

Title: Seed ecology of endemic winter annual species of the eastern Mojave Desert

Authors: Lesley A. DeFalco, Sara J. Scoles-Sciulla, and Alex Stosich

Affiliation: U.S. Geological Survey, Western Ecological Research Center, Boulder City Field Office, 500 Fir St., Boulder City, NV 89005

Abstract

Studies on seed ecology are paramount for the conservation of rare and sensitive plant species by providing basic biological information that can inform practical approaches to propagation and maintaining long term seed collections. Studies on the germination ecology of two endemic winter annuals of the eastern Mojave Desert were initiated to understand germination temperature requirements and seed dormancy and guide *ex situ* propagation techniques to manage species in habitat. We studied the seed type, dormancy strategy, soil seed bank dynamics and the mating system of *Eriogonum viscidulum* (sticky buckwheat), a narrowly distributed species that is associated with public lands around the confluence of the Muddy and Virgin rivers and south along Lake Mead. We initiated similar studies on *Astragalus triquetrus* var. *geyeri* (three-corner milkvetch), an endemic winter annual with a broader overlapping distribution, currently under review for federal protection under the Endangered Species Act. A prolonged drought in the region impacted our studies on this species in particular. Consistent with rare plant surveys conducted in the region, we found no *A. geyeri* var. *triquetrus* plants in habitat during 2021 and 2022 to acquire seed for study. We were also unable to propagate plants of this species from the seed bank because no seedlings emerged during a 9-mo greenhouse incubation of soils collected from habitat, and a subsample of soils that we sieved at the end of incubation did not contain any *A. geyeri* var. *triquetrus* seeds.

The drought also impacted our studies on *Eriogonum viscidulum*. Even though plants were rare (2020) or absent (2021, 2022) during our scouting trips, we raised plants in the greenhouse from soils collected from habitat to obtain seeds from propagated adults. Our studies on these seeds demonstrate that *Eriogonum viscidulum* is a facultative winter annual that naturally after-ripens with > 1 – 3 mo exposure to summer temperatures following dispersal, which shifted seeds with primary dormancy into nondormancy. As seen in our seed bank, seed burial, and germination trials, seeds were unable to germinate under extreme summer (≥ 30 °C) or winter (0 °C) temperatures. The ability of *E. viscidulum* seeds to germinate at broad temperatures between these extremes (≥ 10 °C, ≤ 25 °C) allows the species to emerge during autumn and winter months. Unexpectedly, the duration of exposure to cold, moist conditions for pretreatments and incubations during our germination trial (30 d – 80 d) and seed burial trial (December – March) did not induce secondary dormancy. More study is needed to confirm how *E. viscidulum* temperatures regulate dormancy during spring and summer months when the species is expected to re-enter the persistent seed bank.

Conservation seed collections of *Eriogonum viscidulum* can be made during spring (May – June) following winter months of adequate rainfall. Seeds collected directly from adult plants will be dormant and require warm, dry conditions for after-ripening prior to propagation; seed can remain nondormant under cold storage (5 °C). When plants are too rare or absent in habitat to make direct collections during drought, seed bank propagation may be a means to make conservation seed collections, particularly at historic sites without recent surveys but where a viable seed bank may persist.

Introduction

Winter annual plant species depend on favorable environmental cues to trigger seed germination and support seedling growth to reproduction so that seeds can replenish the soil seed bank. Seed dormancy is an adaptation allowing species persistence in variable and unpredictable environments by creating a persistent seed bank to buffer populations when conditions would otherwise cause catastrophic mortalities (Philippi 1993, Venable 2007) and preserving genetic variability (Evans and Dennehy 2005). Annual species in desert environments can be nondormant (i.e., germinate over broad range of temperatures), conditionally dormant (i.e., germinate over a narrow range of temperatures), or dormant (delayed germination to another season), shifting among phases within the year (Baskin and Baskin 1981, 1983a, 1984a, Roberts and Neilson 1982a,b). Seventy percent of annual species representing a diversity of families in hot semi-desert and desert regions have physiological dormancy (i.e., seeds are permeable to water, possess physiological inhibitors within the seed that prohibit radicle growth, and/or have low radicle radial pressure for the embryo to emerge); however, conditions for dormancy release and dormancy induction have not been elucidated for the majority of species, particularly in North American deserts (Baskin and Baskin 2014).

Eriogonum viscidulum J.T. Howell (sticky buckwheat) is an endemic winter annual restricted to the eastern Mojave Desert with a global distribution largely confined to lands administered by Bureau of Land Management (BLM), National Park Service (NPS), and Bureau of Reclamation (BOR) within Clark County, southern Nevada and peripheral populations in adjacent southeastern Lincoln County, Nevada and northwestern Mohave County, Arizona. Thirteen population groups span the confluence of the Muddy and Virgin rivers and south along Lake Mead (The Nature Conservancy 2007). Due to population declines in an already narrow distribution along with threats from urban development, *E. viscidulum* is listed as a critically endangered species by the state of Nevada, occurs on the sensitive species list for Bureau of Land Management, is listed on the Nevada Natural Heritage Programs Sensitive List (imperiled status), and is a species of concern covered under the Clark County Multiple Species Habitat Conservation Plan and Environmental Impact Statement. Potential threats to *E. viscidulum* populations include trespass cattle (Bangle 2012a, 2012b) and competition with invasive annual species such as *Brassica tournefortii* (Sahara mustard), *Tamarix* sp. (saltcedar), *Salsola* sp. (Russian thistle), and *Schismus* sp. (Mediterranean grass) (Bangle 2012b). Population monitoring of *E. viscidulum* demonstrates large interannual variation in abundance (Bangle 2012b), which is characteristic of winter annual species whose seed germination and seedling emergence is triggered by rainfall pulses during September – December (Beatley 1974).

Seeds of desert winter annual species are known to reside in surface soils for long periods of time, forming a persistent seed bank. The exogenous (temperature, moisture, light) and endogenous factors (embryo development, physiological changes) that influence seed dormancy at dispersal and the seasonal shifts in these factors that regulate germination from the seed bank are not well understood for many desert annual species, including *E. viscidulum*. For annual congeneric species in the Mojave Desert, seedling densities in native habitat fluctuate across years: over a six-year period, seedling densities of *E. maculatum* were highest after heavy rainfall during November – December (41.4 mm) and the highest densities for *E. nidularium*, *E. thomasi*, and *E. trichopes* followed exceptional rainfall in January – February (98 mm) (Bowers 1987). These patterns of seedling emergence indicate annual *Eriogonum* species have physiological seed dormancy. Physiological dormancy is also known for perennial *Eriogonum* species in California (*E. crocatum*, *E. fasciculatum*, *E. gracile*, *E. parvifolium*, *E. wrightii*; Cole

1967), temperate semideserts and deserts of Utah (*E. corymbosum*; Meyer and Paulsen 2000), and north-temperate conifer-dominated montane forests (*E. brevicaulis*, *E. heracleoides*; Meyer and Paulsen 2000). In contrast, perennial *Eriogonum* species in some arid and semi-arid habitats are known to have seeds that are nondormant at dispersal (*E. alatum*, *E. ovalifolium*, *E. racemosum*; Meyer and Paulsen 2000; *E. cinereum*, *E. elongatum*; Cole 1967). Nondormant seeds of desert winter annuals typically germinate in autumn, while ungerminated seeds either enter secondary dormancy as temperatures decline under low soil moisture (“obligate winter annuals”) or remain germinable through winter, but only under low temperatures, a transition called “conditional dormancy” (“facultative winter annuals”; Baskin and Baskin 1993).

For narrowly distributed species, augmentation or re-introduction of wild populations using individuals propagated *ex situ* may reduce the risk of extinction and provide a means to conserve threatened populations or species (Bowes 1999, Cerabolini et al. 2004, Gimenez-Benavides et al. 2005). An understanding of seed dormancy, the environmental cues that break dormancy, and identifying the mating system are all essential for propagation success. Temperature control of seed dormancy and germination is the primary environmental cue regulating seedling emergence (Baskin and Baskin 1988). Therefore, our goal was to understand the seed ecology of *Eriogonum viscidulum* through temperature and moisture treatments under laboratory incubations, germination of seeds buried in habitat and seasonally exhumed, and seedling emergence from soils collected from habitat and incubated in a greenhouse. We adapt traditional approaches to address several research questions: Which dormancy type does *E. viscidulum* possess based on seed anatomy and seed coat permeability (*sensu* Baskin and Baskin 2004)? What is the annual dormancy status (dormant, conditionally dormant, and/or nondormant) beginning at seed dispersal? Does this species possess seasonal dormancy cycling (e.g., conditionally dormant to nondormant, or dormant to nondormant)? To inform *ex situ* propagation, we also tested seed fill for flowers in a shade house setting to identify the mating system for this species. Collectively, we intend for these experiments to guide how to make conservation seed collections and manage seeds for propagation.

Methods

Study sites

Eriogonum viscidulum

Out of the 13 population groups recognized by The Nature Conservancy (2007), we focused our seed ecology and mating system studies on four populations of *Eriogonum viscidulum*. We scouted most population groups in Clark County, and focused our work on Toquop Wash and Upper Muddy River (BLM), and the Black Mountain and Lime Wash population groups (NPS). The latter two groups had the highest recorded numbers of individuals in 1995 surveys (Niles et al. 1995, *sensu* The Nature Conservancy 2007).

Astragalus geyeri var. *triquetrus*

We designed parallel studies on seed ecology and mating system for *Astragalus geyeri* var. *triquetrus*. However, under the prolonged drought, no *A. geyeri* var. *triquetrus* seeds could be acquired directly from plants at any of the 17 population groups for this species (The Nature Conservancy 2007). Seeds were also absent from soil samples collected in habitat of four population groups – California Wash and Mud Lake in 2020, Bark Bay and Sandy Cove in 2021.

The former two population groups had scattered numbers of plants during spring 2020, but permits had not yet been issued for us to make seed collections. Therefore, the remaining Methods, Results, and Discussion focus exclusively on *E. viscidulum*. A summary detailing our efforts to study *A. geyeri* var. *triquetrus* occurs in Appendix A.

Seed characteristics

We examined the internal seed anatomy of *Eriogonum viscidulum* seeds grown out from the soil seed bank and the germination trial to determine whether the embryo is fully developed at dispersal (i.e., embryo size relative to the volume of the seed) and/or differentiated (i.e., identifiable cotyledons, hypocotyl, and radicle). We determined seed coat permeability by weighing 20 seeds both before and after soaking in deionized water at 5 °C for 1 h, 2 h, 4 h, 6 h, 20 h, 28 h, 36 h, 60 h, 84 h and 128 h (seeds were collected from wild Toquop Wash plants and after-ripened under a companion study, 2021-USGS-2075A). These characteristics, in combination with germination trials, were used to determine seed dormancy class based on Baskin and Baskin (2004).

Soil seed bank, 2020 – 2022

Soil seed bank samples were collected around 20 senesced *E. viscidulum* plants at each of two populations on 28 July 2020 (Lime Wash and Black Mountain on NPS land, accessed by boat at Lake Mead National Recreation Area) and at 17 and 20 points, respectively, within historic locations at each of two populations on 22 September 2021 (Toquop Wash and Upper Muddy River on BLM land). Four 10.5 cm × 10.5 cm metal frames were placed at cardinal directions around each plant or point, and soil was collected from 0 cm – 2.5 cm depth within the frames (441.0 cm² per sampling point). Soils from two frames per point were placed in a plastic zip top bag for cold, dry storage (refrigerator at 5 °C), while soils from the other two frames were stored in a zip top bag at the USGS, Boulder City, Nevada shade house under ambient, summer/autumn conditions (warm dry storage; daytime maximum 26 – 45 °C and nighttime minimum 15 – 30 °C in 2020 and daytime maximum 18 – 36 °C and nighttime minimum 7 – 24 °C in 2021).

Soils were incubated in the USGS greenhouse starting in autumn: 17 October 2020 and 29 October 2021. The greenhouse is semi-controlled to allow for seasonal fluctuations in temperature but ensuring minimum temperatures remain above freezing and maximum temperatures below 30 °C. Soil samples were split with a soil splitter across multiple bulb pots (15 cm diameter, lined with weed blocker cloth) to maintain 2.5 cm depth and randomized over six benches (blocks) within the greenhouse. A thin layer of vermiculite was added to the surface of each pot to maintain moisture (medium grain, Therm-O-Rock). We modified our approach from emergence methods developed for the Great Basin (Young *et al.* 1969, Young and Evans 1975, Young *et al.* 1981) and specifically used for seed bank studies of Mojave Desert species (Esque 2004, DeFalco *et al.* 2009, Scoles-Sciulla and DeFalco 2009, DeFalco and Esque 2014). All pots were subjected to four wetting cycles known to promote germination of seeds (Meyer and Poljakoff-Mayber 1982, Baskin and Baskin 2014) alternating with approximately 3 wk intervals when soils were not watered and allowed to air dry. During the first and second wetting phases, pots were watered with tap water using a hand-held sprayer, and water was applied until it drained from the bottom of the pots, but no standing water remained. In the third and fourth wetting phases, 50 mL of a 0.01 M solution of potassium nitrate and 50 mL of a 6.5×10^{-4} M solution of gibberellic acid, respectively, was initially added followed by daily watering with tap

water. During each wetting phase, seedlings of all species were counted and harvested as soon as they could be identified. Unknown seedlings were transplanted to pots and given time to develop so they could be identified to species. *E. viscidulum* seedlings were transplanted into pots and allowed to grow for use in laboratory germination and habitat seed burial trials (see below). Watering ceased and dry-down began once emergence declined and no new germinants appeared for a week.

Germination trial, 2022

Due to the unprecedented drought gripping the Desert Southwest during our studies (Williams et al. 2022), seeds of *E. viscidulum* were either too low in number for us to collect and remain in compliance with our permits or were entirely unavailable (see Appendix A). We therefore used seeds that were produced by three adult plants propagated from the soil seed bank (F1 generation, Lime Wash population, see *Results, Seed bank* below), harvested on 1 October 2021, and stored under prevailing temperatures within our shade house in a dry, airtight container October 2021 – July 2022. Viability on a subsample of these seeds tested with a cut test was > 80%. During the pre-treatment phase (1 – 31 August 2022), six replicate Petri plates each containing 50 seeds with equal representation across maternal plants were exposed to one of four moisture-temperature treatments: dry storage at 0 °C, moist stratification at 0 °C, dry storage at 25 °C (after-ripened), and moist stratification at 30 °C (N = 24 total plates). Seeds for both moist pre-treatments were dipped in a 10% bleach solution for 20 s and rinsed for 20 s in tap water prior to beginning the pre-treatment phase; seeds from both dry pre-treatments were sanitized in the same manner prior to beginning the incubation phase (31 August – 20 October 2022). For the incubation phase, the six replicate plates of each pre-treatment were randomly assigned to 10 °C/moist or 25 °C/moist chambers (n = 3 plates per pre-treatment/incubation combination). The eight pre-treatment/incubation combinations in the germination trial were: after-ripened/10 °C, after-ripened/25 °C, 0 °C (dry)/10 °C, 0 °C (dry)/25 °C, 0 °C (moist)/10 °C, 0 °C (moist)/25 °C, 30 °C (moist)/10 °C, 30 °C (moist)/25 °C.

During the incubation phase, seeds were placed on moist filter paper (Whatman 1001-110) in Petri plates sealed with Parafilm® (Bemis Co. Inc., Neenah, WI), and placed in the 10 °C or 25 °C chamber with LED lights programmed for a 12-h photoperiod. The temperature in each chamber was monitored using a Kestrel® DROP datalogger (Nielsen-Kellerman Co., Boothwyn, PA). Filter papers were kept moist with distilled water and seeds were monitored every 2 d. A seed was considered germinated if an elongating radicle emerged from the seed coat. The seed coat of some seeds, particularly in the 30 °C moist pre-treatment, split open and the embryo expelled. We considered these seeds viable but not germinated, as an elongating radical did not emerge normally. Seeds remaining after 50 d of incubation were tested for viability using a tetrazolium staining protocol for species in the Polygonaceae (AOSA 2010).

Seed burial, 2022 – 2023

Using the same seed source as the germination trial (F1 generation), we prepared 1,250 seeds of *E. viscidulum* and maintained seeds in paper envelopes under dry seasonal conditions until the start of seed burial on 6 September 2022 (daytime maximum 40 – 50 °C and nighttime minimum 20 – 30 °C). Sealed nylon mesh packets containing 50 seeds each were prepared and subsequently buried in shrub interspaces at five locations spaced 200 m – 300 m apart within the Overton Arm population group. At each location, four replicate bags were buried at 2 cm depth and secured with landscape pins in separate holes radiating 2 m from a center stake at the 3

o'clock, 6 o'clock, 9 o'clock and 12 o'clock positions. We exhumed one bag per location on 6 December 2022 (3-mo), 6 March 2023 (6 mo), 9 June 2023 (9 mo), and 6 September 2023 (12-mo). Bags were transported to the laboratory for germination and viability tests. Testing of the 0-mo unburied seeds began on 20 September 2022.

Each mesh bag was opened in the laboratory, and seeds were equally and randomly divided into two Petri plates (one for 10 °C and one for 25 °C incubation). Seeds were sanitized prior to incubation by dipping in 10% bleach solution for 20 s and rinsing in deionized water for 20 s. Seeds were placed on moist filter paper (Whatman 1001-110), sealed with Parafilm[®] (Bemis Co. Inc., Neenah, WI), and placed in the germination chambers with LED lights programmed for a 12-h photoperiod. The temperature in each chamber was monitored using a Kestrel[®] DROP (Nielsen-Kellerman Co., Boothwyn, PA). During each 30-d incubation, filter papers were kept moist with distilled water, and seeds were monitored every 2 d. Seeds with a radicle were recorded as germinated, seeds with only seed coats remaining were recorded as germinated during burial, and moldy seeds were recorded as dead and discarded. Seeds remaining after 30 d were tested for viability using a tetrazolium staining method for species in the Polygonaceae (AOSA 2010).

Mating system, 2021 – 2022

Because few to no plants emerged in habitat of *Eriogonum viscidulum* in 2021 and 2022, we used greenhouse-raised plants maintained in our open shade house for determining the mating system to inform propagation. Initial attempts to manipulate single flowers or to bag whole flowering branches on six *E. viscidulum* plants (F1 generation) raised from the 2020/2021 soil seed bank failed because the flowers for this species were too small and delicate to attempt pollen transfer by hand. These plants were thereafter maintained at prevailing temperatures in the shade house until after they set seed and senesced on 1 October 2021. Although we were able to collect seed directly from only 3 of the 6 plants, we collected the top 5 cm of soil and debris from the surface of all six pots and incubated these soils at the same time as the 2021/2022 seed bank samples. The seeds in topsoil from these original six plants created 491 seedlings, and 29 plants survived transplanting and matured to adult stage (F2 generation). In early spring 2022, we imposed bagging treatments around these plants to determine whether the species is an obligate outcrosser or can self-fertilize.

We assigned all 29 *E. viscidulum* plants in bud on 2 February 2022 to one of two inflorescence bagging treatments – bagged canopy to exclude invertebrate pollinators and bagged with canopy left accessible. A hole in the bottom of a 5-gal fine mesh bag was fit over each inflorescence before elongation so that at maturation, the open end of the bag at the top of the canopy could be closed to prevent access to pollinators (closed access) while maintaining a tight seal at the base to exclude pollinators. Open access plants were similarly placed in bags, but the top remained open to allow access to pollinators while imposing a similar microclimate around the inflorescence. We counted the number of buds and flowers on each plant on 29 March, 18 April, 28 April, 11 May, and 19 May. Flowering was complete by 25 May 2022, and all bags with dried inflorescences were collected and stored in paper envelopes in the shade house under prevailing summer temperatures to after-ripen. After two weeks, viability of seeds was determined with a seed cut test.

Statistical analyses

We analyzed the responses of *Eriogonum viscidulum* using SAS statistical software (Cary, NC, version 9.4). For the seed germination trial, we used the LOGISTIC procedure to test the fixed effects of the pre-treatments (4 levels), incubation temperatures (2 levels), and their interaction on the “event/trial” response (i.e., germinated seeds/total viable seeds at 80 d). Due to quasi-complete separation in the data (i.e., when particular treatment levels are confounded when the event = all non-germinated or all germinated, such as in the shadehouse pre-treatment level incubated at 10 °C) we included a Firth’s bias adjustment to account for penalty in likelihood estimation. We used a Tukey-Kramer adjustment on *P*-values when comparing odds ratios among the pretreatment-incubation temperature combinations. We used a general linear model using the GLIMMIX procedure to compare the same main effects and interaction on seed mortality (i.e., those seeds discarded during trial that were deemed “dead”).

Likewise, in the seed burial data, excessive zeros in the germinable fraction occurred at the 6 mo – 12 mo levels because seeds that germinated during burial (i.e., empty seed coats) by definition could not germinate during the incubations, and few viable ungerminated seeds remained. This situation made maximum likelihood techniques unable to estimate zero for those levels due to complete separation (i.e., germinants occurred in the 0-mo and 3-mo exhumed dates and no germinants in the later dates). We used the LOGISTIC procedure to test the fixed effects of burial duration on the two levels that had viable seed (0 mo and 3 mo), on incubation temperature (2 levels, 10 °C and 25 °C), and the interaction on the probability of individual seeds germinating during incubation (event = “germinated” during incubation); we included a Firth’s bias adjustment to account for penalty in likelihood estimation.

Soil seed bank counts of *E. viscidulum* seedlings were analyzed to compare the fixed effects of population (4 levels), storage condition (2 levels), and their interaction using GLIMMIX procedure; we used a negative binomial distribution and a log link function with storage*replicate as a random effect. The link function generated estimates and confidence limits on the mean seedling count scale. Means and upper and lower confidence limits were converted to number of seedlings/1 m² for illustration.

To examine the mating system, we calculated the proportion of seeds set as #filled seeds/maximum flower count and analyzed the proportion using a beta distribution and a logit link function using GLIMMIX procedure with bagging treatment as a fixed effect (2 levels). The link function generated estimates and confidence limits on the mean proportion scale.

In all models, we used the Laplace method to minimize the -log likelihood function. We illustrated when treatment levels were significant at the $\alpha = 0.05$ level with Tukey’s adjustment as different uppercase letters (see figure captions for details). Convergence criterion was satisfied for all models, and data were not over-dispersed based on Pearson Chi-square/DF values near 1.0.

Results

Seed characteristics

Eriogonum viscidulum seeds are formed in a superior ovary with one seed produced per flower (Fig. 1A, 1B). Mature seeds are approximately 0.385 mg (based on weight of 20 seeds), are spherical and shiny brown with a pronounced beak at the distal end from the flower receptacle (Fig. 1B, 1C). The large peripheral, fully developed embryo is folded into the seed with minimal endosperm (Fig. 1D, 1E). Emerging seedlings have oval cotyledons covered with viscid, glandular hairs on the surface and margins (Fig. 1F). The stems and leaves of young and

adult plants are covered with these same hairs, giving this species its characteristic “sticky” texture. Seeds of *E. viscidulum* increased in mass from pre-soak (dry) by ~50% within the first 24 h when soaked in water and maintained this mass for the duration of the permeability trial (Fig. 2).

Seed bank

Forty-two *E. viscidulum* seedlings emerged from soil collected in habitat and subjected to nine months of alternating wetting and drying cycles (20 seedlings in 2020 and 22 seedlings in 2021) with the majority emerging during the first wetting cycle (14 seedlings in 2020, 22 in 2021). For the 2020 seed bank, a small number of seedlings emerged during the second wetting cycle (2 seedlings), the potassium nitrate cycle (2 seedlings), and the gibberellic acid cycle (2 seedlings). The majority of emergence during the first wetting cycle occurred immediately after minimum greenhouse temperatures falling briefly below 15 °C (Supplemental Fig. S1). Dry storage of soils in the shade house under prevailing summer temperatures prior to watering resulted in more than 5 times the number of seedlings emerging from the seedbank compared with soils placed in dry storage at 5 °C (Storage: $F_{1,38} = 5.66$, $P = 0.023$, Fig. 3). Seedling numbers also differed among populations ($F_{3,108} = 78.92$, $P < 0.001$) with higher numbers in samples collected at Toquop Wash and Lime Wash compared with Black Mountain and Upper Muddy River populations (Fig. 3; interaction not significant).

Germination trial

The germination fraction of *E. viscidulum* differed among seed pre-treatments (df = 3, Wald’s chi-square = 52.87, $P < 0.001$) but not between incubation temperatures (df = 1, Wald’s chi-square = 1.747, $P < 0.186$), and the interaction between the pre-treatments and temperatures was statistically significant (df = 3, Wald’s chi-square = 26.949, $P < 0.001$; Fig 4). Germination fractions were high among all pre-treatments, except the warm stratification regardless of incubation temperature (Fig. 4). Seed mortality during the trial – denoted by seeds that became moldy or tested negative with tetrazolium staining – was highest for both moist stratification pre-treatments compared with both dry pre-treatments ($F_{3,12} = 23.33$, $P < 0.001$); neither the incubation temperature ($F_{1,12} = 1.85$, $P = 0.199$) nor the interaction was significant ($F_{3,12} = 0.75$, $P = 0.544$).

Seed burial

Seeds of *E. viscidulum* stored dry under prevailing summer temperatures had the same probability of germination (i.e., germination fraction) for the 10 °C and 25 °C incubation temperatures at the time bags were buried in September and at 3-mo burial in December (Burial date × Temp; $F_{1,153} = 10.80$, $P = 0.001$, Fig. 5). Nearly all seeds germinated while buried in habitat prior to the March collection, resulting in negligible numbers for viable seed left in the bags collected; thus, March – September 2023 dates couldn’t be included in the analysis. This mass germination in habitat by the March exhumation date coincided with a period when maximum/minimum daily temperatures remained below 20 °C/10 °C (starting 3 November 2022) and rainfall totaled 79 mm (Fig. 5).

Mating system

During visits to water plants in the shade house, we observed *Bombyliidae* flies visiting *Eriogonum viscidulum* flowers that were open to pollinators; no pollinators were observed in the

closed bags. Removing three plants from analysis where the number of filled seed exceeded our count of maximum number of flowers, the proportion of seeds set was not statistically different ($F_{1,24} = 0.79$, $P = 0.384$) between bagged plants (95% CI proportion = 0.2742 – 0.5696) and unbagged plants (0.2216 – 0.4631) (Fig. 6). Plants in the F1 generation of *Eriogonum viscidulum* grown out from seed bank samples produced 2,792 filled seeds with 96% average viability, while the 29 plants in the F2 generation produced 25,215 filled seeds with 70% average viability. For the F1 generation, at least 491 additional viable seeds were produced and germinated from the topsoil.

Discussion

An early study on germination of Mojave Desert and Colorado Desert annual species concluded that “probably the whole genus *Eriogonum* is winter-germinating” (Went 1948). Janice Beatley (1967) later observed that annual species of Polygonaceae including *Eriogonum* are “well represented as following autumn germination”. Our collective studies provide evidence that the rare Mojave Desert annual *Eriogonum viscidulum* is a facultative winter annual that likely has primary dormancy at dispersal, becomes nondormant (ND) following exposure to warm summer temperatures, and is nondormant during autumn and winter. Mature *Eriogonum viscidulum* seeds have large, peripheral embryos, a characteristic of the family Polygonaceae (Finch-Savage and Leubner-Metzger 2006, Fig. 1). Water uptake through a permeable seed coat occurs within 24 h for after-ripened seeds, indicating that *E. viscidulum* has physiological seed dormancy (Fig. 2). We did not discriminate among the five types of physiological dormancy by incubating excised embryos and examining if normal seedlings were produced or by testing germination response to gibberellic acid (Baskin and Baskin 2004). However, mature seeds did not require additional time for development but only needed a short time to imbibe water and favorable temperatures for the embryo to elongate, the radicle to penetrate the thin seed coat, and the cotyledons to break free and establish as a new seedling, a process that we observed occurring as soon as two days after incubation began. These characteristics indicate *E. viscidulum* seeds have non-deep physiological dormancy, a common dormancy type in annual species of unpredictable habitats found in deserts worldwide (Baskin and Baskin 2014).

Seeds of *Eriogonum viscidulum* that disperse May – June require exposure (>1 mo – 3 mo) to hot, dry conditions to prime them for germination during cooler late autumn months (September – October). Seed bank soils collected in habitat during July or September and exposed to summer heat resulted in a more than five-fold increase in seedling emergence in our greenhouse compared with soils stored dry at 5 °C following collection (Fig. 5). After-ripening at high, often dry, temperatures that shifts the dormancy state from primary dormancy to conditionally dormant (CD) or nondormant (ND, see below) is well documented for seeds of winter annuals (Baskin and Baskin 1976, 1986), including those of North American deserts (Baskin et al. 1993). The majority of *E. viscidulum* seedlings emerged from the seed bank during the first wetting cycle when daily maximum/minimum temperatures declined below 25 °C/20 °C in 2020 and 27 °C/15 °C in 2021 (Fig. S1). Greenhouse temperatures remained < 28 °C during the nine months that seedling emergence was observed (Fig. S1), while maximum/minimum daily temperatures known to after-ripen winter annuals under moist or alternating moist-dry conditions are at least 30 °C/15 °C or 35 °C/20 °C (Basin and Baskin 1976). Thus, the low temperature of soils stored at 5 °C immediately following July collection prevented seeds from after-ripening further and greenhouse temperatures were also too low for after-ripening to occur during the watering-drying cycles.

Our germination trial demonstrates that *Eriogonum viscidulum* seeds that after-ripened with summer heat were non dormant (ND). Seeds had overall high germination under both 10 °C and 25 °C incubations, except in the case of the 30 °C moist pre-treatment (Fig. 3). Whereas moist 30 °C conditions can occur in habitat during unpredictable, typically brief summer monsoon storms in July – August, *E. viscidulum* seeds had lower germination at this temperature in our trial, consistent with seeds degrading during longer exposure than they would typically experience in habitat (Baskin and Baskin 1986).

Eriogonum viscidulum seeds were also nondormant during autumn in our seed burial trial, and longer exposure to winter temperatures and ample moisture germinated the majority of seeds during burial; thus, we did not detect whether colder seasonal temperatures shifted seeds into a CD or D state. Seedlings of other desert winter annuals in the family Polygonaceae are known to emerge in response to rains in September and October (Beatley 1974). The absence of empty seed coats for bags exhumed in early December indicates germination did not occur for *E. viscidulum* following heavy rain in early September (34.7 mm predicted by PRISM) when minimum daily temperatures hovered around 20 °C, but maximum temperatures remained above 30 °C (Fig. 4). Even though soil moisture was not limiting following this exceptional autumn storm, temperatures were too warm to stimulate germination of the buried *E. viscidulum* seeds. During our germination trial, we similarly saw a consistently lower germination fraction for the 30 °C moist pre-treatment regardless of incubation temperature. An early November storm occurred during a period of lower temperatures likely more suitable for germination (maximum/minimum < 18 °C/3 °C), consistent with the high germination fraction we observed during our germination trial for the 0 °C (moist) pre-treatment/10 °C incubation; however, 11.2 mm of rain is not adequate for triggering germination of most Mojave Desert winter annuals (Beatley 1974). In contrast, considerable rain during December to early March (66.7 mm), falling in multiple storms when maximum/minimum temperatures were lower than 20 °C/10 °C, triggered complete germination in habitat by 6 mo of burial (i.e., empty seed coats in bags retrieved March – September 2023). This mass germination reflects how ND *E. viscidulum* seeds in autumn (Fig. 4), all germinated because of the prevailing optimal cold, moist conditions.

Germinability of seeds can change seasonally as environmental cues promote dormancy loss (CD to ND states in late autumn) and/or dormancy induction (ND to D states in winter; Baskin et al. 1993, Baskin and Baskin 2014). Furthermore, geographically widespread members of species within the same genera will likely have the similar patterns in temperature cues for after-ripening, germination, and dormancy (Baskin and Baskin 1988). Two common eastern Mojave Desert winter annual species – *E. deflexum* and *E. nidularium* – showed patterns ranging from ND to conditionally dormant (CD) following exposure to warm, dry conditions (Earsom 1979). Seeds of *E. abertianum*, a facultative winter annual of the Chihuahuan Desert, germinate across a wide range of temperatures at dispersal in autumn (ND), are CD during winter, and become ND again the following autumn (Baskin et al. 1993), a notable example of dormancy cycling. Herbaceous and woody species of the genus *Eriogonum*, including the Mojave Desert shrub *E. corymbosum*, had seeds that were ND or became ND after moist chilling at 2 °C for 5 – 16 weeks, but there was no evidence for secondary dormancy induced by longer chilling (Meyer and Paulsen 2000). Our seed burial trial occurred during a prolonged drought, yet punctuated rainfall events were sufficient to germinate the majority of *E. viscidulum* seeds buried in soils longer than 3 mo. These results imply that *E. viscidulum* did not have the opportunity to enter secondary dormancy as seen for other winter annual species (Baskin et al. 1993) because they all germinated in a ND state. Burial of *E. viscidulum* seeds in habitat exposed to the changing

seasonal temperature cues, but protected from rainfall, would clarify the temperature cues that regulate dormancy cycling in this winter annual species as it maintains a persistent seed bank.

Acquiring conservation seed collections for endemic winter annuals is particularly challenging under our changing regional climate. The current megadrought gripping the southwestern U.S. is bringing less rainfall to the region (Williams et al. 2022), with cool season rainfall specifically declining and more variable, and the duration of dry periods increasing in the Desert Southwest (Zhang et al. 2021). Conservation of rare plant species may eventually depend on reintroduction into habitat, although consideration of genetics for source material and extant populations and the current or imminent threats are essential for successful implementation (Maschinski and Albrecht 2017). Our seed bank emergence trial detected seeds in habitat at historic points where adult plants were absent (Toquop Wash in 2021) yet sampling around senescing *E. viscidulum* plants didn't always assure seeds would emerge during our soil seed bank grow-out (Black Mountain in 2020). Population differences in seedling emergence might explain local extirpation of *E. viscidulum* from historic occurrences at Upper Muddy River (BLM, Southern Nevada), although seed densities were quite low even for populations with plants present at the time of collection (Lime Wash and Black Mountain, Lake Mead NRA).

Although *E. viscidulum* had low seed densities in the soil seed bank overall, we showed that mature seed-bearing plants can be produced through seed bank propagation in a greenhouse or nursery. Seeds require a dry/hot after-ripening period, preferably by collecting soils after seed dispersal and storing soils protected from rain and rodents under the prevailing summer temperatures until autumn. While we incubated after-ripened samples in our greenhouse, soils could also be incubated under a protected outdoor nursery setting in the Mojave Desert so samples could be after-ripened in place (no watering, June – September) and incubated to trigger *E. viscidulum* germination and seedling growth to maturity. Whereas we found that this species is self-compatible (Fig. 6), growing seed bank samples in the nursery might allow for naturally occurring invertebrate pollinators to exchange pollen among flowering plants and maintain population genetic diversity.

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Literature

AOSA/SCST (2010) Tetrazolium Testing Handbook. Association of Official Seed Analysts.

- Bangle, D. (2012a) Monitoring *Eriogonum viscidulum* (Sticky Buckwheat) and *Astragalus geyeri* var. *triquetrus* (Threecorner Milkvetch) within Lake Mead National Recreation Area – 2008 Update. Report of the Lower Colorado River Multi-Species Conservation Program, May 2012.
- Bangle, D. (2012b) Monitoring *Eriogonum viscidulum* (Sticky Buckwheat) and *Astragalus geyeri* var. *triquetrus* (Threecorner Milkvetch) within Lake Mead National Recreation Area – 2009 Update. Report of the Lower Colorado River Multi-Species Conservation Program, May 2012.
- Baskin, C.C., and J.M. Baskin (2014) Seeds: Ecology, biogeography, and evolution of dormancy and germination. 2nd edition, Academic Press, San Diego, California.
- Baskin, J.M., and C.C. Baskin (2004) A classification system for seed dormancy. *Seed Science Research* 14:1-6.
- Baskin, C.C., P.L. Chesson, and J.M. Baskin (1993) Annual seed dormancy cycles in two desert winter annuals. *Journal of Ecology* 81:551-556.
- Baskin, C.C., and J.M. Baskin (1988) Germination ecophysiology of herbaceous plant species in a temperate region *American Journal of Botany* 75:286-305.
- Baskin, J.M., and C.C. Baskin (1986) Temperature requirements for after-ripening in seeds of nine winter annuals. *Weed Research* 26:375-380.
- Baskin, J.M., and C.C. Baskin (1976) High temperature requirement for afterripening in seeds of winter annuals. *New Phytologist* 77:619-824.
- Beatley, J.C. (1974) Phenological events and their environmental triggers in Mojave Desert ecosystems. *Ecology* 55:856-863.
- Beatley, J.C. (1967) Survival of winter annuals in the northern Mojave Desert. *Ecology* 48:745-750.
- Bowers, M.A. (1987) Precipitation and the relative abundances of desert winter annuals: a 6-year study in the northern Mohave Desert. *Journal of Arid Environments* 12:141-149.
- Cole, N.H.A. (1967) Comparative physiological ecology of the genus *Eriogonum* in the Santa Monica Mountains, southern California. *Ecological Monographs* 37:1-24.
- DeFalco, L.A., T.C. Esque, J.M. Kane, and M.B. Nicklas (2009) Seed banks in a degraded desert shrubland: influence of soil surface condition and harvester ant activity on seed abundance. *Journal of Arid Environments* 73:885-893.
- DeFalco, L.A., S.J. Scoles-Sciulla, C. Engel, N.A. Custer, O.K. Niziolek, and T.C. Esque. (2014) Assessment of post-fire rehabilitation of desert tortoise habitat, Final project report. Deliverable D34 for Clark County Desert Conservation Program, Project #2009-USGS-808A.
- DeFalco, L.A., and T.C. Esque (2014) Soil seed banks: Preserving native biodiversity and repairing damaged desert shrublands. *Fremontia* 42:20-23.
- Earsom, J.R. (1979) The effects of selected pre-germination treatments on six species of summer and winter annual plants of the east Mojave Desert. Theses Digitization Project. 120. <https://scholarworks.lib.csusb.edu/etd-project/120>
- Esque, T.C. (2004) The role of fire, rodents and ants in changing plant communities in the Mojave Desert. Dissertation. University of Nevada. Reno, Nevada. 168 pp.
- Evans, M.E., and J.J. Dennehy (2005) Germ banking: bet hedging and variable release from egg and seed dormancy *Quarterly Review of Biology* 80:431-451.
- Fish and Wildlife Service, Interior (2021) Endangered and threatened wildlife and plants; 90-day findings for three species. *Federal Register* 86(55):15637-15639.

- Finch-Savage, W.E. and G. Leubner-Metzger (2006) Seed dormancy and the control of germination. *New Phytologist* 171:501-523.
- Maschinski, J., and M.A. Albrecht (2017) Center for Plant Conservation's Best Practice Guidelines for the reintroduction of rare plants. *Plant Diversity* 39:390-395.
- Meyer, A.M., and A. Poljakoff-Mayber (1982) *The Germination of Seeds*. Pergamon Press. 4th Edition. New York, NY. 96 pp.
- Meyer, S.E., and A. Paulsen (2000) Chilling requirements for seed germination of 10 Utah species of perennial wild buckwheat (*Eriogonum* Michx. [Polygonaceae]). *Native Plants* 1:18-24.
- Philippi, T. (1993) Bet-hedging germination of desert annuals: beyond the first year. *The American Naturalist* 142:474-487.
- Scoles-Sciulla, S.J., and L.A. DeFalco (2009) Seed reserves diluted during surface soil reclamation in eastern Mojave Desert. *Arid Land Research and Management* 23:1-13.
- The Nature Conservancy (2007) A conservation management strategy for nine low elevation rare plants in Clark County, Nevada. Report of the Nature Conservancy, Nevada Field Office, Reno, Nevada, 17 April 2007.
- Venable, D.L. (2007) Bet hedging in a guild of desert annuals. *Ecology* 88: 1086-1090.
- Went, F. (1948) Ecology of desert plants. I. Observations on germination in the Joshua Tree National Monument, California. *Ecology* 29:242-253.
- Williams, A.P., B.I Cook, and J.E. Smerdon (2022) Rapid intensification of the emerging southwestern North American megadrought in 2020-2021. *Nature Climate Change* 12:232-234.
- Young, J.A., R.A. Evans, and R.E. Eckert, Jr (1969) Population dynamics of downy brome. *Weed Science* 17:20-26.
- Young, J.A., and R.A. Evans (1975) Germinability of seed reserves in a big sagebrush community. *Weed Science* 23: 358-364.
- Young, J.A., R.A. Evans, B.L. Kay, R.E. Owen, and J. Budy (1981) Collecting, processing, and germinating seeds of western wildland plants. U.S. Department of Agriculture, Science and Education Administration, Agricultural Reviews and Manuals. ARM-W-3.
- Zhang, F., J.A. Biederman, M.P. Dannenberg, D. Yan, S.C. Reed, and W.K. Smith (2021) Five decades of observed daily precipitation reveal longer and more variable drought events across much of the western United States. *Geophysical Research Letters* 48: e2020GL092293. <https://doi.org/10.1029/2020GL092293>.

Figure captions

Figure 1. Characteristics of *Eriogonum viscidulum*. Flower at anthesis (A), seed maturing on receptacle as tepals and filaments whither (B), filled seeds released from receptacle (C), lateral view of developed and differentiated embryo enclosed within seed coat (D), embryo released from seed coat (E), and cotyledons of germinant from seed bank (F). Labels include: “seed coat” = seed coat, “endo” = endosperm, “cot” = cotyledon, “hyp” = hypocotyl, “rad” = radicle. Photo credits: 1A, 1F (Lesley DeFalco), 1B, 1D, 1E (Mikaela Gaskill), and 1C (Alex Stosich).

Figure 2. Permeability of *E. viscidulum* seed collected from wild plants at Toquop Wash in July 2023 and after-ripened for 1 mo. Rate of water uptake is reflected in seed weight change for 20 seeds soaked in deionized water at 5 °C and patted dry before weighing at hourly time intervals.

Figure 3. *E. viscidulum* seedlings that emerged from soil seed bank samples maintained at two storage conditions before watering (left panel) and for four populations in southern Nevada (right panel). Each soil sample was split and stored in either a refrigerator at constant 5 °C or under prevailing summer temperatures in the shade house immediately after collection (left panel). Soil samples were collected from two populations with plants present in 2020 (Lime Wash, n = 20; and Black Mountain, n = 20) and from two historic populations in 2021 where plants were not present (Toquop Wash, n=17, and Upper Muddy River, n = 20). Different uppercase letters denote significant differences between storage conditions and among populations.

Figure 4. Final germination fraction for *E. viscidulum* seeds initially after-ripened under seasonal conditions in a shade house (October 2021 – July 2022) before exposure to pre-treatment conditions (continued after-ripening, 0 °C dry storage, 0 °C moist stratification, or 30 °C moist stratification) for 30 d. After pre-treatment, seeds were incubated at 10 °C or 25 °C and germination fraction determined. Significantly different pre-treatment/incubation combinations are denoted with different lowercase letters.

Figure 5. The fate of viable *E. viscidulum* seeds buried for one year in habitat. Seed bags were exhumed at 3 mo intervals and incubated for 30 d at 10 °C and 25 °C and germinating seeds counted. Only 0 mo and 3 mo exhumate dates were included in analysis. The “*” denotes 6 mo – 12 mo dates when bags exhumed contained too few viable seeds for analysis after nearly complete field germination of seeds occurred (empty seed coats in bags, denoted with cross-hatch; see text for details). Maximum and minimum daily ambient temperatures (red and blue lines, respectively) and rainfall (black bars) were downloaded from PRISM (Oregon State University, data downloaded 11/17/2023).

Figure 6. Seed set for *E. viscidulum* individuals grown in the USGS shade house and enclosed with either an open mesh bag to allow access by invertebrate pollinators or a closed mesh bag to exclude pollinators. Seed set was not statistically different between the pollinator treatments.

Figure S1. Maximum and minimum greenhouse temperatures (red and blue lines, respectively) recorded during the 2020/2021 (upper panel) and 2021/2022 (lower panel) seed bank germination trials. Grey zones denote periods of watering/chemical treatments interspersed with periods of dry-down in white.

Figure 1

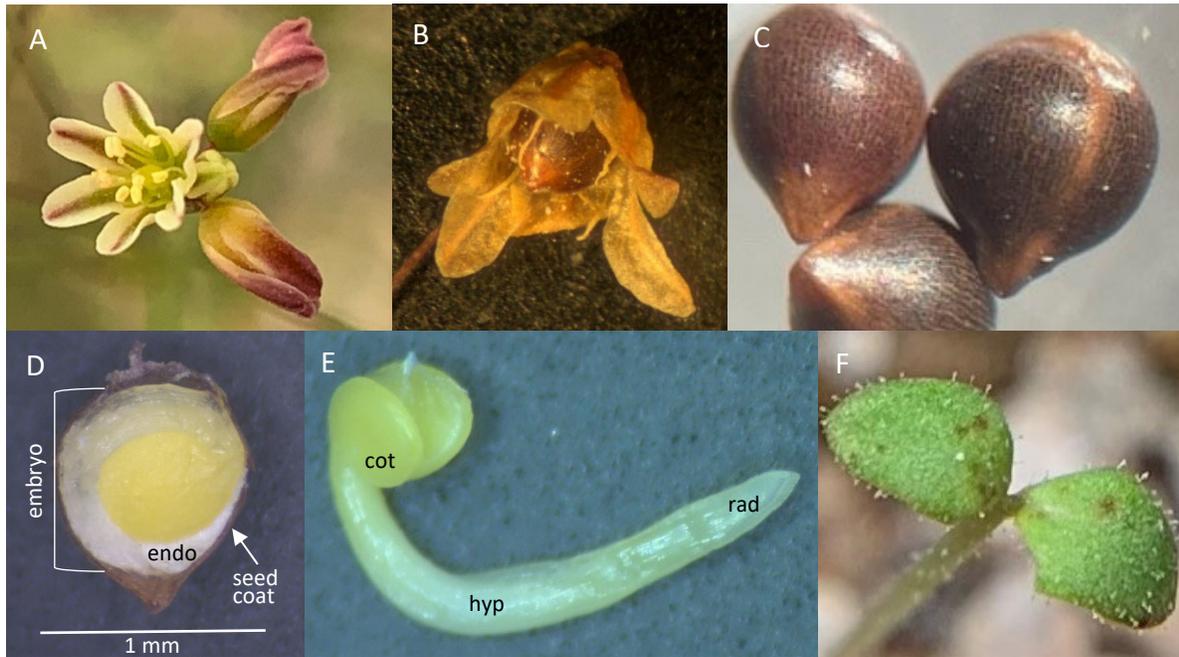


Figure 2

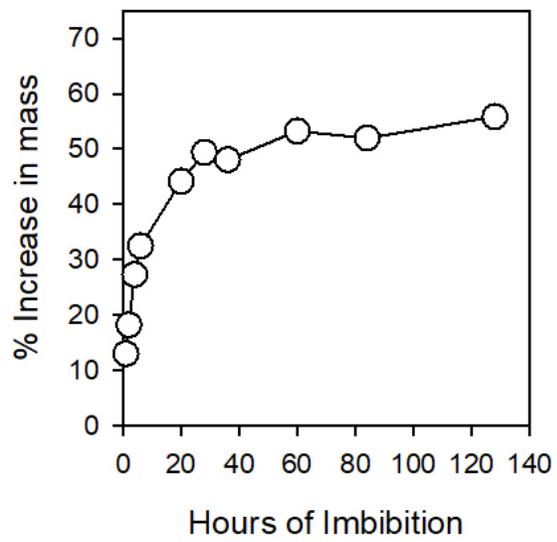


Figure 3

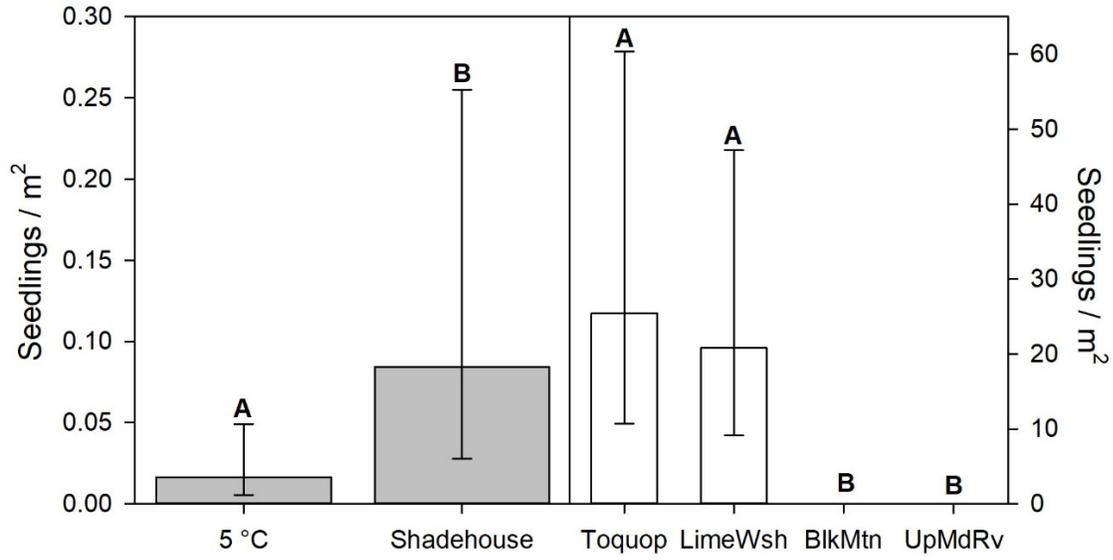


Figure 4

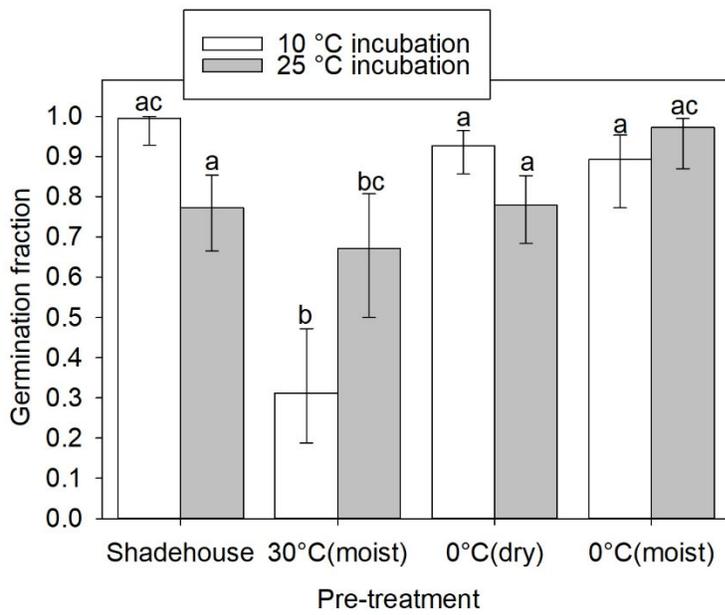


Figure 5

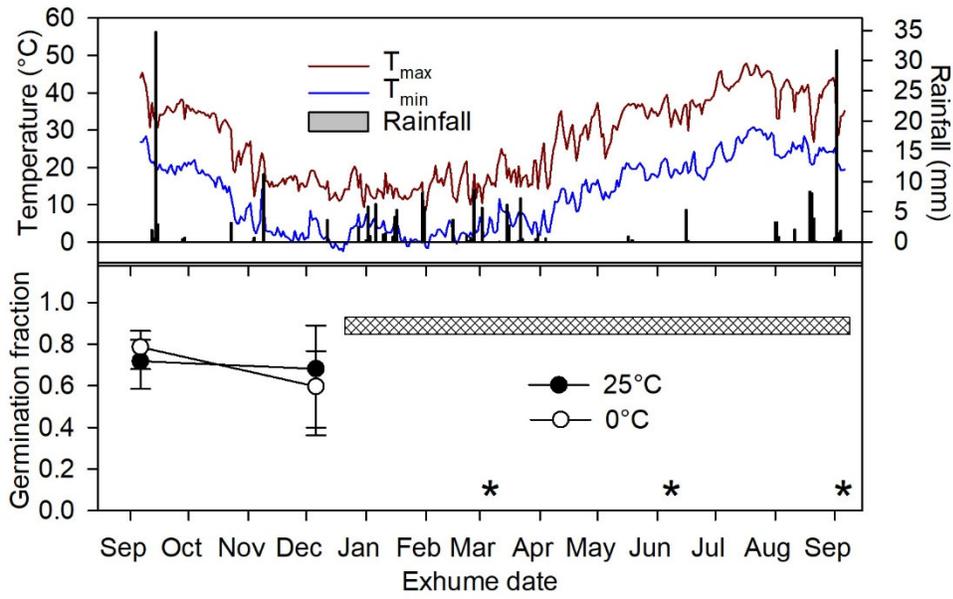


Figure 6

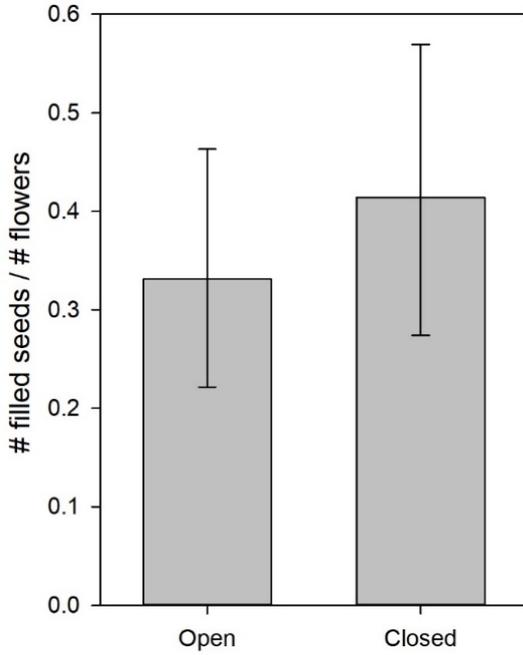
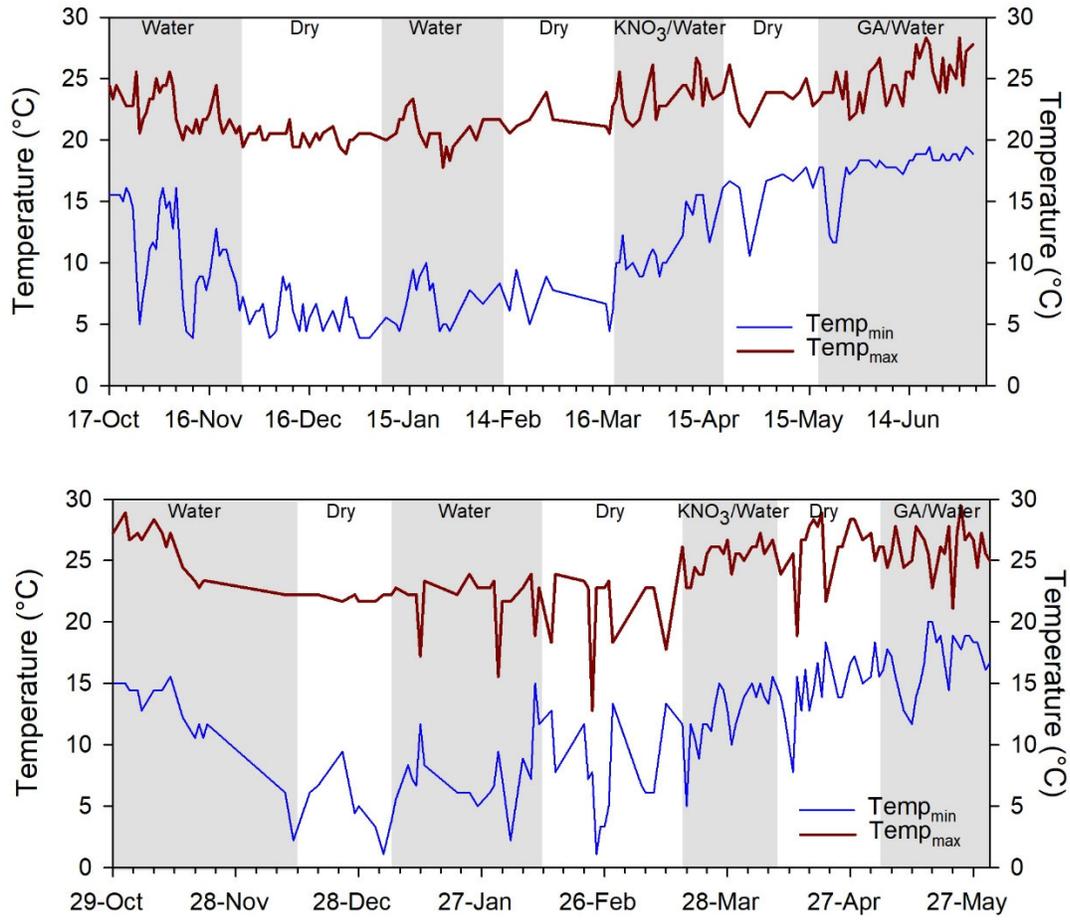


Figure S1



Appendix A. Research Activities on *Astragalus geyeri* var. *triquetrus*

Scouting Populations

During springs 2020 and 2022 we made multiple scouting trips to search for live *Astragalus geyeri* var. *triquetrus* plants from which to collect seed for seed ecology experiments (Table A1). We found sparse occurrences of *A. geyeri* var. *triquetrus* during spring 2020, but we were unable to collect seeds because of the timing for our collection permits in June, after plants had fully senesced and seeds had dispersed. No plants were observed at the Bark Bay and Sandy Cove populations (see next section) when we attempted to identify *A. geyeri* var. *triquetrus* plants for our autumn soil seed bank collection trips; consequently, we did not conduct additional scouting trips during spring 2021. Following low precipitation during the winter of 2021/22, no live *A. geyeri* var. *triquetrus* plants were found in our surveys during spring 2022. Ironwood Consultants likewise found limited numbers of plants in 2020 (250 plants at 14 sites), zero plants in 2021 and 2022, and only 14 plants at two sites in 2023 (Ironwood Consultants, 2023 Clark County Progress Review, Las Vegas, Nevada).

Table A1. *Astragalus geyeri* var. *triquetrus* scouting efforts. The number of soil seed bank collection trips is denoted in parentheses and the number of plants found denoted in bold.

Population ¹	2020	2021	2022
Mud Lake	3 (1) 5	-	1 0
California Wash	3 (1) 25	-	1 0
Weiser Wash	2 0	-	1 0
Logandale	2 0	-	1 0
Muddy River	2 0	-	1 0
Toquop Wash	1 0	-	1 0
Ebony Cove	2 0	-	-
Valley of Fire Wash	1 0	-	-
Sandy Cove	1 0	1 (1) 0	-
Bark Bay	-	1 (1) 0	-
Mormon Mesa	-	-	1 0

¹ Population unit names follow The Nature Conservancy (2007)

Soil seed bank

Soil seed bank samples were collected at 20 points within known *A. geyeri* var. *triquetrus* habitat at each of two populations on 16 July 2020 (Mud Lake and California Wash, on BLM land), within the Sandy Cove population (on NPS land, accessed by boat) on 14 September 2021, and within the Bark Bay population (on NPS land) on 20 September 2021. Ten of the 20 sampling points at Mud Lake were placed around senesced plants identified during the spring, and the other 10 were located at historic points provided by Clark County DCP. For California Wash, 16 of 20 points were at senesced plants and 4 were at historic points, while all 20 sampling points within both Sandy Cove and Bark Bay populations were at historic points due to lack of plants in 2021 (see Data Deliverable D28 Appendix B for locations).

Four 10.5 cm × 10.5 cm metal frames were placed at cardinal directions around each plant or point, and soil was collected from 0 – 2.5 cm depth within the frames (441.0 cm² per sampling point) and transported to the laboratory. Soils from two frames per point were placed in

a plastic zip top bag for cold, dry storage (refrigerator at 5 °C), while soils from the other two frames were stored in a zip top bag at the USGS, Boulder City, Nevada shade house under ambient, summer/autumn conditions (warm dry storage; daytime maximum 26 – 45 °C and nighttime minimum 15 – 30 °C in 2020 and daytime maximum 18 – 36 °C and nighttime minimum 7 – 24 °C in 2021).

Soils were incubated at the USGS greenhouse starting in autumn: 17 October 2020 and 29 October 2021. Soil samples were split across multiple bulb pots (15 cm diameter, lined with weed blocker cloth) to maintain 2.5 cm depth and randomized over six benches (blocks) across the greenhouse. A thin layer of vermiculite was added to the surface of each pot to maintain moisture (medium grain, Therm-O-Rock). We modified our approach from emergence methods developed for the Great Basin (Young *et al.* 1969, Young and Evans 1975, Young *et al.* 1981) and specifically used for seed bank studies of Mojave Desert species (Esque 2004, DeFalco *et al.* 2009, Scoles-Sciulla and DeFalco 2009, DeFalco and Esque). All pots were subjected to four wetting cycles known to promote germination of seeds (Meyer and Poljakoff-Mayber 1982, Baskin and Baskin 2014) alternating with approximately 3 wk intervals when soils were not watered and allowed to air dry. In the first and second wetting phases, pots were watered with tap water using a hand-held sprayer, and water was applied until it drained from the bottom of the pots, but no standing water remained. In the third and fourth wetting phases, 50 mL of a 0.01 M solution of potassium nitrate and 50 mL of a 6.5×10^{-4} M solution of gibberellic acid, respectively, was initially added followed by daily watering with tap water. During each wetting phase, seedlings of all species were counted and harvested as soon as they could be identified. Unknown seedlings were transplanted to pots and given time to develop so they could be identified to species. Watering ceased and dry-down began once emergence declined and no new germinants appeared for a week.

Although germinants of native and nonnative species emerged during the seed bank assays, no *A. geyeri* var. *triquetrus* individuals emerged. We have reported *Astragalus* spp. emerging from previous seed bank emergence studies (*A. acutirostris*, *A. lentiginosus*, DeFalco *et al.* 2009; *A. nuttallianus*, DeFalco *et al.* 2014), and we found *A. sabulonum* from *A. geyeri* var. *triquetrus* habitat in the current study, which would indicate this method is suitable for triggering germination and emergence of *A. geyeri* var. *triquetrus*. We subsequently sorted a subset of the soil samples to determine whether *A. geyeri* var. *triquetrus* seeds were extremely rare in habitat or if seeds remained ungerminated through the 9-mo emergence study due to physical dormancy (Soltani *et al.* 2020). Only seeds of *Ambrosia dumosa*, *Bromus* sp., and *Stipa hymenoides* were recovered, and 98% tested inviable based on tetrazolium testing; no seeds of *A. geyeri* var. *triquetrus* were found in these same samples. These results indicate that the seeds of *A. geyeri* var. *triquetrus* were rare in the habitat during the period of study (during a prolonged megadrought, Williams *et al.* 2022). We are investigating why this species is rare in previously identified habitat through studies of competition with invasive annual species, herbivory, and pollination limitation in future phases.