AN INVESTIGATION OF HERBIVORY AND SEED PREDATION ON SLICKSPOT PEPPERGRASS

by

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A thesis
submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Biology
Boise State University

August 2016

BOISE STATE UNIVERSITY GRADUATE COLLEGE

DEFENSE COMMITTEE AND FINAL READING APPROVALS

of the thesis submitted by

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Thesis Title: An Investigation of Herbivory and Seed Predation on Slickspot Peppergrass

Date of Final Oral Examination: 29 April 2016

The following individuals read and discussed the thesis submitted by student Michelle I. Jeffries, and they evaluated her presentation and response to questions during the final oral examination. They found that the student passed the final oral examination.

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ACKNOWLEDGEMENTS

My first, and most important acknowledgement belongs to my advisor, Dr. Ian Robertson for allowing me to develop my own ideas and guiding my thinking back to our core research objectives. I would also like to thank my committee members, Dr. Julie Heath and Dr. Marcelo Serpe, for advice in aspects of planning and completing my project. The three of you have pushed me to evolve my scientific mind and I greatly appreciate everything.

This project would not have been completed if not for my many volunteers who include: John Young, Leo Ohyama, Derekka Baker, Jessie Bell, Lindsey Bird, Jennica Compton, Riley Johnson, Nick Mills, and Kristen Flemmer. An additional dedication is needed for John Young and Leo Ohyama for their devoted support throughout the majority of the research.

The Idaho Army National Guard, U.S. Fish and Wildlife Service, and the Department of Biological Sciences at Boise State University provided funding for my thesis research.

ABSTRACT

This study was completed during the summers of 2014 and 2015 to continue research on the relationship between harvester ants (*Pogonomyrmex salinus*) and slickspot peppergrass (*Lepidium papilliferum*). Slickspot peppergrass is a rare mustard endemic to south western Idaho. Over the past century, the species has declined in abundance, largely as a result of habitat degradation and fragmentation. In addition to these reasons for decline, seed foraging by harvester ants and plant destruction by harvester ants and small mammal herbivores have been recently indicated as potential factors. This study confirmed that harvester ants remove large numbers of *L. papilliferum* seeds. Specifically, we found that 90–100% of the seeds produced by individual plants were often removed by ants, regardless of the number of seeds within the foraging range of a colony. Nevertheless, although intensive seed foraging was common, we estimated that the number of seeds available to individual ant colonies often exceeded the colony's capacity for seed removal. Thus, when seed numbers are high, predator satiation may be a viable mechanism for *L. papilliferum* seeds to escape seed removal by harvester ants.

In addition to documenting the occurrence and intensity of seed removal by ants, we confirmed that small mammals and harvester ants sometimes act as herbivores on leaf and (in the case of mammals) root tissues. Mortality as a result of herbivory was at times high, although understanding the causes of variation in patterns of herbivory will require further study. In addition to investigating seed predation and herbivory on naturally

occurring plants, we evaluated whether seed introductions represent a viable tool for recovery of slickspot peppergrass. We were successful in germinating *L. papilliferum* seeds, although our efforts were severely hampered by seed predation by harvester ants. In order for seed introductions to become a feasible approach to recovery efforts for *L. papilliferum*, a method to mitigate the effects of seed predation will be needed.

Finally, because this document was written as publishable chapters that reflect the contributions of multiple authors, it has been written in first person plural (i.e., we) rather than first personal singular (i.e., I).

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Abstract

Seed predation can be disproportionally harmful to populations of rare plants, and has the potential to drive local extinction of species. The current study sought to determine whether surface texture and seed availability influenced seed predation by harvester ants (*Pogonomyrmex salinus*) on a rare species, slickspot peppergrass (*Lepidium papilliferum*). Within slick spots, we found that *L. papilliferum* occurred disproportionately in areas of roughly textured (complex) soil. However, complex soils did not make seeds less vulnerable to seed predation, which suggests some other mechanism, such as seed drift, explains the patterns. Seed loss by harvester ants averaged 82.1±4.1% per plant (N=29, range = 31.6-99%). Contrary to our prediction, we found no evidence that the percent of seed loss by individual plants declined as a function of total seed availability within the ant colony's foraging range, despite situations where it was clear that seed loss to ants could not be maintained uniformly at the detected levels. We maintain the predator satiation is a plausible mechanism by which *L. papilliferum* seeds avoid consumption by harvester ants. Further research is warranted to clarify the satiation hypothesis.

Introduction

Seed predation reduces the fitness of individual plants, and has the potential to alter the structure and composition of plant communities. When herbivory is directed toward propagules (e.g., seeds), as well as other reproductive structures (e.g., flowers), it has immediate negative consequences on the reproductive success and fitness of the parent (Janzen 1971, Louda and Potvin 1995, Weppler and Stöcklin 2006, Leja et al. 2015). By contrast, when herbivory is focused on leaves and other vegetative structures, the detrimental consequences to fitness often are less immediate and severe (Hawkes and Sullivan 2001, Maron and Crone 2006). Compared to vegetative structures, seeds typically pack higher nutritional value (Crawley 2000), which makes them targets of herbivores. Post dispersal seed predation rates on individual plants and their populations vary widely, and in some cases involve complete seed loss (Anderson and Ashton 1985, Crawley 2000, Albert et al. 2005, White and Robertson 2009a). The consequences of high seed removal may be especially severe to rare plant populations, where any effect on growth, survival, or offspring recruitment could limit the plants' recovery or even drive it to extinction (Crawley 2000, Ancheta and Heard 2011).

In response to the negative fitness consequences of seed predation, many plant species have evolved defenses that mitigate their losses to foragers. For example, seeds with stronger seed coats were less likely to be consumed by foraging ants and served to limit the vulnerability of seeds to predators (Rodgerson 1998). In other species, chemical defenses produced as secondary metabolites serve as effective deterrents to seed and fruit predation (Ahuja et al. 2010, Mithöfer and Boland 2012, Samuni-Blank et al. 2012). In contrast to the use of mechanical and chemical defenses, some plants compensate for seed loss by producing large numbers of seeds to overwhelm a predator’s capacity to consume them. A unique example of this is masting–the synchronous production of large amounts of seed within a species, followed by long periods of low seed production in non-masting years (Kelly 1994). Masting species are typically long lived, wind-pollinated plants with seeds that predators can readily discover (Crawley 2000). By contrast, most annual and perennial plants vulnerable to seed predators are unable to undergo this landscape level coordination of reproductive effort, and thus are often subjected to consistently high levels of seed predation (Crawley 2000). The production of large amounts of seed may be in part a mechanism to compensate for high seed loss (Andersen 1987), as well as a mechanism to replenish depleted seed banks when conditions are favorable (Price and Joyner 1997, Meyer et al. 2005).

Harvester ants, particularly those in the genus *Pogonomyrmex,* play a primary role of seed removal in many arid and semiarid ecosystems in the Americas (Crist and Friese 1993, MacMahon et al. 2000, Johnson 2001, Beattie and Hughes 2002). Seed foraging and nest building by harvester ants have the capacity to alter plant communities and their associated ecological connections (Reichman 1979, Whitford 1988, MacMahon et al. 2000, Nicolai and Boeken 2012, Ostoja et al. 2013). Individual colonies can persist for up to 30 years (Porter and Jorgensen 1988) and maintain mostly non-overlapping home ranges (Gordon 1991, Brown and Gordon 2000, Howell and Robertson 2015). Colonies are typically uniformly distributed across the landscape with densities as high as 164 colonies/ha (Blom et al. 1991), although densities are typically much lower (Porter and Jorgensen 1988, Blom et al. 1991, MacMahon et al. 2000, Robertson 2015). Foraging trails that radiate away from the nest are selected daily by patrolling ants based on a number of factors that include food availability, surface substrate, and the activity of neighboring colonies (Gordon 1991, Johnson 1992, Greene and Gordon 2007, Flanagan et al. 2012, Howell and Robertson 2015). Foraging typically extends from spring to fall when seeds are abundant and soil temperatures are sufficiently warm (Whitford and Ettershank 1975). Prime temperatures for foraging occur mid-morning to the early afternoon and again in the evening.

Here we investigate the extent to which seed predation by harvester ants affects offspring recruitment in slickspot peppergrass, *Lepidium papilliferum* [(L. Henderson) A. Nels. & J.F. Macbr] (Brassicaceae), a rare mustard endemic to southwest Idaho. Previous studies have shown that harvester ants readily consume this plant's seeds (White and Robertson 2009a; Schmasow 2015), and in some cases completely denude individual plants of their seed-bearing fruits (I. Robertson, personal communication). Slickspot peppergrass has been proposed for protection under the endangered species act several times, most recently in 2009 (USFWS 2009). Over the past century, the species has declined in abundance, largely as a result of habitat degradation and fragmentation, which have been attributed to wildfire, exotic species invasions, irrigated agriculture, livestock grazing, urban development, and off-road vehicle use (Moseley 1994). Slickspot peppergrass plants are restricted to growing microsites called slick spots. Slick spots are small, shallow depressions in the landscape comprised of compacted clay layers and a high salt content that are largely void of other vegetation (Fig. 1.1) (Fisher et al. 1996). Slickspot peppergrass plants have a dual life strategy. Often they grow as a vegetative rosette their first year and mature into a dome-shaped, biennial plant the second year that produces large numbers (often thousands) of seeds that can remain viable in the soil seed bank for up to 11 years. Slickspot peppergrass is also capable of flowering during the first year, but these annuals do not produce as many flowers as the second year biennials (Meyer et al. 2005, White and Robertson 2009b). Seeds drop from the parent plant when the silicle fruits dehisce, usually in July and August (Meyer et al. 2005).

Owyhee harvester ants, *Pogonomyrmex salinus* [Olsen], have the capacity to remove large portions of fruits and seeds directly from individual *L. papilliferum* (White and Robertson 2009a), as well as seeds that drop to the ground (White and Robertson 2009a, Robertson and Crossman 2012). These ants are found throughout the range of *L. papilliferum* and their populations frequently overlap (Robertson 2015). Schmasow (2015) found *L. papilliferum* seeds were overrepresented in the diet relative to the availability of alternative food sources. This preference for *L. papilliferum* seeds is likely caused by a number of factors. First, *L. papilliferum* seeds have high nutritional values and a small size (~1.4 mm), which allows ants to easily collect a quality resource (Fewell 1988, Schmasow 2015). Additionally, *L. papilliferum* drop thousands of seeds into slick spots, thereby creating a clumped resource on a substrate that is easily navigable for ants (Crist and Wiens 1994, Bernadou and Fourcassié 2008, Bernadou et al. 2011, Flanagan et al. 2012). Not surprisingly, seed predation by harvester ants has emerged as a concern for the long-term viability of *L. papilliferum*, and the effectiveness of rehabilitation efforts.

When assessing the role of seed predation by harvester ants on the recruitment and survival of *L. papilliferum*, it is important to determine whether there are mechanisms or situations that promote the retention and survival of seeds on the ground. A cursory examination of slick spots reveals that *L. papilliferum* are often situated near the margins, and often on substrates that are rough in texture (Fig. 1.1). This growth pattern may result from the differential discovery of seeds by harvester ants as a function of surface texture. Specifically, margins may disproportionately contain substrate that enables seeds to evade seed foragers. Conversely, areas in slick spots that have smooth, hard-pan surfaces may increase the foraging efficiency of harvester ants (Bernadou and Fourcassié 2008, Bernadou et al. 2011), thereby resulting in disproportionate losses. One of the objectives of our study was to establish whether a relationship exists between substrate type and the occurrence of *L. papilliferum* within slick spots. We also addressed whether seeds deposited on roughly textured soils are less prone to discovery by ants than seeds deposited on smooth substrates.

In addition to the role that soil surface texture might have on seed survival, the total number of seeds available to ants in a given year might influence rates of seed loss. For example, Andersen (1987) showed that simulated post-fire increases in two Australian woodland plant species resulted in lower overall seed removal rates by ants (i.e., satiation effect). A similar effect in *L. papilliferum* is possible if in favorable years the plants within slick spots produce more seeds than harvester ants can remove. Such events may allow for the replenishment of *L. papilliferum* seed banks in favorable years. By contrast, in less favorable years, harvester ants may consume most or all of the seeds produced by *L. papilliferum*. Therefore, a third objective of our study was to determine whether the proportion of seeds lost by individual plants to harvester ants declines as the number of seeds available to ants increases.

Methods

Study Area

We conducted this study at a population of *L. papilliferum* located in near Melba, Idaho (element occurrence 018 [Kuna Butte SW], as designated by the Idaho Natural Heritage Program) during the summers of 2014 and 2015. Fire destroyed most of the big sagebrush (*Artemisia tridentata*) overstory at this site in the late 1990's, leaving behind a relatively open landscape that is currently dominated by Sandberg's bluegrass (*Poa secunda*), cheatgrass (*Bromus tectorum*), tumble mustard (*Sisymbrium altissimum*), with only an intermittent and sparse overstory of big sagebrush (*Artemisia tridentata*) and rabbitbrush (*Chrysothamnus viscidiflorus*). Slickspot peppergrass occurs sporadically in slick spots that dot the landscape at this site.

Surface Texture, Harvester Ants, and the Distribution of *L. papilliferum* in Slick Spots

In the summer of 2014, we surveyed 10 slick spots to assess the distribution of *L. papilliferum* rosettes as a function of surface texture. In an effort to establish whether seed predation by harvester ants influences the distribution of *L. papilliferum* within slick spots, five of the slick spots we selected were located within the foraging range of at least one harvester ant colony (i.e., 20 m) with active foraging in the slick spot, and five were located beyond the foraging range of a harvester ant colony. Using a 1 m^2 plot frame that was subdivided into 20 x 20 cm squares, we surveyed the entire area of each slick spot systematically and recorded the total coverage area of the following four substrate categories: grass covered, complex (lichens, mosses, pebbles), simple (smooth clay-pan), and intermediate (pebbled surface, frequent fissures in soil, rocks). The plot frame was laid on the ground with minimal disturbance to soil and plants. While assessing surface type we also counted all *L. papilliferum* within the plot frame, and noted for each the life history stage (vegetative rosette, flowering annual, flowering biennial) and the type of substrate in which it was growing. We calculated the proportion of each surface substrate within each slick spot. These values represent the available surface substrate for each slick spot. We additionally calculated the proportions of plants in specific surface substrates. These values represent the used surface substrates. Paired t-tests were used to determine if plants were disproportionally growing in any of the surface substrates.

The area values for the slick spots and the number of plants within them were log transformed to meet normality. A t-test was utilized to distinguish a difference in the overall size of the slick spots where we did and did not observe harvester ants. A two factor ANOVA was used to determine if the numbers of plants varied between slick spots where we did and did not observe harvester ants. A Tukey test was then used to evaluate the group means.

Surface Texture and the Vulnerability of *L. papilliferum* Seeds to Predation by Harvester Ants

In July of 2014, we selected 10 slick spots, each of which was located within the foraging range of a harvester ant colony, to determine whether surface texture influences the vulnerability of *L. papilliferum* seeds to discovery and removal by harvester ants. At each of the slick spots, we placed three cages (15 cm diameter flower pots with the base removed) on complex substrate (i.e., those areas that included lichens, mosses, and small rocks) and three cages on simple substrate (i.e., smooth clay-pan). One of the cages placed on complex substrate and one on simple substrate were fixed tightly to the ground to prevent access by ants. The top rim of each cage was coated with a thin band of

Tanglefoot® to deny ants access over the top of the cage. These cages functioned as controls. The remaining four cages were elevated approximately 2 cm above the soil surface to allow access by ants. Wire mesh was placed over the top of the cage and around the elevated base to exclude seed predators other than ants. Two of the cages (one in complex substrate and one in simple substrate) were placed 0.5 - 2 m away from the ant colony. The other pair was placed 6 - 10 m away. We deposited 200 *L. papilliferum* seeds onto the soil surface within each cage, and lightly misted the seeds with distilled water to help them adhere to the surface. Three days later the top centimeter of soil from within the cages was collected and returned to the laboratory. We sifted each sample through a 500μm sieve to remove silt and other fine particles. The remaining material was meticulously searched for *L. papilliferum* seeds. To evaluate if we were able to recover equal amounts of seeds from both soil types, we used a Wilcox Test for the control cages. The numbers of seeds recovered from the beneath the treatment cages were log transformed to meet normality. We used a two-factor ANOVA to test for an effect of treatment on the number of seeds present in our samples.

<u>Seed Predation as a Function of Seed Availability</u>

We conducted a study to evaluate whether the intensity of seed predation on individual plants by harvester ants varies inversely as a function of total seed availability. We began by selecting 20 ant colonies whose foraging ranges included areas of flowering *L. papilliferum*. In making these selections, we attempted to encompass the full range of *L. papilliferum* seed availability to ants that year. Once sites were selected, we estimated the number of slickspot peppergrass seeds available to each harvester ant colony. This estimate was completed by taking an inventory of the slickspot peppergrass plants within the foraging home range of the colony. We measured the total flowering surface area of *L. papilliferum* located within each ant colony's foraging range, and while doing so incorporated the curvature of each plant in our assessment. To translate flowering surface area measurements into estimates of *L. papilliferum* seed abundance, we first selected 10 *L. papilliferum* plants from a different area of the study site and counted the total number of seeds produced on inflorescences that occupied a 5 x 5 cm area of each plant's flowering surface. Based on the average number of seeds produced per 5 x 5 cm area of a plant's surface (1,160 seeds), and total flowering surface area within each ant colony's foraging range, we estimated total seed production available to each of the 20 ant colonies.

At each of the 20 study sites, we selected one to three pairs of individual *L. papilliferum*, depending on the size of the colony's foraging range in areas where it overlapped with flowering *L. papilliferum* to estimate seed removal by harvester ants. Each pair of plants consisted of individuals that were similar in size, proximity, flowering phenology, and distance from the ant colony. One plant from each pair was randomly assigned to the treatment (ants present) and the other to the control (no ants). In late June, we fixed a 15 cm high, 40 cm diameter plastic barrier flush to the ground around each control plant. Ants could not ascend the barriers or travel beneath them, and thus were denied access to control plants. The same type of barrier was placed around treatment plants; however, these barriers were elevated 2 cm above the ground on small stilts, thereby allowing access by ants (Fig. 1.2). Chicken wire was secured over the tops of all barriers to exclude mammals and birds while allowing access by pollinator insects. The barriers remained in the field for the duration of the experiment. We routinely checked the control cages for ant intrusions, and in cases where intrusions were found, we corrected for any obvious points of entry past the barrier. To estimate the foraging intensity on plants accessible to ants, we visited each treatment plant daily (except on weekends) and over a 30-second time interval tallied the number of ants inside each cage. We visited the cages between 0830-1230 in order to capture the peak harvester ant foraging hours, and altered the order of our visitations each day.

In late August, once plants had senesced and dropped the majority of their seeds, we collected the top centimeter of soil located within the confines of each barrier, placed the samples individually in brown paper bags, and returned them to Boise State University. We sifted the soil samples through a 500 µm sieve to remove silt and fine grain sand, and meticulously searched the remainder of each sample material for individual *L. papilliferum* seeds. The percent seed removal for each pair was calculated by the following equation:

(1- (seeds remaining in the treatment)/(seeds remaining in the control) * 100).

We used generalized linear mixed modeling with a negative binomial distribution to investigate what variables were important in determining how many seeds escaped predation by ants. Before analysis, we tested our variables for multicollinearity. For each treatment cage, we used the estimated number of seeds available at the slick spot level, the linear distance between the plant and the colony, and the average number of ants observed in the treatment cages to predict the number of seeds that the ants were unable to collect from the treatment plant. The number of seeds available in the paired control cage (an index of plant size) was accounted for by including an offset variable of the natural log number of seeds in each of the paired control plants. A random effect of “ant colony" was included to account variation among colonies. The fixed effects were scaled to standardize their units before analysis. We considered an effect to be statistically significant if the predictor variable from the model had a p-value <0.05 following a Type II Wald Chi-square Test.

All statistical analyses were conducted in R (R Development Core Team 2012). We fit our model using the package "lme4" (Bolker et al. 2013). Means ± SE are reported.

Results

Surface Texture, Harvester Ants, and the Distribution of *L. papilliferum* in Slick Spots

The five slick spots associated with harvester ant foraging were no different in area than the five without (t_8= -0.756, p=0.472). There was a significant interaction between the categorical value of ant presence and life history stages of plants within the slick spot ($F_{2,24}$=13.5, p=0.00012). Slick spots without ants had significantly more *L. papilliferum* rosettes (p=0.00369, Fig. 1.3), about the same number of annuals (p=0.148, Fig. 1.3), and fewer flowering biennials (p=0.0193, Fig. 1.3) than slick spots with ants.

Lepidium papilliferum were not distributed in proportion to the availability of the four types of substrate we recognized within slick spots (Fig. 1.4), except in the case of intermediate substrates (α= 0.0125, t_9=-0.96 p=0.36). Plants were underrepresented in grass covered areas (α= 0.0125, t_9=5.56, p=0.00035) and simple, clay-pan surface substrates (α= 0.0125, t_9=6.05, p=0.00019), and overrepresented in complex substrates (α= 0.0125, t_9=-4.36, p=0.0018).

Substrate Texture and the Vulnerability of *L. papilliferum* Seeds to Predation by Harvester Ants

Nine of the 60 cages we placed in slick spots were disturbed during the experiment and were therefore removed from the analysis. There was no significant effect of surface substrate on our ability to recover seeds from control cages (Wilcox Test, W=34, p=0.885). From the control cages, we recovered an average of 152.2 ± 4.7 of the 200 seeds we started with. The results of the ANOVA included no significant effect of distance to the colony ($F_{1,30}$=0.005, p=0.94) or soil type ($F_{1,30}$=3.1, p=0.088) on the number of seeds we recovered from the cages. From the treatment cages, we recovered an average of 11.9 ± 5.4 of the 200 seeds we started with.

Seed Predation as a Function of Seed Availability

We selected a total of 29 pairs of *L. papilliferum* biennials at 20 ant colonies to evaluate the level of seed removal by harvester ants. The estimated number of available *L. papilliferum* seeds available to an individual ant colony ranged from 45,725-7,890,227 seeds (mean=1,711,268 ± 456,846). The distance between cages and colonies ranged from 2-20 meters. The average number of ants under the treatment plants was 2.32 ± 0.14 ants with a range from 0-60 ants. The number of seeds the ants were unable to remove ranged from 15-5,469 seeds (mean=731.45 ± 205.8). The average number of seeds available in the control cages, and our estimate of what was available to harvester ants at the plant scale (offset variables) was 5,840.45 ± 1,295.38 with a range of 149-23,500 seeds.

Comparing the total number of seeds found in the soil beneath treatment plants to their paired counterparts, we determined that, on average, harvester ants removed 78.8 ±

7.1% of the seeds that dropped to the ground (range = 0 - 99%). There was one instance where no seed loss was detected at a treatment plant. Observations confirmed that this particular pairing of *L. papilliferum* went undiscovered by ants throughout the experiment. When we removed this pairing from the analysis, mean seed loss to harvester ants increased to 82.1 ± 4.1% (range = 31.6 - 99%) (Fig. 1.6). We included all data points in further analyses.

Contrary to our prediction, the number of seeds available to individual ant colonies over the course of the season did not have a significant effect on the percent of seeds depredated from individual plants by harvester ants (Fig. 1.6, Table 1.1). Likewise, distance between a treatment plant and the ant colony was not a significant predictor of seed loss (Table 1.1). The only variable that contributed significantly to seed loss was the level of ant activity recorded at treatment plants (Table 1.1). Specifically, seed loss was higher on plants that had higher levels of ant activity (Fig. 1.7).

Discussion

This study provides compelling evidence that large numbers of *L. papilliferum* seeds lost are to predation by Owyhee harvester ants. However, contrary to expectation, we found no evidence that the proportion of seeds lost by individual plants to seed predation by ants declined as a function of increasing seed availability. Within slicks spots, *L. papilliferum* occurred disproportionately in areas of roughly textured (complex) soil compared to areas with smooth, clay-pan surfaces. However, complex soils did not make seeds less vulnerable to predation. Slick spots where harvester ants foraged contained significantly fewer *L. papilliferum* plants than slick spots without foragers, as well as significantly fewer first year biennial rosettes. Together these findings suggest that harvester ants likely have an important influence on *L. papilliferum* populations through intensive seed removal.

Anecdotally, it appears that *L. papilliferum* grow preferentially in rough, pebbly, often lichen-covered areas of slick spots, which corresponds with our definition of “complex” surface substrates. We hypothesized that complex substrates may provide seeds physical refuge from harvester ants, thereby leading to the observed pattern of colonization within slick spots. Indeed, our analysis of individual slick spots confirmed that *L. papilliferum* are overrepresented in areas with complex surface substrate, and underrepresented in areas with smooth hardpan surfaces. However, this pattern does not appear to be a result of differential discovery rates of seeds–we found no difference in the ability of ants to discover and collect seeds in rough versus smooth surfaces within slick spots. It is possible that complex surface features on soils in our experimental manipulation did not deter ants from searching for seeds in these areas, and that presence of physical refuges in complex soils were negated by chemical cues detectable by ants. An alternative explanation for the overrepresentation of *L. papilliferum* in complex soils is that these areas are preferential for growth relative to simple soils. This possibility is addressed in Chapter 3, although the results were inconclusive. Finally, the pattern of *L. papilliferum* growth within slick spots may reflect seed drift. Robertson and Jeffries (2015) found that *L. papilliferum* seeds are capable of drifting over the winter, likely via wind and water. Seeds dropped on clay-pan soil may drift to the margins of slick spots and become lodged in complex substrates, thereby leading to the observed pattern. Experiments to address this possibility are ongoing.

Our surveys of slick spots revealed that ants were foraging in slick spots that contained large numbers of flowering biennials. Moreover, there were significantly more first year biennial plants (vegetative rosettes) in slick spots where we did not observe ant foraging. Harvester ants may be selecting to forage in areas where *L. papilliferum* seeds are plentiful. Not mutually exclusive to the prior statement, another intriguing question of the aforementioned pattern is: does the presence of large numbers of biennial rosettes in slick spots indicate the absence, or at least low intensity, of seed predation by ants in previous years? If true, it would indicate that the foraging activities of harvester ants are sufficient to limit slickspot peppergrass populations. An ant exclusion experiment carried out over multiple years would help to address this question.

Although it is premature to claim that harvester ants are limiting populations of slickspot peppergrass, it is clear that ants are capable of taking large numbers of *L. papilliferum* seeds from individual plants, even when there are extremely large numbers of seeds available in their foraging area (Fig. 1.6). We found that, on average, harvester ants removed 82% of seeds from individual plants (N=28, range=32-99%, excluding one case where no seed removal occurred), similar to the results of earlier studies (White and Robertson 2009a, Robertson and Crossman 2012). Intensive seed foraging by harvester ants is likely to be most impactful in slick spots with low number of flowering plants, where harvester ants could eliminate an entire generation from the seed pool. By contrast, when conditions favor seed production that exceeds the capacity for removal by ants (e.g., Andersen 1987), the seed bank may be replenished. Periodic replenishment of seed banks may serve as a buffer against severe seed loss to predators in less favorable seed production years.

It is difficult to estimate the number of seeds a *Pogonomyrmex* colony can consume in a season given that the number and types of seeds available, colony size, and competition with neighboring colonies, among other factors, will influence the number of seeds collected. *Pogonomyrmex rostratus* colonies in Argentina have been documented to remove about 60,000 seeds per season (Pirk and Lopez de Casenave 2006), while *Pogonomyrmex occidentalis* colonies in Wyoming have been documented to remove around 81,000 seeds in a season (Crist and MacMahon 1992). However, it is difficult to relate these estimates directly to our study. The seed intake capacity for a colony is largely driven by what type of seed they are foraging on. Slickspot peppergrass seeds are small (1.4 mm) relative to the grass seeds that make up the bulk of ant diet in studies that have estimated total seed intake. Larger seeds take longer to collect (Weier and Feener 1995, Morehead and Feener 1998, Pirk, and Lopez de Casenave 2010, Schmasow 2015) and a colony needs fewer of them to meet their dietary needs (Kelrick et al. 1986). A study by Schmasow (2015) documented the seed intake rate for Owyhee harvester ant colonies in Idaho from 2009-2011. The diet of these ants included several mustard and grass seeds, including large numbers of *L. papilliferum*. Based on the maximum intake rates recorded for *P. salinus* in Schmasow's study, and extrapolating these values to accommodate 6 hours over foraging by ants per day over a 3-month period, we estimate that Owyhee harvester ant colonies could remove 98,000-300,000 seeds in a season. For many of the slick spots included in our study, our estimates of *L. papilliferum* seed availability far exceeded the upper threshold of consumption by a harvester ant colony (we estimated that the foraging ranges of individual harvester ant colonies overlapped an average of 1.7×10^6 *L. papilliferum* seeds [range: 45,725-7,890,227]). Thus, despite intense seed predation by harvester ants on *L. papilliferum*, it seems likely that large numbers of seeds would have escaped predation in our study.

Although many slick spots in our study were estimated to contain more *L. papilliferum* seeds than could be reasonably consumed by a harvester ant colony in a season, we failed to find an effect of total seed availability on the percent of seeds lost per plant to seed predators. The significant correlation between seed loss and ant activity we found confirms that ants were responsible for the seed losses we observed. However, the lack of support for the satiation hypothesis should be viewed with caution because the high levels of seed loss we recorded at many of the plants in slick spots with high numbers of *L. papilliferum* seed would not be sustainable across all plants in the slick spot. High levels of seed removal in slick spots with large numbers of seeds may indicate sampling bias. For example, the cages in our study may have inadvertently been placed in areas of high foraging activity while plants in other areas of the ants' foraging range were subjected to much lower levels of seed removal. Because harvester ants forage more intensively near trunk trails than in other areas of their foraging range (Mull and MacMahon 1997), future studies should sample more widely throughout the foraging range of individual colonies. Given the effort-intensive nature of the sampling procedure, a different technique may be needed.

Understanding the factors that promote seed survival in the face of intense seed removal by harvester ants is critical to the development of effective management strategies for slickspot peppergrass. The present study detected a pattern in the occurrence of *L. papilliferum* with respect to surface texture, but fell short of identifying a mechanism to explain the pattern. The study also confirmed that harvester ants have the capacity to consume large numbers of *L. papilliferum* seeds from individual plants, but did not detect a satiation effect even when seed numbers were in excess of what a colony would be expected to consume in a season. Despite this outcome, it remains reasonable to suggest that *L. papilliferum* can experience reduced rates of seed predation in years of high seed production, and that this may be a mechanism by which slick spots can periodically replenish their seed banks. However, additional research is needed to confirm this mechanism. Rather than conducting experiments that focus on counting how many seeds escape predation by ants (as in the current study), it may be more feasible to measure the effects of ant colony removal on *L. papilliferum* survival and productivity within slick spots.

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Tables

Table 1.1 The results of a generalized linear mixed model with a negative binomial distribution was used to predict the number of seeds remaining after harvester ant foraging. The fixed effects were the number of available seeds in the slick spot, the distance the plant was from the ant colony, and the average number of ants observed in the treatment plant cage. We included an offset in the analysis from the total number of seeds from the paired control plant and the random effect of slick spot. We considered effects with a $p<0.05$ to be significantly different from zero.

Fixed Effect	**Wald X^2**	**df**	**p**
Total number of seeds available	0.04	1	0.85
Distance to the ant colony	1.92	1	0.17
Mean n ants in treatment cages	20.6	1	$<0.001^*$

Figures

Figure 1.1 Photo of a typical slick spot with flowering *L. papilliferum*. Note the preponderance of plants growing in the complex soil (i.e., lichens, mosses, small stones) along the margin of the slick spot, and the absence of plants on the simple soil in the center of the slick spot.

Figure1.2 Pairs of slickspot peppergrass plants were matched for size and then caged within a plastic barrier. One plant was exposed to harvester ants by elevating the cage slightly (on right), whereas the other plant was protected from ants by fixing the cage tightly to the ground (on left). The barriers remained in place for the duration of the experiment.

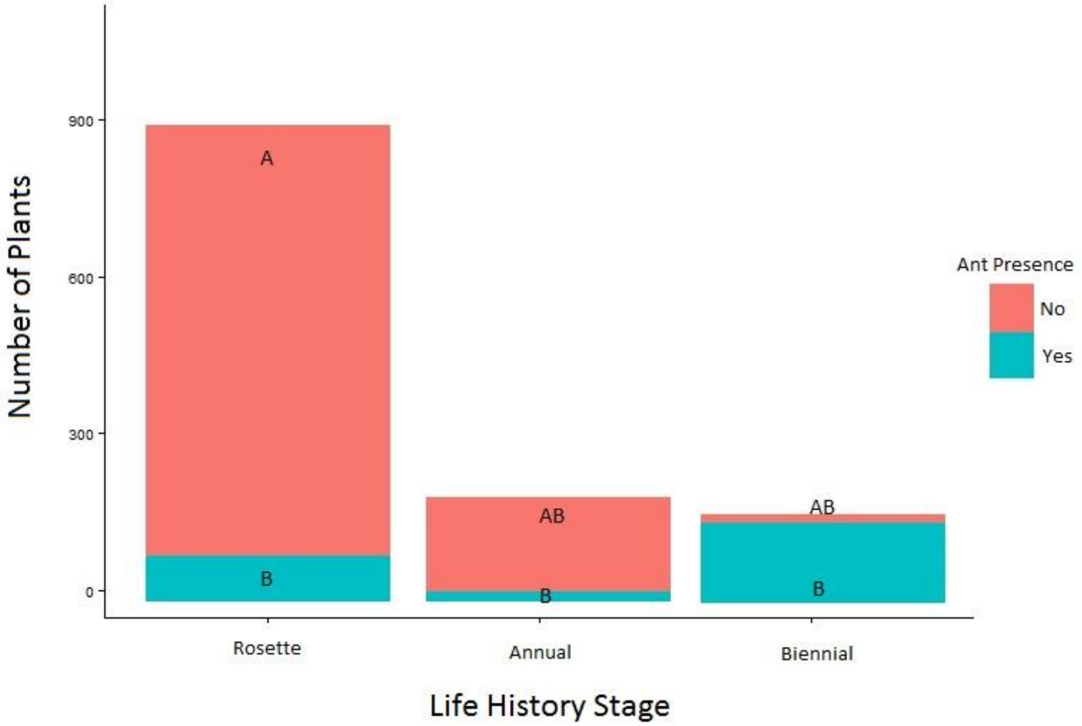

Figure 1.3 Bar plot showing the number and life history stages of plants found in slick spots with (blue) and without (red) harvester ants present (N=5 of each). Three life history stages were noted: Rosette (vegetative rosette, first year biennial), Annual (flowering annual), and Biennial (flowering biennial). Different letters indicate significant differences. Slick spots without ants had significantly more vegetative rosettes than slick spots with ants (p=0.004).

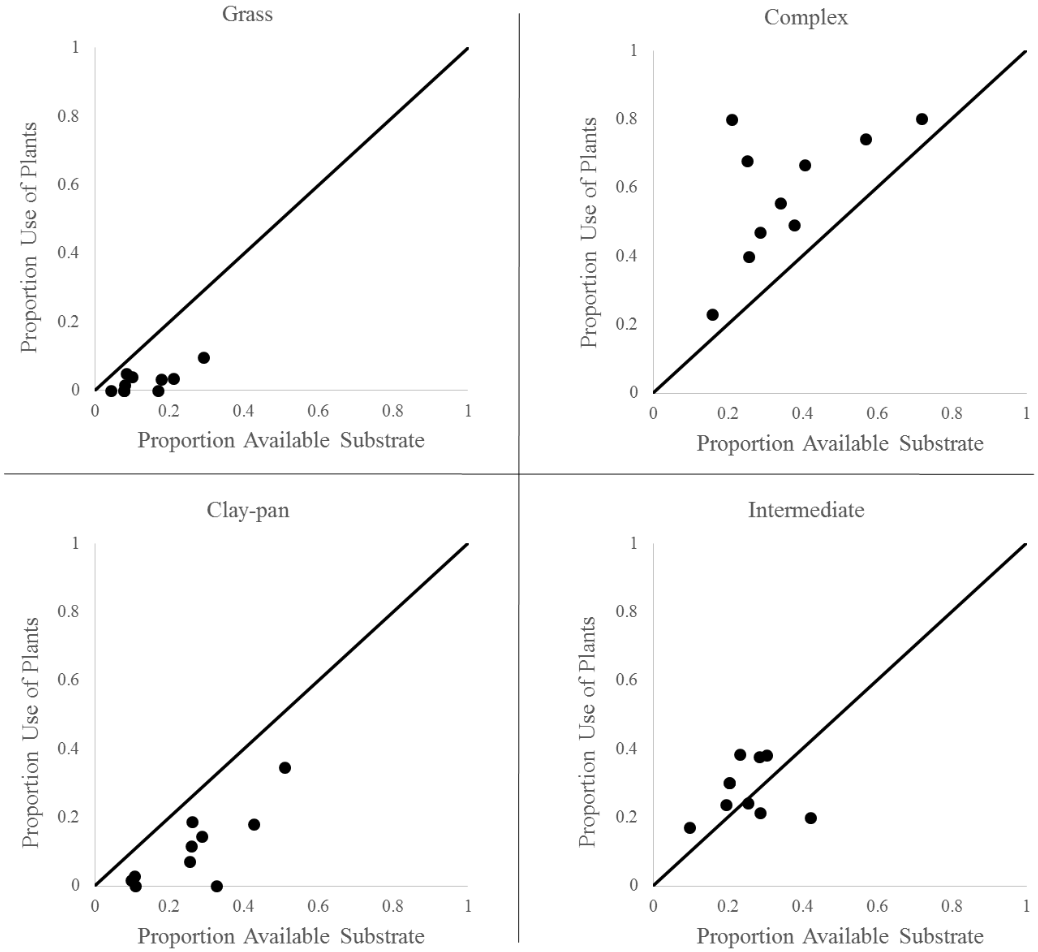

Figure 1.4 The proportional use of four surface substrate types (grass, simple soil, complex soil, and intermediate soil – see text for details) as a function of their availability within slick spots. The diagonal line on each graph represents a 1:1 match between availability and use. Plants avoided grass covered areas (α= 0.0125, t_9=5.56, p=0.00035) and simple clay-pan substrates (α= 0.0125, t_9=6.05, p=0.00019), occurred disproportionately in complex substrate (α= 0.0125, t_9=-4.36, p=0.0018), and proportionally in substrates intermediate between simple and complex (α= 0.0125, t_9=-0.96 p=0.36).

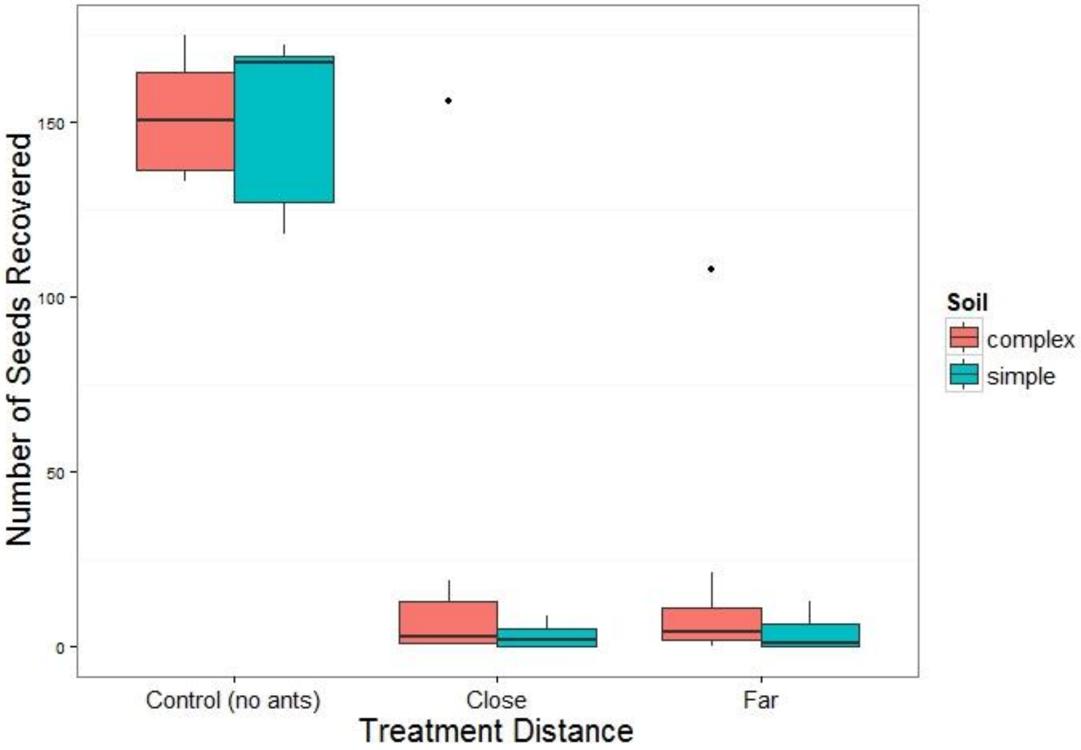

Figure 1.5 Results of seed refuge experiment. The horizontal bar within each box represents the median. The boxes represent the interquartile range. Vertical lines show total range. The red boxes indicate data from cages on complex surface features and the blue on simple surfaces. Ants removed most of the seeds in cages exposed to predation, regardless of soil type ($F_{1,30}$=3.1, p=0.088) or distance from the colony ($F_{1,30}$=0.005, p=0.94). Seed recovery was much higher in control cages (i.e., where there was no seed predation by ants).

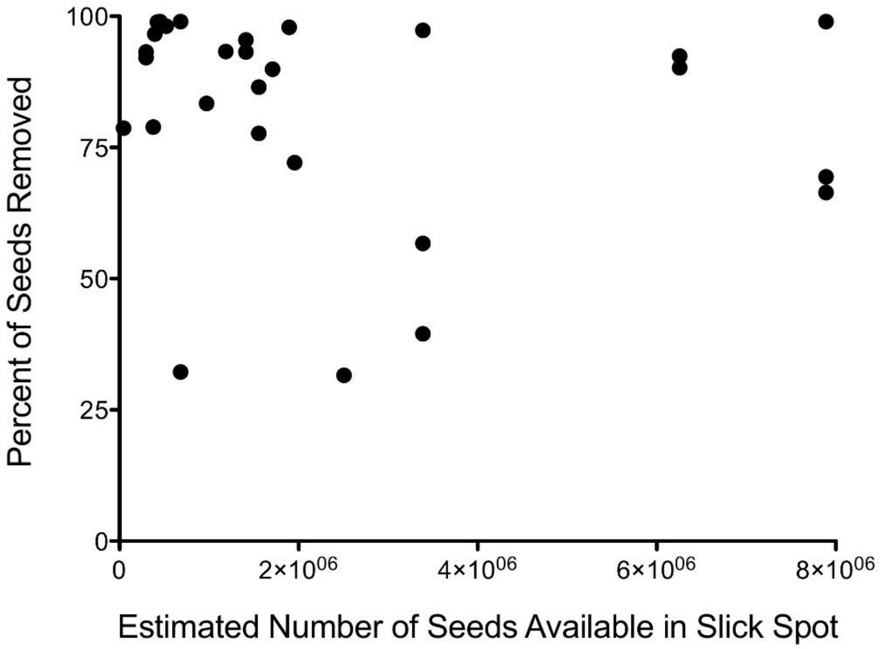

Figure 1.6 Percent seed loss due to herbivory by harvester ants as a function of estimated seed abundance within slick spots. The one case in which ants did not discover the treatment plant is not shown.

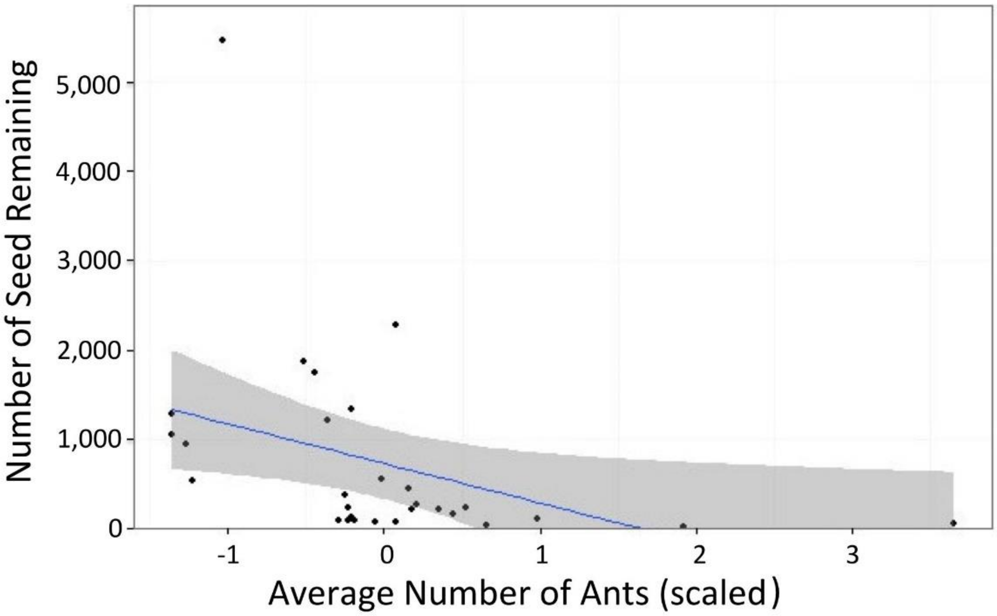

Figure 1.7 The number of seeds remaining in soil as a function of ant foraging activity on and beneath individual treatment plants. Ant activity is scaled to allow for comparisons of effect size among the three fixed effects. Gray bars represent 95% confidence intervals.

CHAPTER TWO: EVIDENCE OF HERBIVORY ON SLICKSPOT PEPPERGRASS BY SMALL MAMMALS AND HARVESTER ANTS

Abstract

Small mammals and insect herbivores can influence the structure of plant communities. The effects of herbivory can be particularly influential on rare plant species. In the present study, we documented and quantified herbivory on a rare mustard found in south-western Idaho, slickspot peppergrass (*Lepidium papilliferum*). We confirmed that Ord’s kangaroo rats (*Dipdomys microps*) are responsible for removing *L. papilliferum* plants, and that vole (*Lemmiscus curatus* or *Microtus montanus*) and mountain cottontail (*Sylilagus nuttallii*) may also be contributors to herbivory. Herbivory by small mammals was higher in 2014 (64%) than in 2015 (<1%), and in both years plants with signs of herbivory suffered higher mortality than those without. In 2015, we expanded our efforts to include harvester ant defoliation. Defoliation by harvester ants (*Pogonomyrmex salinus*) was found at 18.7% of *L. papilliferum* plants that we monitored, and the occurrence declined as a function of distance from the ant colony. As with small mammal herbivory, a significant number of plants with defoliation by harvester ants did not survive. Patterns of herbivory varied by year and study plot, and it is unknown what factors drive these patterns. Further study is needed to understand if herbivory by small mammals and harvester ants are detrimental to the persistence of this rare species.

Introduction

Herbivory can have important consequences for the structure and composition of plant communities (Bakker et al. 2006, Ohgushi 2008, Stam et al. 2014). The magnitude of these consequences can vary as a function of the herbivore community. For example, in some ecosystems, rodents have a major influence on plant communities (Hulme 1996, Howe et al. 2006), whereas in others insects play a more significant role (Bigger and Marvier 1998, Stam et al. 2014). Herbivory, broadly defined, takes many forms and may include direct removal of leafy, woody, root, or reproductive tissue, siphoning of sugars, pollen collection, seed predation, and parasitism. Depending on the specific structure(s) targeted by herbivores, plants may compensate for their losses through growth and repair (Hawkes and Sullivan 2001, Maron and Crone 2006), or they may suffer a direct loss of fitness when reproductive structures are compromised (Louda and Potvin 1995).

The impact of herbivory on a plant species is often influenced by the health and integrity of the habitat (Hawkes and Sullivan 2001, Maron and Crone 2006). Plant communities in areas of ecological disturbance are often more vulnerable and sensitive to the effects of herbivory than plant communities in undisturbed habitat (McEvoy and Coombs 1999). Such may be the case in sagebrush-steppe habitat within the Great Basin of the United States, where the invasive European grass, *Bromus tectorum* (cheatgrass), has altered plant communities (Novak and Mack 2001) and natural fire cycles (Link et al. 2006, Taylor et al. 2014) to such an extent that sagebrush stands in many areas have been replaced by grassland. Studies have shown that the prevalence of *B. tectorum* on the landscape has reduced the availability of preferred foods of many small herbivores (Hall 2012, Rottler et al. 2015, Lucero et al. 2015), and in the process exposed other plant species to higher levels of herbivory than they would normally experience (Beckstead et al. 2008). One plant that may be vulnerable to such a shift in herbivore diet is slickspot peppergrass, *Lepidium papilliferum* [(L. Henderson) A. Nels. & J.F. Macbr], a rare mustard (Brassicaceae) endemic to sagebrush-steppe habitat in southwest Idaho. As a rare plant living in disturbed habitat (Kinter et al. 2013), slickspot peppergrass may be particularly vulnerable to the effects of herbivory because any source of mortality could limit its ability to maintain or expand populations (Ancheta and Heard 2011).

Within sagebrush-steppe habitat, *L. papilliferum* is restricted to growing in slick spots–areas of compact clay characterized by higher water retention and salt content than surrounding areas (Moseley 1994). The plant's numbers have declined since its discovery in 1892 (Moseley 1994), which resulted in its proposal for protection under the endangered species act (USFWS 2009). There are currently about 80 known sites where the plant persists (USFWS 2009) and at least 21 additional sites have been extirpated since the species was discovered (Moseley 1994). Population declines have been attributed to habitat fragmentation and degradation, largely as a result of human expansion, livestock grazing, and wildfire (Moseley 1994). More recently, seed predation by Owyhee harvester ants, *Pogonomyrmex salinus*, has been identified as an important factor that may significantly limit the ability of the species to regenerate (Chapter 1; White and Robertson 2009, Schmasow 2015). Here we examine the occurrence of (non-seed related) herbivory on *L. papilliferum* in an effort to assess whether herbivory represents a serious challenge to this plant's conservation and management.

Herbivory by small mammals and insects has the potential to influence the performance of many grass and forb species, as well as overall plant community structure. For example, Hulme (1996) found that rodents are significant contributors to plant mortality in several grassland species, largely because herbivory in these cases often involves the removal of entire plants. At larger scales, foraging by small mammals can alter entire plant communities (Howe et al. 2006), and the same is true for many insect herbivores in plant communities (Andersen 1988, Louda and Potvin 1995, Ohgushi 2008, Ancheta and Heard 2011, Stam et al. 2014). In the case of slick spot peppergrass, harvester ants, *Pogonomyrmex salinus* (Hymenoptera: Formicidae), have been shown to consume large numbers of seeds (White and Robertson 2009, Robertson and Crossman 2012, Schmasow 2015), and there are accounts of the ants engaging in herbivory on the plant's leaves as well (I. Robertson, personal communication). Harvester ants exhibit intensive nest clearing behavior that can have drastic, albeit localized, community level effects (Willard and Crowell 1965, Clark and Comanor 1975, MacKay 1981, Jorgensen and Porter 1982, Kugler 1984). Herbivory related to nest clearing may be problematic for *L. papilliferum* given that harvester ant colonies are often found in close proximity to slick spots where the plant resides (Robertson 2015).

Because little has been documented concerning defoliation of slickspot peppergrass by small mammals and harvester ants, we set out to (1) document the extent of mammal herbivory on *L. papilliferum,* as well as the identity of the herbivore species, and (2) quantify the extent to which harvester ants contribute to vegetative loss on *L. papilliferum*, particularly as it relates to nest clearing behavior by the ants.

Methods

Study Area and Site Selection

The study was conducted at a population of slickspot peppergrass located near Melba, Idaho (Kuna Butte SW, Idaho Natural Heritage Program element occurrence #018) during the summers of 2014 and 2015. The site burned in the late 1990's and is now dominated by Sandberg's bluegrass (*Poa secunda*), tumble mustard (*Sisymbrium altissimum*), and cheatgrass (*Bromus tectorum*), with a sparse overstory of big sagebrush (*Artemisia tridentata*) and rabbitbrush (*Chrysothamnus viscidiflorus*). Slickspot peppergrass (*Lepidium papilliferum*) is scattered within slick spots throughout the area.

Herbivory by Small Mammals

In 2014, we conducted a pilot study to document the incidence of herbivory by small mammals on *L. papilliferum*. A total of four plots, each approximately 1-2 m^2 in area, were established in two slick spots where signs of mammalian herbivory had been noted (e.g., chewed leaves, sudden disappearance of individual plants). We counted the total number of *L. papilliferum* plants within each plot, and monitored the plots daily from late July to mid-August. A Moultrie M-880 motion-sensitive infrared trail camera was focused on each of the slick spots for several days in an effort to determine the identity of herbivores foraging on *L. papilliferum*. In addition, we tallied how many plants in each plot were lost to herbivory, or in some cases, to unknown causes, during the monitoring period.

In 2015, we expanded the analysis of mammalian herbivory to include plots in 13 separate slick spots occupied by *L. papilliferum*. All slick spots included in this year of study had at least 500 individual *L. papilliferum* at the start of the summer. Within each slick spot, we established a single 1-2 m^2 plot, the borders of which were defined with flagging tape. We counted the total number of plants in each plot, and selected 15 of them for detailed monitoring. These 15 plants included a range of sizes within each plot, and were selected only if they appeared healthy. At the base plant, we fixed a uniquely numbered aluminum tag for identification purposes. We checked the plants daily, excluding weekends, for evidence of rodent disturbance. These disturbances were categorized as follows: (1) plants removed entirely with no visible remains, (2) plants removed with leaves scattered about, and (3) plant with evidence of leaf removal or digging at the base, but otherwise intact (Fig. 2.1). Categories included plants that were entirely removed with no plant remains, removed plants with leaves scattered about, and plants that remained intact with a few clipped leaves or evidence of mammal digging at the base.

At the end of the monitoring period, we recorded the fate of each of the tagged plants: alive, dead, or withering. The withering category was used for plants that were alive but showed signs of water stress (i.e., browning, curled leaves as opposed to fresh green leaves). These plants were capable of continued growth following precipitation, but vulnerable to death if adverse conditions continued. A Pearson’s Chi-squared test was used to determine whether plants that showed evidence of herbivory had lower survival rates than those that did not.

In addition to daily checks of the plots, four Moultrie M-880 trail cameras were set up at plots on a rotational basis from late June through early August of 2015. Cameras remained in place at individual plots for 1 week, after which time they were moved to a different plot. As before, the cameras were used to establish the identity of mammals foraging on or within *L. papilliferum*. Sequential photos of animals were counted as single events unless a new (i.e., obviously different) individual entered the frame or more than five minutes elapsed between frames. Mammal activity was classified as herbivory on *L. papilliferum* if there was direct evidence of the animal consuming a plant. In cases where an animal was photographed while engaged in activities suggestive of herbivory (e.g., digging among *L. papilliferum*, or head depressed within *L. papilliferum*), we scored the activity as a possible instance of herbivory. If a photograph revealed no clear indication of an animal's activity (e.g., the animal was only partially in the frame or otherwise obscured), the activity was scored as unknown. Finally, in cases where an animal was captured in sequential frames as it traversed a slick spot, we scored the event as a case of no herbivory.

Defoliation by Harvester Ants

In the summer of 2015, we selected 10 slick spots occupied by *L. papilliferum* that also had an active harvester ant colony located within the slick spot or along its margin. Within each slick spot we established one to four linear transects, depending on the number of *L. papilliferum* present (i.e., more transects were established when the ant colony was surrounded by *L. papilliferum*). Along each transect we placed a 20 cm X 20 cm plot at 1 m intervals from the ant colony, up to a distance of four meters. One corner of each plot was anchored at a *L. papilliferum* rosette. If there were no rosettes with the designated location of a plot, the plot was omitted. At each plot, we noted whether individual *L. papilliferum* present showed signs of harvester ant herbivory, and whether the plant was alive or dead. A third category of unknown was included for plants of which we were unable to assume a fate. Within each of the slick spots, we also noted the maximum distance (from the ant colony) of herbivory on *L. papilliferum* perpetrated by ants. We used a Kruskal-Wallis rank sum test to determine whether the incidence of herbivory by ants declined as a function of increasing distance from the colony. A Pearson's Chi-squared test was used to test whether herbivory lowered the survival rate of individual plants.

In addition to noting the number of *L. papilliferum* rosettes that showed sign of herbivory, we collected data on how quickly and efficiently ants remove leaves from the plant. We selected six colonies where we were able to observe ants while they removed leaves from individual rosettes. We followed individual ants for five consecutive minutes, during which time we recorded the number of attempted leaf removals, successful leaf removals, and rosettes involved. A different ant was used for each observation to ensure independence of samples. Observations were recorded mid-morning and early afternoon during peak harvester ant activity.

A separate set of observations was used to determine the fate of *L. papilliferum* leaves once they were collected by harvester ants. Using five different ant colonies as a source for ants, we located and followed individual ants that had a *L. papilliferum* leaf in their mandibles, and noted whether the leaf was returned to the nest or discarded by the ant. A different ant was used for each observation to ensure independence of samples. A Welch two sample t-test was used to determine if ants discarded leaves more often than they returned them to the colony. We also conducted a single 10-minute observation at each of the five ant colonies to quantify the types of leaves returned to the nest by harvester ants, and more specifically, whether *L. papilliferum* leaves were returned more often than other types of vegetation. These data were analyzed using a Welch two sample t-test.

All statistics were completed using R (R Development Core Team 2012). Standard errors were calculated and reported as ± from the mean.

Results

Herbivory by Small Mammals

Of the 78 rosettes we monitored in 2014, small mammals removed 50 (64%) (Fig. 2.1A,B). An additional 18 (23%) rosettes were lost to unknown causes. Only 10 plants survived to the end of the season (Table 2.1). Mortality across the four plots that resulted from confirmed cases of herbivory by small mammals averaged 61 ± 0.91% (range = 50 to 73%). Percent mortality from unknown causes averaged 27 ± 4.2% (range = 3.9 to 50%). Trail cameras confirmed a single case of an Ord's kangaroo rat (*Dipdomys microps*) consuming slickspot peppergrass (Fig. 2.2).

In 2015, 22 of the 2,684 (<1%) plants we monitored across 13 plots showed signs of disturbance by small mammals (Table 2.2). Disturbances included plants that had been extracted from the ground with no remnants left behind, extracted plants with leaves scattered nearby, and intact plants with a few removed leaves or signs of digging at the base of the plant. Eight were dug out of the ground with only leaves remaining (Fig. 2.1A), and one was removed completely (Fig. 2.1B). The remaining 13 individuals showed signs of herbivory, but the plants remained intact in the soil (Fig. 2.1C). In total, 195 plants were closely monitored on a daily schedule, excluding weekends. Only 12 of the 22 disturbed plants were tagged and just three of those tagged plants were surviving at the end of the season (75% mortality). Tagged rosettes that were not disturbed suffered only 28% mortality to other factors. A chi-squared test showed that rosettes were more likely die if a rodent disturbance occurred to that plant (Pearson's Chi-squared test: X_2^2=11.70, p=0.0029, Fig. 2.3). Evidence of disturbance by rodents on individual plants often escalated over successive days. In most cases plants were first observed to have a few leaves removed and/or evidence of digging near their base. Several days later the plant was removed from the ground. In many cases, the taproot disappeared completely, but leaves were found uneaten.

The trail cameras recorded 213 still shots of vertebrates in slick spots (Table 2.3). Non-herbivores, such as badgers (*Taxidea taxus*) and burrowing owls (*Athene cunicularia*) were removed from the analysis, leaving a total of 205 unique photos of herbivores. Only one confirmed case of herbivory on *L. papilliferum* was documented. As before, the animal involved was an Ord's kangaroo rat (Fig. 2.4A). An additional seven photos showed possible, but inconclusive, cases of herbivory on *L. papilliferum* by kangaroo rats (*Dipdomys microps*), a mountain cottontail (*Sylilagus nuttallii*), and voles (*Lemmiscus curatus* or *Microtus montanus*) (Table 2.3).

Defoliation by Harvester Ants

At least some level of defoliation by harvester ants was detected on 109 of the 584 (18.7%) rosettes located in plots placed along transects from ant colonies (Table 2.4, Fig. 2.5). Plots associated with two of the ant colonies did not contain rosettes with any sign of herbivory; however, each of the 10 slick spots included rosettes outside of the plots that did show signs of herbivory by ants. Although defoliation was noted as far as 10 m from a colony, rosettes located closer to ant colonies were significantly more likely to suffer leaf removal than those growing further away (Kruskal-Wallis rank sums test:

X_1^2=96.89, p<0.0001, Fig. 2.6). Additionally, rosettes with some or all of their leaves removed by ants were significantly less likely to survive than plants without signs of leaf removal (Pearson's Chi-squared test: X_2^2=54.54, p<0.0001, Fig. 2.7). Ants were successful at removing leaves in 31% of their attempts, and they snipped 0.76 leaves per rosette attempted. Ants were as likely to discard an *L. papilliferum* leaf in transit as they were to return it successfully to their nest (Welch two sample t-test: n=33, $t_{4.7}$= -0.48, p=0.65). Ants returned significantly fewer slickspot peppergrass leaves to their colony than other types of vegetation (Welch two sample t-test: n=211, $t_{4.03}$=-4.88, p=0.008).

Ants exhibited a highly standardized routine when clipping *L. papilliferum* leaves. The ant would first work for several minutes to snip a leaf from the plant and transport it to the ground. It then dropped the leaf at the base of the plant and returned to clip another. Ants that removed leaves did not take them to the nest. The leaves that were brought to the colony were typically dried leaves that had been removed some time earlier. Ants that removed leaves from plants were only observed completing this task and not contributing to other activities such as seed collection.

Discussion

This study provides the first empirical documentation of non-seed related herbivory on *L. papilliferum*, and implicates both small mammals and harvester ants in the activity. The study also shows that herbivory significantly increases mortality of affected plants. However, the intensity of herbivory was variable on both a temporal and spatial scale. For example, large numbers of rosettes in the study areas were affected by herbivory by small mammals in 2014 (64%), whereas only a small number were in 2015 (<1%). Given this magnitude of variability among years, and the short duration of our study, it remains unclear whether herbivory represents an important source of mortality for the long-term viability of *L. papilliferum* populations.

Herbivory by harvester ants was concentrated in the immediate vicinity of colonies, which is consistent with typical nest clearing behavior exhibited by harvester ants (Willard and Crowell 1965). Materials collected and removed from the vicinity of nests often include leaves and twigs, as well as animal products such as feces and the corpses of invertebrates (Willard and Crowell 1965, MacKay 1981, Jorgensen and Porter 1982, Kugler 1984). While the exact reason for vegetative clearing by harvester ants remains debated, thermoregulation, lowered predation risk, ease of travel, and water competition have all been proposed as possibilities (Seeley and Heinrich 1981, Jorgensen and Porter 1982, MacKay 1982, MacMahon et al. 2000, Bucy and Breed 2006). Although we found that herbivory was concentrated near nests, the intensity of herbivory varied among colonies, ranging from intense (e.g., 46.4% of rosettes at one colony) to nonexistent (within plots at two of the colonies). The colony responsible for the highest proportion of plants subjected to herbivory also caused the highest rate of mortality among the rosettes they defoliated (84.9%), and removed leaves from rosettes as far away as 10 m from the nest. Establishing the circumstances responsible for variation in intensity of herbivory will require further study. Nevertheless, given the intensity of herbivory that often occurs when rosettes are situated near harvester ant colonies, the frequent association of harvester ant colonies within or near slick spots (Robertson 2015), and the toll that seed predation by harvester ants has on *L. papilliferum* recruitment (Chapter 1, White and Robertson 2009), it seems clear that the foraging activities of harvester ants have the potential to adversely affect *L. papilliferum* populations.

Herbivory by small mammals, although inconsistent in its prevalence between years, was often devastating to individual *L. papilliferum*. In 2014, small mammals dug up 64% of the plants we monitored, and perhaps more if the unknown cases of loss were also attributable to mammals. However, these estimates may have been biased by our selection protocol. Plots in 2014 were selected after putative instances of herbivory had been observed in the area, since the objective at the time was simply to establish whether small mammals were responsible. This selection process may have inadvertently resulted in plots being situated in areas of higher herbivore activity than was occurring across slick spots in general. In 2015, when observation plots were selected in greater numbers across the landscape, and without regard to prior mammal activity, we recorded much lower levels of plant removal (<1%). Unfortunately, it is impossible to determine with our data whether differences in herbivory between 2014 and 2015 were the result of differences in how plots were selected, differences in levels of herbivory on *L. papilliferum* as a result of mammals abundance or food selection, or a combination of factors. It is noteworthy that in 2015 there was an outbreak of plague that affected small mammals in the study area—rodent corpses were frequently encountered within and around slick spots throughout the summer. Although the trail cameras recorded many instances of small mammal activity in slick spots, it is possible that mammal populations were substantially lower in 2015 than 2014, and that this may account in part for the lower levels of herbivory on *L. papilliferum*. However, further study is needed to determine the diet preferences of small mammals, and whether *L. papilliferum* is an important component of diet.

Ord’s kangaroo rat was the only mammal for which we have clear evidence of herbivory on *L. papilliferum*. Kangaroo rats are opportunistic foragers, although a large portion of their diet consists of seeds (Johnson 1961, Brown 1973, Davidson 1977). As desert inhabitants, kangaroo rats are adapted to conserve water through the production of concentrated urine and dried out feces (Schmidt-Nielsen et al. 1948, Schmidt-Nielsen and Schmidt-Nielsen 1951, Carpenter 1966). Much of the water they acquire comes from vegetation. Specifically, individuals supplement their granivorous diet with water-rich alternative food sources such as taproots (Tracy and Walsberg 2002). Kangaroo rats also consume leaf tissue as a source of nutrients and water (Bradley and Mauer 1971); however, my observations indicate that they often avoid consuming *L. papilliferum* leaves while digging down for the taproot. Aversion to *L. papilliferum* leaves may be in response to their presumably lower water content compared to taproots, or because of chemical defenses in leaves. Species within the Brassicaceae, wherein *L. papilliferum* belongs, produce glucosinolates, defensive compounds that might explain herbivory patterns of small mammals (Bones and Rossiter 1996, Meyer et al. 2005). In *Arabidopsis thaliana,* another species within Brassicaceae, roots and leaves produced early in development contain the lowest concentration of toxins (Brown et al. 2003). Toxin levels also vary throughout the day (Rosa et al. 1994) and with water stress (Jensen et al. 1996, Champolivier and Merrien 1996, Zhang et al. 2008, Schreiner et al. 2009). Together, these may explain the observed herbivory patterns by Ord’s kangaroo rats, both between tissues and seasons.

The extent to which herbivory by ants and mammals contributes to mortality of *L. papilliferum* at the population level remains an open question in need of further study.

The present study demonstrated that *L. papilliferum* is subjected to (non-seed) herbivory and that mortality often results. Small mammals and harvester ants both contribute to herbivory, but the spatial distribution of these activities, as well as their intensity, varies within slick spots and across years. The potential impact of herbivory on *L. papilliferum* populations should not be ignored. When a healthy rosette is removed by an herbivore, it represents the loss of thousands of potential seeds from the next generation. Such losses may be particularly detrimental to rare species like *L. papilliferum* where any impacts on growth, survival, or offspring recruitment could limit a plants' recovery or potentially push it to extinction (Crawley 2000, Ancheta and Heard 2011). However, before conclusions can be drawn about the significance of non-seed herbivory to *L. papilliferum* populations, it will be important to learn more about diet selection in small mammals and the causes of variation in the intensity of herbivory.

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Tables

Table 2.1 Results of mammalian herbivory observations in 2014.

Plot	Initial No. of Plants	Remaining Plants	No. Plants Removed by Mammals	No. Plants: Unknown Loss	Mammal Caused Death Rate	Unknown Death Rate
1	17	4 (23.5%)	10	3	58.8%	17.7%
2	6	0 (0%)	3	3	50%	50%
3	26	6 (23.1%)	19	1	73.1%	3.9%
4	29	0 (0%)	18	11	62.1%	37.9%
Total	**78**	**10 (12.8%)**	**50**	**18**	**64.1%**	**23.1%**

Table 2.2 The incidence of mammalian herbivory on *L. papilliferum* at plots located within slick spots in 2015 (N=13).

Plot	**No. of plants in plot**	**No. of plants with signs of herbivory**	**Evidence of mammal herbivory within the slick spot but outside of plot**
1	227	0	No
2	66	1 (1.5%)	No
3	166	0	No
4	235	1 (0.43%)	No
5	195	1 (0.51%)	No
6	289	0	No
7	218	5 (2.3%)	Yes
8	153	3 (2.0%)	Yes
9	261	8 (3.1%)	Yes
10	120	2 (1.7%)	Yes
11	434	0	Yes
12	140	1 (0.71%)	Yes
13	180	0	Yes
Total	**2,684**	**22 (0.82%)**	--

Table 2.3 The identity of small mammals photographed by trail cameras at *L. papilliferum* plots in 2015, and the number of cases of herbivory on *L. papilliferum*. Herbivory was scored as "confirmed" when the photograph showed the animal eating *L. papilliferum*. "Possible herbivory" refers to images where the animal was photographed digging among *L. papilliferum* or with its head among rosettes or flowering plants.

Type of Mammal	**Total No. of Images***	**Confirmed Herbivory on *L. papilliferum***	**Possible Herbivory on *L. papilliferum***
Black-tailed Jackrabbit (*Lepus californicus*)	141	0	0
Kangaroo rat (*Dipdomys microps*)	22	1	5
Deer Mouse (*Peromyscus maniculatus*)	13	0	0
Vole (*Lemmiscus curatus* or *Microtus montanus)*	2	0	1
Mountain Cottontail (*Sylvilagus nuttallii*)	5	0	1
Pocket Gopher (*Thomomys* sp.)	1	0	0
Unknown	21	0	0
Totals:	205	1	7

* Cases in which the same type of animal appeared in sequential frames on the camera were counted as a single occurrence unless the interval between images exceeded five minutes.

Table 2.4 Incidence of leaf removal on *L. papilliferum* rosettes by harvester ants in 2015, and a comparison of condition between plants with defoliation and those without. A plant was considered in good health if its leaves were uniformly green and fleshy.

Slick spot	**Total No. of rosettes within plots**	**No. of rosettes with signs of defoliation**	**No. of rosettes with defoliation that survived**	**No. of rosettes without defoliation that survived**
1	158	73 (46.2%)	11 (15.1%)	39 (45.9%)
2	100	10 (10%)	6 (60%)	40 (44.4%)
3	47	6 (12.8%)	1 (16.7%)	19 (46.3%)
4	41	1 (2.44%)	1 (100%)	35 (87.5%)
5	36	2 (5.56%)	2 (100%)	34 (100%)
6	57	9 (15.8%)	0 (0%)	21 (43.8%)
7	9	3 (33.3%)	0 (0%)	6 (100%)
8	67	0 (0%)	--	30 (44.8%)
9	43	5 (11.6%)	1 (20%)	33 (86.8%)
10	26	0 (0%)	--	22 (84.6%)
Total	**584**	**109**	**22 (20.2%)**	**279 (58.7%)**

Figures

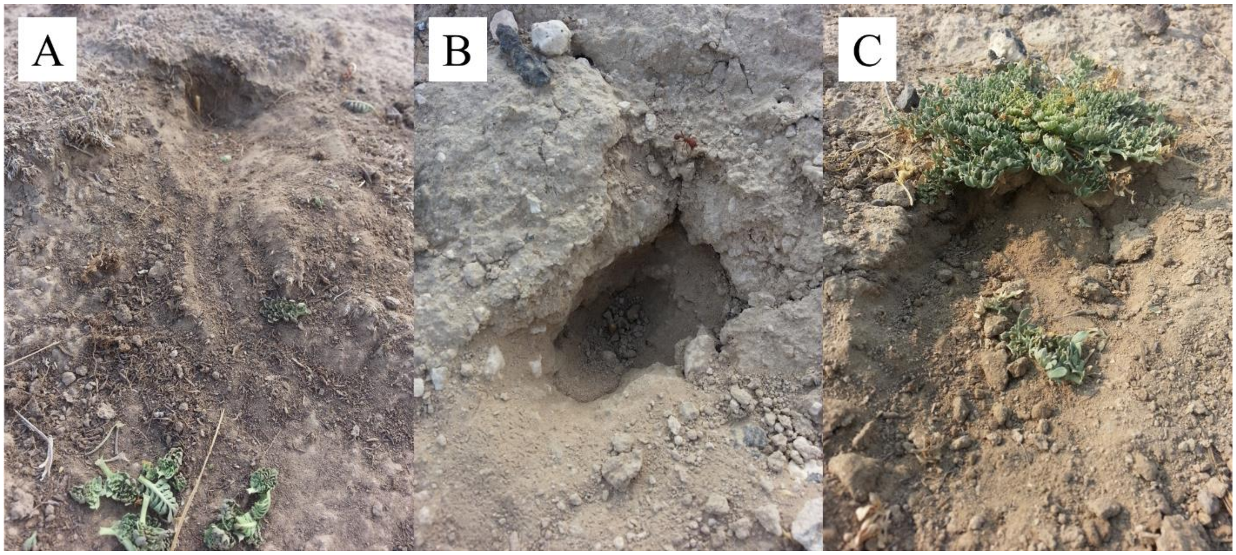

Figure 2.1 Evidence of mammalian herbivory on *Lepidium papilliferum* rosettes. A) The rosette has been completely removed, the taproot exposed, and leaves are scattered about. B) The rosette has been completely removed, the taproot exposed, and no trace of leaves remain. C) The rosette is mainly intact, but there is evidence of leaf removal.

Figure 2.2 An Ord's Kangaroo Rat (*Dipdomys microps*) caught chewing the stem of a slickspot peppergrass plant. The photograph was taken at night with a Moultrie M-880 trail camera in the summer of 2014.

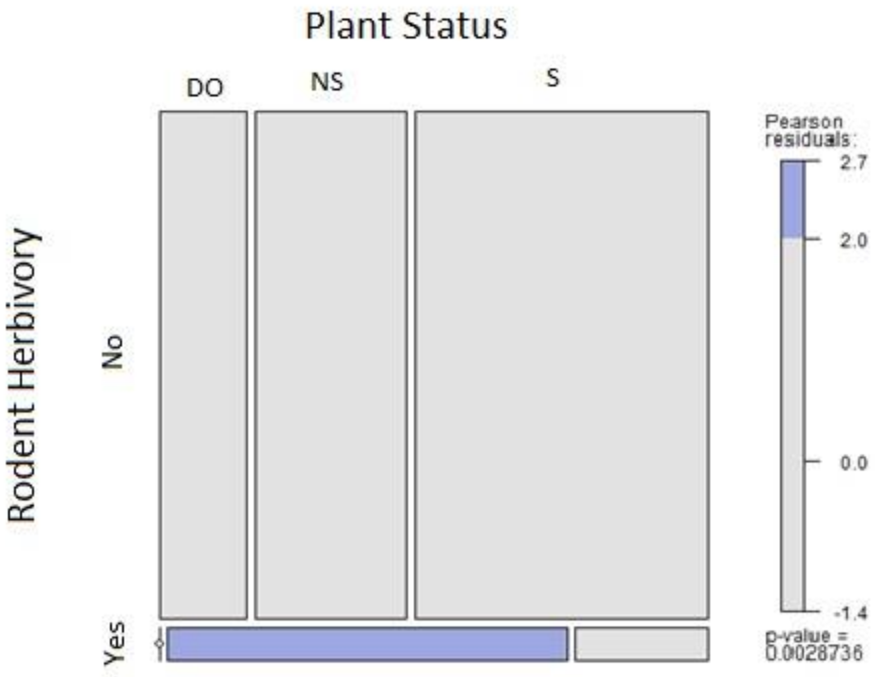

Figure 2.3 A structure plot showing *L. papilliferum* plants grouped by the presence or absence of herbivory by small mammals in 2015. These subgroups are then grouped by their observed condition: DO - Drying out, NS - Not surviving, S - Surviving. The Pearson residual shows how different the data are compared to what would be expected by chance. A significant chi-squared test was being driven by the observed number of defoliated rosettes not surviving (shown in dark blue). The larger the residual (darker blue) means the larger the deviation from what we would expect from chance.

Figure 2.4 Representative photographs of mammal activity within slick spots occupied by *L. papilliferum*. A) An Ord's kangaroo rat (*Dipdomys microps*) eating a slickspot peppergrass rosette. B) An Ord's kangaroo rat, possibly digging at the base of a slickspot peppergrass rosette. C) A vole (*Lemmiscus curatus* or *Microtus montanus*), possibly digging at the base of a slickspot peppergrass rosette. D) A mountain cottontail (*Sylilagus nuttallii*), possibly digging at the base of a slickspot peppergrass rosette. The photographs were taken at night with a Moultrie M-880 trail camera in the summer of 2015.

Figure 2.5 Owyhee harvester ants actively clipping leaves from a slickspot peppergrass rosette. Recently removed leaves are visible on the ground near the rosette.

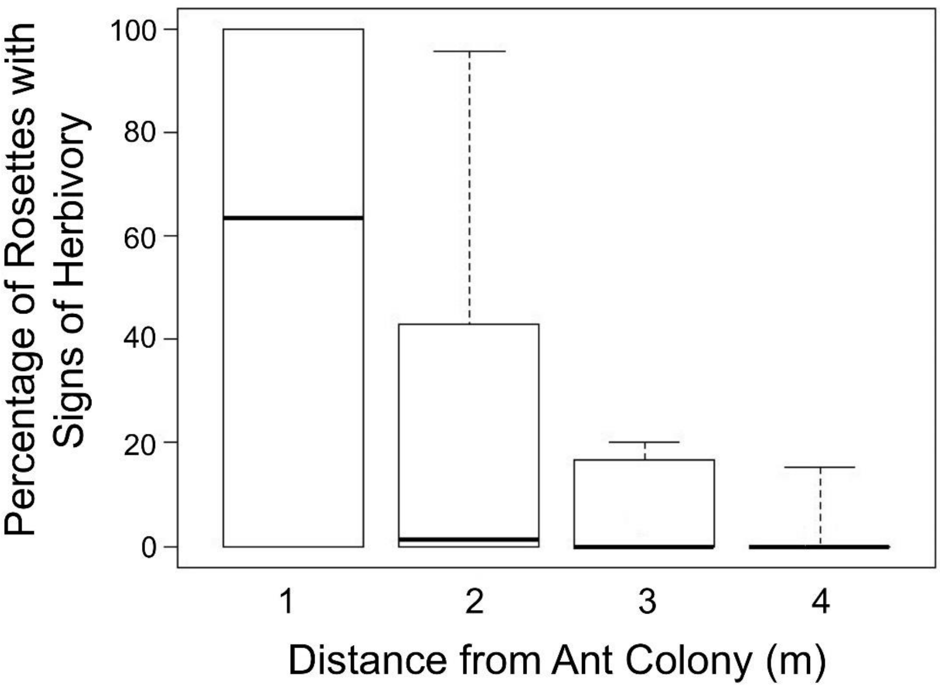

Figure 2.6 Box and whiskers plot showing the proportion of *L. papilliferum* rosettes with signs of herbivory as a function of distance from harvester ant colonies (N=10). The horizontal bar within each box represents the median. Upper and lower limits of the boxes represent the 75th and 25th percentiles, respectively. Vertical lines show the 90th percentiles.

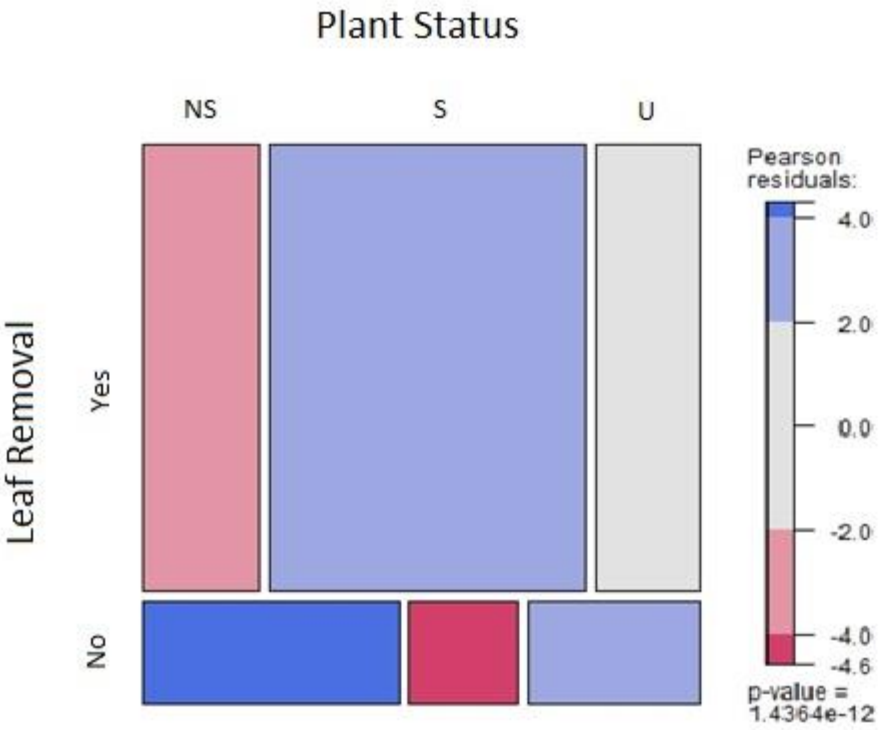

Figure 2.7 A structure plot showing *Lepidium papilliferum* rosettes grouped by the presence or absence of leaf removal by harvester ants. These subgroups are then grouped by their observed fate: NS - Not surviving, S - Surviving, U - Unknown. The Pearson residual shows how different the actual data are compared to what would be expected by chance. The larger the residual (darker blue or darker red) means the larger the deviation from what we would expect if there was no effect of leaf removal. A significant chi-squared test was being driven by the observed number of defoliated rosettes not surviving (shown in dark blue), and conversely the low number that were defoliated to survive (shown in dark red).

CHAPTER THREE: SEED PREDATION BY HARVESTER ANTS HINDERS THE SUCCESSFUL INTRODUCTION AND RECOVERY OF SLICKSPOT PEPPERGRASS

Abstract

We evaluated the extent to which the foraging activities of Owyhee harvester ants (*Pogonomyrmex salinus*) hinder the success of seed introductions of slickspot peppergrass (*Lepidium papilliferum*), a rare mustard endemic to south-western Idaho. Starting in 2014 we introduced *L. papilliferum* seeds to a site that contained slick spots within a matrix of sagebrush and grasses, but had no documented historical population of *L. papilliferum*. Seed introductions were made in the summer, fall, and following spring. The summer introduction mimicked the timing of natural seed release in *L. papilliferum*, whereas the fall and spring introductions were designed to avoid seed predation by ants. Within each of 12 slick spots, 200 *L. papilliferum* seeds were deposited on simple soil (i.e., clay-pan) and complex soil (along margins of slick spots), either in the presence or absence of ant foraging activity. Seed survival and germination was significantly higher on simple soil than on complex soil, but only when ants were denied access to seeds. Very few seeds exposed to ants survived to germinate. Of the 160 seeds that successfully germinated, four went on to produce flowers in their first year, and one of those produced seed-bearing fruits. Based on these results, we conclude that seed introductions in

recovery efforts for *L. papilliferum* show promise; however, seed losses to harvester ants and issues concerning insect-mediated pollination must be addressed to improve success.

Introduction

Seed introductions are commonly used to rehabilitate or augment plant populations. However, evaluating and comparing the success of introduction efforts has proven difficult because of inconsistencies in introduction methodology and subsequent monitoring protocols (Godefroid et al. 2011, Guerrant 2013). While there are some clear examples of successful introductions (Maschinski et al. 2004, Guerrant 2012), Godefroid et al. (2011) cautions that, overall, a large proportion of introduction efforts are unsuccessful, and that seed introductions are no more successful than other introduction methods. On the other hand, alternatives to seed introductions are often more effort intensive and costly. Thus, a prudent approach to introductions may be to explore the success of seed introductions before considering more expensive and time consuming alternatives. Here we evaluated the success of seed introductions of slickspot peppergrass, *Lepidium papilliferum* [(L. Henderson) A. Nels. & J.F. Macbr], a rare cruciferous plant endemic to sagebrush-steppe habitat in southwestern Idaho.

Within sagebrush-steppe habitat, *L. papilliferum* is restricted to microsites known as “slick spots”–shallow depressions of soil devoid of most other plants and characterized by high levels of clay and salt as well as by soil water retention that is higher than that of surrounding areas (Quinney 1998). Following germination late in spring, the plants follow one of two life history trajectories: annuals flower, set seed, and die within a few months whereas biennials forgo reproduction in the first year, overwinter as vegetative rosettes, and reproduce in their second season before dying (Quinney 1998). White and

Robertson (2009) identified a third, albeit uncommon, life history pattern in which individuals flower to a limited extent late in their first year and then again in their second year if they survive the winter. Biennials that survive to reproduce are typically much larger than annuals, and produce many times the number of seeds (Meyer et al. 2005, personal observations). Seeds that drop to the soil can remain viable in the soil seed bank for up to 11 years (Meyer et al. 2005).

Currently there are about 80 known sites where the plant persists (USFWS 2009), many of which support no more than a few hundred individuals (Kinter et al. 2013). Over the past century, *L. papilliferum* has declined dramatically in abundance due to habitat degradation and fragmentation attributed to wildfires, livestock grazing, irrigated agriculture, exotic species invasions, urbanization, and off-road vehicle use (Moseley 1994). Twenty-one sites known from historical records dating back to 1892 are now considered extirpated (Moseley 1994, Colket 2005). Owing to the limited distribution and declining numbers of slickspot peppergrass, and its importance as an indicator of sagebrush-steppe habitat integrity, there is considerable interest in developing measures to ensure the plant's long-term survival. The use of seed introductions to establish new populations in suitable habitat, as well as augment existing populations where numbers are low, is one such measure being considered.

One of the potential impediments to successful introduction and establishment of *L. papilliferum* is the detrimental effect of seed predation by Owyhee harvester ants, *Pogonomyrmex salinus*. Indeed, harvester ants in general have been linked to decreases in the success of seeding in land restoration projects (Anderson and Ashton 1985, Ireland and Andrew 1995, Crawley 2000, Ancheta and Heard 2011, DeFalco et al. 2012). More locally, studies have shown that harvester ants regularly incorporate *L. papilliferum* seeds into their diet (Schmasow 2015) and have the capacity to remove as much as 90% of the fruits/seeds produced by *L. papilliferum*, either directly from the plant or by scavenging seeds that drop to the ground (White and Robertson 2009, Robertson and Crossman 2012). Given the widespread distribution of *P. salinus* within *L. papilliferum* habitat (Robertson 2015), high levels of seed predation could hamper or prevent the establishment of viable populations of *L. papilliferum* in otherwise favorable areas.

To address the question of how best to introduce *L. papilliferum* seeds when harvester ants are present in the environment, we examined whether the timing of seed introduction (i.e., late summer, when seeds normal drop to the ground, versus fall, once ants reduce activity for the winter, and spring) and the presence of harvester ants influences the number of seeds that germinate and grow the following season. We predicted that seeds placed in cages during the summer season would suffer dramatic declines when ants had access to those seeds, whereas seeds placed in cages late in the fall and early in the spring would avoid seed predation entirely and thus have higher success rates.

In addition to evaluating the effect of timing and the presence or absence of ants on the success of seed introductions, we examined whether certain soil surface features within slick spots offer physical refuges to seeds from seed predators, thereby mitigating losses to ants. From a distance, slick spots often appear uniform and barren within the landscape. However, closer inspection reveals their surfaces to be quite variable. Some areas have smooth, hardpan surfaces, whereas other areas contain lichens, mosses, rocks and assorted vegetative debris that add to the complexity of the soil surface. We tested whether these "complex" surfaces within slick spots provide *L. papilliferum* seeds with a physical refuge from harvester ants, thereby making the seeds less vulnerable to discovery and predation. This design also allowed us to assess the suitability of the various substrates for germination and growth of *L. papilliferum* in the absence of seed predation.

An additional way in which seeds may escape predation is by becoming difficult to remove from their environment. When wetted, *L. papilliferum* seeds, like the seeds of many desert plants, imbibe with water and form a mucilaginous coating (Gutterman and Shem-Tov 1997, Gu et al. 2008). Once this coating dries, the seed becomes stuck firmly to the ground (personal observations). In a separate experiment than described above, we investigated whether the addition of water to recently introduced *L. papilliferum* seeds reduces their vulnerability to removal by harvester ants. We hypothesized that seeds sprinkled on soil without subsequent wetting would be more vulnerable to seed predation by ants than seeds that were wetted following introduction.

Avoidance of seed predators, followed by germination, growth, and flowering are only the first steps in the establishment of viable *L. papilliferum* populations through seed introductions. Another key consideration is pollination and seed production. *Lepidium papilliferum* is a primarily outcrossing species that relies on insects for pollination (Robertson and Klemash 2003, Robertson and Ulappa 2004). The plant's small, white flowers, which grow in raceme inflorescences and bloom from late April to late June, attract a wide variety of insects, of which only a subset contribute substantially to pollination (Robertson and Leavitt 2011). The viability of newly established *L. papilliferum* populations requires the presence of a suitable pollinator community to ensure pollination and seed production for subsequent generations. Therefore, in addition to documenting the initial success of seed introductions in terms of the number of plants that germinate and survive to flower, we examined whether pollination success and the production of seed-bearing fruits is comparable to levels found elsewhere in well-established *L. papilliferum* populations.

Methods

Seed Source

The seeds used in this study were collected in July 2014 at the Kuna Butte SW population of *L. papilliferum*, located near Melba, Idaho (element occurrence 018, as designated by the Idaho Natural Heritage Program). We harvested seeds from ~30 plants across several slick spots to ensure genetic diversity at the introduction site, and we stored them in an open air jar at room temperature until needed. An isozyme analysis by Stillman (2006) showed that *L. papilliferum* growing at the Kuna Butte SW population are genetically very similar to *L. papilliferum* growing at the Orchard Combat Training Center, OCTC, thereby making these seeds an ecologically appropriate source for the introduction.

Seed Wetting

In the summer of 2015, we conducted an experiment at the Kuna Butte SW field site to determine whether wetting of *L. papilliferum* seeds reduces their vulnerability to predation by ants (by virtue of the mucilaginous coat that forms after wetting). We filled a total of 30 petri dishes with locally sourced soil and then scattered a total of 100 *L. papilliferum* seeds per dish. Ten of the dishes were designated as controls and received no further attention prior to being placed in the field. The remaining 20 dishes were divided equally into one of two treatments: wetted (seeds were misted with water immediately prior to being placed in the field), and wetted/dried (seeds were misted with water and then allowed to dry thoroughly in the sun before being placed in the field) (Fig. 3.1). As soon as the dishes were ready we placed each within 1-2 m of an active harvester ant colony. Each dish was associated with a different colony. Observations confirmed that ants visited each of the dishes placed in the field. One day after the dishes were placed in the field we removed them and placed their contents into individual zip-lock bags. In the laboratory, we sifted through each sample and counted the number of seeds that were present. Kruskal-Wallis rank sum test was performed using R (R Development Core Team 2012) to determine whether ants removed fewer seeds from treatments than controls and whether there were differences in seed number between the two treatments.

Seed Introduction Experiment

We conducted a seed introduction experiment from July 2014 to August 2015 at the northwest corner of the OCTC, located near Boise, Idaho. This location contains numerous slick spots within a matrix of sagebrush and grasses, but has no documented historical population of *L. papilliferum*. Owyhee harvester ant colonies are scattered throughout the site.

In July 2014, we selected 12 slick spots for the experimental release of *L. papilliferum* seeds. We made certain that there was an active harvester colony located within or along the margin of each slick spot. In late August 2014 (summer introduction), we placed four cages in each slick spot (Fig. 3.2). Each cage consisted of a 10 cm high plastic ring cut from a 15 cm diameter flowerpot. We covered the top of each flowerpot with 1-cm hardware cloth (wire mesh) to prevent access by rodents, rabbits, and seed-eating birds. Two of the four cages in each slick spot were placed on “simple” (i.e., hardpan) soil, and two were placed on “complex” soil (i.e., surfaces that included lichens, mosses, rocks). The latter were usually located along the margins of slick spots. Within each soil type, one of the cages was elevated ~2 cm off the ground to allow unfettered access by foraging harvester ants, whereas the other was fixed tightly to the ground to prevent access by ants. Tanglefoot® was applied in a 2 cm band along the top rim of each of the fixed cages to help ensure that ants could climb over the barrier. On simple soils, we found it difficult to seal the flowerpots tightly to the ground, so a second plastic barrier (15 cm high, 30 cm diameter) was placed around those flowerpots as a further impediment to ants. Once all the cages were in place, we scattered 200 *L. papilliferum* seeds onto the soil surface within each flowerpot. We then misted the seeds with distilled water to help them settle onto the soil.

We repeated the experimental protocol in mid-October 2014 (fall introduction) and again in mid-April 2015 (spring introduction) using additional complements of cages within each of the slick spots. Because *L. papilliferum* seeds require a period of winter dormancy in order to germinate, we treated the spring introduction seeds to a procedure intended to break dormancy. First, we scarified the seeds by rubbing them gently between two sheets of 320-grit sandpaper. We then imbibed the seeds with deionized water on filter paper in Petri dishes, and placed them in cold stratification at 4°C for 6 weeks with no light. This technique was reasonably effective in a previous study at breaking seed dormancy (Billinge and Robertson 2008), although Stillman (2006) achieved higher germination success by piercing individual seed coats with a needle rather than scouring the seed between sandpaper. This latter technique was deemed impractical for the current study given the number of seeds involved.

The cages from each of the three introduction times remained undisturbed through May 2015, at which time we counted the number of *L. papilliferum* germinants present in each. We continued to monitor the cages periodically throughout the summer; however, a number of the cages were knocked over after the first assessment in May, likely by cattle grazing in the area (cattle footprints and dung were present in the immediate area of cages).

We evaluated the effects of timing of introduction and soil surface type on the number of seeds that germinated using a Generalized Linear Model with a Poisson distribution (Zuur et al. 2009). The response variable in the model was the difference between the number of germinating rosettes in the cage exposed to ants from the cage that denied ant access because we treated the cages as matched pairs for each soil type and introduction season. We created models using combinations of our fixed effects (timing of introduction, soil type) and the random effect of slick spot. We ranked models using the Akaike Information Criterion adjusted for small sample size (AIC_c, Burnham and Anderson 2002). We measured an effect if the coefficient for a given predictor was represented in the top model and the top model was ranked above the null model (Burnham and Anderson 2002). We evaluated the fixed effects within the top models using their parameter estimate and 85% confidence intervals to achieve full AIC compatibility (Arnold 2010). We considered parameters with 85% confidence intervals that did not overlap 0 as biologically informative. Additionally, we used a Kruskal-Wallis rank sum test to determine if ant access to cages correlated with fewer germinating rosettes.

Pollination and Fruit Production

It was our intention to use the protocols of Robertson and Leavitt (2011) to document the diversity and relative abundance of insect pollinators visiting flowering *L. papilliferum* at the introduction site. However, the small number of plants that actually flowered in 2015 prompted us to take the more practical approach of simply noting the types of insects observed on flowers during our periodic assessments of germination, and whether these plants produced seed bearing fruits.

Results

Seed Wetting

Seed wetting had no statistically significant effect on the number of seeds that escaped predation by ants one day after seeds were added to soil in petri dishes (Kruskal Wallis Test, X_2^2=1.37, p=0.50). The average number of seeds remaining on the control, wetted, and wetted-dried, petri dishes was 7.1±1.6, 5.0±1.1, and 19.9±9.2, respectively.

Seed Introduction Experiment

Three of the 12 slick spots at our study site failed to support *L. papilliferum* rosettes, regardless of treatment or introduction time. Because these slick spots may have been unsuitable habitat for *L. papilliferum*, we removed them from the analysis. In the nine slick spots that remained, *L. papilliferum* rosettes (N=160) were found in at least some of the cages from both the summer and fall introduction times, confirming that the site contained slick spots suitable for *L. papilliferum* growth (Fig. 3.3). No rosettes were found in any of the cages associated with spring introductions, leading us to conclude that the procedure we used to break seed dormancy was ineffective (Table 3.1, *n.b.*, laboratory results achieved low levels of germination success using a subset of these seeds). Because the spring introductions were uninformative with respect to treatment effects, we eliminated them from our analyses.

Focusing on the summer and fall seed introductions, there were two top models (Table 3.2). The models including soil only and soil and season were ranked as the top models. The 85% confidence intervals for soil type did not overlap with 0, whereas for season they did. This result indicates that season was uninformative and that soil type was responsible for predicting the number of germinating plants. More specifically, simple soil was associated with more germinating rosettes. Moreover, harvester ants negatively influenced the number of rosettes that germinated regardless of soil type (Kruskal Wallis Test, X_1^2=21.2, p<0.0001). Rosette production was highest on simple soils when ants were excluded, and suffered dramatic declines when ants had access to seeds (Fig. 3.3). Fifteen of 24 cages placed on simple soil contained rosettes (132 in total) when ants were excluded whereas only 1 of 24 cages contained rosettes (8 in total) when ants had access to seeds. Cages placed on complex soil produced few rosettes (N=20 rosettes across 48 cages), and the effect of ant exclusion was not as dramatic as in the cages placed on simple soil (Table 3.1). Cages situated on complex soils frequently became filled with grasses and other vegetation, whereas those on simple soils did not (Fig. 3.4).

Of the 160 rosettes produced in cages, 156 remained in their vegetative form (indicative of first-year biennials) throughout the summer. Eighteen percent of the rosettes wilted and died by mid-July, whereas the rest remained green throughout the summer. Four of the 160 rosettes that germinated went on to produce flowers the same year, which is consistent with the annual life history pattern of this species. Three of these individuals were located in the same cage. Both cages that contained flowering individuals were on simple soil protected from ants. The individual that bloomed on its own within a cage produced a full complement of seed-bearing fruits (Fig. 3.5). The three flowering plants that shared a cage showed very low levels of pollination, as indicated by flowers that remained open for several weeks without developing fruits (see Robertson and Klemash 2003). Although a couple of fruits were observed on these plants, the vast majority of flowers went unpollinated (Fig. 3.6). By early July, these plants had withered and died, even though vegetative rosettes in the same cage remained healthy. Gelechiid moths were observed on the flowers of the three plants that failed to produce substantial numbers of fruits (Fig. 3.7).

Discussion

We have shown that *L. papilliferum* introduced as seeds to slick spots can successfully germinate, grow, and in some cases flower and produce fruit. We also offer compelling evidence that harvester ants represent a serious impediment to the success of seed introduction efforts. Survival and growth of *L. papilliferum* was by far the best on hardpan (simple) soil within slick spots, but only when harvester ants were denied access to the seeds. In the presence of ants, very few seeds survived to germinate. This impediment to the success of seed introductions is consistent with previous work showing that harvester ants regularly remove large numbers of *L. papilliferum* seeds under natural conditions (Chapter 1, Schmasow 2015). Wetting seeds in an effort to make them less vulnerable to collection by ants had no effect on survival and thus can be discarded as a method to improve seed survival.

Cages placed among lichens and mosses along the margins of slick spots (i.e., complex soils) failed to support many rosettes, regardless of whether or not ants had access to the seeds. We predicted that complex soils would offer *L. papilliferum* some measure of relief from seed predators by providing physical refuges for seeds, but any such effect was obscured by lower germination success and/or rosette survival. Our selection of complex soil was likely not ideal for *L. papilliferum* germination due to resource competition with mosses and other vegetation within cages. In retrospect, it would have been better to limit our definition of complex soils to surfaces with cracks, crevices, rocks, and vegetative debris. These surfaces could potentially provide *L. papilliferum* seeds with refuge from seed predators, without the complications arising from competition for resources with other plants. Additional experiments are therefore warranted to determine whether a more limited definition of complex soils produces conditions favorable to seed survival.

Even under ideal conditions for seed survival and growth (i.e., hardpan soil, ants excluded), only 132 rosettes were produced from the 4,800 seeds we distributed. However, this low rate of production is difficult to interpret given our lack of knowledge about *L. papilliferum* germination rates in established populations. Low germination rates may be the norm for the species, or at least the norm under certain environmental conditions. Slickspot peppergrass seeds can remain viable in the soil seed bank for up to 11 years, and seeds produced in a given cohort do not all germinate in the same year (Meyer et al. 2005). It would therefore be worthwhile to revisit the introduction site in

2016 (and beyond) to determine whether subsets of the seeds we distributed in 2014 continue to germinate. Revisiting the site will also provide an opportunity to determine the fates of plants that survived their first year as vegetative rosettes. The individuals that successfully germinated and survived their first year as vegetative rosettes are expected to flower in their second year.

The relatively small numbers of seeds placed on the ground may have made those seeds particular vulnerable to removal by harvester ants. In established populations of *L. papilliferum*, large numbers of seeds dropping to the ground over a short period of time might overwhelm the capacity of ants to remove and consume them, thereby ensuring that some survive to germinate. Such “satiation effects” have been documented in other seed-eating ant species (Andersen 1987). However, it is not clear how many *L. papilliferum* seeds would have to be distributed on the ground to achieve such an effect. In a separate study, Jeffries (Chapter 1) found no clear relationship between the percentage of seeds lost to harvester ants and the total abundance of seeds on the ground. In all likelihood, the number of seeds necessary to mitigate losses to ants would exceed the number available for most introduction/augmentation efforts. Therefore, alternative measures to limit seed predation by ants at introduction sites may be needed.

The most effective way to limit seed losses to harvester ants is to prevent ants from accessing seeds, either through avoidance in time or by releasing seeds in areas without harvester ants. We tested various introduction times in an effort to avoid peak periods of harvester ant foraging activity. The summer introduction period was selected to match the natural timing of seed release by *L. papilliferum* and, as expected, high levels of seed loss occurred. The fall introduction treatment was intended to place seeds on the ground at a time when harvester ant activity had declined for the season in response to cooler temperatures. Unfortunately, shortly after we added seeds in mid October 2014, conditions on the ground warmed sufficiently for ants to resume their foraging activities, and ants were observed entering raised cages and removing seeds. For this treatment to be successful, seeds should be added later in the season when there is little or no chance of ants resuming foraging activity until spring. To this end, we repeated the fall introduction protocol in November 2015, and observations confirmed that ant colonies remained dormant afterward. Data from this seed release will be gathered in 2016 and used to augment the results of the present study.

The lack of seed production by three of the four plants that flowered in 2015 suggests either that effective pollinators did not visit the plant's flowers, or that the insects that visited the flowers did not carry outcrossed pollen. Gelichiid moths, which are inefficient pollinators of *L. papilliferum* (Robertson and Leavitt 2011), were the only insects we observed visiting flowers. Because slickspot peppergrass is primarily an outcrossing species (Billinge and Robertson 2008), selfing or cross pollination with a genetically similar plant could have contributed to low fruiting success in our study. However, a sufficient variety of plants was included to ensure genetic diversity in our samples (see Stillman 2006). Thus, the low levels of fruit production among the few flowering plants in our study were likely caused by the lack of effective pollinators visiting those flowers. Given that a variety of native insects serve as effective pollinators of *L. papilliferum* (Robertson and Leavitt 2011), and that, in general, pollinator numbers (and pollination success) tends to increase with the density of flowers available to pollinators (Kunin 1993, 1997; Karron et al. 1995), introduction efforts of *L. papilliferum* should focus on creating suitably dense patches of flowering individuals in order to ensure successful pollination.

Based on the results of our study, the use of seed introductions to rehabilitate or initiate *L. papilliferum* populations seems feasible if the effects of seed predation by harvester ants can be mitigated and if sufficient numbers of flowering individuals are available to pollinators. Releasing *L. papilliferum* seeds in areas without harvester ants could be achieved either by selecting sites that lack harvester ants, or by spot-killing harvester ant colonies that pose a threat to seed survival. Targeted removal of ant colonies through the use of granular baits (Borth 1986, Robertson, unpublished data) may be the more feasible of the two options given the widespread abundance of harvester ants throughout *L. papilliferum*'s range (Robertson 2015).

Finally, the use of seed introductions to rehabilitate or initiate *L. papilliferum* populations must be sensitive to the ecological suitability of the source material. For example, when seed introductions are used to augment current populations, foreign seeds may introduce invasive traits that could alter the genetic integrity of the existing population (Mueller and Hellmann 2008, Ricciardi and Simberloff 2009). Conversely, seeds introduced into novel areas may be maladapted to their new environment and unlikely to produce robust populations (Knapp and Rice 1994, Kramer and Havens 2009, Weeks et al. 2011). Kramer et al. (2015) addressed these concerns by creating seed transfer zones - areas where seeds from a particular source can be distributed with little concern of maladaptation or invasive traits. We addressed this concern in our study by using seeds from a nearby population of *L. papilliferum* that was genetically very similar to existing populations near the release site (Stillman 2006). Future seed introductions of

L. papilliferum should continue to use ecologically appropriate seed sources to reduce the risk of unintended harmful consequences.

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Tables

Table 3.1 Summarized results of the seed introduction experiment. Each row of data represents the cumulative number of plants from nine independent slick spots included in the study.

Season	Soil Type	Ant Access	# Germinating rosettes
Summer	Complex	Yes	1
Summer	Complex	No	6
Summer	Simple	Yes	0
Summer	Simple	No	73
Fall	Complex	Yes	2
Fall	Complex	No	10
Fall	Simple	Yes	8
Fall	Simple	No	59
Spring	Complex	Yes	0
Spring	Complex	No	0
Spring	Simple	Yes	0
Spring	Simple	No	0

Table 3.2 We modeled the difference in germinating rosettes from each matched pair using a Poisson distribution with Generalized Linear Mixed Models. The top model included soil type as the sole predictor for the number of germinating rosettes. More specifically, simple soil was associated with more germinating rosettes.

Model	**K**	**ΔAIC_c**	***w_i***
Soil	3	0	0.58
Soil and Season	4	1.6	0.27
Soil, Season, Soil x Season	5	2.9	0.14
Intercept only	2	36.5	0.00
Season	3	37.9	0.00

Figures

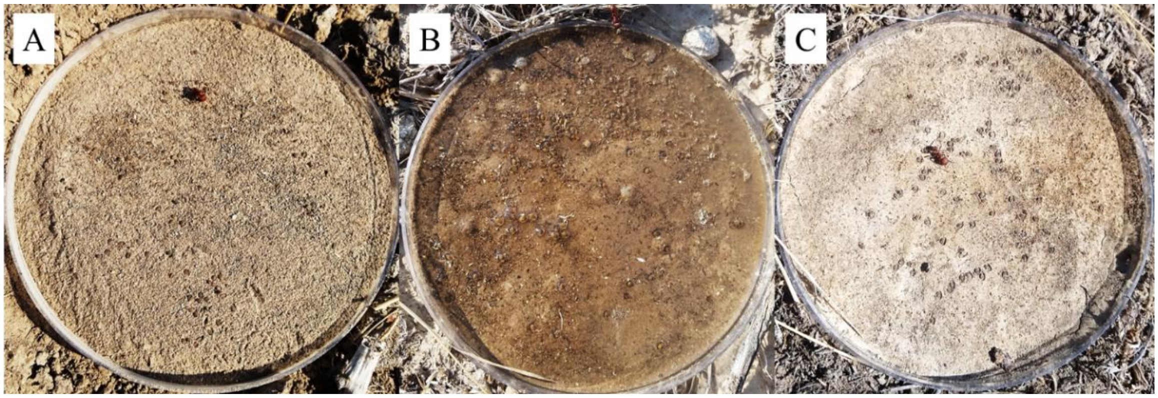

Figure 3.1 Images of the three types of seed wetting used in the experiment: A) Unwetted controls. B) Wetted treatment. C) Wetted /dried treatment. Each petri dish used in the experiment was buried flush with the ground 1-3 m from an ant colony. Treatment had no effect on the number of seeds removed by ants (Kruskal Wallis Test, $X^2=1.37$, $p=0.50$).

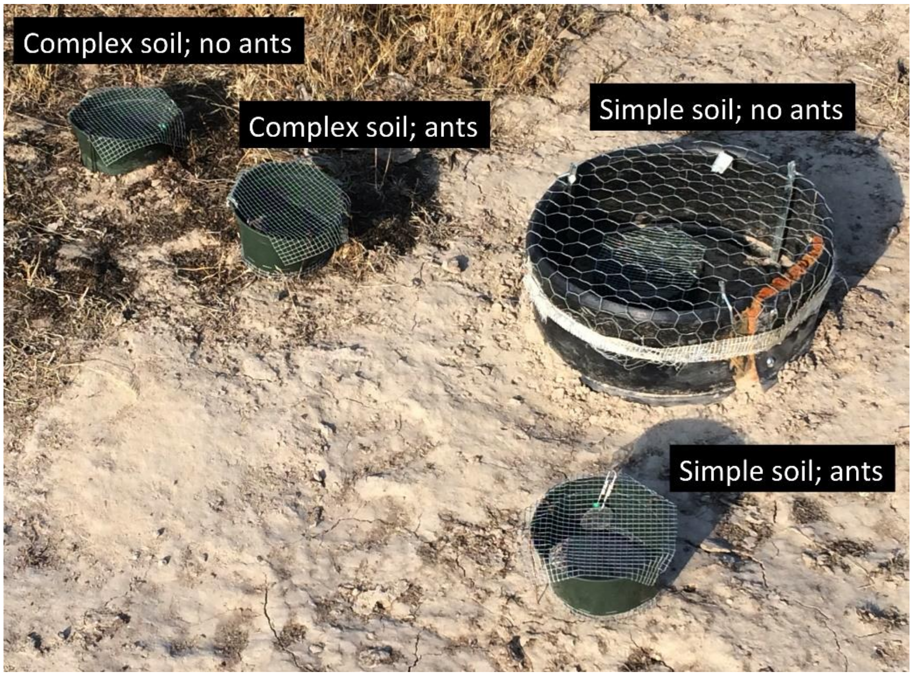

Figure 3.2 Representative photograph showing the four treatment cages placed at each slick spot. Note that the cage for labeled ‘simple soil, no ants’ is surrounded by a larger cage to ensure ants could not access the seeds. This was not necessary on complex soil because it was easier to seal the cage tightly to the ground. Photo credit: Ian Robertson

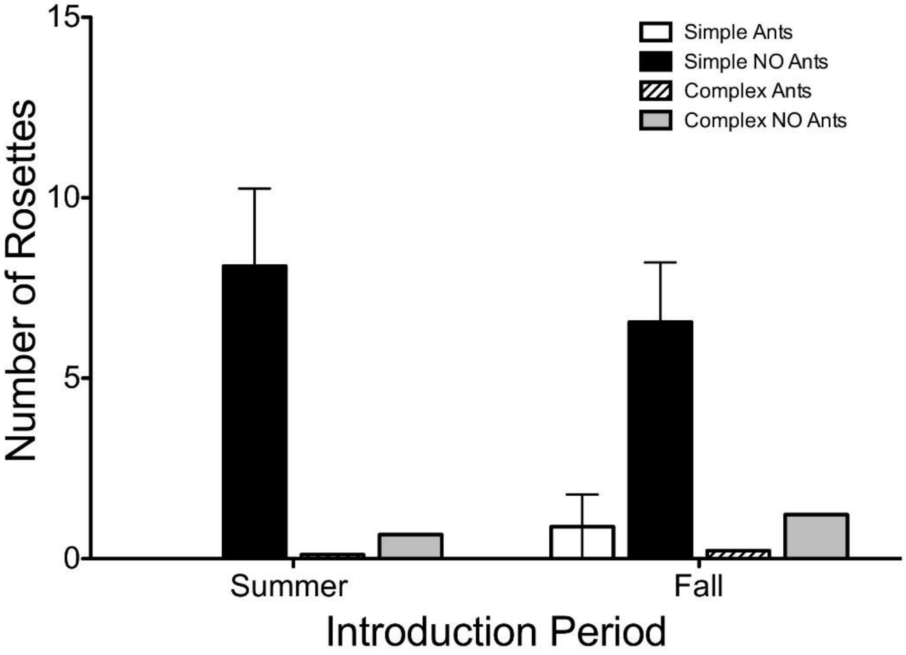

Figure 3.3 Number of *Lepidium* seeds that germinated and produced vegetative rosettes as a function of treatment and introduction time. Production was highest on simple soil when ants were excluded (dark bars).

Figure 3.4 Many of the cages placed on complex soil became inundated with grasses and other vegetation. This was not the case on simple soil. Photo credit: Ian Robertson

Figure 3.5 The lone individual that flowered in a cage produced a full complement of seed-bearing fruits. The pollen source for these fruits is unknown. The cage was located on simple soil and was denied access by ants. Photo credit: Ian Robertson

Figure 3.6 The three individuals that flowered in the same cage failed to produce many fruits, which indicates that pollination did not occur. All three plants withered and died several weeks after flowering began, even though their vegetative counterparts remained green and healthy within the cage. The cage was located on simple soil and was denied access by ants. Photo credit: Ian Robertson

Figure 3.7 Gelechiid moths were observed on the flowers of the plants that went unpollinated. Although these moths are capable of pollinating *L. papilliferum*, they are relatively poor pollinators compared to other insect visitors (Robertson and Leavitt 2011). Photo credit: Ian Robertson