

Getting to the Root of It: Effects of *Castilleja* Root Hemiparasitism on Plant Community Structure and Function

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Abstract

Castilleja is a diverse genus of flowering plants in the Orobanchaceae family. Members of this family commonly are root hemiparasites, including *Castilleja*. Root hemiparasites form connections to their host plants underground through organs called haustoria. These parasites take sugar, water, and other nutrients from the host plant via these haustoria. Host identity of the *Castilleja* species in the RMBL region is poorly known. Studies of European *Rhinanthus spp.* have shown that root hemiparasites can increase community diversity by decreasing the competitive ability of their preferred host, allowing less competitive species to enter the community. The community effects of root hemiparasitism are poorly studied in North America. This project addressed these research gaps by conducting host identity and community analyses of *Castilleja* near RMBL. Additionally, we investigated some of the physiological effects of *Castilleja* root hemiparasitism.

Introduction

Among plants, parasitism has evolved in many disparate lineages. Angiosperms make up the vast majority of plant parasites, though non-flowering plants can also exhibit heterotrophy such as the liverwort *Aneura mirabilis* or the podocarp *Parasitaxus usta* (Wickett & Goffinet, 2008; Seyfullah et al., 2016). Within angiosperms, total or partial parasitism occurs in over 270 genera and more than 4750 species (Nickrent, 2020). Some plants parasitize fungal mycelium such as *Corallorhiza spp.* (Orchidaceae), but parasitism on other plants is more common. Parasitic plants are categorized as either root or shoot parasites depending on where the host-parasite connections (called haustoria) are located and as either hemi- or holo-parasites depending on whether the parasitic plant retains its ability to photosynthesize. Hemiparasites can be further classified as facultative or obligate depending on whether they can survive with or without their host (Mutuku et al., 2021). Parasitic plants use their hosts to obtain key resources such as water, sugar, mineral nutrients, and even secondary metabolites as in one study with *Lupinus argenteus* and *Castilleja miniata* (Ducharme & Ehleringer 1996, Adler & Wink, 2001).

Parasitic plants have recently been considered keystone species in the ecosystems they occupy through two main pathways (Watson, 2009). First, these plants are hypothesized to directly alter the competitive dynamics of their community. In one study, the root hemiparasite *Rhinanthus serotinus* (Orobanchaceae) was shown to decrease the biomass and reproductive success of its hosts (Salonen & Lammi, 2001). Whether the host species of root hemiparasites are competitively dominant or subordinate can change whether the parasite has a net increase or decrease in biodiversity within the system via the altered competitive ability of the host (Gibson

& Watkinson 1992; Press & Phoenix, 2005). Additionally, root hemiparasites have been shown to increase rates of nutrient cycling within their communities (Fisher et al., 2013). Root hemiparasites typically have extremely high stomatal conductance, sometimes an order of magnitude higher than their host (Phoenix & Press, 2005). High conductance rates lead to increased accumulation of sugars and other nutrients from their hosts. The tissues of root hemiparasites are extremely nutrient-dense as a result, increasing the rate of nutrient cycling once these tissues decompose. These changes in nutrient cycling can indirectly alter the productivity and competitive dynamics of the parasite's ecosystem (Press 1998).

The Orobanchaceae is a large, monophyletic family with over 90 genera and more than 2,000 species (McNeal et al. 2013). Root parasitism is ubiquitous in the family, occurring in all but one genus (Schneeweiss, 2013). The family, therefore, represents the perfect system for studying the community effects of root parasitism, given the ubiquity of the family across a wide geographic area. *Castilleja* is a widespread genus in the Orobanchaceae with around 200 species, occurring in North and South America as well as in Asia, though the genus is most diverse in Western North America (Tank et al., 2009). *Castilleja* are facultative root hemiparasites and can create haustorial connections with many different species (Heckard, 1962).

The effects of *Castilleja* on plant communities in North America remain poorly studied. One short-term study found that the removal of *Castilleja spp.* do reduce species richness in a montane community, although this was only examined over one growing season (Reed, 2012). Another study in 2012 found that *C. occidentalis* promotes faster nutrient cycling in an alpine ecosystem in the Front Range of Colorado (Spasojevic & Spuding 2012). More studies are needed to confirm these findings and investigate the role of *Castilleja* concerning its effects on community-level biodiversity and structural organization (Watson, 2009). There is little to no information on *Castilleja* host identity and preference, which is critical for inferring the effects of *Castilleja* on competitive dynamics. The effects of parasitism on host physiology in situ are poorly studied as well.

My project aims to close some of these research gaps via three main research objectives. First, we collected *Castilleja*-host root sods and generated a library of confirmed host species. We also collected data on the rates at which different species grew directly next to *Castilleja* in order to indirectly measure host affinity. We hypothesized that *Castilleja* host association would change depending on the habitat to match the most prevalent or competitively dominant species. Next, we collected cover data on *Castilleja* plant communities. With these data, we were able to infer the effects of *Castilleja* on community evenness and diversity. We hypothesized that areas surrounding *Castilleja* plants would have higher species richness. Lastly, we ran a pilot study in which we collected data on the physiology of preferred hosts in areas with and without *Castilleja* present. Various stresses (drought, temperature, herbivory, eg.) alter the phenology of flowering plants (Kazan & Lyons, 2016). We hypothesized that the physiological stresses induced by *Castilleja* parasitism would be visible via altered flowering times.

Methods

Study Sites:

Research was conducted in the vicinity of The Rocky Mountain Biological Laboratory in Gothic, Colorado, USA. Our research sites spanned an elevation gradient of 2700 m to 3190 m and a gradient from sagebrush steppe to subalpine meadows.

Castilleja chromosa

Castilleja chromosa grows at lower elevations and flowers throughout June. Our study site for this species was roughly 11 km east of CO-135 along Jacks Cabin Cutoff at 2705 m (38.72235, -106.81619 $\pm$ 300 m). We named this site ‘Almont’. Dominant members of this plant community included *Chrysothamnus depresus*, *Artemisia arbuscula*, *Chrysothamnus vaseyi*, and *Phlox multiflora*. This site was used in 2023 and 2024. Coordinates and a species list for this site can be found in the Appendix (Table 1, Table 9).

Castilleja linariifolia

Castilleja linariifolia grows at higher elevations than *C. chromosa* and flowers all summer. Two sites for this species were on a rocky slope above the Deer Creek Trail roughly 1.5 km from Gothic at 3005-3025m (38.95312, -106.97474 $\pm$ 300 m). We named these sites ‘Deer Creek 1’ and ‘Deer Creek 2’. Dominant members of the plant community at both Deer Creek sites included *Artemisia tridentata*, *Symphoricarpos rotundifolius*, *Eriogonum umbellatum majus*, and *Festuca thurberi*. These sites were used in 2023 and 2024. A species list and map of this site can be found in the appendix (Table 9, Figure 20).

Another site was located on a Mancos Shale slope 125 m northeast of ‘Stupid Falls’, 1.75 km south of Gothic at 2850 m (38.94352, -106.98258 $\pm$ 10 m). This site was named ‘Stupid Falls’. Dominant members of the plant community included *Eriogonum lonchophyllum*, *Juniperus communis*, *Erigeron speciosus*, and *Populus tremuloides*. This site was used in 2024 for phenology data only. A map of this site can be found in the appendix (Figure 16).

The last site for *Castilleja linariifolia* was located in the Gothic townsite, roughly 50 m east of Ruby Lounge and above Johnson House at 2925 m (38.95993, -106.98721 $\pm$ 25 m). We named this site ‘Johnson Hill’. Dominant members of the plant community included *Populus tremuloides*, *Berberis repens*, *Heliomeris multiflora*, *Lathyrus lanszwertii*, and *Bromus frondosus*. This site was used in 2024 only. A species list and site map for this site can be found in the appendix (Table 9, Figure 21).

Castilleja septentrionalis

This species grows at moderate to higher elevations around Gothic and flowers all summer. The first site we used for this species was a meadow just north of the Avery Picnic parking lot at 2940 m (38.97659, -106.99841 $\pm$ 25 m). This site was named ‘Avery Picnic’. Dominant members of the plant community included *Taraxacum officinale*, *Potentilla pulcherrima*, *Poa pratensis*, *Erigeron subtrinervis*, and *Ipomopsis aggregata*. A rare *Botrychium*

fern was also found in some plots. This site was used in both 2023 and 2024. A species list and site map can be found in the appendix (Table 10, Figure 19).

The next *C. septentrionalis* site was located roughly 1.5 km east of Gothic along the Copper Creek trail at 3090 m (38.96840, -106.96781 ± 15 m). This site was named ‘Copper Creek’. Dominant members of the plant community included *Eremogone congesta*, *Taraxacum officinale*, *Poa pratensis*, *Thalictrum fendleri*, and *Maianthemum stellatum*. This site was used in 2024 only. A species list and coordinates for this site can be found in the appendix (Table 10, Table 11).

The last *C. septentrionalis* site was located in a meadow 50 m southeast of Emerald Lake at 3190 m (39.00924, -107.04057 ± 25 m). We named this site ‘Emerald Lake’. Dominant members of the plant community included *Potentilla pulcherrima*, *Fragaria virginiana*, *Senecio crassulus*, *Deschampsia cespitosa*, and *Anemone multifida*. This site was used in both 2023 and 2024. A species list and site map for this site can be found in the appendix (Table 10, Figure 18).

Host Identity:

Root sods of *Castilleja* plants surrounded by potential host species were dug up and taken back to the RMBL Herbarium to soak in water. These sods were taken primarily from our study sites. Soil with more clay was soaked for longer, usually over 12 hours. The root sods were gently agitated to rinse out the soil, leaving only roots behind. The roots were taken to a dissecting microscope to look for haustoria on host roots. If haustoria were found, the potential host species was recorded as a confirmed host. When the haustoria couldn't be traced back to a host plant, the roots and haustoria were removed and will be sent to the University of Wyoming's Tank Lab to be sequenced. These sods were then vouchered as an extended specimen for the RMBL Herbarium. The specific *Castilleja* plants we chose to dig up generally grew in sandier soil to make cleaning the roots easier. We also tried to choose *Castilleja* which were growing next to as few potential host species as possible so that the root density was less, making confirming the species identity of the roots easier.

Community diversity and ‘Nearest Neighbor’ sampling:

Community composition and species abundance were estimated using quadrat sampling methods. *Castilleja* "Treatment" plots were centered on a focal *Castilleja* plant and Control plots were placed nearby in an area without a *Castilleja* plant. We did community sampling at Almont for *C. chromosa*, Avery Picnic, Copper Creek, and Emerald Lake for *C. septentrionalis*, and Deer Creek 1+2 and Johnson Hill for *C. linariifolia*.

The methods used for determining what *Castilleja* plants were to be sampled varied based on local site conditions, mainly the density of *Castilleja* plants. At sites with low *Castilleja* density such as Johnson Hill, all individuals within the population were sampled. In areas with higher densities, a transect was laid across the population. Transects were laid parallel to the slope so that every plot was at the same elevation. Every *Castilleja* plant that touched the transect was sampled in moderate-density populations. In areas with high-density populations,

every few plants that touched the transect were sampled. Control plots were selected by moving the quadrat in a consistent direction adjacent to the *Castilleja* plot. We tried to locate control plots at least 10 cm away from any *Castilleja* plants. In areas with higher densities of *Castilleja*, the nearest location without any *Castilleja* was chosen for the control plot.

In all plots (*Castilleja* and Control), the percent cover and count of every species were estimated in a .5 m x .5 m quadrat (.25 m²). A clear plastic sheet of a specific size (1% and 5% of the total plot area) was used to help calibrate our estimates. The percent bare ground of every plot was also recorded. Samples of plant community members were taken back to the Rocky Mountain Biological Laboratory Herbarium (the Herbarium) to be identified and vouchered to ensure the accuracy of species identifications. In *Castilleja* plots, we noted the identities of the species growing within ~10 cm of any *Castilleja* in the plot, termed ‘Nearest Neighbors’ (NNs). The number of *Castilleja* stems in *Castilleja* plots was also recorded. The number of paired plots surveyed from each site is shown in Table 1.

Table 1. Number of paired plots at each site

Hemiparasite	Site	2023 Plots	2024 Plots
<i>Castilleja chromosa</i>	Almont	15	20
<i>Castilleja linariifolia</i>	Deer Creek 1	20	20
<i>Castilleja linariifolia</i>	Deer Creek 2	20	20
<i>Castilleja linariifolia</i>	Johnson Hill	0	20
<i>Castilleja septentrionalis</i>	Avery Picnic	20	20
<i>Castilleja septentrionalis</i>	Emerald Lake	20	20
<i>Castilleja septentrionalis</i>	Copper Creek	0	20
	Yearly sum:	95	140
	Total sum:		235

Instantaneous Phenology sampling:

Three species were examined to gauge the effect of *Castilleja* parasitism on the phenology of the host species. *Eremogone congesta* was used at Deer Creek 2. *Eriogonum lonchophyllum* and *Erigeron speciosus* were used at ‘Stupid Falls’. These species were chosen because they were on the cusp of bud/flower at the time of sampling and were either confirmed hosts via haustorial analysis or were obviously hosts because of their prevalence as NNs of *Castilleja* plants in areas with a high proportion of bare ground. Hosts were chosen for sampling if they were growing within ~5 cm of a *C. linariifolia*. Every host in the population that met this criteria was sampled. 20 samples were measured for *Eremogone congesta*, 9 for *Eriogonum lonchophyllum*, and 10 for *Erigeron speciosus*. A 28 cm diameter wire circle was placed over the host and the number of buds and flowers of that species within the ring were counted. In host species with an umbellate/composite flower structure, if any flowers were open, the entire head was counted as 1 flower. If no flowers were open, the entire head was counted as 1 bud. Control

plots were located by moving the ring to the nearest host species that was more than ~15 cm away from any *Castilleja*.

Data analysis methods:

Data analysis for our phenology data was done in RStudio. Data were analyzed using a Wilcoxon signed rank test to determine if there were any significant differences between treatments.

Our NN analysis was done in JMP. Frequency distributions were made by computing relative frequencies of each species by cover and by the proportion of total NN instances of that species. A linear regression was computed for the relative frequencies, and a 95% confidence interval for the individual points was generated. Species that fell outside of that confidence interval were considered NN outliers. These species either were a NN many more or many fewer times than we expected based on their relative cover.

Analysis for community-level diversity effects was done in RStudio. Richness, Shannon's diversity, and evenness were calculated for each plot. Cover data, rather than count data were used for these metrics. Because of the differences in size between the smallest annuals and largest perennials, cover, rather than count, gave a better depiction of community composition and dominance. A Linear mixed effect model was used to estimate changes in richness, diversity, and evenness. An Anova was then used to calculate the deviance and to compare the inclusion of each model factor. This analysis was done pairwise in order to help control for micro-site effects. We allowed *Castilleja* to contribute to richness scores in our plots because they are also members of the community, not just the treatment. We ran these tests without *Castilleja* contributing to richness and found changes in the magnitude but not significance of the effect, and so decided to exclude these more conservative results from our paper. *Castilleja chromosa* was excluded from these analyses because no cover or count data (only presence/absence from plots) were collected in 2023.

Results

Host Identity:

Haustoria:

We were able to find haustorial connections on 4 *Castilleja* species: *C. chromosa*, *C. septentrionalis*, *C. miniata*, and *C. linariifolia*. A total of 11 unique host species were identified with direct host-parasite connections. *Viola adunca* and *Fragaria virginiana* were found to be host species for 2 *Castilleja* species. The 11 detected host species include a range of functional groups including 7 forbs, 2 grasses, and 2 shrubs. Three of the confirmed hosts were from the Asteraceae family, 2 from the Poaceae, 2 from the Rosaceae, 2 from the Violaceae, 1 from the Caryophyllaceae, and 1 from the Celestraceae. Table 2 summarizes the findings of our haustorial analysis.

Table 2. A summary of host-parasite confirmed haustorial connections. Forbs are colored in red, grasses in yellow, and shrubs in blue.

Hemiparasite	Host	RMBL Herbarium Collector Numbers
<i>Castilleja chromosa</i>	<i>Achillea millefolium</i>	CFW-3450
<i>Castilleja chromosa</i>	<i>Chrysothamnus vasseyi</i>	CFW-3446, CFW-3486, CFW-3493
<i>Castilleja chromosa</i>	<i>Antennaria parviflora</i>	CFW-3484
<i>Castilleja chromosa</i>	<i>Achnatherum pinetorum</i>	CFW-3495
<i>Castilleja septentrionalis</i>	<i>Viola adunca</i>	CFW-3519
<i>Castilleja septentrionalis</i>	<i>Fragaria virginiana</i>	CFW-3519
<i>Castilleja miniata</i>	<i>Fragaria virginiana</i>	CFW-3522
<i>Castilleja miniata</i>	<i>Potentilla pulcherrima</i>	CFW-3523
<i>Castilleja linariifolia</i>	<i>Viola praemorsa</i>	CFW-3524
<i>Castilleja linariifolia</i>	<i>Koeleria macrantha</i>	CFW-3535
<i>Castilleja linariifolia</i>	<i>Eremegone congesta</i>	Anders Hastings #58
<i>Castilleja linariifolia</i>	<i>Viola adunca</i>	Anders Hastings #84
<i>Castilleja linariifolia</i>	<i>Paxistima myrsinites</i>	Anders Hastings #84

‘Nearest Neighbors’:

Figures 1-10 show the frequency distributions of relative abundance by cover and NN instances for all sites and years. All of these distributions were right-skewed with a few common and many uncommon/rare species. NNs were predominantly associated with the most abundant species in each community. We were able to find a few species that were outliers based on what we expected their NN frequency to be based on their relative abundance (outside the 95% confidence intervals for the regression). We found seventeen instances of NN outliers among 17 taxa across all our sites and years (Table 3). 16 of these outliers were from taxa that were recorded as NNs more often than we expected based on their relative abundance in the community. *Artemisia arbuscula* was the only species we found that had a lower number of NN instances than we expected. Of the positive outliers (NN frequency was higher than expected), 9 were grasses, 7 were forbs, and 0 were shrubs. Poaceae was represented 7 times, followed by Cyperaceae with 2 representatives. The rest of the represented forb plant families included Asteraceae, Boraginaceae, Caryophyllaceae, Plantaginaceae, Ranunculaceae, Rosaceae, and Violaceae with 1 representative each.



Figure 1. Frequency distributions of relative abundance by cover and ‘Nearest Neighbor’ occurrences at Almont in 2023

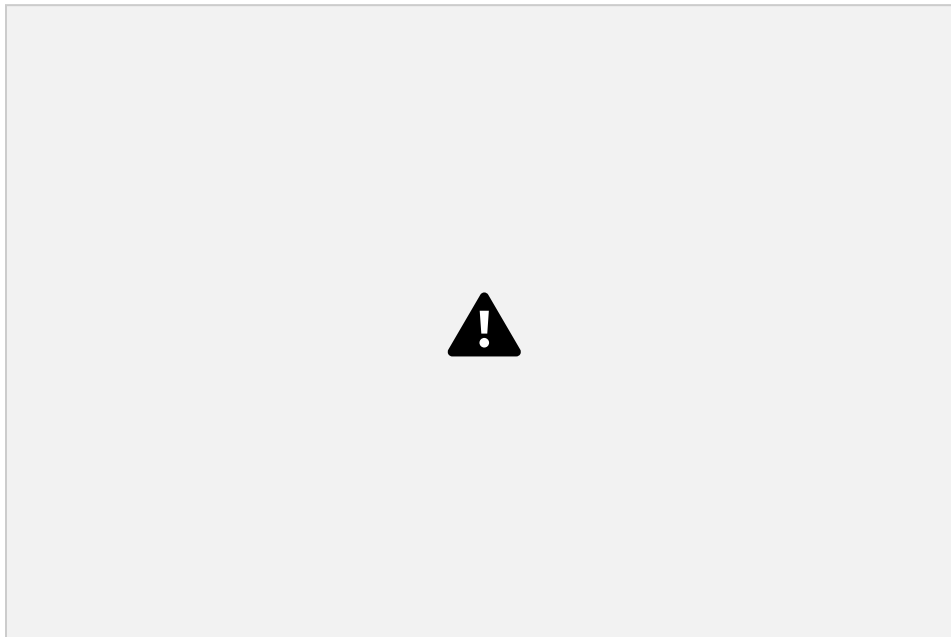


Figure 2. Frequency distributions of relative abundance by cover and ‘Nearest Neighbor’ occurrences at Almont in 2024

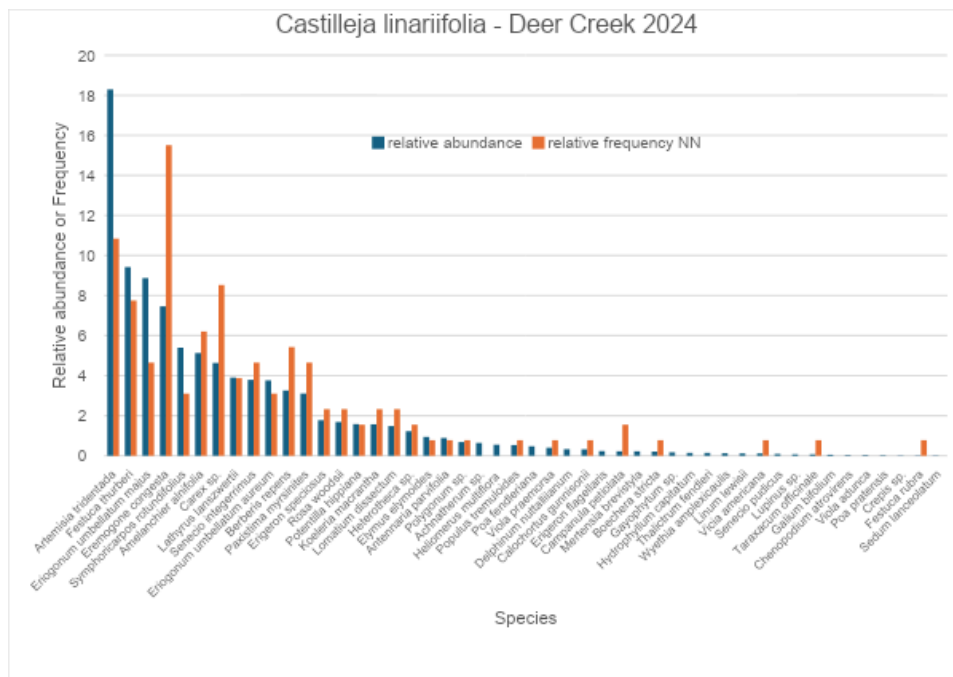




Figure 5. Frequency distributions of relative abundance by cover and ‘Nearest Neighbor’ occurrences at Johnson Hill in 2024

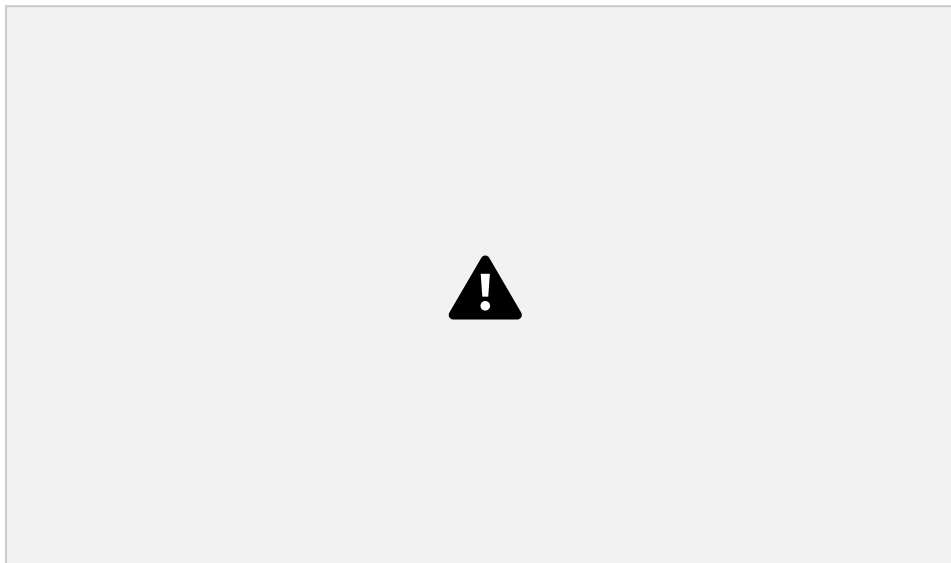


Figure 6. Frequency distributions of relative abundance by cover and ‘Nearest Neighbor’ occurrences at Avery in 2023



Figure 7. Frequency distributions of relative abundance by cover and ‘Nearest Neighbor’ occurrences at Avery in 2024

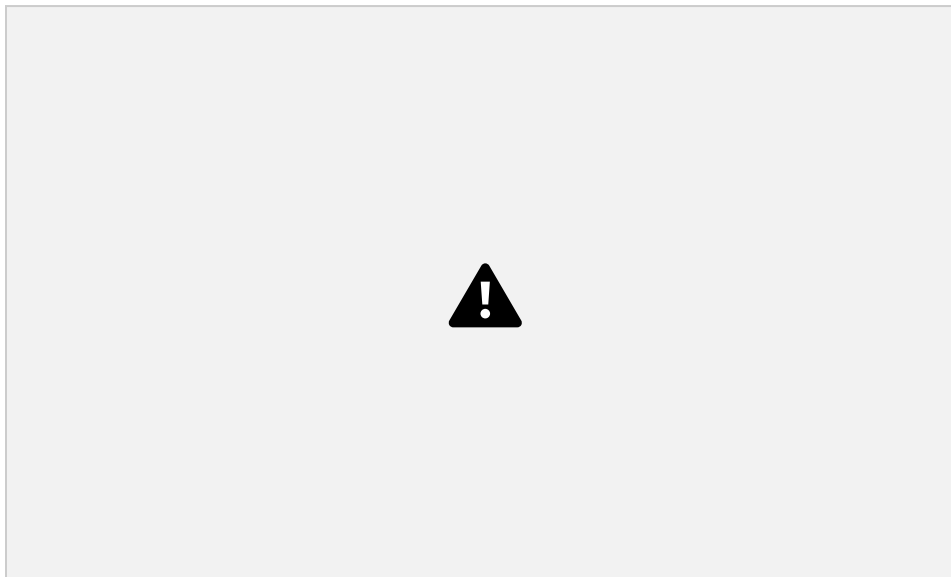


Figure 8. Frequency distributions of relative abundance by cover and ‘Nearest Neighbor’ occurrences at Emerald Lake in 2023

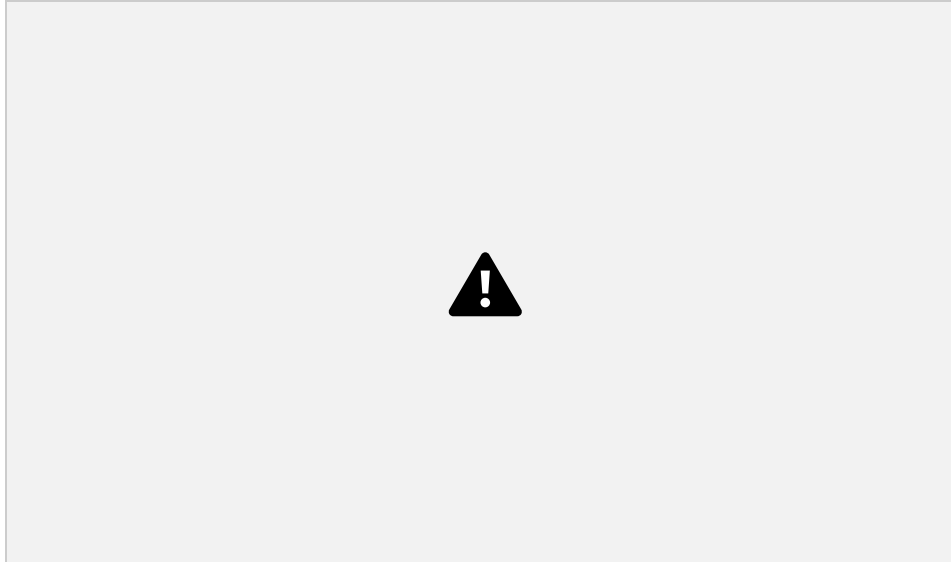


Figure 9. Frequency distributions of relative abundance by cover and ‘Nearest Neighbor’ occurrences at Emerald Lake in 2024

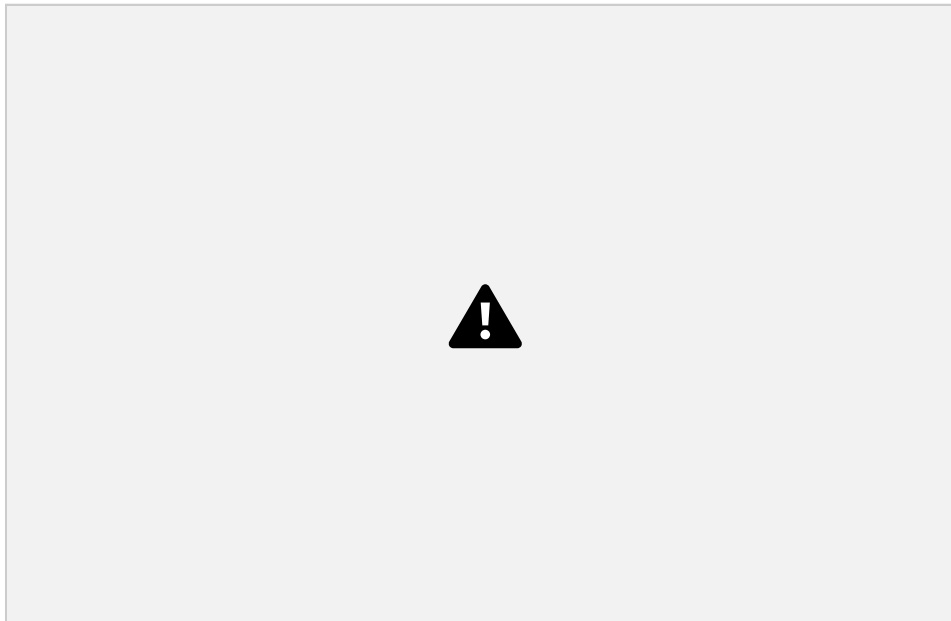


Figure 10. Frequency distributions of relative abundance by cover and ‘Nearest Neighbor’ occurrences at Copper Creek in 2024

Table 3. A summary of ‘Nearest Neighbor’ outliers by site and year. Forbs are colored in red, grasses in yellow, and shrubs in blue.

Site	Year	Parasite	Outliers	Trend
Avery	23	<i>Castilleja septentrionalis</i>	<i>Poa pratensis</i>	NN higher
Emerald Lake	23	<i>Castilleja septentrionalis</i>	<i>Deschampsia cespitosa</i>	NN higher
Emerald Lake	24	<i>Castilleja septentrionalis</i>	<i>Fragaria virginiana</i>	NN higher
Copper Creek	24	<i>Castilleja septentrionalis</i>	<i>Thalictrum fendleri</i> , <i>Mertensia brevistyla</i>	NN higher
Deer Creek 1+2	23	<i>Castilleja linariifolia</i>	<i>Carex sp.</i> , <i>Achnatherum sp.</i> , <i>Bromus inermis</i>	NN higher
Deer Creek 1+2	24	<i>Castilleja linariifolia</i>	<i>Carex sp.</i> , <i>Eremegone congesta</i>	NN higher
Johnson	24	<i>Castilleja linariifolia</i>	<i>Bromus frondosus</i> , <i>Viola praemorsa</i>	NN higher
Almont	2023	<i>Castilleja chromosa</i>	<i>Poa fendleriana</i> , <i>Penstemon caespitosus</i>	NN higher
Almont	2024	<i>Castilleja chromosa</i>	<i>Stipeae</i> , <i>Crepis sp.</i> , <i>Artemisia arbuscula</i>	NN higher, lower for <i>Artemisia arbuscula</i>

Host identities obtained from haustorial analysis and NN analysis are summarized in Table 4. In total, we found 21 unique host taxa. 8 were grasses or sedges, 11 were forbs, and 2 were shrubs. 11 plant families were represented. Poaceae had the most instances of host detection at 9, followed by Asteraceae with 4. Rosaceae and Violaceae were detected twice. Boraginaceae, Caryophyllaceae, Celestraceae, Cyperaceae, Plantaginaceae, and Ranunculaceae all had only 1 representative. *Eremegone congesta*, *Fragaria virginiana*, and *Viola praemorsa* were the only species detected as hosts using both methods.

Table 4. A summary of host identities obtained from haustorial and ‘Nearest Neighbor’ analysis. Forbs are colored in red, grasses and sedges in yellow, and shrubs in blue. Bolded species were detected as a host using both methods

Hemiparasite	Host Identity	Hauatoria	NN
<i>Castilleja chromosa</i>	<i>Achnatherum pinetorum</i>	Yes	No
<i>Castilleja linariifolia</i>	<i>Achnatherum sp.</i>	No	Yes
<i>Castilleja linariifolia</i>	<i>Bromus frondosus</i>	No	Yes
<i>Castilleja linariifolia</i>	<i>Bromus inermis</i>	No	Yes
<i>Castilleja linariifolia</i>	<i>Carex sp.</i>	No	Yes
<i>Castilleja septentrionalis</i>	<i>Deschampsia cespitosa</i>	No	Yes
<i>Castilleja linariifolia</i>	<i>Koeleria macrantha</i>	Yes	No
<i>Castilleja chromosa</i>	<i>Poa fendleriana</i>	No	Yes
<i>Castilleja septentrionalis</i>	<i>Poa pratensis</i>	No	Yes
<i>Castilleja chromosa</i>	<i>Stipeae</i>	No	Yes
<i>Castilleja chromosa</i>	<i>Achillea millefolium</i>	Yes	No
<i>Castilleja chromosa</i>	<i>Antennaria parviflora</i>	Yes	No
<i>Castilleja chromosa</i>	<i>Crepis sp.</i>	No	Yes
<i>Castilleja linariifolia</i>	<i>Eremogone congesta</i>	Yes	Yes
<i>Castilleja septentrionalis, miniata</i>	<i>Fragaria virginiana</i>	Yes	Yes
<i>Castilleja septentrionalis</i>	<i>Mertensia brevistyla</i>	No	Yes
<i>Castilleja chromosa</i>	<i>Penstemon caespitosus</i>	No	Yes
<i>Castilleja miniata</i>	<i>Potentilla pulcherrima</i>	Yes	No
<i>Castilleja septentrionalis</i>	<i>Thalictrum fendleri</i>	No	Yes
<i>Castilleja septentrionalis, linariifolia</i>	<i>Viola adunca</i>	Yes	No
<i>Castilleja linariifolia</i>	<i>Viola praemorsa</i>	Yes	Yes
<i>Castilleja chromosa</i>	<i>Chrysothamnus vasseyi</i>	Yes	No
<i>Castilleja linariifolia</i>	<i>Paxistima myrsinites</i>	Yes	No

Community diversity indices

Figure 11 shows the changes in richness, evenness, and Shannon’s diversity for all sites with *Castilleja linariifolia*. Shannon’s diversity showed a consistent trend where diversity was

higher in *Castilleja* plots. The p -values for these changes were 0.0549, <.0001, and 0.0260 for Deer Creek 1, Deer Creek 2, and Johnson Hill, respectively. Similar trends were observed for species richness with p -values of 0.0016, <.0001, and 0.1853, respectively. Johnson Hill was the only site where changes in species richness were not significant. We found some increases in evenness in *Castilleja* plots for our *C. linariifolia* sites with p -values of 0.9823, 0.0218, and 0.0325, respectively (Tables 5 and 6).

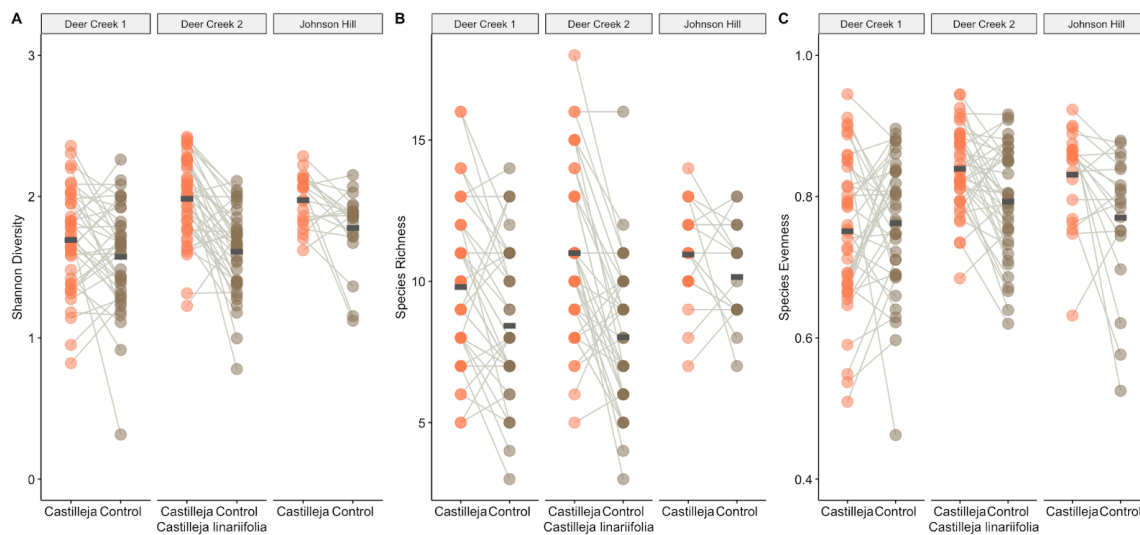


Figure 11. Shannon diversity index, species richness, and species evenness by treatment for *Castilleja linariifolia* across all three study sites.

Figure 12 shows the changes in richness, evenness, and Shannon's diversity for all sites with *Castilleja septentrionalis*. Shannon's diversity showed a consistent trend where diversity was higher in *Castilleja* plots. The p -values for these changes were 0.0018, 0.0240, and 0.0004 for Avery Picnic, Copper Creek, and Emerald Lake, respectively. The same trends were observed for species richness with p -values of 0.0006, 0.0092, and 0.0001, respectively. We found no effect on evenness for *C. septentrionalis* with p -values of 0.6847, 0.3634, and 0.2763, respectively (Tables 5 and 6).

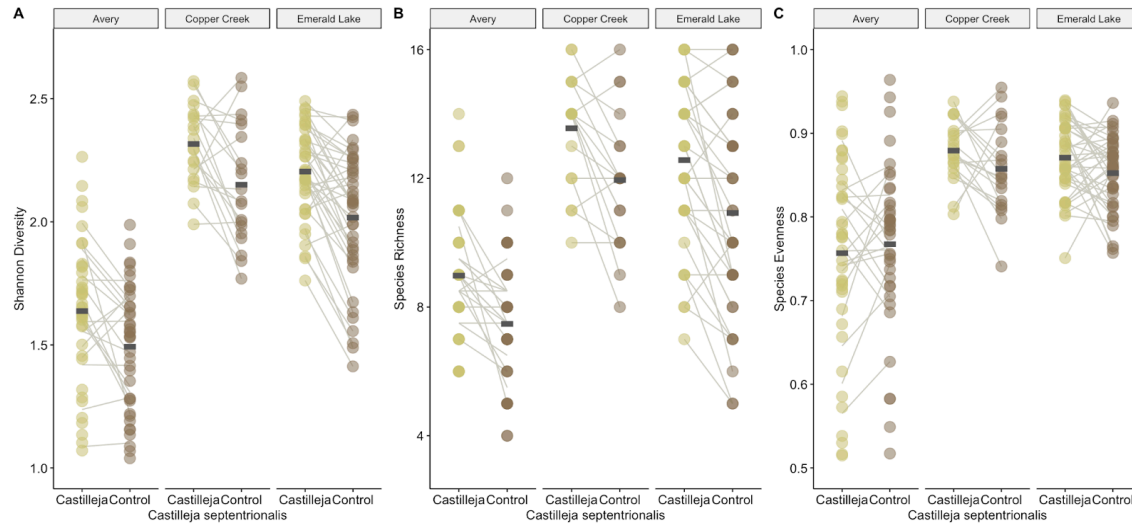


Figure 12. Shannon diversity index, species richness, and species evenness by treatment for *Castilleja septentrionalis* across all three study sites.

Table 5. A summary table of trends with species richness for *Castilleja linariifolia* and *Castilleja septentrionalis* across all study sites.

Hemi-parasite	Site	Richnes trend	Δ Richness	SE	<i>p</i> -val
<i>Castilleja linariifolia</i>	Deer Creek 1	<i>Castilleja</i> >Control	1.38	0.42	0.0016
<i>Castilleja linariifolia</i>	Deer Creek 2	<i>Castilleja</i> >Control	2.98	0.42	<.0001
<i>Castilleja linariifolia</i>	Johnson	not significantly different	0.8	0.60	0.1853
<i>Castilleja septentrionalis</i>	Avery	<i>Castilleja</i> >Control	1.5	0.43	0.0006
<i>Castilleja septentrionalis</i>	Copper Creek	<i>Castilleja</i> >Control	1.6	0.61	0.0092
<i>Castilleja septentrionalis</i>	Emerald Lake	<i>Castilleja</i> >Control	1.73	0.43	0.0001

Table 6. A summary table of trends with evenness and Shannon's diversity for *Castilleja linariifolia* and *Castilleja septentrionalis* across all study sites.

Hemi-parasite	Site	Evenness trend	<i>p</i> -val	Shannon trend	<i>p</i> -val
<i>Castilleja linariifolia</i>	Deer Creek 1	No trend	0.9823	Castilleja>Control (marginally significant)	0.0549
<i>Castilleja linariifolia</i>	Deer Creek 2	Castilleja>Control	0.0218	Castilleja>Control	<.0001
<i>Castilleja linariifolia</i>	Johnson	Castilleja>Control	0.0325	Castilleja>Control	0.0260
<i>Castilleja septentrionalis</i>	Avery	No trend	0.6847	Castilleja>Control	0.0018
<i>Castilleja septentrionalis</i>	Copper Creek	No trend	0.3634	Castilleja>Control	0.0240
<i>Castilleja septentrionalis</i>	Emerald Lake	No trend	0.2763	Castilleja>Control	0.0004

Bare ground:

Bare ground is the reciprocal of plant cover and a higher percentage of bare ground suggests a reduction in plant cover and possibly plant growth and productivity. Changes in bare ground based on treatment are summarized in Table 7. We found that 5 out of the 9 sites/years had significantly higher bare ground in control plots ($P < .05$). Only one site, Almont 24, had higher bare ground in *Castilleja* plots, though this was non-significant ($P = .504$).

Table 7. A summary table of changes in bare ground between *Castilleja* treatments at each site and year. Bolded lines represent significant changes.

Species	Year	Site	Δ (Con-Cast)	Anova <i>p</i> -val
<i>Castilleja chromosa</i>	24	Almont	-0.035	0.504
<i>Castilleja linariifolia</i>	23	Deer Creek 1 + 2	0.1313	0.0172
<i>Castilleja linariifolia</i>	24	Deer Creek 1 + 2	0.105	0.0293
<i>Castilleja linariifolia</i>	24	Johnson	0.02505	>.7
<i>Castilleja septentrionalis</i>	23	Avery Picnic	0.1105	0.036
<i>Castilleja septentrionalis</i>	23	Emerald Lake	0.0595	0.2323
<i>Castilleja septentrionalis</i>	24	Avery Picnic	0.043	0.1109
<i>Castilleja septentrionalis</i>	24	Emerald Lake	0.1045	0.034
<i>Castilleja septentrionalis</i>	24	Copper Creek	0.0905	0.0206

Phenology:

Of the three host species we assessed for phenology, *Eremegone congesta*, *Eriogonum lonchophyllum*, and *Erigeron speciosus*, we found a consistent but non-significant trend in their phenology scores (Figures 13, 14, and 15). The mean differences in phenology scores for each host by treatment were 0.07, 0.13, and 0.07 % earlier in *Castilleja* plots, respectively. That is, hosts in *Castilleja* plots bloomed later on average than in Control plots. *p*-values for these species were 0.436, 0.221, and 0.286, respectively (Table 8).

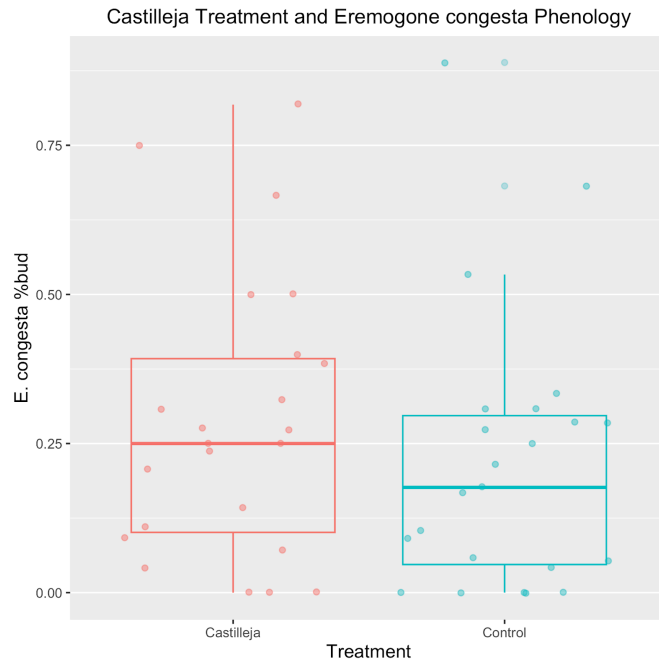


Figure 13. The effect of *Castilleja* treatment on the phenology scores of *Eremogone congesta* expressed as % flower stalks in bud. $p\text{-val} = 0.436$

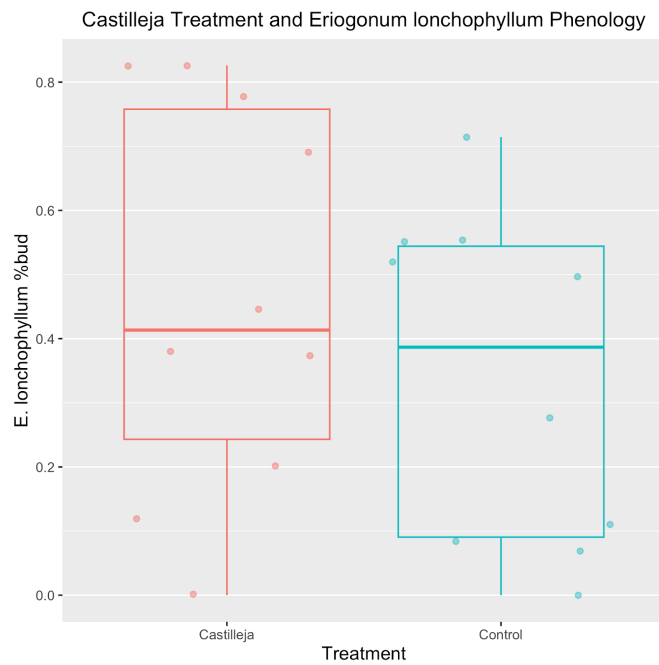


Figure 14. The effect of *Castilleja* treatment on the phenology scores of *Eriogonum lonchophyllum* expressed as % flower stalks in bud. $p\text{-val} = 0.221$

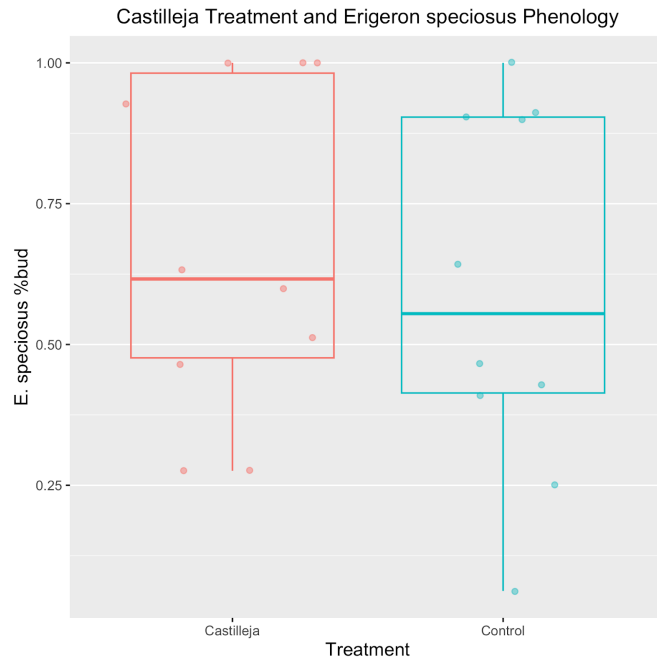


Figure 15. The effect of *Castilleja* treatment on the phenology scores of *Erigeron speciosus* expressed as % flower stalks in bud. $p\text{-val} = 0.286$

Table 8. A summary of phenology scores for all three species expressed % stalks in bud (# buds/(#buds + #flowers))

Host	Hemi-parasite	Sample size	<i>Castilleja</i> Mean (%)	<i>Castilleja</i> SD (%)	Control Mean (%)	Control SD (%)	Difference of Means (%)	Trend	$p\text{-val}$ (Wilcoxon signed rank)
<i>Eremogone congesta</i>	<i>C. linariifolia</i>	20	0.29	0.23	0.22	0.23	0.07	<i>Castilleja</i> earlier	0.436
<i>Eriogonum lonchophyllum</i>	<i>C. linariifolia</i>	9	0.46	0.30	0.34	0.26	0.13	<i>Castilleja</i> earlier	0.221
<i>Erigeron speciosus</i>	<i>C. linariifolia</i>	10	0.67	0.29	0.60	0.32	0.07	<i>Castilleja</i> earlier	0.286

Discussion

In this study, we have added substantially to our knowledge of *Castilleja* host specificity. We further found that the presence of *Castilleja* had significant effects on surrounding plant communities in terms of diversity, growth, and phenology. Each of these effects and their implications are discussed below.

Host Identity:

Overall, we found that the three species studied, *Castilleja septentrionalis*, *C. linariifolia*, and *C. chromosa* are generalists in terms of host specificity. We were able to infer 22 unique host taxa. This list had representatives from all functional groups (grass, forb, shrub), and included 11 plant families (Table 4). These results align with previous literature which indicates that *Castilleja* as a genus can parasitize a wide range of hosts (Heckard 1962).

Interestingly, we did see differences between our two host detection methods. Our haustorial analysis methods were likely very biased. We were only able to sample in areas where clay content was low enough that we were able to easily rinse out the soil from our root balls. We also chose to collect in areas where root densities were low in order to make the microscope work easier. Additionally, we likely undersampled grasses because identifiable reproductive structures were absent during some of the time we collected root samples. Grass roots are often dense with many fine roots near the soil surface, making finding haustoria for grasses more difficult than for many forbs. Using this method, we were able to find 2 grass host species compared to 7 forb species (Table 2). It is possible we also undersampled shrubs with this method, given we only detected 2 shrub host species (Table 2). We were only able to collect root samples from the upper ~ 10 cm of soil. Many of the shrub species in our study areas are deep-rooted and don't have high fine root density near the soil surface, decreasing the chance of finding haustoria which we only found on roots without any periderm (bark). I don't think that the undersampling of shrubs misrepresents our study sites, given that no shrubs were detected as positive NN outliers (Table 3). Interestingly, some of the *Castilleja* we sampled for haustoria were found parasitizing multiple hosts at the same time. Perhaps these *Castilleja* are able to moderate their parasitic draw between these different hosts depending on the season, using a certain host more strongly during the season where it is most productive, but more research needs to be done.

Our NN methods eliminated all of the sampling biases from our host detection, given all species were analyzed simultaneously. Unfortunately, this is an indirect method, so species that were detected as NNs might not be parasitized. Given the wide range of taxonomic diversity *Castilleja* can parasitize given our haustorial analysis and the fact that three NN outliers were also confirmed hosts via haustoria analysis, it is a safe assumption that NN frequencies are a good proxy for the frequency at which different community members are being parasitized (Table 4). Our NN data indicates that in general, our *Castilleja* seem to parasitize community members roughly in proportion to how abundant they appear in the community, though there were exceptions to this pattern at nearly every site. Those exceptions were almost always species that were more dominant in the community in terms of percent cover (Figures 1-10).

Taking our two host detection methods together, we found that our focal *Castilleja* species prefer to parasitize grasses and forbs over shrubs. During our haustorial analysis, we noticed that *Castilleja* root systems rarely extended farther than 15 cm away from the plant. All of our study sites were drier areas relative to the surrounding area. Because of this, shrub root systems were likely out of reach of the *Castilleja* most of the time. Anecdotally, *C. miniata* (a common *Castilleja* species around RMBL, but outside of the focus of our study) was often found

growing in dense stands underneath *Salix spp.* thickets (iNaturalist observations 224748911, 224748939, 224748817, 224748811, 171963565, and 171963823). These areas have much higher water tables, so the fine roots of these shrubs are much closer to the surface. Another study found that *C. linariifolia* is able to parasitize *Artemisia tridentata* (Ducharme & Ehleringer 1996). Perhaps our *Castilleja* would prefer to parasitize shrubs because of their large biomass, but are simply unable to in our systems because of the incompatibility between the rooting depth of these species.

Community effects:

We found that the presence of *Castilleja* had a consistent, significant effect on species richness at all but one site for *C. septentrionalis* and *C. linariifolia* (Table 5). The magnitude of this change ranged from .8-2.98 more species on average in *Castilleja* plots, indicating that the effect of *Castilleja* on species richness is site/community specific. If *Castilleja* are excluded from richness counts, the trend remains significant for most sites, though the magnitude of the diversity effect was reduced. The presence of *Castilleja* did not affect evenness for *C. septentrionalis* but evenness was affected by the presence of *C. linariifolia* (table 6). Our study sites were already relatively even to begin with; only two sites had any species which accounted for more than 15% of the total cover among all plots. Taking these two diversity metrics together with Shannon's diversity index, we found consistent, though slightly less significant increases in *Castilleja* plots. This makes sense given Shannon's diversity is simply a combination of richness and evenness. We did find slight differences in the strength of the richness, diversity, and evenness effects between sites and between species, indicating that the impacts of parasitism are site-specific and depend on the specific *Castilleja* species you are looking at.

The trend we found in species richness and Shannon's diversity could be driven by a few factors. First, *Castilleja* might simply grow in micro-sites that support higher species richness regardless of *Castilleja* presence. Unfortunately, this study design can't differentiate causative vs correlative effects, but there is some support for alternative hypotheses. One alternative is that *Castilleja* parasitism is acting as a mechanism for competitive release because of reduced fitness in dominant community members (Gibson and Watkinson 1992). Almost all of the species we found as NN outliers were species with relatively high percent cover. Averaged together, the NN outliers are in the 82nd percentile in terms of cover, indicating a higher association with more dominant community members. Another possible explanation for the diversity effect is increased nutrient cycling rates from nutrient-dense *Castilleja* litter (Fisher et al. 2013). Increased nutrient cycling may allow more species to coexist because of decreased competition for nutrients. We found that bare ground was generally higher in control plots than in *Castilleja* plots (table 7). If the increase in cover in *Castilleja* plots is driven by changes in nutrient cycling, those changes might also explain increases in richness.

Castilleja is associated with higher diversity driven by an increase in species richness. Even if we can't prove the cause, our evidence suggests, at the very least, that *Castilleja* is associated with high plant species diversity. These findings add to the growing body of literature

which indicate that parasites, despite their relatively low biomass, have outsized impacts on their communities; regulating energy flow, competition, nutrient cycling, and biodiversity provisioning (Hudson et al. 2006).

Phenology:

Our pilot study on the effects of *Castilleja* parasitism on the phenology of three host species found no significant differences between ‘parasitized’ and ‘unparasitized’ hosts (table 8). We did see a consistent but weak trend where parasitized hosts were delaying their phenology among all three hosts. We likely had too few samples to detect any effect. In the future, larger sample sizes and continuous rather than instantaneous phenology measurements would better be able to answer our question. RMBL has many long-term phenology data sets, and finding a way to integrate *Castilleja* presence/absence data with these data sets might provide another opportunity to interrogate the effect of parasitism on phenology.

Conclusions

We found that *Castilleja chromosa*, *C. septentrionalis*, and *C. linariifolia* are generalists in terms of host choice. These species were able to parasitize all functional groups and a wide range of taxonomic groups. We also found that these species parasitize community members roughly in proportion to their abundance, though almost every site we looked at had outliers that were parasitized more often than expected. These outliers were generally more dominant members of the plant community, indicating some preference for those dominant species. These outliers were different at each site, indicating that host preference is site-specific.

We found that *C. linariifolia* and *C. septentrionalis* are associated with areas of higher richness and diversity. Our study design was unable to find a causal relationship, but we did find some evidence in support of both the competitive release hypothesis (higher association with more dominant plant species) and the nutrient cycling hypothesis (decreased bare ground in *Castilleja* plots). More work needs to be done to understand the mechanism driving these patterns.

Lastly, we were unable to make any conclusions about the ability of *Castilleja* to alter host physiology via altered flowering times.

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Appendix

Table 9. Species lists for Almont, Deer Creek 1+2, and Johnson Hill. Almont species are listed alphabetically, the others by relative abundance in terms of percent cover

Almont	Notes	Deer Creek 1+2	Notes	Johnson Hill	Notes
<i>Achillea millefolium</i>		<i>Artemisia tridentata</i>		<i>Berberis repens</i>	
<i>Agoseris glauca</i>		<i>Symphoricarpos rotundifolius</i>		<i>Heliomerus multiflora</i>	
<i>Allium geeyeri</i>		<i>Eriogonum umbellatum majus</i>		<i>Lathyrus lanszwertii</i>	
<i>Amelanchier alnifolia</i>		<i>Festuca thurberi</i>		<i>Bromus frondosus</i>	
<i>Antennaria dimorpha</i>		<i>Castilleja linariifolia</i>		<i>Eriogonum umbellatum majus</i>	
<i>Antennaria parviflora</i>		<i>Eremogone congesta</i>		<i>Castilleja linariifolia</i>	
<i>Antennaria rosea</i>		<i>Amelanchier alnifolia</i>		<i>Artemisia dracunculul</i>	
<i>Artemisia arbuscula</i>		<i>Lathyrus lanszwertii</i>		<i>Viola praemorsa</i>	
<i>Artemisia frigida</i>		<i>Carex</i> sp.	<i>obtusata, geeyeri</i>	<i>Erigeron speciosus</i>	
<i>Artemisia tridentata</i>		<i>Berberis repens</i>		<i>Poa pratensis</i>	
Asteraceae		<i>Erigeron speciosus</i>		<i>Thalictrum fendleri</i>	
<i>Astragalus drummondii</i>		<i>Senecio integerrimus</i>		<i>Lomatium dissectum</i>	
<i>Astragalus miser</i>		<i>Eriogonum umbellatum aureum</i>		<i>Helianthella quinquenervis</i>	
<i>Astragalus</i> sp.	<i>drummondii, miser</i>	<i>Achnatherum</i> sp.	<i>nelsonii, lettermannii</i>	<i>Collomia linearis</i>	
<i>Balsamorhiza sagittata</i>		<i>Paxistima myrsinites</i>		<i>Elymus trachycaulus</i>	
<i>Berberis repens</i>		<i>Rosa woodsii</i>	<i>Rosa blanda</i> subsumed	<i>Vicia americana</i>	
<i>Boechera consanguinea</i>		<i>Heterotheca</i> sp.		<i>Polygonum</i> sp.	most likely <i>douglasii</i> , <i>kelloggii</i> possible
<i>Boechera stricta</i>		<i>Potentilla hippiana</i>		<i>Populus tremuloides</i>	
<i>Calochortus gunnisonii</i>		<i>Lomatium dissectum</i>		<i>Dactylus glomeratus</i>	
<i>Carex pityophila</i>		<i>Koeleria macrantha</i>		<i>Chenopodium atrovirens</i>	
<i>Carex</i> sp.	<i>Obtusa, geeyeri</i>	<i>Agastache urticifolia</i>		<i>Potentilla pulcherrima</i>	
Caryophyllaceae		<i>Heliomerus multiflora</i>		<i>Campanula petiolata</i>	
<i>Castilleja chromosa</i>		<i>Lupinus</i> sp.	most likely <i>polyphyllus</i> , some <i>argenteus</i> and <i>bakeri</i> possible	<i>Hydrophyllum capitatum</i>	
<i>Chrysothamnus depresus</i>		<i>Wyethia amplexicaulis</i>		<i>Viola adunca</i>	
<i>Chrysothamnus vaseyi</i>		<i>Poa pratensis</i>		<i>Galium boreale</i>	

Chrysothamnus viscidiflorus		Bromus inermis		Achnatherum sp.	nelsonii, lettermannii
Crepis sp.	intermedia, occidentalis	Elymus elymoides		Carex sp.	obtusata, geyeri
Cyrtorhyncha ranunculina		Antennaria parvifolia		Festuca thurberi	
Delphinium nuttallianum		Populus tremuloides		Phacelia sp.	
Dicot		Viola praemorsa		Rosa woodsii	
Elymus elymoides		Arctostaphylos uva-ursi		Linum lewisii	
Eremogone congesta		Polygonum sp.	most likely douglassii, kelloggii possible	Ipomopsis aggregata	
Erigeron eatonii		Gayophytum sp.	likely diffusum	Delphinium nuttallianum	
Erigeron sp.		Poa fendleriana			
Eriogonum racemosum		Delphinium nuttallianum			
Eriogonum umbelatum		Juniperus communis			
Galium boreale		Calochortus gunnisonii			
Krascheninnikovia lanata		Vicia americana			
Lupinus polyphyllus		Boechera stricta			
Mertensia brevistyla		Galium bifolium			
Packera streptanthifolia		Crepis sp.	intermedia, occidentalis		
Pediocactus simpsonii		Erigeron flagellaris			
Penstemon caespitosus		Campanula petiolata			
Penstemon strictus		Mertensia brevistyla			
Phlox multiflora		Linum lewisii			
Poa fendleriana		Hydrophyllum capitatum			
Poa sp.		Thalictrum fendleri			
Polygonum sp.		Ipomopsis aggregata			
Potentilla hippiana		Poa sp.			
Potentilla pulcherrima		Erigeron sp.	likely flagellaris		
Pulsatilla nuttalliana		Senecio pudicus			
Purshia tridentata		Taraxacum officinale			
Sedum lanceolatum		Collomia linearis			
Stipeae	Achnatherum pinetorum, Achnatherum sp., Hesperostipa comata	Chenopodium atrovirens			
Symphoricarpos rotundifolius		Viola adunca			
Taraxacum officinale		Festuca rubra			
Vicia americana		Sedum lanceolatum			

Wyethia x magna		Elymus glaucus			
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Table 10. Species lists for Avery Picnic, Copper Creek, and Emerald Lake. Species are listed by relative abundance in terms of percent cover

Avery Picnic	Notes	Copper Creek	Notes	Emerald Lake	Notes
Taraxacum officinale		Eremogone congesta		Potentilla pulcherrima	
Potentilla pulcherrima		Taraxacum officinale		Fragaria virginiana	
Poa pratensis		Poa pratensis		Senecio crassulus	
Erigeron subtrinervis		Thalictrum fendleri		Deschampsia cespitosa	
Castilleja septentrionalis		Maianthemum stellatum		Anemone multifida	
Ipomopsis aggregata		Galium boreale		Achillea millefolium	
Valeriana occidentalis		Castilleja septentrionalis		Carex sp.	chalciolepis, ebenea, egglestonii, hoodii
Agoseris sp.	glauc, aurantiaca	Erigeron speciosus		Taraxacum officinale	
Dactylis glomerata		Campanula petiolata		Castilleja septentrionalis	
Thalictrum fendleri		Helianthella quinquenervia		Elymus lanceolatus	
Achillea millefolium		Ipomopsis aggregata		Erigeron leiomerus	
Erigeron coulteri		Anemone multifida		Cirsium griseum	
Elymus lanceolatus		Potentilla pulcherrima		Erigeron coulteri	
Poa compressa		Mertensia brevistyla		Potentilla glaucophylla	
Achnatherum sp.	nelsonii, letermannii	Cymopterus lemmonii		Agoseris sp.	glauc, aurantiaca
Bromus inermis		Populus balsamifera		Thalictrum fendleri	
Erigeron speciosus		Solidago multiradiata		Viola adunca	
Fragaria virginiana		Carex sp.	obtusata, geyeri	Oreochrysum parryi	
Viola adunca		Boechera stricta		Draba spectabilis	
Lathyrus lanszwertii		Eriogonum umbellatum majus		Poa alpina	
Artemisia dracunculul		Frasera speciosa		Phleum alpinum	
Carex sp.	obtusata, geyeri	Lathyrus lanszwertii		Trisetum canescens	
Solidago multiradiata		Agoseris sp.	glauc, aurantiaca	Pyrrocoma crocea	
Hymenoxys hoopesii		Viola adunca		Valeriana edulis	
Boechera stricta		Achnatherum sp.	nelsonii, letermannii	Hymenoxys hoopesii	
Mertensia brevistyla		Astragalus alpinus		Noccaea fendleri	
Trisetum spicatum		Polygonum sp.	most likely douglassii, kelloggii possible	Aquilegia coerulea	
Botrychium sp.		Achillea millefolium		Gentiana parryi	

		Poa alpina		Erigeron speciosus	
		Sedum lanceolatum		Boechera stricta	
		Vicia americana		Lupinus argenteus	
		Fragaria virginiana		Poa fendleriana	
		Lomatium triternatum		Danthonia parryi	
		Elymus lanceolatus		Androsace septentrionalis	
		Androsace septentrionalis		Carex pityophila	
		Chenopodium atrovirens		Delphinium barbeyi	
		Moehringia sp.	macrophylla or lateriflora	Heterotheca sp.	
		Delphinium nuttalianum		Sibbaldia procumbens	
		Erigeron subtrinervis			
		Silene drummondii			

Figure 16. ‘Stupid Falls’ site maps

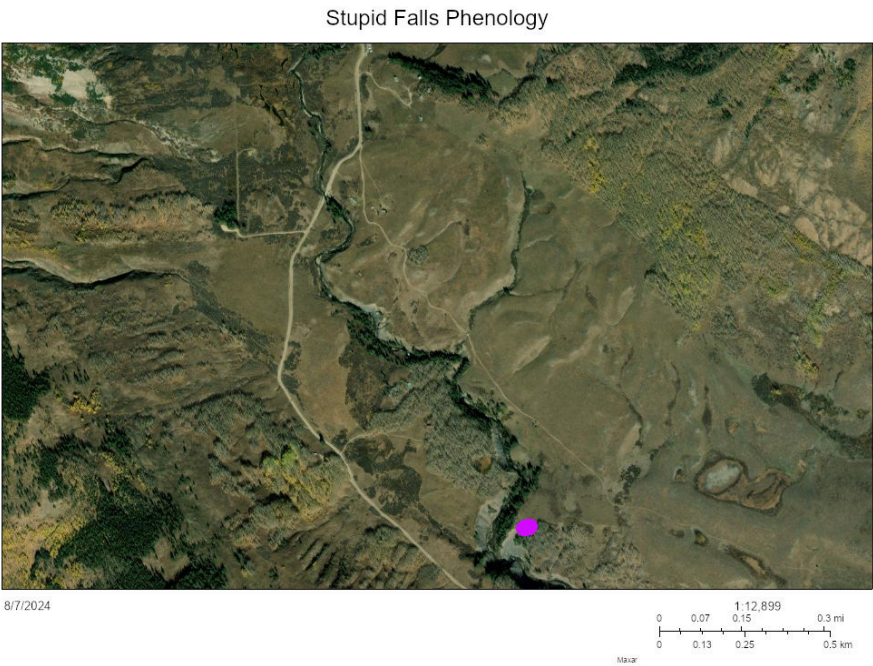
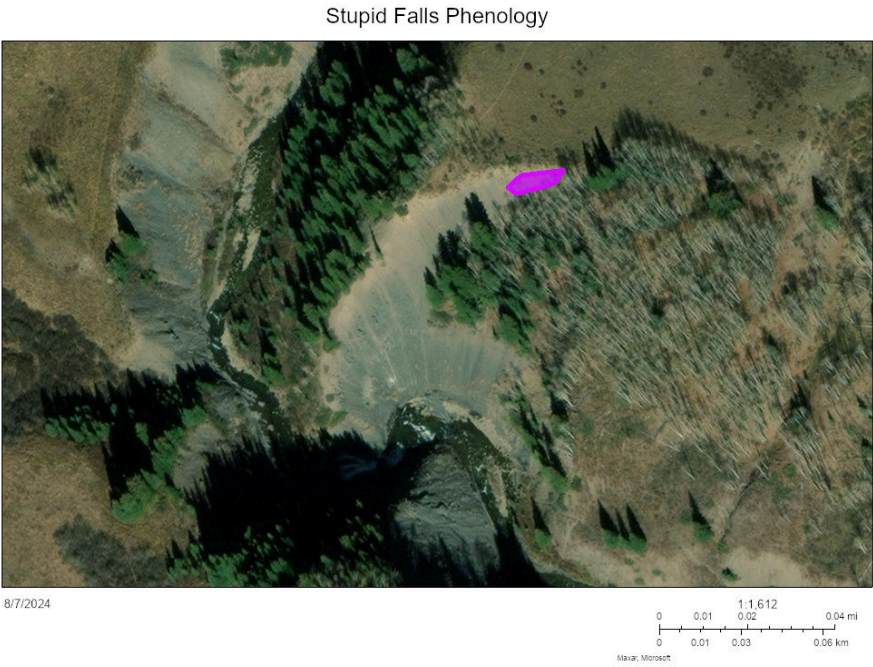


Figure 17. Deer Creek 2 phenology site maps

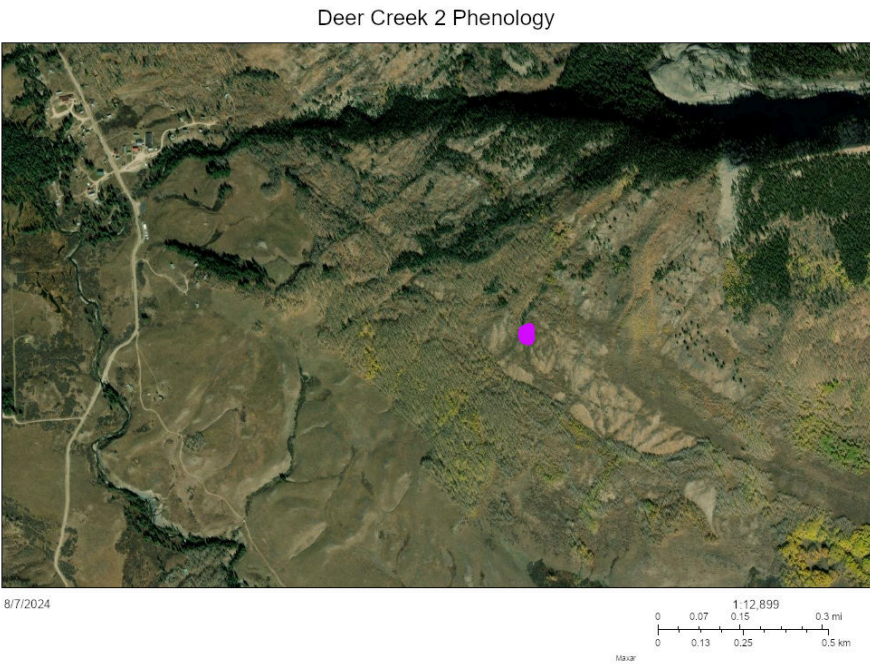
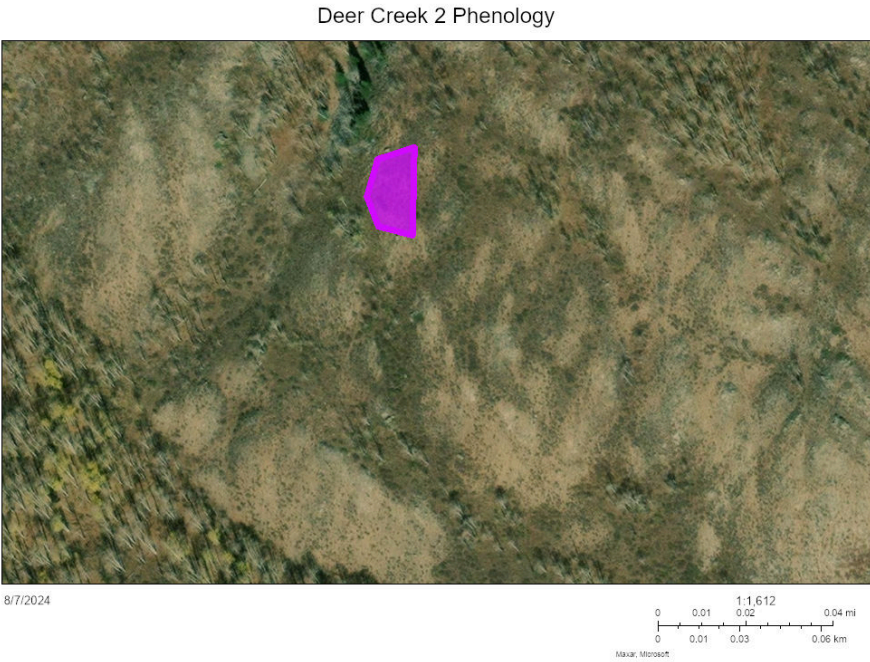


Figure 18. Emerald Lake site maps



Figure 19. Avery Picnic site maps

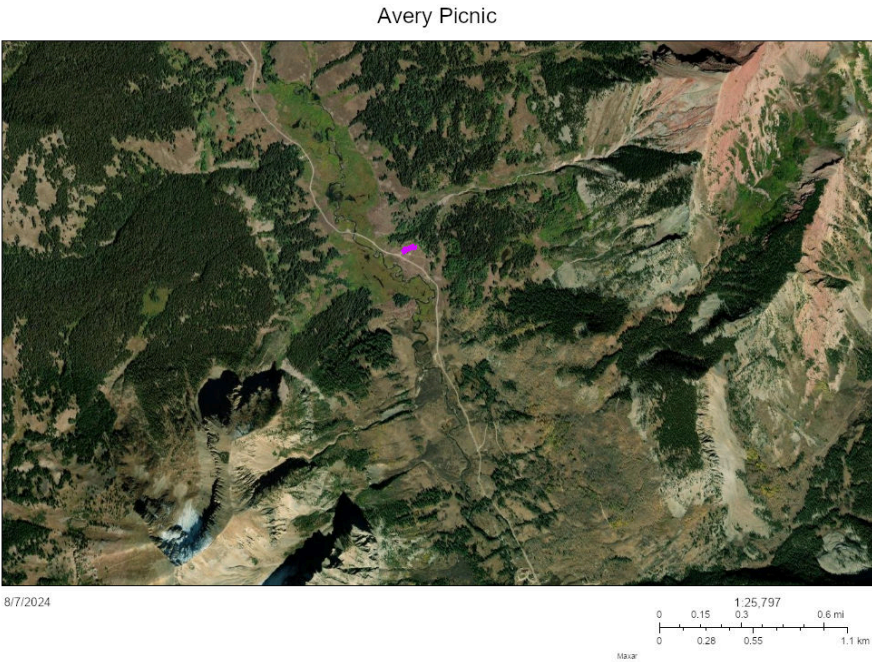
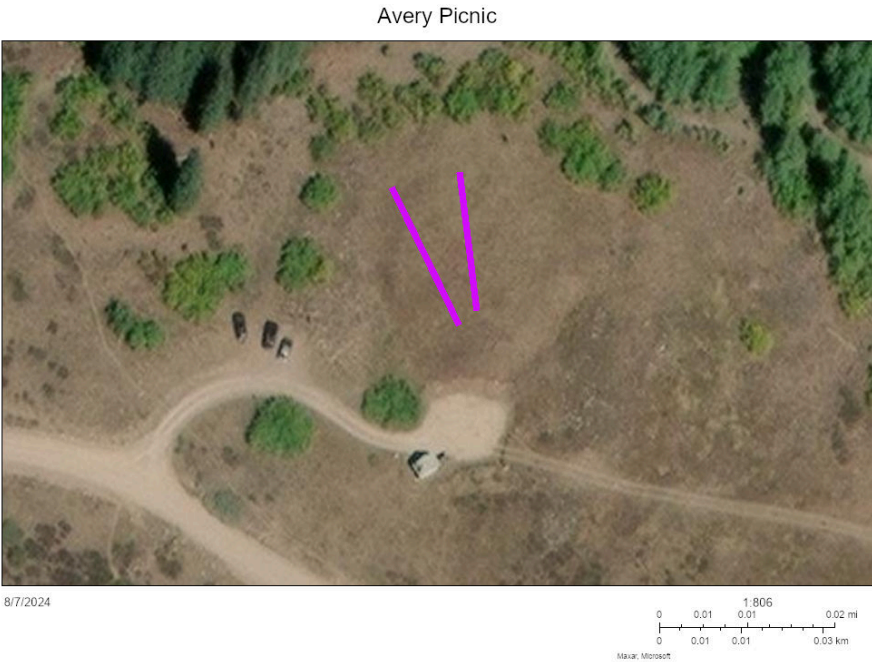
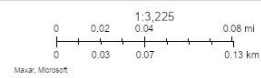


Figure 20. Deer Creek 1+2 site maps. Deer Creek 1 is in the bottom right. Deer Creek 2 is in the top left

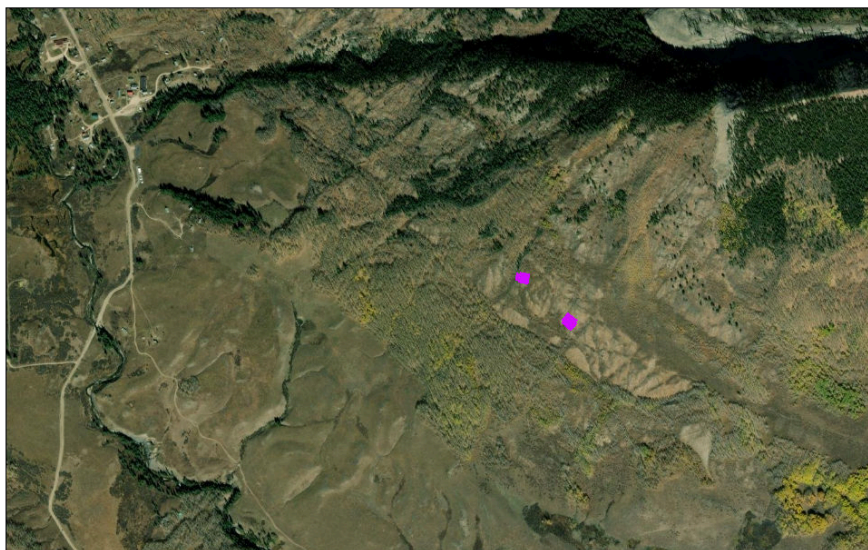
Deer Creek 1+2



8/7/2024



Deer Creek 1+2



8/7/2024

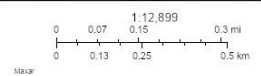


Figure 21. Johnson Hill site maps



Table 11. Coordinates for Copper Creek and Almont sites

Site	Transect	Start	Finish
Copper Creek	1	38.96834, -106.96789	38.96841, -106.96776
Copper Creek	2	38.96837, -106.96784	38.96842, -106.96778
Copper Creek	3	38.96836, -106.96800	38.96843, -106.96779
Almont	1	38.72369, -106.81584	38.72376, -106.81611
Almont	2	38.72045, -106.81617	38.72052, -106.81646
Almont	3	38.72329, -106.81600	38.72328, -106.81590