ylab =
"Species richness 2014")
plot(Rich14 ~ CA_TotalPlants, data = GH.dens.13, xlab = "CALE density 2013", ylab =
"Species richness 2014")
```