Threats research and monitoring on the invasive species Sahara mustard (*Brassica tournefortii*)

D 39 FINAL PROJECT REPORT FOR HYPOTHESIS #6

and

SUMMARY REPORT FOR HYPOTHESES #1-6

2009-2011

Work performed by the National Park Service

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EXECUTIVE SUMMARY

The goal of MSHCP 2005-NPS-532 is to increase knowledge of the biology and ecology of Sahara mustard to aid in efficient eradication and management of this species. This study will provide useful information for land managers in their management of Sahara mustard. Threats research and monitoring on the invasive species Sahara mustard (*Brassica tournefortii*), MSHCP 532 has six hypotheses:

1. Unripened seed pods from Sahara mustard plants will continue to ripen in the field once pulled and will become viable.
2. Sahara mustard seeds remain viable after host plant is sprayed with herbicide during the early fruiting stage while fruits are still immature.
3. There is genetic variation between small and large Sahara mustard plants.
4. Sahara mustard seed germination is inhibited by light.
5. Sahara mustard is not capable of self-fertilization.
6. Sahara mustard seeds are short lived in the soil seed bank.

There were several findings important for successful management of Sahara mustard. The treatments of breaking and pulling plants are only partially effective for reducing germination of seed, reducing only the germination of undeveloped seed. Seed pod removal was the most effective treatment, eliminating seed germination entirely from plants with developing or undeveloped seeds, and reduced germination of developed seeds by half.

Herbicide can be used effectively on Sahara mustard in all stages of development (rosette, bolting, flowering, and early to mature fruiting) to prevent seed development and viability, and to reduce the future soil seed bank. Mechanical removal of fruiting plants is not only more expensive, but also may generate excessive soil disturbance and removes biomass from the site.

Light does significantly inhibit Sahara mustard seed germination at temperatures below 25° C. We can infer that Sahara mustard seeds must be buried below the soil surface or covered in some manner in order to germinate in any quantity and that seeds remaining on the soil surface will not germinate or will at very low rates. Soil seed banks of Sahara mustard appear short-lived at shallow soil depths with commonly greater than 90% depletion by 12-18 months of burial. Deeper burial depths increase seed persistence, but these deeper soils are unlikely to be important for Sahara mustard recruitment unless disturbance bring these soils closer to the surface.

Due to difficulties growing Sahara mustard plants to maturity in a controlled greenhouse setting, Hypotheses 3 and 5 were not completed, and project funding was reduced proportionately.

This work was supported by the Clark County Desert Conservation Program and funded by Southern Nevada Public Land Management Act as project # 2005-NPS-532, to further implement or develop the Clark County Multiple Species Habitat Conservation Plan. The results of this experiment will be incorporated into the Lake Mead Exotic Plant Management Plan for managing Sahara mustard in the future.

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INTRODUCTION

DESCRIPTION OF THE PROJECT

The goal of MSHCP 2005-NPS-532 is to increase knowledge of the biology and ecology of Sahara mustard to aid in efficient eradication and management of this species. This study will provide useful information for land managers in their management of Sahara mustard. There are six hypotheses for NPS 532 - Threats research and monitoring on the invasive species Sahara mustard (*Brassica tournefortii*). The hypotheses are:

- Hypothesis 1--Un-ripened seed pods from Sahara mustard plants will continue to ripen in the field once pulled and will become viable.
- Hypothesis 2--Sahara mustard seeds remain viable after host plant is sprayed with herbicide during the early fruiting stage while fruits are still immature.
- Hypothesis 3--There is genetic variation between small and large Sahara mustard plants.
- Hypothesis 4--Sahara mustard seed germination is inhibited by light.
- Hypothesis 5--Sahara mustard is not capable of self-fertilization.
- Hypothesis 6--Sahara mustard seeds are short lived in the soil seed bank.

MANAGEMENT ACTIONS ADDRESSED

The following MSHCP (Clark County, 2000) conservation management actions were addressed by this project:

- Conservation Management Action NPS (3) - Cooperate in the identification, development, and implementation of research projects located on Federal lands. Emphasis shall be placed on research that addresses management concerns and the conservation of Covered and Evaluation Species.

HCP ECOSYSTEM/HABITAT THREATS ADDRESSED

The following MSHCP (Clark County, 2000) ecosystem/habitat threats were addressed by this project:

- Habitat degradation and population decreases resulting from introductions, competition, and encroachment of exotic species (such as tamarisk, vallisneria, fan palm invasion [upper Muddy River], red shiners, tilapia, and other species) – 1501.
- Development and implementation of site-specific species-specific control or eradication programs - 1501c.

BACKGROUND AND NEED FOR THE PROJECT

Sahara mustard is native to semi-arid and arid deserts of North Africa and the Middle East, as well as Mediterranean lands of southern Europe in habitats similar to those it now occupies in North America (Sanders & Minnich, 2006). It was first collected in the United States in 1927 in

California, where it was believed to have been introduced with date palms brought from the Middle East (Bossard, Randall, & Hoshovsky, 2000). Sahara mustard is an annual weed throughout southern California, southern Nevada, southern Utah, Arizona, New Mexico, west Texas, and northwestern Mexico. It is found as high as 3,300 feet (1,000 m) elevation, but is especially abundant below 1,000 feet (305 m) (Sanders & Minnich, 2006). It thrives on beaches and sand dunes and has established itself as a highly invasive alien species at Lake Mead National Recreation Area.

Sahara mustard has spread rapidly into a variety of habitats from sandy beaches to gravelly washes, and is one of the top non-native plants of concern for managers in the deserts of the southwestern United States. Once a plant has senesced it typically breaks off and tumbles with the wind to a new location. Sahara mustard plants usually disperse their seeds directly below the parent plant, but some seeds remain in the siliques and are dispersed across the landscape. Rodents have been documented caching seeds and may be capable of transporting seeds over 300 feet (Graham, Johnson, & Powell, 2005). Sahara mustard can also remain in place once senesced creating a monoculture of standing dead material. New Sahara mustard seedlings will germinate underneath the dead canopy and grow up through the previous year's standing dead, but native plants have difficulty establishing under these conditions (Figure 1). Native annuals will grow under the dead Sahara mustard canopy, but a study done by Barrows et al. (2009) measured an 80-90% reduction in native annuals' flowering and seed production.

Lake Mead National Recreation Area has been actively controlling Sahara mustard for approximately 11 years. Control efforts have been time consuming and labor intensive, consisting of hand-pulling (Figure 2), bagging, and removal off-site (Figure 3). Only now are we seeing a small decline in Sahara mustard numbers due to the constant control effort every year at one location (Norman, C., personal observation). Sahara mustard will have a high reproduction year when rainfall is above average; in California this can be as little as 1.5 inches of precipitation (Bossard, Randall, & Hoshovsky, 2000). The typical germination time frame for Sahara mustard is December to January, after the first winter rains (Bossard, Randall, & Hoshovsky, 2000). By the time most native annuals start to germinate Sahara mustard is already in a rosette stage, bolting, or even flowering. This gives Sahara mustard a huge advantage over native plants because it will uptake available nutrients and water before the natives have a chance to establish. At Lake Mead National Recreation Area, it flowers as early as January and sets seed by February, senescing by April.

Sahara mustard was placed on the Nevada noxious weed list as a category B weed in 2005 (Marsh, 2005). Category B represents weeds: established in scattered populations in some counties of the state; actively excluded where possible; actively eradicated from nursery stock dealer premises; and control is required by the state in areas where populations are not well established or previously unknown to occur. It is currently on the Arizona Department of Transportation Natural Resources Noxious Weed Priority List (AZ DOT).

Sand dunes and sandy areas contain psammophile (sand-loving) species that are unique to this habitat and are not found on any other soil type in the park. Lake Mead NRA has two rare plant species (*Astragalus geyerii* var. *triquetrus* and *Eriogonum viscidulum*) that are covered by the

Figure 1. Previous season's hanging seed bank of Sahara mustard on the shoreline of Lake Mead National Recreation Area. The senesced plants can be very thick and sunlight may not penetrate through to the soil to allow native annuals to germinate.

Figure 2. Hand-pulling Sahara mustard on the beaches surrounding Sandy Cove. This area is prime habitat for Threecorner milkvetch, a threatened and endangered rare plant.

Figure 3. Crews removing bags of Sahara mustard seeds off-site.

Clark County Multiple Species Habitat Conservation Plan (CCMSHCP). These plants require moving sand and active sand dunes to maintain their populations and recruitment, and can be heavily impacted by infestations of Sahara mustard. Both of these plants are listed as critically endangered in Nevada in lieu of federal listing under the Endangered Species Act (Clark County, 2000). These species are also listed as critically endangered by the Nevada Natural Heritage Program.

The southern most extent of *Astragalus geyerii* var. *triquetrus* is located at Sandy Cove on Lake Mead (Niles, Holland, & Landau, 1995). The sand dunes supporting the Sandy Cove populations are at risk of stabilization by invasive exotic plants, primarily Sahara mustard and Mediterranean grass. The southern and western extensions of *Eriogonum viscidulum* are located at Middle Point on Lake Mead (Niles, Holland, & Landau, 1995). These sand dunes are at risk by invasive exotic plants as well, primarily Sahara mustard, tamarisk, and Russian thistle. With the declining water levels of Lake Mead there is more exposed ground surrounding these dunes for Sahara mustard and other invasive plants to establish. This study will help develop more efficient methods of Sahara mustard control that will enable land managers to control more infested acres and possibly eradicate this weed from critically sensitive sites.

METHODS AND MATERIALS

HYPOTHESIS 1

Four treatments - hand pulling entire plant, breaking plants at the base, seed pod (silique) removal, and no manipulation (control) were applied to Sahara mustard at three different developmental stages (below) to determine effects on seed development and viability:

- Stage 1: undeveloped--siliques with only liquid endosperm without visible embryos; field identification: small and pliable seed siliques
- Stage 2: developing--siliques with partially developed embryos in liquid endosperm; field identification: somewhat firm but still pliable siliques
- Stage 3: developed--siliques are firm and have fully developed embryo with green cotyledons that fill the seed coat; field identification: hard siliques

A 50-m transect line was established in the center of a geographically distinct Sahara mustard population approximately 1 hectare in size at Boxcar Wash, Lake Mead National Recreation Area. Along this transect, the nearest 100 Sahara mustard plants were selected and tagged when the plant was in the rosette/seedling stage. The four treatments were then randomly assigned to 25 plants each from this pool of 100 plants. The random selection was made by numbering the plants and using "Microsoft Excel" (2010) random number generator to randomly assign the treatment. At treatment time, seed pod developmental stages were labeled using different colored embroidery thread.

All plant material remained in the field, where researchers visited regularly to check on ripening status. Seed pods were collected over a period of approximately two weeks during the spring of 2010, as they ripened or dried out, but before the seed pods split apart. Collected seed pods were placed in labeled paper bags and transported to the lab where seeds were separated from seed pods and labeled.

The Echo Bay weather station within Lake Mead National Recreation Area reported an average monthly temperature of 51 degrees F and 0.9 inches of rain in February, the month when Sahara mustard seed and plants began maturing (Western Regional Climate Center). A HOBO climate data logger also was installed directly on the experimental site. Data recording began when Sahara mustard plant materials were being collected in early April. The average daily air temperature in April was 72 degrees F, and the average soil temperature at a depth of 5 cm into the ground was 75 degrees F.

Seed pods and seeds were measured to assess treatment effects on seed pod and seed development. Seed pod size was determined by measuring 1 to 10 individual seed pods per developmental stage per plant. The number of pods varied because the number of pods on plants was not always the same. A total of 1,054 seed pods were measured. Seed size measurements were taken on 10 seeds from a pool of seeds collected from each seed pod in different developmental stages. These pods were selected simply by scooping the seeds randomly out of a container, akin to random selection by randomly drawing from a hat. A digital caliper was used to measure size of seed pods and seeds. The average size of seed pods and seeds was used to describe main features in the data set.

Germinability was assessed by placing seeds on filter paper (#1 Whatman type filter paper, 90 mm) moistened with distilled water in a laboratory under ambient lighting, maintaining the filter paper moist by adding additional water, and allowing up to four weeks for germination. For each of the 12 treatment by developmental stage combinations, more than 600 seeds in lots of 25 seeds were assayed, for a total of more than 7,000 seeds. The germination data were analyzed statistically using a nested, two-factor analysis of variance including three levels of seed developmental stage and four levels of treatment. Percent germination was arcsine transformed as the response variable and analyzed using SAS (SAS, 2001).

HYPOTHESIS 2

Four treatments (three herbicide and one control) were applied to Sahara mustard plants in this experiment: glyphosate at 1.5% solution of glyphosate and water; metsulfuron methyl at 1 gram of metsulfuron methyl in solution with one gallon of water; 2, 4-D at 1.5% solution of 2, 4-D and water; and a no herbicide treatment (control). Herbicides were applied by spot treatments using a 3-gallon backpack sprayer and therefore do not have a spatial application rate. Each treatment was replicated twice along a 50 meter transect (Figure 4). Transects were placed in a dense patch of Sahara mustard located in Callville Wash in the Boulder Basin region of Lake Mead National Recreation Area. Starting at the west edge of the wash, 2 transects were placed heading north and then 2 transects heading south. Two more transects were placed on the east edge of the wash. These transects were placed on ledges and not in the middle of the wash to minimize potential flood damage to the plots. The four treatments were placed 20 meters or more from neighboring transects to reduce herbicide drift onto another treatment. Each transect start/end were recorded with a Trimble Juno GPS.

Along each transect 60 siliques per developmental stage were selected using a random number generator table ranging from 0-50 meters. Once a location on the 50 meter transect was selected then a 0-5 meter number was used to select a plant perpendicular to the transect. The line intercept method was then used to select the branch on Sahara mustard to mark each silique. No more than 5 siliques per plant were selected. Plant locations were marked with pin flags and siliques were labeled using color coordinated tape for each developmental stage, as follows:

- Stage 1: undeveloped--siliques with only liquid endosperm without visible embryos; field identification - small and pliable seed siliques
- Stage 2: developing--siliques with partially developed embryos in liquid endosperm; field identification - somewhat firm but still pliable siliques
- Stage 3: developed--siliques are firm and have fully developed embryo with green cotyledons that fill the seed coat; field identification - hard siliques

Approximately 3–4 weeks after treatment, (after the siliques had matured and dried, but prior to seed dispersal) they were collected into labeled paper bags. A germination test using a Percival I-36 Series Germination Chamber for 9 days at 25°/15° C in complete darkness was conducted to test all seeds for viability as indicated by visible radical protrusion (Figure 6). Seeds were randomly selected for germination by separating all the seeds from the siliques by treatment and stage and placing them into a bowl, then scooping out seeds with a spoon until the required

amount was reached. Each developmental stage per treatment used 9 petri dishes (100 X 15 mm with a Whatman filter paper moistened with 4 ml distilled water) with 25 seeds per dish, totaling 225 seeds. Additional water was added as needed to keep the seeds moist. Each petri dish was labeled with the appropriate treatment type and developmental stage. The number of germinates were recorded each day and then removed from the petri dish.

Figure 4. Setting up 50 meter transect in a dense patch of Sahara mustard.

Figure 5. Sahara mustard plant three weeks after herbicide treatment.

The active ingredients of the three herbicides used in this experiment have different modes of action. Glyphosate is an amino acid inhibitor-aromatic (EPSP). Metsulfuron methyl is an amino acid inhibitor-branched chain (AHAS/ALS). 2, 4-D is an auxin growth regulator. Weed Science Society of America published an herbicide handbook that describes in detail what the properties of active ingredients are in herbicides (WSSA, 2002). The following specifics for the three types of herbicides used in this experiment can be found in the WSSA handbook:

Glyphosate

Growth of treated plants by glyphosate is inhibited soon after application followed by general foliar chlorosis and necrosis within 4-7 days. Glyphosate inhibits 5-enolypyruvylshikimate-3-phospate (EPSP) synthase. EPSP leads to depletion of the aromatic amino acids tryptophan, tyrosine, and phenylalanine, all needed for protein synthesis or for biosynthetic pathways leading to growth. Glyphosate rapidly and tightly adsorbs to soil which results in low mobility into the surrounding environment. Organic matter, clay, silt, or sand content and soil pH have minimal effect on adsorption rates. The typical half life of glyphosate is 47 days, but lab experiments show <25 days.

Metsulfuron methyl

Growth of treated plants by metsulfuron methyl is inhibited within hours after application, but injury symptoms usually appear > 1-2 weeks later. Meristematic areas gradually become chlorotic and necrotic, followed by a general foliar chlorosis and necrosis. The plants have a rapid foliar and root absorption rate and accumulate in the meristematic areas. Metsulfuron methyl translocates extensively in the xylem following root absorption, and less so in the phloem after foliar application. It inhibits acetolactate synthase (ALS), also called acetohydroxyacid synthase (AHAS), a key enzyme in the biosynthesis of the branched-chain amino acids isoleucine, leucine, and valine. Metsulfuron methyl has a low adsorption to clay, but a greater adsorption to organic matter. There is a moderate residual of the herbicide with a ½ life of 30 days, but ranging from 1-6 weeks.

2, 4-D

Growth of treated plants by 2, 4-D exhibit symptoms that include epinastic bending and twisting of stems and petioles, stem swelling (particularly at nodes) and elongation, and leaf cupping and curling. Leaf shape and venation often appear abnormal. This is followed by chlorosis at the growing points, growth inhibition, wilting, and necrosis. Death of susceptible plants occurs slowly, usually within puckered areas of the leaves. The tips of new leaves may develop into narrow extensions of the midrib. The active ingredient is transported primarily via the symplastic pathway (including the phloem) and accumulates principally at the growing points of the shoot and root. Average persistence of phytotoxicity is generally 1-4 week in warm, moist soil. The typical half life is 10 days. Dissipation studies indicate that >95% of applied 2, 4-D moves <15 cm. However, 2, 4-D has moved to 30-46 cm in sandy soils in California with heavy amounts of applied water.

Statistical analysis was not performed as a majority of the measurements equaled zero in the three herbicide treatments, versus high germination from the controls. Visually the data show huge differences between germination rates in the four treatments.

HYPOTHESIS 3

Three size classes of Sahara mustard were determined by a line intercept along a 20 meter transect. The start of the transect was randomly chosen in a heavy infestation of Sahara mustard by tossing a metal stake, then 20 meters was stretched out due east. Each plant that touched the tape was measured for height of plant, diameter of basal rosette, and height of first fruit on plant. These measurements were grouped into three distinct size classes to be used in the rest of this experiment.

A 50 meter transect (replicated twice, resulting in a sample size of N=40 per size class) was then laid within the same Sahara mustard infestation. The plots were placed in sandy, gravelly loam soils. Twenty random plants, chosen using a random number chart, per size class were labeled with a unique identification and measurements collected included height of plant, diameter of basal rosette, number of seed pods, number of seeds, seed weight, and seed size. Seed pods were collected prior to seed dispersal and placed into manila envelopes and labeled accordingly.

Many attempts at Sahara mustard growout to flowering and seedset were made (see hypothesis 5 results), however, they were unsuccessful and this hypothesis could not be completed.

HYPOTHESIS 4

Sahara mustard seeds were collected from mature dried plants in three densely infested areas at Lake Mead National Recreation Area over three growing seasons: April 23, 2008 from Katherine Landing; April 22, 2009 from Sandy Cove; and May 25, 2010 from Overton Beach. The seed pods were collected into paper bags and kept at room temperature until the pods could be cleaned and seeds removed. The dried and fully mature seeds were then stored in plastic containers in darkness at room temperature until these germination tests were conducted.

The first germination test used seeds from the 2008 and 2009 collections and was conducted between April 1, 2010 and June 4, 2010; resulting in 1 year (2009) and 2 year old seed (2008) being tested. The second germination test incorporated 2010 seeds as well as the 2008 and 2009 season seed and was conducted between October 21, 2010 and February 15, 2011; resulting in 1 year (2010), 2 year (2009), and 3 year old seed (2008) being tested.

The experiment used a Percival I-36 Series Germination Chamber for maximum control. Sahara mustard seeds were randomly pulled from a container from each corresponding growing season (2008, 2009, and 2010). There were 225 seeds germinated per treatment per year. The seeds for each year were subjected to six germination treatments: 25°/15°C, 20°/12°C, or 15°/9°C alternating temperatures, each under 12 hour light/dark or total dark conditions. In the light/dark treatments, the higher temperature corresponded with the light period. Twenty-five seeds were placed in a petri dish (100 X 15 mm) with one #1 Whatman type filter paper (90 mm) pre-

moistened with 4 ml of distilled water. Additional water was added when needed to keep the seeds moist. Each treatment was conducted nine times per year tested per germination test. Seeds were removed from the petri dish once they germinated as determined by visible radical protrusion (Figure 6). The number of seeds that germinated each day was recorded on data sheets, and testing was terminated after nine days for all treatments.

Data were analyzed using a two-factor analysis of variance with SAS (SAS, 2001), consisting of three levels of temperatures (25°/15°C, 20°/12°C, and 15°/9°C) and two levels of light (dark and light/dark).

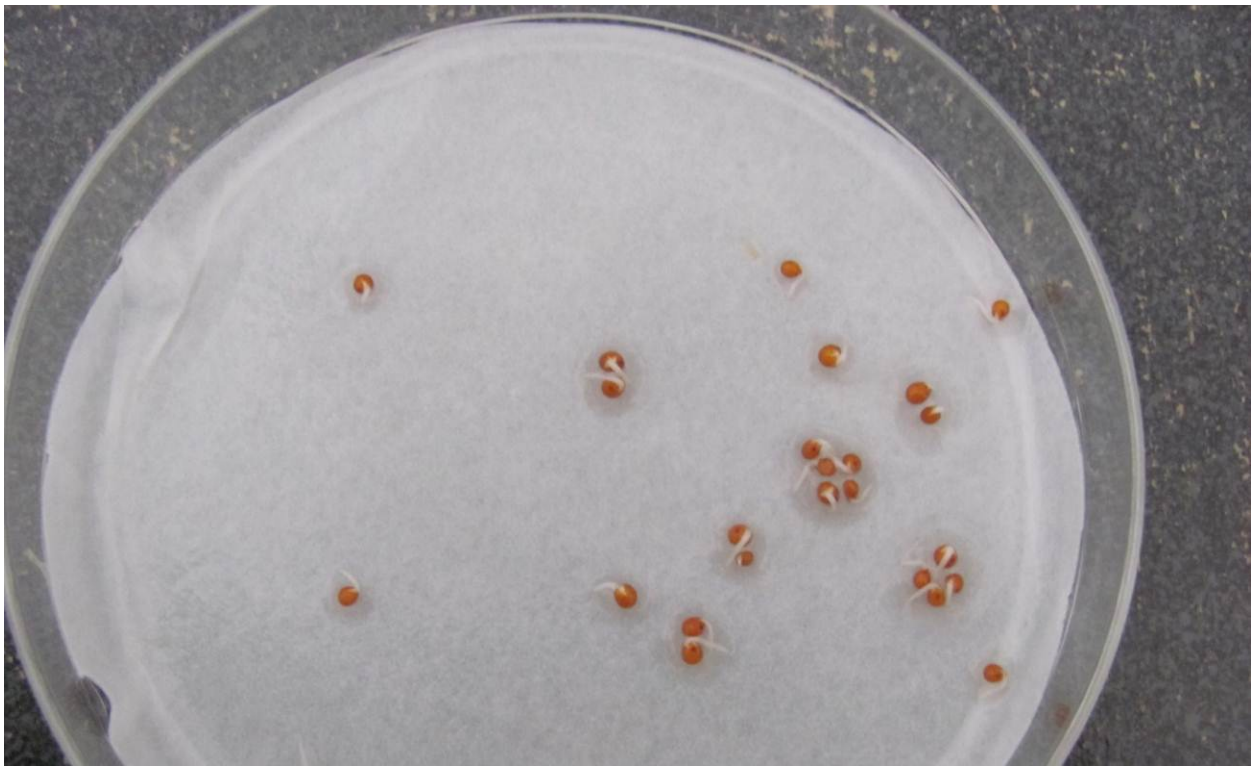


Figure 6. Viable Sahara mustard seeds with radicle protrusion

HYPOTHESIS 5

A total of 80 Sahara mustard plants were propagated from previously collected seed. Many combinations of soil mixtures, watering regimes, and container size were used to attempt to grow Sahara mustard to maturity, but all were unsuccessful. The soil was a commercially-available decorative sand and cactus mix, using either 50% sand/50% cactus mix, 100% sand, or 75% sand/25% cactus mix. The watering regimes tried were misting 3 times per day for 2 minutes; 2 times per day for 1 minute; 3 times per day for 1 minute; and 2 times per day for 5 minutes. The container sizes used in this experiment were 5 gallon pots, 1 gallon pots, and books (1 square inch wide by 4 inches deep) which were then transplanted into 1 gallon pots once the plants grew their first leaves. Due to the inability to grow Sahara mustard plants successfully to maturity in a controlled greenhouse setting this hypothesis was not completed (see hypothesis 5 results).

HYPOTHESIS 6

Four study sites containing Sahara mustard infestations about 1-2 hectare in size were identified in Lake Mead National Recreation Area. Each site supported areas of sandy and alluvial soils that contained creosote/white bursage plant communities. We established a 100 × 100 m (1 ha) plot at each site spanning the soil types. To assess Sahara mustard seed longevity in the soil and potential influences on longevity, we tested for the effects of soil type (sandy and alluvial), microhabitat (interspace and undershrub), burial depth (2, 5, and 15 cm), and time since burial (6, 12, 18, and 21 months).

Within each 1-ha plot, seed packets containing about 100 Sahara mustard seeds mixed in 160 ml of sterile soil were buried at the appropriate depths in randomly selected open and undershrub microhabitats (directly below the center of canopies of either creosote or bursage). Microhabitats were selected based on randomly selecting coordinates within plots and locating the nearest microhabitat to the coordinate. Seed packets were housed in cages to prevent seed removal by rodents.

A germination assay was conducted to evaluate what proportion of seeds placed in packets were germinable at burial time zero. Germinability was assessed by placing seeds on filter paper (#1 Whatman type filter paper, 90 mm) moistened with distilled water in a laboratory under ambient lighting, maintaining the filter paper moist by adding additional water, and allowing up to four weeks for germination. Radicle emergence was used to indicate germination. Of 720 seeds used in the assay, 712 (99%) germinated, so for our analyses we assumed that all seeds were germinable and that this was constant across treatments because seeds were randomly allocated to treatments.

Sahara mustard seeds were buried in February 2010 and packets were exhumed after 6 months (August 2010), 12 months (February 2011), 18 months (August 2011), and 21 months (November 2011). Exhumed seeds were recovered from the soil in seed packets with the aid of 1-2 mm mesh sieves. Recovered seeds were placed in Petri dishes (100 × 15 mm) with moistened blotter paper on a lab bench to test for germinability for up to four weeks using the same procedure as for the time zero assay.

There was one seed packet that for an unknown reason was not able to be recovered in the field. This packet was for site 2, alluvial soil, open microhabitat, 15 cm burial depth, on the 6-month date. This observation simply was treated as ‘missing’ in analyses.

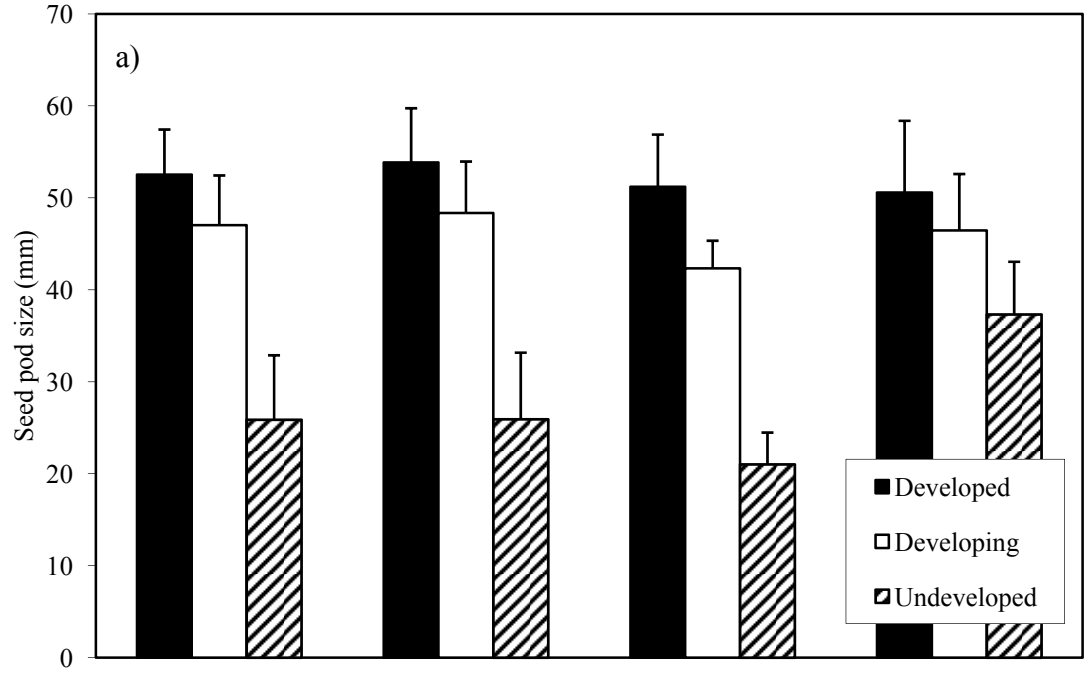
For statistical analysis, the response variable was the percent of germinable seed out of the original seed placed in packets for each date. This percent germinable seed was arcsine transformed for analysis. To reflect the repeated measurement and nested design, a repeated measures analysis of variance, including burial date as a repeated measure and site and soil type (and their interactions with microhabitat, burial depth, and time) as random effects, was used to test the influences on seed persistence of soil type, microhabitat, burial depth, and time (and all interactions). The residuals were subjected to a normal probability plot to assess the distribution of the residuals. SAS software was used to conduct the analyses (SAS, 2001). For the reporting of means and standard deviations, the untransformed percent of germinable seed remaining is used for ease of interpretation.

RESULTS

HYPOTHESIS 1

Seed pod size and seed size and germination varied with developmental stage and treatment (Figs. 7, 8). Seed pods in the undeveloped stage in any treatment except for the control were only about half the size of developed seed pods (Fig. 7a). The control, however, contained undeveloped seed pods about twice as large as treated plants. Trends were similar for seed size (Fig. 7b). Breaking and pulling had little effect on the germination percentage of developed or developing seeds, but these treatments did reduce germination of undeveloped seeds by about 3-5-fold compared to the control (Fig. 8). Seed pod removal reduced the germination of developed seeds by about half, and entirely eliminated germination of developing and undeveloped seeds.

These data were analyzed statistically as a required project deliverable. Seed stage and treatment interacted significantly ($F = 27.43$, $P < 0.0001$), so multiple comparisons of means were performed on the interaction term. Developing and undeveloped seeds in the removal treatment showed statistically significantly lower germination than all other stage × treatment combinations.


a)
Seed pod size (mm)
70
60
50
40
30
20
10
0
Developed
Developing
Undeveloped

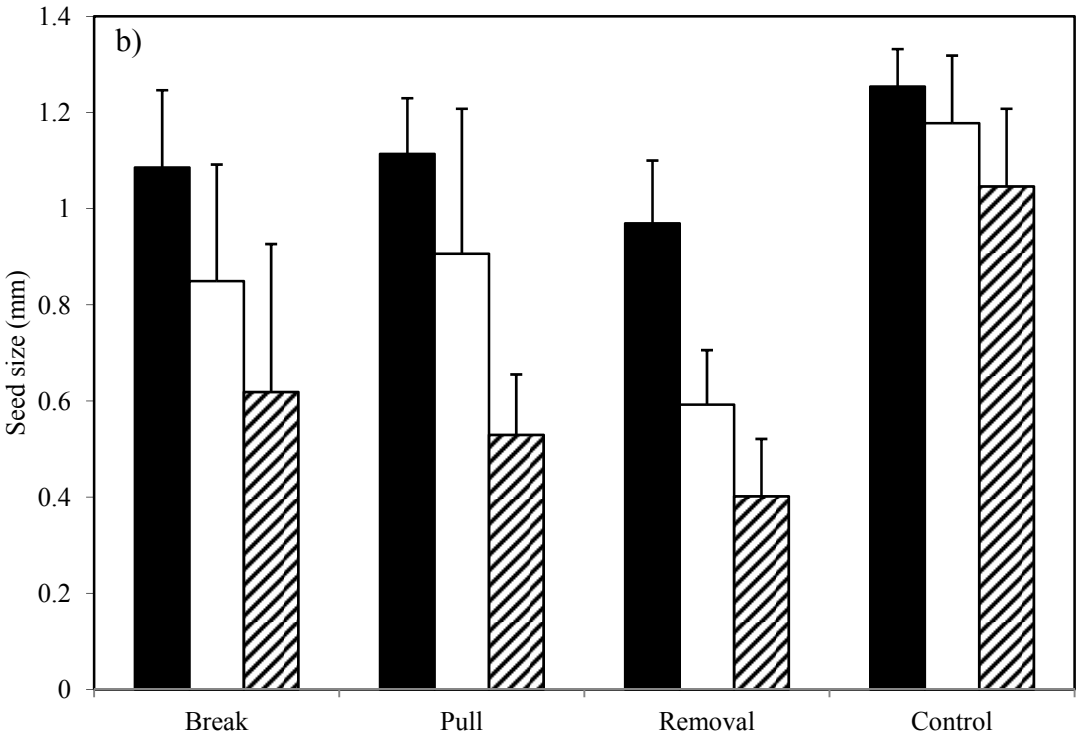

b)
Seed size (mm)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Break
Pull
Removal
Control

Figure 7. Treatment and seed development effects on Sahara mustard a) seed pod size and b) seed size. Values are means and the error bars represent 1 standard deviation.

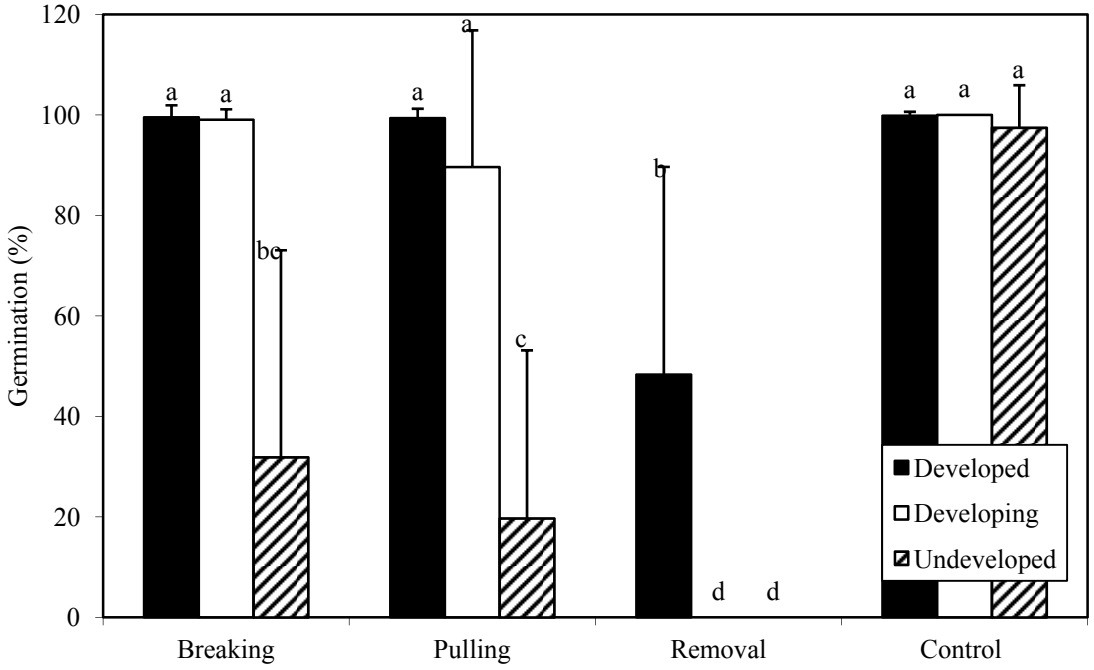


Figure 8. Treatment and seed development effects on Sahara mustard germination. Values are means and the error bars represent 1 standard deviation. Means without shared letters differ at P < 0.05.

HYPOTHESIS 2

All three tested herbicides in this experiment inhibited almost all seed germination in all three stages as shown in Figure 9. The control treatment showed the highest germination rate for all of the three developmental stages; stage 1 germinated at 86%, stage 2 and 3 at 94%. The three other treatments which involved herbicide applications showed virtually no germination, except for the 2, 4-D application on stage 3 siliques at 1%.

Three weeks after herbicide treatment, the seeds were shriveled and did not fully develop (Figure 10, Figure 11, and Figure 12A), as opposed to control seeds which received no herbicide treatment (Figure 12B). Control seeds were lighter in color and very spherical compared to herbicide treated seeds. Control seed germination can be seen in Figure 13. Figure 14 shows herbicide treatment effects on siliques for all three stages.

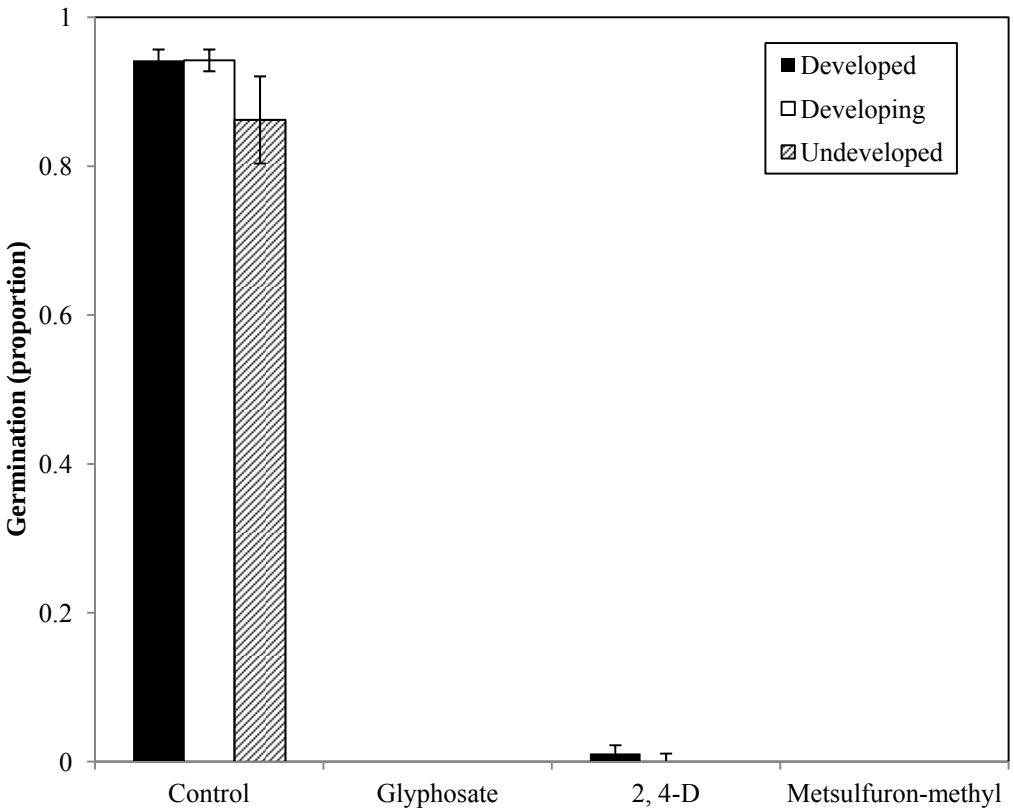


Figure 9. Percentage of total seeds germinated for each treatment per developmental stage. Mean (± 90% CI) of Sahara mustard seed germination, N=120.

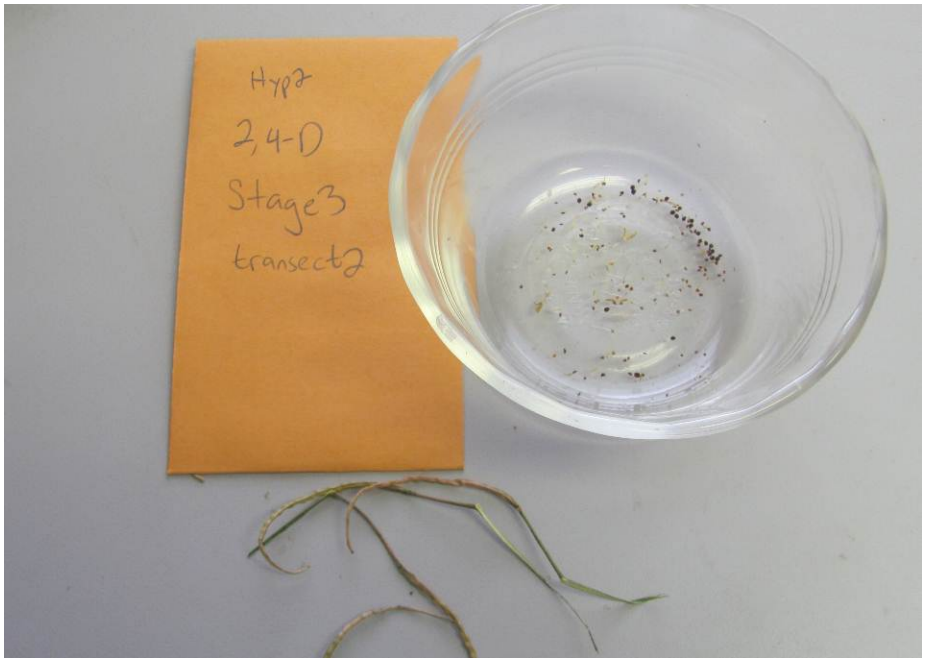


Figure 10. Stage 3 seeds sprayed three weeks prior with 2, 4-D. The herbicide effected the seed development which in turn decreased seed viability.

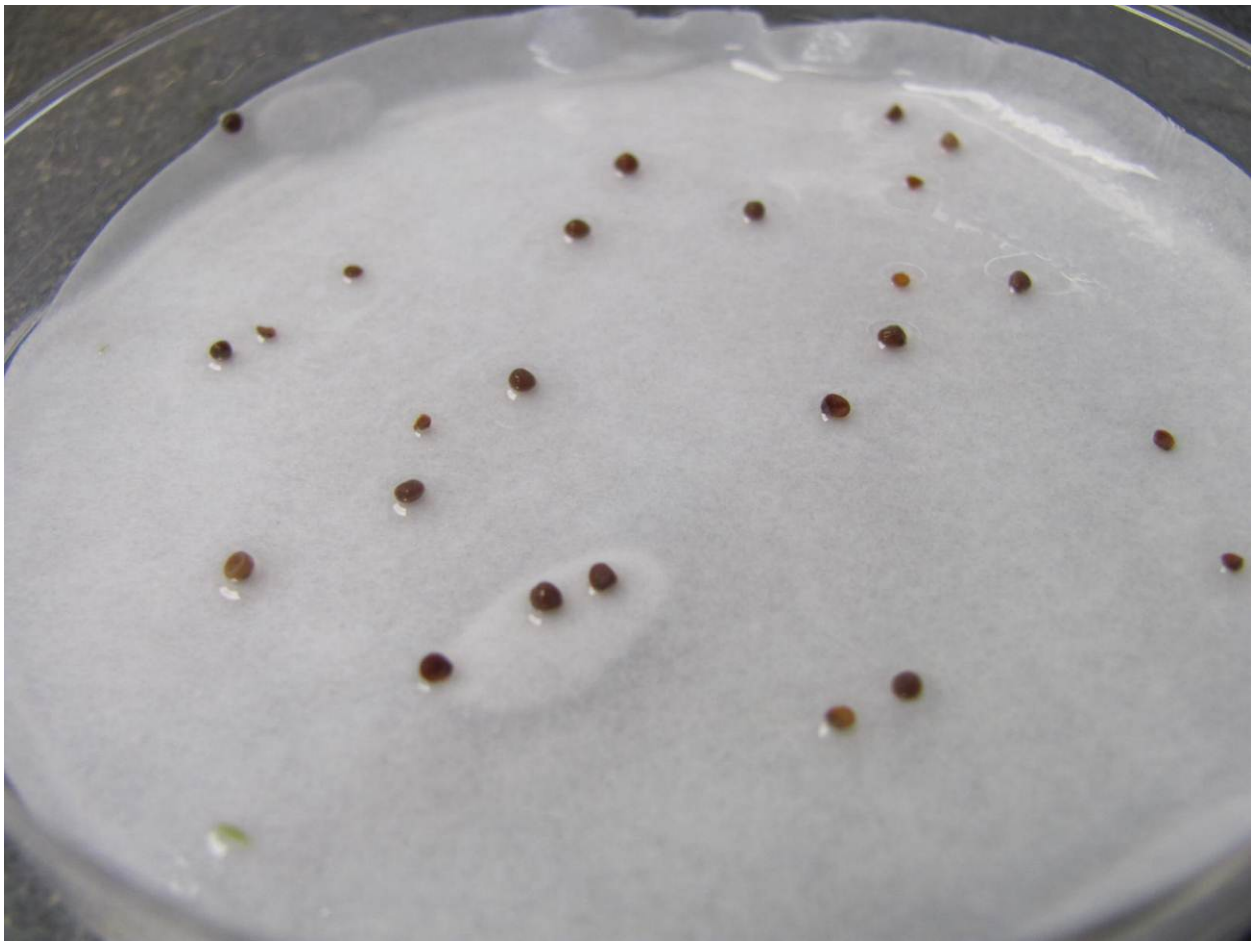
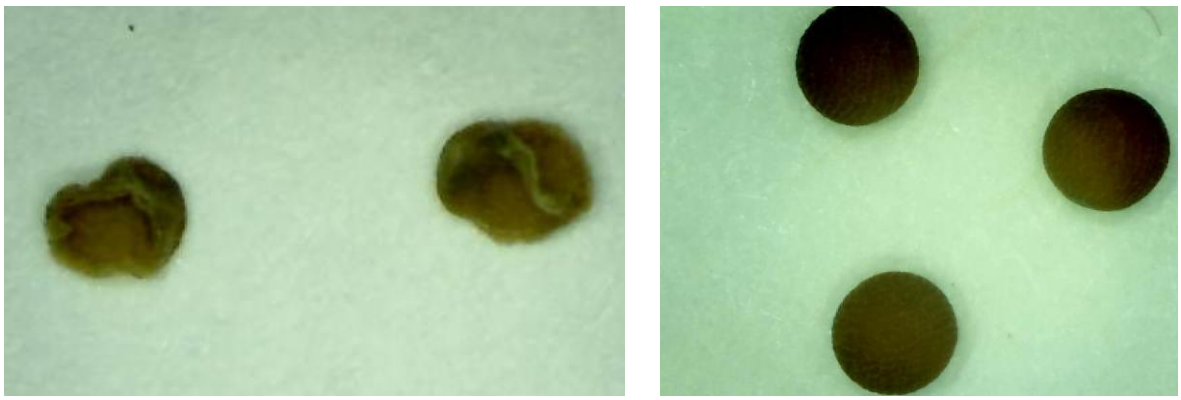


Figure 11. Typical stage 3 seeds after herbicide treatment, Metsulfuron methyl, in petri dish.



A B

Figure 12. Enlarged view of Sahara mustard seeds. A-Typical stage 3 seeds, herbicide treatment. B-Typical stage 3 seeds, control treatment.

Figure 13. Typical control treatment, showing radicle protrusion which indicated seed viability.

A

B

C

Figure 14. Typical Sahara mustard siliques three weeks after any of the herbicide treatments. A-Stage 3 siliques, B-Stage 2 siliques, C-Stage 1 siliques.

HYPOTHESIS 3

We were unable to grow Sahara mustard plants in a greenhouse (see hypothesis 5 results) so there are no results to report on correlating genetic variation between small and large Sahara mustard plants, but useful information on seed characteristics was gained. The size classes small, medium, and large were designated by height. Small plants ranged from 0-52.8 cm, medium plants ranged from 52.9-83.2 cm, and large plants were anything over 83.3 cm. The sample size started out with N=40, however field herbivory decreased the sample size down to N=26 for small plants, N=36 for medium plants, and N=38 for large plants. Figure 15 below shows the difference for average number of seeds per plant, seed pods per plant, and seed size between the small, medium, and large plants.

Size Class		Seed pods/plant	Seeds/plant	Seed size (mm)
Small	Min.	0	0	0
N=26	Max.	471	10362	1.39
	Avg.	23	425	0.6035
Medium	Min.	0	0	0
N=36	Max.	3090	64890	1.31
	Avg.	289	5718	1.06475
Large	Min.	0	0	0
N=38	Max.	6794	169850	1.28
	Avg.	1375	30380	1.1085

Figure 15. Minimum, maximum, and averages for 3 size classes of Sahara mustard for number of pods per plant, number of seeds per plant, and seed size.

The number of seeds per plant ranged from zero to 10,362 for the small size class. On average the number of seeds in a seed pod ranged 13-25 within the three size classes. These average numbers were extrapolated out with the number of pods per plant to equal the average number of seeds found on a plant. The largest Sahara mustard plant had an estimated 169,850 seeds, with 6,794 pods on that one plant.

HYPOTHESIS 4

Sahara mustard seeds germinated best under all dark conditions and at temperatures ranging from a high of 15° to 25° C to a low of 9° to 15° C. One year seeds germinated at a rate of 96-100% in total darkness; two year seeds germinated at a rate of 88-100% in total darkness; and three year seeds germinated at a rate of 82-99% in total darkness. Introducing 12 hours of light to each treatment had a significant effect on seed germination. At 15°/9° C and 20°/12° C the light/dark treatments resulted in 0-2% germination rate for all three years. As the temperature increased to 25°/15° C the light/dark treatment increased the germination rate to a range of 10-46%.

Figure 16 represents one and two year old Sahara mustard seeds germinated between April and May 2010. Figure 17 represents one, two and three year old seed germinated between October 2010 and February 2011. No seed dormancy was evident, and even three year old seeds retained high viability.

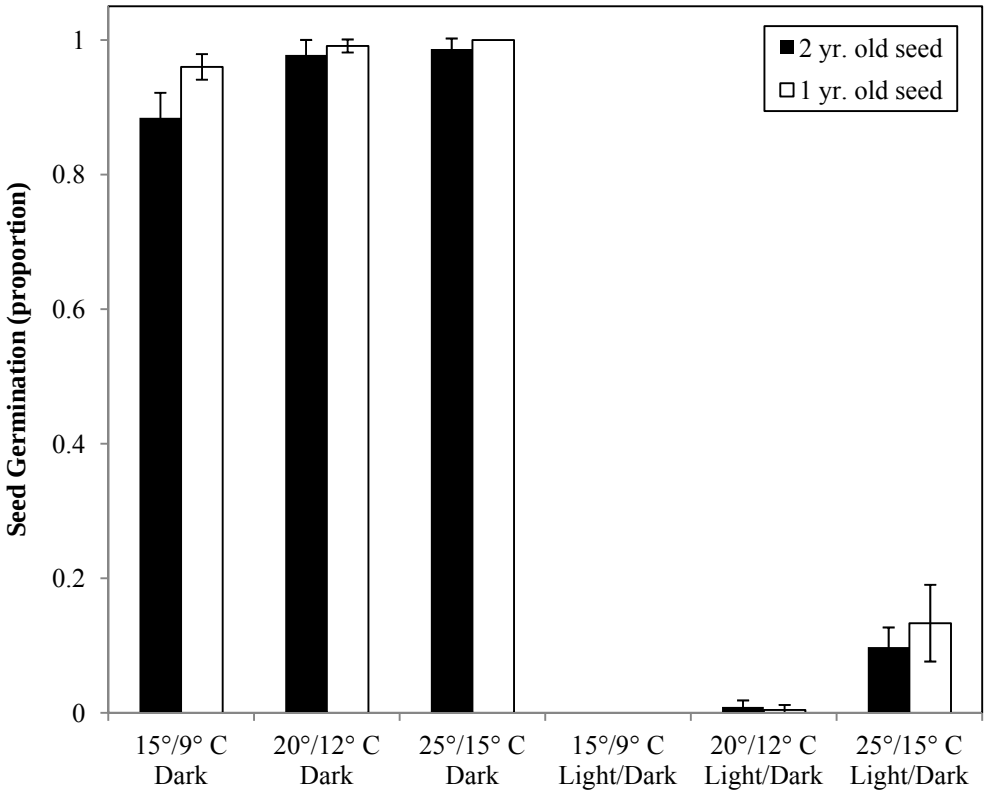


Figure 16. Effect of temperature and light on germination of Sahara mustard seeds. Mean (± 90% CI) of Sahara mustard seeds that germinated at different temperatures, N=9.

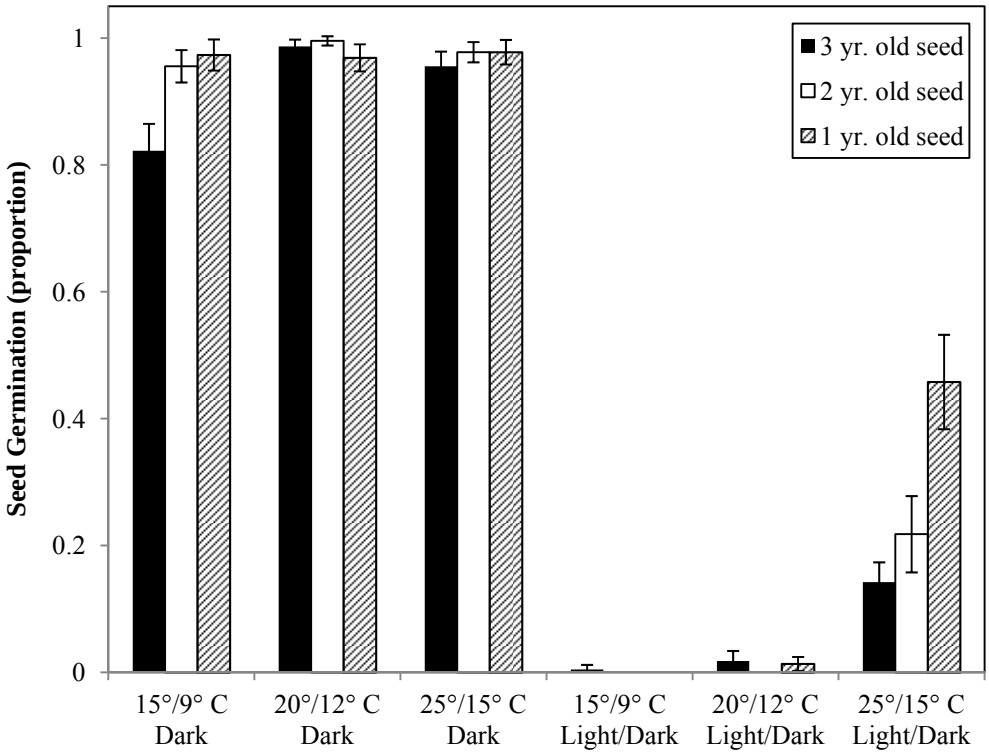


Figure 17. Effect of temperature and light on germination of Sahara mustard seeds. Mean (± 90% CI) of Sahara mustard seeds that germinated at different temperatures, N=9.

HYPOTHESIS 5

Many combinations of soil mixtures, watering regimes, and container size were used to grow Sahara mustard to maturity, but all were unsuccessful. Plants germinated to just past the cotyledon stage, but usually declined and died within 6 weeks of germination. The greenhouses may have been too hot for the Sahara mustard seedlings, as the Lake Mead Nursery recorded temperatures for the greenhouses during our growing out process were well above normal growing conditions. This idea seems to be supported from the summary information below. The batch started in November 2010 progressed the farthest, which was also the time when ambient greenhouse temperatures were lowest.

- February 22, 2010 – all seedlings dead within four weeks
- March 22, 2010 – all seedlings dead within six weeks
- November 15, 2010 – seedlings progressed to rosette stage, some bolted, but all died after 90 days. None progressed to flowering stage.
- March 24, 2011 – all seedlings dead within six weeks
- May 4, 2011 – not doing well after three weeks

Figure 18. Sahara mustard plants growing in books at the nursery.

Figure 19. Five Sahara mustard seedlings planted in a 5 gallon pot.

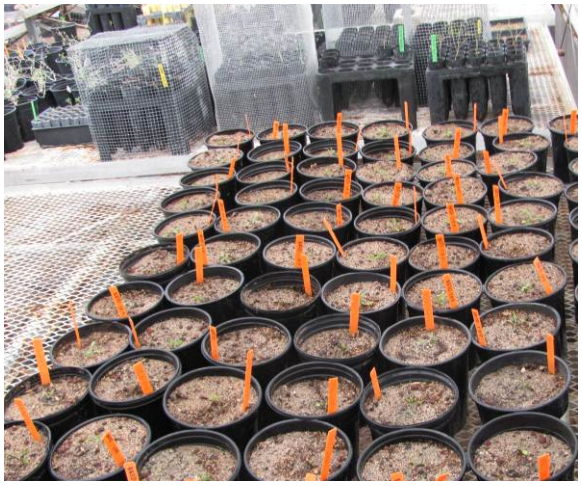


Figure 20. Sahara mustard growing in 1 gallon pots.

HYPOTHESIS 6

There were several interactions in the data, including a four-way interaction of soil type, microhabitat, burial depth, and time (Figure 21). Statistically, therefore, data should be presented on the basis of this four-way interaction, which we show in Figure 22 for the mean percent (and standard deviation) of germinable seed remaining as a function of each of the interacting treatment factors. Because the main effects have important biological meaning, we also discuss main effects and lower-order interactions.

The importance of the treatment effects varied. One of the most important lower-order interactions was a significant depth by time interaction, where fewer germinable seeds remained at shallower burial depths over time compared to deeper burial depths. For example, fewer than 6% of seeds were left at the 2-cm depth at 18 months across microhabitats, compared to 51-90% of seeds remaining at a 15-cm depth. Depth and time were the most important main effects influencing seed remaining. In contrast, soil type and microhabitat were not significant main effects.

Effect	Num DF	Den DF	F Value	Pr > F
Soil	1	6	2.36	0.1756
Microsite	1	6	1.74	0.235
Soil*Microsite	1	6	1.34	0.2905
Depth	2	24	68.29	<.0001
Depth*Soil	2	24	1.72	0.1997
Depth*Microsite	2	24	1.97	0.1619
Depth*Soil*Microsite	2	24	0.09	0.9127
Time	3	36	50.56	<.0001
Soil*Time	3	36	2.49	0.0761
Microsite*Time	3	36	3.29	0.0317
Soil*Microsite*Time	3	36	0.2	0.8924
Depth*Time	6	71	8.25	<.0001
Depth*Soil*Time	6	71	0.23	0.9641
Depth*Microsite*Time	6	71	5.14	0.0002
Dept*Soil*Micro*Time	6	71	2.34	0.0401

Figure 21. Influences of treatment factors on Sahara mustard seed longevity in soil.

Average of % seed remaining

			Time month			
Soil type	**Microsite**	**Depth (cm)**	**6**	**12**	**18**	**21**
Alluvial	Open	2	28	0	2	2
		5	37	29	35	32
		15	81	72	67	62
	Shrub	2	32	1	3	1
		5	69	13	13	11
		15	80	75	84	82
Sandy	Open	2	42	2	14	9
		5	73	35	51	41
		15	79	75	51	50
	Shrub	2	74	14	6	4
		5	88	55	36	31
		15	96	75	90	83

StdDev of % germination

			Time month			
Soil type	**Microsite**	**Depth (cm)**	**6**	**12**	**18**	**21**
Alluvial	Open	2	13	0	2	3
		5	31	20	30	26
		15	5	20	18	15
	Shrub	2	17	1	3	1
		5	7	18	17	17
		15	7	33	7	9
Sandy	Open	2	21	2	5	5
		5	32	24	38	30
		15	22	29	33	33
	Shrub	2	17	14	4	4
		5	13	46	41	42
		15	3	38	5	16

Figure 22. Average percent of germinable seed remaining for seed of Sahara mustard buried in the soil as a function of multiple treatment factors over time. Standard deviations (1 SD, in %) are provided below the means.

DISCUSSION

Many effects on seed germination were investigated; various herbicide applications, various mechanical treatments, effects of light and temperature, and burial at several depths, with important implications for the management of Sahara mustard. This study has expanded the window of time for effective control Sahara mustard, which can result in reducing the future seed bank, and allowing biomass to remain on site. Although hand-pulling and bagging for removal is the most common method of control at Lake Mead NRA it is inadequate and expensive for managing large-scale invasions. Bagging and removing whole plants may also remove substantial nutrients from a system that are not replaced. Treatments are most effective before Sahara mustard seeds are developed, but this is not always possible because multiple seed stages are often present within the same stand of plants. Breaking off and pulling whole plants is somewhat effective in reducing seed viability but only at the earliest stage of seed development. Treatments effective at reducing seed viability at all phenologic stages are needed for management of this invasive plant.

HYPOTHESIS 1

These results have important implications for the management of Sahara mustard. According to (Brooks, Draper, & Trader, 2006), who studied Sahara mustard populations in the western Mojave Desert, individual Sahara mustard plants produce on average 1,000 seeds/plant and the largest plants can produce over 15,000 seeds per plant. (See Hypothesis 3 for seeds counts substantially higher.) This means that giving consideration to seed numbers, seed viability, and methods to mitigate them, is essential to any management strategy for this species.

The results show that the different treatments (breaking, pulling, or removing seed pods) have variable results on the germination of Sahara mustard seeds. All treatments reduced the germination of undeveloped seeds, but the amount of the reduction varied among treatments. As seeds became more developed, however, treatments effectiveness was reduced. For developed seeds, breaking and pulling had no effect on germination while seed pod removal only reduced germination by about half. Overall, seed pod removal was the most effective treatment for reducing germination; removing seed pods completely eliminated germination for developing and undeveloped seeds. However, removing seed pods from individual plants is not likely to be a practical management strategy. If plants have developed seeds, then treatments would need to remove entire plants from the site or develop techniques (such as using herbicide) to treat plants that will prevent germination. Strategies such as burning the plants to kill seeds or carefully bagging and removing the plants could be used.

If possible, treatments should be conducted before Sahara mustard seeds are developed. This is not always possible because multiple seed stages are often present within the same stand of plants, but timing treatments prior to seed maturity is likely to be most effective.

HYPOTHESIS 2

Little to no research on herbicide effects on seed development and seed viability of Sahara mustard has been conducted; previous studies have focused on herbicide effects on the plants themselves. This study indicates a larger window of time is available to effectively control Sahara mustard, reduce the future seed bank, and allow biomass to remain on site. Integrating

herbicide use with mechanical control methods may also reduce safety related problems that arise from continuous hand-pulling actions.

Although hand-pulling is the most common method of control it is inadequate and expensive for managing large-scale invasions. Herbicides are consistently more cost-effective than hand-pulling but their use in desert wildlands has been limited by concerns about impacts on sensitive species (Murshia, Cadotte, & Holt, 2010; Brooks, Draper, & Trader, 2006). Herbicides disturb the soil less than hand-pulling and soil disturbance often promotes invasive exotic plants. Hand weeding can encourage rather than discourage some exotic species (Murshia, Cadotte, & Holt, 2010).

In Brooks, Draper, & Trader (2006), there was no significant difference between an applied 1.6% glyphosate solution and mechanical removal in controlling plants at the late rosette to early bolting stage. Typical herbicide control expenses include herbicide purchase, training and certification of applicators, and backpack sprayer and personal protective equipment purchase. Mechanical methods require only a semi-skilled labor source and no special equipment except garden hoes. Brooks (2006) noted that treating 10 acres using herbicide methods cost $1694.40 compared to $3333 for mechanical treatment (hoeing), factoring in labor hours, equipment and herbicide expenses. Labor hours were significantly different between methods; 24 hours per person per acre for mechanical treatment, versus 8 hours per person per acre for herbicide treatment (Brooks, Draper, & Trader, 2006). Even though herbicide use requires more expensive skilled labor, material and equipment, the cost per acre can be less than mechanical treatments.

Evidence of herbicide resistance for two of the three herbicides used in this experiment, glyphosate and metsulfuron methyl has been found (Boutsalis, Karotam, & Powles, 1999). Sahara mustard has also been found to be resistant to ALS inhibitors in Australia (Bowran & Gill, 2000). However, Jander, et al. (2003) mentions resistance to glyphosate is less frequent than resistance to sulfonylurea (i.e. metsulfuron methyl) and imidazolinone herbicides. Jander also noted that as of 2003, 261 herbicide-resistant weed biotypes, involving at least 17 different herbicide modes of action, have been found. Best management practices should alternate methods of control each year or season to prevent Sahara mustard from becoming resistant to any one particular herbicide. Herbicides should employ different modes of action, and mechanical removal should be included as one of the alternating methods.

Sahara mustard is invading and impacting biological soil crust habitats and possibly the sensitive species associated with them, but no studies addressing long-term herbicide impact on biological soil crusts are known. Two different glyphosate herbicides were applied on moss-dominated biological soil crusts which resulted in no short-term negative impact on bryophyte cover (Youtie, Ponzetti, & Salzer, 1999). Mature stage Sahara mustard treatments may cause more non-target herbicide applications to soil crusts than rosette stage treatments because of higher potential for drift. A long-term (at least three years post-treatment) soil crust monitoring protocol should be implemented if herbicides are used in these biologically sensitive areas.

HYPOTHESIS 3

Although Hypothesis 3 could not be completed, we did discover that average numbers of seeds per plant are high, and that a single plant produced almost 170,000 seeds. This strongly emphasizes the need to reduce the soil seed bank as much as possible, as neglected control efforts could quickly be reversed and wasted. Reduced competition from other Sahara mustard plants could allow remaining plants to become very large and produce huge numbers of seed. If these remaining plants are herbicide treatment survivors, all seed produced would overwhelm other susceptible genotypes, creating optimum conditions for herbicide resistance to develop.

HYPOTHESIS 4

Multiple studies on optimum germination rates and conditions for Sahara mustard have been conducted, and except for Chauhan, Gill, & Preston (2006), with similar results: Sahara mustard germination is inhibited by light. An experiment done by Delipetrou, Georghiou, & Thanos (1993) showed Sahara mustard seed germination was inhibited by light, with an optimum temperature range of 15°-25° C and a germination rate of >90%. Another experiment by Thanos, Georghiou, Douma, & Marangaki (1991) also showed optimum germination temperatures for Sahara mustard at 15°-25° C, with a photoperiod of 11 hours light and 13 hours dark. After 1 week, their all dark treatments plateaued at 80% germination, and their light/dark treatments plateaued at approximately 10% after 1 to 2 weeks. Their seeds all germinated within several days, and they found that Sahara mustard germination was sharply suppressed above 20° C. This study's photoperiod was 12 hours of each light and dark, with results very similar to their 20/12° C light/dark and all dark treatments. Bangle, Walker, & Powell (2008) showed that optimum germination temperatures for Sahara mustard were 16°-28° C. This resembles this study's results and the two previously mentioned.

Sahara mustard seeds collected in Australia were not influenced by light conditions at the optimum temperature of 20/12° C, however, seed germination was inhibited by light at lower temperatures of 15/9° C (Chauhan, Gill, & Preston, 2006). Our results showed an inhibition due to light at these same temperatures.

Some literature shows conflicting results regarding Sahara mustard seed dormancy. Cousens, Baweja, Vaths, & Schofield (2006) showed one to two year old Sahara mustard seeds did not germinate in water alone, but required pre-rinsing in 1% sodium hypochlorite to induce germination. Batanouny (1974) used gibberellic acid to break seed dormancy and obtained up to 100% germination, but experienced very low germination with water alone: 18% in dark and 2.5% in light at 25 ° C. Washing the seeds with water or treating them with sulphuric acid had no significant effect on germination in either light or dark, however, alternating temperature promoted the germination to 31% in dark and 12% in light. These results were not consistent with the results of this experiment. Our seeds did not show dormancy, and germinated within four days for all the dark treatments. Cousens, Baweja, Vaths, & Schofield (2006) indicated that seeds were collected and stored at room temperature, much the same way our seeds were treated. Batanouny (1974) did not elaborate on either seed storage methods, or seed age at germination trials, but suggests that alternating temperatures would promote germination. Germination methods in this study used alternating temperatures, which may explain why no evidence of seed dormancy was found.

Increased germination rates in light under higher temperatures suggest that Sahara mustard could technically demonstrate summer recruitment; however, this is highly unlikely due to extremely high temperatures in southern Nevada during the flowering and fruiting stages of the plant. Sahara mustard plants have been reported growing at Lake Mead National Recreation Area in the rosette stage during the summer season, but they have never been seen bolting or flowering (Norman, C., personal observation). The high temperatures in southern Nevada during this time may be enough to wilt succulent stems and desiccate pollen, preventing flowers or fruits from setting or maturing.

HYPOTHESIS 5

Although hypothesis 5 could not be completed, we did discover that there are many variables effecting the growing out process of Sahara mustard in a controlled greenhouse setting, such as light conditions, air and soil temperature, moisture, and container size. As stated in the Results section, ambient air and soil temperatures may have been too high for success. Unfortunately, low temperatures that may be conducive to Sahara mustard growth and maturation may also be too low for many other plant species to tolerate without inducing dormancy. Growing Sahara mustard out to maturity may require dedicating an entire greenhouse to them, which may not be practical or economically feasible to do.

HYPOTHESIS 6

Results provide new knowledge on the basic biology of Sahara mustard and have important implications for the management of this species. The results suggest that Sarah mustard seed banks are short lived in the upper 2 cm of soils, with commonly less than 5% (and sometimes 0%) of buried seeds remaining after 12 months. This upper surface soil layer is likely the most biologically important in undisturbed systems, as Guo, 1998 reported that 91% of viable seeds of Mojave Desert soil seed banks occur within the upper 2 cm of the soil. Moreover, in a greenhouse experiment, Abella (2011) found that Sahara mustard has limited ability to emerge when buried at depths below 2 cm. Emergence was 50% at the soil surface, 10% when seeds were buried at 2 cm, and < 10% for burial depths greater than 5 cm. Unless deeper soils are brought to the surface, such as by anthropogenic disturbance or biopedturbation by animals (Whitford, 1999)), upper soils are the soils critical for providing propagules for Sahara mustard recruitment.

As burial depth increased, Sahara mustard seed persistence increased. This effect persisted through time. The observed persistence at deeper depths could result from several factors. For example, seeds deeper in soils may have been better protected from the elements, such as by experiencing less dramatic temperature fluctuations, or were not as exposed to decomposition by soil microbes. Additionally, burial at the deeper depths may have induced seed dormancy, which would serve to curtail germination deep in the soil to result in seed death and seed bank depletion. If this mechanism occurred, then Sahara mustard would differ from some other annual weeds infesting southwestern arid lands. For example, Gleichsner (1989) found that ripgut brome (*Bromus rigidus*) readily germinated at deep burial depths (up to 30 cm depths tested), thus killing the seeds.

We were surprised that microhabitat (below shrub versus interspace) had minimal influence on Sahara mustard seed bank persistence. We had anticipated that below-shrub microhabitats might

better protect seeds than would interspaces, thus allowing seed banks to persist longer below shrubs. On the other hand, decomposition might be more rapid in the more organic-rich shrub microhabitat, which could hasten seed decomposition. Apparently these factors either averaged out differences between microhabitats or other factors resulted in little difference in seed persistence between microhabitats. However, the no difference in seed persistence between microhabitats is consistent with the observation that Sahara mustard can invade interspaces as well as below-shrub microhabitats (Barrows et al., 2009).

The relatively rapid decay of Sahara mustard seed banks in upper soils within 12-18 months provides information for management. These results strongly suggest that if the seed production of Sahara mustard stands can be reduced or eliminated through management, then soil seed banks will become depleted. The problem for management, however, is that even under good control of Sahara mustard where the majority of plants are killed, seed production of even a few Sahara mustard plants is so prodigious as to be capable of replenishing soil seed banks (Trader et al., 2006). This is consistent with the common observation by managers that several years of control are needed to achieve reductions in Sahara mustard (A.C. Newton, C. Norman, personal observations). Nevertheless, if seed production is reduced this should directly translate to reduced soil seed banks as seed banks become depleted over time.

Results also highlight another important consideration: if Sahara mustard seeds become buried, disturbances that bring this soil back to the surface might facilitate Sahara mustard establishment. Apparently burial prolongs seed persistence, as even after 18 months seeds buried at 15 cm remained germinable. This observation suggests caution in situations such as roadside construction that might result in the piling, moving, and subsequent redistribution of soil.

CONCLUSION

The treatments of breaking and pulling plants are only partially effective for reducing germination of seed, reducing only the germination of undeveloped seed. The germination of developed and developing seed did not differ significantly from the controls. Seed pod removal was the most effective treatment, eliminating seed germination entirely for plants with developing/undeveloped seeds, and reduced germination of developed seeds by half. However, this control method is impractical for large-scale implementation.

Herbicide can be used effectively on Sahara mustard in all stages of development (rosette, bolting, flowering, and early to mature fruiting) to prevent seed development and viability, and to reduce the future soil seed bank. Mechanical removal of fruiting plants is not only more expensive, but also may generate excessive soil disturbance and removes biomass from the site. It is important to monitor any type of herbicide treatment to determine if the species may be developing resistance, but rotation of herbicide compounds with different modes of actions may prevent herbicide resistance from occurring.

Light does significantly inhibit Sahara mustard seed germination at temperatures below 25° C. We can infer that Sahara mustard seeds must be buried below the soil surface or covered in some manner in order to germinate in any quantity and that seeds remaining on the soil surface will not

germinate or will at very low rates. Soil seed banks of Sahara mustard appear short-lived at shallow soil depths (e.g., 2 cm), with commonly ≥ 90% depletion by 12-18 months of burial. Deeper burial depths increase seed persistence, but these deeper soils are unlikely to be important for Sahara mustard recruitment unless disturbance bring these soils closer to the surface. The factors of soil type (alluvial vs. sandy) and microhabitat (below shrub vs. interspace) were less important in influencing seed persistence than were burial depth and time.

RECOMMENDATIONS

Implementation plans with rotating treatment methods should combine different herbicide modes of action and mechanical methods. During drought years land managers may choose to utilize hand-pulling or other mechanical methods instead of herbicide because there should be fewer plants emerging, native or non-native. During an above average precipitation year land managers may choose to implement herbicide applications only in order to treat as many plants as possible.

Herbicides that utilize different modes of action than the ones used in this experiment should be applied to the same three seed developmental stages of Sahara mustard to determine how seed development and viability are affected. A wide array of effective herbicides will enable land managers to vary treatments so that the potential for Sahara mustard herbicide resistance is lowered. A long term monitoring protocol should be implemented post-herbicide treatment to determine herbicide resistance and any negative effects on native vegetation, such as a decrease in native species diversity or quantity of plants. If herbicide is applied in biologically sensitive soil crust areas then a monitoring protocol should be implemented at least for three years post-treatment to assess herbicide effects on biological soil crusts and mosses.

The timing of physical treatments (pulling and breaking plants) prior to seed development can help reduce viable Sahara mustard seed on targeted sites. If these treatments occur during seed development or after seeds have developed, entire plants should be removed from the site to prevent the dispersal of viable seed. A useful and practical topic for future work would be to test the effectiveness of different treatments (such as burning) for killing seeds on site without herbicides.

Land managers should reconsider the use of mechanical means such as hand-pulling or hoeing as options for control. Mechanically removing Sahara mustard plants creates a considerable amount of disturbance from foot prints, plant uprooting, and other actions. This may bring seeds deep in the ground close to the surface, and cover seeds that are on the surface, resulting in optimum conditions for germination next season.

Land managers should be prepared to commit considerable resources to control and eradication efforts in any particular site. High seed production rates among treatment survivors can reverse or negate previous years' work very quickly, resulting in wasted resources.

No treatments on dried mature Sahara mustard seed to determine if herbicides will penetrate the seed coat to prevent germination are known. Successful results would give land managers a longer window of effective treatment, perhaps extending to hanging seed banks.

Previous control methods included bagging all plants with mature seed pods and disposing of them in the landfill. However, seed viability of this material a few days after bagging has not been researched. If the seeds are still viable then this method should no longer be used because seeds are merely being transported to another location for potential infestation.

The 12 hour photoperiod and the temperatures used in this experiment were based on other research studies. A more suitable photoperiod and temperature regime that mimics Mohave Desert conditions at Lake Mead National Recreation Area should be tested in the future. Sahara mustard begins germination in December or January when day length is an average of 9 hours and 53 minutes. The average high temperatures during these two months are 18°-21° C, and 0°-4° C for the average low. Changing the photoperiod to 10 hours light and 14 hours dark, and using temperatures of 20° C high and 5° C low would mimic optimum growing conditions in low elevations in southern Nevada.

Choosing higher treatment temperatures and photoperiods that mimic summer conditions may allow land managers to determine whether Sahara mustard will germinate in the summer and how climate change may affect Sahara mustard invasions. During the months of May to September the typical average high/low temperatures at Lake Mead are 32°-46°/12°-27°, suggesting higher test temperatures of 32°/12° C, 39°/20° C, and 46°/27° C, with appropriate corresponding photoperiods.

The specific mechanisms underlying Sahara mustard's ability to persist at deeper depths warrants future research, as this might provide better insight into its germination ecology in the soil.

Understanding why there was little difference in seed persistence between below-shrub and interspace microhabitats might be valuable for understanding spatial relationships of recruitment of Sahara mustard and relationships with native vegetation.

While the percent of viable seed remaining was low after 12 months at shallow burial depths, the sheer amount of Sahara mustard seed in soil can make 1% persistence significant. For example, if initial seed banks are 1,000 seeds/m^2, even 1% remaining translates to 10 seeds/m^2, likely sufficient to produce at least one plant which in turn will produce prodigious seed. Investigation into the longer term persistence (beyond 21 months) of Sahara mustard seed banks is warranted. This would help understand the recruitment of this species during longer term droughts or cessation of management control actions.

Implementation of Hypotheses 3 and 5 experienced difficulties growing out Sahara mustard under controlled conditions. Much is unknown regarding growing Sahara mustard seeds to mature plants. Soil mixtures, watering cycles, pot size, and temperatures ideal for successful propagation of Sahara mustard seed should be further investigated if additional research is to occur.

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