Title: Seed dormancy, germination requirements, and seed banks of *Penstemon albomarginatus* (Plantaginaceae), a rare herbaceous perennial of the northeast Mojave Desert

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ABSTRACT

We conducted laboratory, greenhouse, and habitat experiments to understand seed dormancy dynamics and the mating system for the rare herbaceous desert perennial *Penstemon albomarginatus* (white-margined beardtongue) for informing conservation and *ex situ* propagation for a species under threat from land use and urban expansion. Despite the drought conditions during our study that affected reproduction, we collected seeds exposed to summer temperatures at dispersal from plants at Ivanpah Valley, Nevada and incubated them under varying seasonal temperatures in a laboratory germination trial. The highest germination occurred under cold (winter) stratification and the transition from warm to cold stratification (autumn to winter), compared with seeds incubated under warm (summer or spring) stratification. Seeds from the same source were buried in habitat, exhumed over 3 mo intervals, and incubated in the laboratory at 5 °C. Little rain fell during the year-long burial trial. The highest fraction of germinable (non-dormant) seeds were those that after-ripened under summer temperatures, but the fraction declined with cooling temperatures during autumn through the following spring, and rebounded the following summer, demonstrating seasonal dormancy cycling in this species. Comparing seed set for bagged and unbagged flowers in habitat where natural pollinators occurred, we found that fruit production was lower in bagged flowers compared with bags allowing access; thus, similar to other congenerics, *P. albomarginatus* is an obligate outcrosser. Surface soils containing the seed bank were collected near plants after seed dispersal and placed in cold, dry storage (5 °C) or warm, dry storage in a shade house (26 ºC – 45 ºC maximum and 15 ºC – 30 ºC minimum); soils from both were subsequently maintained in a greenhouse under a series of wetting/drying cycles to promote germination. The number of *P. albomarginatus* seedlings emerging from soils under warm, dry storage was 3× greater than that from soils placed under cold, dry storage, confirming that seeds required summer after-ripening to become nondormant in autumn.

Collectively, our studies shed light on the seed ecology and mating system for this rare Mojave Desert *Penstemon*. We show that *P. albomarginatus* can be propagated from soils collected in habitat, and seeds can be collected directly from plants in habitat and used for propagation once high temperature after-ripening and cold incubation conditions are met. As with any stored seed germplasm with physiological dormancy, periodic seed testing should be performed during long term storage of conservation collections.

INTRODUCTION

Seed dormancy is an adaptation for many desert and semidesert species to prevent premature germination under unfavorable soil moisture and temperature conditions while maintaining a persistent seed bank that allows germination under periods of more favorable conditions. These sometimes complex dormancy strategies can challenge conservation and management of many rare desert species because species-specific dormancy strategies have not been elucidated. Temperature control of seed dormancy and germination is the primary environmental cue regulating seedling emergence (Baskin and Baskin 1988). Environmental cues that break seed dormancy are species- and habitat-specific, and these dormancy patterns are also genetically controlled in some species (Baskin and Baskin 1998, Meyer et al. 1995, Meyer and Kitchen 1992). Seed dormancy prevents all viable seed from germinating together during "optimal" conditions – conditions that may quickly turn unfavorable for seedling growth and survival in unpredictable environments (Philippi 1993, Baskin and Baskin 1998, Venable 2007).

White-margined beardtongue (*Penstemon albomarginatus* M.E. Jones; Plantaginaceae) is a rare herbaceous perennial restricted to the northeastern Mojave Desert, with a central cluster of populations in southern Nevada and disjunct populations in eastern California and southwestern Arizona (The Nature Conservancy 2007, Everett 1950). The species is a psammophile restricted to soils with little profile development, ranging from sand dunes to sandy/gravelly washes, mainly in small drainages, on valley floors, or on gentle foot-slopes at 363 m – 1057 m elevation (The Nature Conservancy 2007). Seed dispersal is likely by gravity, and juvenile recruitment events are rare, likely tied to episodic and successive wet years for seed production and germination (Etyemezian et al. 2010). The species is threatened by urban and energy development in many parts of its range (Anderson 2001, Smith 2001 cited in The Nature Conservancy 2007, Zitzer et al. 2010), is currently listed as a BLM sensitive species, and was recently petitioned for federal protection (Center for Biological Diversity 2023). Mitigation and conservation efforts are important for protecting this species under its current distribution and areas essential for the long term persistence of the species. Understanding seed dormancy, germination requirements, and the mating system for the species is important for guiding nursery propagation work and for informing the timing and optimal seed treatments for potential re-introduction efforts, particularly under rapidly changing temperature and precipitation patterns that may impact the establishment of new plants.

Seeds with physiological dormancy (PD) readily imbibe water and germinate in response to specific temperature cues in variable environments (Baskin and Baskin 1998). PD is a common type of seed dormancy, including for many species in Plantaginaceae (Baskin and Baskin 1998, Meyer 2006, Baskin and Baskin 2020). Seeds that have nondeep PD can shift from dormant or conditionally dormant states – when the temperature, moisture, or light requirements are not met for all or part of the seed population – to a non-dormant state where seeds germinate at most temperatures when rainfall is not limiting (Baskin and Baskin 1998). *Penstemon* species from high elevation/cold zones typically produce seeds that are dormant when they disperse in summer and require stratification (cold, moist conditions) to germinate (e.g., *P. utahensis, P. acuminatus,* and *P. moffattii*, Allen and Meyer 1990; *P. haydenii*, *P. gibbensii*, Tilini et al. 2016). In contrast, low elevation/warm desert species produce seeds that are nondormant at dispersal and enter dormancy through a cold stratification period (e.g., *P. bicolor, P. palmeri, P. petiolatus*) (Meyer et al. 1995, Meyer and Kitchen 1992). Species whose ranges experience cold winters required long cold stratification periods for dormancy loss, while species with a broad distribution range lack a cold stratification requirement in warm desert populations and require cold stratification in high-elevation populations (Meyer et al. 1995).

Our study aimed to characterize the germination and seed dormancy patterns of the narrow Mojave Desert endemic *Penstemon albomarginatus* through controlled experimental trials. We identified the temperature requirements for germination and how changes in seasonal temperatures regulate dormancy by incubating seeds under a range of temperatures in the laboratory. Our *in situ* seed burial and retrieval study sheds light on whether seeds of this rare species cycle between dormant and nondormant states and identifies the temperature cues that regulate these shifts in dormancy. We also examined how to propagate the species from soils collected from habitat (soil seed bank) and incubated in a greenhouse, and identified the mating system for this species by manipulating pollinator access when flowers were in bloom in habitat. These studies together can inform conservation seed collections and storage, and guide propagation of the species from seed.

METHODS

Study sites

Penstemon albomarginatus is endemic to the southeastern Mojave Desert with southern Clark County, Nevada at the center of distribution and additional disjunct populations in Nye County, Nevada, San Bernardino County, California, and Mohave County, Arizona (The Nature Conservancy 2007). The four populations in Clark County, Nevada are approximately 48 km southwest of Las Vegas near Jean, Nevada and occur within 20 km of one another: Ivanpah Valley (35°45' N 115°19'W), Hidden Valley (35°50' N 115°09'W), Roach Lake (35°40' N 115°19'W), and Jean Lake (35°47' N 115°13'W). The 30-yr normal of annual precipitation at the site is 143 mm/yr (range 29 – 272 mm/yr) and during this period, 76% of precipitation fell during cool-season months (October – April) while 21% fell during warm-season months (July – September) (PRISM Climate Group, Oregon State University, accessed 12/04/2023). *P. albomarginatus* seeds disperse from adult plants in May – June, with summer monsoonal moisture unpredictable and variable in its effect on surface soil moisture during July – August before temperatures decline and cool-season rains begin in September/October.

Seed characteristics

We examined the internal seed anatomy of *Penstemon albomarginatus* seeds from 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). These characteristics, in combination with germination trials, were used to determine seed dormancy class based on Baskin and Baskin (2004). Photo documentation of the cotyledons as they emerged from the soil seed bank was also taken for future reference when searching for seedlings in habitat or during propagation.

Seed germination and dormancy, 2020

Seed availability for *P. albomarginatus* at the four populations was unpredictable over the study period due to extended drought in the region. A total of 1,676 seeds were collected from eight maternal plants at least 5 m apart at the Ivanpah Valley population on 4 June 2020. Most flowers dried out without developing fruit at the Roach Lake, Jean Lake, and Hidden Valley populations, so we avoided collecting from those populations to ensure we impacted < 10% of the standing seed crop. We isolated hard, dark intact seeds by lightly rubbing dried capsules to remove debris. Seeds were stored in envelopes at ambient temperatures in the USGS shade house for 28 d (daytime highs 40 °C – 50 °C and nighttime lows 20 °C – 30 °C) until the start of seed burial and laboratory germination studies.

We used 726 seeds collected on 4 June 2020 to determine dormancy patterns for *P. albomarginatus* across multiple incubation environments (Baskin and Baskin 2014). Our environments were established to represent four seasonal controls and two different seasonal shifts in temperatures for these seeds, transitioning across incubation environments approximately every 28 d (summer → fall → winter → spring and winter → spring → summer → fall). Seeds were divided among three replicate Petri plates for each incubation environment, with approximately 40 seeds per plate. Starting on 1 July 2020, seeds were moved onto moist filter paper (Whatman 1001-110), Petri plates sealed with Parafilm® (Bemis Co. Inc., Neenah, WI) and placed in their respective incubation environments: winter (refrigerator, constant 5 °C), spring (growth chamber, 20 °C/10 °C), summer (shade house, prevailing Boulder City, NV temperatures), and fall (greenhouse, semi-controlled to stay warmer than 10 °C and cooler than 29 °C). Filter papers were kept moist with deionized water during daily germination checks. To further prevent seeds from drying out in the greenhouse and shade house, where humidity was low, the plates were set on top of a 4 cm layer of gravel in plastic tubs, which were regularly filled with water to a depth of 2 cm – 3 cm. Plates were monitored daily until 3 November 2020: seeds were considered germinated upon emergence of a radicle and moldy seeds were recorded and discarded. Remaining ungerminated seeds at the end of the trial were tested for viability using a tetrazolium staining protocol for species in the Plantaginaceae (AOSA 2010) and germination fraction determined. Germinated seeds were transplanted out of plates and into pots in the greenhouse.

Seed burial, 2020 - 2021

For the seed burial experiment, 950 seeds collected on 4 June 2020 were sorted by maternal line and evenly stratified across 25 fine nylon mesh packets (5 cm × 5 cm). Because of differences in seed numbers among maternal plants, we prepared 2 – 9 seeds per matriline for each of the 5 burial intervals (0 mo, 3 mo, 6 mo, 9 mo, 12 mo) and 5 replicate packets, which resulted in 38 seeds per replicate (190 seeds per interval) and proportional representation across maternal lines. When placing seeds into packets, handling revealed poor seed fill for some seeds (i.e., seed disintegrated when rubbed between index and thumb finger); thus, we performed a "rub" test on all seeds to remove unfilled seeds: mean ± SD, 54.8 ± 10.9% filled seed. Remaining numbers for each treatment combination ranged from 13 – 32 seeds per replicate bag, while total seed numbers pooled over replicates for each interval ranged from 101 – 106 seeds.

Seed packets were buried at five random locations situated in shrub interspaces within the Ivanpah Valley population on 13 July 2020. 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. On 5 August 2020, we began germination and viability tests on the 0 mo seeds; the other seed bags were exhumed and tested on 13 October 2020 (3 mo), 13 January 2021 (6 mo), 12 April 2021 (9 mo), and 13 July 2021 (12 mo). At each collection time, one packet from each burial location was exhumed and transported to the laboratory for germination and viability tests.

Seeds were removed from each mesh bag, counted, placed in a Petri plate on moist filter paper (Whatman 1001-110) and sealed with Parafilm® (Bemis Co. Inc., Neenah, WI). Because of limited seed availability, all plates were incubated under a single temperature regime: 5 °C in a refrigerator for 90 d and then moved to a growth chamber set at 20 °C day/10 °C night (12 h photoperiod) for 17 d. Filter papers were kept moist with deionized water and seeds were monitored every 2 d. Seeds were considered germinated upon emergence of a radicle, while moldy seeds were recorded and discarded. Seeds remaining at the end of incubation were tested to determine viability using a tetrazolium staining protocol for species in the Plantaginaceae (AOSA 2010). Germinated seeds were transplanted to pots in a greenhouse (50:50 sand:potting soil mix) to document establishment as seedlings and mature plants.

Soil seed bank, 2020 – 2021

Soil seed bank samples were collected under the drip line of 20 senesced *P. albomarginatus* plants at each of the four Clark County populations on 13 – 14 July 2020, after fruits had dehisced. Four 10.5 cm × 10.5 cm metal frames were placed at cardinal directions around each plant, and soil collected from 0 cm – 2.5 cm depth within the frames (441.0 cm^2 per sampling point). Soils from two frames per plant were placed in a plastic zip top bag for cold, dry storage (refrigerator at continuous 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 ºC – 45 ºC and nighttime minimum 15 ºC – 30 ºC).

On 17 October 2020, soil from each bag was spread to approximately 2 cm depth in two bulb pots (15 cm diameter) lined with weed blocker cloth to prevent soil loss and covered with 78 ml of vermiculite (Therm-O-Rock West Inc., Chandler, Arizona) to help retain moisture. Control pots were filled with coarse, washed sand and covered with vermiculite to detect seeds drifting into the greenhouse and pots. All samples (n = 320 pots) were randomized across six benches (blocks) in a temperature semi-controlled greenhouse and subjected to four wetting cycles known to promote germination for species that have physiological dormancy (Meyer and Poljakoff-Mayber 1982), the most common type of seed dormancy and typical of *Penstemon* species (Baskin and Baskin 2014). Our seed bank emergence method was modified from the Great Basin (Young *et al.* 1969, Young and Evans 1975, Young *et al*. 1981) and specifically used in seed bank studies for Mojave Desert species (Esque 2004, Scoles-Sciulla and DeFalco 2009, DeFalco et al. 2009). During the first and second wetting phases, pots were watered with tap water using a hand-held sprayer 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 to each pot followed by daily watering with tap water. Wetting cycles lasted until no more seedlings emerged for one week, alternating with approximately 3 wks – 4 wks when soils were not watered and allowed to air dry. 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. Seedlings of *P. albomarginatus* were easily identified by the white margins of their first true leaves (see *Results*) and transplanted to pots with a 50:50 mix of sand and organic mulch.

Seedling production, 2020 – 2022

We transplanted germinants as they emerged during the soil seed bank, seed germination, and seed burial trials into 10 cm wide × 10 cm tall pots with a 50:50 mix of washed sand and potting soil. On 21 December 2020, live *P. albomarginatus* seedlings from the germination trial and seed bank emergence were transplanted to 10 cm diameter × 30 cm tall cylindrical pots to encourage deep rooting in one of two soil mixes (50:50 or 75:25 sand:potting soil). We then initiated the first phase of testing watering method (deliver water to pots from above or below through capillary action) and the two soil mixtures in a factorial experiment with initial plant size randomly assigned to treatment combinations ($N = 31$ plants total). Watering from above occurred every 2 d – 3 d on half the number of plants to maintain pot capacity, while below watering was similarly maintained on the other plants by adding water every 3 d – 4 d to a tub containing the pots and allowing the tub to dry out between waterings until 12 January 2021.

The seedlings that were alive from the watering trial moved on to a second phase of testing ($n = 22$ plants total). Continuing from initial transplant, we counted pairs of live leaves produced weekly from 21 December 2020 – 28 January 2021 and monthly thereafter when plants were non-dormant until 22 February 2022. During this phase, we first evaluated whether soil mixture affected leaf pair growth rate at the time of peak activity on 17 June 2021 (approximately 6 mo old plants). We also investigated the effect of soil mix and propagation environments by moving half the number of *P. albomarginatus* potted plants to the shade house on 1 October 2021 and maintaining the other half in the greenhouse. We then evaluated whether soil mixture and propagation environment affected leaf pair growth rate at peak activity on 22 February 2022 (approximately 14 mo old plants).

Mating system, 2022

We imposed flower bagging treatments on eight *P. albomarginatus* plants at the Ivanpah Valley population on 31 March 2022 to determine whether the species is an obligate outcrosser or can self-fertilize. On each plant, three similar sized stems with early flower buds received one of three treatments: entire stem enclosed in a 15 cm × 20 cm green fine-mesh organza bag (bag treatment) that denied access to pollinators, entire stem similarly enclosed but with large slits cut into bag to allow access to pollinators while controlling for potential microclimate effects of the mesh bag (bag control), and one stem without a bag to allow full access to pollinators (open treatment). When a stem had a few flower buds already open, that stem was assigned the open treatment if no other stem with unopened buds was available. Numbers of buds/flowers on stems were not statistically different among the treatments at the time bagging treatments were implemented ($F_{2,16} = 0.21$, $P = 0.816$). Flowering was complete by 18 April 2022 with only dead flowers and immature fruits attached to stems, and all treatment stems were bagged in place to allow fruits to mature and capture seed from shattering capsules. On 25 May 2022, all bags were collected and stored in paper envelopes in a shade house under prevailing summer temperatures. Two weeks after collection, viability of seeds was determined with tetrazolium staining as for other experiments.

Statistical analyses

We analyzed the responses of *Penstemon albomarginatus* 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 incubation environment (6 levels) on the "event/trial" response (i.e., germinated seeds/total viable seeds); the 28-d, 56-d, 84-d and 125-d intervals were tested separately to illustrate how the transitions in temperatures (e.g., winter to fall, and summer to spring) compared with the steady seasonal temperatures. Due to quasi-complete separation in the data (i.e., when particular treatment levels are confounded because the event = all ungerminated or all germinated, such as in the shade house at 28-d) 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 incubation temperatures.

For the seed burial trials, we tested the fixed effects of burial duration (5 levels) on the probability of individual seeds germinating during incubation (event = germinated) using the LOGISTIC procedure. One seed bag was not recovered from the 12-mo burial interval due to rodent activity. The ilink function generated estimates and 95% confidence limits on the mean probability scale. Odds ratios compared burial durations and *P*-values for multiple comparisons among mean probabilities were adjusted using Tukey-Kramer.

Soil seed bank counts of *P. albomarginatus* seedlings were analyzed in a generalized linear mixed effects model to compare the fixed effects of population (4 levels), storage condition (2 levels), and their interaction; we used a negative binomial distribution and a log link function with storage*replicate as a random effect. The ilink function generated estimates and 95% confidence limits on the mean seedling count scale.

Seedling survival and growth during propagation were analyzed during two phases. The probability of survival during the initial watering phase was analyzed using the GLIMMIX procedure to test the fixed effects of watering (2 levels) and soil mix (2 levels) and the interaction; we also included the number of leaf pairs at the beginning of the trial as a covariate of plant size. Similarly, we analyzed the effects of soil mix on relative leaf pair growth rate for the various phases of soil and propagation environment trials (log differences between leaf pairs at the beginning and end of the growth period).

RESULTS

Penstemon albomarginatus fruit is a capsule that splits lengthwise to release numerous seeds (Fig. 1A, 1B). Embryos are well-developed and differentiated into plumules, hypocotyl, and radicle at dispersal during summer (Fig. 1C). Cotyledons that emerged from soil seed bank were strap-shaped (Fig. 1D), and true leaves emerged in pairs with the distinctive white margin that gives this species its name (Fig. 1E).

Seed germination and dormancy

Seeds collected from *Penstemon albomarginatus* plants at Ivanpah Valley were initially stored dry for 25 d in the shade house prior to the germination trial. Exposure to the range of seasonal temperatures showed *P. albomarginatus* had its highest germination under winter conditions compared with spring, autumn, or summer temperatures during the first 28 d (Fig. 2). At 56 d, the continued winter temperature exposure again had higher germination than the other seasons, and was also statistically higher than the winter season that shifted to spring. At 84 d, the germination fraction response was complex due to an unintended shift in the incubation set points: specifically, the autumn temperature – represented by the greenhouse – had a sharp 8 °C decrease in maximum temperature, causing a rapid rise in the germination fraction. In contrast, the germination fraction of seeds that transitioned from summer → autumn → winter temperatures did not increase as precipitously. By 125 d, seeds in the original autumn treatment with its unplanned decrease in daytime high temperatures were largely nondormant, and the germination fraction in the summer treatment – represented by the shade house – began a steady increase as temperatures slowly changed, particularly as the minimum temperatures began declining (Fig. 2).

Seed burial

Germination fraction for *P. albomarginatus* was highest for seeds immediately after dispersal during summer (July), declined gradually in soils exposed to cooling temperatures in autumn (August – October) and winter (November – January), and reached lowest germinability (increased dormancy) as soil temperatures increased in spring (February – April); germination fraction rebounded with warm temperatures the following summer, May – July ($F_{4,115} = 4.36$, $P = 0.003$; Fig. 3). No germinated seeds from the 6-mo and 9-mo burial (n = 19) produced cotyledons – they exhibited low vigor and produced only radicles before dying. Germinated seeds from the 3-mo burial (n = 13) also largely produced only radicles before dying, although at least one did produce cotyledons. In contrast, the majority of germinated seeds in 0-mo and 12-mo burial samples (n = 38) produced radicles and cotyledons and developed into young seedlings.

Seedling emergence from seed bank

A total of 123 *P. albomarginatus* seedlings emerged from surface soils collected in habitat and subjected to 9 mo of alternating wetting and drying cycles; 13 of these seedlings died before they could be transplanted. The majority of seedlings emerged during the first wetting cycle (119 seeds, average maximum/minimum greenhouse temperatures = 22 °C/10 °C), no seedlings emerged during the second wetting cycle (21 °C/7 °C) or the potassium nitrate cycle (23 °C/11 °C), and a small number germinated during the gibberellic acid cycle (4 seeds, 25 °C/18 °C). The surface area per sampling point (441.0 cm^2) captured seeds at almost half or more of the number of sampling points at all populations: Hidden Valley (16 of 20 points), Roach Lake (11 of 20 points), Ivanpah Valley (10 of 20 points), and Jean Lake (8 of 20 points). Warm, dry storage of soils in the shade house for 3 mo prior to watering resulted in more than 3 times the number of seedlings emerging from the seedbank compared with soils under dry, cold storage (Storage: $F_{1,38}$ =7.84, $P = 0.008$, Fig. 4). Seedling numbers also differed among populations ($F_{3,114} = 4.90$, $P = 0.003$) with highest numbers collected at Hidden Valley, followed by Roach Lake, and Ivanpah Valley and Jean Lake populations (Fig. 4; no significant interaction).

Seedling production

After growing in smaller pots for 1 mo – 2.5 mo, we transplanted *Penstemon albomarginatus* seedlings to 10 cm diameter × 30 cm tall cylindrical pots to evaluate propagation potential. Transplants varied in initial size from cotyledons only (0 leaf pairs) to 3 leaf pairs (6 true leaves). During the first 22 d (21 December 2020 – 12 January 2021), we observed a declining status of plants watered through capillary action, and the initial size of the transplant also influenced survival. A one unit increase in the number of leaf pairs increased the odds of survival by 8.794 (leaf pairs, $F_{1,26} = 6.67$, $P = 0.016$), and overhead watering of seedlings increased odds of survival by 11.398 compared with watering from below (water, $F_{1,26} = 4.63$, $P = 0.0410$). Soil mixture ($F_{1,26} = 0.48$, $P = 0.495$) and the interaction between watering and soil mix ($F_{1,26} = 0.15$, $P = 0.702$) were not statistically significant. When we tested the soil mix effects on the seedlings that survived the watering treatments, we found that soil mix had no significant effect during the first (Soil; $F_{1,14} = 1.08$, $P = 0.317$) or second phase (Soil; $F_{1,10} = 0.57$, P =0.4683); propagation location also had no effect on the growth rate during the second phase (Location; $F_{1,10} = 1.89$, P =0.199). Following our propagation trials, we maintained the 22 surviving *P. albomarginatus* plants through March 2022 (15 mo old plants), after which time they began entering a dormant, leafless state by July 2022. Only two plants remained actively growing through March 2023 (2 yr old plants).

Mating system

The proportion of fruits formed from flowers was significantly lower for bagged stems (95% CI = 0.0169 – 0.1244) compared with the bag control (0.1472 – 0.3241) and the unbagged treatments (0.1613 – 0.3458) ($F_{2,16} = 5.16$, $P = 0.018$); bag control and unbagged treatments were not statistically different (Fig. 5). The negligible proportion of fruits for the bagged treatment resulted from one of the eight plants where two fruits formed five seeds. Insects visited open flowers on other stems when we first set up the experiment: a small number of bees, likely from the genus *Ashmeadiella* (family Megachillidae, Joseph Wilson, Utah State University, pers. comm.) were observed fitting their bodies snugly into the tubular flowers and foraging solely on *P. albomarginatus* in the area. A potter wasp (family Vespidae) was also observed, but did not venture inside the flowers, possibly stealing nectar from the base of the flowers (AS, pers. obs.).

DISCUSSION

Collectively, our experiments show that *Penstemon albomarginatus* seeds have non-deep physiological dormancy, which is the most common type of seed dormancy among angiosperms (Baskin and Baskin 2022). Seeds have a fully developed and differentiated embryo (Fig. 1C) and are likely nondormant or conditionally dormant at the time they disperse in late May, as indicated by high seedling emergence from our seed bank trial (shade house versus cold storage soils, Fig. 2) and seed burial trial (high germination fraction in July, Fig. 3). We found low germination fraction under warm incubation, indicating that warm summer temperatures limit germination in habitat, similar to other North American *Penstemon* species (*P. palmeri*, Meyer and Kitchen 1992; *P. eatonii*, *P. strictus*, Allen and Meyer 1990; *P. bicolor* and *P. petiolatus*, Meyer et al. 1995). Rainfall was insufficient during prolonged drought to promote germination in habitat during our seed burial trial (i.e., no empty seed coats to indicate germination during burial, Fig. 3). Seeds then acquired dormancy with the onset of winter and early spring temperatures and became nondormant after warm summer temperatures returned, an indication of dormancy cycling important for species of unpredictable environments (Baskin et al. 1993).

Our studies overlapped a period of drought during the past two decades that has gripped the southwestern U.S., including the Mojave Desert (Williams et al. 2022). Consequently, reproduction for *P. albomarginatus* was low, and we could not collect seeds across all four Clark County populations. Because of low seed availability, we could not test whether *P. albomarginatus* seeds from plants in habitat are dormant, conditionally dormant, or nondormant immediately upon dispersal during late spring/early summer. During our seed bank trial, lower seedling emergence in the soils exposed to 3 mo of storage implies either cold temperatures induced secondary dormancy in seeds that were nondormant at dispersal (Meyer et al. 1995), or summer after-ripening at high temperatures before collection shifted seeds from a (primary) dormant to nondormant state by autumn (Baskin and Baskin 1986). The same seed source that we collected for the seed burial and germination trials sheds light on dormancy state at dispersal. The cold, moist incubation during the germination trial had a moderate germinable fraction (< 0.50, Fig. 2) compared to the almost completely nondormant seed at the beginning of the seed burial trial (> 0.80, Fig. 3), the only difference being a 2-wk longer exposure to high temperatures in the shade house for the latter trial. We initially expected that seeds of *P. albomarginatus* were likely nondormant at dispersal consistent with other low elevation/desert *Penstemon* species (Meyer et al. 1995). Given the different germination fractions for germination and burial trials, and the greater seedling emergence from the soil seed bank, a period of warm, dry after-ripening (>1.5 mo after seed shatter) is more likely necessary to increase the germinability of seeds following dispersal. Chilling during a period with too little rain for the seeds to germinate induced dormancy, as seen in the reduced probability for germination during October – April burial. Other *Penstemon* species from warm desert populations had almost complete germinability under autumn temperatures, including the rare *P. bicolor* (93% – 97% germination without chilling) and *P. petiolatus* (84% – 87%) combined with dormancy induction through short chilling (Meyer et al. 1995). Seeds for these *Penstemon* species were stored under laboratory conditions for an unspecified amount of time, which could have a similar effect on dormancy release as high temperature after-ripening (Baskin and Baskin 2014). Seeds with summer after-ripening requirements are common for other herbaceous species so they are primed to respond to late autumn/winter rainfall (Baskin and Baskin 1986, Beatley 1974).

Seed dormancy that confers long-term survival in the soil seed bank can impede plant conservation seed collections or reintroduction to habitats when population numbers for rare species are already low. Forbs in southwestern deserts do not generally require cold stratification but do well with warm after-ripening periods or warm stratifications (Pendleton and Pendleton 2014). Although we did not directly test the effects of exogenous chemicals on *P.*

albomarginatus seed germination, application of growth regulators such as gibberellic acid or potassium nitrate can increase rates of germination and broaden the temperature window suitable for germination (Allen and Meyer 1990). We did not see a notable increase in germination after applying these chemicals during our seedbank study (4 germinants during gibberellic acid phase only), but it is possible that the majority of potential germinants had already emerged under favorable germination conditions during the first watering phase.

Maintenance of pollinators will be important for promoting reproduction in habitat, as our pollination study confirms that *Penstemon albomarginatus* is an obligate outcrosser. Whereas Griswold et al. (2005) found that pollinator visitors were infrequent to *P. albomarginatus* flowers during observations on cool, cloudy, and/or windy days in 1998, 2004, and 2005, species of *Penstemon* are normally attractive to bees and are well visited. *Penstemon* specialists have not been observed at *P. albomarginatus* flowers (Griswold et al. 2005), potentially due to their atypically small diameter flowers. However, multiple potential pollinator species have been observed visiting *P. albomarginatus* flowers in the southern Nevada populations, including *Anthidium paroselae*, *Ashmeadiealla gillettei*, *A. holtii*, *A. xenomastax*, and *Lasioglossum sisymbria* (Smith 2001, Griswold et al. 2005). We also confirmed that *Ashmeadiealla* spent time visiting the flowers of *P. albomarginatus* in habitat.

For narrowly distributed species, the potential for 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). There are recent concerns that *P. albomarginatus* is at high risk of extinction under the current climate conditions (Miller 2021), and there is additional concern that this risk is compounded by growing exurban development pressures and projected warming climate (Center for Biological Diversity 2023). Laboratory germination, greenhouse and seed burial studies that identify dormancy strategies and germination requirements are useful for predicting the timing and dormancy characteristics of seed populations as they respond to weather patterns (Allen and Meyer 2008), and potentially for predicting rare plant persistence in a changing climate (Bandara et al. 2019).

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Figure legends

Figure 1. Characteristics of *Penstemon albomarginatus*. Fruit capsules containing numerous small seeds (A), mature seeds released from capsule (B), developed/differentiated embryo (C), strap-shaped cotyledons (D), and first true leaf pairs (E). Labels include seed coat, hypocotyl ("hyp"), radicle ("rad"), and plumules ("pls"), and primary ("1°") and secondary ("2°") leaf pairs with visible white margins characteristic of the species. Photo credits: 1A, 1D, 1E, (Lesley DeFalco); 1B, 1C (Alex Stosich).

Figure 2. Cumulative germination fraction of *P. albomarginatus* for seeds subjected to different incubations in a move-along experiment. Seeds were collected from plants at the Ivanpah Valley population and stored dry in a shade house for 25 d prior to treatments. Incubations included controls for winter, spring, summer, and fall temperatures held as constant as possible (see text), and two incubations that varied temperature seasonally from winter through following fall, or summer through following spring. Arrows represent seasonal transitions occurring at 28 d, 56 d, and 84 d. Analyses on cumulative fractions were conducted separately for each day of incubation, and significant environment effects are denoted as different lowercase letters; significance is not interpretable across days of incubation. See text for changes in environmental controls during the 125-d period of incubation.

Figure 3. Soil temperature at 2 cm depth (average of 2 iButtons) and rainfall (PRISM, Oregon State University, downloaded for Ivanpah Valley population, 3/7/2023) measured for *P. albomarginata* seeds following 3 mo, 6 mo, 9 mo, and 12 mo of burial. Germination fractions (i.e., probability of germination, 95% CI) differed among burial durations (different uppercase letters denoted at $P < 0.05$ after Tukey-Kramer adjustment). Most rain fell during two storms: over 23 – 30 January (11.9 mm) and 11 – 13 March (10.0 mm).

Figure 4. *P. albomarginata* seedlings (95% CI) emerging from the soil seed bank after 9 mo of alternating wetting/drying cycles that included water only (first and second cycle), potassium nitrate treatment (third cycle), and gibberellic acid treatment (fourth cycle). Different uppercase letters denote differences among populations (left panel) located geographically north to south (Hidden Valley, Jean Lake, Ivanpah Valley, Roach Lake) and between storage conditions (right panel) where samples were stored dry for 102 d after collection in a refrigerator (cold, 5 °C) and in a shade house (warm; maximum 26 – 45 ºC and minimum 15 – 30 ºC) prior to wetting/drying cycles.

Figure 5. Seed production per flower (left panel) for stems of *P. albomarginatus* assigned to one of three pollination treatments: Unbagged, Control (enclosed with a bag slit with large holes to allow access by invertebrate pollinators) or Bagged (enclosed with a closed bag to exclude pollinators). Significantly different treatments are denoted with different uppercase letters. Bagging treatments shown for one plant in habitat (right panel). Photo credit: A. Stosich

Figure 1

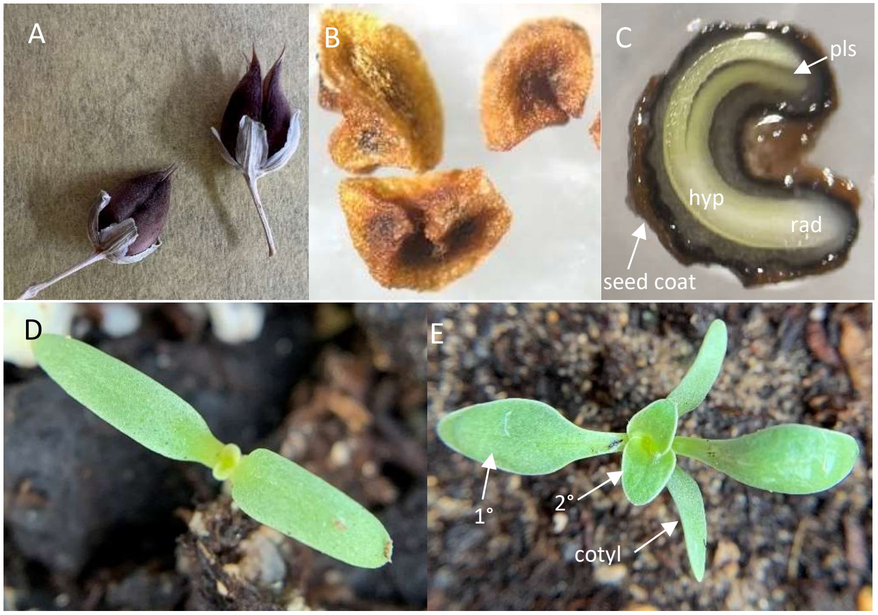

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Figure 2

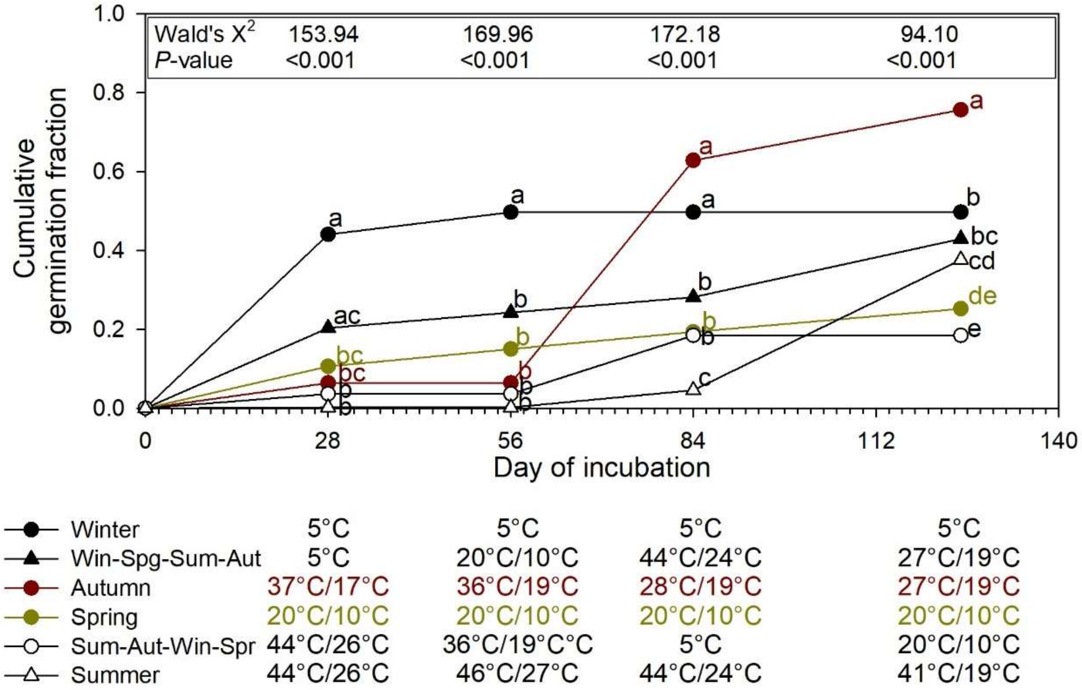
Wald's X² 153.94 169.96 172.18 94.10
P-value <0.001 <0.001 <0.001 <0.001
Cumulative germination fraction
0.0
0.2
0.4
0.6
0.8
1.0
Day of incubation
0
28
56
84
112
140
a
ac
bc
bc
b
b
a
b
b
b
b
b
a
a
b
b
b
c
a
b
bc
cd
de
e
Winter 5°C 5°C 5°C 5°C
Win-Spg-Sum-Aut 5°C 20°C/10°C 44°C/24°C 27°C/19°C
Autumn 37°C/17°C 36°C/19°C 28°C/19°C 27°C/19°C
Spring 20°C/10°C 20°C/10°C 20°C/10°C 20°C/10°C
Sum-Aut-Win-Spr 44°C/26°C 36°C/19°C°C 5°C 20°C/10°C
Summer 44°C/26°C 46°C/27°C 44°C/24°C 41°C/19°C

Figure 3

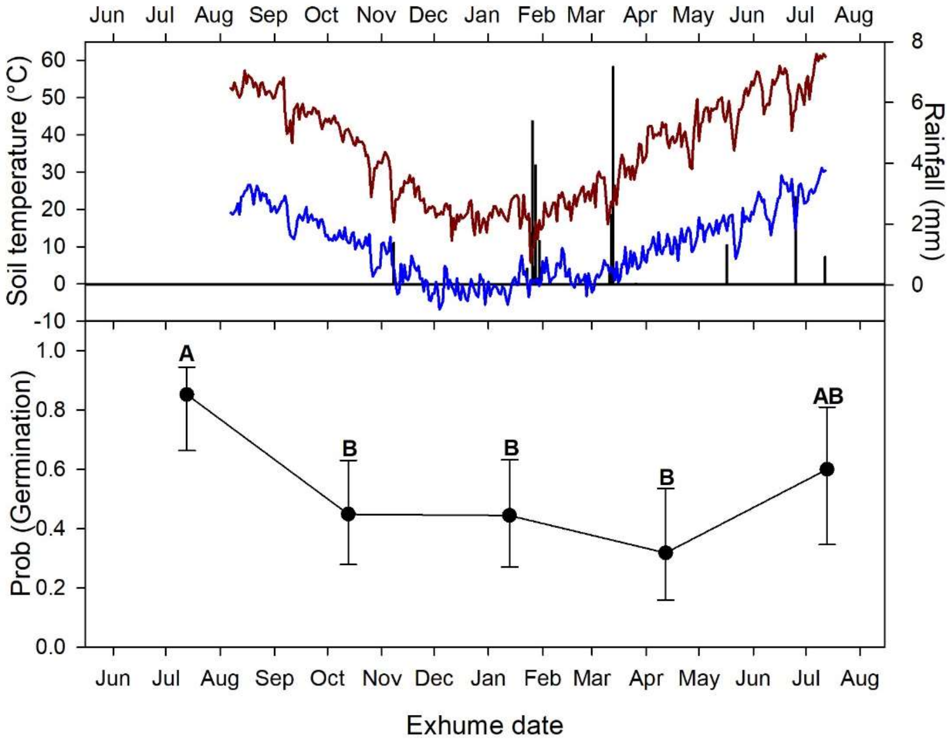
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Soil temperature (°C)
60
50
40
30
20
10
0
-10
Rainfall (mm)
8
6
4
2
0
Prob (Germination)
1.0
0.8
0.6
0.4
0.2
0.0
A
B
B
B
AB
Exhume date

Figure 4

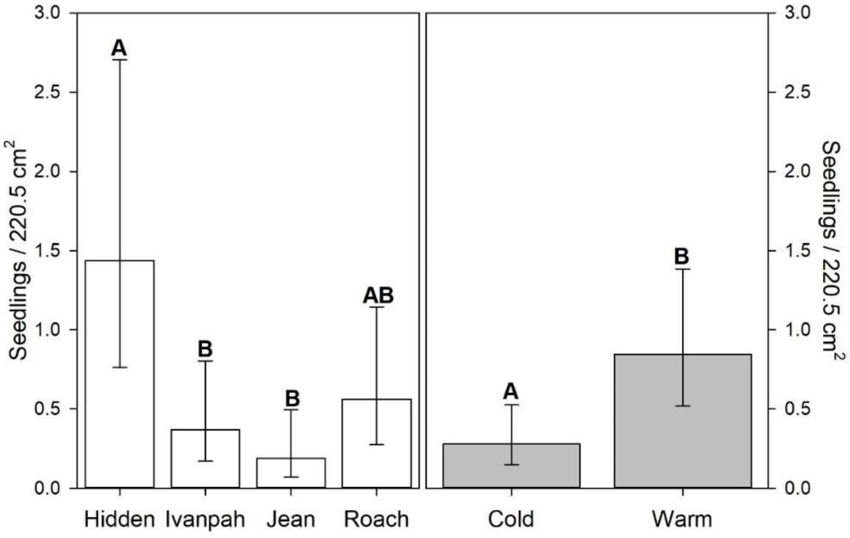
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Seedlings / 220.5 cm^2
A
B
B
AB
Hidden
Ivanpah
Jean
Roach
A
B
Cold
Warm
Seedlings / 220.5 cm^2

Figure 5