SEED PREDATION BY OWYHEE HARVESTER ANTS AND THE POTENTIAL OF

SEED INTRODUCTIONS IN RECOVERY EFFORTS FOR SLICKSPOT

PEPPERGRASS

by

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ABSTRACT

Slickspot peppergrass (*Lepidium papilliferum*) is a rare plant endemic to the sagebrush-steppe habitat in southwestern Idaho. Within sagebrush-steppe, the plant is restricted to microsites known as "slick spots"– shallow depressions of soil characterized by distinct clay layers and surface water retention that is higher than that of surrounding areas. Having one of the highest extirpation rates among rare plant taxa in Idaho, and considering its unique habitat requirements, limited range, and declining numbers, land managers and conservationists have voiced concern regarding the species' long-term viability. While range-wide declines in slickspot peppergrass have been attributed largely to the loss of and disturbance to suitable habitat, seed predation by Owyhee harvester ants (*Pogonomyrmex salinus*) has recently been identified as another potential threat to *L. papilliferum* survival. However, the extent to which harvester ants remove seeds from slick spots is an unanswered question. To address this question, I conducted a field experiment to examine Owyhee harvester ant foraging behavior and to quantify the loss of seed by individual slickspot peppergrass plants. Additionally, I examined the potential for a dilution effect where the proportion of seeds lost per plant would be inversely related to the total number of flowering plants found in a slick spot. The study showed that seed predation by harvester ants represents a significant threat to seed recruitment in *L. papilliferum* populations, as individual plants sustained an average seed loss of 73.2% (N=20, range = 0–97.7%). In slick spots with >150 flowering plants, seed loss was proportionally lower compared to slick spots that contained fewer plants, suggesting that

harvester ant colonies may be reaching a threshold of consumption when the quantity of available seeds exceeds their capacity to collect and consume those seeds.

In a separate experiment, I examined the potential of seed introductions as a recovery tool for conservation and management efforts aimed at slickspot peppergrass. I demonstrated that *L. papilliferum* can successfully germinate, flower, and fruit when seeds are released into unoccupied slick spots. The total number of plants produced (N=9) was very low compared to the number of seeds released (N=19,800), although some of the seeds were exposed to seed predation by ants. Because poor climatic conditions in the year of study may have contributed to the low numbers of seedlings, further investigation into the use of seed introductions in recovery efforts of *L. papilliferum* is warranted. Overall, my research speaks to a plant species that living in a changing environment where the interactions between the plant and its natural enemies such as harvester ants are shifting, and it has highlighted the need for further investigations aimed at recovery tools such as introductions in the management and conservation of this rare plant species.

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Abstract

Seed predation can significantly reduce the reproductive success of individual

plants and their populations. The consequences of seed predation often are most

pronounced for rare plant species, where the loss of seeds can have a disproportionate

effect on populations when compared to common plant species. The present study

examined the impact of seed predation by Owyhee harvester ants (*Pogonomyrmex

salinus*) on slickspot peppergrass (*Lepidium papilliferum*), a rare mustard endemic to

sagebrush-steppe habitat in southwest Idaho. Within sagebrush-steppe, the plant is

restricted to microsites known as "slick spots"– shallow depressions of soil characterized

by distinct clay layers and surface water retention that is higher than that of surrounding

areas. Harvester ants frequently nest within *L. papilliferum* habitat and readily consume

the plant's seeds. I conducted a controlled field experiment to quantify seed loss by

individual plants as a result of seed predation by harvester ants, and whether the

proportion of seeds lost per plant was inversely related to the total number of flowering

plants within a slick spot. Individual plants exposed to harvester ants experienced an

average seed loss of 73.2% (N=20, range = 0–97.7%) relative to those individuals

shielded from ant activity. Seed loss was proportionally lower in slick spots with >150

plants than those with 150 or fewer, suggesting that within the natural range of plant

densities found in slick spots, there is an upper threshold to the number of seeds that can

be collected and consumed by ants. Thus, while seed predation by harvester ants

represents a threat to offspring recruitment in *L. papilliferum* populations, populations

may be less affected by seed predation if cumulative seed output within slick spots

exceeds the capacity of ants to consume those seeds.

Introduction

Many plants experience large losses of biomass to herbivores (Cyr and Pace

1993) that can influence the reproductive success and survival of individual plants and

their populations, as well as the evolution of life history traits (Fletcher et al. 2001a,b,

Kettenring et al. 2009, Vergeer and Kunin 2011, Martin and Meinke 2012, Sharp

Bowman et al. 2017). Measuring the extent of herbivory, and its fitness consequences to

individual plants is a critical first step in the assessment of how herbivory impacts plant

populations. These impacts may vary widely in response to the unique physical,

physiological, and/or behavioral characteristics associated with each herbivore and plant

species, as well as the environmental conditions present in a particular ecosystem. In

some instances, herbivory appears to enhance plant fitness by stimulating growth and

reproduction (Inouye 1982, Abhilasha and Joshi 2009), inducing defense mechanisms

(McArt et al. 2013) and increasing the rates of seed dispersal (Wilson et al. 2012). In

most instances, however, herbivory has unfavorable effects on growth, survival,

dispersal, and fitness of plants (Bruelheide and Scheidel 1999, Rand 2002, Poveda et al.

2003, Barber et al. 2011). In addition to the costs generally associated with herbivory,

(i.e., loss of photosynthetic area), herbivory on seeds (commonly referred to as seed

predation or granivory) can adversely affect individual plants and their populations

because seed predation imposes an immediate cost on offspring recruitment (Crawley

1989, Weppler and Stocklin 2006, Kolb et al 2007, Burgos et al 2008).

The consequences of herbivory may be particularly severe for rare plant species,

where any adverse effects of herbivory on survival and recruitment could limit or prevent

population maintenance or recovery (Kettenring et al. 2009, Ancheta and Heard 2011,

Martin and Meinke 2012, Leonard and Auken 2013). Herbivory that results in damage or

loss of seeds is a major contributing factor to low recruitment of individuals into rare

plant populations (Crawley 2000, Méndez et al. 2004, Albert et al. 2005, Raju et al.

2009). Thus, rare species, which by definition generally experience limited abundance,

limited habitat availability, and restricted geographic ranges (Rabinowitz 1981), are at a

greater risk of population decline and extinction in the face of herbivory than are more

common species (Gaston 1994, Johnson 1998, Crawley 2000, Matthies et al. 2004,

Ancheta and Heard 2011). In the present study, I examine the effects of seed predation by

Owyhee harvester ants, *Pogonomyrmex salinus* (Olsen) (Hymenoptera: Formicidae), on

slickspot peppergrass, *Lepidium papilliferum* [(L. Henderson) A. Nels. and J.F. Macbr.]

(Brassicaceae), a rare and threatened plant endemic to southwestern Idaho.

Harvester ants in the genus *Pogonomyrmex* are voracious seed predators that have

the capacity to remove large numbers of seeds from their environment (Tschinkel 1999,

MacMahon et al. 2000). While some *Pogonomyrmex* species forage individually with

little or no coordination among nestmates (Gordon 1991, Gordon 1995, MacMahon et al.

2000), others, including *P. salinus*, forage collectively along trunk trails that lead to food

patches (Janzen 1971, Hölldobler and Wilson 1990, Taber 1998, Hölldobler et al. 2001).

The success of collective foraging relies upon the ability of workers to recruit nestmates to profitable food patches. Using pheromones laid along trunk trails by returning foragers, as well as chemical cues produced by seeds in the vicinity of nests, harvester ants actively recruit nestmates to increase the number of foragers exploiting patches of food (Brown et al. 1979, Hölldobler 1976, Hölldobler et al. 2001, Johnson 2000, Johnson 2001, Greene et al. 2013). Mobilization of foragers to food patches decrease mean individual search times and increase the rate of harvest as well as the cumulative number of seeds collected from within a patch (Brown et al. 1979, Hölldobler 1976, Hölldobler et al. 2001, Johnson 2000, Johnson 2001, Greene et al. 2013). Although the proportion of seeds removed from patches by harvester ants may vary for a variety of reasons (i.e. seed species, seed abundance, alternative seed availability, nutritional requirements of colony, etc.), the removal of preferentially harvested seed species can be as high as 100%, and may lead to reductions in plant abundance and shifts in plant distributions (Anderson and Ashton 1985, Hobbs 1985, Crist and MacMahon 1992, Ireland and Andrew 1995).

Lepidium papilliferum is a rare plant endemic to sagebrush-steppe habitat in southwestern Idaho. Within sagebrush-steppe, the plant is restricted to microsites known as "slick spots" (Moseley 1994) – shallow depressions of natric soils characterized by distinct clay layers and surface water retention that is higher than that of surrounding areas (Fisher et al. 1996). The unique habitat requirements of *L. papilliferum*, along with the plant's limited range and declining numbers (see Mancuso and Moseley 1998, Menke and Kaye 2006, Sullivan and Nations 2009, Bond 2017) has raised concern among land managers and conservationists regarding the species' long-term viability. Range-wide declines in *L. papilliferum* have been largely attributed to the loss of suitable habitat as a

result of urbanization, agriculture, livestock grazing, the spread of invasive species, and

an increase in wildfire frequency (Moseley 1994, United States Fish and Wildlife Service

2016). More recently, seed predation by Owyhee harvester ants has been identified as a

potentially significant source of seed loss to *L. papilliferum* (White and Robertson 2009a,

Robertson 2015). Seeds of *L. papilliferum* are preferentially harvested by *P. salinus*

(Schmasow and Robertson 2016), and in a preliminary analysis of seed loss to harvester

ants, White and Robertson (2009a) reported that ants can remove >40% of mature fruit

directly from plants and up to 90% of seeds experimentally placed on the ground beneath

plants. However, this latter statistic was based on the removal of only a small number of

seeds (N=10) placed beneath plants. The extent to which harvester ants collect and

consume total seed output of individual plants and within slick spots remains unknown.

For many plant species, the production of large seed crops may ensure the

survival of enough seeds to sustain a population despite intense seed predation and/or

extreme environmental conditions. While high-density resource patches have the

potential to attract a greater number of predators (i.e., a positive numerical response),

predation risk to individual seeds in high-density patches may be offset through a dilution

effect (Lehtonen and Jaatinen 2016, Wenninger et al. 2016), particularly if seed

availability increases above a consumption threshold for colonies (Janzen 1971, Kelly

1994) and there is limited capacity for a numerical response. In Owyhee harvester ants,

foraging ranges of neighboring colonies do not overlap (Howell and Robertson 2015).

This lack of overlap means that any numerical response to increased food availability will

be limited to an individual colony's ability to recruit foragers. It has been estimated that

individual colonies of *Pogonomyrmex* ants collect and consume 50,000 to 81,000 seeds in

a given foraging season (Crist and MacMahon 1992, Pirk and Lopez de Casenave 2006), although based on the seed intake rate of *P. salinus* reported by Schmasow (2015), Robertson and Jeffries (2016) suggest that the total number of seeds collected by colonies may be considerably higher when small seeds, such as those produced by *L. papilliferum*, are prevalent in diet. However, the density-dependent effects of seed predation on *L. papilliferum* individual fitness has not been explored.

I conducted a manipulative field experiment to quantify seed predation by Owyhee harvester ants on individual *L. papilliferum* and an observational study of ant foraging behavior. Also, I investigated density-dependent effects of seed predation on *L. papilliferum* fitness by examining whether the proportion of seeds collected from individual plants was inversely related to the total number of flowering plants within their respective slick spots. I predicted that the proportion of seeds being harvested would decrease as the number of *L. papilliferum* plants increased within a slickspot, at least after a threshold of available seed numbers was met.

Materials and Methods

<u>Study Area</u>

The study was conducted from June-August in 2012 at a population of *Lepidium papilliferum* located near Melba, Idaho (43°23'14.49" N / 116°28'44.59" W. Kuna Butte SW population, Idaho Natural Heritage Program element occurrence #018A; Figure 1.1). This site was chosen because it supports a relatively large population of *L. papilliferum* and has an abundance of harvester ant colonies (~25.5 colonies/ha). Overstory vegetation at the site consisted of sparsely distributed patches of *Artemisia tridentata* (big sagebrush) and *Ericameria nauseosa* (gray rabbitbrush), while the understory was

dominated by *Poa secunda* (Sandberg bluegrass), *Bromus tectorum* (cheatgrass), and *Sisymbrium altissimum* (tumble mustard).

<u>Study Species</u>

<u>The Owyhee Harvester Ant (*Pogonomyrmex salinus*)</u>

Pogonomyrmex salinus is the northernmost member of the genus and occurs from southwestern Canada through Idaho, Washington, Oregon, northeastern California, Nevada, and western portions of Utah, Montana, and Wyoming (Figure 1.2; Cole 1968, Taber 1998). Population densities as high as 164 colonies per hectare have been recorded (Blom et al. 1991), although densities below 40 colonies per hectare are more typical (Porter and Jorgensen 1988, Blom et al. 1991, Robertson 2015). A mature *P. salinus* colony typically consists of 5,000 to 10,000 workers (MacKay 1981, Johnson 2000) and may survive about 20 years (Porter and Jorgensen 1988, MacMahon et al. 2000) as long as the founding queen survives and continues to lay eggs (Gordon 1991).

Harvester ants forage diurnally from spring to autumn whenever surface temperatures are sufficiently warm (MacKay 1981, Hobbs 1985, Crist and MacMahon 1991, Taber 1998). Daily foraging activity usually occurs in the morning and late afternoon, with periods of inactivity during the hottest portions of the day (Whitford et al. 1976, Hobbs 1985, Crist and MacMahon 1991). *Pogonomyrmex* ants are single-load, central place foragers (Brown et al. 1979, Stephens et al. 2007). They forage up to 20 m from their nest, with a majority of foraging occurring within 12 m (MacMahon et al. 2000, Burris 2004, White and Robertson 2009a). When nests are in close proximity to one another (i.e., <20 m), neighboring colonies share non-overlapping boundaries in the areas between their nests (Howell and Robertson 2015).

Although *Pogonomyrmex* ants collect seeds from a wide variety of plant species, they tend to specialize on abundant, small-seeded species (Crist and MacMahon 1992, MacMahon et al. 2000, Pirk et al. 2009, Pirk and Lopez de Casenave 2011, Ostoja et al. 2013, Schmasow and Robertson 2016). Many species also incorporate arthropods (living and dead), fungi, feces, and assorted vegetation into their diets (Hölldobler and Wilson 1990, Taber 1998, Belchior et al. 2012). Owyhee harvester ants are known to forage on seeds from a variety of plant species that include, but are not limited to, *Poa secunda* (Sandberg bluegrass), *Vulpia spp.* (fescue grass), *Bromus tectorum* (cheatgrass), *Sisymbrium altissimum* (tumble mustard)*,* and *Lepidium papilliferum* (slickspot peppergrass) (White and Robertson 2009a, Schmasow and Robertson 2016, Robertson and Schmasow 2018).

Harvester ants do not gather seeds in direct proportion to the availability of seeds on the soil surface (Hobbs 1985, Detrain and Pasteels 2000, Pirk et al. 2009, Schmasow 2015, Schmasow and Robertson 2016). In the case of *P. salinus*, the seeds of *L. papilliferum*, *P. secunda* and *S. altissimum* are preferred to those of *B. tectorum*, as indicated by the consistent overrepresentation of *L. papilliferum*, *P. secunda* and *S. altissimum* seeds in the diet, and the underrepresentation of *B. tectorum* seeds even when they are abundant on the soil surface (Schmasow and Robertson 2016). The relatively long length of *B. tectorum* seeds, along with their persistent bristled awn, makes them difficult for ants to transport. By contrast, the seeds of *L. papilliferum*, *P. secunda,* and *S. altissimum* are small enough to be transported to nests with relative ease (Schmasow and Robertson 2016).

Slickspot Peppergrass (*Lepidium papilliferum*)

Lepidium papilliferum is rare mustard endemic to sagebrush-steppe habitat in southwestern Idaho. There are approximately 91 sites containing known populations of slickspot peppergrass throughout five counties within the state of Idaho (Figure 1.3; United States Fish and Wildlife Service 2016). Another 21 populations are considered extirpated (Moseley 1994, Menke and Kaye 2006). The extirpation rate of *L. papilliferum* is the highest among rare plant taxa in Idaho (Meyer et al. 2005). Currently, the plant is federally listed as a threatened species (United States Fish and Wildlife Service 2016).

Lepidium papilliferum exhibits two main life history patterns – annuals that germinate, reproduce and die within a single season, and biennials that exist as vegetative rosettes in their first year and reproduce and die in the second year (Meyer et al. 2005). A small percentage of individuals exhibit a third life history pattern characterized by limited flowering late in the first year and a second bout of flowering the following summer (White and Robertson 2009b). For annuals and biennials, flowering typically occurs from May through July, with seed drop occurring from mid-June through August. Seed production is positively correlated with plant size. An average-sized biennial produces about 8,000 seeds, whereas annuals typically have seed sets at or below 215 seeds (Schmasow 2015). Seeds that drop to the ground become part of a persistent seed bank that in some years may represent the majority of the population (Mancuso and Moseley 1998). Current estimates are that *L. papilliferum* seeds remain viable in the seed bank for about 10 years (Meyer et al. 2005), although in laboratory studies most seeds remain viable for at least double that time (I.C. Robertson, personal communication).

Seed Predation Experiment

In June 2012, I initiated a study to quantify the amount of *L. papilliferum* seeds collected by harvester ants from individual plants. I selected 20 slick spots that were occupied by flowering *L. papilliferum* and located within 8 m of an active *P. salinus* colony. This distance is well within the 12-m foraging range typical of harvester ants (Jorgensen and Porter 1982, MacMahon et al. 2000, personal observations). Throughout this study, I considered slick spots as the experimental unit.

Within each slick spot, I selected two flowering *L. papilliferum* plants that were similar in size, flowering phenology, and distance from the ant colony. The size of the plant was recorded by its height and the overhead surface area of the flowering portion of the plant, assuming the flowers were arranged in a disk. One plant was randomly assigned to the treatment (access by ants) and the other to the control (ants excluded). A 15 cm high, 30-35 cm diameter plastic barrier was fixed 2 cm deep in the soil around the base of each control plant. Metal stakes were used to secure each barrier firmly to the ground. A similar plastic barrier was placed around treatment plants; however, these barriers were elevated on supports 3-5 cm above the ground to permit access by ants as they foraged. While small mammals (i.e. rodents) could potentially access the treatment plants, there has been no evidence of seed predation by animals other than harvester ants (I.C. Robertson, personal communication; personal observations). Metal stakes were again used to hold the barriers in place. I attached chicken wire over the tops of all barriers to deter vertebrate herbivores while allowing insect pollinators access to the plants' flowers (Figures 1.4 and 1.5).

I conducted a seed drift experiment to determine whether elevated barriers facilitated dispersal of seeds away from the plants, thereby biasing high my estimates of seed predation by ants. At 10 of the 20 slick spots used in the seed predation experiment, I selected a third *L. papilliferum* plant that I matched with the others for size, flowering phenology, and distance from the ant colony. I placed an elevated 30-35 cm diameter barrier around each of the plants, as described for the previous experiment. Then, I placed a second plastic barrier, approximately 60 cm in diameter and 15 cm in height, evenly centered around the elevated barrier (Figure 1.5). This larger barrier was fixed 2 cm deep in the soil and secured in place with metal stakes. Any seeds drifting beyond the perimeter of the inner barrier would be confined to the soil located between the inner and outer barriers.

To determine whether the proportion of seeds removed by harvester ants was dependent on the density of plants in a given slick spot, I recorded the total number of individual flowering *L. papilliferum* in each slick spot (Table 1.1). While I did not estimate plant size, I noted that the majority of flowering plants within each slick spot were medium to large sized biennials. In mid-October, once most fruits had dehisced and dropped their seeds to the ground, I collected the upper 1 cm of soil located within the perimeter of the barrier surrounding each plant using a small garden shovel. Immediately prior to collecting the soil, I shook each plant to allow any seeds from undehisced fruits to drop. The soil samples were placed individually in paper bags and returned to the laboratory. At a later date, I sifted the samples through a series of increasingly finer sieves (1.4 mm, 850 μm, 710 μm, 500 μm, and 250 μm diameter mesh, Hogentogler and Co., Inc.) to separate the *L. papilliferum* from other seeds and debris. Then, while blind to

the treatment, I searched the remainder of each sample for individual *L. papilliferum* seeds.

<u>Foraging Observations</u>

I conducted frequent observations (i.e., 3-4 times per week) of harvester ant activity at each of the 20 slick spots included in the seed predation experiment to verify the role of harvester ants as seed predators of *L. papilliferum*. These observations were initiated in June 2012 and continued through September 2012. During each set of observations I noted whether harvester ants were present in the slick spot, whether there was evidence of *L. papilliferum* fruits having been clipped by ants (see White and Robertson 2009a; Figure 1.6a), whether ants were seen transporting *L. papilliferum* fruits, seeds, or both to their nest (Figure 1.6b), the presence of *L. papilliferum* fruit husks in the midden of the ant colony, and the general status of *L. papilliferum* within the slick spot (i.e. plants flowering, fruiting, dropping seed, etc.). In mid-July, at 16 of the 20 ant colonies included in the study, I randomly aspirated between 15 and 30 harvester ants as they returned to their nest; activity at four of the colonies was too low to sample. Aspirated ants were immediately placed in glass vials (one vial per colony) along with any items they were carrying (note: when aspirated, harvester ants steadfastly hold onto food in their mandibles). The samples were returned to the laboratory and viewed under 10x magnification. To confirm the occurrence of seed predation by harvester ants on *L. papilliferum* in the experimental slick spots, I recorded the number and identity of seeds present in each sample, as well as the number of *A. tridentata* leaves, arthropods, and unidentified organic fragments that were present.

Statistical Analyses

All statistical analyses were conducted in R version 3.1.3 (R Development Core Team 2013). The confidence interval was set at 95% and the results were considered statistically significant with a $p \leq 0.05$.

Data from the seed predation experiment were analyzed using a likelihood ratio test with the R packages 'car' and 'lme4'. The data were fit into a general linear mixed model with the "*number of seeds in soil*" as a function of barrier treatment (i.e., access by ants; ants excluded; seed drift). I considered experimental slick spots which were associated with an individual ant colony as a random effect and plant height and overhead flowering area as fixed effects. A planned comparison of the least square means of the three treatment levels was performed using a Tukey's HSD test in the R package 'lsmeans'. Prior to analysis, the number of seeds counted in the soil samples were log-transformed to achieve a normal distribution of the residuals and homogeneity of variance.

As plant size can affect the number of seeds produced by an individual, I compared the sizes of plants assigned to different treatment groups (predation, control, and seed drift), using general linear mixed models with plant height and flowering area as a function of treatment group with experimental slick spot as a random effect. The values for flowering area were not distributed normally in any of the groups (i.e., treatment, control or seed drift), nor were the values for plant height in the treatment (access by ants) group. Squaring flowering area values worsened the distribution in all three groups, whereas log-transforming the values created a normal distribution in all but the treatment group. Thus, the log-transformed values for flowering area were used in the analysis. In

the case of plant height, squaring failed to normalize the distribution in the treatment group, and log transformation worsened the distributions of all the data. Therefore, I used untransformed data for plant height in the model. Both flowering area and plant height met the assumption of homoscedasticity prior to and after log transformation of their values.

To ensure that my estimates of seed removal were effective based on my pairing of the treatment and control plants by size, I used linear regression analysis to examine whether plant height was an accurate indicator of seed number in samples taken from beneath plants in the control (ants excluded) (N=20) and "seed drift" (N=10) groups. A similar analysis was used to examine whether plant flowering area was an accurate indicator of seed number produced by individual plants. A Cook's distribution was used to identify any significantly influential outliers in both data sets.

The number of flowering *L. papilliferum* plants found in each of the experimental slick spots and the proportion of seeds removed from plants experimentally exposed to ants were used to examine the potential for a satiation threshold. The experimental slick spots were binned into various plant density groups (i.e. ≥ 50, ≥ 75, ≥ 100, etc.). Paired groups were then compared using the R package 'coin' with a Wilcoxon rank-sum tests to determine the density of flowering plants where the data showed a significant difference in the proportion of seeds removed (i.e. was the proportion of seeds removed from treatment plants within slick spots containing <50 plants significantly different from those in slick spots with ≥ 50 plants?).

Results

Foraging Observations and Seed Predation Experiment

I observed harvester ants foraging in all 20 of the slick spots, and all 20 harvester

ant colonies associated with a slick spot had *L. papilliferum* fruit husks in their middens.

In 16 (80%) of the slick spots I observed ants climbing on *L. papilliferum* plants, and in

11 slick spots, I found individual plants with signs of depredated fruit in the form of

clipping. No clipping was observed in the remaining nine experimental slick spots (Table

1.2). Among the 370 ants collected via aspirator, *L. papilliferum* fruits and seeds

represented 74% of the 127 items returned to the nests. The percentage of *L. papilliferum*

fruits and seeds recovered in individual samples from colonies where I aspirated ants (N

= 16 colonies) ranged from 0% to 100% with a mean of 70% (Table 1.3).

I found no evidence of clipping on plants within sealed barriers, which confirms

that the control barriers were effective at preventing seed predation by harvester ants. The

use of barriers to exclude harvester ants from access to *L. papilliferum* had a statistically

significant effect on the number of seeds remaining on the soil surface (Figure 1.7; χ^2 [df

2] = 31.53, p <0.0001). Specifically, significantly more seeds were present in the top

layer of soil beneath plants that had ants excluded than those that were exposed to ants

(means comparison, p <0.0001) resulting in an average seed removal of 73.2% (N=20,

range = 0–97.7%) from plants with raised barriers. Likewise, significantly more seeds

were present in the top layer of soil beneath plants in the seed drift treatment (i.e., plants

with a raised inner barrier and sealed outer barrier) than those that were exposed to ants

(p = 0.0002). By contrast, there was no significant difference in the number of *L.*

papilliferum seeds present in the top layer of soil beneath plants where ants were

excluded from those used in the seed drift experiment (p = 0.47). This latter result indicates that seed drift from beyond the perimeter of barriers does not explain the low number of seeds found on the soil surface when individual *L. papilliferum* were exposed to ants. Combined with my foraging observations, the seed predation experiment indicates that seed predation by harvester ants, and not seed drift, is responsible for the reductions in seed number beneath plants exposed to ants.

There were no statistically significant differences in the flowering area (χ^2 [df = 2] = 0.602, p = 0.74) or height (χ^2 [df = 2] = 5.21, p = 0.07) of plants selected for the treatment, control, and seed drift groups. Thus, the plants selected in each experimental slick spot were reasonably matched for size and there was no systematic size bias in assignment of treatments. Likewise, there was no significant difference in plant height or the number of seeds in the top layer of soil between control and seed drift groups (i.e., when ants were denied access to the plant) (R^2 (adj) = 0.0098, $F_{1,28}$ = 1.287, p = 0.27). As expected, there was a significant positive correlation between overhead flowering area and the number of seeds recovered from the top layer of soil when ants were denied access to plants (Figure 1.8a; R^2 (adj) = 0.0968, $F_{1,28}$ = 4.107, p = 0.05). In one of the slick spots (No. 4), seed totals for the control and seed drift plants were found to be significant outliers. When these data points were removed, the positive correlation between flowering area and the number of seeds recovered from the top layer of soil increased in statistical significance (Figure 1.8b; R^2 (adj) = 0.495, $F_{1,26}$ = 27.5, p <0.0001).

A Wilcoxon rank-sum test was used to analyze several plant density levels within the experimental slick spots and revealed a threshold of 150 flowering plants as a

potential satiation level. Therefore, slick spots with ≥150 plants (N = 6, range = 181-647 plants) were considered high density, whereas those with <150 plants (N = 14, range = 28-137 plants) were considered low density. Comparisons of seed numbers between matched pairs within slick spots revealed that the proportion of seeds lost to ants was significantly lower for plants in slick spots with high *L. papilliferum* density than those in slick spots with low *L. papilliferum* density (Figure 1.9; -0.51±0.17 versus 0.87±0.03, respectively; Wilcoxon rank sum test, the mean ranks of high density and low-density treatments were 6.0 and 12.43 respectively, z = -2.23, p = 0.024).

Discussion

Harvester ants are important seed consumers in the arid and semi-arid grasslands of North America (MacMahon et al. 2000), and at the landscape scale, their foraging activities have the potential to influence plant communities and the population dynamics of the species they contain (Reichman 1979, Inouye et al. 1980, MacMahon et al. 2000). Compared to common plant species, rare species are particularly vulnerable to herbivory and seed predation because of their already low abundance and susceptibility to population decline (Crawley 2000, Ancheta and Heard 2011, Inouye et al. 1980, Combs et al. 2011). In the case of slickspot peppergrass, harvester ant colonies can be found within most populations, although their numbers vary with the composition of vegetation at the site. Areas with limited sagebrush cover and an abundance of non-*Bromus* grasses support the highest densities of *P. salinus* colonies (Robertson 2015). Because individual ant colonies can survive for many years (Porter and Jorgensen 1988), those situated near or within slick spots occupied by *L. papilliferum* represent a persistent source of annual seed mortality.

Although *P. salinus*, like other *Pogonomyrmex* ants, consume seeds from a variety of plant species (Crist and MacMahon 1992, MacMahon et al. 2000; Pirk et al. 2009, Pirk and Lopez de Casenave 2011, Ostoja et al. 2013), they exhibit a strong preference for *L. papilliferum* seeds when those seeds are available (Schmasow and Robertson 2016). The reason for this preference may partially be explained by the specialized habitat requirements of *L. papilliferum*. Because *L. papilliferum* are restricted to growing within the boundaries of slick spots, their seeds are concentrated in dense patches, often in close proximity to harvester ant colonies. Patches of seed deposited on the easily traversed surfaces of slick spots may facilitate rapid removal by ants because of the capacity of ants to recruit foragers from their colony to profitable patches (Brown and Gordon 2000, Gorb and Gorb 2000, Guarino et al. 2005, Flanagan et al. 2012).

While the specific habitat requirements of *L. papilliferum* may exacerbate the plant's vulnerability to seed predation by harvester ants, other factors likely play an important role in determining the population level consequences of seed predation. In the present study, Owyhee harvester ants removed, on average, 73.2% of seeds produced by individual *L. papilliferum* across a naturally occurring range of plant densities within slick spots. However, the proportion of seeds individual plants lost to harvester ants was lower in patches that contained more plants, suggesting that a satiation threshold (i.e., upper limit of seed consumption) was being reached within the range of plant densities included in my study. Thus, the consequences of seed predation by harvester ants are expected to be most pronounced in slick spots that contain relatively small numbers of flowering plants. By contrast, in slick spots that contain large numbers of plants, it is likely that many seeds will escape predation because the cumulative number of seeds

overwhelms an ant colony's capacity to consume them (see O'Dowd and Gill [1984] and

Andersen [1987] for similar examples). Moreover, the territorial nature of *P. salinus*

colonies with respect to their foraging boundaries (Howell and Robertson 2015) limits the

potential for ants to mount a strong numerical response in terms of colony number when

L. papilliferum numbers are high. The inability to mount a strong numerical response

caps the effect harvester ants can have on *L. papilliferum* seed banks.

Schmasow (2015) estimated that *P. salinus* return approximately 400,000 seeds

(comprised mainly of *L. papilliferum*, *Sisymbrium altissimum*, and *Poa secunda*) to their

nests each year. This number is considerably higher than earlier estimates of annual seed

intake by harvester ants. For example, Crist and MacMahon (1992) and Pirk and Lopez

de Casenave (2006) estimated that *P. occidentalis* and *P. rostratus* collected ~60,000 and

~81,000 seeds, respectively, per season. However, the seeds harvested by ants in these

earlier studies were larger than those typically collected by *P. salinus*. Larger seeds are

associated with increased handling time, which decreases the rate of return to the nest

(Schmasow 2015). Moreover, because larger seeds are generally higher in energy content

than smaller seeds (Kelrick et al. 1986), fewer are required to support a colony. Thus,

estimates of annual seed collection in *Pogonomyrmex* may be influenced by the size of

seeds in the diet. Assuming the value Schmasow's (2015) estimate is a reasonable

approximation for annual seed collection by *P. salinus*, slick spots that produce seed

numbers in excess of 400,000 will exceed the maximum capacity for intake by ants (note:

the actual number is undoubtedly smaller because ants do not consume *L. papilliferum*

seeds exclusively). While this may seem like a large number of seeds, Robertson and

Jeffries (2016) estimated that some slick spots at this field site produce in excess of 2

million seeds, and in a few cases up to 8 million. Thus, conditions exist where *L. papilliferum* seed production greatly exceeds the capacity for ants to collect and consume them.

Lepidium papilliferum numbers fluctuate widely year to year, largely in response to the amount and timing of precipitation during the previous winter (Kinter et al. 2013, Bond 2017). For example, Robertson (personal communication) estimates that in some years there are tens of thousands of flowering *L. papilliferum* at this study site. However, in 2013, after an unusually dry winter, only 19 flowering individuals were found, and during my study in 2012, range-wide *L. papilliferum* numbers were similarly low (Kinter and Miller 2016). Although *L. papilliferum* seeds are vulnerable to high levels of predation when plant numbers are low, the presence of a persistent seed bank may buffer the species against such periodic losses. In favorable years, *L. papilliferum* may yield enough flowering individuals to replenish the seed banks of slick spots even when ants are present and actively foraging on *L. papilliferum* seeds. In this respect, favorable years for *L. papilliferum* are analogous in their effect to mast seeding, the synchronous production of seeds by a population of perennial plants. Mast seeding is viewed as a reproductive strategy to reduce the impact of seed predation by overwhelming the predator's ability to harvest seed due to a satiation threshold (Janzen 1971, Kelly 1994). While *L. papilliferum* does not exhibit seed masting per se, stochastic events that produce favorable conditions for growth can result in greater numbers of flowering plants with a higher than average seed production (Moseley 1994, Mancuso and Moseley 1998, Meyer et al. 2005). These favorable reproductive seasons may result in a predator satiation effect allowing a higher proportion of seeds to survive and replenish the seed bank.

While the data I collected during this study indicate that seed losses were proportionally lower when there was a higher number of plants in a slick spot, several improvements to the experimental design should be considered in future studies. For example, while the number of flowering plants present in a given slick spot allows for a rough approximation of seed availability, the number of seeds produced by individual plants varies considerably as a function of the plant's size (Schmasow 2015). Thus, measures that estimate seed production within a slick spot more directly are needed. Likewise, the technique I used to quantify seed numbers in soil samples has a seed recovery rate that is lower than expected given our understanding of seed production by individual slickspot peppergrass plants (Schmasow 2015). While this technique allowed for a reasonable quantification of seed loss due to predation, new techniques should be explored in the interest of obtaining more accurate estimates.

Combined with the ongoing threats of habitat degradation caused primarily by invasive plants and increased frequency of wildfire (Moseley 1994, Mancuso and Moseley 1998, Menke and Kaye 2006), seed predation by harvester ants represents a potentially serious threat to the long-term survival of slickspot peppergrass. Specifically, when *L. papilliferum* numbers are low, seed predation by harvester ants may be very costly to offspring recruitment. By contrast, when favorable conditions produce large numbers of *L. papilliferum*, the plant likely is able to replenish its seed bank, at least to some extent. The ability to periodically replenish seed banks likely serves as a buffer to years when seed predation by ants severely limits individual reproductive success. In the long term, survival of *L. papilliferum* will depend largely on the abiotic conditions that influence reproductive success. Conditions that adversely affect *L. papilliferum*

productivity will make the plants more vulnerable to the effects of seed predation by

harvester ants. This possibility is a concern given the ongoing degradation of *L.*

papilliferum habitat and the potential consequences of climate change on precipitation

patterns that influence *L. papilliferum* populations. Given these expected changes, it is

important to continue assessing the role that seed predation by harvester ants has on *L.*

papilliferum survival and reproductive success. Future studies should also investigate

further the potential importance of herbivory by mammals (see also Jeffries 2016) on

plant survival and reproductive success.

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Figures
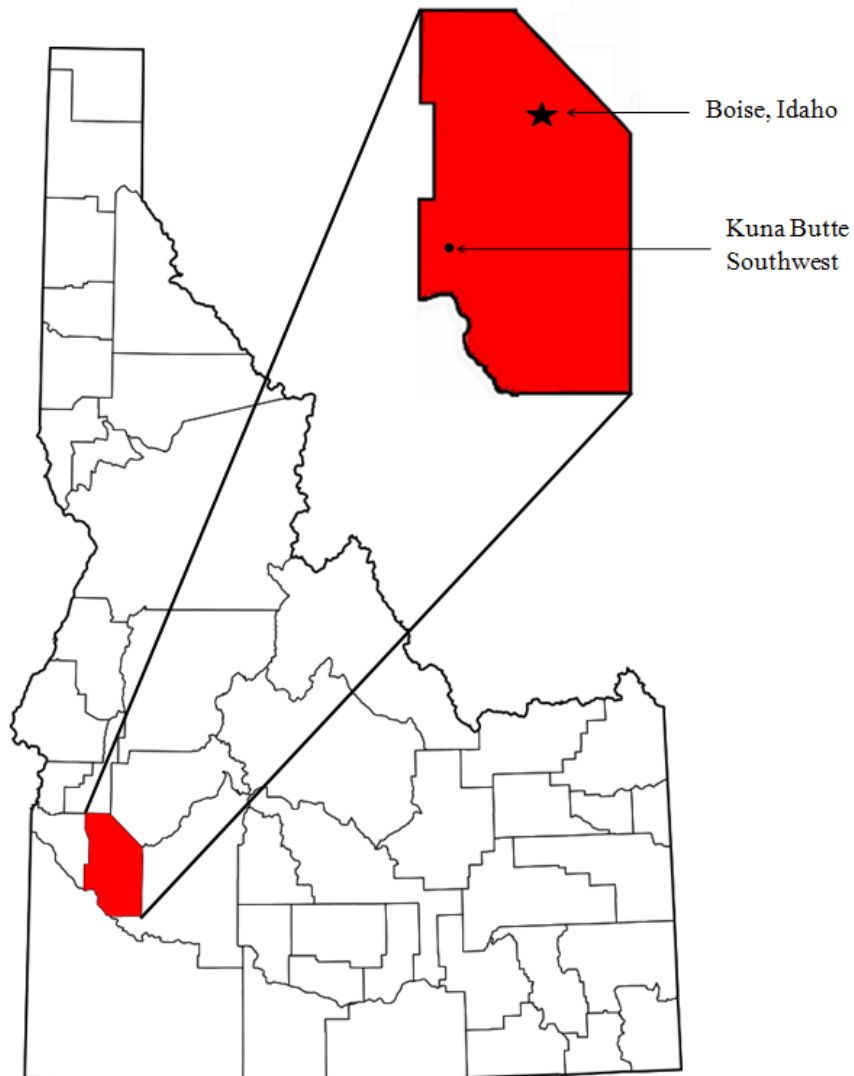
Figure 1.1. Map showing the location of the Kuna Butte Southwest study site. "Map of Idaho highlighting Ada County.svg" by David Benbennick/ Ada county highlighted and labeled with site identifiers within the original map.

Figure 1.2. **The general distribution range of *Pogonomyrmex salinus* (Owyhee harvester ant). "Blank Map-USA-states-Canada-provinces, HI closer.svg" by Lokal_Profile is licensed under CC BY-SA 2.5/ *P. salinus* distribution range layered upon the original map; Hawaii has been cropped.**
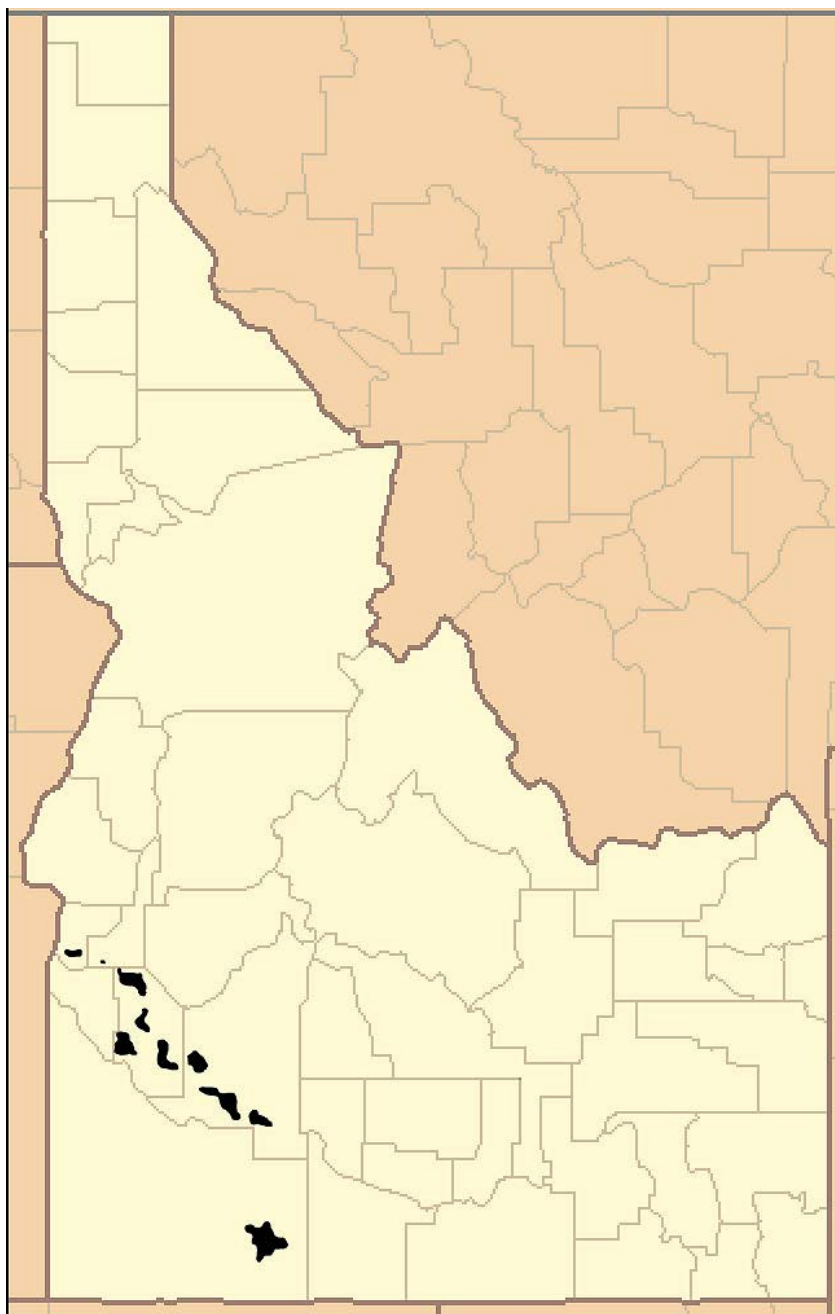
Figure 1.3.   The general distribution range of *Lepidium papilliferum* (slickspot peppergrass). "Idaho Locator Map.PNG" by U.S. Census, Ruhrfisch is licensed under CC BY-SA 3.0/ *L. papilliferum* distribution range layered upon the original map.

Figure 1.4. Photographs of "Ants excluded" treatment barrier preventing access of *P. salinus* to the selected *L. papilliferum* plants and "Access by ants" treatment barrier which allowed access of harvester ants to the selected *L. papilliferum* plant used in the seed removal experiment.
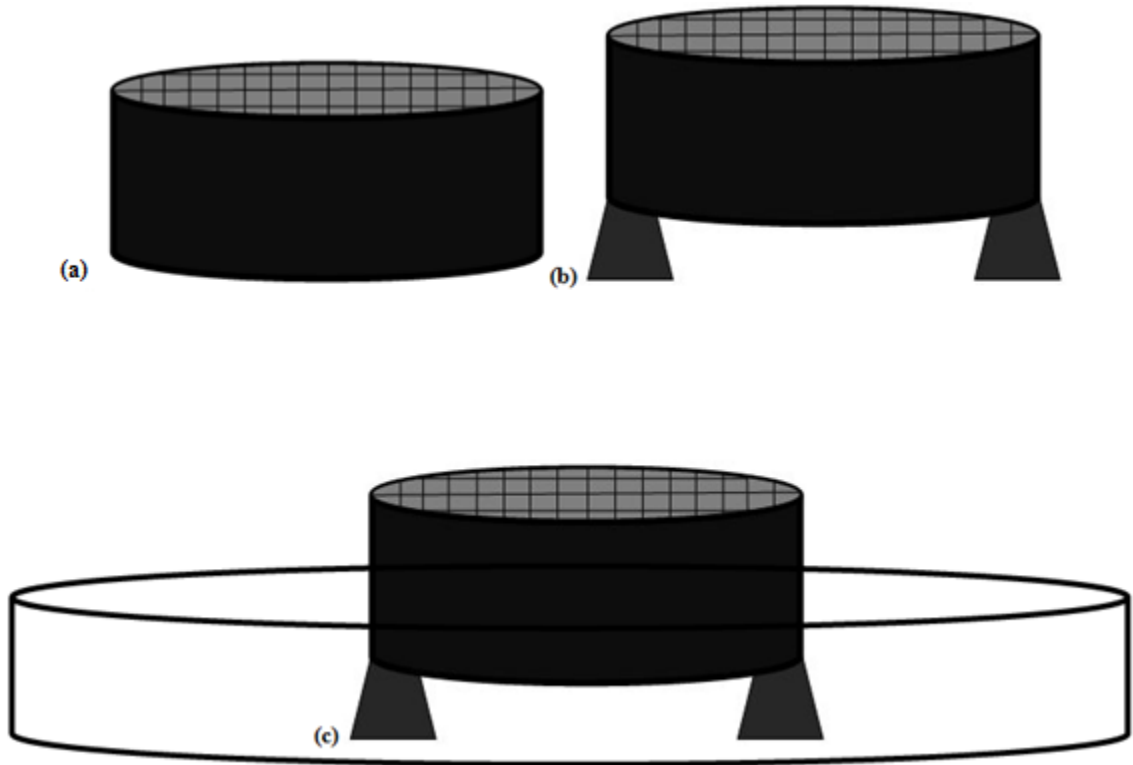
Figure 1.5. Design for barriers used in the seed removal and seed drift experiments. (a) "Ants excluded" treatment barrier preventing access of *P. salinus* to the selected *L. papilliferum* plant. (b) "Access by ants" treatment barrier which allowed access of harvester ants to the selected *L. papilliferum* plant. (c) "Seed drift" control barrier consisting of an elevated barrier surrounded by a larger fixed barrier to prevent access of harvester ants to the selected *L. papilliferum* plant accounting for the dispersal of seeds by wind and/or water. It should be noted that while this figure illustrates the larger seed drift barrier in a different color, these barriers were constructed using the same material as the smaller barriers.

Figure 1.6. (a) *P. salinus* removing mature *L. papilliferum* fruit. The arrows indicate locations where fruit has been previously clipped. (b) *P. salinus* returning to the nest with *L. papilliferum* fruit. Photographs were taken by Ian Robertson.
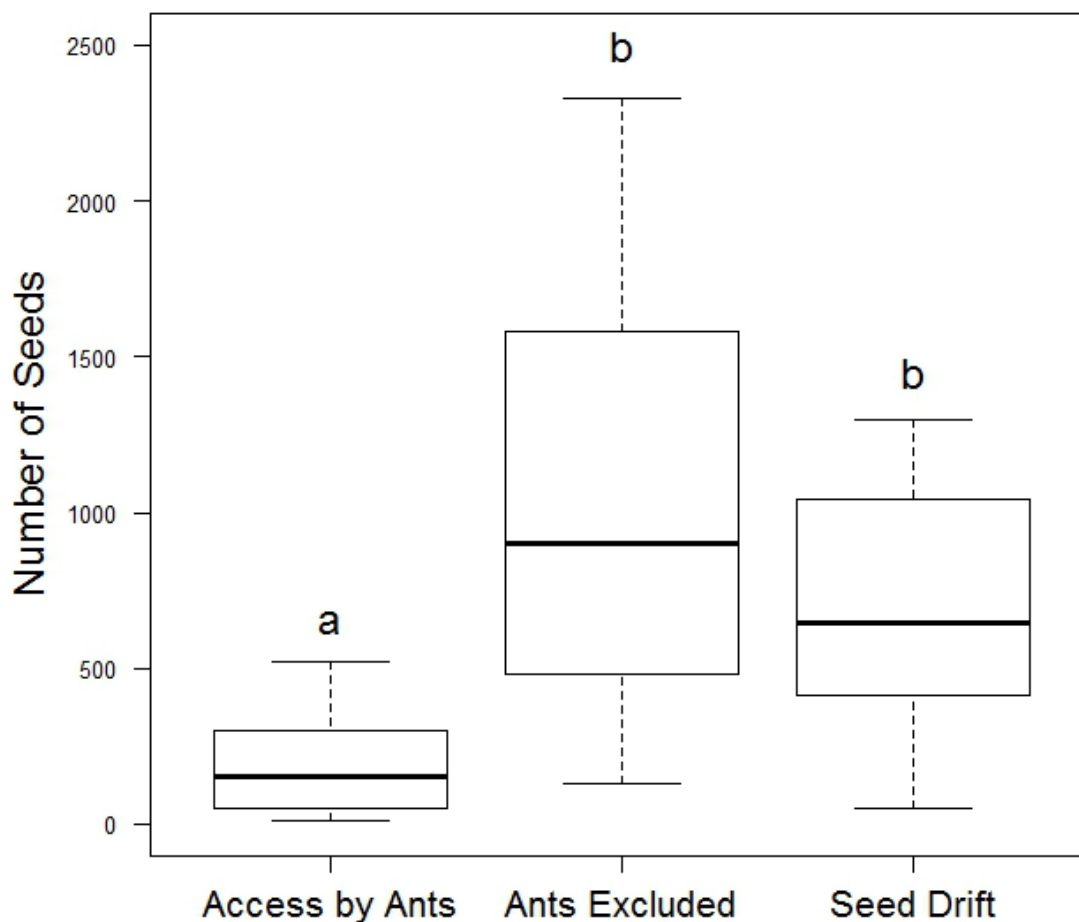
Figure 1.7. Boxplot chart showing the number of *L. papilliferum* seeds remaining as a function of barrier treatment. The 75th and 25th percentiles are indicated by the upper and lower limits of each box, respectively. The upper and lower ends of the whiskers represent the 90th and 10th percentile, respectively. The thick horizontal line within the box represents the median. While the boxplot reflects the distribution of the data prior to log transformation, the letters above the bars indicate significant differences among the groups based on a means comparison of log-transformed values. The effect of barrier treatment was significant (p <0.0001) where the exposure of *L. papilliferum* to foraging ants had a significant effect on the remaining number of seeds indicating that *P. salinus* is an influential seed predator of the rare plants.
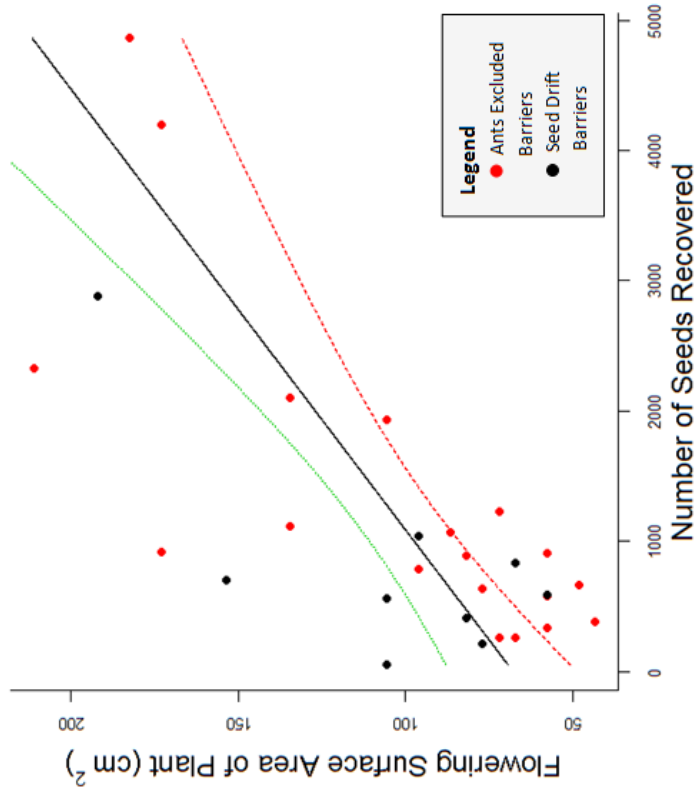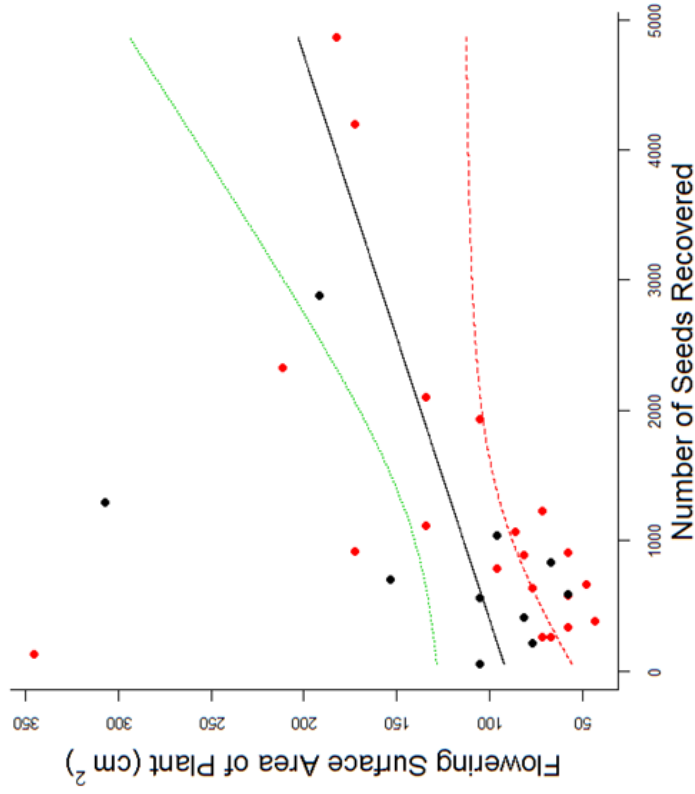
Figure 1.8. **The flowering surface area of *L. papilliferum* plants as a function of the number of seeds recovered under plant where ants were denied access. The complete data set is shown in 1.7(a), and the results when outlying values from experimental slick spot #4 have been removed and are shown in 1.7(b). The best fit line is shown in black, and the 95% confidence levels are indicated with the green and red lines.**
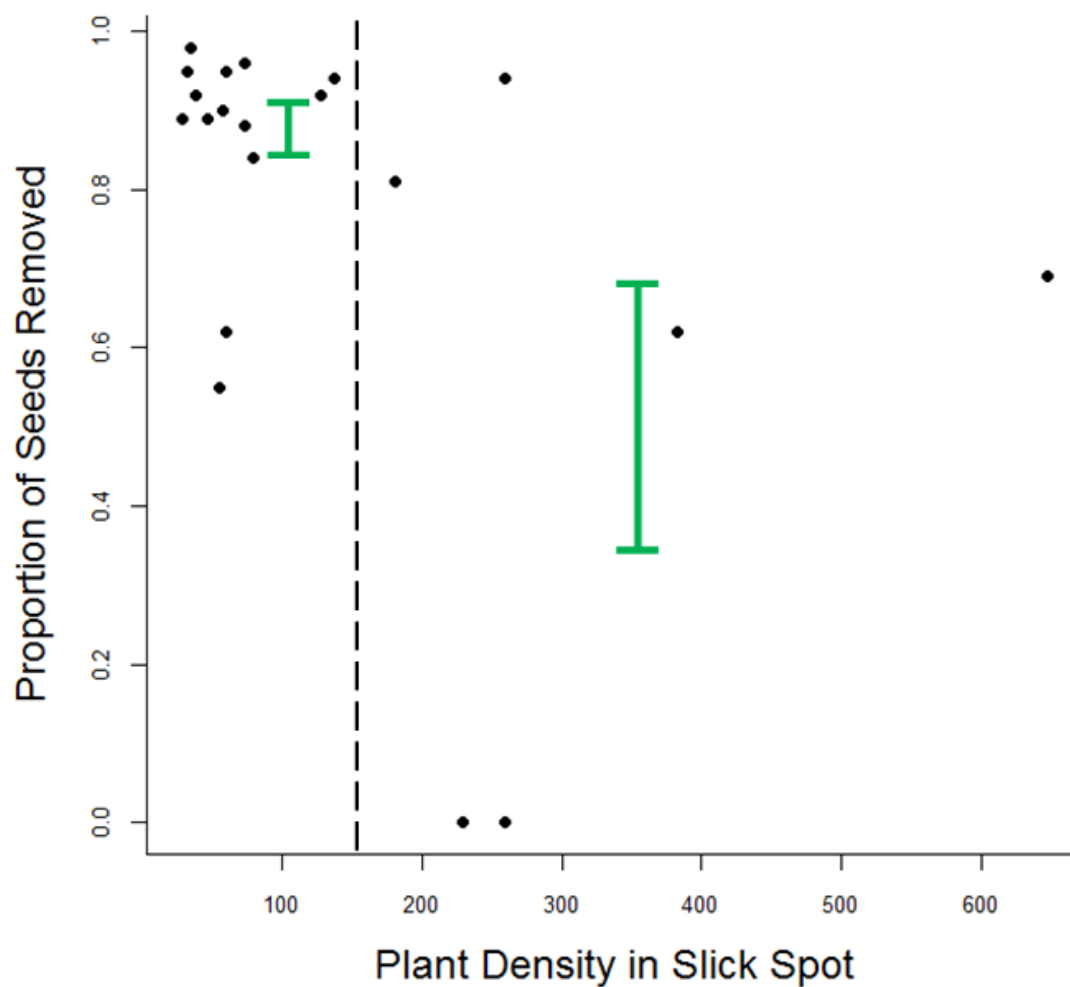
Figure 1.9. **The proportion of seeds removed from individual *L. papilliferum* plants as a function of the density of flowering *L. papilliferum* in a slick spot. The vertical dotted line indicates the point where the proportion of seeds removed was statistically significant between high density and low density slick spots (≥150 plants and <150 plants respectively) based on a Wilcoxon rank sum test (p = 0.024). The green bars show the mean removal of seeds and SE for both the high and low density slick spots.**

Tables

Table 1.1.   **Data measurements associated with each experimental slick spot. The distances reflect the distance measured from the nest to the experimental barriers contained in each slick spot.**

Experimental slick spot	Distance of nearest colony (m)	Number of flowering plants
1	6.2	647
2	5.2	229
3	3.2	38
4	2.6	259
5	6.4	259
6	4.1	35
7	4.6	28
8	3.6	32
9	3.9	73
10	6.7	181
11	5.8	60
12	6.5	57
13	6.6	60
14	2.5	137
15	4.2	128
16	2.7	79
17	5.7	382
18	4.8	55
19	7.7	73
20	5.6	46

Table 1.2.   Behavioral and other observational data related to foraging and predation collected 2-3 times a week throughout the 2012 field season.

Experimental slick spot	Fruit in colony midden	Ant activity in slick spot	Ant activity on plants in slick spot	Clipping of fruit in slick spot
1	yes	yes	yes	no
2	yes	yes	yes	no
3	yes	yes	yes	yes
4	yes	yes	yes	yes
5	yes	yes	yes	yes
6	yes	yes	yes	no
7	yes	yes	yes	yes
8	yes	yes	yes	no
9	yes	yes	yes	yes
10	yes	yes	yes	no
11	yes	yes	yes	yes
12	yes	yes	yes	no
13	yes	yes	no	no
14	yes	yes	yes	yes
15	yes	yes	yes	yes
16	yes	yes	yes	yes
17	yes	yes	no	yes
18	yes	yes	yes	no
19	yes	yes	no	yes
20	yes	yes	no	no

Table 1.3. Items carried by ants returning to the nest at the experimental slick spots. Slick spots 2, 3, 19, and 20 were not sampled because of low ant activity during the sampling period.

Experimental slick spot	Number of ants	*Lepidium papilliferum*	*Artemisia tridentata*	*Poa secunda*	*Bromus tectorum*	Insects	Organic fragments
1	20	3	3	0	0	0	2
2	NA	NA	NA	NA	NA	NA	NA
3	NA	NA	NA	NA	NA	NA	NA
4	24	6	0	1	0	0	0
5	24	12	0	0	0	0	0
6	20	5	0	0	0	1	1
7	22	7	0	1	3	0	0
8	17	4	0	0	0	0	0
9	21	4	2	1	0	0	0
10	29	7	0	0	0	0	1
11	21	0	0	1	2	0	0
12	26	6	0	0	0	0	4
13	21	0	1	1	0	1	0
14	26	12	0	1	0	0	0
15	30	7	0	0	1	0	0
16	26	7	0	3	0	0	0
17	20	6	0	0	0	0	1
18	23	8	0	1	0	0	0
19	NA	NA	NA	NA	NA	NA	NA
20	NA	NA	NA	NA	NA	NA	NA
Total	**370**	**94**	**6**	**10**	**6**	**2**	**9**

CHAPTER TWO: EXPLORING THE POTENTIAL OF SEED INTRODUCTIONS AS

A MANAGEMENT TOOL FOR SLICKSPOT PEPPERGRASS (*LEPIDIUM*

PAPLLIFERUM) RECOVERY

Abstract

Seed introductions can be an effective tool for establishing new or augmenting existing plant populations. As a rare mustard endemic to the sagebrush-steppe habitat in southwestern Idaho, the conservation and management of slickspot peppergrass (*Lepidium papilliferum*) has been a focus and concern for both federal and state management agencies. The present study investigated the use of seed introductions as a potential approach to the conservation and recovery of *L. papilliferum* populations and the extent to which seed predation by Owyhee harvester ants (*Pogonomyrmex salinus*) might hamper those efforts. In 2012 and early 2013, I introduced *L. papilliferum* seeds into 22 slick spots at a site with no record of *L. papilliferum* presence over the past 20 years. The experimental design within each slick spot included three seed introduction times (summer, fall, and spring) and three treatments related to the risk of seed predation (protected from ants, access by ants and small mammals, and access by ants only). The introduction of seeds during the summer coincided with the natural timing of seed deposition and the maximum intensity of foraging activity by ants. The fall and spring introductions avoided the foraging activity of ants. Because *L. papilliferum* seeds generally require at least one overwintering period to break dormancy, seeds introduced in the spring were first subjected to a laboratory protocol to break dormancy. A total of

nine plants germinated from the 19,800 seeds released in this study (three reproducing annuals, three reproducing biennials, and three rosettes with potential for flowering in the subsequent spring). While the number of germinating plants tallied in this study was very low, this study confirmed that *L. papilliferum* can successfully germinate, flower, and fruit when seeds are introduced into unoccupied slick spots. Efforts to improve the success of seed introductions are needed before seed introductions can be considered as a viable approach to the conservation and management of *L. papilliferum* populations.

Introduction

Biodiversity is a driving force of ecosystem function, and it sustains versatility which promotes stability in natural systems (Lefcheck et al. 2015, Weisser et al. 2017). Climate change, land use, and other anthropogenic influences have resulted in degradation, fragmentation, and loss of habitat in many ecosystems, threatening their biodiversity (Brooks et al. 2002, Millennium Ecosystem Assessment 2005, Giam et al. 2010, Krauss et al. 2010, Ashraf et al. 2012, Alofs et al. 2014, Staude et al. 2018). These effects have also been felt throughout sagebrush-steppe habitat in the western United States and Canada, where anthropogenic disturbance (e.g., irrigated agriculture, livestock grazing, off-road vehicles, and military training), altered wildfire cycles, and exotic species invasions are causing many sagebrush-steppe communities to lose native diversity and be replaced by grasslands (Rosentreter 1992, Watts 1998, Hilty et al. 2003, Yeo 2005, Huntly et al. 2011, Mitchell et al. 2017, Seipel et al. 2018). In the process, native forb species have experienced severe declines (Creutzburg et al. 2015, Mitchell et al. 2017).

Slickspot peppergrass, *Lepidium papilliferum* [(L. Henderson) A. Nels. and J.F. Macbr.] (Brassicaceae), a rare mustard endemic to southwestern Idaho, is an example of a sagebrush-steppe species that has declined in abundance as a result of habitat degradation and fragmentation (Moseley 1994). Currently, there are 91 sites known to contain *L. papilliferum* (United States Fish and Wildlife Service 2016), with only six of these occurrences considered high quality by the Idaho Department of Fish and Game. A further 21 sites are known from historical accounts but are now considered extirpated (Moseley 1994). The loss of populations, coupled with the relatively poor state of many surviving populations, has prompted resource managers to consider the use of seed introductions to establish new populations in suitable habitat, as well as augment existing populations if plant numbers start to dwindle.

Seed introductions are a potentially effective tool in conservation efforts aimed at recovery of rare plant species (Dalrymple et al. 2011, Godefroid et al. 2011, Guerrant 2012, Atondo-Bueno et al. 2016, Menges et al. 2016). However as previous introduction studies have employed a variety of different methods and measurement criteria, the success of these studies have been varied (Guerrant 2013). While *L. papilliferum* seeds have been germinated successfully in controlled greenhouse environments (Meyer et al. 2005, Stillman 2006, Billinge and Robertson 2008, Loffredo et al. 2010, Traversa et al. 2013), seed introductions in the field (D. Quinney and J. Weaver, unpublished results) have yielded unverifiable results because the introductions were made within existing populations, thereby making it impossible to determine whether observed seedlings originated from introduced seeds or the existing seed bank (C.W. Baun, personal communication). The primary goal of the present study was to introduce *L. papilliferum*

seeds in habitat not currently occupied by *L. papilliferum* to assess whether these introductions have the potential to assist in recovery efforts for the species.

Within sagebrush-steppe habitat, *L. papilliferum* grows within patchily distributed microsites known as "slick spots" – shallow depressions of soil characterized by higher levels of water accumulation, sodium content, and clay content relative to surrounding soil (Moseley 1994, Fisher et al. 1996, Quinney 1998). The plant exhibits two main life history trajectories – annual and biennial. Annuals germinate, flower, set seed and die within a single growing season, whereas biennials exist as vegetative rosettes in the first year and then flower, set seed and die in their second year (Quinney 1998, Meyer et al. 2005). The plant's small, white flowers, which grow on multi-flowered inflorescences that typically bloom from late May to late June, attract a wide variety of insect pollinators (Robertson and Klemash 2003, Robertson and Leavitt 2011). Mature seeds dehisce from their fruits in late summer, at which point they enter the soil seed bank and remain dormant and viable for up to 11 years (Meyer et al. 2005, Meyer et al. 2006), perhaps longer (I.C. Robertson, personal communication). Only a subset of seeds in the seed bank germinate in a given year, even when conditions seem ideal (Meyer et al. 2005).

Slickspot peppergrass has the highest extirpation rate among rare plant taxa in Idaho (Meyer et al. 2005) and is currently listed as a threatened species under the Endangered Species Act (United States Fish and Wildlife Service 2016). Habitat degradation and fragmentation as a consequence of urbanization, agriculture, livestock grazing, invasion of exotic plant species, and increased frequency of wildfires, are thought to be major contributors to population declines (Moseley 1994, Mancuso and Moseley 1998, Menke and Kaye 2006). Intense seed predation by Owyhee harvester ants,

Pogonomyrmex salinus (Olsen) (Hymenoptera: Formicidae), may also contribute to the species' vulnerability and decline (Chapter 1, White and Robertson 2009).

Seed predation by harvester ants represents a potentially serious impediment to the successful use of seed introductions to augment or establish *L. papilliferum* populations. Harvester ants, which frequently nest within *L. papilliferum* populations (Robertson 2015), regularly incorporate *L. papilliferum* seeds into their diet (Schmasow and Robertson 2016) and have the capacity to remove as much as 90% of the fruits and seeds produced by individual plants (White and Robertson 2009, I.C. Robertson unpublished data). Because seed predation by ants could hamper the establishment of *L. papilliferum* populations in otherwise favorable habitat, the timing of seed introductions may be critical to the success of this recovery measure. Seed introductions that coincide with the timing of natural seed deposition for *L. papilliferum* may be exposed to higher levels of seed predation than seeds released in late fall, once ants have ceased foraging for the year, or seeds released in early spring before ants become active. However, it is unknown whether fall or spring introductions would adversely affect germination success. Therefore, the second goal of my study was to determine whether the timing of seed introduction influences the success of introduction efforts. I predicted that, because of the risk of seed predation by harvester ants, seeds introduced during the fall and spring would have a higher likelihood of surviving to germinate than seeds introduced the previous summer. Note that because *L. papilliferum* seeds are dormant upon release from the parent plant and require at least one overwintering period to break dormancy, seeds introduced in the spring were first subjected to an established laboratory protocol to break dormancy.

Materials and Methods

Study Site

The study was conducted from July 2012-summer 2014 at the Idaho Army National Guard's Orchard Combat Training Center (OCTC), south of Boise, ID. The study site (116º8'3.68" W, 43º22'12.67" N; Figure 2.1), which was located approximately 3 km from the nearest active *L. papilliferum* population, contained slick spots that had no record of *L. papilliferum* colonization over the past 20 years (C.W. Baun, personal communication). Harvester ant colonies were scattered throughout the site. The plant community was dominated by *Artemisia tridentata* (big sagebrush), *Ericameria nauseosa* (gray rabbitbrush), *Poa secunda* (Sandberg's bluegrass), *Bromus tectorum* (cheatgrass), and *Sisymbrium altissimum* (tumble mustard).

Seed Source

The seeds used in this study were originally collected in 2008 from greenhouse specimens by Dr. Susan Meyer at the Rocky Mountain Research Center (USFS, Fort Collins CO), and were supplied to me by the Environmental Management Office of the Idaho Army National Guard in Boise, ID.

Seed Introduction Experiment

In designing this experiment, my goal was to assess the effects of seed predation by harvester ants in two ways: (1) by quantifying the number of seeds remaining in the soil at the end of the 2012 season, and (2) by counting the number of *L. papilliferum* that germinated the following summer. Unfortunately, recovery of introduced seeds from soil samples was very low (20-25%), even in situations where no ants were present. Therefore, for the purposes of this analysis, I focus exclusively on germination success in

the field. Although this measure cannot account for seeds that remained dormant in the soil, I assumed that the proportion of seeds that remained dormant was spread evenly across treatments.

I selected a total of 22 slick spots located within 15 m of an active harvester ant colony (Figure 2.2). The experimental design within each slick spot included three seed introduction times (summer, fall, and spring) and three treatments related to the risk of seed predation (protected from ants, access by ants and small mammals, and access by ants only). The small mammal treatment was included to account for the possibility that rodents may also contribute to the predation of *L. papilliferum* seeds (e.g., Anderson and MacMahon 2001).

The summer introduction time coincided with the timing of natural seed deposition by *L. papilliferum*, and thus the seeds were exposed to an extended period of foraging by harvester ants. By contrast, the fall and spring introductions were intended to reduce the exposure of seeds to ant predation since harvester ants are usually inactive at these times. Seeds released in the summer and fall received no preparation because I assumed that the natural overwintering period would be sufficient to break their dormancy. Seeds released in the spring were subjected to a protocol to break dormancy. Following Billinge and Robertson (2008), seeds intended for spring release were stored at room temperature in the dark for three months. I then scarified the seeds by rubbing them gently between two sheets of 320 grit sandpaper, imbibed them with deionized water for 24 h on filter paper in Petri dishes, and placed them in cold stratification at 4°C for 8 weeks. A portion of the seeds (N = 1,000) was retained in the lab to test germination success. These seeds were distributed evenly across 10 Petri dishes lined with filter paper

moistened with deionized water. The dishes were placed in a location with a natural photoperiod and temperatures that ranged from 21-23°C. After 10 days (i.e., one week after the first seedlings were detected) I counted the total number of seeds that had germinated.

Small cages were used for all seed introduction treatments in the field. Each cage consisted of an 8-10 cm high plastic ring cut from a 15-cm diameter flowerpot, and the top of each cage was covered with 1 cm hardware cloth. The "access by ants and mammals" cages were elevated 4-5 cm from the ground using plastic rebar supports, while the "access by ants" cages were elevated 4-5 cm from the ground using a ring of 1.0 cm hardware cloth that prevented access by mammals. The "protected from ants" cages were sealed directly on the ground to prevent access by seed predators (Figure 2.3). I scattered 100 seeds on the soil surface within each cage at the appropriate introduction time (8/6/2012, 10/21/2012, and 4/20/2013, for the summer, fall, and spring introductions, respectively.) I monitored the cages for vegetative rosettes and flowering annuals regularly between 4/22/2013 and 8/16/2013, and again in the summer of 2014.

Results

Of the 1,000 seeds set aside from the scarification protocol to break dormancy, only 45 germinated within their Petri dishes. At the field site, a total of three *L. papilliferum* rosettes were found across all treatments in 2013 (Figure 2.4). Due to the low number of rosettes, no statistical analysis of the results was performed, and no attempt was made to interpret germination success in terms of treatment or introduction time. All of the cages were removed from the site in August 2013, except the two cages that contained the three rosettes.

In May 2014, I revisited the three rosettes produced in 2013. All three had developed into flowering biennials (Figure 2.5d,e). I observed pollinator insects (i.e., various species of Hymenoptera) visiting the flowers on one of the three plants. In addition to the three flowering biennials, I discovered one new flowering annual (Figure 2.5a) and five new rosettes (Figure 2.5b,c,d,f) in slick spots where seeds had been introduced in 2012 and 2013; however, I was unable to establish which treatment the new plants belonged to because I had removed their associated cages late in 2013. In a subsequent and final visit to the site in July 2014, no new *L. papilliferum* plants were discovered. Two of the five new rosettes previously documented had flowered and produced fruit. Seed-bearing fruits were also present on the flowering annual I discovered earlier in the season, as well as on the three second-year biennials. Three of the new rosettes remained in a vegetative state with the potential to flower as biennials in the spring of 2015. Additional field observations made during the course of the study include evidence of trampling by cattle in 31.8% of the treatment slick spots. The damage was noted to have compromised a centimeter or more of the upper layer of soil in each of the affected slick spots, and in four of the slick spots, several treatment barriers were either overturned or damaged (Figure 2.6).

Discussion

Seed introductions offer a promising approach to the conservation and management of rare plant populations (Dalrymple et al. 2011, Godefroid et al. 2011, Guerrant 2012, Atondo-Bueno et al. 2016, Menges et al. 2016). The results of my study confirm that *L. papilliferum* can successfully germinate, flower, and fruit when seeds are introduced into unoccupied slick spots. However, the number of seeds that germinated

was disappointingly low. Although alternative introduction methods, such as transplanting, often have higher success rates in terms of the establishment and survival of individual plants, these approaches can be cost prohibitive, time-consuming, and labor-intensive relative to seed introductions (Guerrant and Kaye 2007, Menges et al. 2016). In the case of *L. papilliferum*, although plants can be successfully grown from seed in greenhouse environments (Meyer et al. 2005, Loffredo et al. 2010), transplanting individual plants into the wild is likely an impractical approach to conservation given the associated cost of both time and labor compared to seed introductions. Moreover, transplanting would require mechanical disruption to the upper layers of soil within slick spots, which could alter soil moisture and other characteristics on which *L. papilliferum* is dependent for growth and survival (Fisher et al. 1996, Traversa et al. 2013).

The low numbers of seedlings produced in my study may reflect a loss of seeds from slick spots, either through dispersal, predation, or death. However, seed dispersal and predation are unlikely explanations given the design of the experiment, and there is little reason to suspect seeds died in high numbers. Seed dormancy is a more likely explanation for the lack of germination, as *L. papilliferum* seeds can remain dormant in the soil seed bank for years (Mancuso and Moseley 1998, Meyer et al. 2005). Seed dormancy and persistent seed banks are common in desert annuals (Went 1949) and are generally viewed as a bet-hedging strategy to decrease the risk of reproductive failure in highly variable and unpredictable environments (Philippi 1993, Tielbörger et al. 2012, Volis 2012). Indeed, the sagebrush-steppe ecosystem occupied by *L. papilliferum* is characterized by large annual variation in the timing and amount of precipitation (Molles 2013).

Between the summer of 2012 and 2013, unusually low spring precipitation levels are thought to have contributed to a range-wide deflation of *L. papilliferum* numbers (Kinter et al. 2013). These same conditions may have contributed to the low germination rates of seeds in my study. In support of this assessment, low germination rates of introduced seeds (in a study initiated in 2016) in 2018 coincided with a range-wide deflation of *L. papilliferum* numbers (I.C. Robertson, personal communication). Given the relationship between annual precipitation and *L. papilliferum* numbers (Bond 2017), the low rate of seed germination encountered in my study may be well within the norm for *L. papilliferum*. It is possible, but unverified, that many of the seeds I introduced in 2012 and spring 2013germinated in subsequent years when conditions were favorable.

Given the persistent seed bank of *L. papilliferum*, and the large fluctuations in plant numbers that can occur across years (Kinter et al. 2013), the success of seed introduction efforts should be assessed over longer periods than just one or two years. It is also important to consider germination success in a particular year relative to the success of the plant elsewhere. If germination rates are low in years when the plant is thriving range-wide, this may indicate that the introduction is not likely to be successful. Success may also be dependent on the number of seeds released; i.e., more seeds will likely improve the chances of success. A study subsequent to mine substantially increased the number of seeds introduced to unoccupied slick spots (5,000 seeds were released in each of 110 experimental slick spots); however, the success of these efforts has yet to be evaluated (I.C. Robertson, personal communication). Large-scale introductions of *L. papilliferum* seeds in recovery efforts should only be considered once the parameters for success have been established through carefully monitored

experiments. Ideally, this assessment will include analysis of the soil chemistry needed to promote germination and growth within slick spots.

The use of protocols to break seed dormancy may increase the likelihood of seed germination following introduction. However, the scarification protocol I used, while moderately successful in a previous study (Billinge and Robertson 2008), was largely ineffective in my study (see also Jeffries 2016). Better success at breaking dormancy has been achieved by pricking the seed coat of individual seeds with a pin instead of using sandpaper to scarify a large number of seeds at once (Stillman 2006); however, this technique is labor intensive and would not be feasible on the scale needed for recovery efforts. Moreover, because desert annuals such a slickspot peppergrass rely upon seed banks and seed dormancy as a strategy for surviving unpredictable and ephemeral environmental conditions (Philippi 1993, Tielbörger et al. 2012, Volis 2012), the release of prepared seeds could result in complete failure if conditions for survival are unfavorable.

Because harvester ants remove large quantities of *L. papilliferum* seed from slick spots (Chapter 1, Jeffries 2016, Schmasow and Robertson 2016, Robertson and Jeffries 2016), the presence of harvester ants is an important consideration in recovery efforts that involve seed introductions. Measures to address this problem include selecting sites without harvester ant colonies, eradicating ant colonies located near slick spots (Robertson et al. 2017, I.C. Robertson, unpublished data), using physical barriers to deny ants access to seeds (Chapter 1, Robertson and Jeffries 2016), and introducing seeds at times when ants are not active, such as late fall (this study, Robertson and Jeffries 2016). Selecting sites without harvester ants and targeted eradication of ant colonies are the most

feasible strategies for large-scale recovery efforts involving seed introductions. Introduction of seeds late in the fall might preclude the initial loss of seeds to ants, but any seeds produced by plants in those slick spots would be vulnerable to predation.

Successful germination is an incomplete measure of success for any seed introduction study. For introductions to be relevant to conservation efforts, the plants must survive to reproduce. Slickspot peppergrass is reliant primarily on outcrossed pollination mediated by insects for reproduction (Robertson and Klemash 2003, Robertson and Ulappa 2004, Robertson and Leavitt 2011). In the present study, pollinator insects were observed on *L. papilliferum* flowers, and successful pollination and fruit production occurred in most cases of flowering despite the small number of plants available for insects to visit and cross-pollinate.

In summary, I have shown that the introduction of *L. papilliferum* seeds to unoccupied slick spots can result in successful germination, growth, and reproduction of the species. However, further research is needed to assess whether these successes can translate into an effective tool for recovery efforts. For seed introductions to be effective in recovery, it will be important to establish the appropriate number and distribution of seeds needed to maximize germination and outcrossing success; and to confirm the availability of pollinators at introduction sites prior to seed release. Research is also needed to determine whether differences in chemical profiles among slick spots influence germination, growth, and reproduction of *L. papilliferum*. Finally, the disruption caused by cattle trampling in my study highlights the need to select sites with little risk of physical disruption to slick spots, as disruption to the integrity of slick spots can

adversely affect the distinct soil profile and characteristics *L. papilliferum* requires for survival (Fisher et al. 1996, Traversa et al. 2013).

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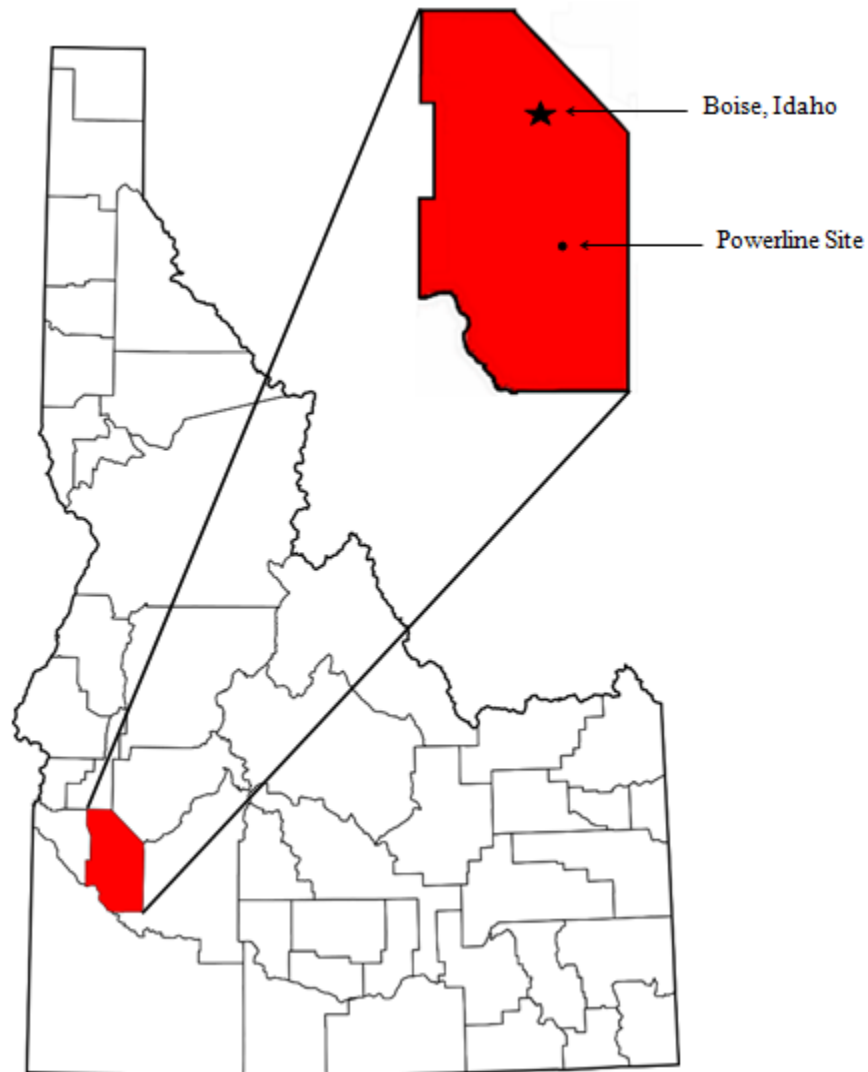
Figures

Figure 2.1. **Map showing the location of the Powerline study site relative to the City of Boise. "Map of Idaho highlighting Ada County.svg" by David Benbennick/ Ada county highlighted and labeled with site identifiers within the original map.**
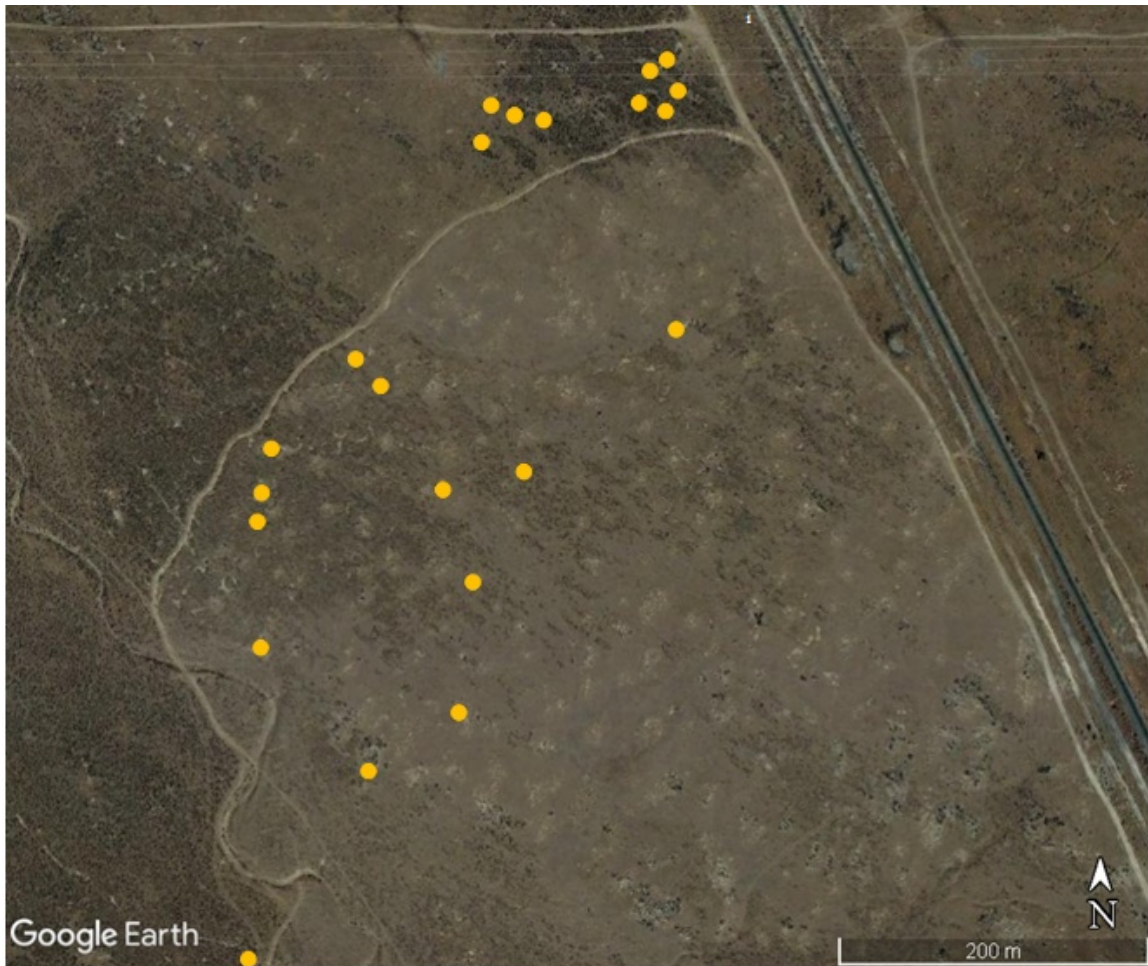
Figure 2.2.   Map of the Powerline study site and experimental slick spots. The map was created using Google Earth Pro and shows the detail and terrain as photographed via satellite on October 5, 2012.
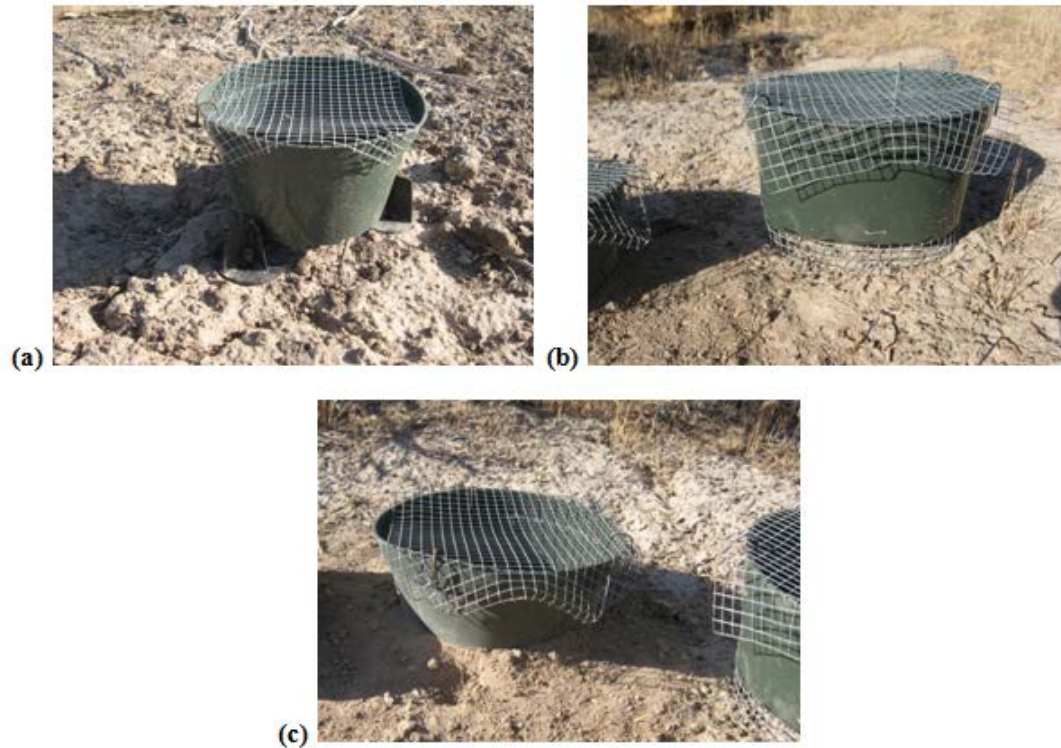
Figure 2.3.   **Photographs of the three types of cages deployed in the experiment. (a) The "access by ants and small mammals" cage which was elevated using plastic rebar supports. (b) The "access by ants" cage which was elevated using 1.0 cm metal hardware cloth. (c) The "protected from ants" cage with the lower edge buried 2.0 cm in the soil.**
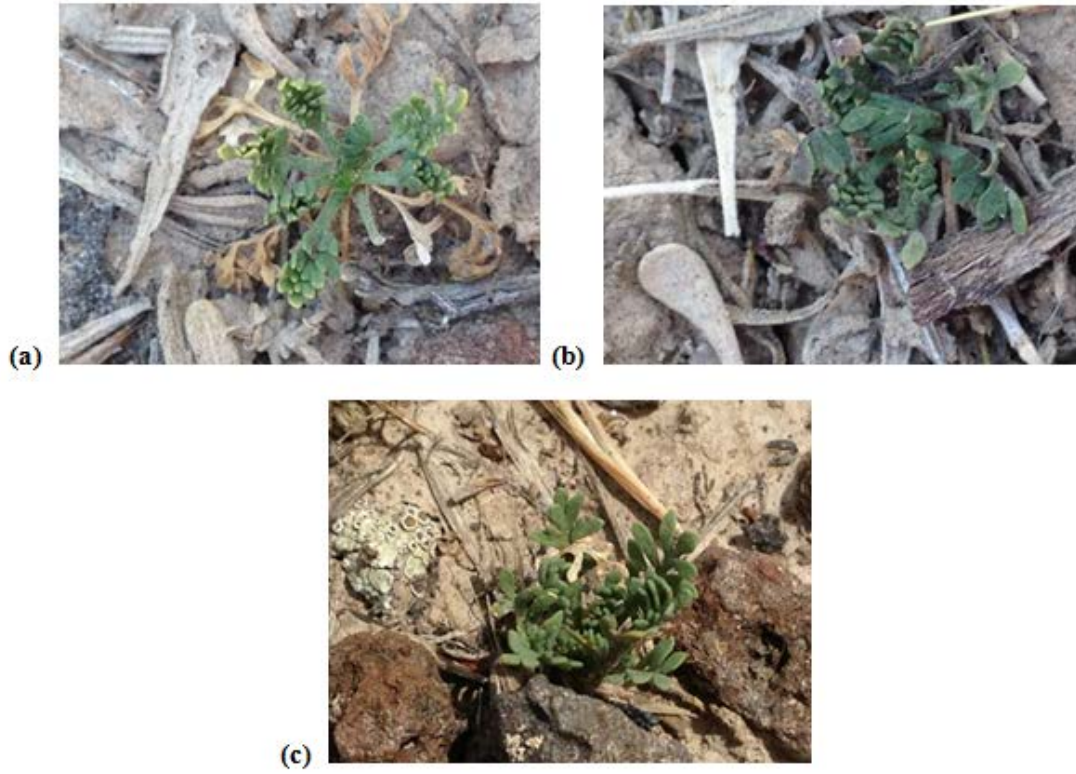
Figure 2.4. Photographs of the three rosettes found in 2013. These photographs were taken by the author on July 5th, 2013. (a,b) Rosettes found in experimental slick spot #7. (c) Rosette found in experimental slick spot #18.

Figure 2.5. *Lepidium papilliferum* resulting from the seed introduction experiment. These photographs were taken on May 22nd, 2014 by Ian Robertson. (a) A flowering annual located in slickspot #2. (b,c) Rosettes found in experimental slick spot #7. (d) Flowering biennials of two plants that were recorded as rosettes in slick spot #7 in 2013. Two new rosettes were also present within the cage. (e) Flowering biennial of a plant that was recorded as a vegetative rosette in slick spot #18 in 2013. (f) A new rosette was found in experimental slick spot #20.

Figure 2.6.   Damage to experimental cage caused by cattle in one of the slick spots. Damage to the slick spot's soil crust can be seen in the upper left-hand corner of the photograph.