THESIS

RESTORING TWO THREATENED *PHYSARIA* SPECIES IN THE PICEANCE BASIN OF NORTHWESTERN COLORADO

Submitted by

Sasha L. Victor

Graduate Degree Program in Ecology

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Summer 2015

Master's Committee:

Advisor: Mark W. Paschke

Jorge M. Vivanco
Carol A. Dawson

ABSTRACT

RESTORING TWO THREATENED *PHYSARIA* SPECIES IN THE PICEANCE BASIN OF NORTHWESTERN COLORADO

Physaria congesta and *Physaria obcordata* are rare plants endemic to the Piceance Basin of northwestern Colorado, USA. Since the Federal listing of both species in 1990, management efforts have focused largely on protecting critical habitat. However, this unique habitat is also a prime energy development area, necessitating additional measures to protect and restore both species. The overall objective of my research is to determine the best approach for establishing new populations of *P. congesta* and *P. obcordata* in suitable but unoccupied habitats in the Piceance Basin.

To address this objective I used 3 methods: a soil feedback experiment, a field ecological survey, and a field establishment experiment. In recent years it has been shown that relative abundance of some species is strongly correlated with plant soil feedbacks and rare species can demonstrate strong negative feedbacks with pathogens from their own root systems (Klironomos 2002). Based on this theory I conducted a 12-week soil feedback study using field soil as inoculum collected from occupied and unoccupied suitable sites. I found no significant differences in plant biomass for either species when inoculated with soil from occupied or unoccupied habitats.

To further investigate the differences between occupied and unoccupied sites I conducted a field ecological survey, building upon previous habitat suitability research, comparing plant cover, soil color, and soil/air temperature differential. I found significant differences between

occupied and unoccupied sites for both species in multiple parameters (*P. congesta* = aspect, elevation, percent bare ground and rock, and soil color; *P. obcordata* = slope and soil color). Within occupied sites I found a negative correlation between *P. congesta* density and slope as well as a positive correlation between *P. obcordata* density with increased forb cover (< 5%) and decreased bare ground and rock cover (between 80 and 90%).

The final phase of my research, which was delayed for a year due to legal issues, was to establish field plots, where I seeded and transplanted both species during fall 2014. Additional plants were transplanted during spring 2015. For each species three sites were located more than 600 meters from existing occupied habitat of the same species (Far Sites) and three within 600 meters (Near Sites). Initial germination and establishment rates, were collected spring 2015 and I found limited germination of seeded plots and moderate survival of fall transplants.

Early trends show that *P. obcordata* is performing better than *P. congesta* and transplants are more successful than seeds. Within *P. obcordata* sites, far sites are performing better than near sites. During spring transplanting and monitoring I developed some initial suggestions to improve the success of transplanting including avoiding hard frosts (possibly by limiting transplanting to spring), ensuring that the soil is thoroughly tamped down during planting as well as planting flush to ground level to minimize impacts from wind, and finally watering at time of planting is essential and additional watering may be required in lower precipitation years.

Long term monitoring is essential to understand the full efficacy establishment treatments as well as monitor these populations for reproduction, fecundity, response to disturbance, and population dynamics. Results from this research will assist land managers to make informed decisions regarding future conservation and restoration of these species.

ACKNOWLEDGEMENTS

I want to start by acknowledging the support and guidance of my advisor Mark Paschke, PhD. Through all of the twists and turns that have accompanied this research he has always been there to provide probing questions, thoughtful criticism, and unconditional encouragement. Additionally, my other committee members, Jorge Vivanco, PhD and Carol Dawson, PhD, provided guidance throughout the process.

I would like to acknowledge the Bureau of Land Management and XTO energy for the financial support that allowed this project to occur. A special thank you to Zoe Miller and Gina Glenne from the U.S. Fish and Wildlife Service for invaluable advice and guidance during site selection, Environmental Assessment development, and throughout the appeal and legal process.

The Restoration Ecology Lab at CSU has been my home for the past three years and many people have contributed to my amazing experience in Fort Collins. First and foremost, Jayne Jonas-Bratten was always available to discuss research and statistics issues (lots of statistics!), listen to my frustrations, and celebrate victories. Additionally, numerous students provided essential assistance in the greenhouse caring for plants as well as in the field collecting seeds, selection sites, and the final seeding and transplanting.

I am also very lucky to have a group of friends that has not only expanded while in Colorado but are always willing to listen and provide encouraging words. My family has supported me since we were kids running through the woods and now as I begin my career to research and restore those natural areas we grew up in. Finally my husband, David, has been my rock for the past seven years. He has been there to celebrate milestones and talk through frustrations during my turbulent tenure at CSU and for this I cannot thank him enough.

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Introduction

Rare species management shares a number of obstacles with general vegetation management including lack of resource as well as inadequate species specific knowledge (Falk and Olwell 1992, Drayton and Primack 2000). Rare plants pose additional challenges including lack of healthy populations, potential damage from monitoring and research activities, and limited suitable habitat (USFWS 2008). Yet in order to effectively manage these types of species it is essential to know their basic biology and ecology. This information is not only required for federal listing and subsequent regulations but without this knowledge it may be impossible to effectively develop and prioritize conservation actions.

Physaria congesta and *Physaria obcordata* are small, rare mustard species endemic to the Piceance Basin of northwestern Colorado. They were originally described in 1982 and listed as federally threatened in 1990 under the Endangered Species Act of 1973. Both species are found only on barren shale outcroppings of the Green River geologic formation, which is also home to one of the most productive energy extraction fields in the United States.

Following their discovery and subsequent federal listing there has been a small flurry of research surrounding *P. congesta* and *P. obcordata* in order to obtain baseline information about their biology, ecology, and population dynamics. The extent of this research is partly due to their suitable habitat being located in a region with energy extraction. The objective of this review is to describe both *Physaria* species and their habitats as well as to provide a summary of research that has been done to date on these two species.

General Description of Habitat and Plants

Located in northwestern Colorado in Rio Blanco and Garfield Counties, the Piceance Basin encompasses over 4,100 km^2 (Taylor 1987). Monthly average temperature ranges from -5.9 °C - 20.5 °C and monthly precipitation levels range from 2.1 – 4.0 cm (Figure 1.1) (Western Regional Climate Data for Meeker, CO 1997-2008). Annual precipitation within the basin ranges from 27-63.5 cm, where about half falls as snow and the remaining during late summer thunderstorms (Tiedman and Terwilliger 1978). Hot, dry summers and cold, snowy winters typify the semiarid climate. Elevation varies between 1,706 and 2,740 meters due to unique geological formations found within the basin. The landscape contains steep shale hills surrounded by pinyon-juniper woodlands and sagebrush dominated rangelands.

P. congesta and *P. obcordata* are rare, perennial mustard species that are endemic to the Piceance Basin of northwestern Colorado. Suitable habitat for both species is limited to barren shale outcroppings of the Green River geologic formation (Table 1.1, Figure 1.2) (Anderson and Jordan 1993). These outcroppings are a mixture of calcareous sandstone and shale strata formed as lacustrine deposits in Lake Uinta (U.S. Office of the Federal Register 1990). Most likely phyto-edaphic classification of suitable habitat is low elevation pinyon-juniper / shallow very gravelly sandy loams (PEU-4; Tiedman and Terwilliger 1978). Main distinguishing characteristic is 60-90 % bare ground with less than 30% rock outcroppings.

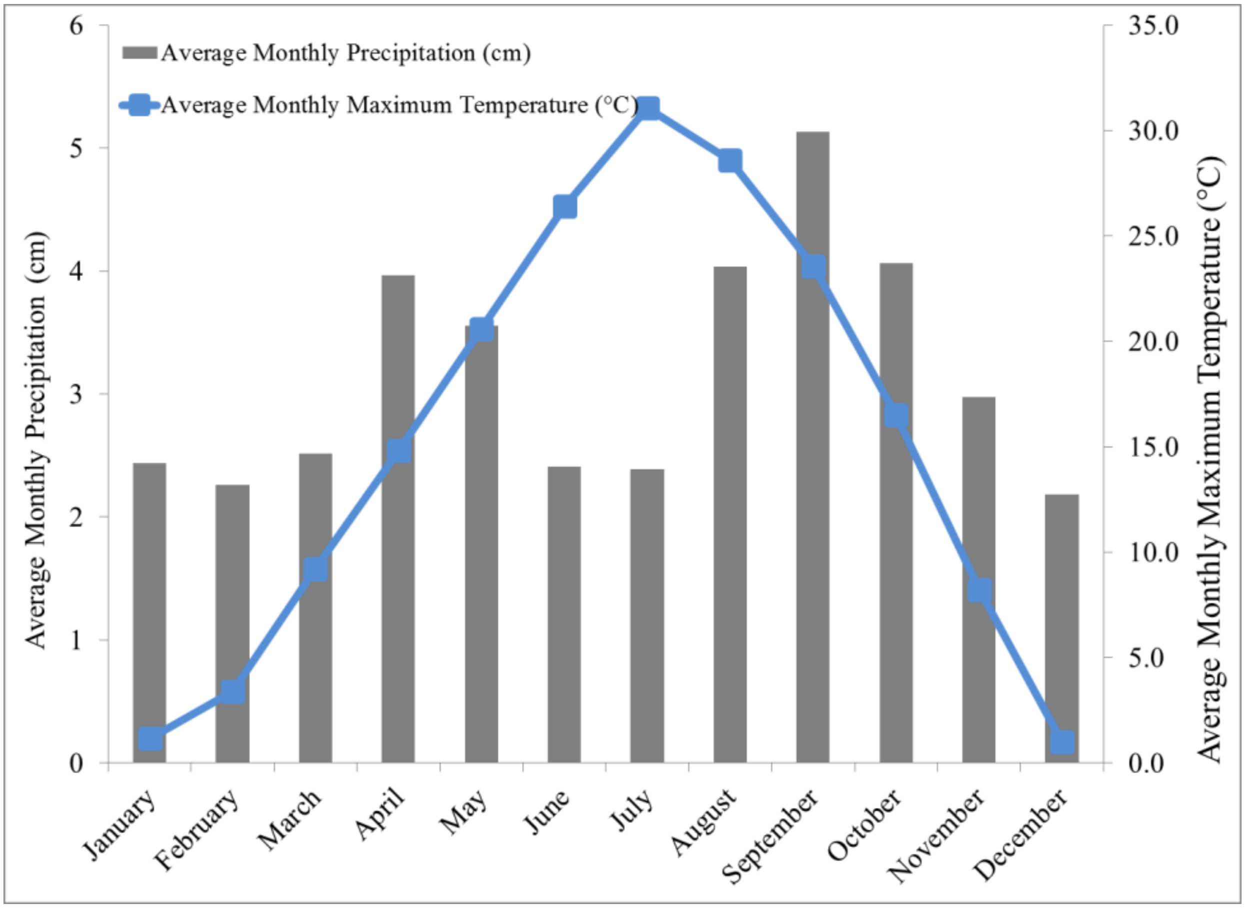

Figure 1.1. Average monthly precipitation and maximum temperature for Piceance Basin, Colorado (Western Regional Climate Data for Meeker, CO 1997-2008). Data taken from Meeker Airport weather stations because it is closest to the Piceance Basin.

Table 1.1. Species specific characteristics of *P. congesta* and *P. obcordata* (Office of Federal Register 1989, Rollins 1993, USFWS 2008) I derived elevation and slope ranges from GIS data provided by the Bureau of Land Management.

Species	***Physaria congesta***	***Physaria obcordata***
Common Name	Dudley Bluff's Bladderpod	Dudley Bluff's Twinpod
Lifeform	Perennial forb	Perennial forb
Size	Diameter: 1-3 cm; Height: 1-3 cm	Height: 12-18 cm
Caudex	Thick caudex, presence of old leaf bases, with minimal branching	Thick caudex, presence of old leaf bases, with thick branching
Leaves	3-4 mm long, silvery, linear-oblanceolate; entire margins; acute to narrowly obtuse	Numerous silvery basal leaves (broadly oblanceolate, acute, entire to sparingly sinuate-dentate); cauline leaves narrowly lanceolate
Trichomes	Stiff radiately branched, fused at center	Leaves, stems, pedicels, and siliques densely covered with lepidote trichomes with numerous rays fused to tips (Required to distinguish from congener *P.acutifolia*)
Flowers	Yellow spatulate petals; 5-6 mm long (late April - early May)	Yellow; 7-9 mm long (May - June)
Seed pod	Infructescenes sessile and congested; siliques pods, erect ovate, flattened at apex and margins, 3.5-4.5 mm long	Fruiting pedicels divaricately ascending and straight, 1-1.5cm long; siliques pendant, obcordate with deep open sinus, slightly inflated, replum broadly obovate to nearly orbicular, 4-5 mm long
Seeds	Plump and wingless; 2-4 per pod	Plump and wingless; 2-4 per pod
Seed Ripening Period	Late May to Mid-June	July
Habitat	Flat ridgelines and outcroppings of Thirteenmile Creek Tongue of Green River geologic formation	Steep slopes and downcutting drainages of Thirteenmile Creek Tongue and Parachute Creek Member of Green River geologic formation
Populations	7 distinct populations est. 550,000-600,000 plants ~207 ha over 16 km range (90% on BLM Land)	10 distinct populations est. 18,000-27,000 plants 57-117 ha over 54 km range (80-85% on BLM Land)
Elevational Range	1860 - 2010 meters	1806 - 2255 meters
Mean Slope	14.75%	42.52%
Photo		

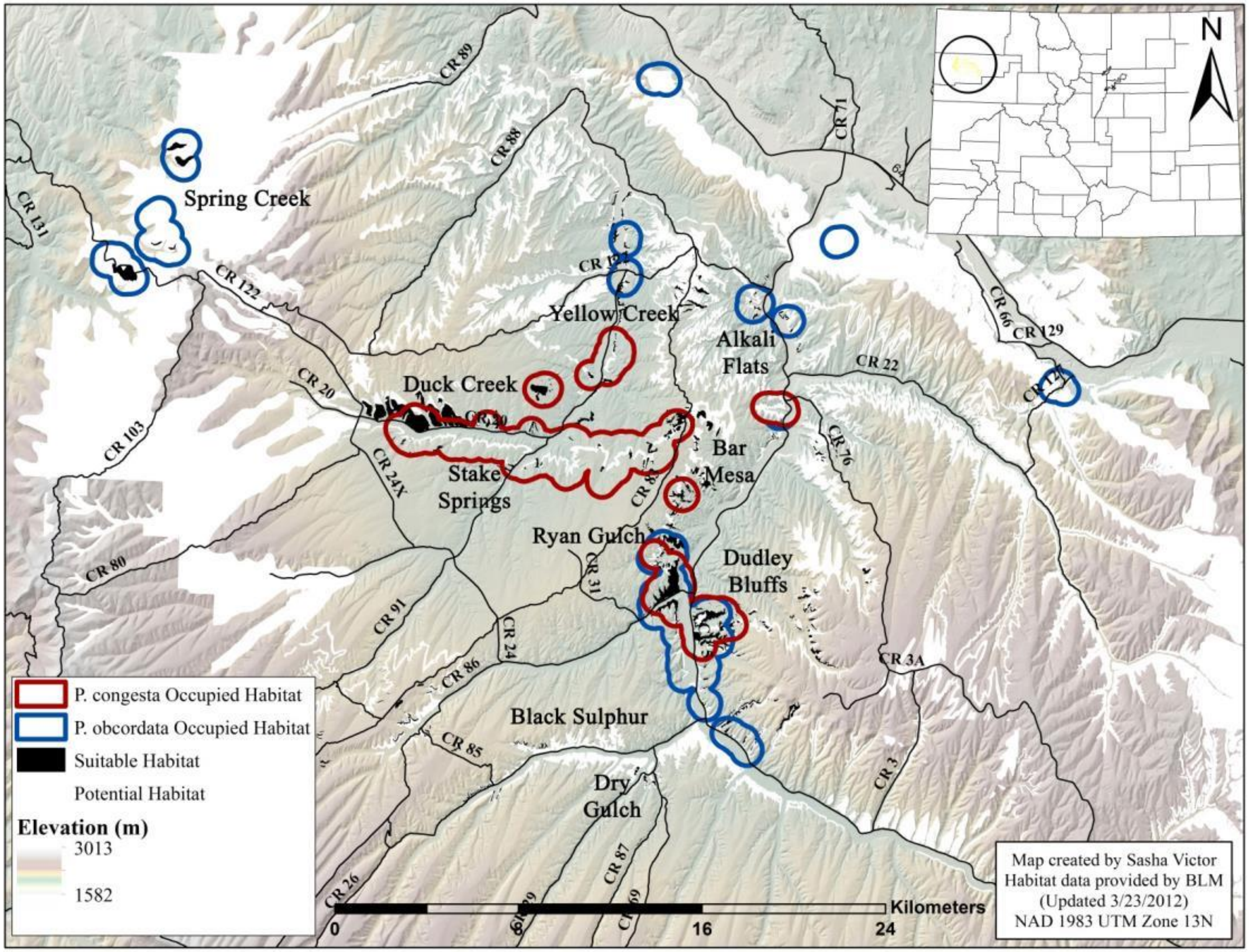

Figure 1.2. Map of *P. congesta* and *P. obcordata* occupied and suitable habitats in the Piceance Basin, Colorado. Potential habitat derived from occupancy modeling and aerial imagery. Suitable habitat is potential habitat that has been ground-truthed.

Federal Listing and Management History

The first collection of *P. congesta* was in 1959 but remained unidentified for over twenty years (U.S. Office of the Federal Register 1990). In the summer of 1982 a Colorado Natural Heritage Program (CNHP) floristic inventory of the Piceance Basin recorded both *P. congesta* and *P. obcordata* for the first time. By 1984, Dr. Reed Rollins, an expert on mustard species, described and named both *Physaria* species based on co-occurring populations at Dudley Bluffs (U.S. Office of the Federal Register 1989, Rollins 1983, Rollins 1984). *Lesquerella congesta* was the original name of *Physaria congesta* at time of listing but the entire *Lesquerella* genus was united with *Physaria* in 2002 (Al-Shehbaz and O'Kane 2002).

Federal action to list both species by the U.S. Fish and Wildlife Service (USFWS) was initiated in 1987. Due to an error in the name of one species and lack of biological information as well as a back log of species with higher priority, the Federal Register did not publish the petition to list until January 24, 1989 (U.S. Office of the Federal Register 1989). Original listing documentation notes five populations of each species *(P. congesta*: approximately 20 ha over 16 km range; *P. obcordata*: approximately 101 ha over 35 km range). In the Endangered Species Act of 1973 there are five possible factors for listing of which each species must show evidence of at least one to be considered. There was evidence to support listing of *P. congesta* and *P. obcordata* based on three factors: 1) present or threatened destruction, modification, or curtailment of its habitat or range, 2) inadequacy of existing regulatory mechanisms, and 3) other natural or manmade factors affecting its continued existence (U.S. Office of the Federal Register 1989).

At time of listing, the most imminent threat to both species was that nearly all occupied habitat (100% for *P. congesta* and 72% for *P. obcordata*) occurs in a multi-mineral oil shale zone and that planning for future projects would significantly impact these species if they were not included during the planning process (Anderson and Jordan 1993). Although there were few regulatory mechanisms in the early nineties, the Bureau of Land Management (BLM) had designated two areas (Dudley Bluffs and Calamity Ridge) of occupied habitat as Areas of Critical Environmental Concern (ACEC) and declared No Surface Occupancy (NSO), prohibiting occupancy or development, protecting approximately 20% of occupied habitat for both species (Figure 1.3).

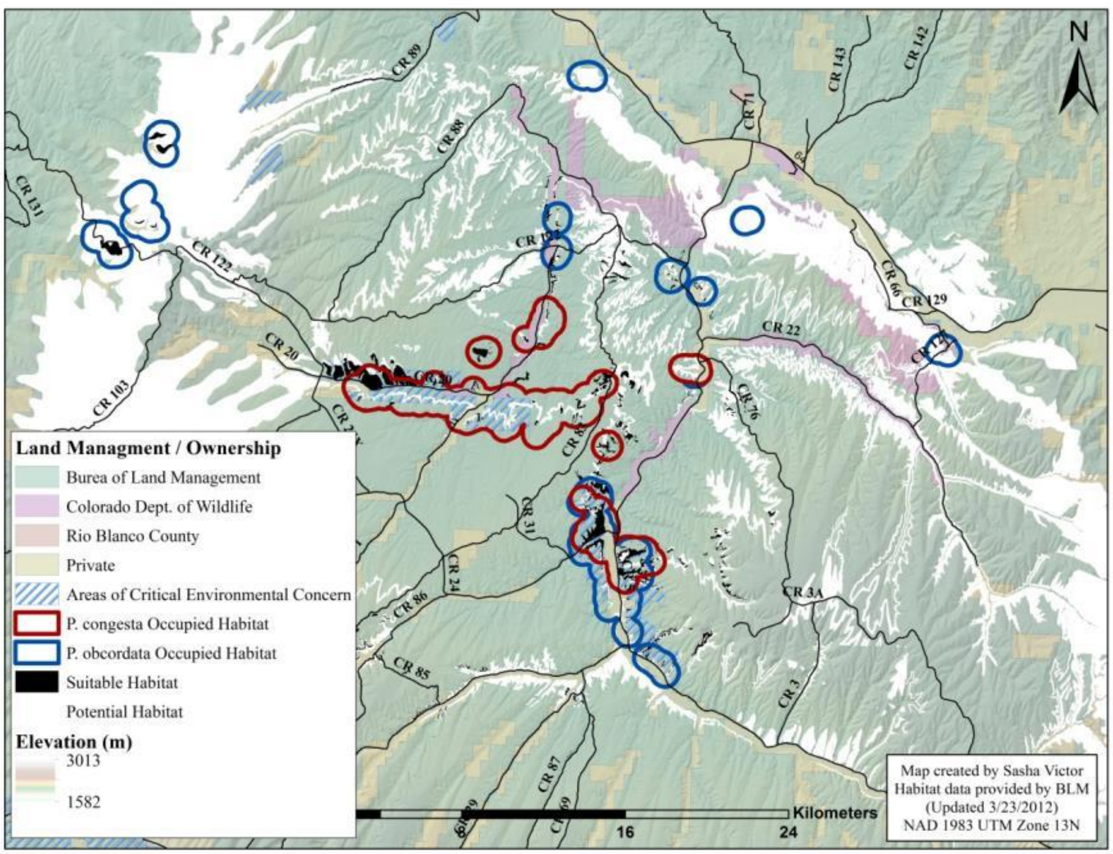

Figure 1.3. Map of *Physaria* habitats in the Piceance Basin, Colorado with land management / ownership and ACEC boundaries as of 2012. GIS data provided by the BLM.

Managers believed that listing these species would give them increased protections including the requirement of Section 7 consultations (under Endangered Species Act of 1973) for all future projects on suitable habitat. Section 7 consultations can be formal or informal and require other federal agencies to engage in interagency cooperation by consulting with the USFWS on any project that may impact a listed species (USFWS and NMFS 1998). After three years, the final threatened listing announcement for both *P. congesta* and *P. obcordata* ran in the Federal Register on February 6, 1990 and became effective on March 8, 1990 (U.S. Office of the Federal Register 1990).

Three years after listing, the USFWS and BLM finalized a recovery plan for both species. The main objective of this plan was to protect *Physaria* populations and habitat through five primary actions: 1) inventory remaining potential habitat, 2) establish formal land management designations, 3) private land exchanges and easements, 4) conduct life history studies, ecology and soil research, and 5) monitor exiting populations' trends (Anderson and Jordan 1993).

Priority tasks included designating three additional ACECs for Ryan Gulch, Yellow Creek, and Duck Creek populations and applying NSO stipulations to all future and reissued leases in these areas, as well as designating off-highway vehicle use restrictions. The BLM has established permanent monitoring plots to look at reproductive success, vigor, tolerances to disturbance, and other life history traits for both species. Additional research needs at that time included soil analysis (narrow down suitability and tolerances), isolation mechanisms and other population genetic research, and pollination/reproductive biology. It also indicated that there was a need to investigate the feasibility of conducting reintroductions on unoccupied, suitable habitats (Anderson and Jordan 1993).

In 2006, USFWS initiated the first review of the recovery plan (USFWS 2008). In the 13 years since the original recovery plan, the number and size of occurrences increased for both species (Table 1.1). Two additional ACECs (Ryan Gulch and Duck Creek), designated by the BLM, increased protected area for both species to 177 ha. Additional survey work during those years found two new occurrences, totaling 180-187 ha, for *P. congesta* and five new occurrences, on 16 ha, for *P. obcordata* (CNHP 2006 and CNHP 2008 in USFWS 2008). Monitoring, although erratic, showed that at two locations, populations increased in size and/or number, which in one case they attributed to the erection of a fence to exclude cattle and wild horses (USFWS 2008). Another site showed a decrease in numbers attributed to trampling and drought, of which the two impacts were inseparable.

One major revelation in the review is that there were a number of serious threats that the recovery plan failed to address. Those included impacts of oil and gas extraction, secondary impacts associated with oil shale development, effects of grazing and trampling, and climate change (USFWS 2008). Since development of technology to effectively and efficiently process oil shale has not occurred as quickly as projected, the major threat has shifted to the tremendous increase in development in the Piceance Basin by the oil and gas industry (Figure 1.4).

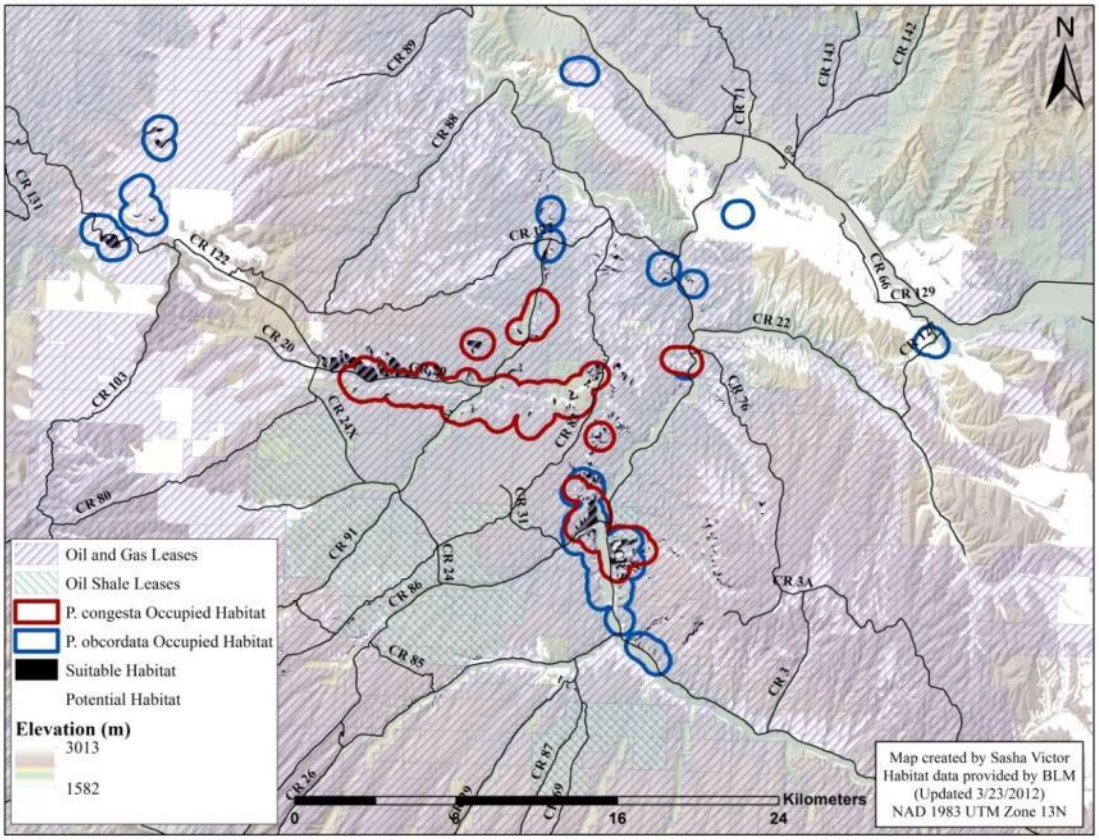

Figure 1.4. Map of *Physaria* habitat in the Piceance Basin, Colorado with energy extraction lease boundaries. GIS data provided by the BLM.

In 2015, the White River field office of the BLM released a proposed amendment to their Resource Management Plan that contained five alternative management plans with oil and gas development projections ranging from 550 well pads with 4,600 new wells to 2,556 new well pads to over 21,000 new wells within the field office boundaries with the majority occurring in the Piceance Basin (BLM 2015). These estimates were projected for 20 years of development from 2009 through 2028 (BLM 2007) and is the most up to date projection information available. In 2006 there were wellpads and pumping stations within 60 meters and pipelines within 50 meters of *Physaria* populations (Kurzel 2006 in USFWS 2008) although the quantity was not reported. Beyond direct impacts of degradation and destruction of habitat or plants, additional impacts from energy development include air and dust pollution (Lewis 2013) as well as possible destruction of pollinator habitat (Graff and Alward 2012, Clark 2013). Drought is also a major issue in the area and climate change is likely to exacerbate its frequency and intensity (USFWS 2008).

The BLM and USFWS have been working to apply appropriate buffer distances around development sites as well as conducting Section 7 consultations on all new or renewed projects including processing facilities, pipelines, and land exchanges. Impact from development and the effectiveness of a buffer zone is unknown but in general managers suggest a 200-meter buffer, although in many cases that is not practical due to site constraints. In the face of additional pressures from oil and gas and oil shale leases across the entirety of their range, protection of these species will continue to rely on Section 7 consultations (USFWS 2008).

The USFWS also suggested a number of improved management regulations including the development and implementation of consistent conservation measures, expand existing and designate new ACECs, and monitor the effects of a 200 meter buffer. Researchers suggested that managers can focus on additional inventories of suitable habitat and monitoring of occupied habitat as well as developing more detailed GIS maps. Additional research opportunities focus on life history of both species as well as pollinator biology and requirements (USFWS 2008).

***Physaria* Research**

In order for managers to effectively protect these unique species it is essential to know about their biology and ecology. Shortly after CNHP recorded these plants in their 1982 floristic inventory, additional surveys and monitoring began. Although there are still a lot of questions to answer, many researchers have contributed significantly to a knowledge base for these species. Research has focused on suitable soil characteristics (Hayden Wing Associates, LLC 2010) and habitat modeling (Decker et al 2006, Decker et al 2013), population genetics (Neale 2013), reproductive biology and pollinators (Tepedino et al 2012, Chesus 2013, Clark 2013), and impacts of development (Graff and Alward 2012). I have continued this progression by focusing my research on the restoration of both *Physaria* species.

Suitable Habitat Modeling

Using element occurrence data for both *Physaria* species, researchers from CNHP used two models, envelope model and DOMAIN, to better understand and delineate species distributions (Decker et al 2006). Envelope models create a new raster dataset focused on areas possessing the same combination of environmental conditions found at known locations. The DOMAIN models result in a data layer that reflects 'distance' to nearest known occupied point where the highest value represents areas most similar to known occupied habitats (Decker et al 2006). They validated both models using 25% of elemental occurrences that they removed from the original modeling dataset.

Overall researchers found all models indicated there may be additional unmapped areas of suitable habitat with *P. obcordata* being less restricted to the Green River formation than *P. congesta*. They cited a lack of finely mapped geology as well as a need for additional, more refined ecological information on limitations to species distribution as being limiting factors to modeling suitability (Decker et al 2006).

DOMAIN models, using four levels for both species, proved to be the most useful and land managers may be able to use these models as a prioritization tool. Within the model, levels 1 and 2 are areas with highest similarity and are appropriate to use for *P. obcordata* populations due to the fact that mapped occurrences are small and well represented by a single sample point. Levels 3 and 4, which account for all habitat that is similar to mapped occurrences, is more appropriate for mapping *P. congesta* due to occurrences being large, internally heterogeneous, and not well represented by a single sample point (Decker et al 2006). However, although these models can be useful they are not a guarantee of species presence or absence and not appropriate to use for land management or conservation planning without field verification.

In 2013, CNHP released a report with updated models for both *Physaria* species following additional research and ground-truthing (Decker et al 2013). Major updates to both models included a decrease in study area coverage and a new grid sampling method in large polygons. Along with environmental variables included in previous model, distances to the Green River formation as well as refined surface geology and soil type information was included. Researchers created draft maximum entropy (maxent) models in 2011 that found distance to Thirteenmile Creek Tongue was the best factor for predicting *P. congesta* occurrence while the model for *P. obcordata* also incorporated a number of climate factors including growing degree days and annual precipitation.

These models were subsequently ground-truthed with very poor results. In 2013 additional revisions were made focusing on improving resolution of digital elevation models and geology inputs. Researchers ran revised models using both maxent and random forest (classification and regression tree) models. Results from maxent models show that, as expected, *P. congesta* habitat is largely determined by presence of Thirteenmile Creek Tongue (70% of model variability) but they also found that tolerable temperatures are more important than precipitation. Predicted *P. obcordata* occurrence continued to show decreased reliance on specific geologic layers and increased importance of winter minimum temperatures and season precipitation patterns (Decker et al 2013).

Random forest models agreed with maxent models about the importance of the Thirteenmile Creek Tongue as well as growing degree days for *P. congesta*. Additional important parameters include distance to the Green River formation, spring precipitation, total annual precipitation, and May minimum temperature. For *P. obcordata*, random forest models found distance to the Green River formation, winter minimum temperatures and soil type to be most influential. Overall researchers were able to narrow down their predictions of suitable habitat but mentioned that they have likely reached a limit of available data. They predict that there is between 3,200-6,000 hectares of *P. congesta* potential habitat and 4,800-13,000 hectares *P. obcordata* potential habitat. Researchers again reiterate that these models do not guarantee presence or absence of either species and managers should not base land management and conservation decisions solely on their results (Decker et al 2013).

Soil Characteristics and Associated Species Analysis

Consultants from Hayden-Wing Associates, LLC (2010) analyzed fifty composite soil samples from occupied and unoccupied sites in the Piceance Basin to determine if suitable habitat could be determined through the use of simple soil analysis tests. They also looked for suitable habitat indicator species. Using classification trees, they included a variety of soil characteristics, soil nutrients/chemistry, and landscape variables with no *a priori* knowledge of importance. They found that three models were considered significant: 1) differences between the eight soil types sampled due to lime and gypsum equivalent measurements as well as nitrate-nitrogen levels, 2) differences between occupied and apparently suitable due to phosphorus, and 3) differences between occupied/highly suitable and apparently suitable due to sodium and phosphorus levels. Co-occurring plant species significantly associated with *P. congesta* included seven species and nine species for *P. obcordata* (Table 1.2).

Table 1.2. Co-occurring plant species associated with *P. congesta* and *P. obcordata*. Recreated from Hayden Wing Associates, LLC (2010).

	Species Name	**Common Name**	**Indicator P-value**
Physaria congesta			
	Juniperus osteosperma	Utah Juniper	0.02
	Phlox hoodii	spiny phlox	0.02
	Linum lewisii	Lewis Flax	0.04
	Tetradymia canescans	spineless horsebrush	0.02
	Asclepias cryptoceras	pallid milkweed	0.05
	Penstemon caespitosus	mat penstemon	0.03
Physaria obcordata			
	Ericameria naueseosa	rubber rabbitbrush	< 0.01
	Mentzelia multicaulis	manystem balzingstar	< 0.01
	Pascopyrum smitii	western wheatgrass	< 0.01
	Atriplex canescens	fourwing saltbursh	< 0.01
	Cirsium barnebyi	Barneby's thistle	< 0.01
	Abronia agrillosa	clay sand verbena	< 0.01
	Achnatherum hymenoides	indian ricegrass	0.02
	Eriogonum longifolium	longleaf buckwheat	0.04
	Sphaeralcea coccinea	scarlet globemallow	0.03

There are many caveats to the use of these models including the exploratory nature of analysis that calls predictive ability into question. Due to the non-linear nature of this data, traditional statistical analysis was not applicable. However the larger issue is lack of replication in soil types other than Thirteenmile Creek Tongue as well as limitations on distance from *Physaria* plants required for soil collection on occupied sites (1-m from any plant) (Hayden Wing Associates, LLC 2010). Although there were issues with the design of this study their conclusions can be useful in guiding future investigations into methods to better delineate suitable habitat.

Reproductive Biology and Pollinators

Research on reproductive biology and pollination of *P. obcordata* began in 1992, shortly after listing. Vincent Tepedino and colleagues at Utah State University collected data on pollinator limitation, hybridization, flower visitors, and pollen collection (Tepedino et al 2012). They found that *P. obcordata* flowers were virtually self-incompatible (only 3 of 39 flowers tested for selfing capability were able to reproduce and researchers claim that was likely due to contamination with outcross pollen) and incapable of reproducing sexually without insects to move pollen although there was no presence of pollinator limitation. Four bee families visited these flowers, with Andrenidae and Halictidae being most common and reliable. It is important to note that the specific species of ground nesting bees are native to the Piceance Basin. Tepedino also estimated flight distances for these species at 20-600 meters for 50% return and up to 1.3 km for 10% return (2012). These results show that it is essential for land managers to consider pollinator habitat protection when making management decision, which will require additional research on bee nesting habitat descriptions and mapping.

Pollinator research on *P. congesta* did not begin until 2010. Sarah Clark, a graduate student at Utah State University, undertook a breeding system and cross pollination study of *P. congesta* as well as analyzing the effect of oil and gas development on pollinator communities of both *Physaria* species (Clark 2013). Results showed that *P. congesta* is also self-incompatible and requires pollen movement through pollinators. Primary pollinators are native ground-nesting bees in the Andrenidae and Halictidae families. Development impacts on pollinator communities were examined at three distances 0, 50 and 150 meters from development but no change in total pollinator community or plant fecundity was detected. Researchers took samples at already developed well-pads or roadsides and suggested that it is necessary to study impacts before, during, and after construction to understand the full range of impacts. They also caution that their sample size may have been too small to detect significant differences. A final recommendation was to include other native plant species near future *Physaria* restoration sites to provide additional foraging material for pollinators (Clark 2013).

An undergraduate research assistant in the Restoration Ecology Lab (REL) at Colorado State University conducted a study on potential competition for pollinators between *P. congesta* and co-flowering plants (Chesus 2013). Working at six populations (three locations and two distances from road, within and greater than 200 m), observers watched a total of 41 plants for twenty minutes on two days, at a different time each day, in 2013. They collected data on visitations as well as number and percentage of open flowers. These plants were tagged and in June, of the same year, researchers returned and collected seeds for reproductive success measurements (I used those seeds in subsequent field establishment studies (Chapter 4)). Researchers found no competitive relationship between co-flowering species (based on visitation and viable seed counts) nor did they find any relationship between number of co-flowering individuals and *P. congesta* visitations (Chesus 2013).

Population Genetics

Denver Botanic Gardens collected tissue samples from both *Physaria* species in 2010 and 2013 across the range of occupied habitats (Neale 2013). Using microsatellite markers developed for each species, researchers found that both species show moderate to high genetic diversity with *P. congesta* having higher diversity estimates than *P. obcordata*. *P. obcordata* demonstrated statistically strong genetic clustering into three regional groups (northern, southern, and western) (Figure 1.5). Not only was there genetic clustering, there was significant variation among and within populations of both species (when analyzed together or in genetic clusters), except for among genetic clusters for *P. congesta*. Researchers recommend *in-situ* conservation of all current populations and that *ex-situ* conservation and restoration should not mix genotypes from different genetic clusters of either species (Neale 2013).

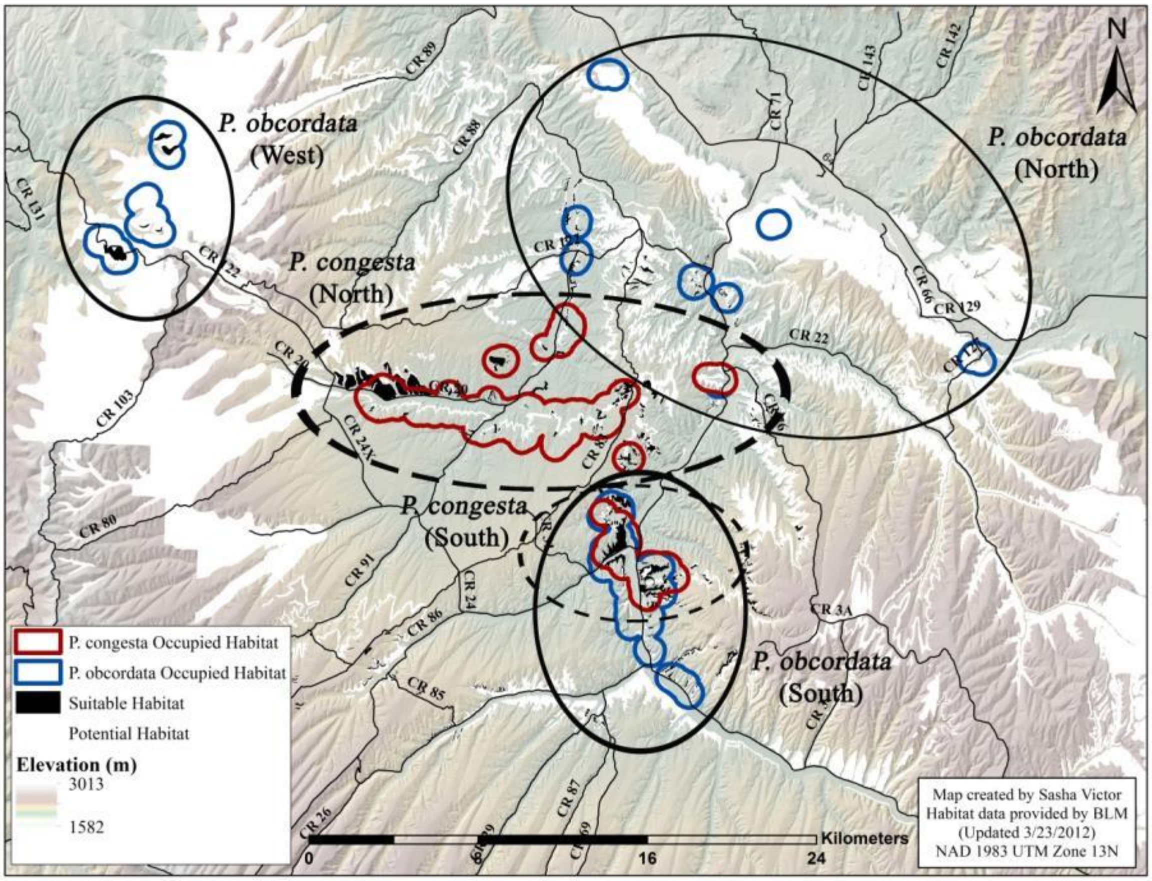

Figure 1.5. Map of *Physaria* habitat in Piceance Basin, Colorado with genetic clusters. *P. obcordata* genetic clusters are statistically significant and noted with black circles. *P. congesta* genetic clusters are noted with black dashed circle. They are not statistically significant however the BLM and USFWS acknowledge the clustering and I was required to use them when developing seed and transplant introduction protocols (Chapter 4) (Neale 2013).

Impacts of Development

During the summers of 2010 and 2011, researchers from BIO-Logic, Inc. investigated indirect effects of oil and gas development on both *Physaria* species. With a main objective of determining if distance from disturbance, mainly unpaved roads, significantly impacted abundance, fecundity, life stage structure, or occupied habitat characteristics of either species

(Graff and Alward 2013). Researchers collected data from four sites for each species. Although the objective was the same for both species, study design for each was slightly different (*P. congesta* – effects as a function of linear distance from development edge, *P. obcordata* – effects studies at two near and two far sites). This resulted in a relatively broad mix of results and conclusions.

Researchers found that *P. congesta* fecundity (number of fruits per flower and number of seeds per fruit) significantly decreased with increasing distance from development, although they did not see the same pattern in other occupied habitat characteristics or life stage structure. Authors cautioned that this does not mean that development promotes reproductive success but that there is likely additional unmeasured factor(s) that are creating these patterns (Graff and Alward 2013). Extreme site differences confounded abundance results due to the small sample size. Variables like slope and rock/litter cover had stronger effects on occupied habitat structure than distance from development. Although statistically significant, fecundity effects were small and subtle and only explain a small proportion of overall variation. Even though this effect is small, researchers note that in the face of additional and compounding pressures these effects may have profound impacts (Graff and Alward 2013). *P. obcordata* populations were also highly variable and researchers found no pattern relating *P. obcordata* density, fecundity, life stage structure, or occupied habitat characteristics to distance from disturbance.

Overall Graff and Alward (2013) found no negative effects of development on either species. However they do caution that lack of resources as well as difficulty in finding suitable sites near enough to active development limited the overall power of study results. They do not suggest changing best management practices for development near these species, either to strengthen or weaken regulations. In fact they had a number of suggestions for continued research to better understand development impacts. These suggestions included long term monitoring of permanent plots created through their study and treating abundance and fecundity as baseline data, impacts of dust on these species (similar to Lewis 2013), monitoring through the lifetime of development, and changing sampling design to include more intensive sampling within fewer sites rather than limited sampling across many sites.

In a different study, dust accumulation samples were taken in *P. congesta* occupied habitats during pollinator sampling (Chesus 2013) to determine if distance from dirt roads had an impact on reproductive success. Dust traps were developed (traps based on Lewis 2013) and set at six populations for two months. Researchers found that there were significant differences in dust collected between near (within 200 m) and far (greater than 200 m) sites, although they did not find any significant impact of dust on reproductive success. Chesus (2013) suggested that due to these results and similar studies on other species (Lewis 2013), it is necessary to more thoroughly investigate effect of dust on both *Physaria* species.

Future Research

Although there has been a lot of work done to increase the knowledge base surrounding these species there are still a lot questions. One of the most pressing issues is a need for more consistent long-term monitoring on plant health, reproduction, fecundity, and dispersal. At this stage there is no information available about how these species disperse and it is essential to understand these mechanisms to effectively manage them. Greater attention also needs to be given to mapping pollinator habitat in the area to allow for conservation and incorporation into Section 7 consultations. Finally, additional monitoring needs to take place on impacts of development and in particular impacts throughout life of multiple types of development projects (Graff and Alward 2013, Clark 2013). As important as collecting this information it is essential for researchers to effectively communicate results with managers to ensure that these species are managed using the most up-to-date science.

Research included in this Thesis

Building on previous and current research, this thesis contains information on three additional studies focused on the restoration of both species. I began by looking at the role of soil feedbacks from occupied and unoccupied habitats on productivity of both *Physaria* species (Chapter 2) as well as a field ecological survey to determine significant differences between two occupied and unoccupied habitats (Chapter 3). The final, and continuing, aspect of my research is assessing the feasibility of creating new populations in suitable, unoccupied habitats in the Piceance Basin (Chapter 4).

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Chapter 2. Role of soil feedbacks on *Physaria congesta* and *Physaria obcordata* productivity

Introduction

Physaria congesta and *Physaria obcordata* are federally threatened plants endemic to the Piceance Basin of northwestern Colorado. Since listing of both species in 1990, management efforts have focused largely on protecting critical habitat (U.S. Office of the Federal Register 1990). By 2008, the Bureau of Land Management (BLM) had designated four Areas of Critical Environmental Concern for both species covering 64%, approximately 180 ha, of occupied habitat found on public land (USFWS 2008). However, the fact that this unique habitat is also a prime energy development area necessitates additional measures to protect and restore both species. In order to create an effective management plan, it is essential to go beyond protecting established populations and further understand what mechanisms have contributed to these species being rare. Researchers have found that the interaction between a species geographic range size, habitat specificity, and local population size help to determine rarity of a species (Kruckeberg and Rabinowitz 1985).

Biotic interactions, such as competition and herbivory, have long been seen as important drivers of plant community composition (Connell 1983, Callaway and Walker 1997, Wardle et al 2004). Many have theorized that the absence of competition in harsh environments can create a refuge for a high number of endemic and rare species (O'Kane and Anderson 1987, Kelso et al 2003, Stohlgren et al 2005). Escape from competition is likely a contributing factor to the narrow habitat range of rare *Physaria* species, although due to presence of other small, non-rare forbs associated with these species it is unlikely the only factor.

P. congesta and *P. obcordata* are not only endemic to the Piceance Basin but they are further limited to unique white shale outcroppings of the Thirteenmile Creek Tongue of the Green River geologic formation. *P. obcordata* populations also can inhabit select areas of the Parachute Creek Member with the vast majority of populations located on the Thirteenmile Creek Tongue (USFWS 2008). Although they have a small geographic range, there is a lot of assumed suitable habitat within that range that is currently unoccupied. Multiple researchers have shown that there is a pattern within narrowly endemic species whereby they occupy isolated portions of their overall geographic range (Kruckeberg and Rabinowitz 1985, Breinholt et al 2009).

Another possible factor is unique substrate qualities that are heterogeneous throughout the suitable habitat range of rare plants, which can result in extreme edaphic endemism (Kruckeberg and Rabinowitz 1985, Stohlgren et al 2005). Exploring this theory, researchers have found that in the Piceance Basin, *Physaria*-associated plant species, along with multiple soil-related (sodium and phosphorus levels) and site (slope) variables differed significantly between occupied habitats of *P. congesta* and *P. obcordata*, as well occupied habitats for both species being significantly different than unoccupied habitats (Hayden Wing Associates, LLC 2010).

Although escape from competition and reliance on very specific, unique substrate both appear to be important factors in the rarity and endemic status of these species. There is another possible factor that may explain the fact that they do not occupy the entirety of their suitable habitat: soil microbes and negative soil feedbacks. The importance of interactions between soil microbial communities and plants in nutrient cycling has been widely recognized (Allen 1991; Wardle et al 2004; Bardgett et al 2005). Early research done on soil microbes focused on agricultural systems and their relative impacts on crop productivity (Turkington et al 1998).

Recently researchers have shifted their focus from the role of these feedbacks solely on plant productivity, to their role in determining plant diversity and community composition (Bever 2003; De Deyn et al 2004). Historically, the role of partitioning of abiotic resources was thought to determine and maintain plant community diversity (Tilman and Pacala 1993). However, this resource partitioning might be a by-product of plant soil feedbacks.

As a plant ages it accumulates a greater number of harmful pathogens that can reduce productivity by directly removing carbon and nutrients from plant tissue or reducing root uptake capacity (Bever et al 1997). Different plant families and in some cases functional groups are adapted to accumulate pathogens at different rates, which is possibly dependent on their ability to produce secondary compounds to protect itself or attack pathogens (Swain 1977, Bernays et al 1989). But their susceptibility also depends on the variability of habitats and climate that can produce large pulses of active pathogens that may be able to overcome plant defenses (Burdon 1991; Reynolds et al 2003). In multiple experiments, researchers found that some plant species were growth inhibited when grown in soil previously occupied by same species relative to soil previously occupied by competitors (Van der Putten et al 1993; Klironomos 2002; Wardle et al 2004; Kardol et al 2007). This negative feedback was strong and long lasting, having a significant effect on dominance patterns in later successional stages (Kardol et al 2007).

Negative feedbacks associated with specialized soil pathogens may be stronger influences for rare plants than positive interactions with microbes, like mycorrhizae and other plant growth promoting microbes, due to their inability to migrate or disperse quick enough to escape them or a lack of suitable habitat. Although there are multiple mechanisms that interact to promote rarity of a plant species, recently researchers have begun to look at importance of negative plant soil feedbacks and soil pathogens that often cause them. Klironomos (2002) was able to show that some rare plants experienced a significant decrease in growth when inoculated with pathogens from their own root systems. He was also able to demonstrate a strong correlation between soil feedbacks and relative abundance of a species, where those with strong negative feedbacks had low relative abundance while plant species with high relative abundance had either low negative feedback or positive feedback. Abundance of pathogens in soil and inability of rare plants to escape these natural enemies contribute to continued rarity of some species and may be a driving force in their ultimate extinction.

The objective of this study was to determine if there were any feedbacks between occupied and unoccupied soil and *Physaria* seedlings as well as if any detected feedback was microbial or nutrient based. Results from this study could help to guide future restoration and introduction site locations. I hypothesized that both species would have decreased aboveground biomass when inoculated with soil from occupied sites relative to unoccupied sites.

Methods

Piceance Basin

Located in northwestern Colorado in Rio Blanco and Garfield Counties, the Piceance Basin encompasses over 4,100 square kilometers (Taylor 1987). Monthly average temperature ranges from -5.9 ºC to 20.5 ºC and monthly precipitation levels range from 2.1 - 4.0 cm (Western Regional Climate Center Data for Meeker, CO 1997-2008). Annual precipitation within the basin ranges from 27 - 63.5 cm, where about half of annual precipitation falls as snow and remaining falls as rain during late summer thunderstorms (Tiedemann and Terwilliger 1978). The semiarid climate is typified by hot, dry summers and cold, snowy winters. Elevation varies between 1,706 and 2,740 meters due to unique geological formations found within the basin.

Seed and Soil Collection

Seeds

Due to the rarity and federally threatened status of both *Physaria* species it was essential to collect seeds directly from the field. In accordance with the Endangered Species Act of 1973, U.S. Fish and Wildlife Service (USFWS) awarded the Restoration Ecology Lab (REL) at Colorado State University (CSU) a permit (#TE76718A) to collect 1,000 seeds per species per year beginning in 2012. The permit contained a number of restrictions on collections including: avoid direct trampling by limiting number of people on site, avoid areas with greater than 35% slope, avoid occupied habitats during and immediately following rain or snow events, and prevent spread of noxious weeds. Along with a seed limit, the permit stipulated that collections should not exceed 50% of annual seed production of an individual or from populations smaller than ten individuals. The BLM and USFWS approved protocols prior to collections.

During 2012, workers from the Restoration Ecology Lab (REL) and the BLM collected seeds from five different populations each of *Physaria congesta* (collected June 4, 5, and 14) and *Physaria obcordata* (collected July 5 and 12). At each site, each collector collected seeds into an individual envelope, which were later collated into one collection per site and assigned a seedlot number. If possible, we collected seeds without pods attached. However, at a few locations it was essential to collect pods and separate later. Processing of seeds included removing from seed pod, cleaning, and conducting a final count. The Colorado Seed Lab, at CSU, tested 25 seeds from each seed lot to determine viability using a TZ (tetrazolium chloride) test (Table 2.1). Remaining seeds were stored at 4 °C in the REL. Based on earlier germination tests I expected a 30% germination rate. Since my target was 210 plants (10 plants per inoculation) per species, I started the experiment with 630 seeds of each species. See Appendix A for more information on germination and survival rates.

Table 2.1. *Physaria* seed collection locations for seedlots used in feedback study. Seeds collected by research assistants from the REL and BLM. Colorado Seed Lab at CSU conducted viability testing using tetrazolium (TZ) testing. Dr. Jennifer Neale at Denver Botanic Gardens conducted genetics testing (Neale 2013).

Species	Seedlot	Date Collected	Number of seeds collected	Population Name	Viability	Genetic Cluster
P. obcordata						
	1	7/12/2012	111	Unknown	75%	-
	2	7/12/2012	36	Unkown	52%	-
	3	7/12/2012	263	Unknown	61%	-
	4	7/5/2012	201	Dudley Bluffs	92%	S
	5	7/5/2012	236	Dudley Bluffs	96%	S
P. congesta						
	12	6/5/2012	306	Duck Creek	100%	N
	13	6/4/2012	264	Duck Creek	90%	N
	14	6/5/2012	392	Yellow Creek	100%	N
	15	6/14/2012	281	Yellow Creek	100%	N
	16	6/14/2012	120	Yellow Creek	65%	N

Soil Inoculum

We collected soil on September 20 -21, 2013 to be used as soil inoculum. For each species we collected soil from ten locations, five sites occupied by that species and five sites that were classified as suitable (by BLM and USFWS) but unoccupied by either *Physaria* species (Table 2.2). Soil was specific to each species and not shared. At each of the 20 collection locations we collected ten small samples (30-45 g for occupied sites; 40-75 g for unoccupied sites) using a 0.4 cm diameter soil corer and then used a 2 mm sieve to ensure no seeds were removed from the site. In occupied sites soil collection occurred in the immediate rooting zone of a *Physaria* plant while in unoccupied sites soil collection occurred in an area of suitable habitat with no plants in the immediate area. We then placed sieved soil into a sterile whirlpak bag and stored in a cooler. During inoculum collection we wore nitrile gloves and in between every plant we sterilized all equipment and gloves with 70% ethanol.

Table 2.2. Soil inoculum collection locations, occupied and unoccupied yet suitable, for soil feedback study. For each location, I composited 10 subsamples into one sample and then divided it in half for sterilization. I randomly assigned an inoculation order for each species prior to start of inoculations.

Species	Location	Occupied Status	Sterile Status	Inoculation Order
P. congesta				
	No Inoculum Control	-	-	PHCO-21
	Duck Creek	Occupied	Live	PHCO-1
	Duck Creek	Occupied	Sterilized	PHCO-16
	Duck Creek	Unoccupied	Live	PHCO-12
	Duck Creek	Unoccupied	Sterilized	PHCO-15
	Dudley Bluffs	Occupied	Live	PHCO-10
	Dudley Bluffs	Occupied	Sterilized	PHCO-20
	Dudley Bluffs	Unoccupied	Live	PHCO-2
	Dudley Bluffs	Unoccupied	Sterlized	PHCO-5
	Ryan Gulch	Occupied	Live	PHCO-18
	Ryan Gulch	Occupied	Sterilized	PHCO-4
	Ryan Gulch	Unoccupied	Live	PHCO-17
	Ryan Gulch	Unoccupied	Sterilized	PHCO-19
	Stake Springs	Occupied	Live	PHCO-14
	Stake Springs	Occupied	Sterilzed	PHCO-3
	Stake Springs	Unoccupied	Live	PHCO-8
	Stake Springs	Unoccupied	Sterilzed	PHCO-7
	Yellow Creek	Occupied	Live	PHCO-13
	Yellow Creek	Occupied	Sterilized	PHCO-11
	Yellow Creek	Unoccupied	Live	PHCO-6
	Yellow Creek	Unoccupied	Sterilized	PHCO-9

Table 2.2. Continued.

Species	Location	Occupied Status	Sterile Status	Inoculation Order
P. obcordata				
	No Inoculum Control	-	-	PHOB-4
	Alkali Flats	Occupied	Live	PHOB-20
	Alkali Flats	Occupied	Sterilized	PHOB-14
	Alkali Flats	Unoccupied	Live	PHOB-7
	Alkali Flats	Unoccupied	Sterilized	PHOB-3
	Dudley Bluffs	Occupied	Live	PHOB-16
	Dudley Bluffs	Occupied	Sterilized	PHOB-1
	Dudley Bluffs	Unoccupied	Live	PHOB-10
	Dudley Bluffs	Unoccupied	Sterilized	PHOB-19
	North Ryan Gulch	Occupied	Live	PHOB-13
	North Ryan Gulch	Occupied	Sterilized	PHOB-18
	North Ryan Gulch	Unoccupied	Live	PHOB-12
	North Ryan Gulch	Unoccupied	Sterilized	PHOB-6
	Ryan Gulch	Occupied	Live	PHOB-15
	Ryan Gulch	Occupied	Sterilized	PHOB-5
	Ryan Gulch	Unoccupied	Live	PHOB-17
	Ryan Gulch	Unoccupied	Sterilized	PHOB-8
	Yellow Creek	Occupied	Live	PHOB-2
	Yellow Creek	Occupied	Sterilized	PHOB-11
	Yellow Creek	Unoccupied	Live	PHOB-21
	Yellow Creek	Unoccupied	Sterilized	PHOB-9

In the lab, I composited the ten soil samples from each site and then divided each composited sample in half. One half of the sample was autoclaved twice for 30 minutes at 121 °C and 120 kPa to sterilize and the other half was placed immediately into a 4 °C fridge. Finally, I randomized the resulting 20 inoculums, and a no inoculum control, per species to determine inoculation order. For duration of experiment I stored all inoculums in a 4 °C fridge.

Germination

I germinated all seeds using an aeration method. Seeds separated by seed lot (see Table 4.1 for number of seeds per seed lot) were placed into glass flasks filled with 400 mL of sterilized water (autoclaved at 121 °C and at 120 kPa for 30 minutes). I then placed flasks into a warm water bath (35 °C) and an aerator connected to air purified through a water purge. Seeds stayed in the aerator until a radicle began to form (3 - 51 days). I added sterile water as needed. At 51 days I planted all remaining seeds whether there was a radicle present or not.

All seeds were planted in conetainers (2.5 cm diameter, 12 cm depth, 49 ml volume, Model: RLC3L, Stuewe and Sons, Tangent, Oregon) with a custom potting mixture (4 parts potting mix (Promix BX, Premier Horticulture, Quakertown, Pennsylvania), 3.5 parts turface (MVP, Profile Products, Buffalo Gap, Illinois), 1 part vermiculite, 1 part perlite). I watered plants with approximately 10 mL every day until they developed true leaves then every 2-3 days for remainder of the experiment. Plants were fertilized (Peters Excel, 15-5-15 CAL-MAG Special) once during week 6. I randomized plants between trays and within inoculum order once per month. Plants were grown in a growth chamber (Percival E-36140, Perry, Indiana) with 14 hours of daylight, at 15 °C (4 °C 'night' temperature), and 40% relative humidity.

Inoculation

I inoculated plants 2, 6, and 10 weeks after planting with the first inoculation occurring two days after soil inoculum collection. Inoculation consisted of putting ~ 1.2 g of specified inoculum at the base of each plant and then watering with approximately 10 mL of water. Uninoculated controls received water only. In between each inoculum all instruments were sterilized using 70% ethanol. For each species, I randomized inoculum treatments, which served as inoculum order and was assigned consecutively at week 2, allowing for emergence and development of true leaves.

Data Collection

Data collection began on week 2, same week as initial inoculations, and every other week until week 12. Data collection always occurred prior to inoculation. Data included survival, height (cm), basal (*P. obcordata*) or crown (*P. congesta*) diameter (mm), and number of live leaves. I did not take basal diameter measurements for *P. congesta* because plants were too small and measurement would likely have damaged the stem. Starting at week 6, I took digital photos to measure leaf area (mm^2) and estimate aboveground biomass (g).

Leaf Area: Estimated Biomass Regression Analysis

Due to the federally threatened status of both *Physaria* species there were restrictions on destructive sampling. I developed a regression equation that correlates leaf area and aboveground biomass for each species. I harvested 15 individuals of each species on week 12 and dried them for seven days at 65 °C. Aboveground biomass was separated from belowground biomass at root collar prior to drying.

I calculated leaf area for weeks 6 and 12 using methods adapted for measuring leaf area in grasses and other species (Tackenberg 2007, Berger et al 2010, Bumgarner et al 2012). Digital photographs were taken of each individual along with a ruler at the same distance and camera settings. I then edited these photos in Adobe Photoshop Elements to create a layer of only plant tissue. I then imported that outline into Image J to calculate the leaf area of each plant.

I ran regression analyses to determine the non-destructive measurement that best predicted biomass for each species separately. Against aboveground biomass, belowground biomass, and combined total biomass I tested leaf area, number of live leaves, average basal or crown diameter, and height. The best correlation (N=15, P <0.0001) for both species was between leaf area and aboveground biomass (*P. congesta* R^2=0.76; *P. obcordata* R^2=0.78; Figure 2.1). Resulting regression equations [*P. congesta*: Estimated Biomass = ((Leaf Area *0.00030098) + 0.00415), *P. obcordata*: Estimated Biomass = ((Leaf Area*0.00038196) + 0.00698)] were used to estimate aboveground biomass of remaining plants.

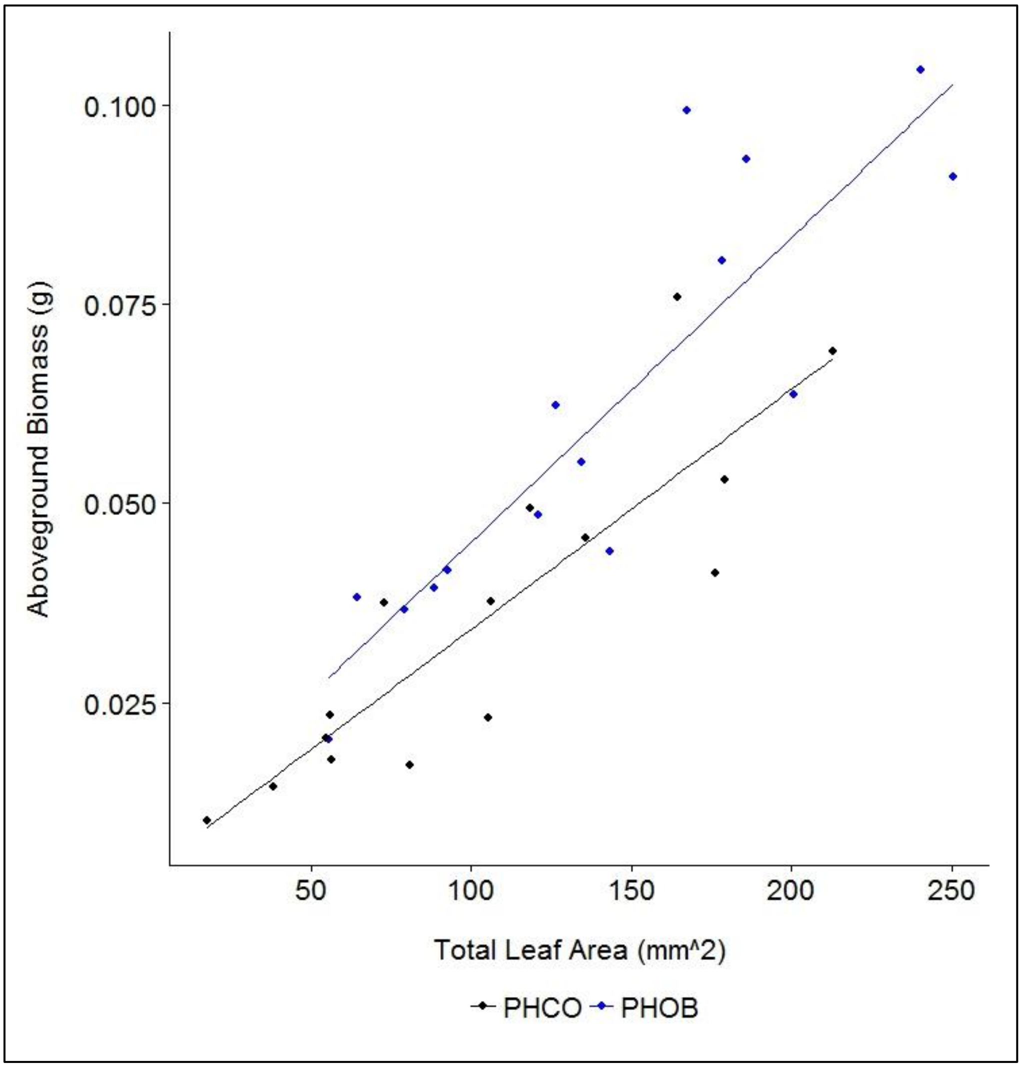

Figure 2.1. Regression analysis showed a significant correlation between leaf area and aboveground biomass (P<0.0001, N = 15) of *P. congesta* and *P. obcordata*. I measured aboveground biomass for 15 individuals per *Physaria* species and calculated leaf area using digital photographs. Resulting regression equation used to estimate biomass for remaining plants, which were then used as transplant stock in field establishment study (Chapter 4).

Statistical Analysis

All statistics were run using SAS 9.3 software (SAS Institute, Cary, NC). I ran a series of restricted maximum likelihood (ANOVA) tests on log transformed estimated biomass measurements from week 12. I performed two analyses: the first analysis compared each treatment to control using Dunnett's multiple comparison adjustment while the second analysis removed control and performed a factorial analysis using Tukey's multiple comparison adjustment to determine if there was a significant difference between occupied status or inoculum treatment. I also ran tests on the difference in estimated biomass between week 6 and 12, scaled by week 6 estimated biomass, to test whether estimated biomass changed over time and if that difference was significant between treatments.

Results

Inoculation with either live or sterile soil reduced biomass of both species relative to uninoculated controls (Figure 2.2, Figure 2.3). The test on difference in estimated biomass for both species showed that there were no significant differences between treatments and control nor were they significantly different than zero.

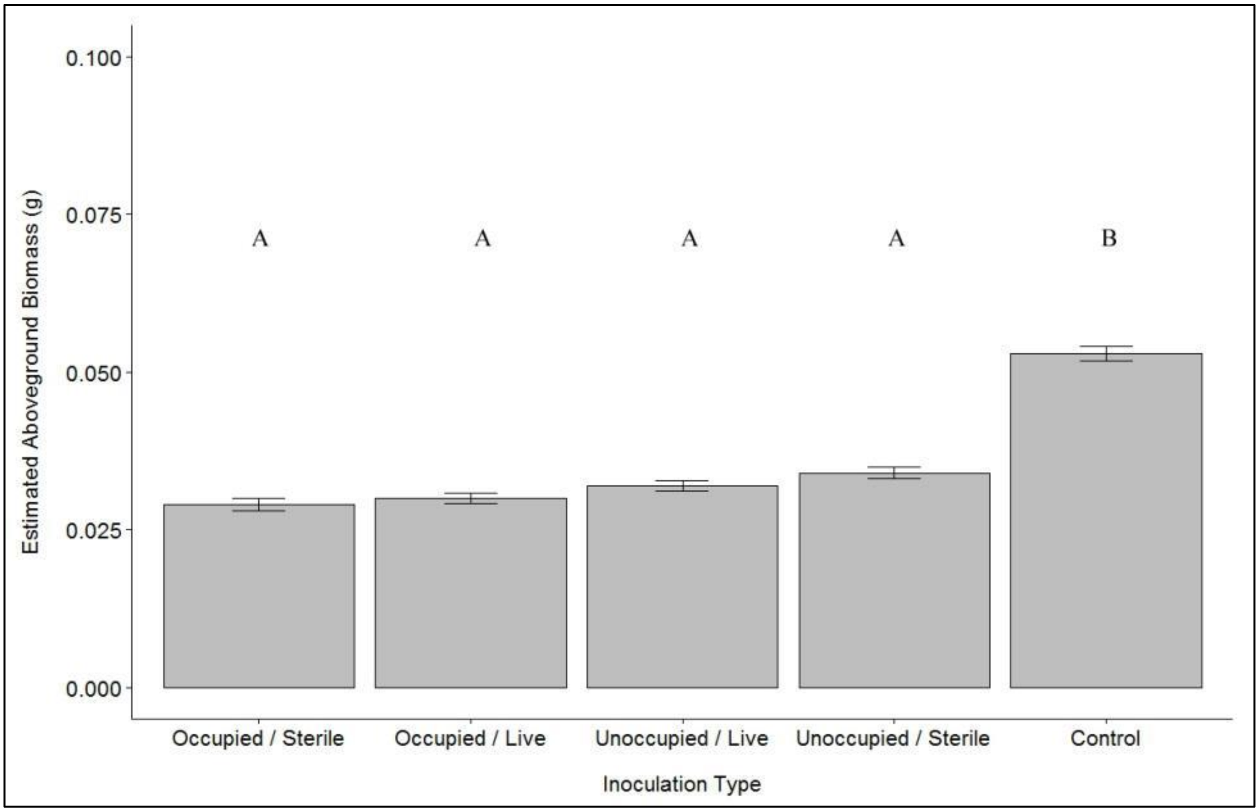

Figure 2.2. Mean estimated biomass of *P. congesta* plants based on inoculation treatment type. Soil inoculum was collected at five occupied and five unoccupied habitats. The composited samples from each collected site was divided into two. One half of the sample was left as is and the other half was sterilized twice at 121 °C for 30 minutes at 120 kPa. Statistical analysis done on log transformed variables but presented in non-transformed variables for ease of interpretation. Means with different letters indicate significant differences ($P < 0.05$; n=150).

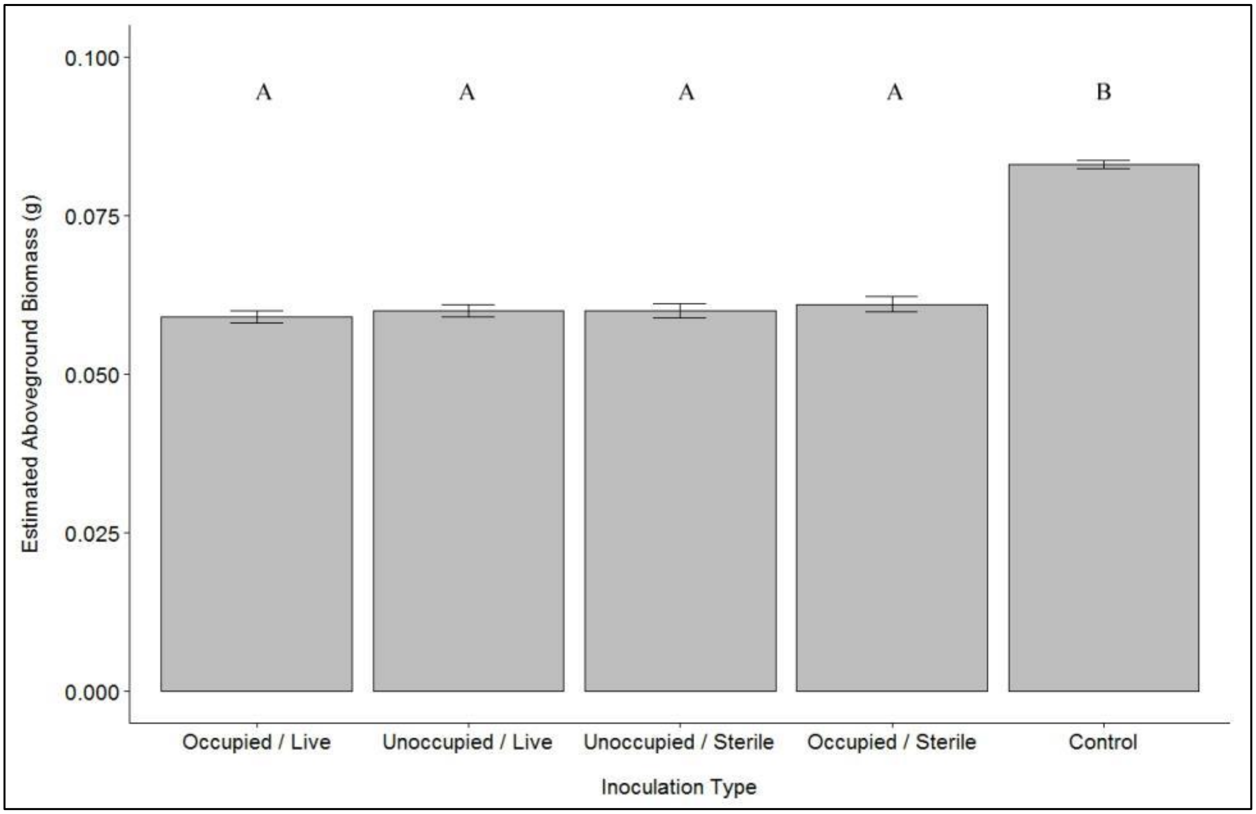

Figure 2.3. Mean estimated biomass of *P. obcordata* plants based on inoculation treatment type. Soil inoculum was collected at five occupied and five unoccupied habitats. The composited samples from each collected site was divided into two. One half of the sample was left as is and the other half was sterilized twice at 121 °C for 30 minutes at 120 kPa Statistical analysis done on log transformed variables but presented in non-transformed variables for ease of interpretation. I removed two seedlots from second analysis due to lack of replication. Means with different letters indicate significant differences ($P < 0.05$; n=208).

Discussion

I hypothesized that both species would have decreased aboveground biomass when inoculated with soil from occupied sites relative to unoccupied sites. However my results do not support this hypothesis. I found that although plants inoculated with either occupied or unoccupied soil inoculum were significantly smaller than those treated with no inoculum, there was no negative effect of live soil inoculum on aboveground biomass.

My hypotheses were based on studies by Klironomos (2002) that showed some rare plants exhibited negative feedbacks when grown in their home soil versus competitor soil.

Although I based my study on the same theory, there are significant differences between the two studies. Klironomos grew plants in soil inoculated with 'home' soil, harvested them, and then grew additional plants in conditioned soil thus recreating home or competitor soil, depending on what plants were grown first. This difference may have impacted the quantity of microbes the seedlings were exposed to between my study and that of Klironomos (2002), which may have impacted possible soil feedbacks. Due to restriction on both *Physaria* species this type of study was not feasible. Also, inoculation method may be more practical in context of determining restoration sites or possibly using field soil from occupied habitats as a soil amendment.

Other similar studies have found strong native plant soil feedbacks for different species, although most of them are trees (Bell et al 2006, Bagchi et al 2010, Mangan et al 2010) or more broadly with plant community composition (Wolfe and Klironomos 2005, Kardol 2007, Reinhart 2012). The fact that trees are such long-lived species may partially explain why these negative plant soil feedback appear to be more prevalent. At this point, it is unknown how long-lived these *Physaria* species are, even though they are perennial, which may impact concentration and diversity of soil microbes depending on how long a site had been occupied. To fully understand differences between *Physaria* occupied and unoccupied habitats it may be necessary to conduct additional microbial community analyses.

The overall negative effect of the soil inoculum (live or autoclaved) on plant biomass might be due to the clay content of Piceance Basin soil. Initial exploratory soil analyses showed that soils are clay, sandy clay, or sandy clay loam. In the field this soil can create a hard crust that may inhibit water infiltration or plant growth and I saw a similar process occur within conetainers. Although I made an effort to ensure that water had infiltrated prior to returning plants to the growth chamber, it is possible that soil inoculum interfered with water infiltration into growth media.

A power analysis determined that comparison between control and inoculum treatments had enough power to detect differences but all other comparisons did not have enough power. Due to the similarities of means and / or variability between the inoculum treatments I would have needed upwards of 30,000 plants, which is unfeasible for common species let alone those that are imperiled.

The fact that I found no significant soil feedbacks suggests that introduction of these species to suitable but unoccupied habitats may be feasible. Based on this result it is likely that other ecological or site parameters determine distribution of these species. It is also possible that the unique methods used to estimate biomass may prove useful to others researching rare or listed species. Increased regulations and restrictions on this class of species can prove a major hurdle to research and these methods allow preserving the majority of plant stock following analysis.

Conclusion

Since I found no presence of soil feedbacks for either species, restoration efforts could take place on all suitable habitats without risk of negative plant soil feedbacks. Such introductions could lead to range expansion efforts beyond current occupied habitats. Limited experimental introductions of these species could lead to insights into the feasibility and efficacy of larger-scale efforts.

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Chapter 3. Field ecological survey to determine significant differences between *Physaria congesta* and *Physaria obcordata* occupied and unoccupied habitats

Introduction

Conservation and management of rare and federally listed plants is wrought with numerous difficulties including increased regulation and permit requirements, lack of detailed ecological and population dynamic information (Brussard 1991, Falk and Owell 1992, Drayton and Primack 2000, Decker et al 2006, Decker et al 2013), and lack of resources. It can prove to be especially difficult to determine suitable habitat for endemics and subsequently map that suitable habitat due to limitations in understanding which physical, chemical or ecological variable is limiting species dispersal and survival (Breinholt et al 1985). Researchers found that endemics can occupy isolated portions of their perceived suitable habitat due to the heterogeneous nature of the limiting substrate or site characteristic (Kruckeberg and Rabinowitz 1985, Breinholt et al 2009), making surveying and mapping even more difficult.

Physaria congesta and *Physaria obcordata* are rare, threatened mustard species that are endemic to the Piceance Basin of northwestern Colorado. Both species are edaphic endemics due to their occupied habitat being limited to barren shale outcroppings of the Green River geologic formation (Chapter 1). Like many other rare plants, management of these species relies heavily upon the designation of suitable habitat, although managers continue to struggle with its classification (Falk and Olwell 1996, USFWS 2008).

Predictive habitat models have proven useful in rare plant management in many ways including predicting suitable habitat (Engler et al 2004, Bourg et al 2005), narrowing down survey locations for discovery of new populations (Wiser et al 1998, Williams et al 2009, Buechling and Tobalske 2011), and guiding locations for restoration or reintroduction efforts

(Pavlik et al 1993, Johnson 1996, Shapcott and Powell 2011, Runk et al 2014). Although there are a lot of different types of models that can be used for predictive applications with rare species there are issues with a lack of true absences (Engler 2004, Phillips et al 2009). Due to lack of detailed monitoring data showing were species were predicted but not found or all areas surveyed where they were not found (true absences) it can be difficult to determine what factors influence rare species occurrence. Recently researchers have developed and tested a number of different models for rare species including generalized linear models (Engler et al 2004), ecological niche factor analysis (Engler et al 2004), logistic regressions (Wiser et al 1998), classification trees (Bourg et al 2005, Decker et al 2013), maximum entropy (Phillips et al 2006, Decker et al 2013), random forest based (Buechling and Tobalske 2011), and Bayesian based (Hamilton et al 2015).

Although there are many options with model selection, success often comes down to quality of data used (increased spatial resolution) rather than quantity of data (Engler et al 2004, Decker et al 2013). It is also important to think about scale both in data collection and sampling parameters because researchers found that different predictive variables were significant at different scales (Wiser et al 1998). There is limited ecological and biological data available for *P. congesta* and *P. obcordata*, which makes habitat modeling difficult (Chapter 1).

Knowing the importance of, as well as a need for, accurately defined suitable habitat to rare plant management I developed a field ecological survey. The objective of this survey was to determine if there were any significant differences in occupied versus unoccupied habitats for rare *Physaria* species in the Piceance Basin of northwestern Colorado. Such differences may aid in the improvement of suitable habitat classification. This study is built off of previous work (Hayden Wing Associates, LLC 2010) that found that the main significant differences between occupied and suitable habitat was soil sodium and phosphorus levels. My study also builds off of recent modeling efforts of the Colorado Natural Heritage Program (Decker et al 2006, Decker et al 2013).

My hypotheses for the field ecological survey were that: 1) occupied sites would have lower plant cover (higher bare ground or rock cover) than unoccupied for both species (refugia from competition: O'Kane and Anderson 1987, Kelso et al 2003, Stohlgren et al 2005), 2) grass cover will be lower in occupied sites due to their potential large impact on the germination and survival of seedlings, 3) density of *Physaria* plants will be lower with increased plant cover (seedling, vegetative, and reproductive life stages may be differentially impacted), and 4) soil color in occupied sites would not only be lighter but that soil temperature (or air and soil temperature differential) would be lower (Brady and Weil 2008). The landscapes these species grow in are very arid and precipitation falls in very patchy patterns across the Piceance Basin. These *Physaria* species, as well as other shale endemics, may be able to escape competition by growing in these barren shale outcroppings due to the possible mediating effects that lighter soil color may have on water evaporation and water retention as well as related to soil temperature. These effects are likely to have large impacts on initial germination and establishment of *Physaria* seedlings.

Methods

Study Area: Piceance Basin

Located in northwestern Colorado in Rio Blanco and Garfield Counties, the Piceance Basin encompasses over 4,100 square kilometers (Taylor 1987). Monthly average temperature ranges from -5.9º C - 20.5º C and monthly precipitation levels range from 2.1 - 4.0 cm (Western Regional Climate Center Data for Meeker, CO 1997-2008). Annual precipitation within the basin ranges from 27 cm - 63.5 cm, where about half of annual precipitation falls as snow and remaining falls as rain during late summer thunderstorms (Tiedemann and Terwilliger 1978). The semiarid climate is typified by hot, dry summers and cold, snowy winters. Elevation varies between 1,706 and 2,740 meters due to unique geological formations found within the basin.

Survey Site Selection

For each species, *P. congesta* and *P. obcordata*, I surveyed ten occupied and ten suitable sites in unoccupied habitat. I selected sites using equal unstratified sample design using GRTS (generalized random tessellation sampling) code in the program R (R Core Team, Vienna Austria). Using GIS (global information system) shapefiles provided by the Bureau of Land Management's (BLM) White River Field Office I created separate files for occupied and suitable habitat of each species. Occupied and suitable areas were then clipped to include only areas that were on public land, not overlapping with occupied habitats of the same species, and within 1,000 meters of a county or main BLM road. Using these shapefiles as an input shape the program then selected 30 random sample points (10 for site selection and 20 for backup) (Figure 3.1). I used backup points if one of the original ten was determined to be unsafe or inaccessible.

Site Set-Up and Data Collection

Surveys were conducted between August 19 - 22 and September 26 - 27, 2014. The first step at each site was to walk the area and determine the longest axis of suitable habitat. Along this main axis a 20-m main transect was laid out and at every 4-m a perpendicular 10-m (5-m on each side) transect was laid out for a total of five transects (Figure 3.2). I used a random number table to determine where to place a ½-m^2 quadrat within the 5-m on each side of the main transect. Within each of the ten quadrats percent cover was determined by lifeform through ocular estimate (tree, shrub, forb, grass, bare ground and rock, and litter).

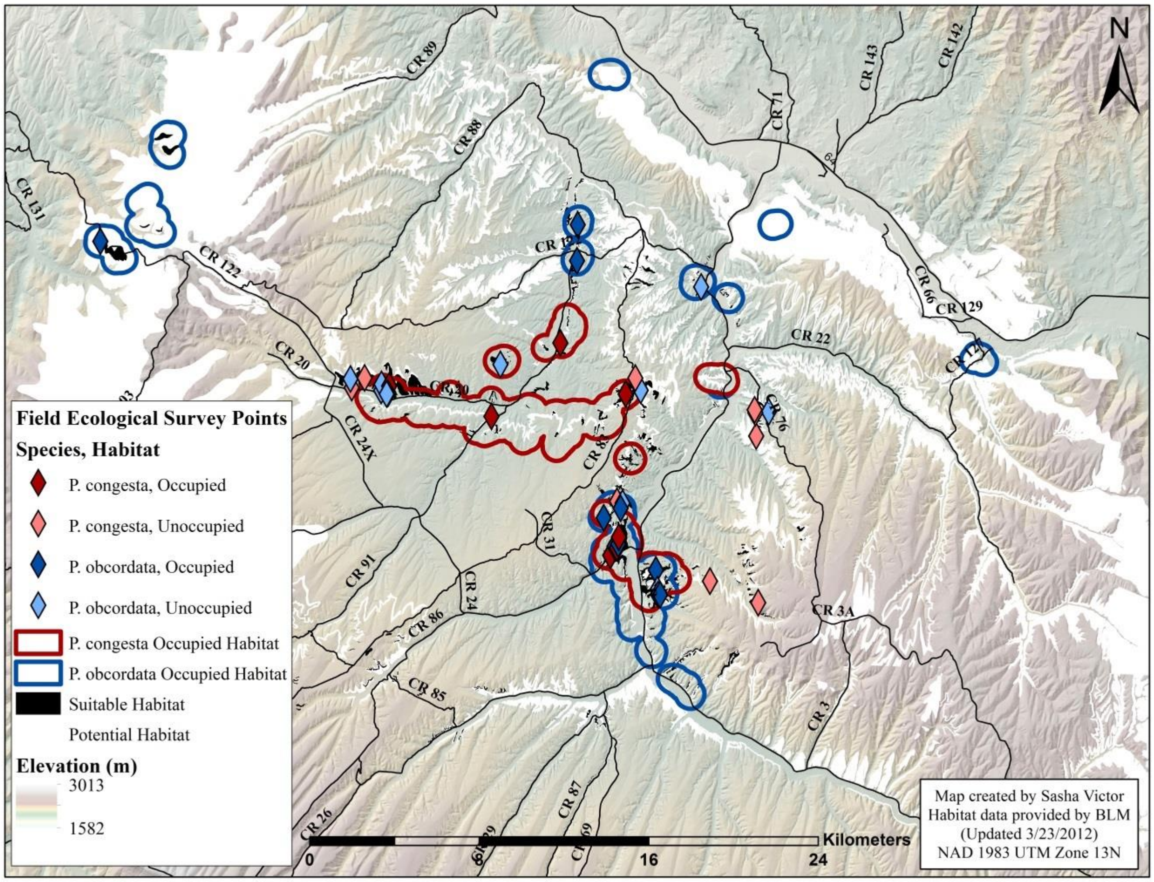

Figure 3.1. Final field survey sites for *P. congesta* and *P. obcordata* in the Piceance Basin of northwestern Colorado. I selected sites using GRTS (generalized random tessellation sampling) code in the program R. Some original sites were replaced due to safety concerns or inaccessibility. *Physaria* habitat information was provided by the BLM.

At the epicenter of each quadrat I measured air temperature using a portable weather station and soil temperature using an infrared thermometer. At the exact spot that soil temperature was taken, I also determined soil color using a Munsell Soil Color Book (2009) and took a digital photo. At occupied sites an additional 10-m^2 belt transect was set up centered on the main transect starting at 5-m and ending at 15-m. Within this belt I counted all *Physaria* individuals and categorized each into one of three life stage categories: reproductive, vegetative, and seedling. I determined reproductive status by the presence of current year's flower/seed stalk and seedlings were those with cotyledons still attached or less than five true leaves.

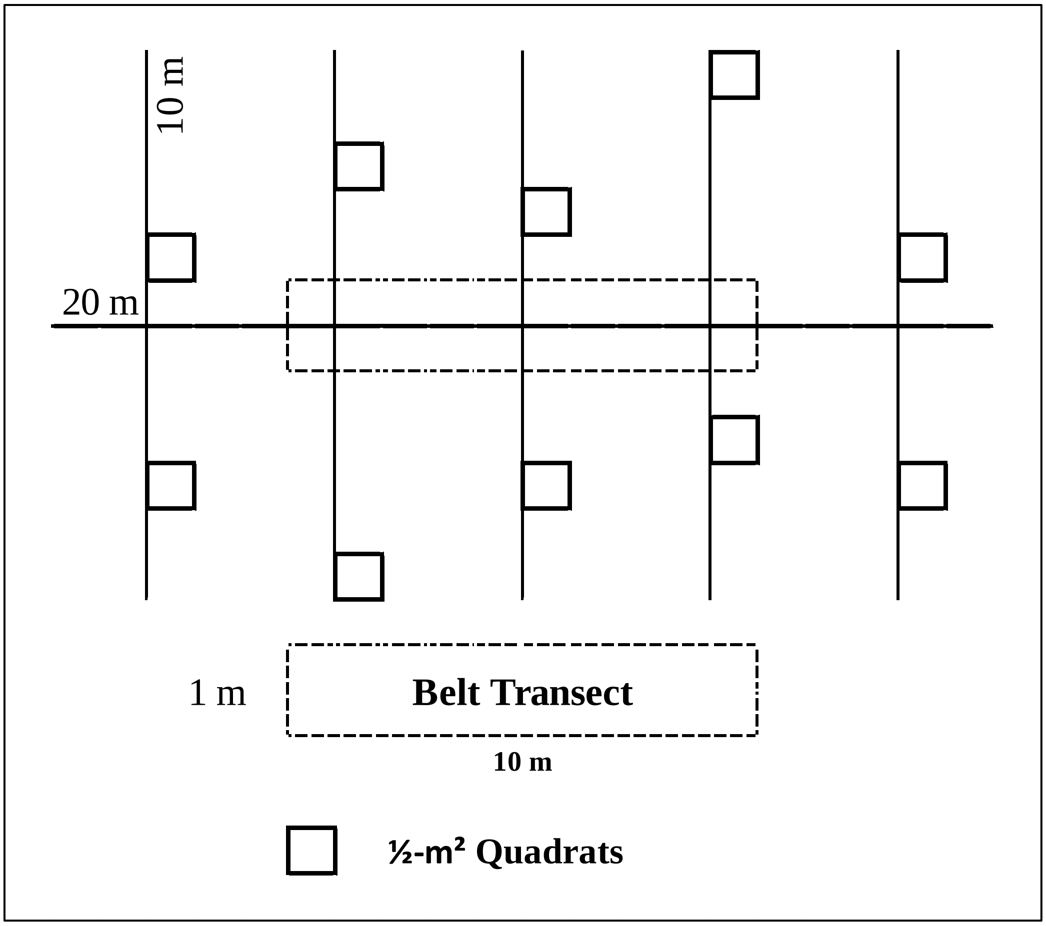

Figure 3.2. Diagram of ecological field survey data collection (not to scale). Main transect was 20-m long with 5, 10-m long perpendicular transects every 4-m. One ½-m^2 quadrat placed randomly between 1-5-m on each side of main transect. Percent cover (by lifeform), soil and air temperatures, and soil color measurements were taken at in each quadrat. A 10-m^2 belt transect was laid along the main transect to measure density of *Physaria* plants by lifestage.

Analysis

All statistics were run using SAS 9.3 software (SAS Institute, Cary, NC). Initial steps for data analysis included transforming all cover data from 0.5 m^2 to 1 m^2 area, calculating mean percent cover of each response variable for each site, and testing data for normality. Nearly all variables were non-normal, due to a large number of zeros in cover and density data, and required a variety of transformations to reach normality including log, arcsine, and square root. I used ANOVA tests to assess if variables are significantly different between occupied and unoccupied, yet suitable, sites for each species separately. In some instances, transformations were inadequate to achieve normality. For those variables, I then ran ANOVA and non-parametric Wilcoxon rank sum tests on raw variables. There was no difference in which variables were significant between normal or nonparametric tests. Thus, I report raw non-transformed results to maintain analytical consistency among all response variables. I also ran a series of correlations using non-parametric Kendall rank correlation coefficient on my data from occupied sites. This allowed me to determine if there were any variables that influence density of *Physaria* plants in occupied habitats.

In order to analyze the soil color data, I transformed the color code from the Munsell Soil Color Book (2009) to the associated color and ran a chi-square test to determine if there were any significant differences between the expected frequency and observed frequency of soil color at occupied versus unoccupied sites. I removed any soil color that occurred with less than 5 % frequency from the analysis, which resulted in six colors for *P. congesta* and seven color for *P. obcordata*.

Results

Occupied *P. congesta* sites had higher bare ground and rock cover with a range of 91.73 - 98.52 % for occupied and 74.50 – 97.63 % for unoccupied sites (Table 3.1). Occupied sites were found at lower elevations (occupied: 1875 -1994; unoccupied: 1921 – 2118) and also had more southern aspects than unoccupied sites which tended to have more southwestern aspects. *P. obcordata* occupied sites had steeper slopes (slope range for occupied site was 21.60 to 55.44 % and unoccupied sites were 4.13 to 40.13 %) than unoccupied sites (Table 3.2). Using a chi-square test I found statistically significant (P = 0.05) differences between soil color in occupied compared to unoccupied sites for both species (*P. congesta*: df = 5, n = 180; *P. obcordata*: df =

6, n=170). Specifically, in *P. congesta* occupied sites there was a higher proportion of white soils than expected and in *P. obcordata* occupied sites I found a higher proportion of white and light gray soils. Looking closer at just occupied sites, I used Kendall's rank correlation coefficient and found three significant correlations. *P. congesta* vegetative density is negatively correlated with slope while *P. obcordata* is positively correlated with forb cover and negatively correlated with bare ground and rock cover (Table 3.3).

Table 3.1. Significant differences between *Physaria congesta* occupied and unoccupied habitats in the Piceance Basin of northwestern Colorado. Mean values for ten or eleven sites (standard error of mean is in parentheses) are presented. Bolded values are significant (P > 0.05; *P. congesta* n=21; *P. obcordata* n=20). Due to non-normality of cover data, I ran ANOVA and non-parametric equivalent tests simultaneous and found no difference in variables determined to be significant. I am reporting non-transformed, non-parametric results for analytical consistency.

Survey Variable	Habitat Type	Mean (SE)	Range
% Bare ground and rock Cover	Occupied	0.954 (0.002)	91.73 - 98.52
	Unoccupied	0.907 (0.006)	74.50 - 97.63
% Forb Cover	Occupied	0.023 (0.002)	0.23 - 5.60
	Unoccupied	0.011 (< 0.001)	0.00 - 2.46
% Grass Cover	Occupied	0.003 (< 0.001)	0.00 - 1.00
	Unoccupied	0.005 (< 0.001)	0.00 - 2.95
% Litter Cover	Occupied	0.004 (< 0.001)	0.00 - 2.50
	Unoccupied	0.027 (0.003)	0.00 - 9.20
% Shrub Cover	Occupied	0.010 (< 0.001)	0.00 - 2.80
	Unoccupied	0.029 (0.003)	0.00 - 8.60
% Tree Cover	Occupied	< 0.001 (< 0.001)	0.00 - 0.72
	Unoccupied	0.021 (0.003)	0.00 - 10.00
% *P. congesta* Cover	Occupied	N/A	0.07 - 1.24
	Unoccupied	N/A	0.00 - 0.00
% *P. obcordata* Cover	Occupied	< 0.001 (< 0.001)	0.00 - 0.37
	Unoccupied	0.000 (0.000)	0.00 - 0.00
% *Physaria* Cover	Occupied	**0.004 (< 0.001)**	**0.08 - 1.61**
	Unoccupied	**< 0.001 (< 0.001)**	**0.00 - 0.10**
Aspect (°)	Occupied	**160 (6)**	**53 - 236**
	Unoccupied	**216 (4)**	**159 - 322**

Table 3.1. Continued.

Survey Variable	Habitat Type	Mean (SE)	Range
Elevation (m)	Occupied	**1936 (3.9)**	**1875 – 1994**
	Unoccupied	**1985 (5.5)**	**1921 – 2118**
Slope (%)	Occupied	16.210 (1.360)	4.87 - 42.67
	Unoccupied	23.700 (1.520)	1.70 - 53.97
Air Temperature (°C)	Occupied	26.270 (0.400)	20.89 - 32.10
	Unoccupied	27.820 (0.400)	20.94 - 37.76
Surface Temperature (°C)	Occupied	24.210 (0.540)	17.42 - 32.87
	Unoccupied	28.340 (0.650)	18.18 - 44.62
Temperature Difference (°C)	Occupied	2.060 (0.310)	-3.49 - 7.22
	Unoccupied	-0.530 (0.310)	-6.86 - 4.00

Table 3.2. Significant differences between *Physaria obcordata* occupied and unoccupied habitats in the Piceance Basin of northwestern Colorado. Mean values for ten or eleven sites (standard error of mean is in parentheses) are presented. Bolded values are significant (P > 0.05; *P. congesta* n=21; *P. obcordata* n=20). Due to non-normality of cover data, I ran ANOVA and non-parametric equivalent tests simultaneous and found no difference in variables determined to be significant. I am reporting non-transformed, non-parametric results for analytical consistency.

Survey Variable	Habitat Type	Mean (SE)	Range
% Bare ground and rock Cover	Occupied	0.908 (0.004)	83.47 - 95.00
	Unoccupied	0.915 (0.007)	74.78 - 97.94
% Forb Cover	Occupied	0.039 (0.003)	1.54 - 9.00
	Unoccupied	0.028 (0.003)	0.27 - 8.50
% Grass Cover	Occupied	0.009 (0.001)	0.00 - 3.00
	Unoccupied	0.007 (< 0.001)	0.00 - 1.69
% Litter Cover	Occupied	0.004 (0.0008)	0.00 - 2.50
	Unoccupied	0.006 (0.002)	0.00 - 4.8
% Shrub Cover	Occupied	0.033 (0.002)	0.50 - 6.40
	Unoccupied	0.030 (0.005)	0.00 - 17.26
% Tree Cover	Occupied	0.0002 (< 0.001)	0.00 - 0.20
	Unoccupied	0.012 (0.003)	0.00 - 9.80
% *P. congesta* Cover	Occupied	< 0.001 (< 0.001)	0.00 - 0.11
	Unoccupied	< 0.001 (< 0.001)	0.00 - 0.01
% *P. obcordata* Cover	Occupied	N/A	0.00 - 1.78
	Unoccupied	N/A	0.00 - 0.00

Table 3.2. Continued.

Survey Variable	Habitat Type	Mean (SE)	Range
% *Physaria* Cover	Occupied	**0.005 (0.0005)**	**0.00 - 1.78**
	Unoccupied	**< 0.001 (< 0.001)**	**0.00 - 0.20**
Aspect (°)	Occupied	165 (5.4)	105 - 271
	Unoccupied	174 (6.1)	88 - 265
Elevation (m)	Occupied	1940 (11.9)	1843 - 2265
	Unoccupied	1947 (5.6)	1821 - 2013
Slope (%)	Occupied	**36.15 (1.201)**	**21.60 - 55.44**
	Unoccupied	**18.59 (1.306)**	**4.13 - 40.13**
Air Temperature (°C)	Occupied	25.500 (0.640)	15.71 - 35.29
	Unoccupied	25.369 (0.560)	15.63 - 35.67
Surface Temperature (°C)	Occupied	25.290 (0.920)	11.21 - 42.44
	Unoccupied	24.140 (0.740)	9.83 - 35.78
Temperature Difference (°C)	Occupied	0.210 (0.340)	-6.93 - 4.50
	Unoccupied	1.230 (0.260)	-1.76 - 5.80

Table 3.3. Relationship between rare *Physaria* abundance and plant cover and site characteristics in the Piceance Basin of northwestern Colorado. Kendall's rank correlation coefficients (r) for field ecological survey parameters are presented from 20 (*P. obcordata*) or 21(*P. congesta*) sites. Bolded values are significant (P ≤ 0.0039, Dunn-Sidak adjusted P-value for multiple comparisons).

	Cover	Density			
Survey Parameters	(%)	Total	Reproductive	Vegetative	Seedling
P. congesta					
Aspect	-0.165	-0.097	-0.070	-0.103	-0.063
Bare ground and Rock Cover (%)	0.239	0.417	0.386	0.459	0.111
Forb Cover (%)	0.096	-0.177	-0.187	-0.218	-0.079
Grass Cover (%)	-0.119	-0.255	-0.236	-0.262	-0.092
Litter Cover (%)	-0.108	-0.097	-0.115	-0.090	0.052
Shrub Cover (%)	-0.159	-0.367	-0.358	-0.385	-0.048
Tree Cover (%)	-0.171	0.247	0.241	0.226	0.475
Elevation (m)	-0.162	0.103	0.111	0.132	-0.008
Slope (%)	-0.209	-0.486	-0.468	**-0.493**	-0.269

Table 3.3. Continued.

Survey Parameters	Cover (%)	Density			
		Total	Reproductive	Vegetative	Seedling
P. congesta					
Air Temperature (°C)	-0.048	0.017	-0.012	0.023	-0.047
Surface Temperature (°C)	-0.117	-0.086	-0.129	-0.080	-0.111
Temperature Difference (°C)	0.196	0.314	0.362	0.333	0.269
P. obcordata					
Aspect	-0.252	-0.160	-0.158	-0.334	-0.117
Bare ground and Rock Cover (%)	-0.226	-0.399	-0.370	**-0.521**	-0.183
Forb Cover (%)	**0.432**	0.296	0.252	0.414	-0.183
Grass Cover (%)	0.110	0.103	0.087	0.312	0.018
Litter Cover (%)	-0.018	0.224	0.161	0.157	-0.152
Shrub Cover (%)	0.121	0.309	0.300	0.295	0.317
Tree Cover (%)	-0.286	-0.267	-0.253	-0.038	-0.094
Elevation (m)	-0.281	-0.189	-0.212	0.007	-0.117
Slope (%)	0.346	0.308	0.299	0.374	0.083
Air Temperature (°C)	-0.0362	-0.0114	-0.0176	0.1870	0.3162
Surface Temperature (°C)	-0.0362	0.0228	0.0411	0.1603	0.3162
Temperature Difference (°C)	0.0327	-0.1596	-0.1701	-0.2137	-0.3162

Discussion

Creating a more accurate definition of suitable habitat for *P. congesta* and *P. obcordata* is a high priority objective for land managers due to increased pressures from energy development and a resulting need for Section 7 consultations (USFWS 2008). More accurate habitat classification would allow managers to prioritize specific projects and areas more efficiently. Currently, mapped suitable habitat is derived from soil studies (Hayden Wing Associated, LLC

2010) and computer modeling (Decker et al 2006, Decker et al 2013). However the soil study had issues with lack of sufficient replication as well as sampling distance from *Physaria* individuals and thus is limited in its practical application. Computer modeling has proven useful however ground-truthing efforts have shown that there is still a need for improvement.

Through these field ecological surveys I was able to detect significant differences for both species between occupied and unoccupied habitats. I found that mean tree and mean bare ground and rock cover as well as aspect and elevation were all significantly different between *P. congesta* occupied and unoccupied sites. Aspect differed only slightly with occupied sites being found more often on southwestern aspects and unoccupied on southern aspects. I also found that occupied sites were lower in elevation than unoccupied (occupied: 1875 – 1994 m; unoccupied: 1921-2118 m) . Soil color was also significantly different with occupied sites having higher proportions of lighter soil (particularly white) than unoccupied sites. Overall *P. congesta* tends to occur on southeast sites lower in elevation and with more bare ground and rock cover that are lighter in color than unoccupied habitats.

Due to the extremely short stature of *P. congesta*, establishment may be inhibited by any amount of shading from other plants or their litter (USFWS 2008) and I found that bare ground and rock cover was significantly different between occupied and unoccupied sites. This may be due to a lack of other species that are able to tolerate the harsh site conditions. The amount of bare ground and rock may also be due to the amount of water and surface soil movement that can damage or destroy plants that are not adapted to such a disturbance. Although valuable information, elevational differences I found may be confounded due in part to the location of multiple unoccupied survey sites being located in a relatively small area, which is higher in elevation than nearly all occupied locations, however this area is classified as suitable.

According to my results *P. obcordata* occupied sites had steeper slopes than unoccupied (occupied: 21.60 – 55.44%; unoccupied: 4.13 – 40.13%). The importance of slope in determining *P. obcordata* occurrence is not surprising since this species tends to grow on steep slopes and down-cutting drainages (U.S. Office of the Federal Register 1989). This parameter is both easily measured using digital elevation models (DEM) and could easily be incorporated into habitat models by land managers. I also found that soil color is significantly different between occupied and unoccupied *P. obcordata* sites with occupied sites again having a higher proportion of lighter soils (particularly white and light gray) than unoccupied sites.

Correlations were used to determine if there were any variables that significantly influence *Physaria* plant density in occupied sites, by species or by life stage. I found that vegetative *P. congesta* density is negatively correlated (r = -0.49) with increasing slope, which is what I expected since they are found on flatter ridgelines and outcroppings (U.S. Office of the Federal Register 1989). Vegetative *P. obcordata* density is negatively correlated (r = -0.52) with bare ground and rock cover which ranged from 83-94% in occupied sites. Mean *P. obcordata* cover was positively correlated (r = 0.43) with forb cover (not including *Physaria* cover) suggesting that overall this species performs better on areas with higher, but still relatively low cover (mean forb cover was less than 0.03% and mean bare ground and rock cover ranged from 83-94% in occupied sites). Overall the correlations showed that vegetative *P. congesta* grows more dense on gentler slopes while vegetative *P. obcordata* density and overall *Physaria* cover are positively correlated with a little more cover (increased forb and decreased bare ground and rock cover).

These results agree with previous habitat characterizations and may provide more accurate estimations for these parameters and increase accuracy of future occupancy and suitable habitat modeling. Effective prioritization of management activities, including population surveys and the creation of new Areas of Critical Environmental Concern, would be positively enhanced by increased performance of these models.

A power analysis determined that this survey had very little power to detect differences for the majority of survey variables with only two variables having power between 0.50 and 0.80 (elevation for *P. congesta*; slope for *P. obcordata*). Taking these results into account it is important to interpret correlation results with caution. When I determined how many sites would be necessary to produce enough detection power there was a very wide range between the variables where the majority were less than 1,000 sites but a few were greater than 10,000. Although these numbers are infeasible due to many factors, the results are still useful. Results from my field survey could be used to create continuous raster data that can be incorporated into suitable habitat and occupancy models if it is known what parameters are important to species of concern. Using GIS raster data allows researchers to samples thousands of locations, which would provide the necessary detection power.

Next step for this data would be to use it to create an occupancy model for each species. It may be possible to use aerial imaging to help delineate bare ground and rock cover and aid in the creation of a GIS raster file. This along with details on aspect, elevation, and slope from digital elevation models can be incorporated in the model developed by the Colorado Natural Heritage Program (Decker et al 2006, Decker et al 2013). The soil color results will likely be more problematic to translate into GIS data due to difficulty in translating Munsell-based colors to computer color values, however it is possible. By improving these models, land managers will have a more robust tool to assist in the conservation and protection of these imperiled species.

Conclusion and Management Implications

This analysis is the first step to creating or improving occupancy models for *P. congesta* and *P. obcordata*. Many of the variables that are significantly different between occupied and unoccupied habitats are easily derived from readily available GIS data including elevation, aspect, and slope, although it may be necessary to get some data with finer resolution (Decker et al 2013). It may also be possible to derive relative bare ground and rock cover and soil color using aerial imagery or remote sensing data. Future work could incorporate these variables into existing occupancy models or create new models and determine if it increases accuracy of suitable habitat delineation. By improving these models, land managers will have a more robust tool to assist in conservation and protection of these imperiled species.

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Chapter 4. Creating new populations of *Physaria congesta* and *Physaria obcordata* in suitable, unoccupied sites in the Piceance Basin, Colorado

Introduction

Creation of new populations of rare or endangered plants through reintroduction or translocation is an important management tool, especially in areas where existing populations are threatened by industrial development (Primack 2006, Fahselt 2007, Johnson and Prodgers 2013). However there are many legitimate concerns about its effectiveness and cost (Davy 2002, Fahselt 2007, Godefried et al 2011, Drayton and Primack 2012). There is very little information about long-term success of reintroduction and what is available shows little success after three or more years (Maunder 1992, Drayton and Primack 2000, Fahselt 2007, Guerrant and Kaye 2007, Drayton and Primack 2012). Many of the failures remain unpublished and the knowledge gained during these experiments lost. Another major issue is the lack of a unifying definition of success. Although many researchers have urged a focus on the presence of a reproducing population that persists multiple generations beyond founders (Primack and Drayton 1997, Fahselt 2007, Menges 2008).

Currently, there are still many unanswered questions about the efficiency and legitimacy of reintroduction or translocation of species. By carrying out reintroduction or introduction plans in a rigorous, experimental structure it may be possible to clarify different aspects that are important for multiple species in multiple habitats (Guerrant and Kaye 2007). Treating reintroduction plans as scientific experiments allows researchers to include biological and project purposes that have the potential to provide valuable insight even if plants are unable to establish (Pavlik 1996). In most cases the ultimate goal is to increase populations and expand ranges with the goal of saving species from extinction. Biological purposes generally focus on establishment, or re-establishment, of individuals in areas that are currently unoccupied, although may have been occupied historically. Project purposes are more likely to focus on technical aspects of reintroduction including determining best propagule type and number, site preparation techniques, and post-planting care (Pavlik 1996, Fahselt 2007).

This restoration structure is likely to include better site evaluations prior to reintroductions to ensure that associated plant and microbe species, as well as soil and light conditions, are present (Subhan et al 1998, Fahselt 2007, Drayton and Primack 2012). Using a larger number and more genetically varied seeds and transplants is also likely to increase the probability of success (Falk et al 1996, Pavlik 1996, Drayton and Primack 2012). Recommendations made from previous reintroduction studies include increased focus on the biology of the rare species, use of more mature adult transplants rather than seeds, and increased long term monitoring (Pavlik 1996, Guerrant and Kaye 2007, Godefroid et al 2011, Drayton and Primack 2012).

In the case of *P. congesta* and *P. obcordata*, little is known about their biology, especially reproduction and dispersal, or ecological site requirements that could provide a framework for a reintroduction plan. I structured this study to have biological and project specific purposes that could improve the conservation and possible future restoration efforts for these species. Prior to beginning field studies, extensive site selection took place, examining potential locations for associated plants species and soil characteristics. I also experimented with different establishment techniques to help determine most efficient and effective method for each species. This research takes into account many suggestions for study improvements made by researchers that have conducted similar experiments (Pavlik 1996, Falk et al 2007, Guerrant

2007, Drayton and Primack 2012, Johnson and Prodgers 2013) and will likely increase the probability of successful population establishment.

My initial plan was to set-up plots and seed them in October 2014 but before that happened the Western Slope Colorado Oil and Gas Association filed an administrative appeal over the approval of the Environmental Assessment required for my study. The appeal centered on the Bureau of Land Management (BLM) and U.S. Fish and Wildlife Service's (USFWS) decision not to list new populations as non-essential, giving them full protection under the Endangered Species Act of 1973. After nearly a year in the court system, the Environmental Assessment received a favorable judgment from the Interior Board of Land Appeals and I was given permission to establish my sites.

The objective of this study was to determine efficacy of creating new populations of *P. congesta* and *P. obcordata* in suitable yet unoccupied sites within the Piceance Basin, as well as testing the efficacy of using seeds versus transplants for establishment. I hypothesized that both species will have higher survival rates when transplanted compared to seeded plots and that plants will have greater success at sites further from existing populations where diverse seedlots could be planted, compared to near sites where seedlot sources were restricted to within resident genetic cluster of each species per the BLM and USFWS (Neale 2013).

Methods

Study Area: Piceance Basin

Located in northwestern Colorado in Rio Blanco and Garfield Counties, the Piceance Basin encompasses over 4,100 square kilometers (Taylor 1987). Monthly average temperature ranges from -5.9 ºC - 20.5 ºC and monthly precipitation levels range from 2.1 - 4.0 cm (Western Regional Climate Center Data for Meeker, CO 1997-2008). Annual precipitation within the basin ranges from 27 - 63.5 cm, where about half of annual precipitation falls as snow and remaining falls as rain during late summer thunderstorms (Tiedemann and Terwilliger 1978). The semiarid climate is typified by hot, dry summers and cold, snowy winters. Elevation varies between 1,706 and 2,740 meters due to unique geological formations found within the basin.

Site Selection

Final site selection took place April 25 – 28, 2013 throughout the Piceance Basin. Based on habitat maps provided by and discussions with the White River Field Office of the BLM, I identified 12 sites for field establishment plots, six sites per *Physaria* species. Selection criteria included presence of white shale substrate, suitable slope, presence of known associated species in the area (Hayden Wing Associates, LLC 2010), and lack of existing vegetation (decrease amount of disturbance necessary during site preparation) (Table 4.1). Selection was also focused around Areas of Critical Environmental Concern (ACEC) designated for the protection of these species by designating No Surface Occupancy.

For each species three sites were located more than 600 meters from existing occupied habitat of the same species (Far Sites) and three within 600 meters (Near Sites). This design takes into account the genetic structure of these two species and dictated the seedlots that were appropriate for placement at each site (Neale 2013). Following genetic analysis, the BLM and USFWS did not want me to mix seedlots from different genetic clusters for either species within 600 meters of occupied habitats (Chapter 1) due to worry about impacts of possible outbreeding depression or other detrimental genetic issues. Within far sites I had permission to seed and transplant from all available seed lots whereas within near sites I was limited to using seedlots from within genetic cluster where site is located. Each potential site was mapped using GPS and provided to the BLM, USFWS, and West Slope Colorado Oil and Gas Association for review.

Table 4.1. Field establishment sites for *P. congesta* and *P. obcordata*. Site characteristics derived from GIS data provided by BLM. Near and Far designation refers to distance from occupied habitat (near < 600 m; far > 600 m) and determine seedlots available for near sites. In near sites, I could only use seeds from the same genetic cluster and in far sites I could mix all available seedlots. Dr. Jennifer Neale at Denver Botanic Gardens conducted genetics testing and found genetic clustering for both species (only clustering for *P. obcordata* was significant but the BLM and USFWS are managing *P. congesta* as if the clustering was significant) (Neale 2013).

Species	Site Name	Genetics	Near/Far	Elevation (m)	Slope (%)	Aspect
P. congesta						
	Alkali Flats (AF)		F	1822	22.7	N
	Bar Mesa / Pondo (BM)		F	1964	17.2	SE
	Dry Gulch (DG)		F	1937	14.0	E
	Duck Creek (DC)	N	N	1951	14.2	S
	North Ryan Gulch (NRG)	S	N	1931	7.0	NE
	Yellow Creek (YC)	N	N	1880	14.2	W
P. obcordata						
	Alkali Flats (AF)	N	N	1829	25.9	SE
	Black Sulphur (BS)		F	1935	45.9	SW
	Dry Gulch (DG)		F	1927	33.4	SE
	North Ryan Gulch (NRG)	S	N	1918	30.6	W
	Stake Springs (SS)		F	1910	40.6	E
	Yellow Creek (YC)	N	N	1854	22.8	SW

Following the initial review, rare plant surveys were conducted that included determining the presence of rare plants, historical remnants, and sensitive wildlife presence (i.e. raptor nests). I conducted rare plant surveys during May 28 – 31, 2013. Surveys were conducted by walking a buffer zone around each potential site looking for suitable habitat and then walking transects within suitable habitat looking for rare plants, mainly *Physaria* species. Far sites required a 600-m buffer while near sites required only a 300-m buffer. Within *P. congesta* suitable habitat, transects were 10-m apart and for *P. obcordata* suitable habitat they were 15-m. This discrepancy is due to the fact that at the time of the surveys *P. congesta* were flowering and thus easier to locate than the non-flowering *P. obcordata*. All sites (Figure 4.1) were cleared of any factors that would preclude establishment of study plots.

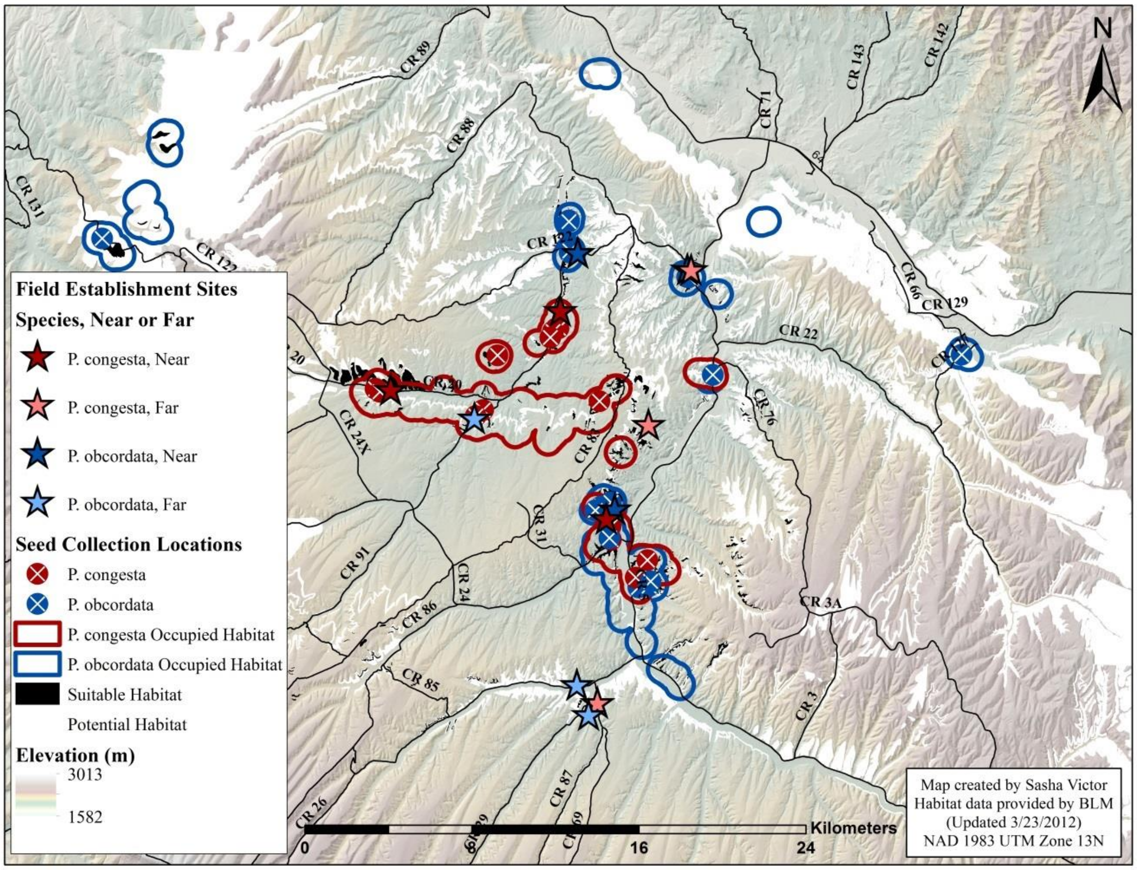

Figure 4.1. Map of *Physaria* habitat in Piceance Basin, Colorado with seed collection and final field establishment site locations. Seeds collected during 2012 and 2013. Field establishment sites established in 2015. Near sites within 600-m and far sites are greater than 600-m from any occupied habitats. At near sites only seeds from the same genetic cluster (Neale 2013) could be used per the BLM and USFWS.

Environmental Assessment

Site selection was essential to develop an Environmental Assessment (EA) that was required to conduct this research. Following public comment periods and stakeholder meetings, the Western Slope Colorado Oil and Gas Association appealed (October 2013) the approval of the EA resulting in the postponement of my research until the lawsuit was decided upon. During

August 2014, I learned that the EA had received a favorable decision from the Interior Board of Land Appeals and I had permission to establish my field plots.

Seed Collection and Germination

The Restoration Ecology Lab (REL) at Colorado State University and the BLM collected seeds across the range of both species during the summers of 2012 and 2013. I based 2013 collection protocols on those used to collect seeds for my soil feedback study (Chapter 2), although they were more conservative by limiting collections to 5-10 seeds from each of 10-20 plants per species. Also by this time, Denver Botanic Gardens reported that they found significant genetic clustering (3 – north, south, and west) in *P. obcordata* (Neale 2013). Using this knowledge I developed a collection scheme that ensured that we collected seeds from genetic clusters as well as to even out collections by cluster when possible (Figure 4.2). I estimated number of seeds per pod at the first collection location for each species. I initially found 2 seeds per pod but this ended up being an underestimation when we began finding three or four seeds per pod regularly. Denver Botanic Gardens provided seedlots 36-41 in October 2014 to supplement seeds already collected (Table 4.2).

Seeds were germinated and grown in the REL. Most seeds were germinated using an aeration method (Chapter 2 - Methods) with a few being cold stratified in moist, sterilized sand at 3 °C for 2-3 months. See Appendix A for more information on germination and survival rates. A subset of the transplant stock was used for a soil feedback experiment (Chapter 2) while all others were grown specifically for the field establishment study. I watered plants every 3-4 days and fertilized (Peters Excel, 15-5-15 CAL_MAG Special) every 8-10 weeks. I kept all plants in a CSU greenhouse until they were hardened-off for transplanting. To harden off transplants, I moved them outside into partial shade (except for a few nights in the beginning that were below

0 °C when they were moved into a covered breezeway in the CSU greenhouse and cut watering to once every 5-6 days). During the wait for final approval of the EA, I transplanted plants into larger conetainers (June 2014 and February 2015). Final conetainer dimensions were 2.5 cm x 16 cm with a volume of 66 ml (Model: RLC4L, Stuewe and Sons, Tangent, Oregon).

Table 4.2. *Physaria* seedlots used in field establishment study. Seedlots 1-35 collected by research assistants from the REL and BLM in 2012 and 2013. Denver Botanic Gardens provided seedlots 36-41 in the fall of 2014. Colorado Seed Lab at CSU conducted viability testing using tetrazolium (TZ) testing. Dr. Jennifer Neale at Denver Botanic Gardens conducted genetics testing and found genetic clustering for both species (only clustering for *P. obcordata* was significant but the BLM and USFWS are managing *P. congesta* as if the clustering was significant) (Neale 2013).

Species	Seedlot	Date Collected	Number of seeds collected	Population Name	Genetic Cluster	Viability
P. congesta						
	12	6/5/2012	306	Duck Creek	N	100%
	13	6/4/2012	264	Duck Creek	N	90%
	14	6/5/2012	392	Yellow Creek	N	100%
	15	6/14/2012	281	Yellow Creek	N	100%
	16	6/14/2012	120	Yellow Creek	N	65%
	22	6/18/2013	50	Pinto Mesa	N	90%
	23	6/18/2013	17	Dudley Bluffs	S	92%
	24	6/18/2013	150	Dudley Bluffs	S	92%
	25	6/18/2013	126	Yellow Creek	N	96%
	26	6/17/2013	188	Duck Creek	N	92%
	27	6/18/2013	411	Stake Springs	N	100%
	28	6/18/2013	251	North Ryan Gulch	S	96%
	29	6/18/2013	170	Ryan Gulch	S	92%
P. obcordata						
	1	7/12/2012	111	Unknown	-	75%
	2	7/12/2012	36	Unkown	-	52%
	3	7/12/2012	263	Unknown	-	61%
	4	7/5/2012	201	Dudley Bluffs	S	92%
	5	7/5/2012	236	Dudley Bluffs	S	96%

Table 4.2. Continued.

Species	Seedlot	Date Collected	Number of seeds collected	Population Name	Genetic Cluster	Viability
P. obcordata						
	30	7/30/2013	235	Yellow Creek	N	84%
	31	7/30/2013	298	Spring Creek	W	92%
	32	7/30/2013	251	Dudley Bluffs	S	88%
	33	7/29/2013	221	North Ryan Gulch	S	96%
	34	7/29/2013	251	Ryan Gulch	S	92%
	35	7/30/2013	206	Alkali Flats	N	100%
	36	7/7/2010	45	Yellow Creek	N	Untested
	37	7/6/2010	45	Alkali Flats	N	Untested
	38	7/6/2010	45	Yellow Creek	N	Untested
	39	7/8/2011	45	Rock School	N	Untested
	40	7/13/2012	45	Yellow Creek	N	Untested
	41	6/28/212	45	Rocky Ridge	N	Untested

Seeding

Seeding of field plots took place October 17 – 19, 2014. Within each seed lot, seeds were evenly distributed between 4 or 5 plots, depending on population genetics (see Table 4.3 for number of seeds and transplants). Upon arrival at each site we assessed the area, especially topography, to determine the best locations for each plot. Selected sites had suitable slope for each species, lack of existing vegetation, and avoided highly erosive areas (including gullies and patches of overland soil movement). Once the location was determined, a 0.25 m^2 quadrat was laid down for each seedlot and nails hammered into 2 corners (when facing up slope, upper left and lower right-hand corners). A plot number was attached in the lower right-hand corner. One person then roughened up the surface, approximately 2-cm depth, using a three claw hoe. Next I spread seeds, from a single seedlot throughout the plot and then tamped down the surface using a board, with special attention made to make sure that no seeds were attached to the bottom. This process was followed for all additional seedlots.

Table 4.3. Seed and Transplant number for field establishment sites for *P. congesta* in the Piceance Basin of northwestern Colorado. Near and Far designations refer to distance from occupied habitat (near < 600 m; far > 600 m) which influences the seedlots available for establishment. In near sites, I could only use seeds from the same genetic cluster and in far sites I could mix all available seedlots regardless of genetic cluster. Dr. Jennifer Neale at Denver Botanic Gardens conducted genetics testing and found genetic clustering for both species (only clustering for *P. obcordata* was significant but the BLM and USFWS are managing *P. congesta* as if the clustering was significant) (Neale 2013).

Species	Site Name	Genetics	Near/Far	# Seeds	# Fall Transplants	# Spring Transplants
P. congesta						
	Alkali Flats (AF)		F	224	12	68
	Bar Mesa (BM)		F	224	12	69
	Dry Gulch (DG)		F	224	12	68
	Duck Creek (DC)	N	N	151	6	41
	N. Ryan Gulch (NRG)	S	N	86	6	28
	Yellow Creek (YC)	N	N	151	6	42
P. obcordata						
	Alkali Flats (AF)	N	N	75	6	25
	Black Sulphur (BS)		F	261	15	60
	Dry Gulch (DG)		F	261	15	62
	N. Ryan Gulch (NRG)	S	N	145	6	29
	Stake Springs (SS)		F	261	15	62
	Yellow Creek (YC)	N	N	75	6	25

Transplanting

Fall

Fall transplanting took place November 7 – 9, 2014. I randomly selected plants from available transplant stock and moved them outside to harden-off (in partial shade and decreased watering) for 3 weeks prior to planting. To ensure that I would be able to test fall versus spring transplanting, only seedlots that had a minimum of six live plants were included for a total of 6 –

15 plants per site (Table 4.3). I packed transplants into buckets or totes filled with slightly moist shredded paper for transportation.

Upon arrival at each site, the location of seeded plots and locations for new plots was determined. Once locations were determined I laid down a 0.50-m^2 quadrat and placed nails in two corners, top left-hand and bottom right-hand (with a plot number attached). A few sites required additional effort to find areas that were easy to dig before plot locations were determined. Each plot was randomly assigned three plants randomly selected from the same seedlot and was organized in a triangle with two plants near the front and the middle plant towards the back, maximizing space available for each plant. Using a dibble, a pointed transplanting tool, to help break through the rocky soil, I made a small hole and in some cases further hand excavation was required to reach an adequate planting depth. I then carefully removed a plant from its conetainer. While holding the plant in the hole, soil was carefully moved in and tamped down. Once the hole was filled and tamped down, I watered the plant with approximately 0.25 liters. If watering left a depression I added a little more soil to even the surface. Finally a unique plant number tag was installed near the lower right-hand side of each plant.

Spring

Spring transplanting took place April 6-9, 2015. I assigned all remaining plants randomly to the six sites per species (Table 4.3). I moved plants outside for four weeks prior to planting to hardened-off by placing in partial shade for one week and then full sun as well as cutting back watering. Again, I packed plants into totes with slightly moist shredded paper during transport. Plot location, set-up, and planting methods were the same as fall transplanting. See Appendix B for a detailed map of each introduction site.

Data Collection

During the spring transplanting trip I also collected initial germination and survival data from fall seeding and transplanting plots. At each site we determined the number of germinants (or possible germinants) at each seeded plot and noted each transplant that had survived winter, which I determined by presence of green tissue. I also took extensive notes on each plant's condition as well as site characteristics, mainly if there was any evidence of animals or water movement through the site. I took photos of all transplants still on site as well as new germinants.

Analysis

Due to the short time span between fall seeding/planting and spring data collection, there was not enough information to run meaningful statistics I will present a summary of data collected as well as qualitative results.

Results

I found that mean germination and mean transplant survival was very low for all *P. congesta* sites (Table 4.4). I found one germinant of *P. congesta* and only one fall- transplanted plant appeared alive in six months after fall seeding and transplanting efforts. *P. obcordata* mean percent germination was also very low for all sites (2 % or less) while mean percent transplant survival at four sites was moderate (20 -60 %). I found new *P. obcordata* germinants at three sites and live transplants at four sites.

Table 4.4. Mean germination and survival percentages for *Physaria* field establishment sites in the Piceance Basin of northwestern Colorado (SE in parentheses). Near and Far designations refer to distance from occupied habitat (near < 600 m; far > 600 m). Mean germination is for plots seed and mean survival is for plants transplanted in the fall of 2014.

Species	Site Name	Near/Far	Mean % Germination	Mean % Transplant Survival
P. congesta				
	Alkali Flats (AF)	F	0.00% (0.0000)	8.00% (0.08)
	Bar Mesa / Pondo (BM)	F	0.00% (0.0000)	0.00% (0.00)
	Dry Gulch (DG)	F	0.00% (0.0000)	0.00% (0.00)
	Duck Creek (DC)	N	0.00% (0.0000)	0.00% (0.00)
	North Ryan Gulch (NRG)	N	0.00% (0.0000)	0.00% (0.00)
	Yellow Creek (YC)	N	2.00% (0.0200)	0.00% (0.00)
P. obcordata				
	Alkali Flats (AF)	N	0.00% (0.0000)	33.00% (0.03)
	Black Sulphur (BS)	F	0.00% (0.0000)	60.00% (0.16)
	Dry Gulch (DG)	F	0.17% (0.0017)	27.00% (0.12)
	North Ryan Gulch (NRG)	N	0.00% (0.0000)	0.00% (0.00)
	Stake Springs (SS)	F	2.00% (0.0180)	20.00% (0.08)
	Yellow Creek (YC)	N	2.00% (0.0180)	0.00% (0.00)

Discussion

From this preliminary monitoring data, I found that overall mean percent of surviving transplants is greater than mean germination from seeds. This pattern also holds true for each species separately (Table 4.4). *P. obcordata* had more new germinates and surviving transplants than *P. congesta*. Among *P. obcordata* populations, the Black Sulphur site was most successful (i.e. number of live germinates and surviving transplants) but the transplants at the Stake Springs site appeared healthiest, where one was beginning to put on new leaves, and had the most germinates. Far sites are producing higher mean percent germination and mean percent survival than near sites, which if this trend continues would provide a large range expansion for these species, especially for *P. obcordata*. Data collection likely occurred too early to for the majority of spring germinates and it is likely that there will be more in subsequent surveys.

Some possible reasons for the low survival rate in fall transplants are frost heave, lack of precipitation, wind shear or a combination. Although rarely talked about in literature, and most often about tree seedlings (Goulet 1995, Sahlén and Goulet 2002), practitioners have found that transplants that experience frost conditions shortly after planting can experience frost heave (Randy Mandel *personal communication*). If this occurs it may be possible to go back a few weeks after planting and re-bury any plants that have been heaved from the ground. This environment can also have very high winds which may significantly impact transplants especially when conditions are dry. Another possibility is soil/rock movement caused by water. At most sites I found evidence of recent erosion in the form of small gullies or rivulets.

Based on these observations, I modified spring transplanting protocols in a few ways. As holes were filled during transplanting, I made sure to fill the hole evenly and tamped down throughout. This is an attempt to avoid frost heave, although this should be less of a concern with spring transplant relative to fall transplants. Another important modification was to ensure that plants were planted as close to the surface as possible to decrease impact of high winds. Winds in this area are inevitable and it may be necessary to build small wind breaks around plants to protect them at least until they become established.

Based on all of my field observations I have a handful of limiting factors that may be important to the success of these seeding and transplanting efforts. First and foremost is uncertainty of precipitation and difficulty of providing water to transplants. Without enough natural spring precipitation it may be necessary to provide supplemental watering. This is the first hurdle for these transplants but the next one will be a long, hot, dry period before late summer monsoons (Figure 1.1). However with more precipitation comes increased likelihood for destructive water and land movement at introduction sites. Luckily these species are well adapted to these movements (USFWS 2008) and thus have a greater chance for survival.

Hard frost events immediately following transplanting is an immediate threat to these species and should be incorporated into any future transplanting plans. Strong winds are also inevitable in the Piceance Basin and it may be necessary to build temporary wind blocks at transplanting sites until plants can begin to grow new roots and better establish. There were animal tracks from multiple species throughout multiple sites but I did not see any loss of plants due to herbivory or trampling. It will be necessary to continue to monitor these impacts and make this assessment again in the future to determine if there are any significant changes.

The final and biggest threat to these introduction sites is the continued legal issues over the Environmental Assessment. Although it has already been up held by the BLM and the Interior Board of Land Appeals, the Western Slope Colorado Oil and Gas Association continues to pursue legal action. If they are successful the worst case scenario would be the destruction of these study plots and forfeiture of any future data.

Conclusion

Although these results are preliminary there are some important lessons learned from this effort to go beyond protecting habitat into active restoration efforts. After six months, I found survival of 27% of fall *P. obcordata* transplants, with one site having 60% survival. Even though it was early April, I did find five new germinates and expect to find more in future surveys. Early trends show that *P. obcordata* is doing better than *P. congesta* and transplants are performing better than seeds. Within *P. obcordata* sites, far sites are doing much better than near sites with Black Sulphur being most successful (most live plants and germinates) and Stake Springs having the healthiest plants.

During my many field visits I have developed a short list of possible reasons for death of transplants including lack of precipitation, frost heave, and high winds. I slightly modified spring transplanting protocols to incorporate lessons from fall transplanting and monitoring. At this point, monitoring should continue on all transplants because I have seen plants that appear to be dead but later had new shoots appear.

The limited survival that I saw in April 2015 provides evidence of some early success of both transplanting and seeding efforts. Long term monitoring is essential to understand the full efficacy of these efforts as possible management techniques. However, for this to happen there must be resolution to all legal issues. If our Environmental Assessment is upheld it is likely to set a precedent for this type of study on other rare and/or listed species. It will provide land managers another tool to protect and conserve these unique species.

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Chapter 5. Synthesis and Management Implications

Results of these three studies have significantly added to the depth of knowledge around *Physaria congesta* and *Physaria obcordata*. I did not find evidence of soil feedback within occupied or unoccupied sites, which means that introduction efforts can take place on any suitable habitat with the expectation that there will not be any immediate soil feedbacks. This is important when considering restoration/introduction efforts, as hypothesized soil feedback could be detrimental to long-term success of introduction efforts.

There is a strong need for a more accurate and measurable definition of suitable habitat for these species and the field ecological survey helped to elicit a few more details. Overall *P. congesta* tends to grow on southeast aspects lower in elevation (occupied: 1875-1994 m; unoccupied: 1921-2118 m) and with increased bare ground and rock cover (occupied: 91.73-98.52%; unoccupied: 74.50-97.63%) and lighter (mainly white) soil color than unoccupied habitats. While *P. obcordata* occupies sites with steeper slopes (occupied: 21.60-55.44%; unoccupied: 4.13-40.13%) than unoccupied sites as well as white or light gray soils. Correlations showed that *P. congesta* has higher population density on gentler slopes (occupied slope range: 4.87 – 42.67%) while *P. obcordata* population density and overall *Physaria* cover is associated with increased forb (occupied forb cover range: 1.54 – 9.00%) and decreased bare ground and rock cover (occupied bare ground and rock cover: 83.47 – 95.00%). Incorporation of this information into existing (Decker et al 2013) or new habitat models should results in refinement of suitable habitat designations.

Finally, although in the preliminary stages, the field establishment study provides a wealth of new information for managers regarding the feasibility of creating new populations of these species as well information on the best establishment method. Beyond these benefits, researchers will be able to monitor these populations for reproduction, fecundity, response to disturbance, and population dynamics. Favorable judgment received on the Environmental Assessment developed for this project will also create a precedent for future projects with other rare and listed plant species. This type of proactive management may be the only hope for some of these species to survive in the face of pressure by increased development and climate change.

There is still a lot of unknown information about *P. congesta* and *P. obcordata* and there are many avenues for future research. I propose that the most immediate needs are to better understand reproduction, fecundity, and dispersal of these species. The new populations I created can serve as an outdoor laboratory to closely monitor their population dynamics and impacts of natural disturbances. After numerous site visits and long hours contemplating these species, I propose that the limiting factors to successful population and range expansion are establishment/recruitment and dispersal. These investigations should be a priority for land managers.

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Appendix A. Seed germination and survival for *Physaria congesta* and *Physaria obcordata*

Physaria congesta and *Physaria obcordata* are both rare mustard species that are endemic to the Piceance Basin of northwestern Colorado. Both species have a very limited range (Chapter 1) and are subject to numerous regulations based on their federally threatened status. In order to complete my soil feedback study (Chapter 2) and field establishment experiment (Chapter 4), I needed to grow hundreds of *Physaria* plants. Due to their rare and threatened status I was required to apply for a permit with the U.S. Fish and Wildlife Service to collect seeds (Chapter 2). I germinated seeds in the Restoration Ecology Lab at Colorado State University and grew them in a growth chamber and a greenhouse. Tables A.1 and A.2 summarize germination and survival data for each species.

Table A.1. Germination and survival percentages for *P. congesta*. All seeds used organized by seedlot and study germinated for as well as method used. Aeration consisted of bubbling seeds in a warm water bath until a radicle is produce and cold stratification was done in moist sand for 2-3 months at 3 °C. Percent germination (number seeds germinated) calculated using number of seeds germinated and seeds used. Percent emergence (number of plants that emerge and produce true leaves) was calculated using number plants that emerged and number of viable seed (number of seeds*viability). I calculated percent survival (number of plants survived and transplanted during field establishment study) two ways: 1) percent survival of all seeds used and 2) percent survival of all seeds that emerged.

	Seedlots	# Seeds Used	% Germination	% Emergence	% Survival of seeds	% Survival of emerged
Initial Germination						
Aeration	12	5	40% (2)	0% (0)	0% (0)	0% (0)
	14	10	60% (6)	20% (2)	20% (2)	100% (2)
	15	5	20% (1)	0% (0)	0% (0)	0% (0)
	16	5	20% (1)	33% (1)	20% (1)	100% (1)
Soil Feedback						
Aeration	12	135	81% (110)	12% (16)	12% (16)	100% (16)
	13	130	65% (84)	32% (38)	9% (12)	32% (12)
	14	135	66% (89)	17% (23)	13% (17)	74% (17)
	15	130	93% (121)	56% (73)	29% (38)	52% (38)
	16	120	58% (70)	54% (42)	10% (12)	29% (12)
Field Establishment						
Aeration	24	71	61% (43)	32% (21)	28% (20)	95% (20)
	25	87	53% (46)	22% (18)	21% (18)	100% (18)
	26	127	46% (58)	22% (27)	20% (26)	96% (26)
	27	133	67% (89)	44% (59)	44% (59)	100% (59)
	28	95	72% (68)	59% (54)	57% (54)	100% (54)
	29	96	72% (69)	58% (51)	53% (51)	100% (51)
Cold Stratification	13	40	33% (13)	3% (1)	3% (1)	100% (1)
	14	38	68% (26)	8% (3)	8% (3)	100% (3)
	15	50	62% (31)	14% (7)	14% (7)	100% (7)
	24	34	47% (16)	3% (1)	3% (1)	100% (1)
	25	37	27% (10)	3% (1)	3% (1)	100% (1)
	26	38	26% (10)	8% (3)	8% (3)	100% (3)
	27	81	62% (50)	14% (11)	14% (11)	100% (11)
	28	82	20% (16)	10% (8)	10% (8)	100% (8)

Table A.2. Germination and survival percentages for. *obcordata*. All seeds used organized by seedlot and study germinated for as well as method used. Aeration consisted of bubbling seeds in a warm water bath until a radicle is produce and cold stratification was done in moist sand for 2-3 months at 3 °C. Percent germination (number seeds germinated) calculated using number of seeds germinated and seeds used. Percent emergence (number of plants that emerge and produce true leaves) was calculated using number plants that emerged and number of viable seed (number of seeds*viability). I calculated percent survival (number of plants survived and transplanted during field establishment study) two ways: 1) percent survival of all seeds used and 2) percent survival of all seeds that emerged.

	Seedlots	# Seeds Used	% Germination	% Emergence	% Survival of seeds	% Survival of emerged
Initial Germination						
Aeration	1	5	40% (2)	0% (0)	0% (0)	0% (0)
	2	5	40% (2)	50% (1)	20% (1)	100% (1)
	3	5	60% (3)	33% (1)	20% (1)	100% (1)
	4	5	40% (2)	0% (0)	0% (0)	0% (0)
	5	10	100% (10)	78% (7)	70% (7)	100% (7)
Soil Feedback Study						
Aeration	1	25	40% (10)	17% (3)	4% (1)	33% (1)
	2	15	47% (7)	71% (5)	0% (0)	0% (0)
	3	180	39% (70)	31% (34)	11% (20)	59% (20)
	4	171	71% (122)	59% (93)	23% (39)	42% (39)
	5	225	63% (142)	49% (105)	16% (35)	33% (35)
Field Establishment Experiment						
Aeration	30	181	80% (144)	64% (98)	54% (98)	100% (98)
	31	150	2% (3)	1% (1)	1% (1)	100% (1)
	34	30	40% (12)	30% (8)	27% (8)	100% (8)
	35	121	65% (79)	35% (42)	35% (42)	100% (42)
Cold Stratification	30	38	26% (10)	3% (1)	3% (1)	100% (1)
	31	78	26% (20)	4% (3)	4% (3)	100% (3)
	32	74	50% (37)	12% (8)	11% (8)	100% (8)
	33	99	61% (60)	21% (20)	20% (20)	100% (20)
	34	93	60% (56)	28% (15)	16% (15)	100% (15)
	35	18	28% (5)	6% (1)	6% (1)	100% (1)