

Seed bank dynamics of blowout penstemon in relation to local patterns of sand movement on the Ferris Dunes, south-central Wyoming

Kassie L. Tilini, Susan E. Meyer, and Phil S. Allen

Abstract: Plants restricted to active sand dunes possess traits that enable both survival in a harsh environment and local migration in response to a shifting habitat mosaic. We examined seed bank dynamics of *Penstemon haydenii* S. Watson (blowout penstemon) in relation to local sand movement. We measured within-year sand movement along a 400 m transect and examined plant density, seed production, and seed density in relation to this movement. Plant densities were highest in areas of moderate sand movement. Annual seed rain averaged 13 seeds·m⁻², whereas persistent seed bank density (0–10 cm depth) averaged only 0.1 seeds·m⁻². A laboratory burial experiment with nondormant (chilled) seeds showed that most deeply buried seeds (>6 cm) were held in enforced dormancy under spring conditions, while seeds at intermediate depths (4–6 cm) were induced into secondary dormancy that was broken by subsequent drying and re-chilling, thus promoting seed carryover until the following spring. Most near-surface seeds produced seedlings. Enforced and secondary dormancy provide mechanisms for maintaining a persistent seed bank of more deeply buried seeds that could become part of the active seed bank as sand movement re-exposes them near the surface. This could facilitate both population persistence and migration as previously occupied habitat becomes unsuitable.

Key words: active sand dune habitat, cyclic dormancy, *Penstemon haydenii*, plant migration, secondary dormancy.

Résumé : Les végétaux confinés aux dunes de sable actives possèdent des traits qui leur permettent de survivre dans un environnement rude et de migrer localement en réponse à une mosaïque d'habitats en mouvement. Les auteurs ont examiné la dynamique de la banque de graines de *Penstemon haydenii* S. Watson (penstémon Hayden) en fonction du mouvement local du sable. Ils ont mesuré au cours d'une année le mouvement du sable le long d'un transect de 400 m et examiné la densité des végétaux, la production de graines et la densité des graines en fonction du mouvement. La densité des végétaux était la plus élevée dans les zones à mouvement de sable modéré. La dispersion aérienne des graines était en moyenne de 13 graines·m⁻², alors que la densité de la banque persistante de graines (0–10 cm de profondeur) était en moyenne de 0,1 graine·m⁻² seulement. Une expérience d'enfouissement en laboratoire avec des graines non dormantes (refroidies) montrait que les graines enfouies plus profondément (>6 cm) étaient gardées en dormance forcée en conditions printanières, alors que les graines enfouies à des profondeurs intermédiaires (4–6 cm) étaient plongées dans une dormance secondaire qui était levée par un assèchement et un refroidissement subséquents, favorisant ainsi une réserve de graines jusqu'au printemps suivant. Les graines les plus près de la surface produisaient des semis. Les dormances forcée et secondaire constituent des mécanismes permettant de maintenir une banque persistante de graines à partir de celles enfouies plus profondément qui pourraient faire partie de la banque de graines actives lorsque le mouvement du sable les réexpose plus près de la surface. Cela pourrait faciliter tant la persistance de la population que sa migration lorsqu'un habitat précédemment peuplé devient inadéquat. [Traduit par la Rédaction]

Mots-clés : habitat de dunes de sable actives, dormance cyclique, *Penstemon haydenii*, migration des végétaux, dormance secondaire.

Introduction

The unique adaptations that permit plants to survive and prosper in active dune environments have been an object of study for many decades (Maun 1998). Many of

these early studies were carried out in coastal or lake-shore dune systems under relatively mesic climatic regimes. Recently there has been intense interest in the ecology of desert sand dunes, particularly in China,

Received 28 January 2017. Accepted 28 March 2017.

K.L. Tilini and P.S. Allen. Department of Plant and Wildlife Sciences, Brigham Young University, Provo UT 84602, USA.

S.E. Meyer. USFS Rocky Mountain Research Station, Shrub Sciences Laboratory, 735 N. 500 East, Provo UT 84606, USA.

Corresponding author: Susan E. Meyer (email: smeyer@fs.fed.us).

This work is free of all copyright and may be freely built upon, enhanced, and reused for any lawful purpose without restriction under copyright or database law. The work is made available under the [Creative Commons CC0 1.0 Universal Public Domain Dedication](https://creativecommons.org/licenses/by/4.0/) (CC0 1.0).

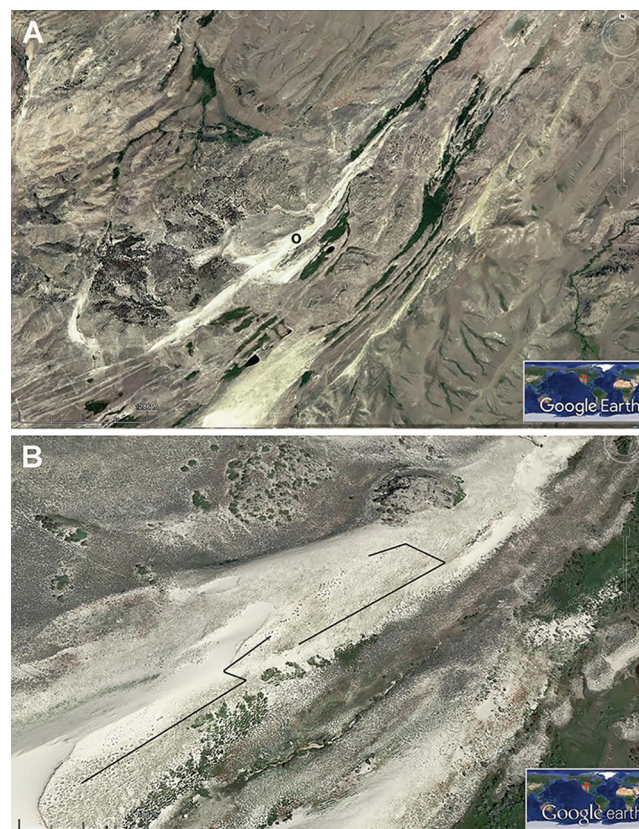
where dune encroachment is a serious management problem (Li et al. 2004; Yan et al. 2005; Liu et al. 2007; Ma and Liu 2008). The harsh abiotic environment of sand dunes imposes many stresses on plants, including low nutrient status, rapidly drying surface layers, and abrasion by windblown sand. Perhaps the pre-eminent stress faced by plants in these environments is sand movement, which poses the dual threat of shoot burial and root excavation.

Adaptations that permit herbaceous perennial plants to survive in the vegetative state on active dunes include the rhizomatous habit or the ability to root adventitiously from buried shoots and tolerate shoot burial. Plants may exhibit compensatory growth in response to burial, sandblasting, and wind, and may also make more efficient use of nutrients and water than plants of less stressful environments (Maun 1998). In spite of these adaptations, individual actively growing plants or local groups of plants may frequently succumb to the burial or excavation events that accompany progressive dune movement. In addition, when dunes become stabilized, the plants that are specialized for the active dune environment are at a competitive disadvantage and are frequently displaced during the process of succession (Lichter 2000). In order for a locally occurring active dune specialist to persist and migrate in response to this shifting mosaic of suitable habitat, a mechanism for recruitment from seed after loss of adult plants must be in place.

Adaptations of sand dune specialists that function during establishment include the ability to emerge from deep burial, an adaptive trait often associated with large seed size (Buckley 1982). Equally important is the ability of seeds to sense depth of burial and respond adaptively. The same sand movement that poses a challenge for established plants can provide the opportunity for a deeply buried seed to be placed in a more advantageous position closer to the surface, if it can remain ungerminated but viable under deep burial conditions. Thus seed germination regulation and seed bank dynamics may be key components of the life history strategy of herbaceous plants that are dune specialists. It remains an open question whether persistent seed banks are important in population persistence for these species (Liu et al. 2007).

Sand dune environments occur across the full gamut of climatic regimes, and particular climatic regimes can add their own challenges to the survival of dune-adapted plants. In this study, we examined the potential importance of the seed bank for the federally endangered dune endemic *Penstemon haydenii* S. Watson (Plantaginaceae) in a stressful climatic environment in the Ferris Dunes, Carbon County, south-central Wyoming (Figs. 1A and 2A). This species was first discovered in Wyoming in 1996; previously it was known only from the Nebraska sandhills, where it occurs under considerably less stressful climatic conditions (Fig. 2B). The species was listed as

Fig. 1. (A) Aerial photograph of the Bear Mountain study location showing the active sand dune that supports a large population of *Penstemon haydenii*, with open circle showing approximate transect area. (B) Close-up of central part of the dune showing the location and orientation of the 400 m transect that was used for monitoring sand movement and for *P. haydenii* in situ seed bank and reproductive output studies (Landsat imagery, Google Earth). [Colour online.]

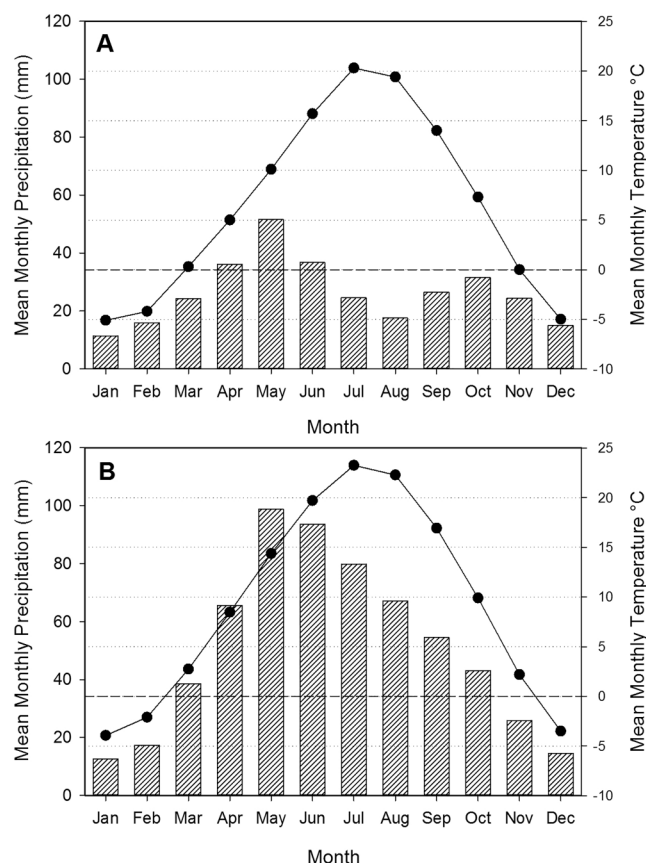


endangered because of habitat loss in the Nebraska sandhills associated with progressive dune stabilization (USFWS 1987; Stubbendieck et al. 1989).

Penstemon haydenii is a nonclonal herbaceous perennial plant (Stubbendieck et al. 1993). It can survive prolonged burial and can root adventitiously from buried stems (Kottas 2008; Heidel 2007, 2012), and is also known to exhibit compensatory growth in response to wind and sandblasting (Stubbendieck et al. 2010). It is likely relatively long-lived for a penstemon (>5–10 years), but life-span is difficult to determine accurately because living plants may survive prolonged burial (Heidel 2012). In Nebraska, the species characteristically occurs in blowouts, i.e., upwind of the dune rim (Stubbendieck et al. 1997), whereas in Wyoming it has primarily been found in the downwind active dune habitat (Fertig 2000; Heidel 2012).

Penstemon haydenii seeds from Wyoming are dormant at dispersal but are rendered completely nondormant under optimum conditions by a relatively short cold stratification of four weeks, suggesting field germination in early spring (Tilini et al. 2016). In a field seeding study

Fig. 2. Climogram showing monthly mean precipitation and mean temperature averages for the period 1980–2010 (A) at the Bear Mountain Study Site, and (B) in the Nebraska sandhills habitat of *Penstemon haydenii* (data from <http://www.prism.oregonstate.edu/explorer/>).



in Nebraska, Kottas (2008) indeed found that 99% of *P. haydenii* germination and emergence occurred in spring. Unlike most penstemons (Meyer et al. 1995), the seeds of *P. haydenii* do not germinate in prolonged chilling. Chilled seeds of *P. haydenii* require cool, alternating temperatures for germination (Tilini et al. 2016). An alternating temperature requirement represents one mechanism for sensing depth of burial (Thompson and Grime 1983) and would likely be advantageous in a dune environment.

The study reported here had the following objectives: (i) determine the relationship between sand movement along a dune transect and *P. haydenii* plant density; (ii) use measured components of *P. haydenii* reproductive output to estimate seed rain; (iii) relate in situ persistent seed bank density to sand movement, adult plant density, and seed rain; and (iv) determine the effects of burial at different depths on seed viability, dormancy, and germination, as well as on seedling emergence in a growth chamber experiment with chilled seeds.

Materials and methods

Study site characterization

This study was conducted on Bear Mountain, one of the largest areas of active sand dune occupied by *P. haydenii*

in the Ferris Dunes of Carbon Co., Wyoming (Fig. 1A). The Ferris Dunes consist of >300 km² of predominantly stabilized parabolic sand dunes downwind of the Great Basin Divide (Stokes and Gaylord 1993; Heidel et al. 2014). *Penstemon haydenii* occupies active sand dune slopes ranging in elevation from 1786–2270 m a.s.l. and occurs on soils consisting of psammments derived from wind-blown Quaternary alluvium (Munn and Arneson 1998). On Bear Mountain, the species is most numerous on the steep slip-faces of “sand streak” dunes that extend parallel to the prevailing wind direction (Stokes and Gaylord 1993; Fertig 2001). These dunes form where sand intercepts steep underlying topography, and commonly have >60% slopes (Heidel 2012).

Mean annual precipitation at the study site over the period 1980–2010 was 315 mm (Fig. 2A, <http://www.prism.oregonstate.edu/explorer/>). Most of the precipitation is received in late spring and summer. Winters are cold and dry, with mean monthly temperatures below 0 °C and very little snow, while summers are relatively temperate, with mean monthly temperatures from 10–20 °C and reasonably frequent rainfall. This is in contrast to the Nebraska sandhills habitat, which is much more mesic (mean annual precipitation 611 mm) and not as cold in winter (Fig. 2B).

Seed size characterization

Seeds of *P. haydenii* were compared with seeds of three other intermountain dune specialists as well as with two generalist penstemon species that represent extremes in seed size for dryland penstemons in the region. We used representative seed collections for each species on file at the USFS Shrub Sciences Laboratory, Provo, Utah, for these measurements. Mean seed mass is based on two replicates of 50 seeds for each collection, while 20 seeds were randomly selected for measurement to obtain a range for maximum seed diameter. Means separations for seed mass are from analysis of variance (ANOVA) on log-transformed data (SAS version 9.4 PROC GLM). Seeds were also photographed for visual comparison.

Measurement of short-term sand movement

We set up a transect line in May 2012 for the purpose of quantifying within-year sand movement along the Bear Mountain dune (Fig. 1B). The south-western three-quarters of the study transect ran along the slip-face of the downwind active dune. The transect then dog-legged back across the top of the dune into an area that was more densely vegetated and relatively stable. Along this transect we placed 55 sampling points, one point every 7 m. Each sampling point was marked by a 1 m plastic planting stake inserted approximately 0.5 m into the sand. Sampling points were labeled with metal tags attached to each stake. The height of each stake was recorded upon insertion and then measured at each visit to the site. This allowed us to determine whether the sampling point had been subjected to burial (lower stake

height) or erosion (greater stake height). Comparing the differences between the heights of the stakes at different points in time allowed us to measure sand movement at each point along the transect line and therefore to relate sand movement to plant density and seed bank estimates. We measured the heights of the stakes on five dates in May 2012, June 2012, July 2012, November 2012, and May 2013.

Plant density, seed rain, and reproductive success

We took a series of four measurements that were used to determine the number of viable seeds produced per unit area in a single season. In June 2012, a tape was used to extend 4.6 m on either side of the 400 m transect described earlier for a total area of approximately 3650 m². The total number of adult plants (i.e., plants that were not obviously juvenile) within this area was counted in each 7 m × 9.2 m plot as we moved from sampling point to sampling point. The ratio of reproductive to nonreproductive plants was obtained by scoring the number of reproductive plants out of a sample of 500 plants encountered along the transect in November 2012. To determine the number of stalks per reproductive plant, we counted the total number of stalks on up to three plants nearest to each sampling point within 4.6 m in July 2012. We then randomly collected one stalk from each of these plants, placed it in a labeled brown paper sack, and took it to the laboratory to determine the number of viable seeds per stalk.

In the laboratory, we also evaluated factors affecting reproductive success using the stalk collection described above. For each stalk, we measured length, number of filled, aborted and insect damaged capsules, and number of filled, aborted and insect-damaged seeds. These measurements allowed us to estimate both ovary (capsule) and ovule (seed) success. We counted as aborted only those capsules from buds that had actually produced flowers. Many more buds on most stalks aborted before opening; these were not included.

Seed rain per unit area was estimated by multiplying plant density by measured values of the following yield components: fraction of plants that flowered, mean stalk number per flowering plant, and mean number of filled seeds per stalk. This estimate is based on dividing the total number of filled seeds by the total number of stalks and gives a slightly different estimate for seed number per stalk than that obtained by multiplying mean capsule number per stalk by mean seed number per capsule.

In situ seed bank estimates

The persistent seed bank of *P. haydenii* was estimated in situ in July of 2012, prior to current-year seed dispersal. Four soil samples were taken at each sampling point along the transect line. However, one stake was completely unburied and lost before soil samples were taken and therefore this point was not included in the study. A total of 216 soil seed bank samples were obtained. Each

soil sample was taken by inserting a 0.1 m² square metal frame into the sand to a depth of approximately 10 cm. Sand was removed from the metal frame and screened on site using round metal sieves with a mesh size of 0.28 cm (7/64 inch). Seeds and debris left on the sieve were collected in a labeled bag and transported to the laboratory for further processing. *Penstemon haydenii* seeds found at each sampling point were counted and recorded. A total surface area of approximately 20 m² of soil was processed to a depth of 10 cm to estimate the persistent shallow soil seed bank. We also evaluated the viability of the seed bank seeds using tetrazolium staining (Grabe 1970). Our original plan was to sample additional depth increments, but this was not possible using our method because the sand was wet below 10 cm and could not be screened in the field.

Data collected on the presence or absence of a *P. haydenii* seed in each sample were analyzed in relation to both sand dune movement and adult *P. haydenii* plant density along the transect using logistic regression. We used linear regression to determine whether there was a significant relationship between sand movement and plant density. Analyses were performed using R 2.15.2 (R Development Core Team 2012).

Laboratory seed burial experiment

We used seeds harvested from plants as part of the seed rain study to perform a laboratory experiment aimed at determining whether burial depth in sand would affect the behavior and fate of *P. haydenii* seeds. After cleaning, seeds were stored in manila envelopes under laboratory conditions (20–22 °C, 6%–8% moisture content) for approximately 2 months before inclusion in burial experiments. Initial viability of the seed lot was determined to be 97% using tetrazolium staining ($n = 4$ replicates of 50 seeds; Grabe 1970).

To break primary dormancy, seeds intended for this study were placed on water-saturated germination blotters in 15 mm × 100 mm plastic Petri dishes. Dishes were then placed in a dark growth chamber at 2–4 °C for four weeks. Following moist chilling, imbibed seeds were removed from Petri dishes and subjected to one of six burial depth treatments: 1, 2, 4, 6, 8, or 10 cm. Seeds ($n = 50$) were placed in plastic planting pots (13 cm × 12 cm) and buried with wet sand from the Bear Mountain site to the assigned treatment depth. Pots were then placed in trays filled with wet sand and buried approximately half-way into the sand. This was done to reduce any oxygen contamination from drainage holes in the bottoms of the pots. The trays with the pots were then placed into a 10–20 °C growth chamber (12 h photoperiod with light at the higher temperature). The four trays, each containing one pot of each of the six planting depth treatments, were treated as blocks in the statistical analysis. Pots were watered every other day to saturation and seedling emergence was recorded daily for six weeks. After the experiment was ended, seeds and unemerged seedlings

were exhumed for each burial depth increment by washing the sand through a fine sieve. Seeds were categorized as ungerminated or visibly nonviable and seedlings were classified as emerged or unemerged (i.e., seedlings that failed to emerge from germinated seeds).

Ungerminated seeds retrieved from each experimental unit in the burial experiment were then placed in 15 mm × 100 mm plastic Petri dishes on saturated germination blotters and placed back into 10–20 °C (12 h photoperiod) incubation for four weeks to test for burial-induced secondary dormancy. Dishes were scored for germination twice weekly. Seeds were categorized as germinated, dormant, or visibly nonviable. Seeds that experienced suppressed germination in burial but then germinated in post-burial incubation were considered to have been in a state of enforced dormancy. Seeds remaining dormant in post-burial incubation were considered to be in a state of secondary dormancy. These secondarily dormant seeds were re-chilled for four weeks (2–4 °C) in an effort to break this dormancy. Following four weeks of re-chilling, these seeds were placed back into 10–20 °C incubation and read twice a week for germination as before. As many of the remaining viable seeds were still dormant after this treatment, we decided to allow the seeds to air-dry at 20 °C for eight weeks to simulate a dry season followed by another round of chilling and post-chilling incubation as described above. Seeds were scored as germinated, dormant, or nonviable (negative tetrazolium staining).

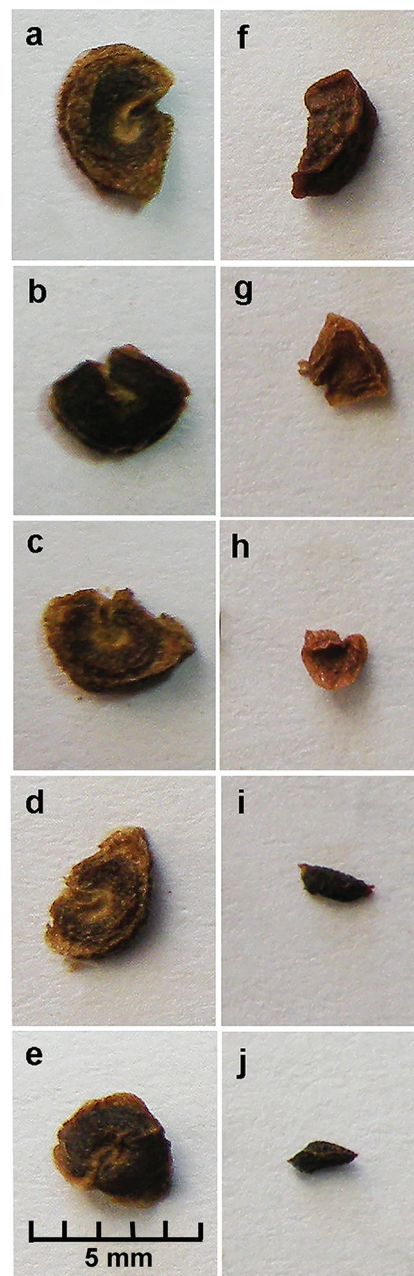
Data were expressed for each experimental unit as proportion of total seeds planted. The response variables were emerged seedlings, germinated but unemerged seedlings, seeds in enforced dormancy (germinated post-burial without additional treatment), seeds in secondary dormancy that responded to chilling, seeds in secondary dormancy that responded to drying-chilling, seeds that remained dormant following chilling treatments, and seeds that were recorded as nonviable (cumulative across all experimental treatments). We also analyzed the chilling data after combining the treatments, i.e., post-chilling plus post-drying chilling. The independent variable in each analysis was depth of burial. Proportional data were arcsine square root transformed to improve homogeneity of variance prior to analysis. All data were analyzed as mixed model randomized block designs in SAS version 9.4 (PROC MIXED).

Results

Seed size study

We detected no pattern for larger, heavier seeds in dune endemic species overall, indicating that large seed size is not a necessary condition for success on sand dunes (Fig. 3; Table 1). *Penstemon haydenii* clearly had the largest, heaviest seeds, however, and to our knowledge it has the largest seeds of any intermountain penstemon. The horseshoe-shaped seeds are flattened and stacked

Fig. 3. *Penstemon haydenii* seeds (a–e), showing the range of typical seed shape and size, compared with seeds of generalist dryland penstemons *P. pachyphyllus* (f) and *P. palmeri* (j) and dune specialist penstemons *P. angustifolius* var. *dulcis* (g), *P. acuminatus* (h), and *P. ambiguus* (i). All photographs are to scale. See Table 1 for seed mass information. [Colour online.]



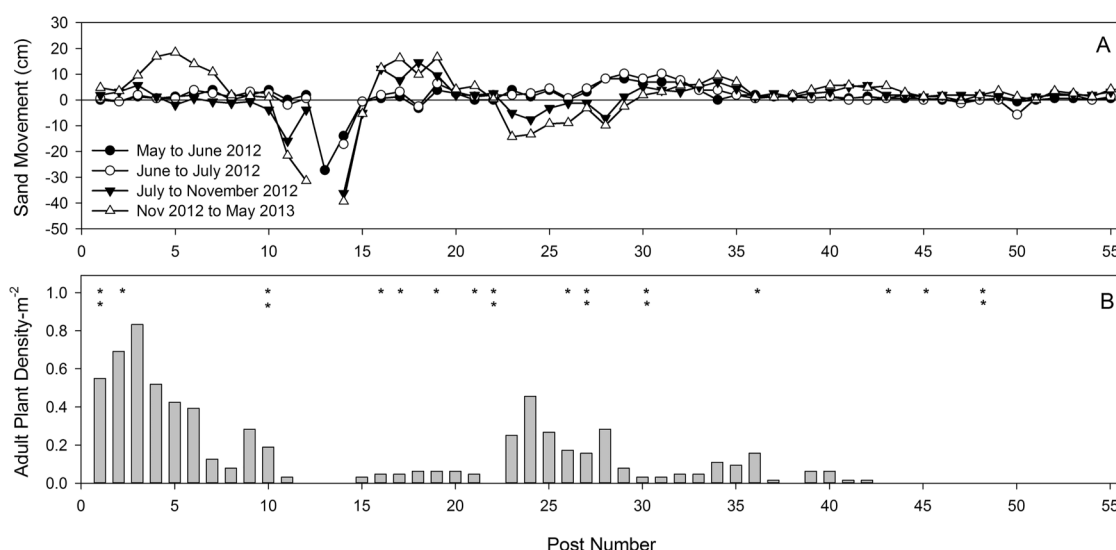
into the two-chambered capsule in a manner reminiscent of yucca seeds. This is in contrast to other large-seeded species, which have irregularly polyhedral seeds (e.g., *P. pachyphyllus*, Fig. 3f). The seeds of *P. haydenii* also possess a more or less distinct membranous wing along the outer rim. These features may compensate for large size in facilitating dispersal by wind, a clear advantage in the shifting dune habitat.

Table 1. Mean seed mass and maximum diameter range for *Penstemon haydenii*, three other dune specialist penstemons, and two generalist species that represent the seed size range of typical dryland penstemon sp.

<i>Penstemon</i> sp.	Collection location	Seed mass (mg) mean $\pm$ SE	Seed max. diam. range (mm)
Dune specialists			
<i>P. haydenii</i>	Ferris Dunes, WY	4.66 $\pm$ 0.08a	3.5–5.0
<i>P. angustifolius</i> var. <i>dulcis</i>	Jericho Dunes, UT	1.17 $\pm$ 0.09c	2.0–3.0
<i>P. acuminatus</i>	Bruneau Dunes, ID	0.79 $\pm$ 0.04d	1.5–2.5
<i>P. ambiguus</i>	Big Water Dunes, AZ	0.91 $\pm$ 0.02d	2.0–3.0
Dryland habitat generalists			
<i>P. pachyphyllus</i>	Burr Trail, UT	2.21 $\pm$ 0.06b	2.5–3.5
<i>P. palmeri</i>	Mt. Carmel Hwy, UT	0.80 $\pm$ 0.01d	1.5–2.0

Note: Values for seed mass followed by the same letter are not statistically different ($P < 0.05$) based on LSMeans separation from analysis of variance. See Fig. 3 for photographs showing seed size and shape for each species. WY, Wyoming; UT, Utah; ID, Idaho; AZ, Arizona.

Fig. 4. (A) Measured sand movement at each of the 55 measurement stations on the Bear Mountain Dune during four periods from May 2012 through May 2013. Movement is expressed relative to the original height of measurement stakes, which are numbered from southwest to northeast (Fig. 1B). (B) *Penstemon haydenii* plant density in plots centered on each of the 55 measurement stakes along the sample transect (bars) and location of *P. haydenii* seeds detected in seed bank sampling (asterisks).



Short-term sand movement

Along the southwestern part of the 400 m transect, which traversed the steep slip-face of the active dune, sand movement alternated between accretion and deflation, with one area, between posts 10 and 16, experiencing dramatic excavation (Fig. 4A). Along the remainder of this section, net movement over 12 months ranged from ca. 15 cm of deflation to ca. 20 cm of accretion. Most of the sand movement took place in winter and spring. Along the dogleg part of the transect at the northeastern end, where vegetation had stabilized the sand, there was minimal sand movement during any season.

Plant density, reproductive success, and seed rain

Plant density in the sampled area averaged 0.126 adult plants per square metre (Table 2), but the plants were not evenly distributed along the 400 m transect (Fig. 4B). There was no direct relationship between net sand move-

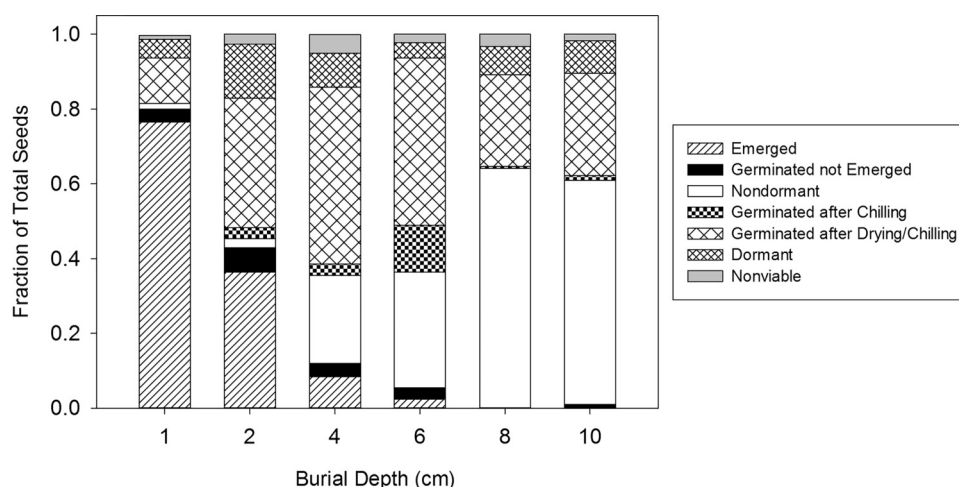
ment at a post and plant density within the surrounding plot (linear regression; $P = 0.781$). However, there were no adult plants present at the northeast end of the transect (Fig. 1B), where the sand dune had nearly stabilized and almost no net sand movement was measured. Plants were also absent from plots surrounding posts 11–14, where erosion was most severe, but regularly occurred where sand movement was moderate, whether accretion or deflation (Figs. 4A and 4B).

The seed stalks of *P. haydenii* averaged 6.23 cm in length in 2012 at Bear Mountain and bore an average of 6.08 filled capsules (Table 2). Capsule abortion rate was high; only about one-third of the flowers produced capsules with viable seeds. Only a few capsules were completely ravaged by pre-dispersal insect predators in 2012. Seed set was considerably higher than fruit set, averaging about two-thirds of the ovules in a capsule. Insect

Table 2. Reproductive output components and seed rain estimate for *Penstemon haydenii* at the Bear Mountain study site in 2012.

Parameter	Mean	SE	%	Sample (n)
Plants·m ⁻²	0.126	1.60	—	55 plots (459 plants)
Reproductive plant fraction	0.613	0.73	—	15 plots (500 plants)
Stalks per reproductive plant	2.61	0.35	—	71 reproductive plants
Stalk length (cm)	6.28	0.34	—	73 stalks
<i>Capsules per stalk</i>				
Filled	6.08	0.62	34.5	73 stalks
Aborted	10.88	1.08	61.8	73 stalks
Insect damaged	0.66	0.30	3.7	73 stalks
<i>Seeds per filled capsule</i>				
Filled	10.74	0.73	63.4	63 stalks*
Aborted	5.04	0.35	29.7	63 stalks*
Insect damaged	1.17	0.17	6.9	63 stalks*
Filled seeds per stalk	70.01	8.31	—	73 stalks
Filled seeds·m ⁻²	14.11	—	—	—

*Stalks with no filled capsules were not included in the calculations.

Fig. 5. Fate of *Penstemon haydenii* seeds during a six week growth chamber sand burial experiment and subsequent post-burial incubation treatment. See Table 3 for statistical analyses.

predation was again relatively low. Using these estimates, the average filled seed production per flowering plant at Bear Mountain in 2012 was 170 seeds per plant, approximately 22% of the maximum seed production possible given mean capsule and ovule numbers per plant. Tetrazolium staining determined that 97% of filled seeds were viable. Seed rain in 2012 was estimated at 14.11 seeds·m⁻² using the mean number of seeds per stalk as the multiplier, and at 13.16 seeds·m⁻² when all the yield components were included as multipliers (Table 2).

In situ seed bank estimates

A total of 21 *P. haydenii* seeds were recovered from the sampled surface area of approximately 20 m². Only two of the seeds were viable, for an estimate of approximately one viable seed per 10 m² of surface area in the shallow persistent seed bank for this population of *P. haydenii* in summer 2012. There was no significant relationship between seed presence/absence and average

plant density in the surrounding plot even when nonviable seeds were included (logistic regression; $P = 0.78$).

Average net sand movement for posts with samples that had any seed present, whether viable or nonviable, was slightly greater (2.8 cm.) than for posts with samples without a seed present (0.5 cm). However, this relationship was also not statistically significant (logistic regression; $P = 0.199$). These results suggest that very few viable seeds of *P. haydenii* persist across years in the surface layer of the sand (<10 cm depth), and that even nonviable seeds are present at very low densities. This resulted in an essentially random distribution of seeds in the surface soil relative to the presence of adult plants and also relative to the net amount of sand movement (Figs. 4A and 4B).

Laboratory seed burial experiment

Seeds emerged successfully in the burial experiment only from the shallower planting depths, with little or

Table 3. Analysis of variance of the growth chamber experiment on *Penstemon haydenii* seed fate after sand burial at different depths using SAS 9.4 Proc Mixed for a randomized block design.

Response variable	df	F value	P value
Emerged	5,15	25.85	<0.0001
Germinated, not emerged	5,15	2.91	0.0498
Nondormant	5,15	35.39	<0.0001
Germinated after chilling	5,15	3.07	0.0420
Germinated after drying/chilling	5,15	2.22	0.1064
Germinated after chilling, combined	5,15	3.14	0.0389
Dormant	5,15	1.61	0.2171
Nonviable	5,15	0.62	0.6878

no emergence from depths >4 cm, and emergence decreased progressively as a function of depth (Fig. 5; Table 3). Very few seeds germinated but failed to emerge, and there was no clear pattern with depth. Most of the remaining ungerminated seeds were viable. Their dormancy status varied as a function of depth. Ungerminated seeds closer to the surface were more likely to enter secondary dormancy than seeds buried more deeply, most of which germinated without further treatment after exhumation. Most of the seeds that entered secondary dormancy during burial were released from dormancy through chilling. Chilling without a period of desiccation was much less effective (<3% average germination) than chilling that followed an eight-week desiccation period (32% average germination). After the desiccation plus chilling treatment, there was still a fraction of seeds that remained in secondary dormancy (8% average), but there was no interpretable pattern as a function of burial depth. Even at the shallow planting depth of 1 cm, almost 20% of the seeds failed to germinate, and most of these entered secondary dormancy that could be broken by desiccation plus chilling. Essentially no seeds died during the experiment, as the average nonviable seed percentage (3%) was similar to the nonviable percentage in the seed lot prior to initiation (97% viability as determined by tetrazolium testing, reported earlier).

Discussion

Seed bank dynamics

The species has been thought to capable of forming persistent seed banks (Stubbendieck et al. 1997), and there is some field data to support this claim. In a retrieval experiment with artificial burial at 22 cm in the Nebraska sandhills, >50% of *P. haydenii* seeds remained viable and ungerminated after three winters (Kottas 2008).

In an artificial seeding experiment with protection from predation in the Nebraska sandhills, Kottas (2008) found that surface-sown seeds experienced varying degrees of burial both prior to germination and after seedling emergence, which ranged from 14% to 50% across three sowing years, while viable seed remaining in the soil after emergence the first spring ranged from 11% to

30%. This demonstrates the ability of seed sown on the surface in a manner mimicking natural dispersal to persist in the seed bank at least one year. An estimated 57%–60% of originally viable seed germinated the first year.

Our burial experiment suggests that *P. haydenii* has mechanisms for ensuring both appropriate within-year germination timing and carryover of seeds across years. First, seeds placed close to the surface can germinate and produce emerged seedlings under spring conditions (chilling followed by cool, alternating temperature). Second, most seeds placed too deep to germinate are held in enforced dormancy at least until sand movement again exposes them to germination-permissive conditions. And third, even seeds placed at depths where considerable emergence is possible can enter secondary dormancy that is relieved by desiccation plus chilling, a scenario that mimics seed carryover until the subsequent spring. Even for more deeply buried seeds, a sizeable fraction entered secondary dormancy and is thus likely to carry over across years. Lastly, not all the seeds rendered secondarily dormant were released from dormancy even by the desiccation plus chilling treatment, suggesting that carryover across multiple years is possible.

The burial experimental results also suggested that viable seeds should be able to carry over across years at depths <10 cm, yet our field seed bank study failed to detect many seeds in this surface layer. We kept the sand in our experiment continuously saturated, but this is not likely to occur under field conditions at Bear Mountain, where winters are very dry (Fig. 2A) and sand movement is considerable (Fig. 4A). The physics of water movement in sand is such that the surface layer dries rapidly and acts as a mulch to keep the lower layers close to saturation (Modaihsh et al. 1985). As this drying front moves progressively downward, there is usually a relatively sharp boundary between saturated and dry sand (Shokri et al. 2008). This means that seeds could be fully imbibed at a given depth, but much of the overlying sand could be dry. A major factor thought to maintain buried seeds in enforced dormancy is low oxygen availability (Benvenuti and Macchia 1995). The saturated sand in our experiment probably limited oxygen to laboratory seeds more than would be experienced by seeds at the same depth in the field, which likely would be in relatively close proximity to oxygen-rich dry sand. This means that field seeds would be much more likely to germinate at a given depth than seeds in the laboratory experiment. Because of their relatively large size, coupled with the decreased resistance to emergence offered by a layer of dry sand, germinated *P. haydenii* seeds could potentially emerge from depths that were completely suppressive to germination in the laboratory, thus depleting the seed bank in the 10 cm layer. Confirmation of the hypothesis that surface drying enhances germination of buried *P. haydenii* seeds would require seed burial experiments in the field. In

any case, our failure to find many seeds in the top 10 cm does not negate the possibility of a more deeply buried persistent seed bank. Most of the persistent seed bank on active dunes is likely to be buried much more deeply (Liu et al. 2007).

Seeds buried more shallowly in our experiment were more likely to enter secondary dormancy, while those buried more deeply were more likely to experience only enforced dormancy. Some of the constraints thought to enforce dormancy of buried seeds include anoxia as mentioned earlier (Benvenuti and Macchia 1995), darkness (Baskin and Baskin 1985), and suppressive temperature conditions (e.g., temperatures too high or too low for germination, or constant temperature conditions for seeds requiring alternating temperature for germination; Baskin and Baskin 1985; Ghera et al. 1992). The same conditions that enforce dormancy of buried seeds can often lead to secondary dormancy, especially with prolonged exposure (Ghera et al. 1992; Zhan and Maun 1994). We hypothesize that perhaps relatively high levels of oxygen are required to induce secondary dormancy in darkness in this species, so that seeds deeply buried in saturated sand and held in enforced dormancy through anoxia would not be as likely to enter secondary dormancy as those closer to the surface. Whether these shallowly buried seeds germinate or enter secondary dormancy might involve two competing processes that proceed at different rates in individual seeds.

The patterns observed in the laboratory suggest that buried *P. haydenii* seeds may experience a seasonal pattern of cyclic dormancy change that potentially contributes to the maintenance of a persistent seed bank. Such cyclic changes in dormancy status are well-documented in annual plants, especially the weeds of arable lands that experience repeated burial and exhumation (Baskin and Baskin 1985). Seeds of perennial plants are much less frequently found to exhibit cyclic dormancy, but the short-lived perennial *Penstemon palmeri* was found to show a seasonal cyclic dormancy pattern (Meyer and Kitchen 1992). The apparent pattern in *P. haydenii* resembles that of a spring annual, with seeds dormant at dispersal but rendered nondormant under winter conditions, restricting their germination to spring. Remaining seeds enter secondary dormancy removed through the sequential cues of summer drying and winter chilling, thus restricting germination of carryover seeds to spring as well.

Reproductive output

Our study supports the work of Tepedino et al. (2007) in demonstrating that *P. haydenii* can successfully set considerable quantities of high-quality seeds even in the harsh environment of the Great Divide Basin in Wyoming. In our 2012 study of naturally pollinated plants, both fruit set and seeds per capsule (Table 2) were lower than in the Tepedino study, which was carried out in 2005, a year of exceptionally high flowering in Wyoming

(Heidel 2012). In comparing reproductive output studies from Nebraska (Tepedino et al. 2006) and Wyoming, the most conspicuous reproductive output difference was in inflorescence height and consequently in number of flowers per inflorescence. This difference was noted by Heidel (2012) and quantified for the Bear Mountain population in the present study. Kottas (2008) reported inflorescence heights of 13–21 cm for Nebraska plants depending on population and year, while in our study inflorescence height (stalk length) averaged only 6.3 cm (Table 2). The shorter inflorescences in Wyoming appear to be related to a more dwarf habit overall, which could be an adaptive response to the harsher abiotic conditions (Figs. 2A and 2B). Genetic dwarfing is a frequently noted phenomenon in high elevation plants relative to their congeners from lower elevation (Körner 2003). Estimated mean seed output per plant was correspondingly lower for the Bear Mountain population in 2012 (170; Table 2) than for Nebraska populations (300–2000; Tepedino et al. 2006; Kottas 2008). Our reproductive output estimate is from a single year and may not be representative of longer term patterns.

We conclude based on results of our study that population persistence of *P. haydenii* in the shifting spatial mosaic of suitable and unsuitable habitat that characterizes active sand dunes is likely enhanced by the maintenance of a persistent seed bank, but expanded sampling at both vertical and spatial scales would be required to confirm this in the field. Seed bank persistence is likely an important life history feature of many other active sand dune species, but detailed studies are available for very few (e.g., Liu et al. 2007). Our approach using field-screening enabled us to evaluate a much larger surface area for the presence of carryover seeds than the usual core techniques, but we were limited in our ability to detect seeds at greater depths.

Acknowledgements

This project was funded in part by a grant from the USDI Bureau of Land Management Wyoming State Office. We thank Frank Blomquist of the BLM Rawlins Field Office and Bonnie Heidel of the Wyoming Natural Heritage Program for seed collection and other logistical support, and students at Brigham Young University for help in the field.

References

- Baskin, J.M., and Baskin, C.C. 1985. The annual dormancy cycle in buried weed seeds: a continuum. *Bioscience*, **35**: 492–498. doi:10.2307/1309817.
- Benvenuti, S., and Macchia, M. 1995. Effect of hypoxia on buried weed seed germination. *Weed Res.* **35**: 343–351. doi:10.1111/j.1365-3180.1995.tb01629.x.
- Buckley, R.C. 1982. Seed size and seedling establishment in tropical arid dunecrest plants. *Biotropica*, **14**: 314–315. doi:10.2307/2388093.
- Fertig, W. 2000. Status of blowout penstemon (*Penstemon haydenii*) in Wyoming. Unpublished report prepared for the Wyoming Cooperative Fish and Wildlife Research Unit, U.S. Fish and

- Wildlife Service, and Wyoming Game and Fish Department by the Wyoming Natural Diversity Database, Laramie, Wyo.
- Fertig, W. 2001. 2000 survey for blowout penstemon (*Penstemon haydenii*) in Wyoming. Prepared for the Bureau of Land Management Wyoming State Office by the Wyoming Natural Diversity Database, University of Wyoming, Laramie, Wyo.
- Ghersa, C.M., Benesh Arnold, R.L., and Martinez-Ghersa, M.A. 1992. The role of fluctuating temperatures in germination and establishment of *Sorghum halepense*. Regulation of germination at increasing depths. *Funct. Ecol.* **6**: 460–468. doi:10.2307/2389284.
- Grabe, D.F. (Editor). 1970. Tetrazolium testing handbook for agricultural seeds. Handbook on seed testing. Association of Official Seed Analysts, Springfield, Ill. 62 p.
- Heidel, B. 2007. *Penstemon haydenii* (blowout penstemon) final monitoring report 2004–2006. Carbon County, Wyoming. Prepared for the Bureau of Land Management — Rawlins Field, Rock Springs Field, and Wyoming State Offices. Wyoming Natural Diversity Database, University of Wyoming, Laramie, Wyo.
- Heidel, B. 2012. Status of *Penstemon haydenii* (Blowout Penstemon) in Wyoming - 2012. Prepared for the Bureau of Land Management — Rawlins Field, Rock Springs Field, and Wyoming State Offices. Wyoming Natural Diversity Database, University of Wyoming, Laramie, Wyo.
- Heidel, B., Cox, S., and Blomquist, F. 2014. Dune habitat trends of an endangered species, *Penstemon haydenii* (blowout penstemon) in Wyoming. Bureau of Land Management Technical Report in cooperation with Wyoming Natural Diversity Database.
- Körner, C. 2003. Alpine plant life: functional plant ecology of high mountain ecosystems. 2nd ed. Springer Verlag, Berlin, Germany. 359 p.
- Kottas, K.L. 2008. Life history and modeling of an endangered plant, *Penstemon haydenii*. Ph.D. dissertation, University of Nebraska, Lincoln, Neb.
- Li, Y., Cui, J., Zhao, X., and Zhao, H. 2004. Floristic composition of vegetation and the soil seed bank in different types of dunes of Kerquin Steppe. *Arid Land Res. Manage.* **18**: 283–293. doi:10.1080/15324980490451410.
- Lichter, J. 2000. Colonization constraints during primary succession on coastal Lake Michigan sand dunes. *J. Ecol.* **88**: 825–839. doi:10.1046/j.1365-2745.2000.00503.x.
- Liu, Z., Yan, Q., Liu, B., Ma, J., and Luo, Y. 2007. Persistent soil seed bank in *Agriophyllum squarrosum* (Chenopodiaceae) in a deep sand profile: Variation along a transect of an active sand dune. *J. Arid Environ.* **71**: 236–242. doi:10.1016/j.jaridenv.2007.03.003.
- Ma, J., and Liu, Z. 2008. Spatiotemporal pattern of seed bank in the annual psammophyte *Agriophyllum squarrosum* Moq. (Chenopodiaceae) on the active sand dunes of northeastern Inner Mongolia, China. *Plant Soil*, **311**: 97–107.
- Maun, M.A. 1998. Adaptations of plants to burial in coastal sand dunes. *Can. J. Bot.* **76**(5): 713–738. doi:10.1139/b98-058.
- Meyer, S.E., and Kitchen, S.G. 1992. Cyclic seed dormancy in the short-lived perennial *Penstemon palmeri*. *J. Ecol.* **80**: 115–122. doi:10.2307/2261068.
- Meyer, S.E., Kitchen, S.G., and Carlson, S.L. 1995. Seed germination timing patterns in Intermountain *Penstemon* (Scrophulariaceae). *Am. J. Bot.* **82**: 377–389. doi:10.2307/2445584.
- Modaihsh, A.S., Horton, R., and Kirkham, D. 1985. Soil water evaporation suppression by sand mulches. *Soil Sci.* **139**: 357–361. doi:10.1097/00010694-198504000-00010.
- Munn, L.C., and Arneson, C.S. 1998. Soils of Wyoming: a digital statewide map at 1:500,000-scale. Agricultural Experiment Station Report B-1069. University of Wyoming, Laramie, Wyo.
- R Development Core Team. 2012. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. url: <http://www.R-project.org/>.
- Shokri, N., Lehmann, P., Vontobel, P., and Or, D. 2008. Drying front and water content dynamics during evaporation from sand delineated by neutron radiography. *Water Resour. Res.* **44**: W06418. doi:10.1029/2007WR006385.
- Stokes, S., and Gaylord, D.R. 1993. Optical dating of Holocene dune sands in the Ferris Dune Field, Wyoming. *Quat. Res.* **39**: 274–281. doi:10.1006/qres.1993.1034.
- Stubbendieck, J., Flessner, T.R., and Weedon, R.R. 1989. Blowouts in the Nebraska sandhills: the habitat of *Penstemon haydenii*. In *Proceedings of the Eleventh North American Prairie Conference*, Lincoln, Neb.
- Stubbendieck, J.L., Flessner, T.R., Butterfield, C.H., and Steuter, A.A. 1993. Establishment and survival of the endangered blowout penstemon. *Great Plains Res.* **3**: 3–19.
- Stubbendieck, J., Fitzgerald, J.B., and Lindgren, D.T. 1997. Blowout Penstemon, Nebraska's threatened and endangered species publication series. Nebraska Game and Parks Commission, Lincoln, Neb.
- Stubbendieck, J., Schmidt, C.D., Hillhouse, H.L., and Landholt, L.M. 2010. Influences of wind and sandblasting on the endangered blowout penstemon. *Endang. Species Res.* **9**: 99–104. doi:10.3354/esr00246.
- Tepedino, V.J., Bowlin, W.R., and Griswold, T.L. 2006. Pollination biology of the endangered Blowout Penstemon (*Penstemon haydenii* S. Wats.: Scrophulariaceae) in Nebraska. *Bull. Torrey Bot. Soc.* **133**: 548–559. doi:10.3159/1095-5674(2006)133[548:PBOTEJ]2.0.CO;2.
- Tepedino, V.J., Toler, T.R., Bradley, B.A., Hawk, J.L., and Griswold, T.J. 2007. Pollination biology of a disjunct population of the endangered sandhills endemic *Penstemon haydenii* S. Wats. (Scrophulariaceae) in Wyoming, USA. *Plant Ecol.* **193**: 59–69. doi:10.1007/s11258-006-9248-7.
- Thompson, K., and Grime, J.P. 1983. A comparative study of germination responses to diurnally-fluctuating temperatures. *J. Appl. Ecol.* **20**: 141–156. doi:10.2307/2403382.
- Tilini, K.L., Meyer, S.E., and Allen, P.S. 2016. Breaking primary seed dormancy in Gibbens' beardtongue (*Penstemon gibbensii*) and blowout penstemon (*Penstemon haydenii*). *Native Plants J.* **17**: 256–266. doi:10.3368/npj.17.3.256.
- United States Fish and Wildlife Service (USFWS). 1987. Endangered and threatened wildlife and plants: Final rule to determine *Penstemon haydenii* (blowout penstemon) to be an endangered species. *Fed. Regist.* **82**(189): 32926–32929.
- Yan, Q., Liu, Z., Zhu, J., Luo, Y., Wang, H., and Jiang, D. 2005. Structure, pattern and mechanisms of formation of seed banks in sand dune systems in northeastern Inner Mongolia, China. *Plant Soil*, **277**: 175–184. doi:10.1007/s11104-005-6836-6.
- Zhan, J., and Maun, M.A. 1994. Potential for seed bank formation in seven Great Lakes sand dune species. *Am. J. Bot.* **81**: 387–394. doi:10.2307/2445486.