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FACTORS CONTRIBUTING TO THE CONSERVATION OF *PHACELIA*  
*SUBMUTICA* (BORAGINACEAE), A THREATENED SPECIES IN  
WESTERN COLORADO: REPRODUCTIVE BIOLOGY  
AND SEED ECOLOGY

by

Alicia M. Langton

A thesis submitted in partial fulfillment  
of the requirements for the degree

of

MASTER OF SCIENCE

in

Ecology

Approved:

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Logan, UT

2015

## ABSTRACT

Factors Contributing to the Conservation of *Phacelia submutica* (Boraginaceae), a Threatened Species in Western Colorado: Reproductive Biology and Seed Ecology

by

Alicia M. Langton, Master of Science

Utah State University, 2015

Major Professor: Dr. Eugene W. Schupp  
Department: Wildland Resources

Conservation and recovery plans for rare species require biological and ecological information to discern how they may be susceptible to human disturbances. *Phacelia submutica* is a threatened annual species in western Colorado. Human activities including energy development, recreation, and livestock grazing are occurring within the species' range. To provide conservation practitioners with a scientific basis for management, this research aimed to elucidate elements of the species' ecology. Chapter 2 describes the reproductive biology of *P. submutica*. Potential insect pollinators were not observed during two years of observations. Floral traits and development ensure self-pollination and reduce the likelihood that insects would be effective pollinators. Hand pollination experiments using varying pollen sources did not result in significant differences in seed number and mean mature seed weight per fruit, two metrics of reproductive success. These results indicate that the species is habitually autogamous. Conservation of this species will not require the protection of pollinators and their

habitat, but should consider the potential impacts of autogamy on the species' genetic diversity. Chapter 3 examines aspects of *P. submutica*'s seed ecology as they relate to the development and maintenance of the seed bank. Observations suggest seeds are limited in their long-range dispersal capacity. Average seed bank density was low (74 seeds per m<sup>2</sup>) and seeds were highly aggregated within sites. Based on three years of seed burial data, the species forms a long-term persistent seed bank that maintains high proportions of viability in drought years, but germinates prolifically in favorable years. Projections of seed depletion rates from this dataset predicted longevity to be between four and six years. Finally, seeds were generally unresponsive to germination trials involving varying degrees of cold-moist stratification, incubation temperatures, and scarification. *P. submutica* shows adaptations that promote its persistence in an arid environment characterized by climatic variability. Appropriate management of this species will require protection of the seed bank and the dynamics involved in its replenishment and maintenance. Chapter 4 presents implications of the two major studies and provides conservation practitioners with an integrated assessment of the results and how they relate to management.

(157 pages)

## PUBLIC ABSTRACT

Factors Contributing to the Conservation of *Phacelia submutica*, a Threatened  
Species in Western Colorado: Reproductive Biology and Seed Ecology

Alicia M. Langton

Conservation and recovery plans for rare species require biological and ecological information to discern how they may be susceptible to human disturbances. *Phacelia submutica* is a threatened annual species in western Colorado. Human activities including energy development, recreation, and livestock grazing are occurring within the species' range. To provide conservation practitioners with a scientific basis for management, this research aimed to elucidate elements of the species' ecology. Chapter 2 describes the reproductive biology of *P. submutica*. Potential insect pollinators were not observed during two years of observations. Floral traits and development ensure self-pollination and reduce the likelihood that insects would be effective pollinators. Hand pollination experiments using varying pollen sources did not result in significant differences in seed number and mean mature seed weight per fruit, two metrics of reproductive success. These results indicate that the species is habitually autogamous. Conservation of this species will not require the protection of pollinators and their habitat, but should consider the potential impacts of autogamy on the species' genetic diversity. Chapter 3 examines aspects of *P. submutica*'s seed ecology as they relate to the development and maintenance of the seed bank. Observations suggest seeds are limited in their long-range dispersal capacity. Average seed bank density was low (74 seeds per m<sup>2</sup>) and seeds were highly aggregated within sites. Based on three years of

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## PREFACE

The format of this thesis follows that of the professional journal *Plant Species Biology* with the necessary adaptations required by the School of Graduate Studies at Utah State University. Chapter 2, “Reproductive biology of *Phacelia submutica*, a threatened species in western Colorado,” is written as a manuscript for submission to *Plant Species Biology*. In the Appendix, species codes are from the United States Department of Agriculture (USDA) plants database (<http://plants.usda.gov>) and soil information follows the National Soil Information System (NASIS).

## CHAPTER 1

### INTRODUCTION

Human activities have led to a significant impact on biodiversity of the Earth's ecosystems (Chapin *et al.* 2000; Goudie 2013). We have entered an age called the "anthropocene" (*sensu* Eugene F. Stoermer, popularized by Crutzen & Stoermer (2000)), a time when the dominant driver of global environmental change is human activities. Taxa worldwide are at risk of being impacted, and this is especially the case for species with restricted ranges or habitats (McKinney 1997; Payne & Finnegan 2007). Plants are especially at risk because, unlike most animals, their mobility is limited (Stein *et al.* 2000). Presently, it is estimated that 20% of all extant plant species may become extinct in the near future (IUCN 2004). In the United States alone, nearly 18,000 species (30% of native species) are imperiled (Natureserve 2010). Habitat degradation, loss and fragmentation, alteration of plant-animal interactions, and changes to the chemical, physical, or biotic environment are the primary drivers of species decline (Wilcove *et al.* 1998; Schwartz & Brigham 2003). The impact of any one or a combination of these threats to a species depends on species-specific characteristics. Studies that elucidate the species' biological requirements, or its autecology, are necessary if conservation practitioners are to create adequate conservation and recovery plans (Simberloff 1988; Heywood & Iriondo 2003).

*Phacelia submutica* (Boraginaceae, formerly Hydrophyllaceae) is a rare herbaceous spring annual. The species is endemic to an ecoregion known as the Colorado Plateau and is confined to an area within Garfield and Mesa Counties, Colorado, USA. Its habitat is described as clay barrens within a landscape of juniper

woodlands, sagebrush flats, and desert pavement habitats. It was first collected in 1911 by George E. Osterhout and formally described decades later as a distinct species by Howell (1944). *P. submutica* is known to occupy an estimated 227 hectares of land within a 20 km radius from the town of De Beque (Colorado Natural Heritage Program 2012). The vast majority (96%) of occupied habitat is on public lands managed for multiple uses (U.S. Fish and Wildlife Service 2012). Increasing development on public lands within the range of the species, including natural gas extraction, recreational activities, and agriculture, are the dominant sources of disturbance and, therefore, potentially pose threats to the species. On 26 August, 2011, *P. submutica* was listed as threatened under the Endangered Species Act (U.S. Fish and Wildlife Service 2011), providing the federal government with the financial and legislative capacity to protect this species from further habitat loss and impending extinction.

One important consideration in the development of a conservation plan is to identify the factors of a species' biology that influence its rarity and susceptibility to certain disturbance activities (Flather & Seig 2007). A species may be intrinsically rare as the result of biological or ecological characteristics that limit its abundance or range (Partel *et al.* 2005). These may include restricted dispersal capacity, low seed production, or phenotypic and genetic inflexibility to adapt to a greater range of conditions (Farnsworth 2007; Flather & Seig 2007). Extrinsic factors may also be operating to reinforce the rarity of a species, such as disturbance, competition, herbivory, and limited habitat availability (Ellstrand & Elam 1993; Brys *et al.* 2004). The intersection of intrinsic and extrinsic factors will determine the range of a species, the



size of their populations, and the dynamics associated with population viability (Richter-Dyn & Goel 1972; Wright & Hubbell 1983; Payne and Finnegan 2007).

The impacts of disturbance to pollinators and pollination have been well documented (Potts *et al.* 2010), and are of key consideration in the conservation of rare plant species (Kearns *et al.* 1998). Land use changes affect the abundance and diversity of pollinators (Aizen & Feinsinger 1994; Kremen & Ricketts 2000), and thus their pollination services to plants (Jennerston 1988; Cunningham 2000). Climate change also threatens to alter the landscape in ways that may be detrimental to local pollinator community assemblages (Hegland *et al.* 2009). In sexually reproducing species, the transfer of pollen within and between flowers influences the abundance, demography, and genetic diversity within and among plant populations (Huenneke 1991; Kearns *et al.* 1998; Takebayashi & Morrell 2001; Kremen *et al.* 2007). Impacts to pollinators may alter their visitation rates or foraging habits, potentially causing a plant to produce fewer or lower quality seeds (Ashman *et al.* 2004). Reproductive assurance through self-pollination can counteract the negative effects of pollinator loss, but may also lead to reductions in fitness and, if habitual, may impair a population's viability through inbreeding depression (Charlesworth & Charlesworth 1987). Inbreeding can also reduce allele richness affecting a loss of evolutionary or adaptive potential (Koehn & Hilbish 1987). Though these impacts are considered a problem to all plant species, rare plant populations may be more vulnerable through the effects of small population size and fragmentation on pollinator visitation (Barrett & Kohn 1991; Ågren 1996), as well as the effect of a small breeding population on genetic diversity (Aguilar *et al.* 2006).

Therefore, assessing the reproductive biology of a species is necessary prior to the design of conservation plans (Hamrick *et al.* 1991; Ellstrand & Elam 1993; Foin *et al.* 1998).

Disturbances within or in close proximity to a species' habitat can displace or remove seeds from the soil, as well as alter the dynamics involved in the development and maintenance of a seed bank (Oostermeijer 2003). Whether or not a disturbance impacts the regenerative capacity of a population depends on the functional significance of the seed bank. For annual species, a seed bank is necessary to store seeds in the absence of adult plants. The persistence of a seed bank through time insures against environmental variability, as well as other disturbances (Thompson 2000; Meyer *et al.* 2005). Seed characteristics such as dormancy, germination requirements, inherent longevity, and dispersal mechanisms are important determinants of the abundance and distribution of adult plants through time and in space (Schupp & Fuentes 1995; Levin *et al.* 2003; Jensen 2004). By studying the ecology of seeds, conservation practitioners can identify potential causes of rarity and deduce how anthropogenic disturbances may further reduce a species' abundance and range.

Populations can only persist if they are able to successfully produce viable offspring that are dispersed into the appropriate habitat, can escape predation and pathogens, successfully germinate, and then reproduce (Richter-Dyn & Goel 1972; Menges 1990; Purvis *et al.* 2000; Oostermeijer 2003). Disruption of one or more of these processes can reinforce rarity or lead to population decline. Adequately protecting a population requires more than simply protecting its habitat, it also requires appropriately managing the ecological interactions that favor or disfavor plant success. Therefore, the

connection between seemingly distinct elements of a species' ecology must be recognized in the development of a conservation strategy (Pärtel *et al.* 2005).

This thesis is the first study on the ecology of *P. submutica*. In Chapter 2, we conduct observations and experiments on the reproductive biology of this species. We describe the reproductive development of flowers from anthesis to senescence and observe plant-pollinator relationships. We also performed hand pollination experiments to test whether pollinators and their foraging behaviors have an effect on seed quantity and quality. Additionally, to assess the impact of pollen source on an additional measure of seed quality, germination experiments were performed. Information is also provided for floral traits that relate to the operation of a breeding system (Pollen-to-ovule ratio (P:O), outcrossing index (OI), and differences between pollen tube growth between different pollen sources). In Chapter 3, we report results from studies on several elements of the species' seed ecology: Observations of dispersal, the spatial distribution and density of the seed bank, the longevity of seeds in the soil through experimental burial, and germination requirements are presented and discussed. At the end of each chapter, a section on conservation implications is included to address the study-specific results in the context of conservation and recovery. In Chapter 4, a conclusion of the results and implications of the two major studies is presented to provide conservation practitioners with an integrated assessment of the results and how they relate to management.

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## CHAPTER 2

THE REPRODUCTIVE BIOLOGY OF *PHACELIA SUBMUTICA* (BORAGINACEAE),  
A THREATENED SPECIES IN WESTERN COLORADO<sup>1</sup>**Abstract**

Human activities, including energy development, recreation, and livestock grazing, in the Piceance Basin of western Colorado threaten many rare plant species endemic to the region. One of these species is *Phacelia submutica* (Boraginaceae, formerly Hydrophyllaceae), listed under the Endangered Species Act as a threatened species in 2011. How *P. submutica* will be affected by these disturbances, as well as how managers can mitigate their threats, depends in part on the species' reproductive strategy. We performed a series of studies in 2011 and 2013 on elements of the reproductive biology of *P. submutica*. Spontaneous self-pollination occurred in the morning within two hours of anthesis and development was marginally protogynous. Breeding system experiments (autogamy, geitonogamy, xenogamy, and an open pollination control) did not result in significant differences in measures of fitness (number of seeds and mean seed weight per fruit) between pollination treatments. Emasculated flowers testing agamospermy did not develop fruits. Pollen tubes entered ovaries within six hours of pollination, and we found no differences in pollen tube growth rates between autogamous, geitonogamous, and xenogamous pollen sources. No floral visitors were seen at *P. submutica* sites during 12.5 hours of watches in 2011 and 2013. These results demonstrate that *P. submutica* has an autogamous breeding system and suggest that pollinators play no role in its reproduction. This reproductive strategy may have evolved

in response to factors including rarity and pollinator loss, and has implications for the conservation of the species.

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<sup>1</sup>This chapter is co-authored by Alicia M. Langton and Eugene W. Schupp.

## Introduction

Conservation and management plans for rare species are only effective if they are able to protect reproductive rates sufficient to ensure a species' survival (Kearns *et al.* 1998). To achieve this goal, these plans must consider the reproductive biology of a species for its influence on the abundance, demography, genetic diversity and, ultimately, the long-term viability of a species (Huenneke 1991; Kearns *et al.* 1998; Takebayashi & Morrell 2001). The global decline in pollinator capacity to provide pollination services is a major threat to the conservation of rare species (Bond 1994; Spira 2001). Habitat degradation and fragmentation can change the abundance and diversity of pollinators, as well as alter their foraging behaviors (Didham *et al.* 1996; Kearns *et al.* 1998; Cunningham 2000; Kevan & Viana 2003; Kremen *et al.* 2007; Winfree *et al.* 2009). A species' response to the effects of disturbance on pollinators will depend, in part, on its breeding system and the degree to which it relies on animals for pollination (Memmott 1999; Navarro & Guitian 2002; Aguilar *et al.* 2006). If the response is the production of fewer or lower quality seeds (pollinator limitation) (Ashman *et al.* 2004), or an increase in the natural rate of inbreeding (Charlesworth & Charlesworth 1987), conservation practitioners will need to include protections for suitable pollinators and their habitat (Foin *et al.* 1998).

Plants use a diverse and often flexible array of strategies to achieve reproduction, from cross- and self-pollination to asexual reproduction through agamospermy and vegetative propagation (Fryxell 1957; Jain 1976). Each strategy affords both advantages and disadvantages to population viability (Ashman *et al.* 2004). Cross-pollination can produce offspring with greater vigor (Darwin 1876) and provide a population with the

genetic diversity necessary for adaptation and evolution (Barrett & Kohn 1991; Hamrick *et al.* 1991). However, obligately outcrossing species reliant on animals as pollen vectors may experience reproductive failure if environmental change or disturbance limits pollination (Lamont *et al.* 1993; Menges 1995). On the other hand, species capable of autogamous self-pollination have an advantage in their capacity to produce seeds in the absence of pollinators (Fausto *et al.* 2001; Kalisz & Vogler 2003). Though reproductive assurance through autogamy can counteract pollinator limitation, it may result in the production of fewer and/or less viable offspring (Kearns *et al.* 1998; Kremen *et al.* 2007). Reduced fecundity and seedling vigor can eventually lead to a reduction in population size and a breakdown of demographic structure (Cheptou 2004; Herlihy & Eckert 2002; Coates *et al.* 2007). Chronic or obligate mating between close relatives can result in the reduction of offspring fitness (inbreeding depression) and may impair the evolutionary potential of the species through homozygosity (Charlesworth & Charlesworth 1987; Huenneke 1991; Ellstrand & Elam 1993). Thus, the frequency of occurrence of the various strategies of reproduction, as well as the flexibility to exploit a variety of strategies, has major implications for population viability.

The operation of a particular strategy is primarily determined by floral traits, which influence the interactions between pollinators and flowers, as well as anthers, pollen, and stigmas. (Schoen *et al.* 1996). At the most basic level, the morphological characteristics of a flower are linked to pollinator attraction and the visitation ability of specific pollinator species (Dafni & Kevan 1996; Galen 1999). Additional features of pollinator attraction include floral scent and nectar (Kevan & Baker 1983; Dobson 1994). Pollen transfer within and between flowers is influenced by the spatial orientation of

male (anthers) and female (stigmas) parts within a flower, which determine the points of contact of these organs with pollinators. If the degree of separation between anthers and stigma(s) is minimal, self-pollination can easily occur (Webb & Lloyd 1986). However, floral development, such as the timing of stigma and anther maturation, mediates the potential for self-pollination (Rick *et al.* 1977; Schoen & Lloyd 1992; Castro *et al.* 2008; Eckert *et al.* 2009). Moreover, the lifespan of a flower may be related to the amount of pollen and the diversity of pollen sources deposited by pollinators (Primack 1985; van Doorn 1997). Additionally, the allocation of resources to male and female reproductive functions, such as the pollen-to-ovule (P:O) ratio, is correlated with the efficiency of pollen transfer (Charlesworth & Morgan 1991), as well as a species' reproductive strategy (Cruden 1977). These functional aspects of a breeding system and their relationship to pollination are useful in identifying the characteristics of a species that influence the natural rates and patterns of pollen deposition, as well as the success of a given pollination strategy (Faegri & Van der Pijl 1979; Bernardello *et al.* 2001; Bernhardt & Edens-Meier 2010).

Successful pollination of a flower may still result in failure to produce seed, and this failure can occur at various stages of fertilization. In some species, pollen from the same individual (self-pollen) may not be able to successfully germinate (Husband & Schemske 1996; de Nettancourt 1997; Lipow & Wyatt 2000). Further, pollen tubes may have variable growth rates depending on their pollen source, influencing the relative success of a given pollination mode (Snow & Spira 1991; Cruzan & Barrett 1993; Eckert & Allen 1997; Kalisz & Vogler 2003). Finally, despite the successful fertilization of an ovule, a seed produced from self-pollen may be sterile (East 1940). These cryptic self-

incompatibility mechanisms can aid in preventing inbreeding depression by reducing or eliminating the number of seeds produced through self-fertilization (Silva & Goring 2001). However, because of its potential impact on fecundity, cryptic self-incompatibility may be mistaken for pollinator limitation or inbreeding depression (Kruszewski & Galloway 2006). Thus, identifying the source of pollination failure is valuable when assessing a breeding system (Lloyd & Schoen 1992).

Rare plant populations may be more vulnerable to disturbance of plant-pollinator interactions. First, rare plant populations often reside in small, sparse, and fragmented populations (Fiedler & Ahouse 1992; Barrett & Kohn 1991; Ågren 1996; Rathcke & Jules 1993; Aizen & Feinsinger 1994). This may cause them to be more susceptible to pollinator limitation through density-dependent foraging behaviors of pollinators (Sih & Baltus 1987; Kunin 1992; Bosch & Waser 1999; Steffan-Dewenter & Tschardt 1999). In addition to the potential loss of fecundity, the genetic consequences of inbreeding depression can develop more rapidly in populations with fewer mates and its effects become more pronounced as populations contract in size (DeMauro 1993; Kwak *et al.* 1998; Brys *et al.* 2004; Anderson *et al.* 2011). The effect of rarity on population viability increases the importance of searching for species-specific traits acting to ensure reproduction prior to the design of conservation strategies.

The rare annual *Phacelia submutica* (Boraginaceae, formerly Hydrophyllaceae) is narrowly endemic to western Colorado, USA. On 26 August, 2011 the species was listed as threatened under the Endangered Species Act as a result of increasing energy development, recreation, and other human activities within its range (U.S. Fish and Wildlife Service 2011). Conservation of this species will require information about its

reproductive biology to protect reproduction and, if necessary, its pollinators. To address this need we performed a series of studies in 2011 and 2013 to identify (i) functional factors involved in the species' breeding system (floral morphology, development, longevity, and nectar and scent production); (ii) floral visitation by potential pollinators and their foraging behaviors; (iii) the breeding system using hand pollination and pollinator exclusion experiments to assess the effect of pollen source on seed quantity (number of seeds) and quality (mean mature seed weight per fruit and the viability and vigor of seeds); (iv) the P:O ratio and outcrossing index (OI); and (v) differences in pollen tube development between pollen sources. These data are then discussed in the context of the conservation and recovery of *P. submutica*.

## **Materials and methods**

### *Study species and area*

*Phacelia submutica* occurs within a 20 km radius from the town of De Beque in Garfield and Mesa Counties, Colorado (Fig. 2.1), and is distributed patchily among 9 populations (U.S. Fish and Wildlife Service 2012). Its highly fragmented and specific habitat is typified by clay barrens within a broader landscape of juniper woodlands, sagebrush flats, and sparsely vegetated desert pavements. Soils are clay-rich (>50% clay) and shallow (<50 cm deep to bedrock). Plants are prostrate growing (2-5 cm tall and 1-5 cm wide) and have several branching inflorescences originating at the base (Fig. 2.2a). Hermaphroditic flowers are 5 mm long and are white with a yellow corolla. The five anthers are contained within the corolla and surround a forked stigma (Fig. 2.2b). Flowers are arranged along the dorsal side of a helicoid cyme.

In 1944, John T. Howell described *P. submutica* as a taxonomically distinct species for a number of characteristics that differentiated it from its more common congener, *P. scopulina*. The primary characteristics are a lack or reduction in terminal apiculation of the capsule, a glabrous style, larger and more densely corrugated seeds, and the predominantly white color of its corolla (Howell 1944). Later treatment of this species by Halse (1981) reported the species to be a variety of *P. scopulina*, proclaiming insufficient evidence to identify it as a full species. However, this treatment by Halse was not considered to have enough support and, therefore, the U.S. Fish and Wildlife Service and others rely on the original description by Howell.

Populations were studied in 2011 and 2013 at five sites across the species' range (Fig. 2.1). Work was not performed in 2012 because a lack of precipitation led to germination failure across the species' range. Multiple sites were chosen to anticipate geographic variability in breeding system attributes across its range (Table 2.1). Sites were chosen for accessibility (<1 km from a road) and required larger populations (> 1000 individuals during 2011 and 2013).

#### *Floral observations*

Flowering was studied March to June in 2011 and 2013. For floral development and longevity, mature buds (n=89) were labeled with colored paint pens and tracked from anthesis to senescence. Flowering was tracked at three populations: Coon Hollow (2011, n=34 flowers), Black Hills (2013, n=34 flowers), and Sunnyside (2013, n=21 flowers). The time of flower opening, pollen release, stigma maturation, and flower senescence were observed (10X magnification). Several time-lapse video recordings of anthesis and



pollination were taken through a digital microscope (Aven 2MP ZipScope 200X magnification) connected to a field computer. A piece of semi-sheer blue fabric was draped over the microscope and plant to reduce glare. Nectar production was visually monitored with a field microscope within each flower receiving a geitonogamous or xenogamous hand-pollination treatment at Black Hills and Sunnyside in 2013 (n=40, per site). Scent was also monitored olfactorily multiple times per day every two weeks during the flowering period.

#### *Pollinator observations*

To identify potential insect pollinators and record their foraging behaviors, floral visitors were observed throughout the flowering period (March to June) at Coon Hollow and Horsethief in 2011 and Black Hills and Sunnyside in 2013. We watched for floral visitors during 30-min bouts arbitrarily selected 1-m<sup>2</sup> patches of plants within each site. Activities were observed at different times and ambient environmental conditions through the season; we watched visitors twice weekly and roughly every two hours between 7:00AM and 3:00PM, totaling about 25 hours per year at each site. Watches were cancelled in the event of precipitation or persistent high winds. We also watched for potential crepuscular and nocturnal pollinators twice in May 2013 at Sunnyside during which we performed 30-min watches at 9:00PM and 7:00AM and 5-min watches at every two hours between, for a total of 2 hours and 40 minutes.

#### *Pollination experiments*

To characterize the breeding system, hand pollination and pollinator exclusion experiments were performed in 2011 and 2013 to detect whether pollen source and

pollinator access impacted the quantity and/or quality of seeds. In 2011 the experiments were conducted at Coon Hollow and Horsethief from 5 through 26 May. At the beginning of the flowering period, 100 healthy appearing juvenile plants with developing inflorescences were haphazardly selected across occupied habitat at each site and one of the following four pollination treatments was randomly assigned to a plant (using a random sequence generator), assigning 25 plants to each treatment:

1. *Autogamy (spontaneous selfing)* – the calyx of the bud was marked with a red sharpie and the entire plant was caged.
2. *Geitonogamy (selfing)* – newly opened flowers were pollinated with fresh pollen from one other flower on the same individual. The calyx of the flower was marked with a silver permanent paint pen and the entire plant was caged.
3. *Xenogamy (outcrossing)* – newly opened flowers were pollinated with fresh pollen from one flower on a plant about 10 m away. The calyx of the flower was marked with a purple permanent paint pen and the entire plant was caged.
4. *Control* – the calyx of the bud was marked with a black sharpie and plants were left uncaged.

Three flowers per plant were treated with the same pollination treatment, with a single flower per plant being treated on a given day. On each treatment day, plants were inspected for a flower that was freshly open (indicated by the bright color of the petals, the aperture of the petal, and the light yellow color of the pollen). Plants assigned to the geitonogamy treatment had to have at least two fresh flowers while all other treatments required only one. For geitonogamous and xenogamous pollinations, a donor flower with

fresh pollen (light yellow) was clipped off and dissected open with a pair of fine-tipped forceps. Anthers were gently rubbed and held on the stigma for at least one minute, until we observed a large number of pollen grains adhering to the stigmatic surface.

Pollinations were performed between 8:00AM and 3:00PM, and each pollination was completed in about 5-min to limit pollen and stigma desiccation. Treatments were not performed on rainy or windy days. We also attempted to evenly distribute pollination treatments during the day by rotating through each of the five of treatments.

To exclude potential pollinators, entire plants were caged because individual flowers were too small, delicate, and clustered to bag singly. Cage frames were made from aluminum plumbers tape, and were covered with white fine (no-see-um gauge) polyester mesh fabric affixed to the frame with hot glue (Fig. 2.3). Plants were gently pushed through a small hole cut into the center of a ~23 x 23 cm square of mesh and the cage was placed on top of the fabric square. The loose fabric from the square was attached to the base of the cage with a rubber band. Cages were then secured to the ground with two 10-cm landscape staples. This design was expected to prevent insects from accessing the plant from above and below the cage. One week after pollination, cages were removed and treated fruits were glued shut to prevent seed loss.

In 2013, experiments were performed at Black Hills and Sunnyside from 29 April through 29 May. Several experimental methods were modified from those used in 2011. In contrast to 2011, only one flower per plant was treated and a total of 20 plants were assigned to each of five treatments. A treatment testing for agamospermy was performed in addition to the autogamous, geitonogamous, xenogamous and control treatments. For this treatment buds were emasculated with a pair of fine-tipped forceps. If the tip of the

forceps damaged any part of the style or ovary, the pollination was cancelled. After pollination, the calyx of the bud was marked and the entire plant was caged. In 2013 treated calyxes were marked with several dabs of red acrylic paint, a medium appreciably more durable than permanent marker paint.

Hand pollination methods were also modified from those used in 2011. Flowers were emasculated prior to geitonogamous and xenogamous hand-pollination treatments to provide better detection of the effect of pollen source on reproductive success. Again, damage to any part of the female reproductive structures resulted in a cancellation of that pollination. Additional measures to increase the accuracy in the timing of hand pollinations were also used. On each treatment day, plants were inspected for the presence of buds close to anthesis. To apply pollen during stigmatic receptivity, emasculated flowers were observed with a field microscope (10X) until the stigma appeared mature (exudates present on stigmatic papillae). A donor flower with recently (<10 min) dehisced anthers was clipped off and the petals removed with a pair of fine-tipped forceps to reveal the stamens.

In both study years, plants were collected from the field in June and July when they had fully senesced. Treated fruits were removed from plants and their development into a capsule was recorded (fruit set). Flowers that did not develop into fruits were extremely small and difficult to find; therefore, if a fruit was not found we assumed that the treated flower did not develop into a fruit. Flowers that had set fruit were carefully dissected, the seeds were removed, and the mature seeds were separated from undeveloped seeds. A seed was considered mature if it was inflated and dark-colored while an undeveloped seed was not inflated, sliver-shaped, or tan and brittle. Mature

seeds from each treated fruit were counted to quantify seed set then weighed together on an electronic analytical scale with a resolution of 0.0001g. The weight was then divided by the number of seeds to estimate the mean mature seed weight. Undeveloped seeds were counted but not weighed since they were too light to register on the scale. Mature seeds were placed into coin envelopes and stored in dry conditions at room temperature (approximately 19°C).

#### *Germination experiments*

Germination experiments were conducted to assess differences in seed viability and seedling vigor between autogamous, geitonogamous, and xenogamous pollen sources. In February 2014, seeds from treated fruits containing more than six seeds at Sunnyside and Black Hills were taken out of dry storage. A total of 535 seeds from 65 plants were used in this study. Seeds from each fruit remained together to ensure a mixed model could be used to analyze treatment effects with seed as the replicating factor and plant as a random effects factor. Seeds were sterilized using a modified *P. submutica*-specific protocol developed by Colorado State University (L. Tembrock, unpublished protocol). Seeds were washed in 70% ethanol, rinsed with autoclaved water twice, immersed in a 15% bleach solution for 20 min (hand-shaken every 5 min), and rinsed another five times with autoclaved water. Seeds from each fruit were placed in a vial with 7 ml of autoclaved water and stored at 4°C for 14 days. After this period of imbibing, seeds from the same plant were plated onto 60 mm Petri dishes with 20 mL of 0.7% phytoagar and dark-cold stratified at 4°C for 30 days. Seeds were then incubated at 23°C with 14 hours daylight/10 hours dark cycle. Once cotyledons were observed,

germinants were transferred onto 60 mm Petri dishes with 200 ml of autoclaved nutrient growth medium containing agar and ½ Murashige & Skoog (MS) nutrient formulation (growing plates) (Murashige & Skoog 1962). Growth was observed and recorded for two weeks.

#### *Pollen and ovule production*

An estimation of the breeding system and the degree of autogamy in flowering plants can be provided by the pollen-to-ovule (P:O) ratio and the Outcrossing Index (OI) (Cruden 1977). In 2013, four flowers from each of two populations (Black Hills and Sunnyside) were collected prior to anthesis and fixed in 70% ethanol. Several weeks later, each flower was dissected in de-ionized water to remove and separate the five anthers and the ovary. Intact immature anthers were removed from their filament and mounted on a glass slide with a drop of 50% glycerin. Using a light microscope at 30X magnification affixed with a digital camera, photos of each of the five anthers were taken so that pollen grains could be counted at a later date. When an anther was missing after dissection, the mean of the other 4 anthers was used to estimate the number of pollen grains for the fifth. Aborted pollen grains were not included in the analysis. Ovaries were dissected in a glass dish with several drops of de-ionized water and ovules were counted under a dissection microscope with 3X magnification.

The OI was calculated as the sum of values for several morphological and developmental traits as assigned by Cruden (1977). These traits included the diameter of the corolla aperture, the temporal development of male and female reproductive functions (dichogamy), and their spatial relationship within a flower (herkogamy). Each class of a

trait is represented by a numeric value (Table 2.2) and their sum serves to estimate the breeding system of the species.

#### *Pollen tube growth experiments*

To test for the relative success of pollen source on pollen tube development, autogamous, geitonogamous, and xenogamous hand pollinations were performed on 31 May 2013 at Sunnyside between 8:00AM and 9:00AM and at Plateau Creek between 9:30AM and 11:00AM. At each site, 12 plants were haphazardly selected and the three pollination treatments were assigned to four plants. One flower on each plant received their designated pollination. Pollination for these three treatments followed the methods developed for the 2013 pollination experiments. Additionally, one bud per site was emasculated and caged to serve as a control. Each flower was collected about 6 hours after treatment and placed immediately into a vial containing 70% ethanol. In the laboratory, procedures followed the protocol of Dafni *et al.* (2005); flowers were softened with 8M sodium hydroxide for 4 hours, rinsed in distilled water, and stained for 10 hours with 0.05% aniline blue solution. Using a surgical knife, the pistil and ovary were dissected from the corolla and placed on a slide with a drop of glycerin. Slides were examined under a microscope fitted with an epifluorescent light (UAV filter A) at 30X magnification to measure the length of pollen tubes in the style, and count the number of pollen grains and tubes.

#### *Statistical analyses*

Pollination data at each site were analyzed separately to account for potential variation in reproductive quantity or quality that may be caused by potential population

differences and site environmental factors such as soil, temperature, and solar radiation.

All models developed for statistical analysis used the GLIMMIX procedure in SAS/STAT<sup>®</sup> release 12.3 for SAS<sup>®</sup> version 9.4 (SAS Institute 2013, Cary, North Carolina, USA).

The 2011 pollination experiments conformed to a completely randomized design with three subsamples per plant replicate. Fruit set for the autogamous and control treatments is presented as the proportion of flowers that set fruit for each pollination treatment. Geitonogamous and xenogamous treatments were not included because the paint used to mark the flowers was prone to wash off after a heavy rain event, resulting in an artificially low proportion of fruits set for these two treatments. Since  $\geq 75\%$  flowers set fruit regardless of treatment, a statistical analysis on treatment effect on fruit set was not performed. Differences between treatments for the response of the number of mature seeds per fruit were analyzed with a generalized linear mixed model that assumed a negative binomial distribution. In this model, plant was identified as a random effects factor, and the number of fruits was log transformed and included as a covariate (offset variable) to account for the impact of the number of fruits used to calculate the plant average. Differences between treatments for the response of the mean mature weight of seeds per fruit were analyzed with a generalized linear mixed model that included repeated measures of the three subsamples on each replicate plant and plant as a random effects factor. The model also assumed a normal distribution. The number of undeveloped seeds per fruit was not analyzed because there were too few data.

The 2013 pollination experiments conformed to a completely randomized design without subsamples. The agamospermic treatment was left out of all analyses since no



fruits were produced with this treatment. Fruit set is presented as the proportion of fruits set for each pollination treatment. Again, since  $\geq 75\%$  of flowers set fruit regardless of treatment, a statistical analysis on treatment effect was not performed. The effect of pollination treatment on the number of seeds per fruit was analyzed with a generalized linear model with a negative binomial distribution while the mean mature weight of seeds per fruit was analyzed with a generalized linear model with a normal distribution. Data for the mean mature seed weight at Black Hills were square root transformed to better meet assumptions of normality and homogeneity of variance. When treatment effects were detected, a Tukey-Kramer multiple comparison of least squares means was used to identify treatment differences between groups. The number of undeveloped seeds per fruit was not analyzed because there were too few data.

Differences in pollen tube development by pollen source at Sunnyside and Plateau Creek were tested by first assessing whether hand pollination treatments resulted in differences in pollen deposition. The number of pollen grains attached to the stigma was pooled over site and analyzed with a generalized linear model for a completely randomized design and a negative binomial distribution. A proportion was then calculated for the number of pollen tubes to the number of pollen grains. To test for significant treatment effects on pollen tube development, a one-way ANOVA for a completely randomized design was used, which included site as a fixed-effects factor and followed a normal distribution.

## Results

### *Floral observations*

Flowers opened between 7:30AM and 12:30PM, peaking between 9:30 and 10:30AM (Fig. 2.4). Flowers are protogynous; stigmatic maturity, determined by the presence of exudates, began 10 to 20 min prior to anther dehiscence. Anthers positioned directly above and adjacent to the stigma deflexed inward to completely cover the stigma during pollination. Such self-pollination was observed while flowers were still opening (aperture 1 to 2 mm wide) (Fig. 2.5) and within 2 hours of anthesis. Flowers were short-lived, remaining open during the first night but beginning to wilt near mid-day the subsequent day and were completely closed 48 hours after anthesis.

Floral development observed using the digital microscope confirmed the timing of self-pollination and the shifting positions of the anthers through time (Fig. 2.5). These recordings were initially confounded by the fabric light diffuser, which delayed the onset of anthesis. To instigate anthesis, flowers were exposed repeatedly to full sunlight so that anthesis could proceed at a normal pace.

Nectar was not observed within the corollas of any of the hand-pollinated flowers. However, plants produce a strong scent (similar to lilac), with musky overtones. We believe this odor is secreted from the glandular hairs distributed on nearly all vegetative surfaces of the plant for two reasons: (1) the odor was perceived even without any open flowers, and (2) the intensity of the odor increased as more oil was visibly secreted from the glandular trichomes during a hot day.

### *Pollinator observations*

During nearly 103 hours of observation, no insect was seen at or near to any *P. submutica* flowers or plants during either diurnal or nocturnal pollinator watches. Several unidentified solitary bee species were seen visiting nearby *Helianthus annuus* and *Grindelia fastigiata* flowers, so ambient conditions during observations was suitable for bee activity and potential pollinators were in the vicinity. Larvae of two species of noctuid moths (*Helicoverpa zea* and *Trichoplusia ni*) were collected from the leaves of *P. submutica* at the Sunnyside site in 2013. There were a large number of plants hosting these and possibly other moth larvae. It is possible that these crepuscular foraging moths visit flowers of *P. submutica* early in the spring while laying eggs on leaves. These larvae also killed an estimated 8% of mature plants at Sunnyside. Larvae targeted both fruits and leaves and encompassed entire small plants (<3 cm diameter) in webbing.

### *Pollination experiments*

A large proportion of flowers set fruit ( $\geq 75\%$ ) after autogamous, geitonogamous, xenogamous, and control pollination treatments (Table 2.3) Agamospermic treatments did not yield fruits; therefore, *P. submutica* is not able to reproduce asexually.

Pollen source and pollinator exclusion did not significantly affect the number of mature seeds per fruit at Black Hills, Coon Hollow, or Horsethief (Table 2.4, Fig. 2.6). Pollination treatment marginally influenced the number of seeds per fruit at Sunnyside in 2013 (Table 2.5, Fig. 2.6). At this site, a pairwise comparison revealed that xenogamous pollination produced marginally fewer mature seeds per fruit than autogamy ( $P=0.04$ ) treatment.

The mean mature seed weight per fruit did not differ significantly between autogamous, geitonogamous, xenogamous, and control treatments at any of the four sites (Black Hills, Coon Hollow, Horsethief, and Sunnyside) (Table 2.4, Fig. 2.7).

#### *Germination experiments*

Only two seeds germinated within two weeks of cold-moist stratification during the imbibing period. These seeds were removed from the glass vial and placed on a growing plate. During the 2-week recording period, these seedlings grew 5 cm long and developed first leaves. No other seeds germinated during the cold-moist stratification or incubation.

#### *Pollen and ovule production*

Anthers most often remained intact and pollen was easy to count when pressed under a coverslip (Fig. 2.8). However, several collected flowers could not be included in the dataset because their anthers had dehisced while in the ethanol solution. As a result, only three flowers from the Black Hills population were included in the dataset. The means and standard deviations for the numbers of pollen grains and of ovules for this population were  $1773 \pm 472$  and  $9 \pm 2$ , respectively. The P:O ratio for this population was  $190 \pm 39$ . Only a single flower contained immature anthers from the Sunnyside population. This flower produced 857 pollen grains and 11 ovules, and thus a P:O ratio of 77. A single aborted pollen grain was excluded from the analysis.

The OI calculated for *P. submutica* flowers was the sum of the following values: The corolla aperture is less than 3 mm wide (2 points); there is direct contact between stigma and anthers (no herkogamy) (0 points); and maturation of anthers and stigma are

(slightly) protogynous (0 points). This outcrossing index value of 2 indicates that the species' floral traits are consistent with breeding systems that are self-compatible and autogamous, and probably outcrossed to only a limited degree (Cruden 1977).

#### *Pollen tube growth experiments*

Pollen attached to the stigma was clearly visible under a microscope and fairly easy to quantify (Fig. 2.9a). The mean and standard deviation of pollen grains was  $22.3 \pm 9.5$ . Pollination treatment did not significantly affect the number of pollen grains attached to the stigma ( $F= 1.33$ ;  $P= 0.29$ ;  $df=2, 14$ ). Examination of the style and ovary under epifluorescent lighting revealed that pollen germination, and penetration of pollen tubes through the style, occurred for autogamous, geitonogamous, and xenogamous pollen sources (Fig. 2.9b). Unexpectedly, tubes had already penetrated the ovary and had fertilized ovules by the time of collection (six hours after pollination) (Fig. 2.9c). This precluded us from assessing prepotency by measuring the length of pollen tubes within the style. The unexpected opacity of the stylar tissue under the epifluorescent lighting, possibly from an overexposure to the Aniline blue stain, would also have likely prevented us from being able to perform this measurement (Fig. 2.9d). Of the 24 flowers treated for this experiment, only 20 were used in the dataset, since four samples were lost by dissection damage. In the analysis of variance on the proportion of pollen grains to pollen tubes in the ovary, there were no significant differences between the treatments at Sunnyside ( $F=1.26$ ;  $P=0.34$ ;  $df=2, 7$ ) or Plateau Creek ( $F=0.03$ ;  $P=0.97$ ;  $df=2, 7$ ).

## Discussion

This research provides information on the reproductive biology of *P. submutica*, a threatened species in western Colorado. The results supply strong evidence that the breeding system of the species is autogamous and that insects do not play a role in its reproduction. Anthesis occurs throughout the morning, the flowers self-pollinating within 2 hours of anthesis. . Flowers readily set fruits and seeds despite the absence of floral visitors (none seen during the two years of study). Fruit set was  $\geq 75\%$  for all treatments except those flowers whose un-dehisced anthers were surgically removed; they failed to set fruit. Similar numbers of seeds per fruit resulted from autogamous, geitonogamous, xenogamous, and control treatments at Black Hills, Coon Hollow, and Horsethief. We did not find evidence of pollinator limitation since the xenogamous hand pollination treatment did not increase fruit set or seed production per fruit compared to the open pollinated control. The P:O ratios and the OI (Cruden 1977) are consistent with an autogamous breeding system. Finally, pollen source did not have a significant effect on the proportion of pollen grains developing tubes. Self-fertilization does not appear to be influenced by this particular mechanism of cryptic self-incompatibility.

Xenogamous hand pollinations at Sunnyside yielded fewer seeds than the autogamous treatment; the difference was only marginally significant. Since xenogamous pollination typically does not reduce fruit or seed set (Lloyd 1992), we believe that these differences were a result of inconsistencies in the hand pollination methods at the Sunnyside site. The terrain at this site was more challenging to navigate than the other sites and completion of pollinations within the 5-min goal was often not possible. Xenogamous pollinations were the most difficult to perform since it required

traversing around steep and fragile terrain to obtain a donor flower at least 10 meters from the treatment plant. This greater period of time that both pollen and stigmas were exposed may have had an impact on pollen viability and the success of the treatment. Alternatively, the difference may not be real but rather due to chance.

Though we did not find differences in the quantity measures of reproductive fitness, inbreeding could still compromise seed and seedling quality through decreased viability and seedling vigor. Unfortunately, with only two germinated seeds, we could not assess the impact of pollen source on seed germination and seedling vigor. The challenge of seed dormancy in *P. submutica* will need to be resolved before land managers can propagate seeds for reintroduction.

#### *Factors influencing habitual autogamy*

*P. submutica* bears a number of morphological and developmental characteristics consistent with an autogamous breeding system. These include: (1) small, inconspicuous flowers with anthers contained within the corolla (Navarro & Guitian 2002); (2) minimal temporal separation between stigmatic maturity and anther dehiscence (homogamy) (Goodwillie *et al.* 2010); (3) minimal spatial separation between anthers and the stigma (herkogamy) (Kalisz *et al.* 2012); (4) short-lived flowers (Ashman & Schoen 1996); (5) a P:O ratio and an OI consistent with the reduced allocation to male function comparable to other autogamous species (Cruden 1977); and (6) equal competitive ability of pollen tubes of outcross- and self-pollen (though these results are inconclusive). One of these characteristics alone may be sufficient to facilitate the occurrence of self-pollination; however, the operation of several in concert in *P. submutica* ensures the habitual nature

of its autogamy. The regular contact of dehiscing anthers and the receptive stigma allows auto-pollination, while the maturation of stigmas and anthers occurring at roughly the same time (approximately 15 min apart) virtually assures self-pollination will occur before any possibility of outcrossing, even in the presence of floral visitors. Further impairing the potential success of outcrossing, the completion of self-pollination occurs before the corolla has opened fully (1-2 mm) preventing entry by all but the smallest pollinators (e.g. thrips) during an appropriate interval for cross pollination.

Evolution of the morphological and developmental traits of autogamy from previously outcrossing populations is supported in theory (Stebbins 1970; Holsinger *et al.* 1984; Goodwillie *et al.* 2005; Eckert *et al.* 2009), and is well-documented in case studies (see Baker 1967; Kalisz *et al.* 2004; Bodbyl-Roels & Kelly 2011). The transition from outcrossing to selfing is thought to be facilitated by a number of factors including: (1) environmental conditions reducing the abundance and reliability of pollinators such as short growing seasons, unsuitable pollinator habitat, few partners, and natural or anthropogenic disturbances (Yamashiro & Maki 2006); (2) fewer pollination visits to small, low-density or fragmented populations (Fiedler & Ahouse 1992; Aizen & Feinsinger 1994; Aizen *et al.* 2002; Ashman *et al.* 2004); (3) changes in pollinator movements that increase selfing rates (Honday & Jacquemyn 2007); (4) unstable or ephemeral environments that select for traits allowing rapid development and reproduction, such as smaller flowers (Jarne & Charlesworth 1993; Mazer *et al.* 2010; Sicard & Lenhard 2011); and the ability to successfully colonize new habitats in the absence of effective pollinators (Baker 1955; Cheptou 2012). During this transition, structures and functions associated with pollinator attraction are reduced or lost through



selection or drift (Mazer 1992; Ågren & Schemske 1995; Karron *et al.* 2012). The loss of traits that promote outcrossing acts to reinforce self-pollination in a population because it reduces the probability of pollinator visitation (Schoen *et al.* 1996; Kalisz *et al.* 2012).

### *Conservation implications*

We have demonstrated that *P. submutica* is autogamous. It logically follows that *P. submutica*'s abundance, demography, and genetic diversity will not be impacted by changes in the abundance, diversity, or foraging behaviors of insect pollinators resulting from habitat disturbances. Therefore, additional protections for insect pollinators and their habitat are not required to ensure the continued survival of this species. Though autogamy provides reproductive assurance in the absence of pollinators, it may still have a negative impact on reproductive fitness (through seed viability, vigor, and recruitment), as well as the long-term viability of a population through its genetic composition.

Conservation plans must consider the consequences of the breeding system on population genetics (Holsinger & Gottlieb 1991). Though we did not detect consequences of inbreeding depression through two elements of reproductive fitness (seed number and seed weight), habitual inbreeding may or may not affect other aspects of quality, such as viability and recruitment (Schemske & Lande 1985; Kearns & Inouye 1997; Larson & Barrett 2000). The production of homozygous individuals through habitual autogamy also reduces the total amount of genetic diversity within a population (Karron 1991; Herlihy & Eckert 2002). Therefore, selfing has been considered an 'evolutionary dead end' because it constrains adaptation and evolution (Lande *et al.* 1999; reviewed in Takebayashi & Morrell 2001). Climate change may pose a significant

threat to *P. submutica* if autogamy has impoverished its genetic flexibility to adapt to the predicted changes in temperature and precipitation regimes (Lande *et al.* 1999). Without genetic exchange between individuals, each population, or even patch of related individuals are in some cases extremely adapted to the particular conditions of its microsite (Levin 1972; Jain 1976; Lacy 1992; Jarne & Charlesworth 1993; Levin 2010). Preserving the range of genetic diversity present across the species range may be an important conservation strategy, but likely would require the protection of a large number of the existing populations (Neel *et al.* 2001).

Though our results indicate that pollinators and their habitats do not need protection to maintain population viability, human disturbances occurring near *P. submutica* habitat may impact reproduction, thereby impairing the survival and recovery of the species. Threats that damage or kill individuals will directly reduce reproductive outputs while disturbances to habitat integrity and function are likely to indirectly affect reproduction by altering the conditions necessary for growth and survival, such as site hydrology and pedogenesis. Development of roads and other disturbances that remove vegetation and expose bare soil will lead to the deposition of fugitive airborne dust on plant surfaces which may interfere with physiological processes such as photosynthesis and stomatal conductance (Farmer 1993; Hirano *et al.* 1995; Sharifi *et al.* 1997; Grantz *et al.* 2003; Lewis 2013), and pollen germination (Harper 1979). The maintenance of optimal reproductive rates is particularly vital to annual species that have a greater dependence on seed production than perennials (Barrett & Eckert 1990; Fischer & Matthies 1997; Wilcock & Neiland 2002). Thorough consideration of the potential

impacts on reproduction from disturbances occurring on a landscape scale will be a crucial element of this species' conservation (Spira 2001).

Moth herbivory found at the Sunnyside site may also lead to reduced reproductive outputs through plant mortality. The species of moth collected from partially eaten *P. submutica* plants at this site were *H. zea* (corn earworm) and *T. ni* (cabbage looper), two major agricultural pests common in North America. Agriculture is present within the range of the species, but is not considered to be a threat since *P. submutica* habitat is not arable. However, agricultural fields regionally may support an abundance of pest moths and promote their presence in greater numbers within natural areas. Monitoring of herbivory by larvae would allow managers to identify if these or other insect species could be a threat.

Managers should be aware that successful reproduction involves a network of ecological factors, including seed bank dynamics, dispersal, and the integrity of habitat characteristics supporting the survival of offspring to adulthood. Data describing these processes would be useful in predicting additional outcomes of disturbances and providing some of the data necessary for a population viability analysis. Genetic studies would allow managers to assess diversity within and among populations to confirm our conclusion that the breeding system is autogamous and identify the potential genetic consequences of habitual autogamy. Additionally, further experiments testing the effect of pollen source on seed quality as measured by seed viability and seedling vigor could be useful in identifying whether its reproductive strategy limit its abundance and distribution. Therefore, the funding of additional research, where appropriate, will

contribute to the knowledge of *P. submutica*, as well as to the broader collection of literature available on rare species conservation.

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**Table 2.1** Information about the *Phacelia submutica* study sites including the population name (U.S. Fish and Wildlife Service 2012) and habitat characteristics.

<b>Year of study</b>	<b>Site Name</b>	<b>Population</b>	<b>Elevation</b>	<b>Slope</b>	<b>Aspect</b>	<b>Size (m<sup>2</sup>)</b>
2011	Coon Hollow	Pyramid Rock	5470	50%	SW	773
	Horsethief	Horsethief Mountain	5940	10%	SE	1,467
2013	Black Hills	Pyramid Rock	6000	<5%	SW	290
	Plateau Creek	Anderson Gulch	5920	<5%	E	472
	Sunnyside	Horsethief Mountain	5830	<5 – 50%	SW, SE	717

**Table 2.2** The Outcrossing Index (Cruden 1977) is determined by a classification system that provides a value for each class of traits that include the width of the maximum aperture of the flower corolla (corolla width), the temporal separation between anther and stigma maturation (dichogamy), and the spatial separation between the anthers and the stigma (herkogamy).

<b>Trait</b>	<b>Class</b>	<b>Value</b>
Corolla width	<1 mm	0
	1-2 mm	1
	2-6 mm	2
	>6 mm	3
Dichogamy	No temporal separation between anther and stigma maturation (Homogamy), or stigma maturation occurs prior to anther maturation (protogyny)	0
	Anther maturation occurs prior to stigma maturation (Protandry)	1
Herkogamy	None	0
	Functionally separated	1

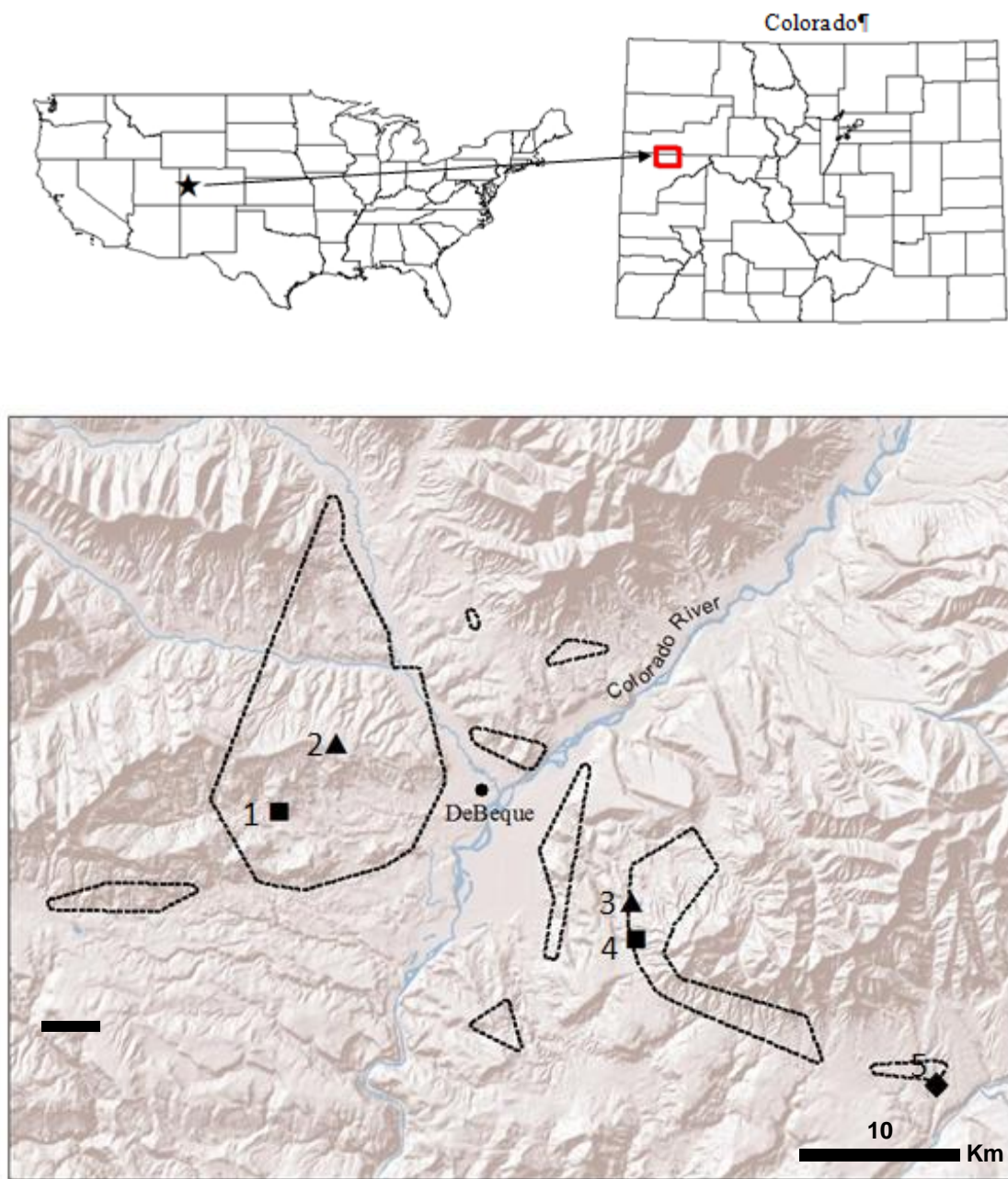
**Table 2.3** The proportion of *Phacelia submutica* flowers setting fruit by treatment and site.

<b>Year</b>	<b>Site</b>	<b>Treatment</b>	<b>Proportion Fruit Set</b>
2011	Coon Hollow	Autogamy	0.94
		Control	1.00
	Horsethief	Autogamy	0.94
		Control	0.91
2013	Black Hills	Agamospermy	0.00
		Autogamy	0.90
		Geitonogamy	0.75
		Xenogamy	0.95
		Control	0.89
	Sunnyside	Agamospermy	0.00
		Autogamy	1.00
		Geitonogamy	0.84
		Xenogamy	0.88
		Control	0.95

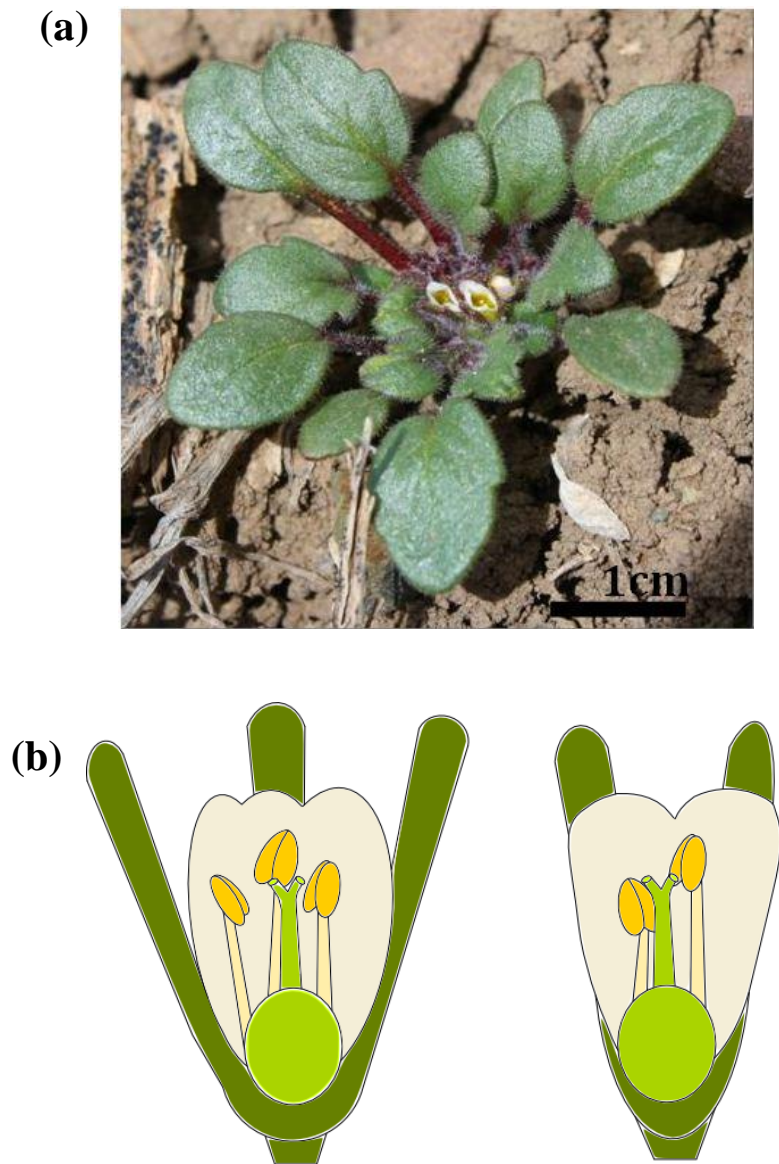


**Table 2.4** ANOVA table for the effect of pollination treatment on the number of mature seeds and mean mature seed weight per *Phacelia submutica* fruit at four study sites. Significant differences at  $\alpha = 0.05$  are in bold.

		Number Mature Seeds			Mean Mature Seed Weight		
		One-way ANOVA			One-way ANOVA		
Year	Site	df	F	P-value	df	F	P-value
2011	Coon Hollow	3,60	1.16	0.33	3,60	0.18	0.91
	Horsethief	3,61	0.23	0.87	3,61	0.84	0.48
2013	Black Hills	3,74	0.38	0.77	3,62	1.29	0.29
	Sunnyside	3,70	2.74	<b>0.049</b>	3,64	0.81	0.49



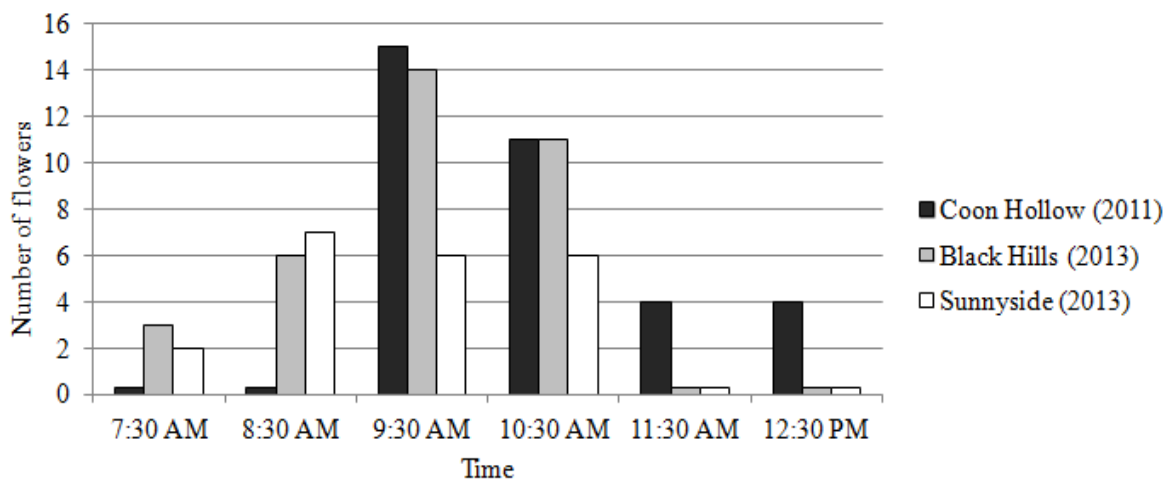
**Fig. 2.1** Distribution of *Phacelia submutica* and location of study sites (1, Coon Hollow; 2, Black Hills; 3, Sunnyside; 4, Horsethief; 5, Plateau Creek). Dashed polygons indicate areas designated as Critical Habitat in 2011. World shaded relief map at 1:50,000 scale for Mesa and Garfield Counties, Colorado, USA (ESRI, Redlands, CA, USA).



**Fig. 2.2** (a) A representative individual of *Phacelia submutica*, and (b) cross section diagram of its floral morphology.

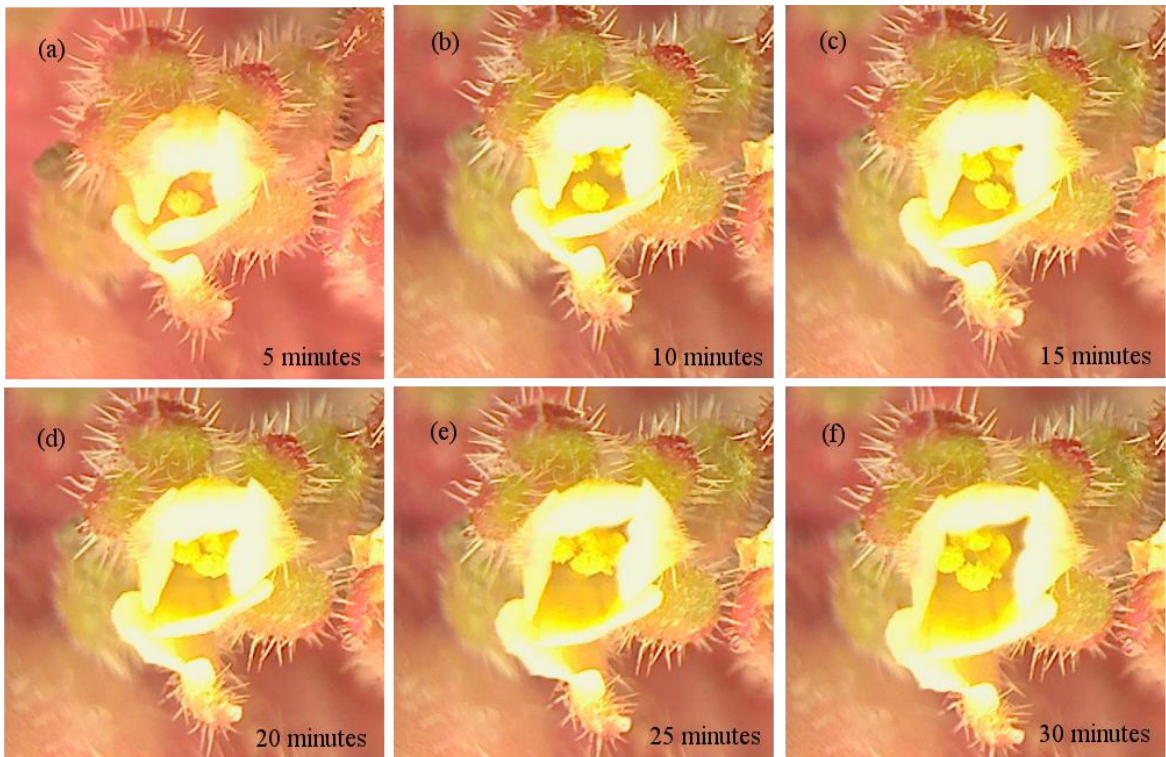


**Fig. 2.3** Cages used to exclude potential pollinators were made from aluminum plumbers tape and fine polyester mesh. *Phacelia submutica* individuals were gently pushed through a small hole in a square of mesh and the edges of the square were secured with a rubber band around the cage. This design was expected to prevent insects from entering the cage.

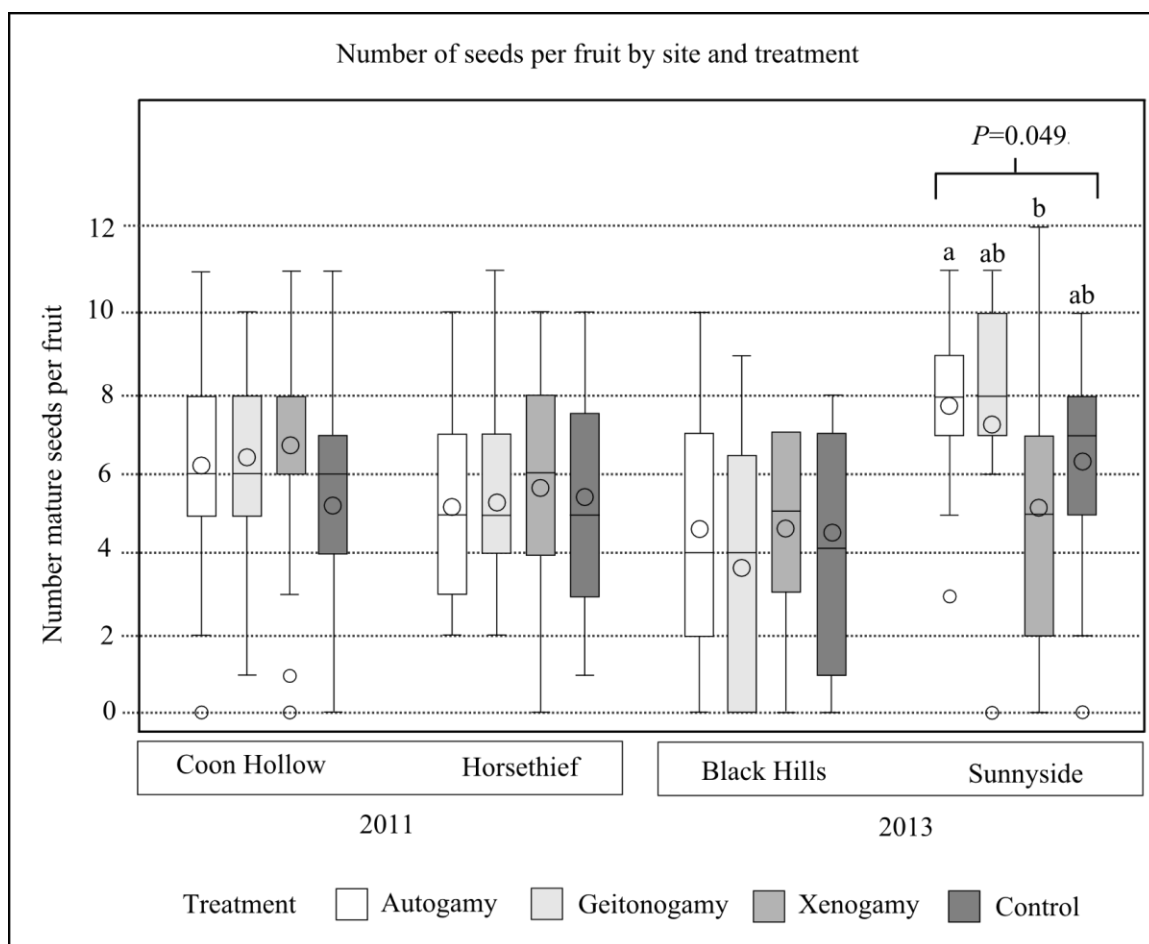


**Fig. 2.4** Frequency of anthesis of *Phacelia submutica* flowers at one hour time intervals between 7:30AM and 12:30PM at Coon Hollow, Black Hills, and Sunnyside.

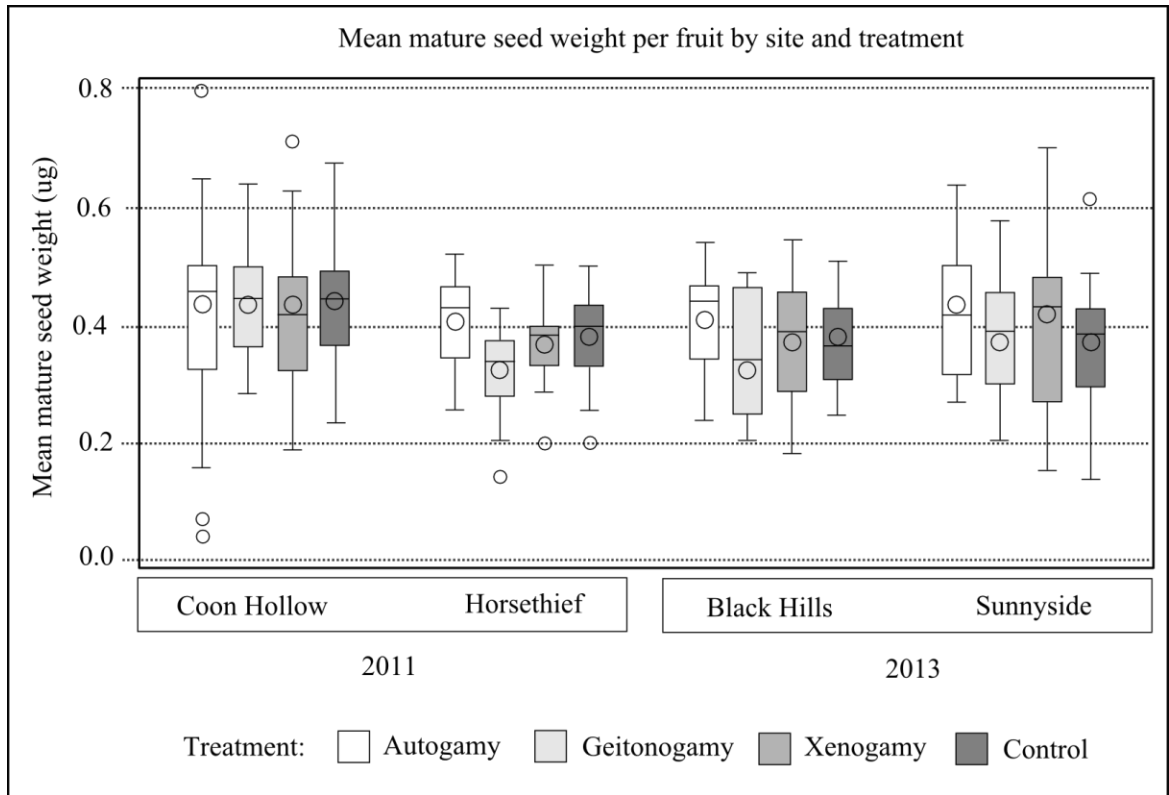




**Fig. 2.5** The process of anthesis and pollination of *Phacelia submutica* flowers, highlighting aperture of the corolla and the position of anthers within the corolla: (a) Aperture open 1 mm, anthers beginning to dehisce, (b) aperture open 1.25 mm, additional anthers can be seen, pollen still attached to anther sac, (c) aperture begins to widen further to 1.5 mm, anthers begin to deflex and surround the stigma, (d) anthers completely surround the stigma, (e) pollen begins to flake from the anther sacs, and (f) aperture opens to 2 mm, movement of anthers deposits pollen onto the stigma.



**Fig. 2.6** Number of mature seeds per *Phacelia submutica* fruit by pollination treatment (autogamy, geitonogamy, xenogamy, and uncaged control) at Coon Hollow and Horsethief in 2011 and Black Hills and Sunnyside in 2013. Large circles indicate mean; horizontal lines, medians; enclosed rectangles, interquartile range (IQR); whiskers, 1.5 times IQR; and small circles, outlier data points. Significant differences detected are presented above the appropriate treatments in bold. Treatments with different letters are significantly different according to Tukey-Kramer multiple comparison of least square means ( $\alpha = 0.05$ ).

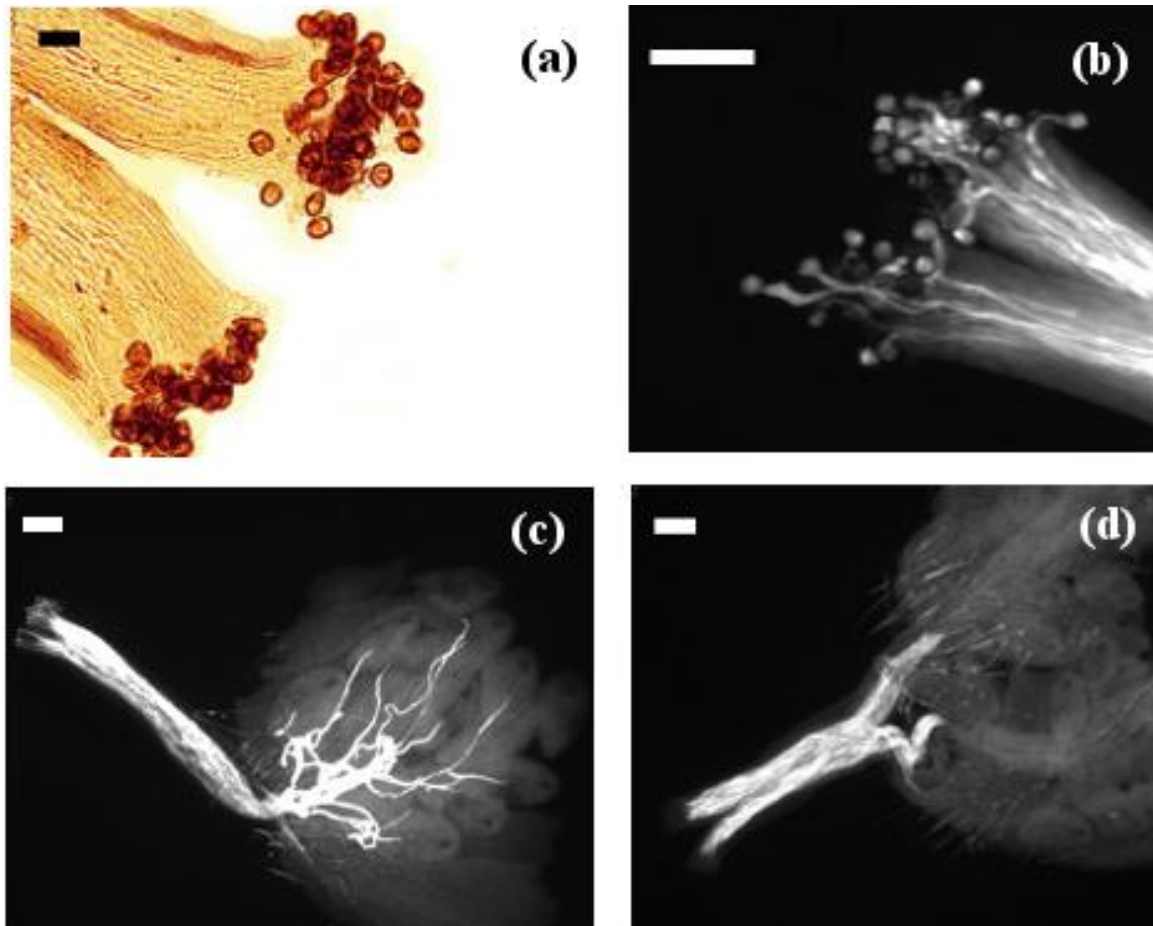


**Fig. 2.7** Mean mature seed weight per *Phacelia submutica* fruit by pollination treatment (autogamy, geitonogamy, xenogamy, and uncaged control) at Coon Hollow and Horsethief in 2011 and Black Hills and Sunnyside in 2013. Large circles indicate mean; horizontal lines, medians; enclosed rectangles, interquartile range (IQR); whiskers, 1.5 times IQR; and small circles, outlier data points.





**Fig.2.8** An immature anther collected from a flower of *Phacelia submutica* and photographed under a light microscope (30X).



**Fig. 2.9.** *Phacelia submutica* pollen tubes were observed using fluorescent microscopy: (a) pollen grains attached to the stigma, (b) pollen tube development at the stigmatic surface and into the style following an autogamous pollination, (c) pollen tube development into the ovary following a geitonogamous pollination, (d) emasculated flower showing opacity of aniline blue staining of stylar tissues. Scale bars, 100  $\mu\text{m}$ .

## CHAPTER 3

THE SEED ECOLOGY OF *PHACELIA SUBMUTICA* (BORAGINACEAE), A  
THREATENED SPECIES IN WESTERN COLORADO**Abstract**

We investigated the seed ecology of *Phacelia submutica*, a threatened endemic annual species in western Colorado, USA. Seeds have no mechanism for long-range dispersal and are dispersed directly below the mother plant between July and September. A network of 2-5 cm cracks that develop as the soil dries appears to constrain secondary seed dispersal. We did not observe post-dispersal seed predation. The estimated density of the seed bank (consisting of both the fresh seed rain and the persistent seed bank) at four sites was low ( $74 \pm 10$  seeds per  $m^2$ ; mean and standard deviation) and seeds were spatially aggregated ( $0.58 \pm 0.08$ ; mean and standard deviation, Morisita index). Seed bank samples also included a high density of other species, including several non-native invasive weed species. Seed longevity was estimated by experimental seed burial at three sites and at two depths (just below the surface and at 5 cm). Seeds recovered 1, 2, and 3 years post-burial revealed that depletion was significantly affected by year and year\*depth interaction. Depth was only significant at one site, where deeper buried seeds (5 cm) depleted at a faster rate than seeds buried at the surface (~3 mm). Logistic seed depletion curves predicted seed longevity from four to six years. Few seeds germinated from 71 combinations of cold-moist stratification periods, incubation temperatures, scarification methods, imbibing, GA3, and heat pre-treatments. Seed traits including a lack of long-range dispersal, a persistent seed bank, and a narrow range of germination

conditions suggest that management strategies must include protections for the seed bank and the dynamics involved in its development and maintenance.

## **Introduction**

The ecology of seeds can significantly influence whether a species is rare or common (Eriksson 1996), as well as how prone it is to extinction (Wilson *et al.* 2004; Hawkins *et al.* 2011). Seed characteristics such as dispersal mechanisms, dormancy, germination requirements, and inherent longevity mediate the development and maintenance of a seed bank, as well as its distribution through time and space (Jensen 2004). These attributes shape the dynamics of the seed bank and control survival at the scale of the microsite, population, or the species (Murray *et al.* 2002). Therefore, research on the ecology of a species' seeds provides valuable insight into its conservation and recovery (Hölzel & Otte 2004; Satterthwaite *et al.* 2007; Megill *et al.* 2011).

A seed is an inconspicuous stage in a plant's life that affords a population with the capacity to regenerate after the natural senescence of adult plants or stochastic removal from disease, disturbance, or herbivory (Menges 2000; Adams *et al.* 2005). A seed bank (also known as a seed pool) is a repository of viable, un-germinated seeds present in the soil, aboveground litter, or attached to plants (aerial seed bank) (Roberts 1981; Baker 1989; Baskin & Baskin 2001). Seed banks can serve a variety of functions, which, in part, depends on the life history of a species (Thompson & Grime 1979). For annual species, a seed bank is especially vital because the species is unable to rely upon other life stages for survival (Bartolome 1979; Chesson & Case 1986; Venable & Brown 1988). Additionally, for species surviving in climates that are characterized by infrequent

or unpredictable precipitation, ephemerally available habitats, or other types of disturbances that may lead to complete seedling or adult mortality in a given year, a seed bank is essential (Slatkin 1974; Guttermann 2000; Adams *et al.* 2005).

Systems of classification have been developed to describe the longevity and, therefore, function of seed banks (Thompson & Grime 1979; Thompson 1992; Poschlod & Jackel 1993; Bakker *et al.* 1996; Walck *et al.* 2005). For the sake of parsimony, transient versus persistent classifications are frequently used in the characterization of a seed bank. A transient seed bank is one where seeds are unable to survive in the soil for more than one growing season. Persistent seed banks, on the other hand, are formed when seeds are able to germinate after more than one growing season (Thompson 2000).

In order for a persistent seed bank to function, seeds must retain their viability and be able to germinate. Seed mortality is common from biotic factors, such as attack by pathogens and predation by a variety of animals (Chambers & MacMahon 1994; Chee-Sanford *et al.* 2006). Seeds also naturally age, and the longer a seed remains in the soil, the greater the probability that it will lose its viability from a failure of enzyme reactions to convey resources or repair DNA (Long *et al.* 2014). Abiotic losses of seeds, including deep burial, physical damage, and flooding, further reduce seed survival. These multiple causes of mortality are a major component of the “environmental sieve” that will eliminate a portion of the seed bank (Harper 1977). Even if a seed escapes mortality, environmental conditions may continue to prevent a seed from germinating if they fail to provide the necessary conditions for seed germination, such as inadequate moisture and oxygen, or suitable temperatures (Baskin & Baskin 2001). Even when these conditions are met, a seed may still not germinate if it is dormant.

Dormancy is an internal or external condition of the seed that prevents germination even when provided adequate light, water, oxygen, and temperature (Vleeshouwers *et al.* 1995; Benech-Arnold *et al.* 2000). Seeds can possess physical, physiological, morphological, or a combination of mechanisms that influence a seeds' germination requirements (Baskin & Baskin 2004). The most effective dormancy strategies involve environmental cues that indicate the availability of favorable germination conditions (Baskin & Baskin 1985). Variability among seeds in response to dormancy-breaking requirements can also drive the dispersal of germination through time, thereby minimizing the risk to any one cohort of seeds from climatic variability, primarily ephemeral moisture availability in arid climates (Walck *et al.* 2005).

Whereas dispersal in time limits the range of climatic conditions a seed faces upon germination, dispersal in space controls the abiotic and biotic conditions that the seed and its subsequent life stages face over its lifetime (Stebbins 1971; Schupp & Fuentes 1995). Common dispersal agents include animals, water, wind, explosive dehiscence, and gravity (Howe & Smallwood 1982). Dispersal away from the mother is necessary for the colonization of new sites and expansion of ranges, and can also aid in the escape from sibling competition (Bakker *et al.* 1996; Nathan & Muller-Landau 2000). On the other hand, dispersal of seeds far from the mother increases the risk of arrival in a site less suitable for germination, growth, and survival (Willson & Traveset 2000). The benefit of any dispersal strategy will be determined by a seed's ability to germinate and establish, as well as the interaction of the seed with the environment at a given microsite (Schupp 1995, Schupp and Funetes 1995). Ultimately, since dispersal controls the fate of

a seed, it is the key unifying factor of seed bank dynamics and population viability (Schupp 1993; Schupp & Fuentes 1995, Nathan & Muller-Landau 2000).

Knowledge of seed ecology and the dynamics of the seed bank are integral to the conservation and recovery of rare species. Seed bank studies have been used to inform management practices (Jacquemyn *et al.* 2011), identify conservation goals (Segura *et al.* 2014), predict the long-term fates of populations (Pavlik *et al.* 1993; Aparicio & Guisande 1997; Satterthwaite *et al.* 2007), and assess the potential success of *ex situ* conservation (Farnsworth *et al.* 2006). Seed bank information is also an essential demographic parameter in population viability analysis (PVA) models (Menges 2000; Doak *et al.* 2002). By studying the ecological processes that contribute to the maintenance of the seed bank, conservation practitioners can also deduce how anthropogenic disturbances can interrupt these processes and influence a species' abundance and persistence.

Our study species is *Phacelia submutica*, a rare annual that is endemic to a high desert ecosystem of the Colorado Plateau in western Colorado that is threatened by anthropogenic disturbances to its habitat. We report elements of the species' seed ecology, including observations of dispersal, the spatial distribution and density of the seed bank, the longevity of seeds in the soil through experimental burial, and germination requirements. Information about the ecology of *P. submutica*'s seeds will lead us to a better understanding of the factors that may influence its rarity and strategy for survival, which are important components of conservation and management plans.

## Materials and methods

### *Study species and area*

*Phacelia submutica* is a small, ephemeral annual species in the family Boraginaceae, formerly Hydrophyllaceae. Seedling emergence occurs between late March and May and adult plants reproduce between April and June (Langton A., unpublished data). Its germination is characterized by high year-to-year variability, resulting in aboveground populations fluctuating between thousands of plants some years and near zero in others (CNHP 2012) likely due to variation in precipitation (O’Kane 1987). Fecundity is also variable from year to year and seems to depend on moisture availability during the growing season (Burt & Spackman 1995). Fresh, mature seeds are 1 to 2 mm in length, weigh between 0.3 and 0.6  $\mu\text{g}$ , are corrugated, and covered in an iridescent coating (Fig. 3.1). Fruits contain between 6 and 12 seeds that are distributed somewhat unequally between the two chambers of the ovary (Langton A., unpublished data).

*P. submutica* is endemic to a high desert ecosystem containing juniper woodlands, sagebrush flats, and sparsely vegetated desert pavements. Its narrow range is located within a 20 km radius from the town of De Beque in the southern portion of the Piceance Basin, a discrete geologic structural basin bounded by uplifts and located in the northeast part of the Colorado Plateau physiographic province (Fig. 3.2a). The topography within the basin is diverse, with high mesas, mountains, low drainages and rolling hills. The species’ edaphic habitat is described as barren, heavy clay exposures of the Wasatch formation (O’Kane 1987) on moderately steep slopes, benches, and ridges. Soils are



clay-rich (>50% clay) and shallow (<50 cm deep to bedrock) with a cracking, self-mulching surface when dry. Elevation, geology, and soil appear to be important factors that constrain the species' distribution (Fig. 3.2b).

The climate is semi-arid with wide seasonal and diurnal temperature ranges. Mean temperatures for De Beque are -4.8°C in January and 21.1°C in July and the mean annual temperature for the period of record is 8.2°C (1959 to 2013; Altenbern, CO, Utah Climate Center 2014). Mean annual precipitation is 421.0 mm, with a minimum of 240.03 and maximum of 614.12 mm (in 2012 and 1985, respectively) (1959 to 2013; Altenbern, CO, Utah Climate Center 2014). Winter months (December to February) deliver a large amount of moisture in the form of snow and sleet. However, most precipitation occurs during May and August, in the form of high-intensity, short-duration monsoonal thunderstorms (based on averages from 1959 to 2013; Altenbern, CO, Utah Climate Center 2014).

#### *Field observations*

Dispersal mechanisms and patterns of seed rain of *P. submutica* were observed between 2011 and 2013 at Black Hills, Coon Hollow, Horsethief, and Sunnyside (Fig. 3.2b). The species was not observed extensively in 2012 because very few plants germinated. Chambers and MacMahon (1994) describe two phases of dispersal that contribute to the spatial distribution of seeds. Phase I (primary) dispersal is the pattern of seed rain that ends the moment the seed reaches the ground while Phase II (secondary) dispersal refers to the horizontal and vertical movements of seeds after the seed reaches the soil surface, and continues until germination. Observations of primary dispersal

included the timing of dispersal and the pattern of seed rain. Inferences about Phase II dispersal mechanisms are based on casual observations between June and October—the period of time when seeds are actively shed through the beginning of soil moisture accumulation, which makes visits to the clayey habitat difficult. The condition of the soil surface during this period of time was described because surface roughness and cracking depth can influence the movement and capture of seeds (Chambers & MacMahon 1994). Informal observations for secondary seed movements by animals were performed during the period of dispersal seeking to detect seed predation or transport.

#### *Seed bank sampling*

Two sites with historically large and two with historically small populations were chosen to sample the seed bank at various locations across its range. The two sites with large populations were at Horsethief and Plateau Creek I, whereas small populations were at Motor Hollow and Plateau Creek II (see Fig. 3.2b). These sites were not chosen at random, but rather were identified by their accessibility and lack of known prior disturbances (primarily cattle trampling and off-highway vehicle recreation). They were located greater than 100 meters from a road. Field sampling was performed in August 2011 so that seed bank samples could contain both the fresh seed rain and a persistent component including seeds from prior years. At each site, three parallel 25-m transects spaced 2 m apart were laid out. A 25 cm x 25 cm sampling frame was centered at each meter along each transect, starting at meter 1 and ending at meter 25. Using a 6 cm diameter x 4 cm deep core (soil tin) we collected soils samples from the four corners and the center of the sampling frame and combined the five samples in a 1 gallon plastic bag

as a composite sample. This resulted in 75 composite soil samples per site. Sample bags were opened to dry out and were stored at ambient room temperature in darkness (approximately 19°C) until samples were processed in December 2011. Soil samples were granulated using a rubber mallet and thoroughly homogenized in a plastic bucket by hand. Rock fragments and most organic material (stems, sticks, and leaves) were removed by sieving through a 2 mm sieve. Since soil properties at each sampling point affected the volume of soil collected, the volume of each sample was measured. Samples contained between 225 mL and 450 mL (1 to 2 cups) of soil.

#### *Seed bank analysis*

To provide a more comprehensive estimate of seed bank density and dispersion we used a combination of both extraction and emergence methods of seed quantification (Conn et al. 1984). First, ½ cup (118.3 mL<sup>3</sup>) of soil was removed from the bulk sample for extraction by sieving. Samples were placed in a 0.25 mm mesh metal sieve and running water was used to remove clay, silt, and fine sand particles. What remained of the sample was rinsed out of the sieve and oven dried at 32°C until samples were dry. Dried samples were spread out over a dry paper towel and, using a magnifying glass (4X), *P. submutica* seeds were removed and counted. The total number of other seeds found in the seed bank sample was also recorded. Viability is often assessed for seeds extracted by this count method. Tetrazolium is often used to determine viability, but it is not a reliable test to distinguish between dormant and inviable seeds, especially in small seeds (Went 1961). In addition, we were unable to develop a reliable germination

protocol despite rigorous testing (see results). Therefore, we considered inflated and undamaged seeds to be viable.

To quantify the germinable seed bank, another ½ cup sub-sample of soil from each composite sample was tied into a fine polyester mesh ‘pouch.’ Pouches were immersed in cool tap water for 2 min until the sample was fully saturated. Excess water was allowed to drain freely for 10 min on a corrugated plastic tray. Soil samples were cold-moist stratified at 4°C in a dark refrigerator for 4 weeks. Individual pouches were placed on top of a 7.6 cm x 7.6 cm pot containing a mixture of perlite and granulated Oasis® hydrocube synthetic foam at a ratio of 1:3. Pouches were opened and the soil was spread out with a metal spoon over the surface of the perlite and foam mixture. The final depth of the soil was approximately 2 cm. Pots were then set in an impermeable plastic tray and automatically watered twice a day for 4 min to maintain a depth of 3.8 cm of water in the tray. This grow-out protocol was designed to maintain a moisture level close to field capacity, the natural soil conditions during the germination period. Emergence was monitored for one month, and the number of seedlings germinating from the samples was recorded by species.

### *Seed longevity*

Experimental burial of seeds yields information about the upper limit of seed longevity under semi-natural conditions (Burnside *et al.* 1996; Hill & Kloet 2005). To experimentally evaluate the decay of the seed bank through time due to germination or mortality, a seven-year field study was initiated in August 2011 at three sites, Black Hills, Coon Hollow, and Horsethief (Fig. 3.2b). The experiment followed a randomized block

design with four replicate blocks per site. To assess the impact of depth of burial on seed fate, seed envelopes were buried at two depths within a replicate block. Shallow envelopes were at the soil surface with only a thin layer of hand granulated soil (~3 mm) covering them while deep envelopes were buried under 5 cm of soil.

Seeds used for this study were collected in August 2010 from Black Hills, Coon Hollow, and Horsethief because they each contained greater than 1000 live plants. An estimated 10% or less of the seed crop at each site was collected, following Center for Plant Conservation (1991) guidelines. Seeds were then sent to the Denver Botanic Gardens for cleaning. The number of seeds collected from a site varied between an estimated 2,000 and 13,000. To ensure that we were able to collect one envelope per depth at each of four replicate blocks, as well as extend the longevity study to seven years (until 2018), the design differed slightly between sites based on seed availability (Table 3.1): (1) At Coon Hollow, only shallow burial was evaluated because seeds collected from this site were relatively few; and (2) two collection years will be excluded at Black Hills and Coon Hollow (2015 and 2017).

Experimental units were made from a 7.6 cm x 15.2 cm piece of 50 mesh (0.0009 gauge) stainless steel woven wire cloth which were then folded in half to create a 7.6 cm x 7.6 cm pre-envelope. Pre-envelopes for the Horsethief and Black Hills sites were filled with 100 healthy appearing seeds while the smaller number of seeds collected at Coon Hollow limited us to 75 seeds. The three open edges of the pre-envelopes were folded over (about 1 cm) and edges were sealed with ground clips to create an envelope.

Envelopes were installed in the field on 1 and 2 August 2011. The four replicate blocks were spaced approximately 5 m apart, and envelopes were installed in a grid with

0.5 m spacing within each replicate block (Table 3.1). For the two sites (Black Hills and Horsethief) with sufficient seeds to evaluate two depths, depth treatment of an envelope was selected with the flip of a coin. Each envelope was secured in the ground with two 10 cm metal landscaping staples and marked with a surveyor flag and a numbered poultry band. Rebar was hammered into the soil at the upper left corner of each replicate for permanent marking.

Our study reports only the first three years of seed longevity results; analyses on the data for the full seven years will be performed at a future date. In August 2012, 2013, and 2014, one envelope per depth per replicate block was harvested at each site. Shortly after collection, envelopes were carefully opened over a fine mesh sieve (0.2 mm) and washed until seeds were clean. As with the seed bank extraction study, seeds that were firm and inflated were considered viable. Viable seeds were counted and placed into a coin envelope for dry storage.

### *Germination trials*

Experiments were performed in 2011 and 2012 to test for effects of cold-moist stratification, temperature, and scarification on germination. *P. submutica* seeds were collected from the Horsethief and Coon Hollow populations after plants had senesced in June of 2010 and 2011, respectively. Seeds were stored at room temperature in coin envelopes until used; at least 3 months of dry storage occurred to provide enough time for after-ripening. A total of 71 experimental trials were performed using non-factorial combinations of conditions including: (1) 0, 1, 2, and 3 months stratification periods at 4°C in the dark; (2) light (abraded for 5 seconds with light pressure) or heavy (abraded

for 5 seconds with heavy pressure) scarification with fine sandpaper (400 grit); (3) imbibing at room temperature in deionized water for 12 hours prior to cold-moist stratification; (4) nicking the dorsal side of the seed coat to the endosperm with a sterilized surgical knife (scarification); (5) incubation in a growth chamber with a 12h temperature cycle at 24, 10-20, 3-20, or 30°C, and providing 12h daily fluorescent lighting; (6) two incubation media; nursery soil in a growth tray or Anchor steel blue seed germination blotters in 60mm Petri dishes; (7) imbibing in Gibberellic acid at a concentration of 100 ppm and applying the solution to the plates once a day for three days; and (8) heating of seeds in a convection oven at 40°C for 60 min to simulate the approximate temperature that seeds may experience in the soil during after-ripening. Additionally, one experiment using seeds remaining dormant after a germination trial was performed to assess whether seeds needed a ‘second season’ of incubation to be released from dormancy. All experimental trials used 100 seeds separated into four replicates of 25 seeds. For trials that used potting soil as a growth medium, soil was a mixture of peat moss, vermiculite, and perlite at a ratio of 1:1:1, and growth trays were watered by hand daily with a fine spray mist until moist. Germination blotters in the Petri plates were moistened once a week during cold-moist stratification and every other day during incubation with deionized water from a mister bottle, and excess water was drained from the plate. During cold-moist stratification, each plate was monitored once a week for fungal growth and infected seeds were removed. During incubation, seeds were monitored every other day and germinated seeds were counted and removed. A seed was considered germinated upon the emergence of the radicle.

*Statistical analyses*

The density of the seed bank was estimated by first calculating the average volume of soil samples at each site. The number of seeds recovered from each ½ cup (118.3 cm<sup>3</sup>) subsample from the extraction method was then scaled to the average volume of soil samples collected at each site. This calculation should reduce biases created by a large range (approximately 150 mL to 200 mL) in soil collection volumes between samples. To estimate the density per m<sup>2</sup>, the scaled number of seeds per sample was then multiplied by 70.77 (a composite sample of 5 cores covered 141.37 cm<sup>2</sup> or 0.014m<sup>2</sup>). For each site, the standardized Morisita's index of dispersion (Smith-Gill 1975) was calculated from the number of seeds extracted from soil samples to test for a random distribution within a 95% confidence interval, which provides a measure of seed aggregation.

Our analyses of seed longevity included models testing the effect of year on the log transformed proportion of viable seeds remaining in the envelopes (the response) at Black Hills, Coon Hollow, and Horsethief. Sites with both shallow and deep replicates (Black Hills and Horsethief) were also analyzed for the effect of depth and year\*depth interaction on the response. Each site was analyzed separately in a random coefficients model following a randomized block design. This model fitted a regression to each replicate block and allowed intercepts and slopes to be variable. To adjust for over-dispersion, the variance among observations (envelopes) was estimated and incorporated into the models developed for Coon Hollow and Horsethief. Site differences were also analyzed using a random coefficients model with the above parameters, but the model instead tested for the effect of site, year and the site\*year interaction at the shallow depth.



The same model was again used to test for site differences that included depth at Black Hills and Horsethief. To estimate the potential longevity of seeds extending past 2014 (our last year of data) logistic regression curves of the predicted proportion of viable seeds were plotted for each site\*depth combination for the time period between 2011 and 2018. All models used the GLIMMIX procedure in SAS/STAT<sup>®</sup> release 12.3 for SAS<sup>®</sup> version 9.4 (SAS Institute 2013).

## **Results**

### *Field observations*

The bulk of seed dispersal occurred between July and September in 2011 and 2013. Dispersal was not observed in 2012 because no *P. submutica* established at any of the study sites. Primary dispersal is most often achieved upon the dehiscence of the fruit capsule along the suture line that is perpendicular to the replum. Early formed capsules shed their seeds earlier in the summer than later formed capsules. Capsules that had been coated in soil after a heavy rain event, however, were unable to release their seeds by September. Upon several additional rain events, these dehydrated plants eventually disappeared, and were either subsumed into the soil or blown away.

During dispersal, soils were most often extremely dry and the surface exhibited a dense network of cracks. Occasionally, convective thunderstorms saturated the clay soils, causing them to swell and become very sticky, but high temperatures and winds dried out the soils within a few days and returned them to a cracked and barren habitat. The plant's low stature likely prevents significant wind-borne dispersal of seeds and the high degree of surface roughness, including cracks, capture seeds, likely greatly limiting

Phase II dispersal. These cracks are usually 2-4 cm deep but occasionally deeper, and 0.5-1.5 cm wide (Langton A., unpublished data). We did not observe potential seed predators interacting with *P. submutica* or other species' seeds at the study sites.

#### *Seed bank density*

The extraction method yielded a total of 106 *P. submutica* seeds from the four sampling sites (Table 3.2; Fig. 3.3-3.6). The number of seeds extracted from any individual sample ranged from 0-15; 84% of samples contained zero seeds (Table 3.2). Seeds were spatially aggregated at all four sites based on the Standardized Morisita Index (Table 3.2). Depending on the site, seeds of other species were one to two orders of magnitude more abundant than seeds of *P. submutica* (Table 3.2).

The germination protocol for the seed bank samples did not result in the emergence of any *P. submutica* seedlings. However, an abundance of other species emerged from the seed bank. These species were *Bromus tectorum*, *Monolepis nuttaliana*, *Lappula occidentalis*, *Ceratocephala testiculata*, *Erymopyrum triticeum*, *Lepidium perfoliatum*, *Schoenocrombe linifolium*, *Erodium cicutarium*, and *Achnatherum hymenoides*; several are nonnative invasive weeds (Table 3.3). The number of seedlings that emerged for each species varied between sites, but 85% of all samples had at least one seedling. The species with the most emergence were *Erymopyrum triticeum* (annual wheatgrass) and *Bromus tectorum* (cheatgrass), two nonnative invasive weeds. Horsethief had the highest number of seedlings, most of which were annual wheatgrass. We expect that seeds of native plants such as *Sarcobatus vermiculatus* (greasewood), *Atriplex confertifolia* (shadscale) and *Artemisia tridentata* (sagebrush), three species

dominant at the edges and sparsely within occupied *P. submutica* habitat, are within these seed bank samples. Perhaps these species' seeds form only transient seed banks. Or, perhaps, like *P. submutica*, our germination protocol did not meet these species' dormancy-breaking and/or germination requirements.

### *Seed longevity*

The proportion of viable seeds remaining was significantly affected by year at all sites (Table 3.4; Fig. 3.7). In the first year of collection (2012) a high proportion of seeds were still present and viable in the envelopes at all sites ( $94.5 \pm 5.5$ ; mean and standard deviation). The second year of collection (2013) showed a large decrease in the proportion of seeds remaining viable ( $52.7 \pm 35.1$ ). By the third year after burial (2014), few seeds were still present and viable in the envelopes; the mean number of viable seeds was 24.0, and five envelopes were completely depleted. However, the standard deviation of the replicates (24.2) was greater than the mean.

The difference between deep and shallow buried bags was not significant in any year at Black Hills and Horsethief, the two sites with both depth levels. However, the analysis of variance detected significant year\*depth interactions at Black Hills (Table 3.4; Fig. 3.7). At this site, the slope of the regression for deeper buried envelopes was steeper than shallow buried envelopes.

Based on the projected years to full depletion, seed longevity is estimated to be from four to six years (Table 3.5). The logistic regression slope of the depletion trajectory and the projected year of full depletion varied between sites and between depths (Table 3.5; Fig. 3.8), but was not significant: There were no significant differences

between the Black Hills, Coon Hollow, and Horsethief sites for the shallow burial samples ( $F= 0.22$ ;  $P= 0.81$ ;  $df= 2, 21$ ) nor for the deep burial samples at Black Hills and Horsethief ( $F= 0.43$ ;  $P=0.52$ ;  $df= 1, 13$ ).

### *Germination trials*

A total of 41 of the 7,100 seeds used in the study germinated, and very few seeds germinated from any one trial (Table 3.6). Due to the extremely small sample sizes germination experiments could not be statistically analyzed.

Optimal germination temperature is unclear. Seeds did not respond differently to incubation temperatures, including alternating periods of 3 to 20°C and 10 to 20°C, the approximate low and high temperatures during March and April, respectively, near De Beque.

Although most germination during incubation took place at 24°C, these results would be biased because 69% of seeds were incubated at that temperature. In addition, higher temperatures appear detrimental in that seeds that produced radicles tended to damp off more readily. Many seeds at the 30°C temperatures rotted within 1 week after the initiation of incubation.

Seeds appeared to benefit, at least marginally, from a period of cold-moist stratification. A total of 24 seeds appeared to respond to a period of stratification (59% of seeds that germinated). Unexpectedly, nine of these seeds germinated on the blotter paper while in cold stratification conditions. These seeds germinated sporadically between 2 and 3 months of cold stratification, and many had grown ~1 cm radicles within a week of germination.

Seeds readily imbibed and were swollen within 12 hours of immersion in water. Imbibing plus light scarification produced 1% germination without a period of cold conditions, compared to zero in the trial without scarification. When a 3 month period of cold-moist stratification conditions were included, 12% of seeds germinated; the most germinants from any one trial. However, the abrasion was not consistent from seed to seed and caused roughly half of the seeds to contract fungal or bacterial infections with a week after the initiation of incubation. Similarly, heavy scarification abraded the seed coat too much and all seeds contracted fungal or bacterial infections within one week of incubation. Nicking the seed coat with a sterilized surgical knife resulted in the mortality of 99.7% of seeds during the first week of incubation. Four seeds survived nicking and germinated within three days of the initiation of incubation at 24°C; all four damped off within another few days.

We used a variety of other methods in an attempt to break the dormancy of these generally unresponsive seeds. Interestingly, 4% of seeds germinated from the three “Wet-Dry (‘season 2’) trials which used seeds from the previous year that had remained ungerminated after a period of no stratification or 1 month cold-moist stratification and incubation at 24°C. Seeds did not respond to either the GA3 application or the heat pre-treatment.

## **Discussion**

Our results demonstrate that *P. submutica* has several seed traits that contribute to its persistence. The species is endemic to an arid landscape in western Colorado, characterized by great variability and unpredictability in precipitation between and within

germination seasons. Seed traits including dormancy, germination, and longevity in the soil are essential adaptations in these types of environments (Cohen 1966). Primary among these strategies for annual species is an ability for a seed to survive in the soil when moisture conditions are not sufficient to promote germination (Saatkamp *et al.* 2013). Our seed longevity experiment revealed that a large proportion of seeds retain their viability during drought years. Coon Hollow was the only site to have two consecutive years of drought and seed depletion was marginal (an average of 10% of seeds were depleted per year in 2012 and 2013). Depletion during these drought years likely represents the proportion removed by natural mortality. During favorable years, however, depletion was substantial, and several seed envelopes were empty at the first favorable year (2013). We presume that the losses of these seeds represent primarily germination. With this high rate of germination, it is expected that adult plants will reproduce and replenish the seed bank. From the three years of seed envelope collection, we predicted the longevity of a seed to be between four and six years, with complete depletion by 2017. Using the seed bank classification system as described by Thompson (1992), *P. submutica* is able to form a long-term persistent seed bank because seeds are projected to survive greater than five years.

Seed longevity, however, is influenced by varying conditions operating at regional and microsite scales (Long *et al.* 2014). The three years of our study included one year of drought conditions at all sites, and two years of favorable conditions at two out of three sites. If our study included three consecutive years of favorable conditions, depletion would likely have been more rapid, perhaps leading to the conclusion that the species has a short-term persistent seed bank. Additionally, disparate conditions within a

site affected seed depletion during favorable years, as evident by the large variability among envelopes within a site (Fig. 3.7). Slight differences between soil conditions such as particle size, structure, topography, and chemistry can impact seed germination, as well as mortality (Pakeman *et al.* 2012; Abedi *et al.* 2014). Since a variety of factors affect seed persistence, it may be useful to determine the inherent longevity of seeds in the absence of germination and mortality through predation (Long *et al.* 2014).

Ultimately, seeds must be able to survive in the soil longer than the maximum period of environmental factors preventing germination, such as burial and drought.

Most studies identifying seed persistence in natural conditions use seed burial experiments. However, artificial seed burial may overestimate or underestimate seed longevity (Thompson *et al.* 1997; Van Mourik *et al.* 2005). Notably, we observed that (1) the interior of our stainless steel envelopes were rusted and, after a rainstorm, appeared to be holding onto water for longer periods of time than the surrounding soils, and (2) both livestock damage and natural erosion on steep slopes actively uncovered buried envelopes. Regardless of the potential errors in the estimation of longevity, *P. submutica* seeds are clearly able to retain their viability in the soil over several years and contribute to the formation of a persistent seed bank.

By spreading germination over several springs that provided ample moisture, *P. submutica* reduces the number of seedlings at risk of mortality. During the first year of favorable conditions in the field, 53% of seeds on average were retained in the envelopes at Black Hills and Horsethief. The second year of favorable conditions at these sites saw an appreciable decline in viable seeds, with 23% of seeds remaining. Approximating the depletion of seeds over these two years, it appears that roughly 50% of seeds will

germinate every favorable year. The delayed germination of seeds employed by desert annual species is a “bet-hedging” strategy, which is well supported by both theoretical and empirical studies (Cohen 1966; Clauss & Venable 2000; Tielbörger & Valleriani 2005; Venable 2007).

The germination proportions of a species in a given year may be influenced by the maternal environment present during seed formation. In study conducted by Pake & Venable (1996) on desert annual species, a greater proportion of seeds germinated from plants that produced seeds during drought years, while the opposite effect was found for seeds produced during favorable years. The mechanisms through which this can occur are not well understood and are likely to be species-specific (Tielbörger & Petru 2010), but in general, abundant resources available during seed development may contribute to thickened seed coats or greater concentrations of germination-inhibiting chemicals (Baskin & Baskin 1998). The possibility of variability in seed longevity implies that some caution should be taken in generalizing our results to all *P. submutica* seed cohorts since seeds used in the longevity study were collected during a favorable growth year (2010).

Concurrently, the dispersion of seeds over time can apply to seeds within the same cohort; accomplished through maternal phenotypic or genetic effects (Roach & Wulff 1987; Allen & Meyer 1998; Baskin & Baskin 2001). Some of our results from the germination trials may suggest that *P. submutica* is able to partition germination of a seed crop through time. This is evidenced by: (1) Only a small fraction of seeds responded from trials that had any response to the conditions they were presented; and (2) a small proportion of seeds that did not germinate in their first germination trial germinated in



their second trial. Both between and within-year variability in germination rates helps to reduce the impact of competition between siblings or other annuals that also are likely to produce copious numbers of seeds during the same favorable year (Tielbörger and Kadmon 2000).

If favorable years are consistently high quality, the risk of high germination proportions must be minimized by the seeds' ability to respond to the appropriate set of environmental cues indicating a favorable year (Tielbörger & Valleriani 2005). From the longevity study, we found that large proportions of *P. submutica* seeds from the same cohort were able to germinate in the field, but had very little response to a wide variety of conditions presented to them in the laboratory. It appears that the temperature and/or stratification conditions we used in the germination trials are suboptimal predictors of a favorable spring, which may also explain our lack of *P. submutica* seedling emergence from the seed bank samples incubated in a greenhouse.

Since the timing of germination influences seedling survival and adult fecundity (Simons & Johnston 2000), dormancy strategies will evolve in response to the environmental conditions that optimize fitness (Clauss & Venable 2000). The rapid imbibition of *P. submutica* seeds indicates that their germination is not constrained by physical dormancy (Baskin & Baskin 1987). Seeds likely have a physiologically-based dormancy strategy that is sensitive to changes in a seed's physical and chemical environment (Baskin & Baskin 2004). Seeds may also have the ability to predict the occurrence of a favorable year, long before the germination season. For example, Pake and Venable (1996) found that December rainfall was highly correlated with vital, yet highly unpredictable spring precipitation during the germination period in February.

Further studies elucidating the potential correlations between temperature and/or rainfall cues outside of the germination season within the range of *P. submutica* would be useful to identify whether this species uses a predictive germination strategy.

Several questions arose from our germination trials, the answers to which would help to identify factors associated with the species' dormancy mechanisms. First, several seeds that did not germinate in their first trial germinated in their second. Are seeds able to germinate in their first spring, or must they experience two winters with cold stratification to release dormancy? Unfortunately, our seed longevity study was unable to provide us this information because the first spring was a drought year. Second, many spring germinating seeds prevent germination in fall by employing a secondary dormancy strategy, where the onset of high summer temperatures reinitiate dormancy, which is then broken by a period of cold in winter (Baskin & Baskin 2006). *P. submutica* seeds germinated at higher proportions while in cold-moist stratification than any other trial not involving seed injury. However, because these germinations were <0.001% of total seeds experiencing 1 to 3 months of cold stratification, we have insufficient evidence that cold stratification is required to alleviate dormancy in the field. Finally, desert annuals have also evolved a number of other strategies to prevent germination until highly saturated conditions are present (Went 1949). One of these traits is a germination inhibiting coating on the surface of the seeds that requires leaching (Went 1949). Does the increase in seed germination after imbibing allude to the presence of such a coating (note the shiny substance of the surface of dry seeds (Fig. 3.1)? Additional studies, especially demographic, will be necessary to determine whether *P. submutica*'s dormancy and germination strategies ensure its survival or contribute to its rarity.

The density of seeds in the seed bank is a function of factors contributing to depletion and replenishment (Pavlik *et al.* 1993), and can vary considerably through time (Parker *et al.* 1989). The estimated average density of *P. submutica* seeds in the soil during dispersal in August across four study sites was 74 seeds per m<sup>2</sup>. The results from the longevity study found that seeds have the capacity to survive in the soil for multiple years, therefore, it is highly likely that these seed bank samples contained both fresh seed rain and dormant seeds produced in previous years. Seed persistence over numerous reproductive years may allow a species to build an extensive reservoir of seeds in the seed bank (Kemp 1989). However, since large proportions of seeds germinate during favorable years, the density of seeds in the seed bank can vary considerably within and between years. The spatial distribution of seeds within sites was highly aggregated and relatively few samples contained seeds. Some sample estimates contained large numbers of seeds, such as 2994 seeds at Plateau Creek I. Aggregation of many seeds will cause density estimates to vary greatly depending on sampling intensity and area (Bigwood & Inyone 1988; Benoit *et al.* 1989). Additionally, a highly aggregated spatial distribution within a site indicates that seeds are constrained by two factors: (1) small-scale patterns or gradients of microsite conditions that support species germination and reproduction; and (2) the patterning of seed dispersal (Guo *et al.* 1998). This suggests that the dispersal of seeds will be the most important factor involved in the longevity of seeds in the seed bank (Schupp & Fuentes 1995).

Dispersal determines the abiotic and biotic conditions that a seed faces in all stages of its life, from seed to reproductive adult. Seed dispersal includes temporal, vertical, and horizontal dimensions that interact to define the dynamics of a seed bank

(Levin *et al.* 2003; Willson & Traveset 2000). *P. submutica* seeds are dispersed in summer and early fall, when temperatures are still high and habitat is predominantly dry. The ambient temperature and moisture conditions a seed experiences during both maturation and after-ripening stages of its development can influence their dormancy and response to environmental cues (Probert 2000; Fernández-Pascual *et al.* 2013). This may possibly contribute to the partitioning of seed germination within and between favorable years (Probert 2000).

A lack of mechanisms facilitating Phase I horizontal dispersal is evident for *P. submutica*, whose small seeds fall by gravity from dehiscent capsules directly below the mother plant into the network of cracks present in habitat during the summer months. The shrinking and swelling of these cracks during heavy precipitation events in the summer, as well as freeze-thaw conditions in the late fall and early spring, would act to incorporate seeds into the soil and reduce the possibility of Phase II dispersal (Chambers & MacMahon 1994). If Phase II dispersal of seeds is occurring, the pattern would most likely be determined by abiotic forces, such as the movement of water funneling through the network of cracks at the soil surface. The accumulation of both sediment and water within micro-topographic depressions would contribute to the sequestration of seeds, as well as the provisioning of germinants and adult plants with enhanced water availability (Harper 1977). Some evidence of this phenomenon has been observed during field studies of the species' edaphic habitat (A. Langton, unpublished data). However, large precipitation events on these erodible slopes may also wash soils containing seeds out of the habitat (Friedman & Stein 1980; García-Fayos & Cerdà 1997).

The presence of cracks of varying depths is likely to be the primary determinant of vertical distribution (Guo *et al.* 1998). Our results show that *P. submutica* seeds are able to germinate at both at the surface and at a depth of 5 cm. Seed bank depletion, however, was more rapid for deeper buried seeds at Black Hills, most likely from germination. Unfortunately, we cannot confirm that seedlings are also able to emerge at this depth, but given the very small size of the seed it is not likely (Grundy *et al.* 2003; Traba *et al.* 2004). Additional studies such as *in situ* seeding are needed to determine proportions of seeds able to emerge at various depths in order to discern how dispersal and disturbance may affect seed bank depletion.

According to theory, a lack of dispersal traits indicates that suitable habitat for reproduction is spatially reliable (Strykstra *et al.* 2002). When the reliability of conditions for establishment at a spatial scale are relatively infrequent, then the species must have the capacity to disperse. If reliability at a temporal scale is low, the seed bank must have the ability to persist until favorable conditions return (Strykstra *et al.* 2002). Applying the model developed by Strykstra *et al.* (2002), *P. submutica* compensates for conditions that are unreliable in time with a persistent seed bank, while its dispersal limitations indicate that conditions should be reliable in space. However, if contemporary habitat conditions are no longer spatially reliable, this strategy may be a factor influencing its abundance and distribution.

### *Conservation Implications*

The factors involved in the formation and maintenance of a seed bank are crucial elements of any conservation strategy (Doak *et al.* 2002; Harper & Van Buren 2004;

Adams *et al.* 2005; Ooi *et al.* 2009; Megill *et al.* 2011). *P. submutica*'s reliance on a seed bank rapidly depleted during favorable years, a restricted dispersal strategy, and a narrow set of environmental conditions that releases dormancy may indicate that its survival in the 'anthropocene' (*sensu* Crutzen 2006) is precarious. One key characteristic of the seed ecology of this species is its persistence in the soil over several years. Our data suggest that seeds are able to remain viable in the soil for greater than six years if conditions at the scale of the site or microsite are not highly favorable to germination for more than two years. Therefore, suitable habitat may be occupied despite the absence of aboveground plants for several consecutive years. Management plans must, therefore, incorporate protections for suitable habitat rather than merely the distribution of the aboveground population because there may be a seed bank.

Seed bank surveys have been proposed as a method to identify occupied areas outside of the growing season or during times of unfavorable climatic conditions. However, we do not advise using seed bank surveys to determine presence-absence of a species that may in a given year have a low density of seeds in the soil. Additionally, surveys would require numerous samples due to the aggregation of seeds within habitat. We recommend that multi-year surveys for aboveground plants during years with favorable climatic conditions be performed prior to disturbance activities that may impact site conditions, the seed bank, or reproductive performance at a site.

Disturbances to the physical features of habitat could have a significant impact on the seed bank and factors ensuring population persistence. Livestock trampling and off-highway vehicle recreation have impacted soils at numerous sites through compaction and destruction of soil structure (Langton A., unpublished data). This type of physical

damage can bury seeds and perhaps inhibit seed germination or seedling establishment (Sheldon 1974). Though seeds were able to germinate at a depth of 5 cm in envelopes, we do not know whether the seed is able to emerge and establish at that depth.

Additionally, these threats can alter the micro-topography supplying water to the discrete patches where *P. submutica* grows, thereby impacting the 'safe sites' that foster growth and reproduction (García-Fayos & Cerdà 1997). Physical disturbances to its fragile habitat may also increase erosion and transport seeds outside of suitable habitat (García-Fayos *et al.* 1995).

The effect of a disturbance will depend partly on the density and spatial distribution of seeds in the seed bank in a given year, which in 2011 we found to be relatively low and highly aggregated. The greater density of seeds in the soil, the greater the buffering capacity of the seed bank may be in the event that aboveground plants are removed. However, successive years of reproductive failure during favorable germination conditions may be catastrophic for this species because a large proportion of seeds are lost to germination during these years. It may take many years for the population to recover because average plant fecundity, even during favorable years, may be fewer than 67 seeds (e.g. Horsethief in 2011) (Langton A., unpublished data). In addition to the spatial aggregation of seeds within a site, the size of most individual *P. submutica* sites are very small, ranging from 1 m to less than fifty meters across (Langton A., unpublished data). Therefore, even small-scale disturbances have the capability of severely depleting a seed bank, and therefore endangering the existence of the species, at that site. To mitigate the potential for this threat, sufficient buffer distances between

human activities and *P. submutica* habitat will be necessary to prevent reproductive impairment and seed bank damage.

Nonnative invasive weeds are a concern in the management of both rare and common species as their impacts are well documented. Threats include reduction in seed production through resource competition (Gurevitch & Padilla 2004); secretion of allelopathic chemicals into the soil (Callaway & Aschehoug 2000; Hierro & Callaway 2003); and alteration of habitat characteristics (Kourtev *et al.* 2002; Ehrenfeld 2003). At the time of listing this species as threatened in 2011, non-native invasive weeds were documented nearby and along the periphery of *P. submutica* habitat, but not considered a factor influencing the continued survival of the species (U.S. Fish and Wildlife Service 2011). During the course of this study, invasive weeds were observed within occupied habitat and at high densities along habitat peripheries. Their presence is well supported by their plentiful emergence from seed bank samples. Since land disturbances, notably the extension of roadways, livestock grazing, and off-highway vehicle recreation, can cause an increase of invasion by nonnative invasive plant species into natural areas (Dukes & Mooney 1999; McKinney & Lockwood 1999), it is possible that the threat posed by these species could become more apparent and warrant intervention. Additional studies should be conducted to reveal the specific impacts that are occurring from the expansion of invasive weeds into *P. submutica*'s habitat.

Since the evolution of seed bank dynamics is a result of selective pressures of the environment (Parker *et al.* 1989), climate change may exert a novel set of selective pressures the species is not adapted to. These changes are likely to affect many rare plant species because seed germination, seed dormancy, and persistence of the seed bank are



all directly dependent on precipitation and temperature patterns (Levine *et al.* 2008). Climate change could be deleterious to *P. submutica* if (1) alterations in precipitation regimes affect seed inputs and outputs to and from the seed bank (Maschinski *et al.* 2006), (2) temperature changes alter dormancy breaking and germination patterns, or steepen the slope of the seed bank depletion trajectory (Ooi *et al.* 2009; Walck *et al.* 2011), or (3) temperature and precipitation changes cause a mismatch between phenology and environmental conditions, thereby affecting seedling mortality or reproductive inputs into the seed bank (Kimball *et al.* 2010).

The threat of climate change may be mitigated by a persistent seed bank. Seed persistence means that the seed bank contains offspring from many generations selected under different sets of environmental conditions (Levin 1990; Bonis *et al.* 1995; McCue & Holtsford 1998). A greater diversity of genes in the seed bank may provide a measure of genetic resilience in the face of climate change (Templeton & Levin 1979; Jump & Peñuelas 2005). Theory also suggests that the aged seeds of *P. submutica* may produce a greater number of genetic novelties through mutations, thereby increasing the species' evolutionary potential (D' Amato 1977). Though this may provide a measure of resilience in the face of climate change, managers must still prepare for the potential unavoidable impacts to a species' seed bank dynamics, predicted to become more apparent in the near future (Thuiller *et al.* 2008; Chen *et al.* 2011).

Range shifts are expected as a result of the shifting bioclimatic envelopes caused by climate change, but are constrained by dispersal ability and the spatial structure of habitat patches (Fordham *et al.* 2012). Without a mechanism to disperse long distances, *P. submutica* is restricted to its current patchy habitat. Conservation managers have

suggested that in the case of such species with dispersal restrictions, human mediated relocation of populations to new habitats may prevent the extinction of rare flora within the next few centuries (Vitt *et al.* 2010). Despite the potential for assisted dispersal by humans, *P. submutica*'s edaphic habitat is constrained to a narrow elevation and geographic range offering limited future habitat opportunities.

The future status of *P. submutica* will depend, in part, on its ability to maintain seed bank dynamics that ensure population persistence. Land managers seeking to conserve this species would, therefore, focus on protecting habitat at a scale that sufficiently prevents damage to the seed bank and alterations to the dynamics involved in its maintenance. Protecting the habitat of a species with a great magnitude of spatial and temporal variability in aboveground populations between years will be unreliable without a clear understanding of suitable habitat. A first, essential step is to identify the edaphic and biotic characteristics associated with suitable (occupied) and unsuitable (unoccupied) habitat (see Appendix). In addition, further research elucidating its germination ecology and demographic processes will provide managers with insight into (1) plant population processes to model extinction probabilities by perturbation (Meyer *et al.* 2006; Keith *et al.* 2008), and (2) effects of increased global temperatures and altered precipitation patterns on the dynamics of its seed bank (Ooi *et al.* 2009, 2012). Long-term monitoring of aboveground populations through time has not been implemented, but would be useful to detect long-term declines in populations and to perform a population viability analysis (PVA). Despite an attempt to conserve the *in situ* populations, it is possible that *if* this species does not have the capacity to genetically navigate the predicted impacts of

climate change, it may follow suit with the estimated 18 to 35% of species predicted to suffer extinction (Thomas *et al.* 2004).

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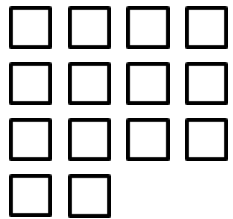
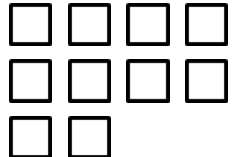
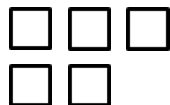
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**Table 3.1** Design of the experimental *Phacelia submutica* seed longevity study installed at three sites in 2011. Presented are the number of seeds per envelope, the levels of the depth factor installed at each site, the number of envelopes per replicate block, the grid pattern for envelope placement, and years that envelopes will be collected from the field at each site.

Site	Number of seeds per envelope	Depths	Number of envelopes per replicate block	Envelope placement for replicate blocks	Years of collection
Horsethief	100	Shallow (~3 mm) Deep (5 cm)	14		2012 2013 2014 2015 2016 2017 2018
Black Hills	100	Shallow (~3 mm) Deep (5 cm)	10		2012 2013 2014 2016 2018
Coon Hollow	75	Shallow (~3 mm)	5		2012 2013 2014 2016 2018

**Table 3.2** Seed bank results from the seed extraction method for each site showing the total number of *Phacelia submutica* (PHSU) seeds recovered from the samples, the number of samples that contained *P. submutica* seeds, the highest estimated density of *P. submutica* seeds within a sample, the estimated mean density of *P. submutica* seeds, the measure of *P. submutica* seed aggregation (Standardized Morisita Index), and the total number of seeds of other species found in the sample. For the Standardized Morisita Index, values <0.5 indicate a random distribution (non-aggregated) within a 95% confidence interval.

Site	Number of PHSU seeds	Number of samples with PHSU seeds (out of 75)	Maximum Estimated Sample Density (m <sup>2</sup> )	Estimated Site Density (m <sup>2</sup> )	Standardized Morisita Index	Number of other species seeds
Horsethief	25	16	1669	69	0.55	3581
Motor Hollow	22	9	1513	63	0.56	5446
Plateau Creek I	32	5	2994	84	0.69	2111
Plateau Creek II	27	18	816	75	0.52	369

**Table 3.3** Results of the emergence method for the seed bank study showing the number of germinations (G) for each species identified and the proportion of samples (S) that contained at least one seedling. Species codes: BRTE=*Bromus tectorum*; MONU= *Monolepis nuttaliana*; LAOC= *Lappula occidentalis*; CETE= *Ceratocephala testiculata*; ERTR= *Erymopyrum triticeum*; LEPE= *Lepidium perfoliatum*; SCLI= *Schoenocrombe linifolium*; ERCI= *Erodium cicutarium*; ACHY= *Achnatherum hymenoides*. Nonnative invasive weed species are indicated with a \*. The total number of seeds and the percentage of samples with germination are also shown. No *Phacelia submutica* emerged from the samples.

Site	Statistic	Species Code									Total
		BRTE*	MONU	LAOC	CETE*	ERTR*	LEPE*	SCLI	ERCI*	ACHY	
Horsethief	G (S)	53 (0.33)	6 (0.07)	4 (0.04)	1 (0.01)	112 (0.63)	0 (0)	2 (0.03)	0 (0)	29 (0.16)	207 (0.93)
Motor Hollow	G (S)	38 (0.33)	31 (0.29)	0 (0)	20 (0.13)	6 (0.07)	0 (0)	2 (0.03)	1 (0.01)	4 (0.03)	102 (0.40)
Plateau Creek I	G (S)	34 (0.31)	1 (0.01)	11 (0.15)	39 (0.23)	23 (0.16)	35 (0.31)	2 (0.01)	0 (0)	5 (0.03)	150 (0.68)
Plateau Creek II	G (S)	3 (0.03)	0 (0)	3 (0.01)	0 (0)	0 (0)	10 (0.05)	0 (0)	0 (0)	0 (0)	16 (0.07)

**Table 3.4.** ANOVA table for the Type III tests of fixed effects on the proportion of *Phacelia submutica* seeds remaining viable at each site by year, depth, and year\*depth interaction. Significant P-values are in bold.

	Site								
	Black Hills			Coon Hollow			Horsethief		
	df	F	Pr >F	df	F	Pr >F	df	F	Pr >F
<b>Year</b>	1,14	485.79	<b>&lt;0.0001</b>	1,7	22.66	<b>0.0021</b>	1,14	12.06	<b>0.0037</b>
<b>Depth</b>	1,6	0.0036	0.9553	----	-----	-----	1,6	1.04	0.3389
<b>Year*Depth</b>	1,14	69.15	<b>&lt;0.0001</b>	----	-----	-----	1,14	0.14	0.7165



**Table 3.5** The intercept and slope of the depletion regression for *Phacelia submutica* seeds, as well as standard errors, calculated for each site and depth. The estimated year when < 1 of the original seeds will remain in the envelopes was calculated with a logistic regression curve.

Site	Depth	Intercept for Year (2012 to 2014)	Standard Error for Intercept Estimate	Slope $\beta_1$ for Year (2012 to 2014)	Standard Error for Slope Estimate	Year with <1 viable seed remaining
Black Hills	Shallow	4478.74	251.94	-2.2246	0.13	2016
	Deep	9905.09	602.02	-4.9202	0.30	2015
Coon Hollow	Shallow	3714.34	780.29	-1.8447	0.39	2017
Horsethief	Shallow	2928.92	1246.94	-1.4551	0.62	2016
	Deep	3629.38	1418.16	-1.8027	0.70	2016

**Table 3.6** Results of the germination trials for *Phacelia submutica* that included the combinations of seed pre-treatments, scarification (gentle [GS], heavy [HS], nicking), periods of cold-moist stratification at 4°C (0, 1, 2, and 3 months), incubation temperatures on a 12-12 hour cycle, and incubation medium. In trials where germination was present, the numbers of seeds that germinated are in bold. Each trial contained 100 seeds. The ♦ symbol denotes the number of seeds germinated during cold-moist stratification.

Pre-treatments	Scarification	Duration stratification	Incubation temperature regime (°C)	Incubation medium	Number of seeds germinated
None	None	0	24	Potting soil	0
None	None	1	24	Potting soil	0
None	None	2	24	Potting soil	0
None	None	3	24	Potting soil	0
None	None	0	10-20	Potting soil	0
None	None	1	10-20	Potting soil	0
None	None	2	10-20	Potting soil	0
None	None	3	10-20	Potting soil	0
Imbibing	None	0	24	Petri	0
Imbibing	None	1	24	Petri	0
Imbibing	None	2	24	Petri	0
Imbibing	None	3	24	Petri	0
Imbibing	GS	0	24	Petri	<b>1</b>
Imbibing	GS	1	24	Petri	0
Imbibing	GS	2	24	Petri	0
Imbibing	GS	3	24	Petri	<b>12</b>
Imbibing	HS	0	24	Petri	0
Imbibing	HS	1	24	Petri	0
Imbibing	HS	2	24	Petri	0
Imbibing	HS	3	24	Petri	0
None	GS	0	24	Petri	0
None	GS	1	24	Petri	0
None	GS	2	24	Petri	0
None	GS	3	24	Petri	0
None	HS	0	24	Petri	0
None	HS	1	24	Petri	0
None	HS	2	24	Petri	0
None	HS	3	24	Petri	0
Imbibing	Nicking	0	24	Petri	<b>4</b>
Imbibing	Nicking	1	24	Petri	0
Imbibing	Nicking	2	24	Petri	0

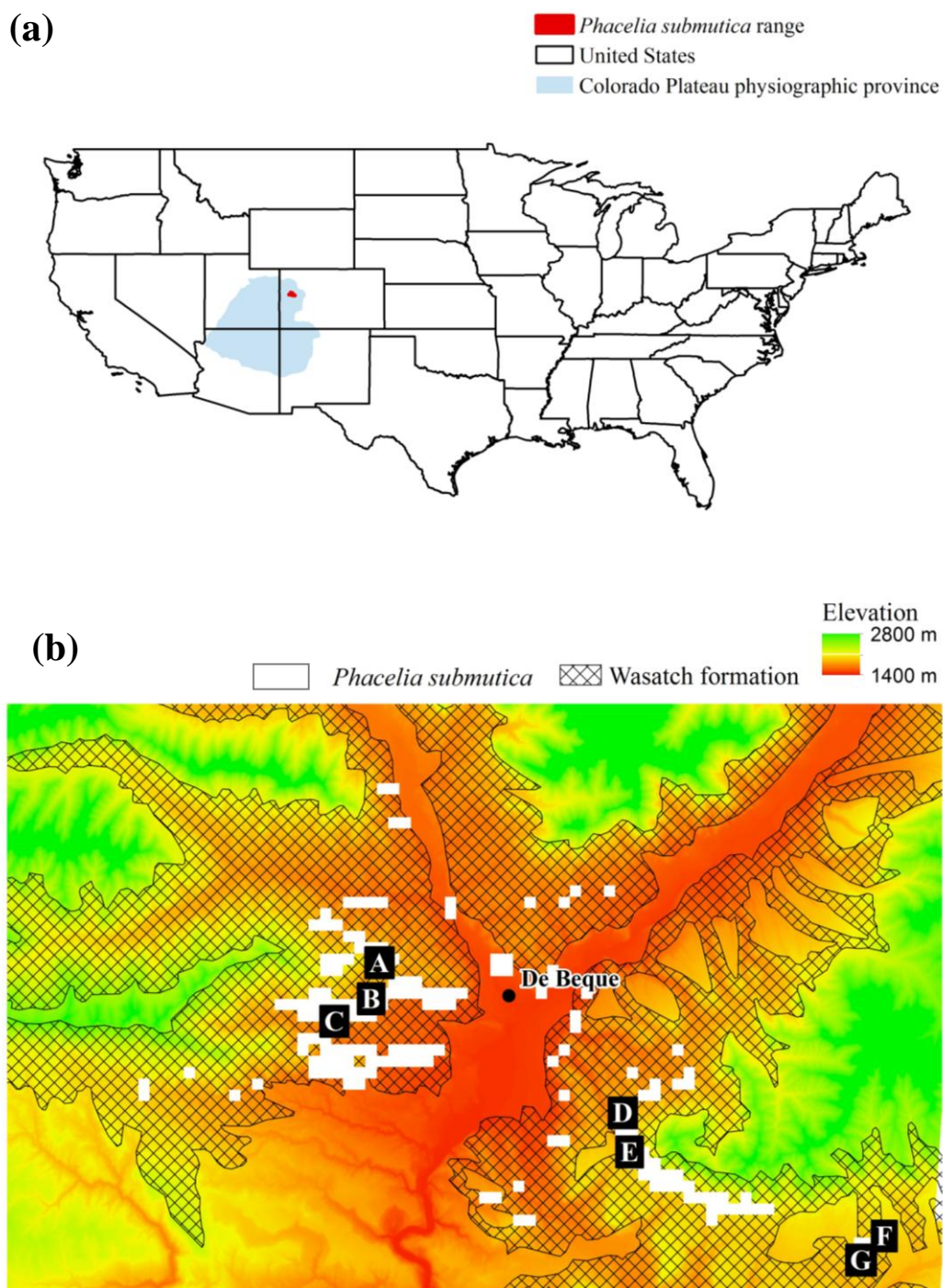
Table 3.6 continued...

Pre-treatments	Scarification	Duration Stratification	Incubation temperature regime (°C)	Incubation medium	Number of seeds germinated
Imbibing	Nicking	3	24	Petri	0
Imbibing	Nicking	0	10-20	Petri	0
Imbibing	Nicking	1	10-20	Petri	0
Imbibing	Nicking	2	10-20	Petri	0
Imbibing	Nicking	3	10-20	Petri	0
Imbibing (GA3)	Nicking	0	24	Petri	0
Imbibing (GA3)	Nicking	1	24	Petri	0
Imbibing (GA3)	Nicking	2	24	Petri	0
Imbibing (GA3)	Nicking	3	24	Petri	0
Imbibing (GA3)	None	0	24	Petri	0
Imbibing (GA3)	None	1	24	Petri	0
Imbibing (GA3)	None	2	24	Petri	0
Imbibing (GA3)	None	3	24	Petri	0
None	None	0	10-20	Petri	0
None	None	1	10-20	Petri	0
None	None	2	10-20	Petri	0
None	None	3	10-20	Petri	0
None	None	0	3-20	Petri	0
None	None	1	3-20	Petri	0
None	None	2	3-20	Petri	1♦
None	None	3	3-20	Petri	2♦
None	None	0	24	Petri	0
None	None	1	24	Petri	2
None	None	2	24	Petri	4♦
None	None	3	24	Petri	2♦
None	None	0	30	Petri	0
None	None	1	30	Petri	0
None	None	2	30	Petri	0

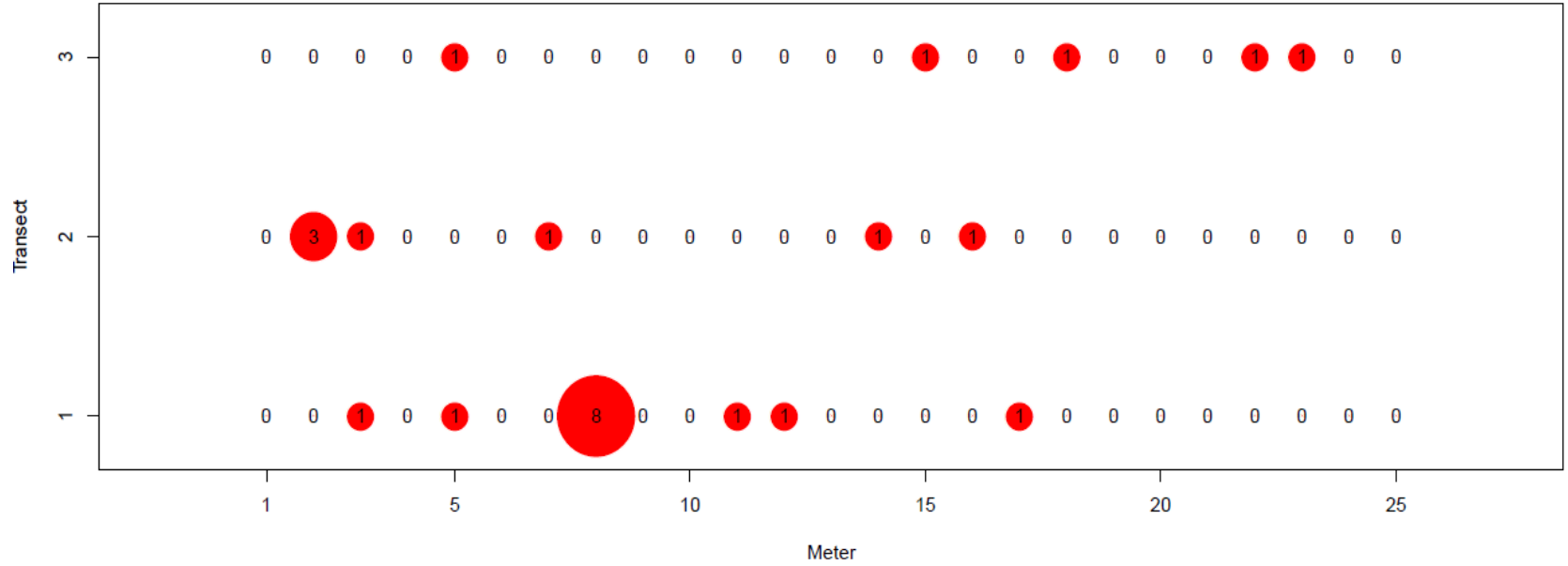
Table 3.6 continued...

<b>Pre-treatments</b>	<b>Scarification</b>	<b>Duration Stratification</b>	<b>Incubation temperature regime (°C)</b>	<b>Incubation medium</b>	<b>Number of seeds germinated</b>
None	None	3	30	Petri	0
Heat	None	0	24	Petri	0
Heat	None	1	24	Petri	0
Heat	None	2	24	Petri	0
Heat	None	3	24	Petri	0
Wet-Dry ('season 2')	GS	0	24	Petri	<b>8</b>
Wet-Dry ('season 2')	GS	0	10-20	Petri	<b>4</b>
Wet-Dry ('season 2')	GS	1	10-20	Petri	<b>1</b>
Imbibing (Heat)	None	0	24	Petri	0
Imbibing (Heat)	None	1	24	Petri	0
Imbibing (Heat)	None	2	24	Petri	0
Imbibing (Heat)	None	3	24	Petri	0

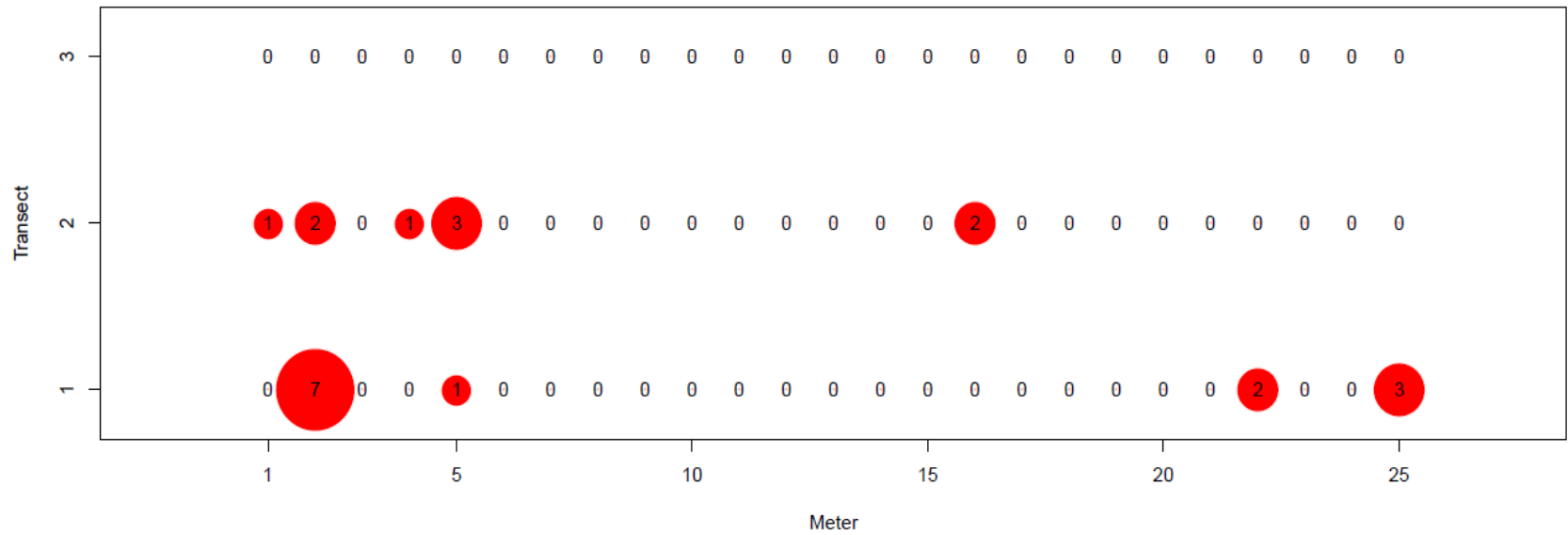




**Fig. 3.2** (a) Colorado Plateau physiographic province and range of *Phacelia submutica*, and (b) a map of geology, elevation, and *P. submutica*'s distribution. Study locations are indicated with letters: A (Black Hills); B (Motor Hollow); C (Coon Hollow); D (Sunnyside); E (Horsethief); F (Plateau Creek I); and G (Plateau Creek II).

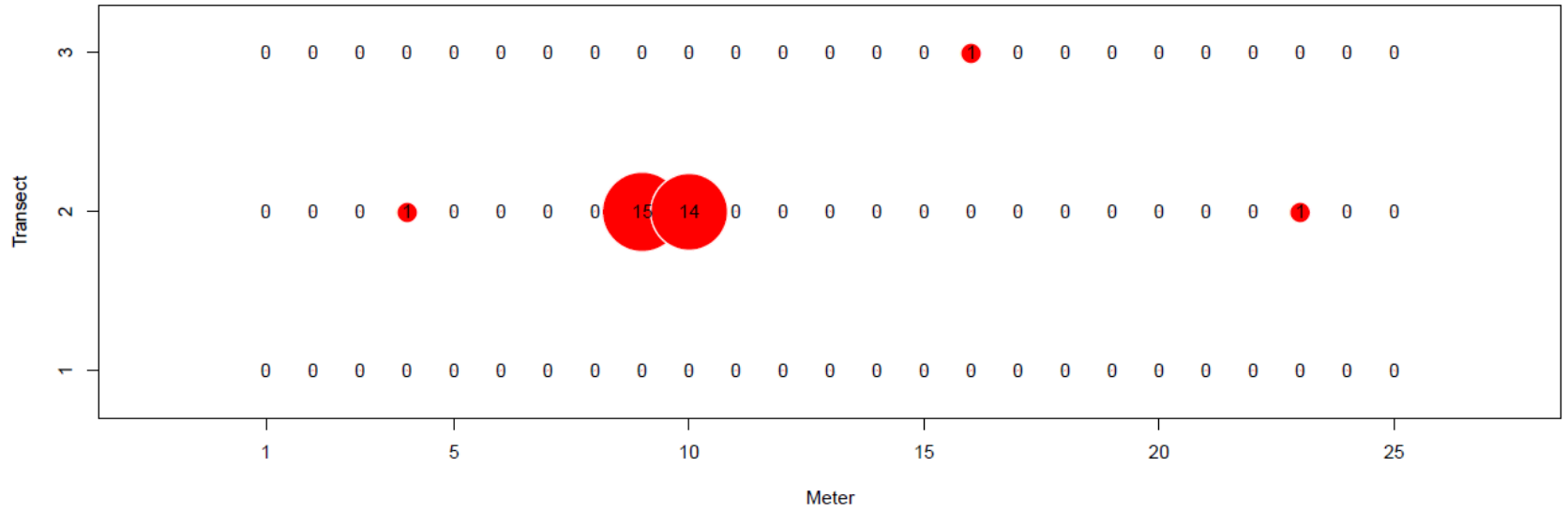


**Fig. 3.3** The number of *Phacelia submutica* seeds extracted from each composite sample along three 25-m transects at Horsethief. Circles are scaled by the number of seeds found in each sample.

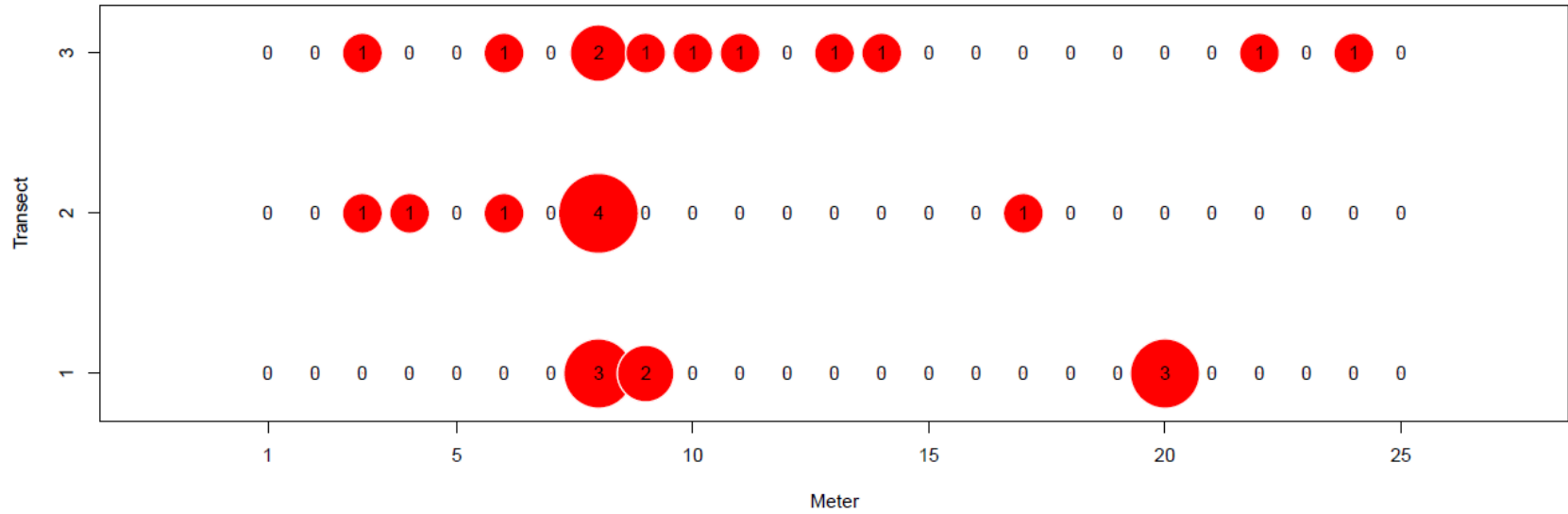


**Fig. 3.4** The number of *Phacelia submutica* seeds extracted from each composite sample along three 25-m transects at Motor Hollow. Circles are scaled by the number of seeds found in each sample.

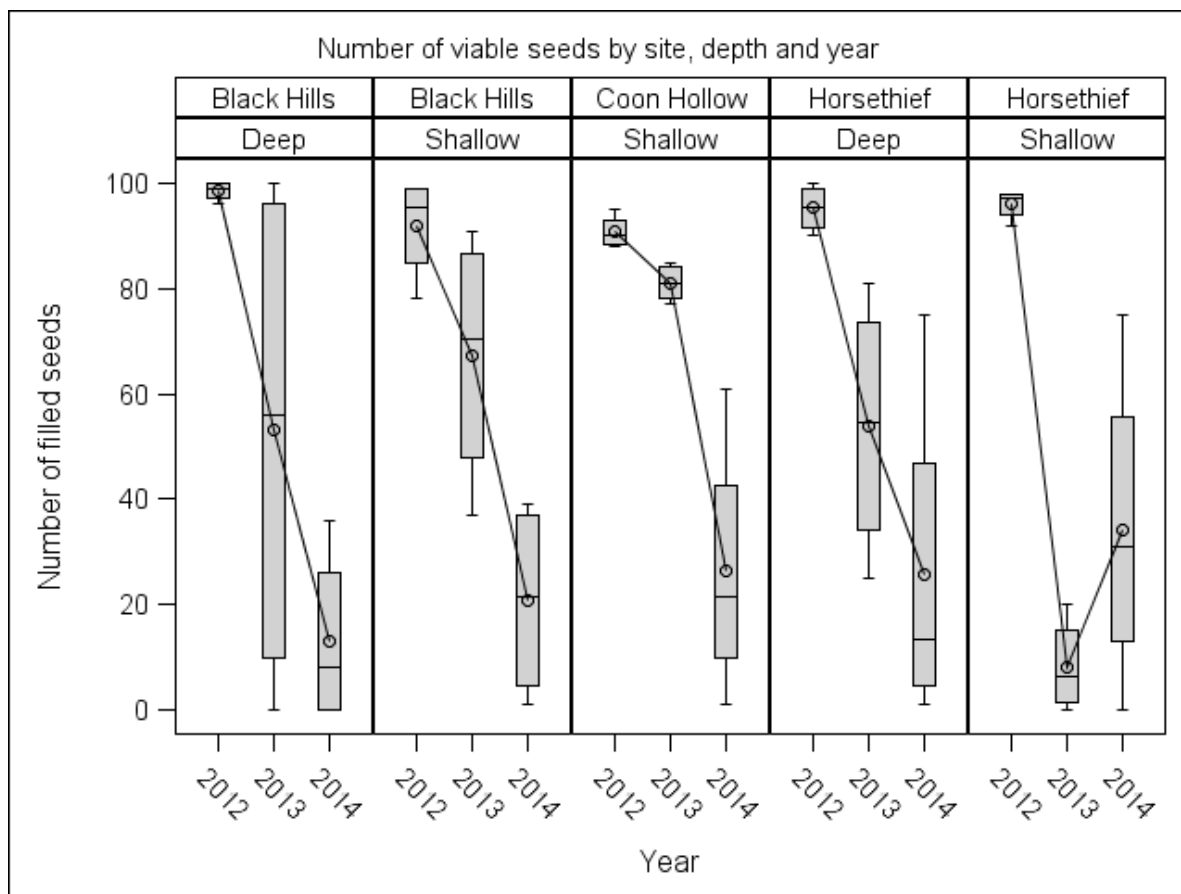




**Fig. 3.5** The number of *Phacelia submutica* seeds extracted from each composite sample along three 25-m transects at Plateau Creek I. Circles are scaled by the number of seeds found in each sample.

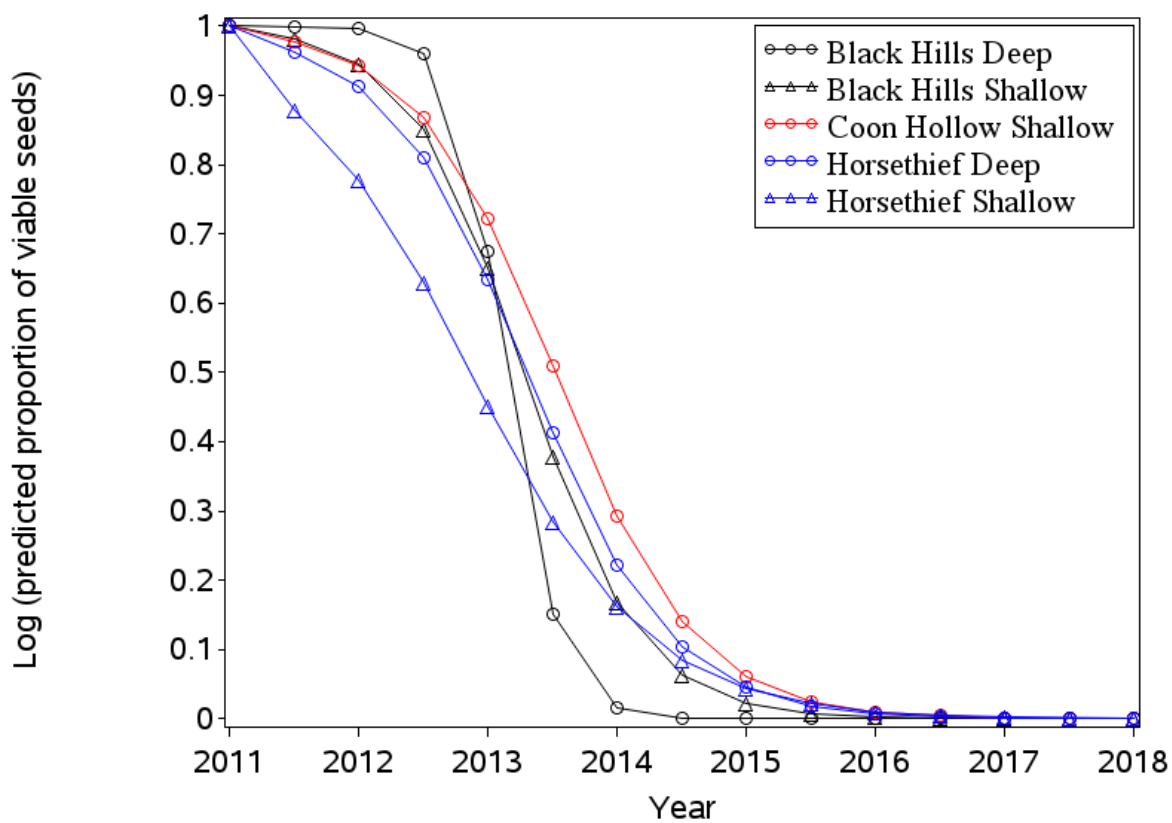


**Fig. 3.6** The number of *Phacelia submutica* seeds extracted from each composite sample along three 25-m transects at Plateau Creek II. Circles are scaled by the number of seeds found in each sample.



**Fig. 3.7** Effect of year and depth on the proportion of filled (viable) *Phacelia submutica* seeds remaining at Black Hills, Coon Hollow, and Horsethief. Large circles represent mean; horizontal lines, medians; enclosed rectangles, interquartile range (IQR); and whiskers, range of the data.

## Regression curves for the predicted proportion of viable seeds



**Fig. 3.8** The predicted rate of *Phacelia submutica* seed bank depletion over an eight-year period for each site\*depth combination.

## CHAPTER 4

### CONCLUSION

Energy development, off-highway vehicle recreation, and livestock grazing are the leading causes of disturbance within the range of *Phacelia submutica*. Land managers aim to balance the needs and interests of a growing human population with the ecological needs of endemic plant species, like *P. submutica*, that occupy tiny ranges and unique habitats on the Colorado Plateau. Our research was funded by the U. S. Fish and Wildlife Service and the Colorado Natural Areas Program to provide critical information to land managers aiming to design adequate conservation and recovery strategies for this species.

The primary goal of endangered species management is to maintain healthy, self-sustaining populations that are resilient to disturbances (Scott *et al.* 2010; Redford *et al.* 2011). A prerequisite to the implementation of any conservation plan for a rare species is to elucidate the autecological factors involved in population persistence (Simberloff 1988; Heywood & Iriando 2003). Our research addressed two key aspects of *P. submutica*'s regenerative capacity; its reproductive biology and seed ecology. With this information, managers will be able to discern the species' susceptibility to disturbances and the recovery objectives necessary to ensure population persistence (Flather & Seig 2007).

A species' reproductive strategy determines its response to altered plant-pollinator interactions (Barrett & Eckert 1990; Fischer & Matthies 1997; Wilcock & Neiland 2002). *P. submutica* reproduces through spontaneous autogamy. Reproductive success (seed

quantity and quality) both in the absence of pollinators and with spontaneous self-pollination was equivalent to success of hand cross-pollination. The functional aspects of *P. submutica*'s breeding system also facilitate autogamy and reduce opportunities for insect pollinators to play a role in reproduction. Consequently, reproduction will occur independent of any potential effects of disturbances to the assemblage of native pollinators and their habitats (see Scott *et al.* 2011). Disturbances that reduce plant population sizes or increase their levels of fragmentation will accordingly have no bearing on the species' vulnerability to pollination failure and the genetic effects of inbreeding depression (DeMauro 1993; Kwak *et al.* 1998; Brys *et al.* 2004; Anderson *et al.* 2011).

Despite the benefit of reproductive assurance, habitual autogamy results in genetic impoverishment (Charlesworth & Charlesworth 1987; Huenneke 1991; Ellstrand & Elam 1993). Though inbreeding depression was not evident in two elements of reproductive fitness (seed number and seed weight), inbreeding may affect seed viability and seedling vigor (Schemske & Lande 1985; Kearns & Inouye 1997; Larson & Barrett 2000). Habitual autogamy also suggests that *P. submutica* populations may also be extremely adapted to site-specific conditions (Levin 1972; Jain 1976; Lacy 1992; Jarne & Charlesworth 1993; Levin 2010). Preserving the range of genetic diversity across the species' range should be considered an important element of a conservation plan because it could represent its evolutionary potential in response to climate change (Neel *et al.* 2001).

Populations that maintain a seed bank are able to recruit infrequently with minimal losses to population viability (Menges 2000). Our results strongly suggest *P.*

*submutica* develops a long-term persistent seed bank that functions to buffer populations from great year-to-year variability in precipitation. Logistic regressions from three years of data projected maximum seed survival to six years. However, because few seeds are lost during unfavorable conditions, it is feasible that seeds can survive for longer in the soil through prolonged periods of drought.

A persistent seed bank is able to develop because *P. submutica* spreads out its germination through favorable years. During the three-year study period, about 50% of surviving *P. submutica* seeds remained ungerminated each favorable year. By spreading germination over several springs that provided ample moisture, *P. submutica* hedges its bets against the risk of seedling mortality in an environment with unpredictable intra-seasonal precipitation. The large proportion of seeds that germinate during favorable years is expected to survive and reproduce, thereby replenishing the seed bank. Reproductive failure through several successive favorable years could severely deplete the seed bank and lead to impairment of long-term population viability.

The naturally small size of *P. submutica*'s habitat, combined with the low density and aggregation of seeds within habitat, increases the species' susceptibility to small-scale disturbances, such as off-highway vehicle damage. Additionally, without the capability to disperse seeds over long distances, the species will not be able to recolonize sites where a population has been lost. Sufficient buffer distances protecting *P. submutica* populations from direct impacts to its habitat, or indirect impacts through hydrological alterations or erosion will be necessary.

Results from the longevity study also found high variability between replicate envelopes within a site during favorable years. This indicates that seed longevity is

influenced by conditions occurring at the scale of the microsite. These results strongly suggest that *P. submutica* seeds and the seed bank will be highly sensitive to changes in habitat quality because conditions occurring at the microsite greatly influence germination and/or mortality rates.

Though spatial buffers between disturbances and suitable habitat can prevent direct disturbances from occurring, climate change is unavoidable and is likely to affect *P. submutica*. Limited dispersal ability may be the greatest factor contributing to its susceptibility to climate change (Fordham *et al.* 2012). Additionally, its breeding system may indicate genetic impoverishment and limited flexibility to adapt to a different set of conditions imposed by climate change. It is possible, however, that multiple generations of seeds in the soil provides for a measure of genetic diversity (Levin 1990; McCue & Holtsford 1998).

In conclusion, *P. submutica* has traits that afford the species with both resiliency and susceptibility to disturbances. At minimum, *P. submutica* populations will require that seeds remain within appropriate habitat, that seeds occasionally germinate and survive to reproduce. The factors associated with the species' seed ecology make it evident that this species will not be able to exist without the presence of a seed bank, and one that is also able to persist through time. Disturbances that eliminate the aboveground population or prevent reproduction in a given year may rapidly lead to decreased population viability. The species' limited dispersal ability means that seeds will be unable to 'escape' disturbances occurring within its habitat. In the absence of pollinator requirements, conservation practitioners must focus on protecting the seed bank and the



dynamics contributing to its maintenance as the primary sources of population regeneration.

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APPENDIX

Table A.1. Field and laboratory data for site #1.

<b>Site Name: Ashmead Draw 01</b>				Soil Unit: Atwell Gulch				Lat:				Long:			
Aspect: 270		Slope: 0		Slope-Length: N/A		Slope Shape: LL		Slope Position: SU		Geomorph. Position: IF					
<b>Plot: Occupied 1</b>				% Surface R.F.: 0				% Veg. Cover: 20				Crack Density:			
Plot Species: BRTE/MONU/SCLI															
Compaction (Number strikes to depth) from (cm): -1				5cm: 3		10cm:6		15cm: 5		20cm:8		25cm: 9		30cm: 11	
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	%R.F.	Bulk Density	pH	Eff.	E.C.	%CCE			
A	1.63		3-F/M-SBK	VF-ABK	5.15	84.16	1.78	1.10	7.7	NE	291u	0.13			
B			3-C/VC-ABK	M-ABK	4.57	85.10	0.00	1.15	8.0	NE	199u	0.14			
<b>Plot: Occupied 2</b>				% Surface R.F.: <1				% Veg. Cover: 5				Crack Density:			
Plot Species: BRTE/MONU/CIPE5															
Compaction (Number strikes to depth) from (cm): 0				5cm: 1		10cm: 5		15cm: 8		20cm:15		25cm: 20		30cm: 27	
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	% CCE			
A	4.32		2-F/M-GR	VF-GR	6.31	81.20	0.50	1.16	8.4	NE	194u	0.19			
B			3-M/VC-SBK	F-GR	6.92	80.85	0.00	1.23	8.3	NE	176u	0.18			
<b>Plot: Not Occupied 1</b>				% Surface R.F.: 0				% Veg. Cover: 10				Crack Density:			
Plot Species: MONU/BRTE/LAOC3/CETE5/ XYVE															
Compaction (Number strikes to depth) from (cm): 0				5cm: 1		10cm:7		15cm: 14		20cm:30		25cm: --		30cm: --	
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	% CCE			
A	2.62		2-F/M-SBK	VF-GR	12.79	70.41	0.11	0.96	8.9	NE	292u	0.39			
B			3-M/C-SBK/MA	F-SBK	13.59	67.09	0.00	1.42	9.4	SL	373u	0.99			
<b>Plot: Not Occupied 2</b>				% Surface R.F.: 0				% Veg. Cover: <5				Crack Density:			
Plot Species: None															
Compaction (Number strikes to depth) from (cm): -2				5cm: 4		10cm: 10		15cm: 12		20cm:15		25cm: 30		30cm: --	
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	% CCE			
A	2.62		3-F/M-ABK	F-ABK	9.62	64.86	1.85	1.14	8.5	NE	348u	0.14			
B			3-M-ABK	F-ABK	5.84	69.09	0.82	1.24	8.2	NE	975u	0.14			

Table A.2. Field and laboratory data for site #2.

<b>Site Name: Baugh Reservoir 01</b>				Soil Unit: Atwell Gulch				Lat:				Long:			
Aspect: 150		Slope: 28		Slope-Length: 21		Slope Shape: VV/LL		Slope Position: BS/SH		Geomorph. Position: SS					
<b>Plot: Occupied 1</b>				% Surface R.F.: 10				% Veg. Cover: <5				Crack Density:			
Plot Species: MONU/LAOC/ LEPE/CETE5															
Compaction (Number strikes to depth) from (cm): -4				5cm: 4		10cm:4		15cm: 5		20cm:14		25cm: 40		30cm: --	
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	%R.F.	Bulk Density	pH	Eff.	E.C.	%CCE			
A	0-3	Wavy	3-F/M-SBK	F-GR	8.98	62.58	0.12	1.02	9.4	VS	314u	0.79			
B	3-18	Smooth	2-C/VC-ABK	NA	9.18	60.36	0		9.7	SL	510u	1.04			
C	18-30		3-C-SBK	VF-GR	7.98	62.77	0	1.27	9.5	VS	768u	0.30			
<b>Plot: Not Occupied 1</b>				% Surface R.F.: 0				% Veg. Cover: 0				Crack Density:			
Plot Species: None															
Compaction (Number strikes to depth) from (cm): 3				5cm: 1		10cm:1		15cm: 2		20cm: 3		25cm: 4		30cm: 4	
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	% CCE			
A	0-3	Wavy	3-F/M-ABK	F-ABK	1.72	63.92	0.13	1.17	9.6	NE	1.2m	1.04			
AB	3-8	Wavy						0.98							
B	8-23	Irregular	2-VTH-PL	PL	1.33	53.36	0	0.94	9.5	NE	3.3m	0.30			
C	23-30		F-SBK	F-GR	3.59	46.09	16.5	1.10	9.3	NE	2.7m	0.23			

Table A.3. Field and laboratory data for site #3.

<b>Site Name: Black Hills 01</b>			Soil Unit: Shire			Lat:			Long:						
Aspect: 200		Slope: 38		Slope-Length: 11.3		Slope Shape: LL		Slope Position: BS/SH		Geomorph. Position: SS					
<b>Plot: Occupied 1</b>			% Surface R.F.: 0			% Veg. Cover: 10			Crack Density:						
Plot Species: ERGO/SCLI															
Compaction (Number strikes to depth) from (cm): 3				5cm: 2		10cm:1		15cm: 4		20cm:5		25cm: 4		30cm: 4	
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	%R.F.	Bulk Density	pH	Eff.	E.C.	%CCE			
A	7.62		3-F/M-SBK	VF-ABK	0.43	66.03	0.0	1.13	9.7	NE	324u	1.32			
B			3-C/VC-SBK	M-SBK	0.37	62.50	0.0	1.28	9.6	SL	345u	1.82			
<b>Plot: Not Occupied 1</b>			% Surface R.F.: 0			% Veg. Cover: 0			Crack Density:						
Plot Species: None															
Compaction (Number strikes to depth) from (cm): 2				5cm: 2		10cm:0		15cm: 0		20cm:1		25cm: 1		30cm: 2	
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	% CCE			
A	7.62		3-F/C-ABK	F/ABK	0.78	61.68	0.03	1.14	9.1	SL	1.5m	4.72			
B			2-C-SBK	VF-GR	0.83	56.92	0.61	1.14	9.2	VS	2.8m	0.68			

Table A.4. Field data for sites #4, #5, #6, and #7

<b>Site Name: Black Hills 02</b>		Soil Unit: Shire		Lat:		Long:	
Aspect: 100	Slope: 10	Slope-Length: 5.3	Slope Shape: LL	Slope Position: SU		Geomorph. Position: IF	
<b>Site Name: Black Hills 03</b>		Soil Unit: Shire		Lat:		Long:	
Aspect: 160	Slope: 35	Slope-Length: 17.3	Slope Shape: LL	Slope Position: BS/FS		Geomorph. Position: SS	
<b>Site Name: Black Hills 04</b>		Soil Unit: Shire		Lat:		Long:	
Aspect: 140	Slope: 42	Slope-Length: 47	Slope Shape: LL	Slope Position: BS		Geomorph. Position: SS	
<b>Site Name: Black Hills 05</b>		Soil Unit: Shire		Lat:		Long:	
Aspect: 160	Slope: 25	Slope-Length: 44	Slope Shape: LL	Slope Position: SH/SU		Geomorph. Position: NS/SS	



Table A.5. Field and laboratory data for site #8.

<b>Site Name: Black Hills 06</b>			Soil Unit: Shire			Lat:			Long:			
Aspect: 100		Slope: 30		Slope-Length: 42		Slope Shape: LL		Slope Position: BS/TS		Geomorph. Position: SS		
<b>Plot: Occupied 1</b>			% Surface R.F.: 0			% Veg. Cover: 25			Crack Density:			
Plot Species: BRTE/LAOC/ERGO/ LOOR/STCO6												
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	%R.F.	Bulk Density	pH	Eff.	E.C.	%CCE
A	3.12		3-M-SBK	F-SBK	1.18	81.40	0	0.88	8.7	NE	380u	NA
B			3-C/VC-ABK	M-SBK	1.13	82.55	0	1.22	8.7	VS	410u	0.59
<b>Plot: Not Occupied 1</b>			% Surface R.F.: 0			% Veg. Cover: 30			Crack Density:			
Plot Species: CIPE/BRTE/ HEAN/LAOC/OECA												
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	% CCE
A	6.12		3-F/M-SBK	VF-GR	0.99	80.61	0	0.88	8.8	VS	191u	0.69
B			1-C/VC-SBK/MA	MA	0.95	80.90	0	1.22		VS		0.99

Table A.6. Field and laboratory data for site #9.

<b>Site Name: Black Hills 07</b>			Soil Unit: Shire			Lat:			Long:					
Aspect: 170		Slope: 25		Slope-Length:		Slope Shape: LL		Slope Position: TS		Geomorph. Position: SS				
<b>Plot: Occupied 1</b>			% Surface R.F.: 5			% Veg. Cover: 25			Crack Density:					
Plot Species: ERTR13/MONU/BRTE/CYBU/ CIPE5														
Compaction (Number strikes to depth) from (cm): -1				5cm: 8		10cm:8		15cm: 24		20cm:25		25cm: 29		30cm: --
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	%R.F.	Bulk Density	pH	Eff.	E.C.	%CCE		
A	5.12	NA	3-M-ABK	F-SBK	1.95	80.00	0.26	1.07	9.2	VS	384u	1.52		
B			3-F/M-ABK	F-ABK	1.87	78.57	1.29	1.03	9.4	VS	563u	1.47		
<b>Plot: Not Occupied 1</b>			% Surface R.F.: 20			% Veg. Cover: <1			Crack Density:					
Plot Species: BRTE/ALTE/LAOC3/MONU/CYBU														
Compaction (Number strikes to depth) from (cm): 0				5cm: 2		10cm:3		15cm: 10		20cm:25		25cm: 30		30cm: --
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	% CCE		
A	6.12		3-M-ABK	F-ABK	5.55	71.17	2.42	1.37	9.3	SL	503u	2.27		
B			3-M/C-ABK	F-ABK	6.33	68.12	1.07	1.30	9.6	SL	554u	2.23		

Table A.7. Field data for sites #10, #11, and #12.

<b>Site Name: Black Hills 08</b>		Soil Unit: Shire		Lat:		Long:	
Aspect: 200	Slope: 35	Slope-Length:	Slope Shape: LL	Slope Position: BS		Geomorph. Position: HS	
<b>Site Name: Black Hills 09</b>		Soil Unit: Shire		Lat:		Long:	
Aspect: 60	Slope: 35	Slope-Length: 48	Slope Shape: LL	Slope Position: BS		Geomorph. Position: NS	
<b>Site Name: Black Hills 10</b>		Soil Unit: Shire		Lat:		Long:	
Aspect: 340	Slope: 35	Slope-Length: 5	Slope Shape: LL	Slope Position: BS		Geomorph. Position: HS	

Table A.8. Field and laboratory data for site #13.

<b>Site Name: Black Hills 11</b>			Soil Unit: Shire			Lat:			Long:						
Aspect: 140		Slope: 25		Slope-Length:		Slope Shape: LL		Slope Position: BS		Geomorph. Position: NS					
<b>Plot: Occupied 1</b>			% Surface R.F.: 10			% Veg. Cover: 15			Crack Density:						
Plot Species: BRTE															
Compaction (Number strikes to depth) from (cm): 0				5cm: 1		10cm:4		15cm: 9		20cm:19		25cm:		30cm: 27	
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	%R.F.	Bulk Density	pH	Eff.	E.C.	%CCE			
A	3.12		3-F/M-SBK	F-GR	3.55	69.61	0	1.01	9.1	ST	280u	NA			
B			3-C/VC-SBK	M-SBK	3.15	65.49	0.28	1.22	9.5	ST	257u	6.25			
<b>Plot: Not Occupied 1</b>			% Surface R.F.: 1			% Veg. Cover: <5			Crack Density:						
Plot Species: BRTE/ELEL5/SPCO/SCLI/CANU/CIPE5/CANU															
Compaction (Number strikes to depth) from (cm): 5				5cm: 0		10cm: 1		15cm: 6		20cm:18		25cm: 28		30cm: 32	
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	% CCE			
A	7.62		1-F/M-SBK	F-GR	4.37	44.51	0.28	1.24	7.6	SL	3.3m	2.36			
B	6.87		1-M/C-SBK	F-GR	3.08	35.73	7.89	1.52	7.7	SL	1.9m	2.45			

Table A.9. Field and laboratory data for site #14.

<b>Site Name: Coon Hollow 01</b>				Soil Unit: Atwell Gulch				Lat:				Long:			
Aspect: 190		Slope: 50		Slope-Length: 57.5		Slope Shape: LL		Slope Position: BS		Geomorph. Position: SS					
<b>Plot: Occupied 1</b>				% Surface R.F.: 25				% Veg. Cover: <1				Crack Density:			
Plot Species: None															
Compaction (Number strikes to depth) from (cm): 15				5cm: 0		10cm:0		15cm: 0		20cm:1		25cm: 4		30cm: 3	
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	%R.F.	Bulk Density	pH	Eff.	E.C.	%CCE			
A	3.12		3-F/M-SBK	F-GR	5.89	77.01	4.67	0.94	9.1	NE	388u	0.57			
B			3-F/M-SBK	F-GR	5.28	77.07	0.57	1.10	8.0	NE	3.1m	0.58			
<b>Plot: Not Occupied 1</b>				% Surface R.F.: 20				% Veg. Cover: 0				Crack Density:			
Plot Species: None															
Compaction (Number strikes to depth) from (cm): 2				5cm: 1		10cm:1		15cm: 3		20cm:4		25cm: 4		30cm: 3	
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	% CCE			
A	3.12		3-F/M-ABK	F-SBK	12.93	64.97	8.26	1.03	8.6	SL	4.2m	2.8			
B			0/1-F/M-SBK/GR	VF-GR	7.69	62.84	3.51	0.96	8.1	VS	4.9m	1.4			
<b>Plot: Not Occupied 2</b>				% Surface R.F.: <5				% Veg. Cover: 10				Crack Density:			
Plot Species: Unidentified perennial bunch grass/ ERGO															
Compaction (Number strikes to depth) from (cm): 2.5				5cm: 1		10cm:1		15cm: 2		20cm:4		25cm: 5		30cm: 13	
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	% CCE			
A	4.12		1-F/M-SBK	VF-GR	9.95	64.11	5.88	1.19	7.9	SL	847u				
B			2-F/M-SBK	F-GR	11.03	59.30	14.29	1.17	7.5	SL	2.8m				
<b>Plot: Not Occupied 3</b>				% Surface R.F.: 30				% Veg. Cover: 0				Crack Density:			
Plot Species: None															
Compaction (Number strikes to depth) from (cm): -2				5cm: 0		10cm:0		15cm: 1		20cm:1		25cm: 2		30cm: 2	
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	% CCE			
A	5.62		3-M/C-ABK	F-GR	5.62	50.56	0.48	1.09	8.7	NE	2.8m				
BC			F-SBK/GR	VF-GR	4.24	44.54	0.32	1.08	7.8	NE	11m				

Table A.10. Field and laboratory data for site #15.

<b>Site Name: Dry Fork Road 01</b>				Soil Unit: Atwell Gulch				Lat:				Long:			
Aspect: 47		Slope: 17		Slope-Length:			Slope Shape: LL		Slope Position: BS			Geomorph. Position: SS			
<b>Plot: Occupied 1</b>				% Surface R.F.: 0				% Veg. Cover: 30				Crack Density:			
												Plot Species: CIPE5/HEAN3/ERTR13			
Compaction (Number strikes to depth) from (cm): -2.5				5cm: 1		10cm:3		15cm: 10		20cm:21		25cm: 36		30cm: --	
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	%R.F.	Bulk Density	pH	Eff.	E.C.	%CCE			
A	3.12		3-M-SBK	F-GR	2.67	82.30	0.49	0.89	8.8	NE	260u	NA			
B			3-M/C-PR/SBK	M-SBK	2.77	79.18	0.03	1.92	9.1	NE	212u	0.81			
<b>Plot: Occupied 2</b>				% Surface R.F.: 0				% Veg. Cover: 25				Crack Density:			
												Plot Species: MONU/ERTR13/			
Compaction (Number strikes to depth) from (cm): 8				5cm: 0		10cm:2		15cm: 6		20cm:9		25cm: 13		30cm: 18	
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	% CCE			
A	3.52		3-F/M-SBK	F-GR	6.54	73.80	0.10	0.86	8.9	VS	169u	1.22			
B			3-M/C-ABK	F-ABK	6.67	76.01	0.67	1.26	9.0	SL	241u	1.39			
<b>Plot: Not Occupied 1</b>				% Surface R.F.: <5				% Veg. Cover: 20				Crack Density:			
												Plot Species: CIPE5/GRFA/ERGO/OECA10			
Compaction (Number strikes to depth) from (cm): 1				5cm: 3		10cm:13		15cm: 34		20cm:--		25cm: --		30cm: --	
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	% CCE			
A	5.12		2-M-SBK	F-SBK	5.35	60.69	1.50		8.8	ST	203u	4.37			
B			3-M/C-ABK	F-GR	8.22	48.22	1.75	1.55	9.1	ST	239u	3.78			
<b>Plot: Not Occupied 2</b>				% Surface R.F.: 0				% Veg. Cover: 10				Crack Density:			
												Plot Species: PLINO			
Compaction (Number strikes to depth) from (cm): 0				5cm: 6		10cm:13		15cm: 20		20cm:32		25cm: --		30cm: --	
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	% CCE			
A	7.62		1-F/M-SBK	VFR-SG	51.73	27.72	0.03	1.27	8.3	VS	129u	2.30			
B			1-TH/PL	F-GR	41.73	32.41	0.31	1.35	8.5	ST	102u	3.42			

Table A.11. Field data for sites #16 and #17.

<b>Site Name: Horsethief 01</b>		Soil Unit: Shire		Lat:		Long:	
Aspect: 140	Slope: 35	Slope-Length: 22	Slope Shape: LL	Slope Position: BS	Geomorph. Position: SS		
<b>Site Name: Horsethief 02</b>		Soil Unit: Shire		Lat:		Long:	
Aspect: 220	Slope: 33	Slope-Length: 22	Slope Shape: LL/VV	Slope Position: SH/SU	Geomorph. Position: SS/IF		

Table A.12. Field and laboratory data for sites #18 and #19.

<b>Site Name: Horsethief 03</b>				Soil Unit: Shire			Lat:			Long:			
Aspect: 240		Slope: 33		Slope-Length: 21		Slope Shape: LL		Slope Position: BS		Geomorph. Position: SS			
<b>Site Name: Horsethief 04</b>				Soil Unit: Shire			Lat:			Long:			
Aspect: 120		Slope: 50		Slope-Length: 25.7		Slope Shape: LL		Slope Position: BS		Geomorph. Position: SS/SH			
<b>Plot: Occupied 1</b>				% Surface R.F.: <5			% Veg. Cover: 10			Crack Density:			
Plot Species: BRTE/ LAOC/													
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	%R.F.	Bulk Density	pH	Eff.	E.C.	%CCE	
A	2.62		3-F/M-SBK	VF-GR	5.71	66.86	0	0.76	9.3	VS	303u	0.99	
B	NA		3-M/C-ABK	F-ABK	4.98	69.17	0.03	1.17	9.5	SL	429u	1.60	
<b>Plot: Occupied 2</b>				% Surface R.F.: 0			% Veg. Cover: 10			Crack Density:			
Plot Species: BRTE													
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	% CCE	
A	2.62		3-F/M-SBK	VF-GR	12.89	62.45	0.52	0.96	9.1	VS	238u	1.05	
B	NA		3-M/C-SBK	VF-GR	12.14	62.90	1.64	1.09	9.6	SL	243u	1.67	
<b>Plot: Not Occupied 1</b>				% Surface R.F.: 0			% Veg. Cover: 20			Crack Density:			
Plot Species: CIPE5/HEAN/BRTE/													
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	% CCE	
A	3.62		1- SBK	F-GR	1.74	59.72	0.08	1.01	8.4	NE	132u	0.67	
B	NA		3-M/C-COL	F-SBK	1.41	58.59	1.32	1.37	8.5	VS	197u	0.88	
<b>Plot: Not Occupied 2</b>				% Surface R.F.: 0			% Veg. Cover: 60			Crack Density:			
Plot Species: Perennial grass													
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	% CCE	
A	2.62		3-F/M-ABK	VF-ABK	0.12	61.85	0	1.05	9.5	NE	1.0m		
B	NA		1-VF/F-GR	F-GR	0.14	56.60	0	1.21	8.8	NE	5.6m	0.11	



Table A.13. Field and laboratory data for site #20.

<b>Site Name: Horsethief 06</b>			Soil Unit: Shire			Lat:			Long:			
Aspect:		Slope:		Slope-Length:		Slope Shape: VV		Slope Position:SU/SH		Geomorph. Position: NS		
<b>Plot: Occupied 1</b>			% Surface R.F.: 0			% Veg. Cover: 10			Crack Density:			
Plot Species: BRTE												
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	%R.F.	Bulk Density	pH	Eff.	E.C.	%CCE
A	3.62		3-F/M-ABK	F-GR	1.12	78.02	0	0.87	8.7	NE	163u	0.01
B	NA		3-M/C-SBK	VF-ABK	0.98	77.13	0	1.06	9.4	NE	368u	0.23
<b>Plot: Not Occupied 1</b>			% Surface R.F.: 0			% Veg. Cover: 0			Crack Density:			
Plot Species: None												
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	% CCE
A	6.62		3-F/M-ABK- SBK	F-GR	2.23	51.56	0.5	0.87	8.1	NE	128u	0.12
B	NA		3-M/C-SBK	F-SBK	2.35	53.46	0.29	1.16	8.8	NE	128u	0.15

Table A.14. Field and laboratory data for site #21.

<b>Site Name: Motor Hollow 01</b>			Soil Unit: Atwell Gulch		Lat:			Long:				
Aspect: 260		Slope: 35	Slope-Length:		Slope Shape: LL	Slope Position: BS		Geomorph. Position: SS				
<b>Plot: Occupied 1</b>			% Surface R.F.: 0		% Veg. Cover: <5		Crack Density:					
Plot Species: MONU/CETE5												
Compaction (Number strikes to depth) from (cm): 0				5cm: 3	10cm:7	15cm: 13	20cm:17	25cm: 34	30cm: --			
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	%R.F.	Bulk Density	pH	Eff.	E.C.	%CCE
A	0-2	Regular	3-F/M-SBK	F-SBK	4.85	82.28	0.29	0.92	7.7	NE	113u	0.13
BA	2-10	Wavy	3-C-SBK	F-ABK	4.79	83.02	0.1			NE		
B	10-30	Wavy	2-C-SBK/PR	F-SBK	4.69	81.09	0		7.8	NE	160u	0.11
<b>Plot: Not Occupied 1</b>			% Surface R.F.: 30		% Veg. Cover: 15		Crack Density:					
Plot Species: BRTE/SCLI/ERICI6												
Compaction (Number strikes to depth) from (cm): 0				5cm: 1	10cm:4	15cm: 4	20cm:15	25cm: 22	30cm: 27			
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	% CCE
A	0-3.5		2-M-SBK	F-GR	16.03	63.85	0	1.10	7.9	NE	103u	0.13
AB	3.5-9.5	wavy	3-C-SBK	F-GR	12.96	68.54	13.8		8.0	NE	194u	0.11
B	9.5-30		2-C-SBK/COL	F-SBK	11.60	70.50	0		8.5	NE	275u	0.15
B (#2)			1-VC-SBK/PR				0					

Table A.15. Field and laboratory data for site #22.

Site Name: Plateau Creek 01			Soil Unit: Shire			Aspect: 190							
<b>Plot: Occupied 1</b>			% Surface R.F.: 5			% Veg. Cover: <1			Crack Density:				
			Plot Species: None										
Compaction (Number strikes to depth) from (cm): 1			5cm: 4		10cm:8		15cm: 18		20cm:35		25cm: --		30cm: --
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	%R.F.	Bulk Density	pH	Eff.	E.C.	%CCE	
A	3.62		3-F/M-SBK	VF-GR	7.52	39.18	0.76	1.04	9.5	ST	210u	4.23	
B	7.62		3-C/VC-ABK	F-GR	11.64	30.67	1.51	1.25	9.9	ST	297u	7.16	
<b>Plot: Occupied 2</b>			% Surface R.F.: 0			% Veg. Cover: 20			Crack Density:				
			Plot Species: ERGO/MONU										
Compaction (Number strikes to depth) from (cm): 1			5cm: 3		10cm:8		15cm: 14		20cm:26		25cm: 43		30cm: --
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	% CCE	
A	1.63		3-VF/M-SBK	F-GR	0.66	74.78	0	1.22	9.0	VS	310u	0.72	
B	NA		3-C/VC-SBK	VF-ABK	0.80	60.84	0	1.24	9.6	VS	382u	1.19	
C	NA		3-C/VC-ABK	F-GR	17.33	32.92		1.33	9.8	SL	650u	0.98	
<b>Plot: Not Occupied 1</b>			% Surface R.F.: <1			% Veg. Cover: <1			Crack Density:				
			Plot Species: ERGO										
Compaction (Number strikes to depth) from (cm): -0.5			5cm: 3		10cm:9		15cm: 12		20cm:10		25cm: 11		30cm: 14
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	% CCE	
A	0-6	Wavy	1-F/M-SBK	VF-SGR	59.75	22.84	3.10	1.32	8.7	VE	83.8u	6.78	
B	6-30	Wavy	3-TN-PL	VF-SGR	66.64	19.29	58.82	1.64		ST			
<b>Plot: Not Occupied 2</b>			% Surface R.F.: 0			% Veg. Cover: 10			Crack Density:				
			Plot Species: GUSA/ Unidentified Astragalus sp.										
Compaction (Number strikes to depth) from (cm): -2			5cm: 0		10cm:1		15cm: 2		20cm:1		25cm: 4		30cm: 3
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	% CCE	
A	1.63		3-VF/M-ABK	VF-ABK	0.73	70.74	0	1.57	9.7	NE	492u	0.33	
B			3-VC-SBK	NA	0.48	64.34		1.05	9.5	VS	1.2m		
C			0-VF/F-GR	NA	0.70	35.23	6.67		9.2	VE	3.2m	0.17	

Table A.16. Field and laboratory data for site #23.

<b>Site Name: Sand Wash 01</b>			Soil Unit: Atwell Gulch		Lat:			Long:							
Aspect: 101		Slope: 70		Slope-Length: 48		Slope Shape: LL		Slope Position: BS		Geomorph. Position: SS					
<b>Plot: Occupied 1</b>			% Surface R.F.: 25			% Veg. Cover: <5			Crack Density:						
Plot Species: MONU/CETE3/PHCR/SCLI/ Unidentified Asteraceae and Poaceae															
Compaction (Number strikes to depth) from (cm): -4				5cm: 5		10cm:11		15cm: 27		20cm:--		25cm: --		30cm: --	
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	%R.F.	Bulk Density	pH	Eff.	E.C.	%CCE			
A	2	Surface	3-F/M-SBK	VF-GR	24.03	40.40	10	0.79	9.3	SL	205u	3.62			
AB	4	Wavy	--	--	--	--	--		--	--	--	--			
B	17	Smooth	3-M/C-SBK	VF-GR	23.94	39.94	10.45	1.26	9.7	SL	273u	2.32			
C	30	Wavy	0-F-GR	F-GR	14.04	32.79	30.76		--	--	--	--			
<b>Plot: Not Occupied 1</b>			% Surface R.F.: <1			% Veg. Cover: 0			Crack Density:						
Plot Species: None															
Compaction (Number strikes to depth) from (cm): -2				5cm: 0		10cm:0		15cm: 1		20cm:1		25cm: 15		30cm: 20	
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	%CCE			
A	<1	Surface	3-VC-SBK	VF-GR	13.35	39.04	0		9.7	NE	NA	0.35			
B	4	Smooth	3-VC-SBK	F-GR	11.44	41.03	0		9.1	VS	857u	0.27			
C?	9	Smooth	2-VK-PL	--			100		--	--	--	--			
Cr	20	Wavy	Rocks	--	--	--	100								

Table A.17. Field and laboratory data for site #24.

<b>Site Name: Sulfur Gulch 01</b>			Soil Unit: Atwell Gulch		Lat:			Long:						
Aspect:		Slope:		Slope-Length:		Slope Shape: LL		Slope Position:BS		Geomorph. Position: SS				
<b>Plot: Occupied 1</b>			% Surface R.F.: <10			% Veg. Cover: 5			Crack Density:					
Plot Species: BRTE														
Compaction (Number strikes to depth) from (cm): 2				5cm: 1		10cm:1		15cm: 1		20cm:2		25cm: 6		30cm: --
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	%R.F.	Bulk Density	pH	Eff.	E.C.	%CCE		
A	2.62		3-F-SBK	F-GR	4.91	79.03	0.11	0.96	9.5	NE	254u	0.42		
B	NA		3-C/VC-ABK	F-GR	4.81	80.34	0.03	1.10	9.2	NE	408u	0.51		
<b>Plot: Not Occupied 1</b>			% Surface R.F.: 20			% Veg. Cover: 0			Crack Density:					
Plot Species: None														
Compaction (Number strikes to depth) from (cm): -4				5cm: 2		10cm:3		15cm: 6		20cm:8		25cm: 8		30cm: --
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	% CCE		
A	6.12		3-M-ABK	F-GR	26.43	52.13	3.38	0.99	9.1	NE	1.7m	0.27		
B			3-M-SBK	C-GR	38.55	44.42	10.7	1.23	9.2	NE	3.9m	0.24		

Table A.18. Field and laboratory data for site #25.

<b>Site Name: New Site (Sunnyside) 01</b>			Soil Unit: Shire			Lat:			Long:			
Aspect:		Slope:		Slope-Length:		Slope Shape:		Slope Position:		Geomorph. Position:		
<b>Plot: Occupied 1</b>			% Surface R.F.: 0			% Veg. Cover: 0			Crack Density:			
Plot Species: None												
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	%R.F.	Bulk Density	pH	Eff.	E.C.	%CCE
A	5.62		3-F/M-SBK	F-GR	4.69	64.14	0.49	0.96	9.3	SL	226u	1.56
B			3-M/C-SBK	F-ABK	2.15	53.57	1.06	1.29	9.6	SL	355u	1.77
C			Rocks				100					
<b>Plot: Not Occupied 1</b>			% Surface R.F.: 0			% Veg. Cover: 0			Crack Density:			
Plot Species: None												
Horizon	Depth	Boundary	Structure	Parting to	%Sand	%Clay	% R.F.	Bulk Density	pH	Eff.	E.C.	% CCE
A	7.62		2-M-SBK	F-GR	4.06	68.78	0.03	1.20	8.1	VS	148u	1.52
B			1-MA	MA	3.07	81.01	0.22		8.8	VS	113u	1.78
C			1-MA	MA	5.38	71.67	0		9.0	SL	166u	2.04