EFFECTS OF LIVESTOCK TRAMPLING ON *LEPIDIUM PAPILLIFERUM*, ITS HABITAT AND SUBSEQUENT HYDROLOGY IN SOUTHWESTERN IDAHO

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AUTHORIZATION TO SUBMIT THESIS

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ABSTRACT

Whether the suggestion that rare species are rare because they are just relicts of past populations applies to *Lepidium papilliferum* (slickspot peppergrass) or not, does not reduce the fact of the rarity of this species and its apparent decline. *L. papilliferum* is an endemic annual or sometimes biennial *Cruciferae* (mustard) of the semi-arid portions of the western Snake River Plain and a portion of the Owyhee uplands. It occurs on slickspot inclusions within the Wyoming big sagebrush steppe. While the subject of several unpublished reports, primary scientific literature is still in its infancy on the autecology of *L. papilliferum*, the relationship between slickspot habitat, the plant, and the potential effects of varying disturbances. This study investigated the relationship that may exist between *L. papilliferum,* its habitat, slickspot hydrology and the mechanical effects of livestock grazing.

Four sites in the Owyhee uplands were chosen with 20 slickspots in each site to be sampled (n=80). A halter-broken heifer was led across one half of the slickspots in each site (n=40) to an ocularly estimated hoof-print cover of 10 percent. Vegetation sampling (density) was completed in ten, 25x50-cm quadrats in each slickspot. The density data was analyzed as a RCBD with repeated measures to test for differences between trampled and un-trampled *L. papilliferum* densities. This analysis was also conducted for 3 exotic annuals to determine if they were being benefited from the disturbance. No significant changes occurred in either analysis. Also, EC and pH measurements were done on soil samples to determine if there was a correlation between these soil properties and *L. papilliferum* density. No relationship was found. Lastly, volumetric water content was measured outside of and within hoof-prints using Decagon Devices, Inc. ECHO probes to determine if slickspot hydrology was being changed by livestock trampling. Results showed that within hoof-print water content was greater than in un-tramped slickspot soils.

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INTRODUCTION

It has been suggested that rare species are rare because they are just relicts of past populations (West 1993). Whether this is the case with *Lepidium papilliferum* (L. Henderson) A. Nels. and J.F. Macbr (slickspot peppergrass) is just speculation, but the rarity of this species and its apparent decline has made it a species of concern. *L. papilliferum* is an endemic annual or sometimes biennial mustard of the semi-arid portions of the western Snake River Plain and a portion of the Owyhee uplands (Moseley 1994) (Figure 1). It occurs on saline and natric soil microsites or “slickspots” as inclusions within the Wyoming big sagebrush steppe vegetation (Fisher *et al.* 1996). These slickspots are, for the most part, devoid of perennial vegetation although several other native and exotic annual plants may be present (e.g., exotic: *Bromus tectorum* L. (cheatgrass), *Sisymbrium altissimum* L. (tumblemustard) *Ceratocephala testiculata* (Crantz) Bess. (bur buttercup), *Lepidium perfoliatum* L. (clasping leaf pepperweed) and *Lactuca serriola* L. (prickly lettuce) native: *Descurania pinnata* (Watt.) Britt. (pinnate tanseymustard).

The U.S. Fish and Wildlife Service had proposed *L. papilliferum* in 2002 for listing as an endangered species under the Endangered Species Act (ESA). The proposal was withdrawn in January 2004 based on the development of a Candidate Conservation Agreement for the species. In 2006, a court order prompted the proposal reinstatement, but in 2007 it was again withdrawn. Potential concerns regarding conservation of this species include the effects of livestock grazing, seed burial or entombment, increased fire occurrence, post-fire revegetation treatments, silting in of slickspots, and competition from other non-native annual plants that can occupy slickspot sites. These concerns result from anecdotal, empirical, and observational data. While the subject of several unpublished reports, primary scientific literature is still in its infancy (although increasing rapidly) on the autecology of *L. papilliferum*, the relationship between slickspot habitat, the plant, and the potential effects of varying disturbances. This study proposes to investigate the relationship that may exist between *L. papilliferum*, its habitat, slickspot hydrology and the mechanical effects of livestock grazing.

LITERATURE REVIEW

The geographical range of *L. papilliferum* encompasses the western Snake River Plain and foothills of Ada, Canyon, Gem and Payette Counties in Idaho as well as a portion of the Inside Desert in Owyhee County (Moseley 1994) (Figure 1). It is typically found in *Artemisia tridentata* Nutt. subsp. *wyomingensis* S.L. Walsh/*Stipa thurberiana* Piper and *A. tridentata* Nutt. subsp. *wyomingensis*/*Pseudoroegneria spicata* (Pursh) A. Löve habitat types (Moseley 1994). These habitat types, previously described by Hironaka *et al.* (1983), are increasingly being converted to annual plant communities on the western Snake River Plain (Peters and Bunting 1994) and restoration has proven to be difficult (Bunting *et al.* 2003).

The conversion of the sagebrush steppe to annual plant communities commenced with the settlement of Euro-American in the mid 1800s (Pyke 1999). This settlement brought farming and livestock. Eventually much of the farming was abandoned, leaving areas devoid of vegetation. The void of vegetation from abandoned fields and overgrazing allowed establishment of nonnative ruderal species brought by Euro-American settlement, such as *S. altissimum, C. testiculata* and *B. tectorum* (Pyke 1999). Of these species, *B. tectorum* is likely the most persistent, ensuing from its role in changing fire frequencies in the Snake River Plain from a historic 35- to 100-year interval to a 3- to 5-year interval in some areas (Whisenant 1990). This change in the fire regime results from the increased fine fuels and fuel continuity that *B. tectorum* and other annuals elicit. Over time, fires have become larger and more uniform, reducing patchiness, seed bank storage and ultimately seed availability of native shrubs and grasses (Whisenant 1990). *B. tectorum* has an advantage over most native perennial grasses and *L. papilliferum* because of its ability to germinate and emerge in almost any season as well as its mechanisms to withstand disturbance (Pyke 1999).

L. papilliferum occurs primarily on low productivity microsites known as slickspots in southwestern Idaho (Moseley 1994). However, the species is not completely restricted to these sites and has been reported in small numbers on adjacent sites. Slickspots, also referred to as playettes, natric sites and panspots, are microsites that are slight depressions that collect runoff from the surrounding landscape. Slickspots have been reported to occur in steppe and shrub steppe throughout the West (Lewis and White 1964, Bakhtar 1977, Hopkins *et al.* 1991, Reid *et al.* 1993). The area of these sites varies from <1 to over 50 m^2. They are characterized by low vegetation cover and by soils with poor water infiltration and relatively high sodium content (Lewis and White 1964, Fisher *et al.* 1996).

The typical soil profile of a slickspot includes a thin vesicular crust, which is an E horizon, and a thin hardpan underlain by an argillic B horizon (Lewis and White 1964, Fisher *et al.* 1996, Meyer and Allen 2005). In most cases the argillic horizon contains enough sodium to qualify as a natric horizon and is generally more saline than the surrounding soils (Fisher *et al*. 1996). The high sodium concentration along with the clayey B horizon prevents water from leaching salts and creates a saline environment. These soil characteristics can be evaluated by the simple measurement of pH and electrical conductivity (EC). A soil pH $\geq$ 8.5 indicates sodic soils and soils with an EC > 4 d S/m indicates a saline soil. Khedr and Lovett-Doust (2000) found a strong soil surface EC correlation to vegetation groups. Water infiltrates slowly within the slickspot soils while the adjacent soils have less restricted water movement (Lewis *et al.* 1959). Conditions of physiological drought may explain the low coverage of vascular plants on the slickspots. Since *L. papilliferum* is strongly associated with slickspots, it is probable that the species has developed physiological adaptations to survive in the saline sodic soils found in slickspots. Therefore, this association may allow for correlation of pH and EC values and *L. papilliferum* abundance.

L. papilliferum may have either an annual or biennial growth form – although short-lived perennial plants have been observed. Plants germinate in early spring and most set seed the same year. A fraction of these plants over-winter as rosettes and, pending summer survival, reproduce the following year (Meyer 1995). Although most plants have the annual form, the biennial form may be 10 times larger and may produce the majority of the seed on a given year (Quinney 1998, Meyer *et al*. 2005). Biennial plants may produce on average six times the seeds of annuals, however, because biennial plant densities are so low that over the long-term they may only represent 8 % of the seed rain over a 100-year period (Meyer *et al*. 2006). It is not known if the biennial form is genetically or environmentally induced, or influenced by both, although Meyer *et al*. (2005) suggest that the biennial form being genotypiclly induced is improbable. Large plants of *Lepidium lasiocarpum* Nutt. have been found to produce seeds with more dormancy than smaller plants (Philippi 1993) but dormancy differences of both growth forms has not been studied in *L. papilliferum*.

L. papilliferum appears to have a long-lived seed bank. Meyer (1995) found that most seeds remain dormant for at least 2 years. With an apparent yearly 9% seed bank loss (6.5% germinating and 2.5% seed death), *L. papilliferum* seeds have about a 12-year life expectancy (Meyer 1995, Quinney 1998, Meyer *et al*. 2005, Meyer *et al*. 2006). Pake and Venable (1996) found that annuals in harsh environments frequently produce large number of seeds and maintain seed banks between high production years to buffer against unfavorable conditions. It has been widely documented that *L. papilliferum* density (recruitment and survival) is highly correlated with spring precipitation (generally February through May) throughout its range (Palazzo *et al*. 2005, Meyer *et al*. 2005, Meyer *et al*. 2006). Therefore, *L. papilliferum* produces copious amounts of seeds in favorable years, which out weighs low seed production years – creating large seed banks (Meyer *et al*. 2005, Meyer *et al*. 2006).

According to a population viability analysis model created by Meyer et al. (2006), an increased variance in late winter (February-March) precipitation decreased the probability of extinction. These factors of seed bank longevity and environmental factors contributing to variable seed production, suggest that *L. papilliferum* needs environmental variance to persist (Meyer *et al*. 2006). Meyer *et al*. (2006) also suggest that *L. papilliferum* could, by natural stochastic environmental variability alone, become extinct during a 100-year period as shown by their population viability analysis. This conclusion brings to light the need for additional understanding on the ecology of *L. papilliferum*.

Elzinga *et al*. (1998) and Palazzo *et al*. (2005) state that monitoring annual plant populations by density, that do not always have yearly expressions, is difficult. They suggest the use of seed bank characterization to study annual plant populations. Despite an apparent abundance of seeds entering the *L. papilliferum* seed bank each year, the distribution of those seeds within and amongst slickspots is minimal. Palazzo *et al*. (2005) found seeds within 2 m outside of slickspots, although in smaller numbers than within slickspots. This shows that some dispersal occurs but it is unclear what maximum distance is possible. Robertson and Ulappa (2004) found that the seeds have no apparent long-range dispersal mechanisms and Moseley (1994) postulated that gravity must be the facilitator of dispersal, but accepted that water and wind must move a portion of seeds – although the seeds have no structures to aid in these methods. Meyer and Allen (2005) establish that seed distribution is patchy and highly variable within slickspots in their seed bank characterization study. Across their three sites, the Orchard Corner site had the highest frequency of seeds, but only 18% of the samples contained any seeds, and of the 700+ samples, 15% contained only 1 or 2 seeds (Meyer and Allen 2005). They concluded that seed bank characterizations of slickspots are not advisable because the high number of samples needed would cause great disturbance to slickspot soil structure and subsequent *L. papilliferum* survival.

The use of *L. papilliferum* as forage by cattle and other large herbivores is uncommon (Popovich 2001). However, limited use by cattle has been reported (U.S. Air Force 2002). Livestock grazing has been shown to both increase and decrease plant species diversity, thereby possibly reducing or increasing the diversity of native insect pollinators (Kearnes and Inouye 1997, Milchunas *et al*. 1988). Loss of insects would be deleterious to *L. papilliferum* since it relies on insect-mediated pollination, but an increase in pollinators could increase fruiting success (Robertson and Klemash 2003, Robertson and Ulappa 2004). Robertson and Ulappa (2004) found that the greater the distance between pollen donors the higher the percent fruit set. This suggests that mating of genetically similar plants could result in lower reproductive success (Robertson and Ulappa 2004).

Mechanical impact (hoof-prints) on slickspots by large herbivores has been noted in several *L. papilliferum* surveys (Moseley 1994, Mancuso 2001, 2002, Popovich 2002).Mancuso (2001) reported that 56% of the 429 slickspots monitored in 2000 had evidence of livestock presence (hoof-prints or feces). Colket (2005) similarly reported that 54% of 71 habitat integrity and population (HIP) transects surveyed across the species' known range had evidence of livestock hoof-prints. Generally livestock use was greater in the southern-most *L. papilliferum* Management Areas (MA), including MA8, MA9, and MA11. The mean livestock hoof-print area per slickspot for *L. papilliferum* in MA 11 (the MA in which this study is located) was 4.6% (range = 0-14.4%, median = 5%).

The mechanical effects of large herbivores and the effects of large animal feces are not known at this time. Livestock feces may increase the amount of organic matter and alter acidity and salinity, allowing other vegetation to colonize the slickspot (Mapfumo *et al*. 2000). It has been suggested that animal prints may reduce slickspot integrity, particularly during winter through spring when the slickspots are wet (U.S. Air Force 2000). Meyer *et al*. (2005) noted that following an extreme trampling event in one of their sites there was an observed reduction of *L. papilliferum* plants for multiple years. These livestock hoof-prints are believed to possibly disrupt or bury the seed bank (Meyer and Allen 2005, Meyer *et al*. 2005, Meyer *et al*. 2006) or alter the hydrologic function of the slickspots due to compaction of the pores in the clay layer (P. Seronko, per. Comm. 2005). Meyer and Allen (2005) found that although slickspots appear homogeneous on the surface, actual depth of the silt E horizon and hardpan layer can vary throughout. They also found that slickspots or areas within slickspots that have a silt layer greater than 3-cm and/or hardpan layer greater than 3-cm, *L. papilliferum* root systems may not reach the clayey B horizon before the summer drought, which may lead to mortality (Meyer and Allen 2005). Meyer et al. (2006) examined the physical disturbance of livestock trampling in their computer simulated population viability analysis, by prognosis of reduced germinant survival, due to: weed invasions, silting and/or disrupted hydrology, and seed burial. They found that simulations for a 15- or 50-year period indicate only slight population declines, at best, even with extreme events and that manifestation of any harmful effects will only be evident over the long term.

The associated compaction of hoof-prints has been shown to decrease water infiltration rates and soil water content (Abdel-Magid *et al*. 1987, Mapfumo *et al*. 2000). One of the major concerns with *L. papilliferum* habitat is the compaction of the micro-spaces or prismatic spaces in the clay layers, which may seal. This process may disturb slickspot hydrology, possibly disrupting seed germination and potentially affecting the ability of the plants taproot to penetrate into the B horizon (P. Seronko, per. Comm. 2005). In determining the water balance or plant available water and therefore nutrients, knowledge of the soil water content is crucial (Bosch 2004). Thus livestock disturbance may alter hydrologic properties in *L. papilliferum* habitat.

APPROACH

The approach taken for this project was both flexible and adaptive since it is studying a plant for which little is fully understood. It encompasses a multi-phase study of the relationship of certain cattle grazing treatments on populations of *L. papilliferum* and slickspot habitat. Phase 1, conducted in May 2002 by The Environmental Company, Inc. and the Idaho Department of Agriculture, was a preliminary investigation or pilot study to identify initial study sites; develop appropriate study methodologies; and better define the relationship between the plant and its habitat. Phase 2 occurred in the summers of 2003 and 2004 where base line population data for each slickspot was collected. Phase 3 involved further identification of study sites and a rigorous testing of effects of livestock mechanical disturbance on *L. papilliferum* populations and its habitat. As this study continues, additional ideas and modifications will be likely added to further increase the understanding of *L. papilliferum* and its habitat.

Four sites were chosen to test simulated grazing impacts on *L. papilliferum* populations, slickspot habitat and hydrology. At these sites, livestock exclosures were constructed to exclude grazing from a portion of the study area. Vegetation response on slickspot habitat and differences in soil physical characteristics of slickspots within the control and treated areas were measured and will continue to be measured over at least a 5-year period. Data from these paired observations were then tested for significant differences between treatments.

STUDY HYPOTHESES

Of the many concerns on the stability of *L. papilliferum* populations, three hypotheses will be tested in this study. Primarily this study hopes to empirically document what effect of livestock (cattle) trampling has on *L. papilliferum* density and if this trampling will affect the invasion of slickspots by exotic species. Associated with this trampling, this study hopes to establish if the hydrology of slickspots is changed by this treatment. Also, this study aims to determine if a correlation can be determined between basic soil factors and L. papilliferum density. The following provides the hypotheses being tested in this study.

Hypothesis 1: Ho - *L. papilliferum* density will be reduced under the trampling treatment.

Ha - *L. papilliferum* density will not be reduced.

Hypothesis 2: Ho - Exotic annual density will increase under the trampling treatment.

Ha - Exotic annual density will not increase.

Hypothesis 3: Ho - Volumetric soil water content will decrease under hoof-prints within slickspots in the trampling treatment.

Ha - Volumetric soil water content will not differ amongst treatments.

Hypothesis 4: Ho - EC and pH values will correlate with *L. papilliferum* density.

Ha - EC and pH values will not correlate with *L. papilliferum* density.

MATERIALS AND METHODS

Study Site and Slickspot Selection

Initial locations for this study have been determined by identifying all known *L. papilliferum* occurrences. This was done by reviewing data from the Idaho Departments of Fish and Game and Agriculture, Idaho Army National Guard, and the U.S. Air Force. Field visits were then conducted to determine if the populations were large enough to implement a paired study, ease of accessibility, and the capability of excluding of grazing at the location. Not all locations are available for the study due to ownership and accessibility issues. In addition, many areas did not have enough slickspots with *L. papilliferum* to serve as a replicate. Study sites were selected in the Juniper Butte area (Figure 2). Four sites were selected: Airbase, Holding Pen Native, Holding Pen Seeded and Three Creek.

Selection of potential locations was done in May 2002 – although Three Creek was not established until 2004. For logistical purposes, study areas are no larger than 40 ha. Barbed wire fencing was used to enclose each site. On Juniper Butte Range (Airbase site), a temporary, two-strand, electric fencing was being used but due to failures and subsequent cattle use, a permanent barbed wire fence was constructed in early 2004.

A set of selection criteria was used to minimize variability across the study areas. Study area selection included the following characteristics to assist in identifying similar sites:

- similar vegetation community type across the potential study area (e.g., *Artemisia tridentata* subsp. *wyomingensis* with mixed native and seeded grass understory);
- similar sizes of slickspots;
- similar vegetative condition of slickspots;
- presence of *L. papilliferum* and/or skeletons indicating recent, previous presence;
- similar topography, including aspect, slope, and elevation.

Twenty slickspot sample units were selected for each sample/study site. A systematic series of steps was used to randomly select ten treatment and ten control slickspots.

Following selection of the study sites and identification of slickspots, each slickspot was mapped utilizing a geographic position system (GPS) using UTM coordinates.

Pretreatment Measurements

Baseline data collection was completed during the implementation of this study (May 2003) and recollected the following year (May 2004). Vegetation measurements completed during the baseline collection included site descriptions of the sagebrush plant community and the slickspot inclusions, and characteristics of slickspot vegetation (Elzinga et al. 1998).

Study site descriptions of adjacent vegetation included percent ground cover by species. Shrub cover was measured with the line intercept method (Canfield 1941) as measured along randomly located, line intercept transects. Coverage of herbaceous species, biological crusts and litter was sampled by ocular percent cover estimates within systematically placed 50x50-cm quadrats along the previously established transect lines. Additionally, a habitat integrity index (HII) was determined in 2003 for each study site following the methods described by Mancuso and Moseley (1998). This system of evaluation was included so that data collected for this study may be compared or extrapolated to other studies that are currently using the HII. Habitat evaluations done in 2005 and 2006 were made using the new habitat integrity and population index (HIP) (Candidate Conservation Agreement 2003).

HIP was not done on the initial three sites (Airbase, Holding Pen Native and Holding Pen Seeded) in the summer of 2004. It was thought by most botanists to be too late in the season to get a meaningful reading. Slickspot vegetation composition was measured by determining density (plants per unit area) of all plant species occupying randomly selected slickspots within each treatment area. Grazing has occurred on all of the sites. Holding Pen Native and Seeded sites were grazed through 2002. Airbase was grazed through 2003 while Three Creek was grazed through 2004.

Data Collection and Sampling Design

Treatment Application

Ten slickspots were randomly selected using random number simulation from the 20 slickspots identified in each of the 4 replications. The mechanical effects of cattle trampling was imposed by repeatedly leading a halter-broken heifer (~410 kg) across each selected slickspot until 8 to 10% of the slickspot is covered with hoof-prints. The number of prints per slickspot varied depending on the size of the slickspot. This treatment level was chosen because it represents the upper end of the range of values found by Colket (2005). She reported that only 7% of the slickspots sampled (5 of 71) across its range had greater than 10% livestock print area. Within MA 11, only 14% (3 of 22) had greater than 10% livestock print area.

Ideally this experiment would have several trampling level treatments and more than a single (per year) treatment date to account for differences in soil moisture. The experimental plants and numbers of occupied slickspots are not available for replicated multiple treatments to be feasible. Therefore, only one treatment level is possible due to limitations on the number of slickspots present in each exclosure area.

Treatments were imposed on May 3, 2005 and April 15, 2006. Snow in April 2005 delayed the treatments until early May. The same slickspots were treated identically during each year of the study. The slickspots and adjacent vegetation were sampled using the methods detailed above at the time of reproductive maturity for *L. papilliferum* (late May-early June 2005 and 2006).

Vegetation

Two primary sampling techniques were used to measure vegetation characteristics in this study. The density method was used to measure vegetation in slickspots, while a line intercept method was used to sample and characterize the adjacent plant community. A full description of these methods is found in Cooperative Extension Service *et al.* (1996). A general description of the application of these methods, used in this study, is outlined below.

Density Sampling of Slickspots

Since slickspots are inconsistent in both shape and size, and plant growth may be discontinuous and sporadic, a flexible sampling system is necessary. For example, while a single 5-m transect may be possible in one slickspot, two transects may be required in another, or it may be impossible to establish 5-m of transect at all while avoiding sampling overlap. Since each slickspot represents 1 sample unit, all sampling within a single slickspot were pooled and averaged to obtain the sample unit data.

The measurement technique used in the field maximized the area of each slickspot sampled. For long, narrow slickspots a single linear transect was established lengthwise through the center of the slickspot. For wider slickspots, multiple transects were established across the slickspot at a distance to prevent quadrat/sampling overlap (Figure 3).

Sampling of slickspots occurred using rectangular 25x50-cm quadrats placed 0.5-m intervals along each transect. Random sampling was introduced in the selection of which side (left or right) by using a digital watch, the random time (minutes) was used to select the side of the first quadrat – even number was right side and odd number was left. The remaining quadrats were then alternated from that initial quadrat. Because slickspots are inherently small in size, for slickspots that were smaller than 5 m in length the opposite side of the initial quadrats was sampled until a total of 10 quadrats per slickspot were established.

When the quadrat extended outside of the slickspot, the quadrat frame was moved into the slickspot, crossing the transect until it was entirely inside the slickspot. Also, when the quadrat did not fit inside the slickspot, that 0.5-m mark was not sampled. For each quadrat placed, the number of plants by species was recorded. In addition, cover estimates of animal prints, the maximum print depth, and average print depth was estimated for each quadrat.

The total number of *L. papilliferum*, both flowering (annual and biennial) and rosette forms, on each slickspot sampled was determined. The *L. papilliferum* HIP monitoring protocol (Candidate Conservation Agreement 2003) was completed for each slickspot sampled.

Line Intercept Sampling of Adjacent Vegetation

Within each study site, a representative area was selected to establish a single 100-m line intercept transect which was randomly placed to sample shrub canopy cover. At every meter mark on the transect tape, the basal and canopy cover of all vegetation was recorded, as well as ground cover classifications (e.g., bare soil, debris, rock, cryptogrammic crusts) using a 50x50-cm quadrat. These data were used to estimate percent cover by species of the vegetation adjacent to the slickspots. Only shrub coverage was recorded in 2003.

Soil Characteristics

Two sampling techniques were be used to measure soil characteristics in this study. Soil characteristic measurements occurred in the least disturbed site, Holding Pen Native and included: 1) Slickspot soil electric conductivity (EC) and 2) pH were measured in the summer of 2005 to establish if a relationship exists between these soil properties and *L. papilliferum* density. In addition, ECHO probes (a dielectric aquameter) from Decagon Devices, Inc. were used to measure volumetric soil water content of slickspots inside and out of hoof-prints in 2006.

EC and pH Measurements

Two samples from all 20 slickspots were extracted with a soil core to a depth of 5 cm. One sample was taken from the middle of the slickspot and the other from the outer edge. These samples were then brought back to the lab and air dried for 24-hours under a hood vent. Each sample was crushed and sieved until no large aggregates remained. Sub samples were then mixed in a 1:1 ratio of 10 g soil and 10 g de-ionized water. Soil pH was measured using the saturated paste and standard pH electrode. EC was measured on the saturated paste extract with a standard electrical conductivity meter.

Volumetric Soil Water Content

A treatment (hoof-print) and a control were used for this analysis. In the treatment a probe was inserted vertically into a hoof-print – all probes were placed to a depth of 5 cm (probe length). The control was inserted approximately 10 – 20 cm away from the hoof-print probe or in the most proximate undisturbed area. Two probes per treatment, per slickspot accounts for 5 slickspots being sampled – for a total of 20 probes (Figure 4). Probes recorded volumetric soil water content percentage every 30 minutes between June 13 and August 15, 2006 on a EM5b Decagon Devices, Inc. datalogger.

Statistical Analysis

Statistical analysis for Hypotheses 1 and 2 – a change in *L. papilliferum* and other exotic forb densities – used a randomized complete block design with repeated measures (years) with 3 replications (sites), 2 treatments (trampled / un-trampled) and 10 samples per treatment. (Since the Three Creek site was not sampled in 2004 and not trampled in 2005, it was excluded from this analysis.) Due to the high variability in *L. papilliferum* density a log transformation was conducted (also, a constant – 0.05 – was added to remove zeros) to normalize the data for the ANOVA (Figure 5). This transformation was also executed for the nonnative species data. Hypothesis 3 – assessing differences of volumetric water content between treatments – initially was designed as Hypothesis 1 but as the study has progressed it was apparent that to truly assess a change in volumetric water content between a hoof-print and the un-trampled soil, independent pairs need to be used for the analysis instead of a randomized complete block design. Hypothesis 4 – assessing correlations between soil EC and pH to *L. papilliferum* density – was tested using Spearman's correlation test. All analysis used SAS 9.1 software (SAS 2003).

RESULTS AND DISCUSSION

Treatment Application

Hoof-print cover on the treated slickspots varied across years and sites, 5.5% (± 2.5) in 2005 and 10.5% (± 1.6) in 2006 (Table 1). This variation between years and slickspots was due to the soil water conditions on the dates of the treatments. In 2005, the sites were relatively dry except for the Airbase site, which had some visibly moist slickspots. In 2006 it rained the night before treatment application. Although there are measured differences between years, hoof-print cover for both years on each slickspot was ocularly estimated to be the same at the time of treatment. This difference is attributed to silting in and the depth of each hoof-print, thus when cover was measured in late May or early June some prints were no longer discernable (Figure 6).

Vegetation

Slickspots

Among the three years of data, entire slickspot *L. papilliferum* density (although quadrat density and entire slickspot density was measured, in all the analyses the quadrat density was used – individuals per square meter) ranged from 0 – 72.2 per slickspot and 0.1 – 9.5 per site (Table 2) and quadrat density ranged from 0 – 144 per slickspot and 0.1 – 17.8 per site (Table 3). It is interesting to note that the density of *L. papilliferum* varied so greatly between treatments, and between years. In 2005 all slickspot densities, in percent change from 2004, were greater except in one treated and one control plot average. In 2006 all but one control and one treated plot average were less than the 2004 baseline data (Tables 2). Similar variability is found in quadrat density results (Table 3). This demonstrates how variable the density of *L. papilliferum* on individual slickspots can be over time and space. It is apparent that 2005 was a favorable year for most sites. This correlates with the precipitation between February and May for that year, with 21.0 cm. In 2004, February through May precipitation equaled 9.2 cm and in 2006, 18.9 cm. Precipitation data was collected by the Mountain Home Air Force Base at the Airbase site on the Juniper Butte Air Force Range (see Figure 2).

Figures 7 and 8 show that the ratio between both measurements of *L. papilliferum* counts – quadrat and entire slickspot – is nearly 2 to 1, suggesting that the quadrat methods results in a *L. papilliferum* count estimate that is twice as great as that of the slickspot count method. This might be useful to extrapolate quadrat data to the entire slickspot in future studies.

There were no significant differences among treatments for either *L. papilliferum* or the three most common introduced species – *B. tectorum, R. testiculatus* and *L. perfoliatum* (Table 4). This indicates that over a two-year period of trampling slickspots with an approximate hoof-print coverage of 10%, *L. papilliferum* density did not decrease, nor was there an increase in nonnative species. There is a significant difference between sites and years, as well as their interaction. This yearly significance further confirms the correlation previously stated between precipitation and *L. papilliferum* density.

Adjacent vegetation

The adjacent vegetation consists of *A. tridentata* subsp. *wyomingensis,* to some degree on all sites, but is less prevalent on the sites that have burned recently – Airbase, 1980, Holding Pen Seeded, 1992 and Three Creek, 1996 (Table 5). Other shrubs present are *Atriplex canescens* (Pursh) Nutt. (fourwing saltbush) and *Chrysothamnus viscidiflorus* (Hook.) Nutt. (green rabbitbrush). Grasses consist of mostly *Poa secunda* J. Presl (Sandburg bluegrass) and in Holding Pen seeded, *Agropyron cristatum* (L.) Gaertn. (crested wheatgrass). The perennial forb with the highest cover was the Phlox species (*Phlox longifolia* Nutt. [longleaf phlox] and *Phlox hoodii* Richards. [Hoods or spiny phlox]). Species richness varied across years and sites with Holding Pen Seeded consistently having the highest and Three Creek having the lowest richness (Table 6).

Comparing the slickspot vegetation to the adjacent sagebrush steppe, species richness is relatively similar. It was an oversight in this study that a consistent measure (i.e., density) was used between the slickspots and the sagebrush steppe to compare and contrast these to habitats. But, data show that the most abundant (density in slickspots and cover in sagebrush steppe) species were the same in both habitats. Future years' data should include density of these prevalent species (Tables 6 and 7) in the sagebrush steppe to further analyze the similarities of these habitats.

Soil Characteristics

EC and pH

Although some have found soil surface EC correlations to vegetation density (Khedr and Lovett-Doust 2000), none was found in this study. EC values ranged from 0.2 to 4.5 d S/m and pH values ranged from 6.9 to 8.6 (Table 8). Spearman's correlation analysis was executed to determine if pH, EC, both together or an interaction of these soil factors were related to *L. papilliferum* densities, none were significant. The greatest correlation was between *L. papilliferum* density and pH in the middle of the slickspots (-.32, 1 being perfectly correlated).

One problem that was not foreseen prior to sampling was that the depth of the B horizon varied and that some samples included the B horizon while some did not. An example of this is demonstrated by the extreme value of 4.5 d S/m in one EC sample, while the average over the 20 slickspots was 2.0 d S/m (Table 8). Any subsequent analysis should collect separate samples of the E and B horizons, as well as the 'hardpan' or restrictive layer.

Volumetric Soil Water Content

Data were averaged daily and nine days over nine weeks were used for the analysis (Tables 9 & 10). As seen in Table 11, using a RCBD reduces the effect of showing differences between treatments due to the great variability of slickspot soils. Meyer and Allen (2005) found that although slickspots appear homogeneous on the surface, actual depth of the silt E horizon and hardpan layer can vary throughout, this study also found that volumetric water content varies greatly in and between slickspots (Tables 9, 10 & 11). These data show that although one may have an apparent blocking feature, such as the slickspot, areas within slickspots can be used as independent samples.

The reason that the RCBD block analysis shows a non-significant treatment effect is due to the large variance within each of these blocks/slickspots, shown by the significant difference in pairs (Table 11). Therefore, although our proposed method (RCBD) did not result in a treatment effect, further analysis of the data shows that an independent pairwise comparison results in a treatment effect. This gives evidence that those areas within hoof-prints have higher volumetric water content than surrounding areas. This is most likely due to the ponding effect that generally occurs in slickspots, which over time, silting in of these hoof-prints may occur and ameliorate this occurrence. This might be measured by leaving some probes for two summers, and others remove after the summer then return and place them back in the following spring and measure through the summer. Since some hoof-prints will not be visible after the winter and subsequent silting in, makers will be needed to guarantee sampling of identical hoof-prints.

Summary

This study did not show a significant change in *L. papilliferum* density as a result of the treatment applied. Also, associated exotic annuals did not show a significant change in density due to treatment applications. EC and pH do not appear to have an effect on *L. papilliferum* density at this study site. Hoof-prints had higher volumetric water content than the un-trampled paired location. This may be due to compacted or reduced pores size resulting in a greater ability of the soil to 'hold' on to the water, or it may be caused by the simple ponding or silting in of the hoof-prints themselves. Further analysis should be done to determine the cause. These results indicate high year-to-year variations in *L. papilliferum* density, consequentially from stochastic environmental factors and not trampling events.

Suggestions for Future Study

These preliminary results indicate what effects may occur when livestock trample slickspots with approximately 10% hoof-print coverage over two years. As this study is currently funded to continue for three more years, cumulative effects may become apparent in future years. Further additions to this study could include other trampling treatments. Since this study is only representing the average, to upper extent of physical disturbance due to livestock that is currently happening on the land (Mancuso 2001), another treatment of 20 to 30% hoof-print coverage could be added to possibly expedite the supposed population declines by excessively trampling a portion of the slickspots. Further analysis should be done to determine the cause for differences in volumetric water content between hoof-prints and un-trampled soil. Other areas of potential interest include; germination in hoof-prints (*L. papilliferum* and other species), silting of hoof-prints and infiltration rates from compaction to silting in or leveling of hoof-prints.

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Table 1. Hoof-print percent cover (and related standard deviation), average and maximum depth (cm) on trampled slickspots.

	2005			2006		
	Percent Cover	Average Depth	Maximum Depth	Percent Cover	Average Depth	Maximum Depth
Airbase	7.4 (2.0)	0.7	1.0	9.5 (3.9)	1.6	2.0
Holding Pen Native	6.5 (4.2)	0.6	0.8	8.8 (2.6)	1.3	1.7
Holding Pen Seeded	2.7 (2.1)	0.6	0.9	11.2 (2.4)	1.4	1.8
Three Creek		Not treated		12.3 (2.8)	1.4	1.8
Average	*5.5*	*0.6*	*0.9*	*10.5*	*1.4*	*1.8*

Table 2: Comparison of entire slickspot *L. papilliferum* mean densities (individuals per m^2) and associated standard deviations for treated and control plots (only treated in 2005 and 2006) and percent change from baseline 2004 data, across all study sites in southeastern Owhyee County, Idaho.

		2004	2005		2006	
		Density	Density	Percent Change	Density	Percent Change
Airbase						
	Control	0.6 (0.7)	0.4 (0.7)	-28	0.2 (0.3)	-63
	Treated	0.3 (0.6)	0.3 (0.5)	10	0.5 (0.8)	75
	Total	0.4 (0.7)	0.4 (0.7)	-16	0.4 (0.6)	-19
Holding Pen Native						
	Control	2.2 (1.8)	15.6 (25.5)	598	2.8 (5.1)	23
	Treated	1.8 (1.0)	3.4 (2.9)	90	1.0 (1.9)	-45
	Total	2.0 (1.4)	9.5 (18.7)	373	1.9 (3.9)	-7
Holding Pen Seeded						
	Control	1.3 (1.7)	4.3 (7.7)	226	0.7 (1.3)	-46
	Treated	1.3 (1.7)	2.0 (1.7)	49	0.6 (0.7)	-55
	Total	1.3 (1.6)	3.1 (5.5)	137	0.7 (1.0)	-51
Three Creek						
	Control	1.2 (1.7)	2.8 (6.2)	146	0.1 (0.1)	-94
	Treated	2.3 (4.1)	0.8 (1.4)	-64	0.2 (0.4)	-91
	Total	1.7 (3.1)	0.8 (4.5)	7	0.1 (0.3)	-92

Table 3: Comparison of quadrat *L. papilliferum* mean densities (individuals per m^2) and associated standard deviations for treated and control plots (only treated in 2005 and 2006) and percent change from baseline 2004 data (2005 for Three Creek), across all study sites in southeastern Owhyee County, Idaho.

		2004	2005		2006	
		Density	Density	Percent Change	Density	Percent Change
Airbase						
	Control	1.3 (2.3)	0.7 (1.2)	-44	0.2 (0.8)	-81
	Treated	0.3 (1.0)	0.9 (1.7)	175	0.8 (1.6)	150
	All	0.8 (1.8)	0.8 (1.4)	0	0.5 (1.3)	-35
Holding Pen Native						
	Control	4.8 (5.8)	31.4 (47.2)	555	5.7 (10.7)	18
	Treated	4.2 (2.4)	4.2 (4.6)	-2	2.6 (5.4)	-40
	All	4.5 (4.4)	17.8 (35.5)	294	4.1 (8.4)	-9
Holding Pen Seeded						
	Control	3.0 (6.3)	13.9 (24.2)	358	2.4 (5.4)	-21
	Treated	3.0 (4.6)	3.1 (2.5)	5	1.0 (1.3)	-68
	All	3.0 (5.4)	8.5 (17.6)	184	1.7 (3.9)	-44
Three Creek						
	Control	No quadrat data collected	7.4 (17.2)		0.2 (0.3)	-98
	Treated		1.6 (2.9)		0.1 (0.3)	-95
	All		4.5 (12.4)		0.1 (0.03)	-97

Table 4. ANOVA with repeated measures - 2004 through 2006 - p-values for quadrat densities of *L. papilliferum* and the three most common nonnative annuals (** denotes significance at alpha = 0.05).

	Lepidium papilliferum	*Lepidium perfoliatum*	*Ranunculus testiculatus*	*Bromus tectorum*
Treatment	0.4863	0.8362	0.5549	0.5550
Site	<0.0001**	<0.0001**	0.0010**	<0.0001**
Site*Treatment	0.8523	0.7003	0.1823	0.7478
Year	<0.0001**	<0.0001**	<0.0001**	<0.0001**
Year*Site	0.0747	<0.0001**	<0.0001**	<0.0001**
Year*Treatment	0.3802	0.8154	0.8762	0.0640
Year*Site*Treatment	0.1522	0.8475	0.9000	0.7343

Table 5. Average shrub percent cover (and related standard deviations) using the line intercept and quadrat methodologies for both 2005 and 2006 data across all sites in Owyhee County, Idaho.

	Airbase	Holding Pen Native	Holding Pen Seeded	Three Creek
Artemisia tridentata subsp. *wyomingensis*	7 (2.9)	20 (2.1)	1 (0.47)	< 1 (0.19)
Atriplex canesenses	< 1 (0.02)	0	1 (2.0)	0
Chrysothamnus visidifloris	6 (1.3)	0	0	< 1 (0.17)

Table 6. Sagebrush steppe species richness and percent cover of the most prevalent species.

	Airbase				Holding Pen Native				Holding Pen Seeded				Three Creek			
	2003	2004	2005	2006	2003	2004	2005	2006	2003	2004	2005	2006	2003	2004	2005	2006
Species richness	No data	No data	26	22	No data	No data	25	24	No data	No data	29	26	No data	No data	19	21
Agropyron cristatum	No data collected	No data collected	1	0	0	0	0	< 1	14	31	27	29	Site not established	Site grazed, no data collected	0	< 1
Bromus tectorum	No data collected	No data collected	< 1	< 1	< 1	< 1	< 1	< 1	0	< 1	1	< 1	Site not established	Site grazed, no data collected	2	3
Elymus elymoides	No data collected	No data collected	< 1	1	5	2	2	1	< 1	0	< 1	0	Site not established	Site grazed, no data collected	3	3
Lepidium perfoliatum	No data collected	No data collected	< 1	< 1	No data	No data	< 1	< 1	No data	No data	0	< 1	Site not established	Site grazed, no data collected	14	1
Phlox species	No data collected	No data collected	6	2	3	5	6	1	1	3	3	< 1	Site not established	Site grazed, no data collected	5	1
Poa secunda	No data collected	No data collected	19	21	18	24	21	15	8	23	16	17	Site not established	Site grazed, no data collected	28	28
Ceratocephala testiculata	No data collected	No data collected	1	< 1	No data	No data	< 1	< 1	No data	No data	< 1	< 1	Site not established	Site grazed, no data collected	1	< 1

Table 7. Slickspot species richness and quadrat density (individuals per square meter) of the most prevalent species.

	Airbase				Holding Pen Native				Holding Pen Seeded				Three Creek			
	2003	2004	2005	2006	2003	2004	2005	2006	2003	2004	2005	2006	2003	2004	2005	2006
Species richness	11	14	13	20	16	17	25	17	13	17	25	17	No data	No data	19	19
Agropyron cristatum	2.3	0.6	0.1	0.1	0.4	0.3	0.5	0.7	23.3	19.0	17.4	18.1	Site not established	Site grazed, no data collected	0.2	0.4
Bromus tectorum	15.0	0.2	0.6	0.3	12.8	14.4	13.4	2.9	7.3	19.8	46.2	10.4			35.8	20.5
Elymus elymoides	13.0	4.7	3.2	3.5	3.4	2.5	1.5	2.2	0.5	0.1	0.5	0.4			2.2	4.3
Lepidium perfoliatum	55.4	119	294	58.9	3.4	6.1	90.6	189	1.6	3.7	86.2	189			287	166
Phlox species	0	0.1	0.6	0.3	0.2	0.3	0.7	0.3	0.9	0.1	0.6	0.1			0.5	0.1
Poa secunda	48.2	20.3	29.6	27.5	10.2	15.6	17.0	16.1	19.6	18.4	25.6	23.7			8.5	9.8
Ceratocephala testiculata	3.4	15.2	179	29.6	40.2	16.5	25.3	5.3	47.9	52.5	54.7	27.9			8.6	56.3

Table 8: Results of pH and EC measurements in the Holding Pen Native site for correlation analysis with average and standard deviation (in parenthesis) values.

	pH		EC	
Slickspot	Middle	Outside	Middle	Outside
1	7.7	7.6	1.6	1.0
2	7.4	7.2	2.0	1.5
3	8.1	7.4	2.8	0.1
4	7.2	7.1	2.6	0.3
5	7.5	8.1	0.9	0.8
6	7.5	7.9	0.7	1.9
7	7.6	7.7	4.5	0.8
8	7.3	7.9	2.4	1.5
9	8.1	8.6	2.2	1.3
10	7.4	7.7	1.2	1.7
11	7.5	7.2	1.7	1.3
12	7.8	7.1	1.8	0.3
13	7.5	7.3	2.4	0.6
14	7.4	7.7	1.6	2.0
15	7.8	7.2	0.9	0.4
16	7.8	7.6	2.0	2.5
17	7.7	8.1	1.4	1.2
18	7.3	8.1	1.8	1.5
19	8.4	6.9	2.7	0.2
20	8.0	8.1	2.0	1.1
Average	7.6 (0.3)	7.6 (0.4)	2.0 (0.8)	1.1 (0.6)

* 'Middle' samples were taken in the center of the slickspot, while 'Outside' samples were taken approximately 10 cm from the edge or rim of the slickspot.

Table 9. Percent volumetric soil water content averages for each treatment (in or out of a hoof-print) for nine days over nine weeks; data used for RCBD analysis.

	Slickspot 6		Slickspot 7		Slickspot 10		Slickspot 11		Slickspot 19	
	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT
June 18	36.5	25.5	40.5	36.1	21.1	21.4	35.5	32.0	54.0	37.9
June 25	22.8	19.9	35.4	31.0	15.8	15.7	21.4	19.9	42.8	29.2
July 2	18.1	17.3	31.6	27.5	13.0	13.4	16.3	15.8	34.9	24.4
July 9	30.1	19.6	37.1	32.1	19.0	18.1	27.4	19.9	44.9	27.0
July 16		16.9	30.4	22.6	14.3	13.4	16.4	14.8	32.1	22.1
July 23	No	15.3	24.4	18.2	11.6	11.9	13.2	12.9	24.4	18.2
July 30	data	14.3	21.0	15.9	10.5	10.8	11.9	11.5	21.3	16.5
August 6		14.2	19.9	15.6	10.2	10.7	11.5	11.4	20.0	16.1
August 13		12.9	17.7	13.9	9.1	9.7	10.2	10.0	17.6	14.4

Table 10. Daily percent volumetric soil water content averages for each probe for nine days over nine weeks; data used for individual pairs analysis.

	Slickspot 6				Slickspot 7				Slickspot 10				Slickspot 11				Slickspot 19			
	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT
	Pair 1		Pair 2		Pair 3		Pair 4		Pair 5		Pair 6		Pair 7		Pair 8		Pair 9		Pair 10	
June 18	26.7	25.6	46.3	25.4	30.5	24.7	50.5	47.4	20.8	23.0	21.4	19.7	39.1	35.6	32.0	28.4	51.5	39.8	56.4	36.0
June 25	18.0	19.7	27.6	20.2	21.3	18.4	49.4	43.6	16.3	17.2	15.4	14.2	25.4	23.2	17.5	16.6	41.8	33.1	43.8	25.2
July 2	14.2	17.5	21.9	17.1	17.6	16.3	45.5	38.8	14.0	14.9	12.0	11.9	19.9	18.4	12.7	13.1	34.9	27.5	34.9	21.2
July 9	19.0	21.5	41.2	17.6	26.2	19.3	48.0	44.9	19.5	18.4	18.4	17.8	34.9	23.0	19.8	16.8	38.4	30.1	51.5	24.0
July 16		17.7	25.6	16.0	18.5	13.9	42.2	31.4	14.9	14.1	13.7	12.6	20.2	17.3	12.7	12.4	28.3	23.9	36.0	20.3
July 23	No	15.9	20.8	14.8	15.8	12.3	33.0	24.1	12.8	13.1	10.4	10.7	16.0	15.0	10.5	10.8	22.3	18.9	26.5	17.5
July 30	data	14.8	19.8	13.7	14.8	10.7	27.3	21.2	11.7	11.8	9.4	9.8	14.3	13.4	9.5	9.7	20.2	17.0	22.5	16.0
August 6		14.7	19.7	13.6	14.8	10.9	25.0	20.3	11.4	11.6	9.0	9.7	13.8	13.2	9.2	9.6	19.6	16.6	20.5	15.6
August 13		13.3	18.5	12.4	13.5	9.7	22.0	18.2	10.3	10.5	7.9	8.8	12.3	11.6	8.1	8.3	17.5	14.8	17.8	14.0

Table 11. Randomized complete block design and independent pairs ANOVA with repeated measures (nine week period) p-values for the volumetric soil water content measurements (** denotes significance at alpha = 0.05).

Variables	RCBD	Independent Pairs
Treatment	0.1504	0.0153**
Block (slickspots)	0.0637	
Pair		0.0022**
Time	<0.0001**	<0.0001**
Time*Block	0.0905	
Time*Pair		0.0001**
Time*Treatment	0.0041**	0.0017**

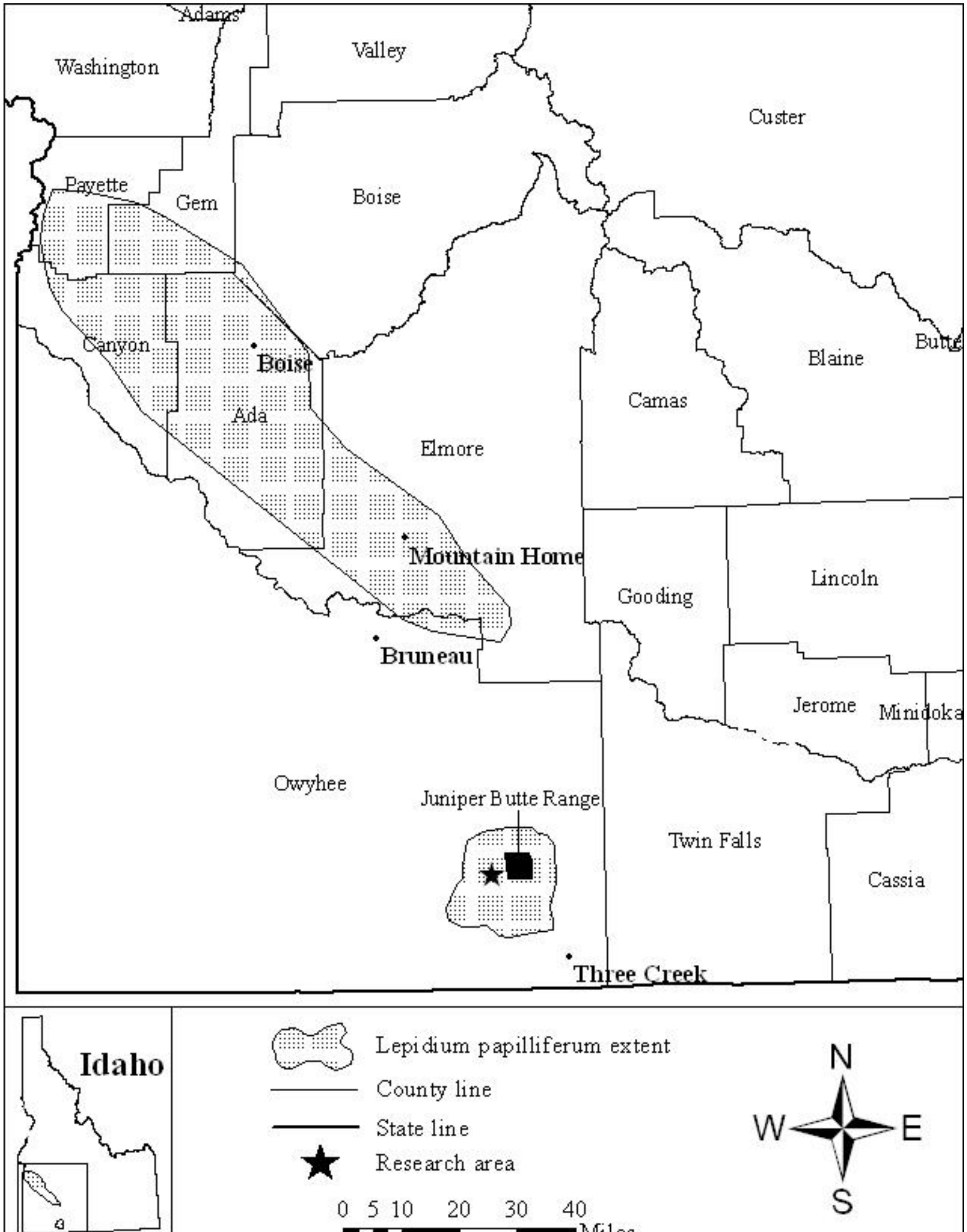

Figure 1. Range of the Snake River Plain and Inside Desert *L. papilliferum* populations.

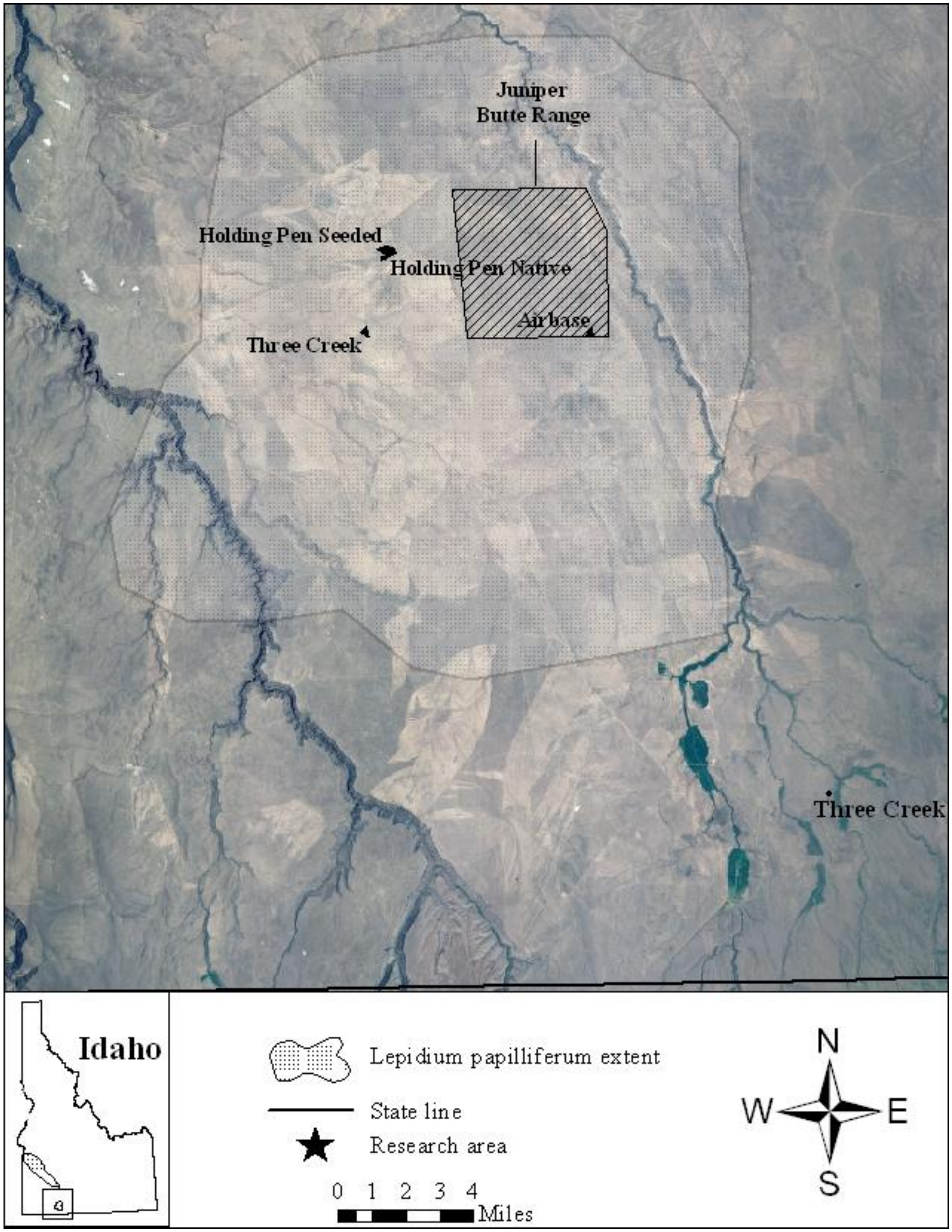

Figure 2. Locations of study sites in the Juniper Butte area, Owyhee County Idaho.

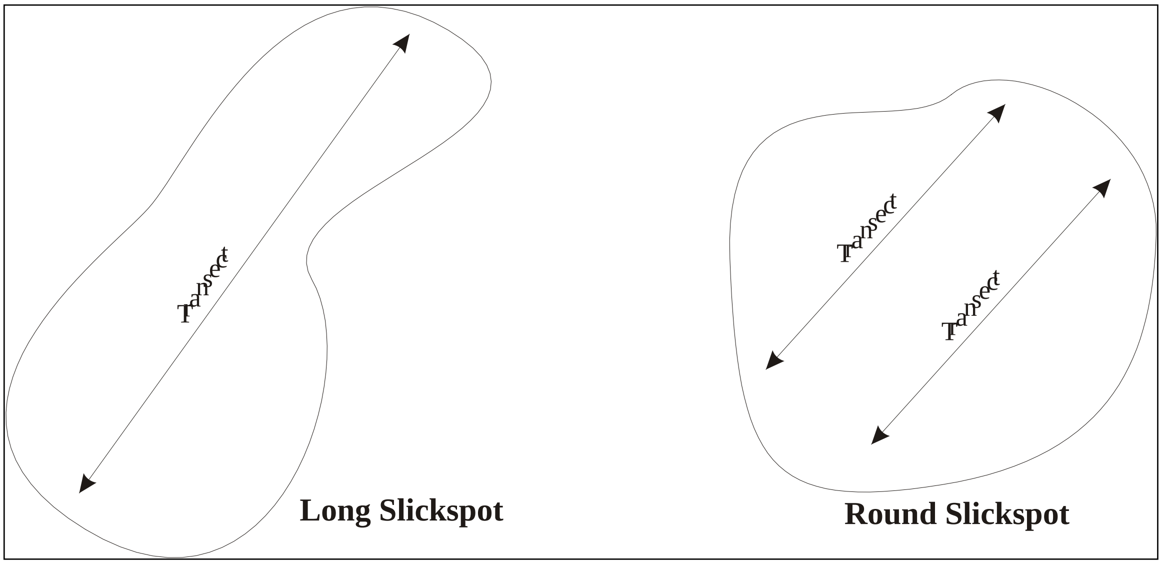

Figure 3. Diagram indicating the procedure for locating transects within each slickspot.

Figure 4. Example of the volumetric soil water content measurements inside and out of hoof-prints.

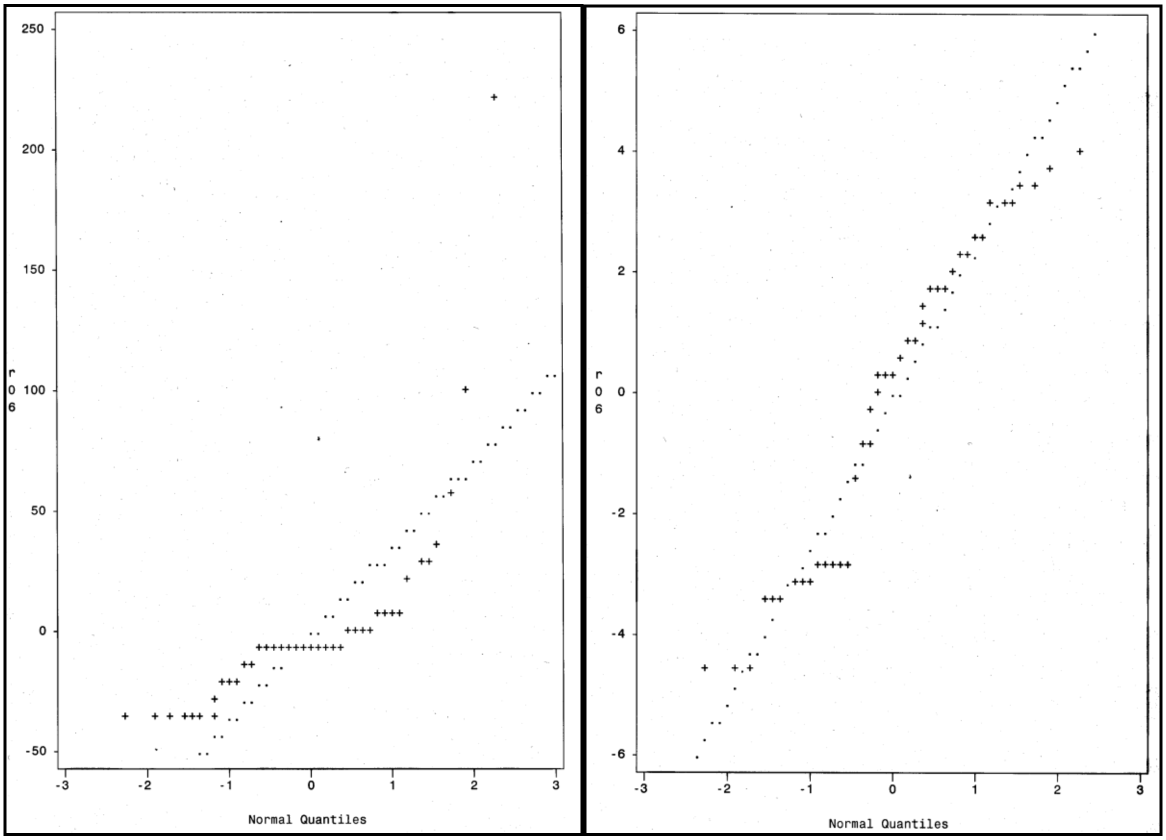

Figure 5. Plots of the residuals (y-axis) by predicted (x-axis) values from SAS (proc plot) of 2006 data. The plot on the left is of the original data, while the plot on the right is of the log-transformed data (2006 data are shown because it was the least normal).

Figure 6. Silting in of hoof-prints just after 3 weeks – maximum depth on day of treatment (upper image) was 3.5-cm and 3 weeks after, 1.5-cm (lower image).

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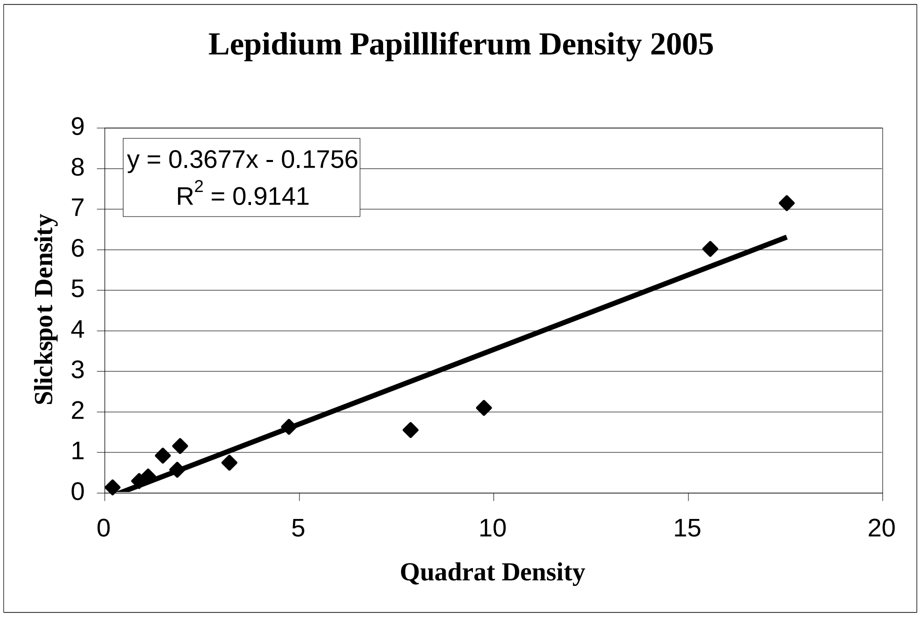

Figure 7. Correlation of total slickspot density and measured quadrat density in 2005.

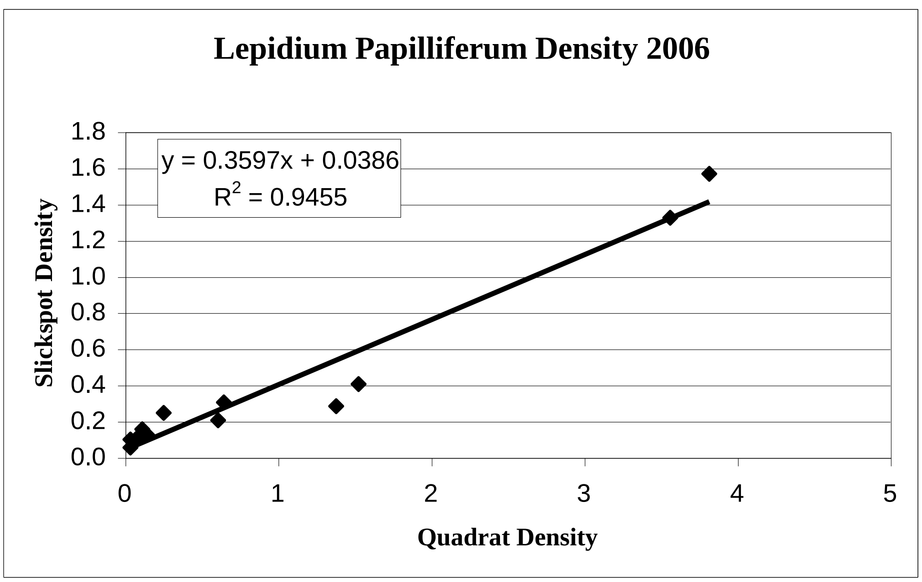

Figure 8. Correlation of total slickspot density and measured quadrat density in 2006.

APPENDIX

Animal Care and Use Committee Protocol Approval

University of Idaho
Animal Care and Use Committee

Date: Friday, August 25, 2006
To: Stephen Bunting
From: University of Idaho
Re: Protocol 2004-31
Effects of livestock trampling on *Lepidium papilliferum* and slickspot composition in souther Idaho

Your requested renewal of the animal care and use protocol shown above was reviewed by the University of Idaho on Friday, August 25, 2006.

This protocol was originally submitted for review on: Thursday, September 18, 2003
The original approval date for this protocol is: Thursday, July 22, 2004
This approval will remain in affect until: Sunday, July 22, 2007
The protocol may be continued by annual updates until: Sunday, July 22, 2007

Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. For the first two years after initial approval of the protocol you will be asked to submit an annual update form describing any changes in procedures or personnel. The committee may, at its discretion, extend approval for the project in yearly increments until the third anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.

Brad Williams DVM

IACUC Representative